Proceedings of the 49th Annual Canadian Ecotoxicity Workshop: October 2 – 5, 2023, Ottawa, Ontario

Carrie Rickwood, Rebecca Dalton, Stacey Robinson, Leana Van der Vliet, Sarah Marteinson, Jennifer Miller

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by

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TABLE OF CONTENTS

PL = Platform presentation; PO = Poster presentation

ABSTRACT ..... **xxx**

RÉSUMÉ ..... **xxxi**

Editors' comments **1**

Remarques des éditeurs **1**

49th Canadian Ecotoxicity Workshop Organizing Committee / Comité organisateur du 49e atelier canadien sur l'écotoxicité **2**

Sponsors / Commanditaires **4**

Dr. Richard C. Playle Awards for Outstanding Theses in Ecotoxicology **1**

M.Sc. Winner: Multigenerational effects of the novel brominated flame retardant 1,2,5,6-tetrabromocyclooctane on reproductive performance in Japanese medaka (*Oryzias latipes*) 1

Chloe Devoy[1]

B.Sc. Winner: Assessing cytotoxicity of legacy and emerging antimicrobial compounds in rainbow trout (*Oncorhynchus mykiss*) RTgill-W1 gill cells 1

Chantel De Lange[1], Hannah Mahoney[1], Markus Brinkmann[1], Natacha Hogan[1]

New Methods and Novel Approaches for Assessing and Monitoring Environmental Contaminant Mixtures or Individual Priority Substances **3**

Minding the (data) gap: Development of toxicity test methods with pulmonate freshwater snails (PL) 3

Ève Gilroy[1], David McNabney[1], Karyn Robichaud[1], Kallie Shires[1], Shelby Ravary[1], Dasha MacKay[1], Victor Pham-Ho[1], Ashley Ferns[1], Maria Villella[1]

High-throughput determination of hepatic clearance as a screening tool for bioaccumulation using isolated perfused trout livers and diverse mixtures (PL) 4

Matthew Schultz[1], Zoey Bourgeois[1], Jordan Comfort[1], Jonathan Challis[1], Jenna Cantin[1], Sophia Krause[2], Markus Brinkmann[1]

Canadian adaptation of the *Ceramium tenuicorne* ISO 10710 standard and preliminary evaluation using pulp and paper mill effluent (PL) 4

Carolyn Martinko[1], Leana Van der Vliet[1], Melanie Gallant[2], Sarah Costantini[2], Cintia Glasner Regis[2], Anna Sobaszek[2], Vanessa Chanes[2], Nadia Pajnic[2], Paige Cochrane[2], Lisa Taylor[2]

Evaluation of a novel toxicity test method with *Hyalella azteca* to optimize reproduction endpoints (PL) 5

Hufsa Khan[1,2], Adrienne Bartlett[2], Amanda Hedges[2], Ryan Prosser[1]

Methodological challenges when conducting sediment toxicity identification evaluations (TIEs): Lessons learned (PL) 6

Kathleen Stevack[1], Lisa Kennedy[1], Tara George[1]

Organic matter decomposition – A new microbial laboratory ISO standard (PL) 6

Stephanie Kvas[1], Ajith Samarajeewa[1], Lee Beaudette[1], Rick Scroggins[1], Juliska Princz[1]

From genes to functions: Illuminating microbial diversity and functional profiles in a highly impacted river system using next-generation sequencing techniques (PL) 7

Milena Esser[1], Cameron Hoggarth[1], Phillip Ankley[1], Helen Baulch[1], John Giesy[1,2,3], Markus Brinkmann[1], Markus Hecker[1]

Exploring environmental thresholds for aquaculture drugs: Assessing the toxicity of spiked sediments on sea urchins using a Central Composite Rotatable Design (PL) 8

Davide Asnicar[1], Lauren Jonah[2], Dounia Hamoutene[2], Benjamin de Jourdan[1]

A new approach methodology that integrates the zebrafish (*Danio rerio*) embryo acute toxicity test with behaviour, metabolic, and transcriptomic assays (PL) 8

Jason O'Brien[1], Jory Curry[1,2], Tyler Nguyen[1], Jan Mennigen[2]

Towards development of an *in vitro* assay of oocyte maturation inhibition to predict reproductive capacity of fishes (PL) 9

Chloe Devoy[1], Yamin Raza[1], Justin Miller[1], Darren VanEssen[1], Dani Jakovljevic[1], Justin Dubiel[1], Makenna Kleiner[1], Markus Brinkmann[2], Paul Jones[2], Jon Doering[3], Steve Wiseman[1]

Low-dose methylmercury promotes prenatal neuronal differentiation and autism spectrum disorder-like behaviours in early adulthood (PL) 10

Allison Loan[1,2], Joseph Wai-Hin Leung[1,2], Chelsea Ko[1,2], David Cook[1,2], Barbara Vanderhyden[1,2], Jing Wang[1,2], Hing Man Chan[2]

Characterization of molecular and apical effects of legacy contaminated groundwater in fathead minnows using the EcoToxChip system (PL) 10

Markus Hecker[1], Bradley Park[2], Laura Gasque-Belz[2], Steven Siciliano[1]

Towards establishing a 24-hour, microplate-based, transcriptomics assay for rainbow trout embryos (PL) 11

Aylish Marshall[1], Hugo Marchand[1], Emily Boulanger[1], Krittika Mittal[1], Jessica Head[1], Niladri Basu[1]

EcoToxChips as a new approach method in chemical toxicity assessment for amphibians (PL) 12

Natacha Hogan[1], Carly Colville[1], Emily Boulanger[2], Jessica Ewald[2]

In vitro screening of UV stabilizers and UV filters: Cytotoxicity, CYP1A activity, and mRNA expression in an immortalized embryonic double-crested cormorant cell line (PL) 13

Tasnia Sharin[1,2], Ramela Koumrouyan[1], Jessica Head[1], Doug Crump[2]

Performance of Japanese quail EcoToxChips in a multi-lab ring test (PL) 13

Jessica Head[1], Elizabeth Brandt[2], Emily Boulanger[1], Natalie Karouna[2]

Incorporation of new approach methods into species sensitivity distributions for ecological risk assessment (PL) 14

Florence Pagé-Larivière[1], Rebecca Dalton[1], Daniel Villeneuve[2]

Addressing endocrine disruption in freshwater: A glimpse into developing recommendations to implement new monitoring methods into policy (PL) 15

Julie Robitaille[1]

Method development and application of walleye as a test species to evaluate the toxicity of constituents of concern from Alberta (PO) 15

Stephanie Schiffer[1], James Elphick[1]

The influence of salinity on the acute lethality of select metals and other deleterious substances to two marine invertebrates (PO) 16

Marriah Grey[1], Pam Howes[1], Melissa Thompson[1]

A high throughput *in vitro* assay using Ultraplex RNA sequencing to derive transcriptomic points of departure (PO) 16

Krittika Mittal[1]

Prospective optimization, evaluation, and application of *in vitro* methods to study biotransformation of organic chemicals in birds (PO) 17

Matthew Schultz[1], Michelle Embry[2], Robert Letcher[3], Christy Morrisey[1], Markus Brinkmann[1]

High-throughput transcriptomic and phenotypic assessments to characterize hazards for per-and poly-fluoroalkyl substances (PFAS) in zebrafish embryos (PO) 18

Hyojin Lee[1], John Stead[2], Andrew Williams[3], Jory Curry[1], Ella Atlas[3], Jan Mennigen[1], Carole Yauk[1]*, Jason O'Brien[4]**

Developing non-lethal techniques for identifying endocrine disruption in fishes (PO) 19

Emily Kennedy[1], David Janz[1], Markus Hecker[1]

The effects of hypoxia on fathead minnow behaviour and 'omics (PO) 19

Raina Hubley[1], Theresa Warriner[1], Aisha Pasha[1], Meghan Allen[1], Denina Simmons[1]

Impact of irradiated and non-irradiated very low sulphur fuel oils on the righting behaviour of common periwinkle snails (*Littorina littorea*) (PO) 20

Danielle Philibert[1]

Dietary exposure of stormwater contaminants in biofilm to two freshwater macroinvertebrates (PO) 21

Gab Izma[1], Moira Ijzerman[2], Melanie Raby[3], Ryan Prosser[2], Rebecca Rooney[1]

Aquatic mesocosms for environmental research: Insights from three studies in six years (PO) 21

Zhongzhi Chen[1], Jim Davies[1], Ryan Melnichuk[1], Brian Eaton[1]

The effects of contaminants on the taxonomic composition of micro-meiofauna present in biofilms (PO) 22

Eugénie Gardebled[1,2], Maelle Baillergeau[1], Antoine Tourret[1,2], Chloé Bonnineau[1], Nicolas Creusot[1], Jacky Vedrenne[1]

Refinement of soil nitrification assessment procedure using genomics tools (PO) 23

Ajith Samarajeewa[1], Derek Smith[1], Renuka Subasinghe[1], Peng Liu[1], Rick Scroggins[1], Juliska Princz[1]

Transcriptomic points of departure using EcoToxChips: A case study with early-life stage rainbow trout exposed to ethinyl estradiol for 24 hours (PO) 23

Emily Boulanger[1], Niladri Basu[1], Aylish Marshall[1], Hugo Marchand[1], Krittika Mittal[1], Jessica Ewald[1], Markus Hecker[2], Jianguo Xia[1], Jessica Head[1]

The use of Japanese quail EcoToxChips to improve the understanding of the mechanism(s) of action of hexabromocyclododecane (HBCD) in early life stage embryos (PO) 24

Emily Boulanger[1], Niladri Basu[1], Doug Crump[2], Jessica Head[1]

The use of Japanese quail EcoToxChips to improve the understanding of the mechanism(s) of action of ethinyl estradiol (EE2) in early life stage embryos (PO) 25

Emily Boulanger[1], Niladri Basu[1], Doug Crump[2], Jessica Head[1]

Incorporating Indigenous Perspectives into Environmental Management: The Importance of Two-Eyed Seeing 26

Bridging Indigenous and western knowledge systems: exploring the importance of Two-Eyed Seeing in environmental management (PL) 26

Jessica Wakefield[1], Karoliina Munter[2], Shyla O'Donnell[2]

Human health and ecological risk assessments in Indigenous communities: A survey of practitioners in Canada (PL) 26

Katherine Chong[1], Gordon Hickey[1], Niladri Basu[1]

Workshop on best practices for recognizing and including multiple ways of knowing in State of Environment reporting (PL) 27

Ashley Mahaffey[1], Mibu Fischer[2], Barry Hunter[3], Breanna Sayles[1], Paula Siwik[4], Kelly Munkittrick[1]

Development of a traditional foods study framework that prioritizes Indigenous community involvement (PL) 28

Mitchell Thorarinson[1], Kara Wolfe[1], Katherine Woolhouse[1], Katie Suek[1]

An academic's perspectives on science communication and teamwork when working with Moose Cree First Nation and Wildlife Conservation Society Canada (PL) 29

*Denina Simmons[1], Keisha Deoraj[1], Claire Farrell[2], Gretchen Lescord[3], Constance O'Connor[2], Jacob Seguin[2], Jennifer Simard[4], Moose Cree First Nation**

Wildlife Ecotoxicology: Exposure, Accumulation, and Effects 30

Target development for molybdenum using wildlife risk assessment techniques – A case study on the challenges associated with the use of uncertainty factors during guideline development (PL) 30

Brett Lucas[1], Colleen Hughes[2], Michelle DesJardins[2], Martin Davies[1]

Mercury exposure in relation to foraging ecology in breeding razorbills (PL) 30

Christina Petalas[1], Rose Lacombe[1], Kyle Elliott[1], Raphaël Lavoie[2]

Unveiling discrepancies in mercury biomagnification through compound-specific stable nitrogen isotope analysis and bulk methods (PL) 31

Rose Lacombe[1], Benjamin Barst[2], David Pelletier[3], Magella Guillemette[4], Marc Amyot[5], Kyle Elliott[1], Raphaël Lavoie[6]

Lead exposure of scavenging birds in Canada due to accidental ingestion of lead ammunition (PL) ... 32

John Chételat[1], Kirsty Gurney[1], Christine McClelland[1], John Elliott[1], Guy Fitzgerald[2], Megan Jones[3,4], Stéphane Lair[2,4], Shane de Solla[1], Fiep de Bie[3,4], Laura Bourque[3,4], Heather Fenton[5], Dayna Goldsmith[4,6], Jane Harms[7], Maud Henaff[7], Sandi Lee[1], Kristenn Magnusson[7], Jamille McLeod[1], Erin Moffatt[4,8], Kyla Rae[9], Leonard Shirose[4,10], Jordyn Stalwick[1], Marnie Zimmer[4,8]

Elements and essential fatty acids in fishes along a large, dammed river (PL) 32

Jenni Velichka[1], Karen Kidd[1,2], Kelly Munkittrick[2,3], Meera Shanmuganathan[1], Philip Britz-McKibbin[1], R. Allen Curry[2,4]

Ecological factors driving differences in PCB bioaccumulation in two Laurentian Great Lakes birds (PL) 33

Shane de Solla[1], Matthew Hammond[1], Kimberley Hughes[2], Craig Hebert[1], Doug Crump[1], Glenn Barrett[1], Kim Williams[1]

Long-term trends of organohalogenated contaminants and mercury in the great blue heron across the St. Lawrence River and Estuary (PL) 34

Raphaël Lavoie[1], Anaïs Kerric[1]

Multi-omic investigation of namew (lake sturgeon) health in an intact and an impacted watershed in the Moose River Basin (PL) 34

Keisha Deoraj[1], Jennifer Simard[2], Constance O'Connor[3], Claire Farrell[3], Jacob Seguin[3], Denina Simmons[1]

Pollutants of concern in aquatic mammals of Western Canada, their interaction with parasitic infection, and effects on host energetic condition (PL) 35

Kyle Shanebeck[1], Nicholas Yarmey[2], Clement Lagrue[1,3], Melissa Todd[4], Phillip Thomas[5], Stephanie Green[1]

A first detailed meta-assessment of PCBs, OCs, and brominated flame retardants in killer whales across the North Atlantic Ocean indicates that diet explains most variation (PL) 36

Anaïs Remili[1], Rune Dietz[2], Christian Sonne[2], Filipa Samarra[3], Robert Letcher[4], Audun Rikardsen[5], Lisa Kettemer[5], Steven Ferguson[6], Cortney Watt[6], Cory Matthews[6], Jeremy Kiszka[7], Aqqalu Rosing-Asvid[8], Melissa McKinney[1]

Tracking flame retardants in air from the back of a gull: The landfill effect (PL) 36

Jonathan Verreault[1], Anaïs Kerric[1], Manon Sorais[1], Marc Mazerolle[2], Jean-Francois Giroux[3]

Assessing the lethal and sublethal effects of oil sands process-affected water in early life stage wood frogs (*Lithobates sylvaticus*) (PL) 37

Katelyn Stenner[1], Blake Danis[1], Asfaw Bekele[2], Neal Tanna[3], Frank Gobas[1], Barry Kelly[1,4], Vicki Marlatt[1]

Quantifying insecticide exposure risk to barn swallows of Saskatchewan (PL) 38

Christy Morrissey[1], Sonia Cabezas[1,2], Dan Garrett[1], Mercy Harris[1], John Headley[2]

Exposure, repellency, and learned aversion to neonicotinoid-treated seeds in granivorous birds (PL) 38

Shuqi Ren[1], Christy Morrissey[1]

Modulatory effects of nutrition on neonicotinoid-induced developmental toxicity in tree swallows (*Tachycineta bicolor*) (PL) 39

Biyao Han[1], Sonia Cabezas[1], John Headley[2], Christy Morrissey[1]

Quantifying responses of American toad tadpoles to microplastics in the environment using an *in-situ* assay (PL) 40

Jihyun Kim[1], Jennifer Provencher[2], Barbara Katzenback[3], J. Drew Thompson[3], Matthew Hoffman[4], Michael Rennie[5], Chelsea Rochman[6], Diane Orihel[1]

Using transcriptomics and early life stage toxicity testing to assess the sensitivity of two avian species to UV absorbents (PL) 41

Ramela Koumrouyan[1], Tasnia Sharin[1,2], Doug Crump[2], Jessica Head[1]

Nontarget screening reveals the presence of multiple plastic related compounds in blubber of polar bear, killer whale, narwhal, and pilot whale from East Greenland (PL) 41

Adam Pedersen[1], Stéphane Bayen[1], Lan Liu[1], Rune Dietz[2], Christian Sonne[2], Aqqalu Rosing-Asvid[3], Steven Ferguson[4], Melissa McKinney[1]

Avian ecotoxicology effects assessment of resident and migratory breeding waterbirds at oil sands tailings ponds in Northern Alberta (PO) 42

Hanna Ulmer[1], Christy Morrissey[1]

Behavioural ecotoxicology of Canadian leeches: Species-specific reactions to UV light and to a pollutant (PO) 43

David Vergote[1], Matthieu Zolondek[1]

Mercury concentrations in marine bird eggs in Lake Melville before and after flooding of the Muskrat Falls hydroelectric reservoir (PO) 43

Margaret Eng[1], Neil Burgess[1]

The importance of riparian vegetation in agricultural ditches for the development and survival of northern leopard frog (*Lithobates pipiens*) tadpoles (PO) 44

Justin Marchand[1], Frances Pick[1], David Lapen[2], Stacey Robinson[3]

Assessing the health of whooping crane migratory wetlands in the Alberta oil sands region (PO) 45

Maureen Freemark[1], Mark Bidwell[1], Lukas Mundy[1], Bruce Pauli[1]

Assessing the health of wood frogs and boreal wetlands in the Athabasca oil sands region of Northern Alberta (PO) 45

Lukas Mundy[1], Danna Schock[2], Maureen Freemark[1], Bruce Pauli[1]

Emerging Contaminants in Wastewater Effluents: Exposure, Effects, and Possible Environmental Risk 47

Antibiotics and antimicrobials in wastewater treatment plants and environmental water samples (PL) 47

Jade Wish[1], Alistair Brown[1], Ayush Kumar[1], Annemieke Farenhorst[1]

Partitioning of micropollutants in biota and environmental samples collected in replicate artificial streams (PL) 47

Daniela Pulgarin-Zapata[1], Leslie Bragg[2], Patricija Marjan[3], Mark Servos[2], Kelly Munkittrick[3], Maricor Arlos[1]

Regional analysis of opioid consumption with wastewater-based epidemiology (PL) 48

Tyler Dow[1], Hélène LeBlanc[1], Denina Simmons[1]

Monitoring endocrine disrupting chemicals in wastewater with bioassays: A proof-of-concept from Québec, Canada (PL) 49

Julie Robitaille[1], Mélanie Desrosiers[2], Marianne Métivier[2], Éloïse Veilleux[2], Isabelle Guay[2], Valérie Langlois[1]

Progress and understanding of genotoxicity and mutagenicity testing of treated water for emerging contaminants (PL) 50

Will Lush[1], Mohammad Sarhan[1]

Direct and indirect effects of flowback and produced water (FPW) from hydraulic fracturing on California blackworm (*Lumbriculus variegatus*) and fathead minnows (*Pimephales promelas*) (PL) 50

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

Comparison of the sensitivity of early life stages of three North American fish species to antimicrobial chemicals (PL) 51

Evan Kohlman[1], Mawuli Amekor[1], Catherine Roberts[1], Niteesh Jain[1], Alper James Alcaraz[1], Junyi Lin[1], Markus Brinkmann[1], Markus Hecker[1], Natacha Hogan[1]

Impacts of antimicrobial exposure on the gut microbiome of early life stage fish: A chemical and species comparison (PL) 51

Phillip Ankley[1], Mawuli Amekor[1], Evan Kohlman[1], Catherine Roberts[1], Yutong Zhou[1], Pu Xia[1], Alper James Alcaraz[1], John Giesy[1], Markus Hecker[1], Markus Brinkmann[1], Natacha Hogan[1]

Enantioselective effects of venlafaxine on the antioxidative defense system in rainbow darters (*Etheostoma caeruleum*) (PL) 52

Sana Gavarikar[1], Sarah Kowalczyk[1], Erika Burton[1], Leslie Bragg[1], Paul Craig[1], Mark Servos[1]

The effect of 17β-estradiol on *Capitella teleta* adult behaviour (PL) 53

Andrea Murillo[1], Lisa Laframboise[1], Andrew Thompson[1], Joanna Wilson[1]

Responses of freshwater invertebrate microbiomes to municipal wastewater effluents (PL) 53

Emilie Diesbourg[1], Brittany Perrotta[1], Karen Kidd[1]

Introducing shinywqbench: An online tool for calculating aquatic life water quality benchmarks for emerging contaminants (PL) 54

Angeline Tillmanns[1], Ayla Pearson[2], Ali Azizishirazi[1]

Toxicological evaluation of emerging antibacterial compounds using the Microtox® test system (PO) 55

Juleanne Flores[1], Junyi Lin[1], Markus Hecker[1], Natacha Hogan[1]

Screening-level assessment of the ecological impacts pharmaceuticals and personal care product contamination to aquatic and terrestrial systems at Alaksen National Wildlife Area (PO) 55

Jeffrey Lam[1], Michael Horton[1], Vicki Marlatt[1]

Are early life stage rainbow trout (*Oncorhynchus mykiss*) sensitive to benzalkonium chloride, an emerging antimicrobial compound of concern? (PO) 56

Evan Kohlman[1], Natacha Hogan[1], Markus Hecker[1], Markus Brinkmann[1]

Transcriptomic changes at the reproductive level of catfish (*Trichomycterus areolatus*) associated with sewage effluents in Greater Santiago, Chile (PO) 57

David Rodríguez[1], Gustavo Chiang[2], Kelly Munkittrick[3], Elvira Vergara[4,5], Paulina Bahamonde[4,5,6]

Transfer of aquatic PPCP contaminants to terrestrial food webs by emergent invertebrates exposed to WWTP effluent (PO) 57

John Fast[1], Karen Kidd[1]

Quaternary ammonium compounds (QACs) in Canadian wastewaters and biosolids, and the potential impact of COVID-19 (PO) 58

Katrina Sullivan[1], Fanny Monteil-Rivera[2], Rachel Bouwhuis[1], Steven Locke[2], Alexander Okonski[1], Shirley Anne Smyth[1], Magdalena Jagla[1], Sarah Gewurtz[1], Don Gutzman[1]

Development of the optimal extraction method of chiral pharmaceuticals from rainbow darter (*Etheostoma caeruleum*) (PO) 59

Sarah Kowalczyk[1], Diana Cárdenas-Soracá[1], Leslie Bragg[1], Sondus Jamal[1], Mark Servos[1]

Estimation of the drugs of abuse consumption in Southern Ontario via wastewater-based surveillance (PO) 60

Diana Cárdenas-Soracá[1], Sandra Salic[1], Lily Warketin[1], Cheryl Chong[1], Rashne Vakharia[1], Leslie Bragg[1], Mark Servos[1]

Mining and the Environment 61

Defining and quantifying stress/disturbance gradients for young wetlands forming in reclaimed oil sands landscapes (PL) 61

Jan Ciborowski[1], Michael Wendlandt[1], Ashlee Mombourquette[1], Elizabeth Gillis[1], Hannah Porter[1], Mir Mustafizur Rahman[1], Jean Birks[1]

Developing monitoring triggers to detect water quality changes associated with unplanned releases from an oil sands tailings pond in the Athabasca oil sands region of Alberta (PL) 61

Prabha Rupasinghe[1], Lisa Boyer[1], Ana Carolina Lima[1], Kelly Munkittrick[1], Garry Scrimgeour[1], Frederick Wrona[1]

Modelling background conditions in the lower Athabasca River to support adaptive monitoring in the oil sands region (PL) 62

Sawyer Stoyanovich[1], Keegan Hicks[2], Catherine Proulx[1], Jonathan Séguin[1], Kelly Munkittrick[3], Mark McMaster[4], Garry Scrimgeour[3], Bruce Kilgour[1]

Toxicity assessment of sediment from an oil sands pilot pit lake containing permanent aquatic storage structure (PASS) treated tailings (PL) 63

Immanuela Ezugba[1], Lorne Doig[1], Banamali Panigrahi[1], Catherine Davila-Arenas[1], Karsten Liber[1]

Spatio-temporal analysis of water chemistry and ecotoxicological risk characterization of a pilot-scale pit lake in the Athabasca oil sands region (PL) 64

Banamali Panigrahi[1], Lorne Doig[1], Immanuela Ezugba[1], Catherine Davila-Arenas[1], Karsten Liber[1]

Putting the puzzle pieces together: Metal modeling, toxicity testing, and tissue uptake to identify cause of effects for a gold mine EEM (PL) 65

Malcolm Stephenson[1], Elena Legrand[1], Mary Murdoch[1], Robyn Knight[1], Paul Mazzocco[1], Barry Wicks[1]

Best practices for establishing exposure and reference sites for metal mining environmental effects monitoring (EEM) studies (PL) 65

Sarah Costantini[1], Jason Plamondon[1], Maria Story[1], Ken Korman[1]

The characterization of natural variability within EEM and subsequent decision-making processes (PL) 66

Kevin Martens[1], Pierre Stecko[1]

Elevated copper and shifts in aquatic and riparian invertebrate microbiomes at a site impacted by historic mining (PL) 67

Karen Kidd[1], Amy Marcarelli[2], Brittany Perrotta[1], David Walters[3], Gordon Paterson[2]

Trace elements in fish from an industrial region located within the Traditional Territory of two First Nations near Sudbury, Ontario (PL) 67

Adam Lepage[1], Gretchen Lescord[1,2], Robert Paishegwon[3], Lori Richer[3], Curtis Assance[3], Thomas Johnston[4], John Gunn[1]

Linking the competition among three rare earth elements (lanthanum, cerium, and yttrium) with the bioavailability to *Chlamydomonas reinhardtii* using the biotic ligand model (PL) 68

Laurianne Pagé[1], Marie-Hélène Brunet[1], Kevin Wilkinson[1]

Environmental monitoring and remediation at Giant Mine – 'It takes a village' (PL) 69

Tamara Darwish[1], Collin Arens[1], Elaine Irving[1], Kate Sinclair[1]

Monitoring to management: A framework for integrating and interpreting complex aquatic monitoring data (PO) 69

Daphne Lewarne[1], Giovanna Diaz[2], Emily-Jane Costa[1], Jennifer Ings[3], Cait Good[2], Mariah Arnold[2], Adrian de Bruyn[4], Todd Hatfield[5], Jason Ammerlaan[5]

Application of novel approach to evaluating acute lethality limits for sulphate (PO) 70

Breda Rahmanian[1], Emily-Jane Costa[1], Kerrie Serben[1], Maíra Mendes[1], Gary Lawrence[1]

General Ecotoxicology: Soil, Sediment, Water, Air, and Biota 71

Confirmation bias and the proliferation of erroneous research and publication bias (PL) 71

Shane de Solla[1], Karen Kidd[1]

Trends in water quality for irregular time series data (PL) 71

Jeff Row[1], Patrick Schaefer[1], Jennifer Ings[1]

Establishing best practices in quantifying aquatic chemistry reference conditions through a simulated approach (PL) 72

Emily Dutton[1], Jeff Row[1], Patrick Schaefer[1], Jennifer Ings[1]

Pairing wild rice and fish farming in a circular economy (PL) 73

Nicholas Blandford[1], Krista Robertson[1], Leah Dickenson[1], Richard Grosshans[2], Bruce Hardy[3], Nazim Cicek[1], Vince Palace[2]

The response of zooplankton communities when exposed to aquaculture wastewater within wild rice

planted mesocosms (PL) 73

Leah Dickenson[1], Krista Robertson[1], Nicholas Blandford[1], Mark Hanson[1], Bruce Hardy[3], Vince Palace[2]

Eutrophication is a cumulative environmental impact problem for watersheds not waterbodies (PL) 74

Lynn McCarty[1], Dean Fitzgerald[2]

Toxicity of oil sands groundwater naphthenic acid fractions in fathead minnow and walleye embryos and larvae (PL) 75

Joanne Parrott[1], Stacey Clarence[1], Christine Lavalle[1], Hufsa Khan[1], Kallie Shires[1], Kaiying Sally Ju[1], Blake Skanes[1], Maegan Rodrigues[1], Adrienne Bartlett[1], Patricia Gillis[1], Greg Bickerton[1], Jim Roy[1], Mark Hewitt[1], Richard Frank[1]

Investigating the barriers to recovery in culturally and ecologically valuable clam populations impacted by an oil spill (PL) 75

Tyler Black[1], Kyle Artelle[2], Diana Chan[2], Benjamin de Jourdan[3], Conrad Pilditch[4], Ryan Prosser[1]

Characterizing the geochemical evolution and subsequent aquatic toxicity of a diluted bitumen spill within the saturated zone of a sand aquifer (PL) 76

Scott Hepditch[1], Jason Ahad[2], Thomas Robert[1], Tuan Anh To[1], Ève Larocque[1], Juan Manuel Gutierrez-Villagomez[1], Gaëlle Triffault-Bouchet[3], Richard Martel[1], Valerie Langlois[1]

Assessing the effect of a minimally invasive oil spill remediation method on shoreline biofilm communities (PL) 77

Hakeem Omilowo[1], Madeline Stanley[1,2], Lauren Timlick[2], Lisa Peters[2], José Luis Rodríguez-Gil[2], Dave Findlay[3], Mark Hanson[1], Vince Palace[2]

Plant-enhanced phenanthrene reduction from freshwater microcosms (PL) 77

Madeline Stanley[1,3], Aidan Guttormson[1,2], Lisa Peters[2], Thor Halldorson[1], Gregg Tomy[1], Blake Cooney[1,2], Michael Opyr[1], Richard Grosshans[1,2], David Levin[1], Vince Palace[1,2]

Behaviour and brain size of larval zebrafish exposed to environmentally relevant concentrations of β-methylamino-L-alanine (BMAA) (PL) 78

Amanda Reside[1], Sana Gavarikar[1], Frederic Laberge[1], Nicholas Bernier[1]

Characterizing the environmental fate and behaviour of diluted bitumen within freshwater systems (PO) 79

Scott Hepditch[1], Juan Manuel Gutierrez-Villagomez[1], Tuan Anh To[1], Qin Xin[2], Heather Dettman[2], Nicole Heshka[2], Gaëlle Triffault-Bouchet[3], Richard Martel[1], Jason Ahad[2], Valérie Langlois[1]

Characterization of chloride exposure levels and ecological risk in the surface waters of Manitoba, Canada (PO) 79

Braedon Humeniuk[1], José Luis Rodríguez-Gil[1,2], Mark Hanson[1]

The role for amphibians in monitoring ecosystem health and environmental change (PO) 80

Sarah Young[1], Jillian Rohonczy[2], Bruce Pauli[1], Stacey Robinson[1,2]

Can analysis of sediments, vegetation, and waters provide differentiation between the sources of anthropogenic and natural contamination in the Tambo River Basin in Southern Peru? (PO) 81

Mackenzie Hobbs[1], Matthew Rennie[1], Lianne Girard[1], Nadia Mykytczuk[1,2], Charles Ramcharan[1], Max Lakanen[1], Julia Anderson[1], Rodolfo Sanchez[3], Agapito Mamani Luis[3], Agapito Flores[3], Gabriela Arias Torres[3], Matilde Palomino Gutierrez[3], Mariela Ramos Ramos[3], Rubliz Huancco Bravo[3], Luis Aguilar[3], Wilver Ramos Farfán[3], Rodrigo Alexis Rojas Villafuerte[3], Alexander Apaza Viza[3], Edy Vanesa Merma Velasco[3], Graeme Spiers[1]

Impacts of land use and ultraviolet radiation on floodplain zooplankton: An integrative ecotoxicity assay with *Daphnia magna* (PO) 82

Shahin Badesab[1], Vincent Fugère[1], Assetou Sacko[1], Andrea Bertolo[1]

Using a passive dosing system to assess the toxicity of individual aromatic compounds to intertidal bivalves (PO) 82

Victoria Loor[1], Benjamin de Jourdan[2], Kyle Artelle[3], Ryan Prosser[1]

Environmental risks of arsenic in irrigated agricultural areas with abnormal arsenic levels in waters from the Paracatu River (Minas Gerais), Brazil (PO) 83

Marina Monteiro Feitosa[1], Ingrid Fernanda Santana Alvarenga[1], Geraldo Jânio Eugênio de Oliveira Lima[2], Fernando José Vilela[2], Thiago Resende[2], Luiz Roberto Guimarães Guilherme[1]

Arsenic speciation in freshwater fish: A systematic review with implications for monitoring and research (PO) 84

Adam Lepage[1], Gretchen Lescord[1,2], Brian Laird[3], Kelly Skinner[3], John Gunn[1]

Evaluating the effect of wastewater treatment plant upgrades on contaminants of emerging concern (CECs) (PO) 84

Leslie Bragg[1], Nivetha Srikanthan[1], Azar Fattahi[1], Katie McCann[1], Hadi Dhiyebi[1], Pam Law[2], Dominika Celmer-Repin[2], Sonya Kleywegt[3], Wayne Parker[1], Mark Servos[1]

Using narcotic and phototoxic target lipid models to develop water quality guidelines for polycyclic aromatic hydrocarbons (PAHs) (PO) 85

Allison Dunn[1], Janet Cermak[1]

nZVI in the field: Remediating mine soils contaminated with a metal(loid) mixture (PO) 86

Emily Bowyer[1], Luba Vasiluk[1], Beverley Hale[1]

Determining the impacts of an aquatic copper exposure to northwestern salamander larvae (PO) 86

Blake Danis[1], Geoffrey Su[1], Vicki Marlatt[1]

Derivation of a nickel benchmark for the protection of aquatic environments using species sensitivity distributions (PO) 87

Jorgelina Muscatello[1], Alan Martin[1], Meghan Goertzen[1]

Tackling the Challenge of Understanding Ecological Effects of Per and Polyfluoroalkyl Substances 88

Potentiation of the PFOA uptake in daphnia (*Daphnia magna*) by TiO_2 nanoparticles (PL) 88

Arian Farajizadeh[1], Greg Goss[1]

Exposure to perfluoroalkyl sulfonic acids (PFSAs) increases liver mass in northern leopard frog (*Rana pipiens*) tadpoles (PL) 88

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

The impact of per and polyfluoroalkyl substances (PFAS) on oxylipin stress markers (PL) 89

Amy Rand[1]

Are individual perfluoroalkyl acids associated with reproductive success of tree swallows? Beyond PFOS and PFOA (PL) 90

Kailee Hopkins[1,2], Melissa McKinney[1], Robert Letcher[2,3], Kim Fernie[1,2]

Sex differentiated biotransformation of 6:2 disubstituted polyfluoroalkyl phosphate in human fecal *in vitro* suspensions (PL) 90

Sierra Peskett[1], Amy Rand[1]

Potential impacts of climate change on the levels of perfluorooctane sulfonate in the Beaufort Sea Shelf food web and implications for human health risk (PO) 91

Elizabeth Wallace[1], Kristen Sora[2], Mi-Ling Li[3], Amanda Giang[2], Emma Gillies[2], Carie Hoover[4], William Cheung[2], Laurie Chan[1]

Proteomic analysis of short-chain perfluorinated alkyl substance (PFAS) exposure in fathead minnows (*Pimephales promelas*) (PO) 92

Cristina Henriques[1], Theresa Warriner[1], Denina Simmons[1]

Characterization of the replacement PFAS, perfluoroethylcyclohexane sulphonate (PFECHS) and perfluorobutane sulphamide (FBSA) *in vitro* individually and in mixture with perfluorooctane sulponate (PFOS) (PO) 92

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

Derivation of Canadian environmental quality guidelines for perfluorooctanoic acid (PFOA) (PO) 93

Janet Cermak[1], Tamzin El-Fityani[1], William Martin[1]

Advancements in Biogeochemistry, Environmental Fate, Ecotoxicology, and Environmental Management of Selenium 94

Guidance for assessing potential impacts of selenium in freshwater aquatic ecosystems (PL) 94

Vince Palace[1], Stephanie Graves[2], Jessica Brandt[3]

The implications of science communication in reporting of the risks posed by selenium in Canadian coal mine effluents* (PL) 94

Guy Gilron[1], Richard Wenning[2], William Adams[3]

Mechanisms of waterborne selenite uptake in freshwater invertebrates and fish are not conserved: Effects of water chemistry on selenium toxicity (PL) 95

Chantelle Klaczek[1], Greg Goss[1], Chris Glover[1,2]

The inverse relationship between ovary selenium concentrations and gonadosomatic index: Implications for sample collection and data interpretation (PL) 96

Nicole Zathey[1], Lucinda Tear[2], David DeForest[2], Katherine Raes[3], Markus Hecker[3], Marko Adzic[1], Kevin Brix[4,5]

State of practice: Selenium bioaccumulation models in Canadian environmental assessment (PL)..... 96

Kate Mill[1], Vince Palace[2], Liz Ashby[3]

Lessons learned in an uncertain climate: Refining a large monitoring program affected by unexpected weather (PL) 97

Cybele Heddle[1], Adrian de Bruyn[2]

Selenium bioaccumulation in lab-cultured and field-collected *Daphnia pulex* via dissolved and dietary exposure routes (PO)..... 98

Catherine Davila-Arenas[1], Lorne Doig[1], Xiaowen Ji[1], Banamali Panigrahi[1], Immanuela Ezugba[1], Xia Liu[1], Karsten Liber[1]

Characterization of lab-maintained periphyton from lotic systems in British Columbia using eDNA metabarcoding and selenium uptake kinetics (PL) 99

William Muzyka[1], Kerstin Bluhm[1], Esteban Gillio Meina[1], Milena Esser[1], Katherine Raes[1], Kevin Brix[2,3], Derek Green[4], Mariah Arnold[4], Karsten Liber[1], Som Niyogi[1], Markus Hecker[1]

Plastic Pollution in Terrestrial and Aquatic Environments – Fate, Impacts, and Management strategies 100

The effects of shape and size on microplastic atmospheric settling velocity and particle entrainment (PL)..... 100

Colette Preston[1], Cheryl McKenna Neuman[1], Julian Aherne[1]

Distribution of microplastics in a background headwater lake during the ice and ice-free period (PL) 100

Brittany Welsh[1], Erin Bennett[1], Andrew Paterson[2], Ken Drouillard[3], Julian Aherne[1]

Spatial distribution and risk assessment of microplastic pollution in surface waters of the St. Lawrence Estuary (PL)..... 101

Noreen Kelly[1]

Long-term fate and remediation of microplastics in subarctic lake sediments in the experimental lakes area (ELA), Canada (PL)..... 102

Hannah Vonberg[1], Tony Walker [1], Andrew Medeiros[1], Rebecca Rooney[2]

The influence of microplastics on leaf litter degradation in oligotrophic freshwater lakes (PL) 102

Rachel McNamee[1], Rebecca Rooney[1], Stephanie Graves[2], Émilie Montreuil Strub[1], Timothy Hoellein[3], Chelsea Rochman[4]

The effects of microplastics on freshwater phytoplankton and zooplankton communities in a boreal lake (PL) 103

Desiree Langenfeld[1,6], Kennedy Bucci[2], Rachel Cable[3], Cody Veneruzzo[4], Rachel McNamee[5], Chelsea Rochman[3], Michael Paterson[6]

Investigating the combined effects of microplastic fibers and temperature on *Daphnia pulex* (PL) .. 104

Natasha Klasios[1], Aurelio Morales Murillo[1], Michelle Tseng[1]

The effects of microplastics on emerging insect and zooplankton communities in a limnocorral experiment (PL) 104

Yael Lewis[1], Rebecca Rooney[2], Chelsea Rochman[3], Jennifer Provencher[4], Matthew Hoffman[5], Michael Rennie[6], Michael Paterson[7], Diane Orihel[1]

Examining aquatic-terrestrial transfer of microplastics sourced from wastewater treatment facilities in the Grand River watershed, Ontario (PL) 105

Colleen Wardlaw[1], M. Dang[1], Karen Kidd[1], Ryan Prosser[2]

The proof is in the poop: Assessing microplastics bioaccumulation and toxicity in freshwater macroinvertebrates (PL) 106

Quinn Allamby[1], Karen Kidd[1], Ryan Prosser[2]

You are what you eat: Microplastic accumulation in *Planorbella pilsbryi* (PL) 106

Émilie Montreuil Strub[1], Rebecca Rooney[1], Rachel McNamee[1], Ryan Prosser[2], Chelsea Rochman[3]

Measuring ingestion of microfibers in different tissues of a freshwater mussel (PL)..... 107

Yaryna Kudla[1], Patricia Gillis[2], Karen Kidd[3], Ryan Prosser[1]

Transcriptomic analysis of developing wood frog larvae following continuous exposure to a microplastic mixture (PL)..... 108

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

The effects of a microplastic mixture on the growth and development of wood frogs (*Rana sylvatica*) in an outdoor mesocosm experiment (PL) 108

Samantha Gene[1], Barb Katzenback[2], Jennifer Provencher[3], Chelsea Rochman[4], Diane Orihel[1]

Microplastics in a chronosequence of biosolid-amended agricultural soils in Southern Ontario (PL) 109

Harriet Walker[1], Julian Aherne[1]

Your #2 is my #1: Assessing the microplastic content of biosolids and agricultural fields (PL) 110

Nicholas Letwin[1], Gladys Stephenson[1], Adam Gillespie[1], Juliska Princz[2], Ryan Prosser[1]

Unveiling nanoplastic biouptake in the environment: Advancing environmental analysis through label-free localization and identification (PL)..... 110

Jun-Ray Macairan[1], Arav Saherwala[1], Florent Blancho[1], Frank Li[1], Owen Armstrong[1], Nathalie Tufenkji[1]

Sorption of three pesticides to weathered and pristine LDPE and PLA microplastics (PO) 111

Liam O'Hara[1], James Longstaffe[1], Adam Gillespie[1]

The role of terrestrial isopods in macroplastic breakdown: Microplastic formation and potential impacts on smaller organisms (PO) 112

Christopher Musgrave[1], Ryan Prosser[1]

Microplastic ingestion by aquatic-emergent insects and an aerial insectivore; observational and experimental field studies (PO) 112

Jessica Wilson[1], Mark Mallory[1], Jennifer Provencher[2]

The impacts of microplastics on physiology, behaviour, and survival of a freshwater gastropod, *Planorbella pilsbryi* (PO) 113

Ana Strbac[1], Quinn Allamby[1], Karen Kidd[1], Ryan Prosser[2]

Impact of microplastics to invertebrate survival and reproduction in a field soil: Direct amendment and biosolid application (PO) 114

Jessica Velicogna[1], Shan Zou[2], Maohui Chen[2], Brian Coleman[2], Branaavan Sivarajah[3], Jesse Vermaire[3], David Lapen[4], Jennifer Provencher[1], Juliska Princz[1]

Testing the effects of a blocking primer on 16S rRNA gene amplicon sequencing of North American wood frog skin swabs (PO) 114

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

Multi-level responses of benthic macroinvertebrates to experimental microplastic pollution in a boreal lake (PO) 115

Natasha Neves[1], Rebecca Rooney[2], Chelsea Rochman[3], Jennifer Provencher[4], Michael Paterson[5], Matthew Hoffman[6], Yohanny Melo[7], Samuel Washington[7], Jacqueline Edwards[1], Diane Orihel[1]

Fate and effect of microplastics on freshwater zooplankton in a whole-lake addition experiment (PO) 116

Madeleine Milne[1], Matthew Hoffman[2], Diane Orihel[3], Jennifer Provencher[4], Michael Rennie[5,8], Chelsea Rochman[6], Michael Paterson[7,8]

Living Laboratories – Advancing Sustainable Production Practices in Canadian Agriculture 117

The Canadian agroecosystem Living Laboratories Network (PL) 117

Pamela Joosse[1], Chris McPhee[1], Eric Page[1], Tracey Ryan[2]

Using aquatic ecological endpoints to assess agricultural practices in the Lake Erie Basin (PL)..... 117

Adrienne Bartlett[1], Sonja Fransen[2], Andrée Gendron[1], Ève Gilroy[1], Lee Grapentine[1], Patrick Handyside[2], Pamela Joosse[2], Colin Little[3], Tatianna Lozier[4], Félix Ouellet[1], Eric Page[2], Chris Parsons[1], Agnes Richards-Blukacz[1], Katie Stammler[5], Zofia Taranu[1], Gerald Tetreault[1]

Assessing the fish community in tributaries of Lake Erie adjacent to agricultural activity (PL)..... 118

Gerald Tetreault[1], Jason Miller[1], Eric Bonk[2], Kirsten Nikel[1], Amy Sett[1], Robert Hanner[2], Adrienne Bartlett[1]

Living labs in Southern Ontario: Stream invertebrate health and watershed agricultural best practices (PL)..... 119

Lee Grapentine[1]

Edge-of-field water quality monitoring for Living Lab-Ontario (PL)..... 119

Patrick Handyside[1], Sonja Fransen[1], Adrienne Bartlett[2], Agnes Richards-Blukacz[2], Félix Ouellet[2], Eric Page[1]

Pesticide occurrence and dynamics in surface waters of two distinct agricultural and ecological regions of Canada (PL)..... 120

Caitlin Watt[1], Jonathan Challis[1], Tobias Laengle[1], Jason Vanrobaeys[1], Swan Lake First Nation Land Governance Office

Soil health: Adapting soil ecotoxicity measures to soil health measures (PO) 120

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

From waste land to retention ecosystem: A success story of the Lake St. Pierre Living Lab, Québec

(PO) 121

Andrée Gendron[1], Jonathan Lafond[2], Georges Thériault[2], Paul Caplette[3], Ghislain Poisson[4], Yasmina Larbi-Youcef[5], Adrienne Bartlett[1], Suzanne Kramer[1], Éloïse Veilleux[6]

Latest Advances in Fate and Effects of Metals in the Natural Environment 122

Canadian water quality guidelines for metals (PL) 122

Sushil Dixit[1]

Evaluation of total aluminum guidelines using chronic toxicity tests with aluminum-spiked mine water (PL) 122

Meghan Goertzen[1], Robert Goldblatt[1]

Development of water quality guidelines for the protection of aquatic life for nickel using the biotic ligand model and other approaches (PL) 123

Andrew Burton[1], Ali Azizishirazi[2]

Characterizing an effect of bicarbonate on chronic nickel toxicity to *Ceriodaphnia dubia* (PL) 124

Jordana Van Geest[1], Jennifer Daley[1], Emily-Jane Costa[1], Melanie Gallant[2], James Elphick[2], Mariah Arnold[3], Adrian de Bruyn[4]

Polar problem: Investigating the nickel sensitivity of Arctic aquatic invertebrates (PL) 124

Connor Stewart[1], Robert Griffin[1], Hannah Lowes[1], Emily Garman[2], Christian Schlekat[2], Elizabeth Middleton[2], Anne Crémazy[3], Tamzin Blewett[1]

A predictive model created to understand how specific water quality characteristics govern the association of cadmium to microplastics in freshwater systems (PL) 125

Lauren Zink[1], Emily Mertens[1], Xingzi Zhou[1], Sarah-Ellen Johnston[2], Matthew Bogard[1], Steve Wiseman[1], Gregory Pyle[1]

Applicability of partial ultrafiltration for determination of critical metals complexation with natural organic matter investigated by HPSEC-ICP-MS (PL) 126

Océane Hourtané[1], Claude Fortin[1], Scott Smith[1]

Lanthanum speciation and bioavailability in aqueous media containing natural organic matter (PL) 126

Marie-Hélène Brunet[1], Laurianne Pagé[1], Houssame-Eddine Ahabchane[1], Scott Smith[2], Kevin Wilkinson[1]

Effects of neodymium, praseodymium, and yttrium: Bioaccumulation studies (PL) 127

Celine Do[1]

Toxic effects of rare earth elements cerium, neodymium, and europium both as single and ternary mixture exposures on tomato and durum wheat (PL) 127

Taylor Vanderyagt[1], Luba Vasiluk[1], Beverley Hale[1]

Assessing the potential risk of lithium mining environments using mineralogy and geochemistry (PL) 128

Becca Kirk[1], Eleanor Berryman[2], Philippa Huntsman[2], Emily Suominen[2], Ted MacKinnon[2], Carrie Rickwood[2], Richard Goulet[2]

Chronic lithium toxicity on the fingernail clam (*Pisidium dubium*) (PL) 129

Awa Mamey Malika Ouedraogo[1,2], Carrie Rickwood[2], Gauri Prabhakar[2], Emily Suominen[2], Frances Pick[1], Richard Goulet[1,2]

Can skin-associated mucous chemically protect against low-level mercury in teleost fish? (PL)..... 129

Ashley James[1], Tracy MacDonald[2], Natalia Dolgova[3], Ingrid Pickering[1], Graham George[1]

Metals impact fathead minnow's ability to regulate oxygen (PL)..... 130

Natalie Nykamp[1], Erin Leonard[1]

Multiple stressors: How metals impact a fish's ability to regulate oxygen (PL)..... 130

Erin Leonard[1], Grant McClelland[2], Jim McGeer[1], Natalie Nykamp[1]

Subchronic impacts of copper and a copper, cadmium, and zinc mixture on the liver and gill proteomes of developing rainbow trout (*Oncorhynchus mykiss*) (PL)..... 131

Michael McKay[1], Laura Baseler[2], Jason Rogalski[3], Renata Moravcova[3], Jordan Beblow[4], Mark Cleveland[4], Vicki Marlatt[1]

Interactions of trace metals on heart mitochondrial reactive oxygen species metabolism (PL)..... 132

Pius Tetteh[1], Zahra Kalvani[1] , Don Stevens[1], Ravinder Sappal[2], Collins Kamunde[1]

Bridging the gap: Estimating heavy metals uptake into native vegetation consumed by Indigenous peoples and wildlife (PL)..... 132

Tania Noble[1], Elyse Doane[2], Kayla Higgins[2], Remi Kennedy[2], Rachel Clark[2], Sophia D'Aurora[2], Elena Legrand[1], Loren Knopper[1]

Proteomic profiles of kidney and liver tissues of rainbow trout (*Oncorhynchus mykiss*) exposed to low concentrations of waterborne nickel (PO) 133

Kylee Ronnenberg[1], Urvi Pajankar[1], Denina Simmons[1]

Ecological risk classification of inorganic substances (PO)..... 134

Rachel Bouwhuis[1], Melissa Ma[1], John Prindiville[1], Michael Beking[1], Rebecca Dalton[1]

Rare earth elements: Toxicological assessment of neodymium (sulfate salt and organometallic) in boreal soil (PO) 134

Patrick Boyd[1], Heather Lemieux[1], Dina Schwertfeger[1], Jessica Velicogna[1], Rick Scroggins[1], Juliska Princz[1]

Iron spiked sediment 42-day survival, growth and reproduction toxicity testing with Twin Lakes *Hyalella azteca* (PO)..... 135

Francesca Farrow[1], Heather Rousselle[1], Grace Chen[1], Amy Festarini[1], Sarah Frye[1], Isabelle Gosselin[1], Dan Festarini[1], Hailly Degagne[1], Kiley Sims[1], Glen Bird[1], Marilyne Stuart[1]

Fate of gadolinium-based contrast agents in the environment: Stability of complexes in aqueous phase and bioavailability (PO) 136

Aissatou Sow[1], Huy Dang[2], Claude Fortin[1]

Development of a predictive model for metal accumulation (copper, nickel, and zinc) in biofilm (PO) 136

Amandine Greil[1], Claude Fortin[1], Isabelle Lavoie[1]

Federal Water Quality Guidelines for iron using a multiple linear regression approach (PO)..... 137

Tamzin El-Fityani[1], Sushil Dixit[1], Rachel Wideman[1]

From Lab to Nature: Applying Environmental Relevance to Standardized Toxicity Testing..... 138

Influence of confounding variables in toxicity tests (PL)..... 138

Josh Baker[1], James Elphick[1]

Flux your stressors! Including diurnal fluctuations in temperature for more realistic stressor exposures (PL)..... 138

Paul Craig[1], Jaycelyn Caklec[1], Sana Gavarikar[1], Allison Weber[1]

A critical evaluation of inherent toxicity and hazard paradigms in regulatory ecotoxicology (PL)..... 139

Lynn McCarty[1], Christopher Borgert[2], Lyle Burgoon[3]

Unraveling the influence of metamorphosis on the fate and effects of contaminants in amphibians (PL)..... 140

Chantal Lanctôt[1], Danielle Hill[1,2], William Bennett[1], Tom Cresswell[2]

Field and lab analysis on the toxicity of lead from recreational fishing gear (PL)..... 140

Sabrina St-Hilaire[1], Gerald Tetreault[2], Richard Frank[2], Ève Gilroy[2], Sheena Campbell[2], Jason Miller[2], Amy Sett[2], Erin Leonard[1]

A laboratory-based chronic toxicity model is predictive of nickel effects on benthic invertebrates in the field (PL)..... 141

Adrian De Bruyn[1]

Twin Lakes *Hyalella azteca* 42-day survival, growth and reproduction toxicity testing of Chalk River Laboratories wetland sediment (PL) 142

Francesca Farrow[1], Samantha Jarron[1], Amy Festarini[1], Glen Bird[1], Marilyne Stuart[1]

Ecotoxicity assessment of petroleum-coke-treated oil sands process-affected water, Part 1: Sublethal toxicity assessment (PL)..... 142

James Elphick[1], Martin Davies[2], Stephanie Schiffer[1], Connor Pattem[3]

Ecotoxicity assessment of petroleum-coke-treated oil sands process-affected water, Part 2: Mesocosm assessment (PL)..... 143

Chris Perrin[1], Martin Davies[2], Morgan Edwards[3], Zachary Mueller[2]

Safety in numbers? Manipulating the social context in the standardized LC50 toxicological assessment (PL)..... 144

Sienna Overduin[1], Tamzin Blewett[1]

Are laboratory organisms still relevant? A comprehensive assessment of *Daphnia pulex* reared in laboratory and wild environments and their responses to anthropogenic contaminants (PL)..... 144

Aaron Boyd[1], Connor Stewart[1], Jenelle McCuaig[1], Sidney Martin[1], Ally Legge[1], Dustin Doty[1], Tamzin Blewett[1]

Comparative acute toxicity of herding agents (ThickSlick 6535 and SilTech OP-40) to multiple aquatic species (PO) 145

Davide Asnicar[1], Tahereh Boloori[1], Jason Durante[1,2], Benjamin de Jourdan[1], Kenneth Lee[3]

Inspiring Science in the Capital – Investigation, Integration, and Implementation 146

Bridging science and policy through environmental quality guidelines (PL)..... 146

Allison Dunn[1], Janet Cermak[1], Sushil Dixit[1], Tamzin El-Fityani[1]

Canada's Chemicals Management Plan – Ecological risk assessment perspectives (PL) 146

Rebecca Dalton[1], Kelly Potter[1], Parisa Ghahreman[1], Delaney Large[1]

Validation and standardization are key design features for a new approach method: Lessons from the EcoToxChip Project (PL)..... 147

Jessica Head[1], Doug Crump[2], Markus Hecker[3], Niladri Basu[1]

Protecting the environment with forethought in environmental impact assessment (EIA) (PL)..... 147

Carolyn Brown[1,2], Kelly Munkittrick[2,3]

Global Atmospheric Passive Sampling (GAPS) Network: Integration and cross-cutting issues (PL) 148

Tom Harner[1], Amandeep Saini[1], John Liggio[1], Jasmin Schuster[1], Egide Kalisa[1], Jacob Mastin[1], Cassandra Johannessen[2], Xianming Zhang[2], Pourya Shahpoury[3], Sabina Halappanavar[4], Anita Eng[1]

Working towards a long-term water monitoring program for pesticides (PL)..... 148

Cassie McLean[1]

Future directions for ECCC's ecological risk assessment program (PO)..... 149

Kelly Potter[1], Rebecca Dalton[1], Parisa Ghahreman[1], Delaney Large[1]

DFO's National Contaminants Advisory Group – a ten-year retrospective on research priorities for investigating the biological effects of aquatic contaminants (PO)..... 150

Sarah Marteinson[1], Judith Leblanc[1], Sarah Sine[1], Trinity Berube[1], Remy Tadonleke[1], Cory Dubetz[1], Amanda Winegardner[1]

Exposure, Effects, and Risk Assessment of Pesticides in the Environment 151

Multigenerational exposure of the earthworm *Eisenia andrei* to two insecticides (PL)..... 151

William Martin[1], Paul Sibley[1], Ryan Prosser[1]

Water monitoring for pesticides pilot program (PL) 151

Cassie McLean[1]

Investigating the potential toxicity of copper nanoparticles from agricultural run-off on *Daphnia magna* (PL)..... 152

Fleur Issac[1]

An assessment of the toxicity of pesticide mixtures in periphyton from agricultural streams to the mayfly *Neocloeon triangulifer* (PL) 153

Moira Ijzerman[1], Melanie Raby[2], Gab Izma[3], Yaryna Kudla[1], Nicholas Letwin[1], Melanie Gallant[4], Stephanie Schiffer[4], Brian Atkinson[1], Rebecca Rooney[3], Paul Sibley[1], Ryan Prosser[1]

Bioaccumulation and physiological/behavioural effects of a common pesticide mix on pond snails (PL) 153

Jared Sparr[1], Patrick Bergeron[1], Isabelle Lavoie[2], Lounes Haroune[3]

Assessing agricultural pesticides risks for aquatic life: Potential health effects of a glyphosate-based herbicide (GBH) on *Elliptio complanata* at environmental concentrations (PL) 154

Yannick Nombré[1], Maxime Gauthier[1,2], Monique Boily[1], Lise Parent[3]

Toxicity of individual and combined effect of mefenpyr diethyl safener and its co-herbicide, fenoxaprop-p-ethyl, to early life stages of zebrafish (PL) 155

Oluwabunmi Femi-Oloye[1,2], Femi Oloye[1], John Giesy[1]

Water temperature influences the sensitivity of larval sea lamprey (*Petromyzon marinus*) to lamprey-specific pesticides (PL) 156

Dejana Mitrovic[1]

Examining effects of neonicotinoids singly and in mixtures using salmonid cell lines (PL) 156

Ania Bula[1], Brenna Hay[1], Amber Feaver[1], Lucy Lee[1]

Characterizing the cytotoxic and molecular effects of environmentally relevant pesticides on human Caco-2 and HepG2 cell lines (PL) 157

Iris (Ke) Xu[1], Krittika Mittal[1], Niladri Basu[1]

Cytotoxic and transcriptomic effects of environmentally relevant pesticides exposed to RTgill-W1 cells (PL) 158

Sophie Emberley-Korkmaz[1]

The environmental health burden of pesticides in the United States (PL) 158

Alan Kolok[1], Eleanor Rogan[2], Jabeen Taiba[2], Naveen Joseph[3]

Toxicity of flupyradifurone and sulfoxaflor commercial formulations to non-target species in soil (PO) 159

Heather Lemieux[1], Patrick Boyd[1], France Maisonneuve[1], Juliska Princz[1]

Pesticides in Québec streams: Mixture toxicity on freshwater organisms and environmental risk (PO) 159

Antoine Faure[1], Isabelle Lavoie[1], Lise Parent[2], Anne Crémazy[1]

Species sensitivity distributions for two bath pesticides used in the finfish aquaculture industry (PO) 160

Dounia Hamoutene[1], Sarah Marteinson[1], Melanie Kingsbury[1], Kathleen McTavish[2]

Screening-level assessment of pesticide and heavy metal contaminated sediments at the Alaksen National Wildlife Area (PO) 161

Michael Horton[1], Jeffrey Lam[1], Vicki Marlatt[1]

Building a biobed: Practical points for effective experiments (PO) 161

Phoenix Nakagawa[1], Alistair Brown[1], Horacio Heinzen[2], Veronica Cesio[2], Natalia Besil[2], Sofía Rezende[2], Francis Zvomuya[1], Annemieke Farenhorst[1]

Assessing biobed efficacy in the Prairies within Canada for processing pesticide rinsate (PO) 162

Phoenix Nakagawa[1], Alistair Brown[1], Horacio Heinzen[2], Veronica Cesio[2], Natalia Besil[2], Sofía Rezende[2], Annemieke Farenhorst[1]

Pollution's Power Play: Effects of Contaminants on Metabolism and Energetics 163

Venlafaxine alters mitochondrial respiration of zebrafish brains, in part due to mitochondrial microRNA regulation (PL) 163

Karyn Robichaud[1], Paul Craig[1]

Naphthenic acids contribute to oxidative stress by eliciting the emission of reactive oxygen species in mitochondria (PL) 163

Zahra Kalvani[1], Collins Kamunde[1], Don Stevens[1], Michael van den Heuvel[1]

The role of animal energetics in contaminant body burdens and biomarkers of health (PL)..... 164

Shane de Solla[1,2], Karen Kidd[2]

Understanding darter (*Etheostoma* spp.) interspecific energetic responses to climate-induced temperature changes (PL) 165

Allison Weber[1], Paul Craig[1]

Rearing temperature influences the development and function of the eye and the behavioural performance of yellow perch larvae (PL) 166

Andrew Thompson[1], Nawal Masood[1], Mellissa Easwaramoorthy[1], Peyton Hartenstein[2], Lisa Laframboise[1], Erica Chow[3], Vivian Choh[3], Daphne McCulloch[3], Richard Manzon[2], Christopher Somers[2], Joanna Wilson[1]

The effect of rearing temperature on the cardiometabolic development of yellow perch (*Perca flavescens*) (PL) 166

Mellissa Easwaramoorthy[1], Andrew Thompson[1], Shamaila Fraz[1], Lisa Laframboise[1], Peyton Hartenstein[2], Richard Manzon[2], Christopher Somers[2], Joanna Wilson[1]

Identifying developmental windows of sensitivity to diluted bitumen exposure in sockeye salmon (*Oncorhynchus nerka*) (PO)..... 167

Derin Calik[1], Amanda Reside[1], Geoffrey Su[2], Christopher Kennedy[2], Sarah Alderman[1], Todd Gillis[1]

The impact of methylene blue on energy metabolism on early zebrafish development (PO) 168

Niepukolie Nipu[1], Lai Wei[1], Jith Thomas[2], Jan Mennigen[1]

Investigating quantitative links between polycyclic aromatic compound (PAC) content and cardiorespiratory performance in sockeye salmon (*Oncorhynchus nerka*) exposed to sublethal concentrations of diluted bitumen (PO)..... 168

Derin Calik[1], Amanda Reside[1], Geoffry Su[2], Christopher Kennedy[2], Sarah Alderman[1], Todd Gillis[1]

Exposure to environmental levels of metformin and guanylurea does not impair the metabolism and energetic stores of zebrafish larvae (PO) 169

Nawal Masood[1], Andrew Thompson[1], Joanna Wilson[1], Oana Birceanu[1], Shemar Williams[1]

A model naphthenic acid reduces oxidative phosphorylation through selective impacts on complex activity (PO) 170

Zahra Kalvani[1] , Collins Kamunde[1], Don Stevens[1], Michael van den Heuvel[1]

Responses of longnose dace and fathead minnow to stormwater exposure in the Nose Creek watershed in Calgary, Alberta (PO) 170

Fateme Taridashti[1], Patricija Marjan[1], Cherrie Chan[1], Kelly Munkittrick[1]

'Omics in Ecotoxicology: Predictive and Diagnostic Applications 172

Characterizing transcriptional responses to three antimicrobial compounds in early life stages of rainbow trout using the EcoToxChip (PL) 172

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

Beyond the prescription: Unveiling the disturbing effects of venlafaxine on aquatic life (PL) 172

Louis Pfeifer[1], Paul Craig[1]

Comparison of *Daphnia magna* and Japanese medaka exposure to pharmaceuticals via targeted mass spectrometry (MS)-based metabolomics (PL) 173

Erico Oliveira Pereira[1], Theresa Warriner[2], Denina Simmons[2], Myrna Simpson[1]

Mucus as a non-lethal alternative: Proteomic evaluation of fathead minnows exposed to nutrient loadings (PL) 174

Krista Robertson[1], Leah Dickenson[1], Nicholas Blandford[1], Ken Jeffries[1], Denina Simmons[2], Markus Hecker[3], Bruce Hardy[4], Vince Palace[5]

Understanding the knowledge gaps in using toxicogenomic approaches for assessing aquatic ecosystem health (PL) 175

Nefertiti Roldán-Wong[1], Laurie Chan[1]

How does tertiary treated municipal effluent affect hepatic transcriptome in longnose dace (*Rhinichthys cataractae*) caged under semi-controlled conditions in artificial raceways? (PL) 175

Patricija Marjan[1], Christopher Martyniuk[2], Maricor Arlos[3], Mark Servos[4], Norma Ruecker[5], Markus Hecker[6], Kelly Munkittrick[1]

Role of toxicokinetics in determining species sensitivity to polycyclic aromatic hydrocarbons in birds (PO) 176

Jonathan Sangiovanni[1], Doug Crump[2], Jessica Head[1]

Non-targeted multi-omic analyses of blood plasma for health exploration of namew (lake sturgeon) in an intact and an impacted watershed in the Moose River Basin (PO) 177

Keisha Deoraj[1], Jennifer Simard[2], Constance O'Connor[3], Claire Farrell[3], Jacob Seguin[3], Denina Simmons[1]

Proteomic analysis of perfluorooctane sulfonate (PFOS) on the freshwater gastropod (*Planorbella pilsbryi*) (PO) 177

Almira Khan[1,2], Jeanne St-Laurent-Guérin[1,2], David McNabney[1], Maria Villella[1], Ève Gilroy[1], Denina Simmons[2], Amila De Silva[1], Adrienne Bartlett[1], Stacey Robinson[1]

Use of transcriptomic points of departure to assess the toxicity of pesticides in early life stage fish (PO) 178

Nicolas Decelles[1], Hugo Marchand[1], Emily Boulanger[1], Nathalie Vachon[2], Jessica Head[1]

Use of transcriptomic dose-response analysis to assess species sensitivity: A case study using 6PPD-quinone, metformin, and guanylurea in a 24-hr microplate-based fish bioassay (PO) 179

Hugo Marchand[1], Niladri Basu[1], Aylish Marshall[1], Emily Boulanger[1], Krittika Mittal[1], Nicolas Decelles[1], Nathalie Vachon[2], Jessica Head[1]

Development, optimization and application of toxicogenomic endpoints for soil invertebrates (PO) 179

Stephanie Kvas[1], Derek Smith[1], Adedamola Adedokun[1,2], Renuka Subasinghe[1], Patrick Boyd[1], Heather Lemieux[1], Steven Siciliano[2], Juliska Princz[1]

Impacts of erythromycin and an antibiotic-mixture on juvenile rainbow trout gut microbiome (PO) 180

Phillip Ankley[1], Jonathan Challis[2], Pu Xia[1], Yufeng Gong[3], Yuwei Xie[4], Yutong Zhou[1], Emily Kennedy[1], Katherine Raes[1], Catherine Roberts[1], Milena Esser[1], John Giesy[1], Markus Hecker[1], Markus Brinkmann[1]

Using transcriptomic dose-response analysis (TDRA) to assess the toxicity of oils in Atlantic cod (*Gadus morhua*) larvae (PO) 181

Jessica Head[1], Elena Legrand[2], Emily Boulanger[2], Tahereh Boloori[3]

Using omics approaches to identify sublethal, chronic effects of artificial sweeteners on rainbow trout (PO) 181

Adam Point[1], Theresa Warriner[1], Denina Simmons[1]

Assessing the sub-lethal metabolomic perturbations of selected carrier solvents used in pollutant exposure studies on *Daphnia magna* (PO) 182

Salwa Hajir[1], Myrna Simpson[1]

The Effects of Contaminants in a Changing Climate 183

Acute thermal stress impacts cardiac function in diploid and triploid Chinook salmon (*Oncorhynchus tshawytscha*) (PL) 183

Ivan Cadonic[1], John Heath[2], Brian Dixon[1], Paul Craig[1]

Degrees of change: Warmer water exacerbates the immediate and latent effects of crude oil exposure on developing salmon (PL) 183

Derin Calik[1], Feng Lin[2], Mackenzie Edgar[2], Christopher Kennedy[2], Elizabeth Johnston[1], Anthony Farrell[3], Todd Gillis[1], Sarah Alderman[1]

The impacts of carbamazepine and temperature on the diurnal and nocturnal behaviour of yellow perch larvae (PL) 184

Andrew Thompson[1], Mellissa Easwaramoorthy[1], Peyton Hartenstein[2], Lisa Laframboise[1], Richard Manzon[2], Christopher Somers[2], Joanna Wilson[1]

Effects of elevated temperatures and exposure to atrazine on the health, immunity, and locomotor performance of larval northern leopard frogs (*Lithobates pipiens*) (PL)..... 185

Melody Gavel[1], Mark Forbes[1], Stacey Robinson[1,2]

Combined effects of manganese and thermal stress on the metabolic capacities of Arctic charr (*Salvelinus alpinus*) (PL) 185

Camille Garnier[1], Pierre Blier[2], Patrice Couture[1]

Does temperature modify the impact of metals on olfactory-mediated behaviours in salmonids? (PL) 186

Derek Alsop[1], Andrew Thompson[1], Joanna Wilson[1]

Environmental DNA (eDNA): Research and Applications to Assess Biodiversity and Supporting Aquatic Ecosystem Health Management 188

A validated and optimized environmental DNA detection assay for Arctic grayling – A Species of Special Concern (PL) 188

Melissa Misutka[1], Chris Glover[2], Greg Goss[1], Heather Veilleux[3]

Comparative analysis of eDNA barcoding and conventional monitoring methods to detect amphibians in Southern Ontario vernal pools (PL) 188

Cailyn Zamora[1], Nathanael Harper[1], Maxwell Bui-Marinos[1], Paul Craig[1], Mark Servos[1], Andrew Doxey[1], John Giesy[2], Barbara Katzenback[1]

Application of novel eDNA methods to determine distribution and estimate biomass of oolichan (*Thaleichthys pacificus*) (PL) 189

Michael Allison[1], Meredith Pochardt[1], Morgan Hocking[2], Mary Lesperance[1], Steve Sharron[2], Francis Juanes[1], Caren Helbing[1]

Investigating key validation steps to optimize eDNA metabarcoding assays targeting rRNA genes of amphibians found in the Grand River watershed (PL) 190

Nathanael Harper[1], Michael Lynch[1], Yuwei Xie[2], Phillip Ankley[2], John Giesy[2], Mark Servos[1], Andrew Doxey[1], Paul Craig[1], Barbara Katzenback[1]

Targeted qPCR-based workflow for detecting fish sedimentary DNA in aquatic systems (PL)..... 190

Mark Louie Lopez[1], Caren Helbing[1]

The use of eDNA in concert with conventional electrofishing in streams adjacent to agricultural activities in the Lake Erin Basin (PL) 191

Eric Bonk[1], Jason Miller[2], Kirsten Nikel[2], Amy Sett[2], Robert Hanner[1], Gerald Tetreault[2]

A data management solution for eDNA research, from field to lab (PL) 192

Alex Borisenko[1], Robert Hanner[1]

Inhibition of RT-qPCR and RT-dPCR of SARS-CoV-2 RNA and fecal indicators in wastewater-based surveillance (PL)..... 193

Hadi Dhiyebi[1], Yash Badlani[1], Heather Ikert[1], Nivetha Srikanthan[1], Samina Hayat[1], Alice Gere[1], Joud Abu Farah[1], Carly Sing-Judge[1], Leslie Bragg[1], John Giesy[2,3], Mark Servos[1]

Wastewater-based surveillance of SARS-CoV-2: Lessons learned in sewersheds that can be applied to watersheds (PL) 193

Heather Ikert[1], Hadi Dhiyebi[1], Meghan Fuzzen[1], Nivetha Srikanthan[1], Samina Hayat[1], Carly Sing-Judge[1], Alice Gere[1], Yash Badlani[1], Chin Ki Anthony Ng[1], Patrick Breadner[1], Leslie Bragg[1], John Giesy[2,3], Mark Servos[1]

Rapid design, validation, and use of RT-qPCR for quantification of SARS-CoV-2 variants in wastewater (PO) 194

Chin Ki Anthony Ng[1], Meghan Fuzzen[1], Nathanael Harper[1], Heather Ikert[1], Carly Sing-Judge[1], Hadi Dhiyebi[1], Rachel Dawe[1], Nivetha Srikanthan[1], Samina Hayat[1], Leslie Bragg[1], John Giesy[2], Mark Servos[1]

Comparing the partitioning behaviour of SARS-CoV-2, PMMoV, and surrogates in liquid and solid fractions of wastewater (PO) 195

Patrick Breadner[1], Hadi Dhiyebi[1], Azar Fattahi[1], Nivetha Srikanthan[1], Samina Hayat[1], Marc Aucoin[1], Scott Boegel[1], Leslie Bragg[1], Paul Craig[1], Yuwei Xie[2,3], John Giesy[3,4], Mark Servos[1]

Wastewater surveillance of influenza (A, B) and respiratory syncytial virus (RSV) (PO) 195

Joud Abu Farah[1], Hadi Dhiyebi[1], Heather Ikert[1], Carly Sing-Judge[1], Dominika Celmer-Repin[2], Arianne Folkema[2], Stephen Drew[2], Mark Servos[1]

Enumeration potential of environmental DNA for Pacific salmon stock assessments (PO) 196

Geoffrey Su[1], Michael Allison[2], Jordan Beblow[3], Kevin Koch[3], Jeffrey Anderson[4], Leithen M'Gonigle[1], Mark Cleveland[3], Caren Helbing[2], Vicki Marlatt[1]

Ecotoxicology of Tire and Road Wear Particles and Related Contaminants – Where Are We Three Years After the Identification of 6PPD-quinone? 198

Toxicity of 6PPD-quinone across fishes of commercial, cultural, and ecological importance (PL) 198

Markus Hecker[1], Catherine Roberts[1], David Montgomery[1], Summer Selinger[1]

Comparison of the toxicity of 6PPD-quinone to early life stage rainbow trout and lake trout (PL) 199

Catherine Roberts[1], Evan Kohlman[1], Niteesh Jain[1], Junyi Lin[1], Mawuli Amekor[1], Alper James Alcaraz[1], Natacha Hogan[1], Markus Hecker[1], Markus Brinkmann[1]

Changes in the blood chemistry of brook trout (*Salvelinus fontinalis*) and Atlantic salmon (*Salmo salar*) after acute exposures to 6PPD-quinone (PL) 199

Danielle Philibert[1], Tillmann Benfey[2], Benjamin de Jourdan[1]

Comparative analysis of cardiometabolic effects of 6PPD-quinone on juvenile salmonids (PL) 200

Summer Selinger[1], David Montgomery[1], Markus Hecker[1], Steve Wiseman[2], Lynn Weber[1], David Janz[1], Markus Brinkmann[1]

Unraveling the molecular mechanism of 6PPD-quinone toxicity using whole transcriptome analysis in lake trout (PL) 201

Junyi Lin[1], Catherine Roberts[1], Evan Kohlman[1], Mawuli Amekor[1], Niteesh Jain[1], Alper James Alcaraz[1], Natacha Hogan[1], Markus Brinkmann[1], Markus Hecker[1]

6PPD-quinone poses a low risk to freshwater invertebrate populations (PL) 201

Jack Salole[1], Ryan Prosser[1]

Acute toxicity of 6PPD-quinone on *Planorbella pilsbryi* and *Megalonaias nervosa* (PL) 202

Spencer Hang[1], Jack Salole[1], Ryan Prosser[1]

Identifying and characterizing tire-related chemical (6PPD-quinone) toxic hotspots in salmon habitat in British Columbia, Canada (PL) 202

Tanya Brown[1,2], Sofya Reger[1], Xiangjun Liao[1], Carys Gallilee[1]

Interlaboratory study on analytical methods for aqueous 6PPD-quinone – on the path towards comparability (PL) 203

Sarah Marteinson[1], Markus Brinkmann[2], Tanya Brown[1], Richard Grace[3], Xiangjun Liao[1], Harold Malle[4], Danielle Philibert[5], Sarah Pomfret[4], Remy Tadonleke[1], Amanda Winegardner[1], Million Woudeneh[3]

Use of toxicity identification techniques to isolate causes of toxicity in stormwaters in British Columbia: Case studies with zinc and 6PPD-Q (PO) 204

Josh Baker[1], Ian Cronshaw[1], Joseph Monaghan[2], Erik Krogh[2]

Investigating environmental sources of the toxic tire-derived chemical 6PPD-Q (PO) 204

Leland Bryshun[1], Kerry McPhedran[1], Markus Brinkmann[1]

A histological assessment of 6PPD-quinone exposed juvenile coho and Chinook salmon (PO) 205

Bonnie Lo[1,2], Vicki Marlatt[1], Gary Marty[3], Tanya Brown[2]

Exposure, Accumulation and Effects of Radionuclides 206

Comparison of radium-226 separation methods based on chromatographic and extraction resins for its determination by ICP-MS in environmental matrices (PL) 206

Marine Roulier[1], Laurie Martin[1], Dominic Larivière[1]

Accumulation of radium-226 in three microalgal species and its effect of growth (PL) 206

Flavie Desreac[1], Anne Crémazy[1], Claude Fortin[1]

Chronic radium-226 toxicity to and bioaccumulation in the aquatic invertebrate *Daphnia magna* (PL) 207

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

Radium-226 water quality guidelines 14-day water-only toxicity test with Twin Lakes *Hyalella azteca* (PL) 208

Francesca Farrow[1], Amy Festarini[1], Alex Hancock[1], Marilyne Stuart[1]

Chronic toxicity of radium-226 to fingernail clams (PL) 208

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

Radium-226 toxicity to the early life stages of the great pond snail *Lymnaea stagnalis* (PL) 209

Léna Guimard[1], Claude Fortin[1], Anne Crémazy[1]

Low radium-226 doses increase growth and condition in rainbow trout (*Oncorhynchus mykiss*) fry at swim-up (PL) 209

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

Baseline environmental monitoring program for a deep geological repository (PL)..... 210

Stacey Fernandes[1], Michelle Nearing[2], Joanne Jacyk[2], Kelly Wells[1], Leah Leon[1]

Extended Abstract/Short Paper 212

The implications of science communication in reporting the risks posed by selenium in Canadian coal mine effluents..... 212

Guy Gilron[1], Richard Wenning[2], William Adams[3]

Author Index..... 225

ABSTRACT

Rickwood, C., Dalton, R., Robinson, S., Van der Vliet, L., Marteinson, S., Miller, J. 2026. Proceedings of the 49th Annual Canadian Ecotoxicity Workshop: October 2 – 5, 2023, Ottawa, Ontario. Can. Tech. Rep. Fish. Aquat. Sci. 3822: xxxi + 234 p. https://doi.org/10.60825/96ab-ce33

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

The 49th annual CEW was held at the Shaw Centre in Ottawa, Ontario, in 2023. After two years of hybrid workshops due to the COVID-19 pandemic, this workshop marked a return to an almost fully in-person CEW event. The workshop included 202 platform presentations and 99 poster presentations. Total participation was 421 attendees.

This workshop was one of a continuing series of annual workshops in Canada on ecological toxicology. covering topics including Indigenous perspectives, plastic pollution, fate and effects of metals, new approach methods, and emerging contaminants. These workshops emphasize an informal exchange of ideas and knowledge on the topics among interested persons from industry, governments, consulting, and academia. Additionally, CEW provides an annual focus on the principles, current problems, and approaches in ecotoxicology. These workshops are administered by a Board of Directors and organized by local organizing committees annually. The Proceedings are published with the support of Fisheries and Oceans Canada.

RÉSUMÉ

Rickwood, C., Dalton, R., Robinson, S., Van der Vliet, L., Marteinson, S., Miller, J. 2026. Proceedings of the 49th Annual Canadian Ecotoxicity Workshop: October 2 – 5, 2023, Ottawa, Ontario. Can. Tech. Rep. Fish. Aquat. Sci. 3822: xxxi + 234 p. https://doi.org/10.60825/96ab-ce33

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

La 49e édition de CEW s'est tenue à le Centre Shaw à Ottawa (Ontario) en 2023. Après deux années d'ateliers hybrides en raison de la pandémie de COVID-19, cet atelier a marqué le retour à un événement CEW presque entièrement en présentiel. L'atelier comprenait 202 présentations de plateformes et 99 présentations d'affiches. Le nombre total de participants était 421.

Cet atelier faisait partie d'une série continue d'ateliers annuels au Canada sur la toxicologie écologique, couvrant des sujets tels que perspectives autochtones, pollution plastique, devenir et effets des métaux, nouvelles méthodes d'approche et contaminants émergents. Ces ateliers mettent l'accent sur un échange informel d'idées et de connaissances sur les sujets abordés entre les personnes intéressées issues de l'industrie, des gouvernements, du secteur du conseil et du milieu universitaire. En outre, CEW met chaque année l'accent sur les principes, les problèmes actuels et les approches en matière d'écotoxicologie. Ces ateliers sont administrés par un conseil d'administration et organisés chaque année par des comités d'organisation locaux. Les actes sont publiés avec le soutien de Pêches et Océans Canada.

Editors' comments

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

The editors would like to thank Carolyn Martinko 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. Les lecteurs sont priés de communiquer directement avec les auteurs pour faire des remarques sur les travaux. Toutes les déclarations et opinions paraissant dans le présent rapport sont entièrement celles des conférenciers; elles ne sont ni approuvées, ni rejetées par les éditeurs. La mention de marques de commerce ou de produits commercialisés ne constitue ni une approbation, ni une recommandation d'emploi.

Les rédacteurs voudraient remercier Carolyn Martinko pour son aide dans la préparation de ces comptes rendus.

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

M.Sc. Winner: Multigenerational effects of the novel brominated flame retardant 1,2,5,6-tetrabromocyclooctane on reproductive performance in Japanese medaka (*Oryzias latipes*)

Chloe Devoy[1]

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The brominated flame retardant, 1,2,5,6-tetrabromocyclooctane (TBCO), is an endocrine disrupting chemical (EDC) that's usage is predicted to increase. It is not known if early-life stage exposure of fish to TBCO causes reproductive impairments, as is the case when sexually mature fish are exposed to this chemical. This thesis demonstrates that TBCO is maternally transferred in Japanese medaka (*Oryzias latipes*), causing multigenerational reproductive impairment. Following parental (F0) exposure, F1 fecundity was decreased by 26.0–56.8%. Plasma concentrations of 17β-estradiol were not significantly decreased. A small decrease in transcript abundance of vitellogenin was observed, but the magnitude was unlikely to have decreased fecundity. However, *ex vivo* maturation of oocytes from the F1 generation was significantly decreased by 20.0–23.4%, and transcript abundances of genes that regulate oocyte maturation were consistently decreased. This is the first study to demonstrate that early-life stage exposure to an EDC caused decreased fecundity via inhibition of oocyte maturation.

B.Sc. Winner: Assessing cytotoxicity of legacy and emerging antimicrobial compounds in rainbow trout (*Oncorhynchus mykiss*) RTgill-W1 gill cells

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Antimicrobials are active chemical ingredients formulated to kill and/or inhibit various pathogens including bacteria and fungi. The amplified reliance on antimicrobial products and increased release through anthropogenic activities has made aquatic life increasingly vulnerable to exposure. The main objective of this research was to evaluate and compare the acute (24-hour) cytotoxicity of six antimicrobials to rainbow trout gill (RTgill-W1) epithelial cells under increasing concentrations. The neutral red uptake assay (indicating lysosomal integrity), following OECD 249 guidelines, was used to determine effective concentrations causing 50% loss in cell viability (EC_{50}). Preliminary data showed that most assayed antimicrobials were cytotoxic at concentrations above 5 $ug \cdot mL^{-1}$ to RTgill-W1 cells

except for chloroxylenol (PCMX), which showed no observable toxicity at any tested concentration. Quaternary ammonium compounds (QACs) were the most cytotoxic to RTgill-W1 cells compared to other groups of antimicrobials, raising concern that the alternatives replacing legacy triclosan may have a greater impact on fish health. In general, the RTgill-W1 cell line cytotoxicity caused by the assayed antimicrobials appear to be limited to higher concentrations that have not yet been measured in the environment. However, these results can be used to inform future studies to assess the impact of QACs and other antimicrobials on the environment.

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

Minding the (data) gap: Development of toxicity test methods with pulmonate freshwater snails (PL)

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Freshwater snails are an important component of aquatic ecosystems. They are present in diverse habitats, can dominate aquatic communities, contribute to nutrient cycling, and are an important food source for predators. Also, along with other mollusks, freshwater snails are amongst the most imperiled invertebrate taxa due to human activities, and they are sensitive to some aquatic contaminants (e.g., some metals, butyltins, bisphenol A). Until recently, freshwater snails were generally overlooked in ecological risk assessments, yet recent efforts to develop internationally standardized toxicity testing procedures have been fruitful. Over the last five years, research in our laboratory has focused on developing standardized testing procedures with freshwater snails, particularly *Planorbella pilsbryi*, a pulmonate freshwater snail common to watersheds across North America. This species is easy to culture and has a short life span relative to other freshwater mollusks, making it an excellent candidate for multi-generation testing. We will present some of our research on the use of freshwater snails for ecotoxicological testing in support of Environment and Climate Change Canada's mandate and commitments under the *Canadian Environmental Protection Act*. Laboratory methods include acute and chronic toxicity tests with various life stages, a partial multi-generation test, and a behaviour assay (under development). We also designed snail cages for in-situ deployment in aquatic habitats. These cages have been used in projects across Southern Ontario, in the Bay of Quinte, and in the St-Lawrence River. Once we have shared our enthusiasm for these cool creatures, we will propose areas for future research.

High-throughput determination of hepatic clearance as a screening tool for bioaccumulation using isolated perfused trout livers and diverse mixtures (PL)

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Chemical risk assessment often focuses on screening substances for criteria of persistence, bioaccumulation potential, and toxicity (PBT). Of these criteria, bioaccumulation potential presents unique challenges due to the difficulty associated with testing compounds that undergo biotransformation, compounded by the diversity of chemical classes of interest. Current assessment frameworks rely largely upon *in vivo* assays (OECD 305). The adoption of new approach methodologies (NAMs) such as *in vitro-in vivo* extrapolation assays (IVIVE; OECD 319) has been limited due to concerns of overprediction, uncertainty, and limited domain of applicability. These concerns can be addressed using the isolated perfused liver model, representing an intermediate level of biological organization. This model delivers a physiologically relevant measure of hepatic clearance and thus can estimate bioaccumulation potential. The present study uses this model to validate prior IVIVE methods by comparing direct clearance measurements with those of IVIVE prediction in a range of reference chemicals. To demonstrate an expanded domain of applicability, similar assays were performed using a pharmaceutical mixture of nine psychotropic drugs, as well as a diverse mixture of chemicals found within the United States Environmental Protection Agency's Non-Targeted Analysis Collaborative Trial (ENTACT) trial. Samples were analyzed using liquid-chromatography-high-resolution-mass-spectrometry (LC-HRMS) to semi-quantitatively measure individual chemicals within mixture and calculate hepatic extraction fraction. Our results serve to both validate prior *in vitro* methods, as well as demonstrate the potential use of the isolated perfused liver model as a tool for high-throughput bioaccumulation screening within a tiered risk assessment approach.

Canadian adaptation of the *Ceramium tenuicorne* ISO 10710 standard and preliminary evaluation using pulp and paper mill effluent (PL)

Carolyn Martinko[1], Leana Van der Vliet[1], Melanie Gallant[2], Sarah Costantini[2], Cintia Glasner Regis[2], Anna Sobaszek[2], Vanessa Chanes[2], Nadia Pajnic[2], Paige Cochrane[2], Lisa Taylor[2]

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The *Pulp and Paper Effluent Regulations* (PPER) pursuant to the *Fisheries Act* require sublethal toxicity testing on effluent discharged into marine or estuarine environments using marine algae species. Historically, reproduction tests using *Champia parvula* were used, but this test is no longer offered by North American labs due to concerns regarding data quality and lack of available new organisms. Environment and Climate Change Canada

(ECCC) is investigating the use of the ISO 10710:2010 7-day growth inhibition test using the red macroalgae *Ceramium tenuicorne*. This genus is native to Canadian coastal waters, and so is ecologically relevant to Canada. *C. tenuicorne* has been continuously cultured in the laboratory and used in European toxicity evaluations for >20 years, and therefore could address the shortcomings of *C. parvula*. ECCC developed an add-on procedure with supplementary conditions for use in a Canadian regulatory context, and Nautilus Environmental Company Inc. has been working with ECCC to refine these conditions. Initial testing demonstrated that *C. tenuicorne* can be successfully cultured in a Canadian lab and produce reliable reference toxicant test results. Recently, reference toxicant tests were conducted using two distinct organism size classes to determine if the sensitivity of the test is impacted by the use of more practical sizes. In addition, the sensitivity of *C. tenuicorne* has been characterized through testing effluent samples voluntarily submitted by Canadian pulp and paper facilities of various processing types. This work will support the improvement of the add-on procedure and inform amendments to the PPER sublethal toxicity testing requirements.

Evaluation of a novel toxicity test method with *Hyalella azteca* to optimize reproduction endpoints (PL)

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Hyalella azteca is a freshwater benthic crustacean used in ecotoxicology because it is ubiquitous in North American freshwater systems and is sensitive to changes in water quality. The standard reproduction toxicity test method for this species outlined by Environment and Climate Change Canada (ECCC) is a 6-week sediment test that begins with juveniles. Reproductive endpoints typically occur in weeks 4–6 of the standard test. It is difficult to achieve robust data for reproduction in *H. azteca* because there is naturally a high biological variability associated with reproductive yield and because effects on reproduction often co-occur with effects on growth. A novel reproduction toxicity test method for *H. azteca* was created to improve the quality and quantity of reproductive data generated. Four-week, water-only, static-renewal reproduction tests were initiated using sexually mature (6-to 7-week-old) organisms in a sex ratio of 7:3 (females:males), based on a previous study that determined the presence of excess females reduced reproductive variability. Standard 6-week reproduction toxicity tests used juveniles and were adapted to water-only, static-renewal conditions. For both methods, amphipods were exposed to sub-lethal concentrations of imidacloprid (0.5, 1, 2, 4, and 8 $ug \cdot L^{-1}$, determined from previous studies), and survival and reproduction were assessed weekly. Growth was measured at the end of the tests. Comparisons between methods focused on reproduction, but also included assessments of survival, growth, and amount of resources invested. Results revealed that the novel reproduction test method can improve toxicological assessments of reproduction in *H. azteca*.

Methodological challenges when conducting sediment toxicity identification evaluations (TIEs): Lessons learned (PL)

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Contaminated sediment has been identified as one of the major impediments to the remediation of many Areas of Concern (AOCs) in the Great Lakes. Within the Thunder Bay AOC, the former Northern Wood Preservers Inc. site resulted in elevated concentrations of wood preservation chemicals on the adjacent land and water. Beginning in the mid-1990s, the process of remediation, known as Northern Wood Preservers Alternative Remediation Concept (NOWPARC), commenced in the impacted area. Long-term monitoring results for water, sediment, and biota have shown elevated contaminant levels (in some cases above environmental quality guidelines). Using sediment from a site shown to consistently result in acute toxicity, a Phase I Toxicity Identification Evaluation (TIE) assessment was conducted to determine the general cause of NOWPARC toxicity (metals vs organics), following the United States Environmental Protection Agency Guidance Document (EPA/600/R-07/080). Since publication of the guidance document in 2007, amendment types recommended for use have been phased out of production or have changed formulation type. Issues encountered when performing TIE manipulations included discontinuation of the recommended carbonaceous resin amendment and sourcing of a replacement, "blank toxicity" in procedural controls due to cation exchange resin addition, and chemically characterizing changes in bioavailability of contaminants after manipulation. Here we document the challenges encountered when trying to identify the chemical classes responsible for the toxicity of sediments adjacent to the Northern Wood Preservers site, and the solutions found in the process.

Organic matter decomposition – A new microbial laboratory ISO standard (PL)

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The soil microbial community is critical to maintaining a healthy ecosystem and as such, methods to include the evaluation of soil microbial processes are needed in risk assessment approaches. Here, we introduce a new International Organization for Standardization (ISO) standard for Soil Quality: ISO 23265 "Soil Quality – test for estimating organic matter decomposition in contaminated soil". This standard is a simplified laboratory-scale method that measures changes to organic matter decomposition in the presence of soil contaminants. The new ISO standard was based on research conducted by Environment and Climate Change Canada, wherein the mass loss of filter papers (as a representative material for organic matter) was measured over time in different soil exposures. The method can be applied to most soils including field-collected,

or those amended with a chemical substance for risk assessment purposes by which a quantitative measure of toxicity (e.g., EC_{50}) can be derived. Standardization of the method required an international ring test involving up to 14 laboratories from across Canada, South Korea, and Europe. We will introduce the methodology involved as the method was developed, and highlight results from the different phases of the ring test, thus demonstrating the applicability of the method under different scenarios. The publication of this new standard contributes to the current gap for inclusion of soil microbial studies in risk-based assessments.

From genes to functions: Illuminating microbial diversity and functional profiles in a highly impacted river system using next-generation sequencing techniques (PL)

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Microbial communities are essential components of freshwater ecosystems and vulnerable to human-induced impacts. To facilitate effective ecosystem management, understanding their responses is crucial. This study utilized next-generation sequencing techniques to investigate the diversity and activity of microbial communities in Wascana Creek, Southern Saskatchewan, Canada, a previously identified highly impacted river system. Integrating targeted chemical analysis, suspect screening, and assessments of nutrients and greenhouse gases, the research aimed to identify specific pollutants and characterize their presence in the creek. Conducted in 2022, the investigation revealed a substantial anthropogenic influence on Wascana Creek. Upstream of the city of Regina, agricultural practices led to elevated phosphorus levels, inducing changes in microbial community composition and activity. Consequently, dissolved oxygen concentrations decreased, resulting in fish kill events. Additionally, a notable increase in methane emissions, a potent greenhouse gas, was observed. Downstream of the Regina wastewater treatment plant, the introduction of wastewater effluent significantly increased anthropogenic pollutants and nitrogen, and triggered notable shifts in microbial community composition. These alterations coincided with elevated emissions of nitrous oxide (N_2O), another potent greenhouse gas. These findings underscore the direct impact of human activities on microbial communities and emphasize the importance of understanding the intricate interactions between anthropogenic activities, microbial communities, and ecosystem functioning. By examining microbial responses to anthropogenic stressors, this research provides valuable insights into the underlying mechanisms of ecosystem disruption.

Exploring environmental thresholds for aquaculture drugs: Assessing the toxicity of spiked sediments on sea urchins using a Central Composite Rotatable Design (PL)

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In Canada, the salmon aquaculture industry is a major economic activity. For fish health control and management, antibiotics and anti-parasitic drugs are used on a regular basis. In-feed products like Emamectin benzoate (EMB), and Oxytetracycline (OTC) enter the marine environment accumulating in sediments, potentially remaining bioavailable to non-target organisms from months to years. Organic matter (OM) is often enriched in sediment surrounding aquaculture sites, and this may affect compounds' toxicity, bioavailability, and degradation. Despite the regular use of chemicals in Canadian aquaculture, there are still knowledge gaps regarding environmental fate and effects of mixtures of contaminants on non-target organisms. In this scenario, there is an urgency to understand the effect of multiple stressors at multiple environmentally relevant concentrations for environmental quality standards determination. This project has the primary objective of validating proposed environmental thresholds for aquaculture drugs, by mimicking real-world field conditions in the lab through the use of an innovative design. The Central Composite Rotatable Design (CCRD) is a response surface methodology developed for optimization studies. Design points are arranged to maximize the data range covered while reducing the number of experimental units required. The CCRD was applied to spike natural sediment with five levels of the three factors: combining environmentally relevant scenarios of EMB (0–34 $ng \cdot g^{-1}$), OTC (0–340 $ng \cdot g^{-1}$), and OM (0–10%), for a total of 30 experimental units. The toxicity of spiked sediment was assessed on multiple life stages of the green sea urchin (*Strongylocentrotus droebachiensis*) both acutely and chronically throughout a 140-day experiment.

A new approach methodology that integrates the zebrafish (*Danio rerio*) embryo acute toxicity test with behaviour, metabolic, and transcriptomic assays (PL)

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We present an integrated new approach methodology based on the zebrafish (*Danio rerio*) embryo/larvae model that combines four assays: the fish embryo toxicity (FET) test, the behavioural photomotor response assay, an alamarBlue-based metabolic energy expenditure assay, and a transcriptomic assay. Embryos were exposed from 6 hours post-fertilization to 5 days post-fertilization, with daily semi-static renewal, to 30 chemicals with diverse mechanisms of action. The assay employed a six-point dose-response

experimental design so that points of departure (PODs) from each endpoint could be derived and compared. Dose-response analysis was based on the benchmark dose method. For the FET, behaviour, and metabolism endpoints we incorporated an empirical approach to identifying biologically meaningful thresholds (i.e., the benchmark response) for determining PODs. For the transcriptomic dose-response analysis we determined both pathway-based and pathway-agnostic PODs. We found that integrating behavioural, metabolic, and transcriptomic endpoints into the FET test increased overall sensitivity of the assay (i.e., more chemicals tested positive or had lower PODs compared to the FET endpoint alone). Generally, the transcriptomic assay was the most sensitive, followed by the behavioural and metabolic assays. Behavioural, metabolic, and pathway responses were generally consistent with the known toxicity mechanisms of the chemicals tested demonstrating that the integrated assays also provided insight into mechanism of action. However, there were instances where chemicals of known toxicity tested negative, indicating that the assay may require further refinement to improve sensitivity. Overall, this muti-endpoint integrated zebrafish assays could become a relatively rapid and informative tool for chemical screening and risk assessment.

Towards development of an *in vitro* assay of oocyte maturation inhibition to predict reproductive capacity of fishes (PL)

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In fish, oogenesis is a dynamic process by which fertilizable oocytes develop from germ cells. Oogenesis can be divided into a growth phase and a maturation phase. Many endocrine disrupting chemicals impair reproduction by disrupting vitellogenesis that occurs during the growth phase. From this research, several adverse outcome pathways (AOPs) have been developed for decreased fecundity due to disruption of vitellogenesis, including AOP 30 (direct estrogen receptor antagonism), and AOP 25 (depression of estradiol (E2) synthesis through inhibition of aromatase). Our lab has been investigating inhibition of the second phase of oogenesis, oocyte maturation, as a mechanism of decreased fecundity. To this end, we use a previously developed *in vitro* assay of oocyte maturation in zebrafish and have developed *in vitro* assays for Japanese medaka and fathead minnows. We quantify inhibition of oocyte maturation using *in vitro* exposures of isolated immature oocytes to candidate chemicals, or by assessments of oocyte maturation following *in vivo* exposure of sexually mature female fish to candidate chemicals. In one study with Japanese medaka, *in vitro* exposure of stage IX oocytes to 1,2,5,6-tetrabromocyclooctane (TBCO) caused a 40% reduction in oocyte maturation, which was approximately equal to the decrease in fecundity of females exposed to dietary TBCO. Lack of changes in plasma E2 or expression of vitellogenin suggest that impairment of oocyte maturation likely caused the decreased fecundity. This study, and others, including

multigenerational studies suggestive of epigenetic disruption leading to inhibition of oocyte maturation, will be presented as evidence that assays of oocyte maturation might be useful to predict impaired fecundity.

Low-dose methylmercury promotes prenatal neuronal differentiation and autism spectrum disorder-like behaviours in early adulthood (PL)

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Methylmercury (MeHg) is a global pollutant that has been shown to elicit a range of adverse health effects, particularly on neurodevelopment, in both humans and wildlife populations. Humans are exposed to MeHg mainly through the consumption of contaminated seafood. Developing fetuses are especially susceptible to the effects of MeHg as it can cross the blood-brain barrier and placenta. We investigated the effects of MeHg *in vivo* throughout development by treating pregnant mice with either 0.2 ppm MeHg or 0 ppm MeHg-treated drinking water starting from conception. In the embryo, single-cell RNA sequencing and immunohistochemical analysis revealed that treatment with 0.2 ppm MeHg results in increased cortical thickness and a simultaneous increase in newborn neurons generated through asymmetric division of radial glial cells to directly form immature neurons. Autism spectrum disorder (ASD) has been linked to prenatal MeHg exposure and is associated with an overgrowth of the cerebral cortex and neuronal overproduction. ASD is a neurodevelopmental disorder that is characterized by impairments in communication and sociability, combined with restricted repetitive behaviours. In early postnatal and adulthood, mice who had been prenatally treated with low-dose MeHg showed reduced sociability and increased restrictive repetitive behaviours. This study is the first to show that prenatal low-dose MeHg exposure triggers ASD-like behaviours in a rodent model. This is a very pertinent topic for Canadian health as women of childbearing age who are frequent fish consumers in northern regions tend to have high MeHg exposure that often exceeds Health Canada's guidelines.

Characterization of molecular and apical effects of legacy contaminated groundwater in fathead minnows using the EcoToxChip system (PL)

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Mechanistic toxicology approaches are seen as a promising alternative to traditional live animal testing in support of ecological risk assessments; however, the often-noted lack of predicted linkages between effects observed at molecular and apical levels curtails the

adoption of such approaches. The objective of this study was to test the utility of a novel transcriptomics tool, EcoToxChips, to characterize the effects of complex mixtures of contaminants in fish and to compare molecular response patterns to apical outcomes. Embryonic and adult fathead minnows (FHM) were exposed for seven and four days, respectively, to groundwater collected from a legacy contaminated site under controlled laboratory conditions and in mesocosms. There were significant apical effects in embryonic and adult FHM after exposure to the groundwater mixtures in the laboratory. However, no such impacts were observed when fish were exposed in mesocosms. EcoToxChip analysis revealed significant changes in transcriptional response in laboratory exposed fish that are involved in biological pathways that could be linked to the apical outcomes observed. However, only two genes were slightly dysregulated in fish exposed during the mesocosm experiment, reflecting the lack of apical responses. Overall, the results observed in this study demonstrated that focused transcriptomics approaches such as the EcoToxChip were indicative of later apical outcomes, and that this system shows promise to support risk assessments of complex contaminated sites.

Towards establishing a 24-hour, microplate-based, transcriptomics assay for rainbow trout embryos (PL)

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Transcriptomic points of departure (tPODs) derived from acute toxicity tests are thought to be protective of chronic effects of chemicals in fish. The objective of this study was to establish a high-throughput toxicity test for larval fish that could simultaneously generate tPOD values and provide basic mortality data. We based our pilot method on recent efforts by United States Environmental Protection Agency researchers to establish a larval fathead minnow high throughput transcriptomics assay. Here, 1- to 2-day post hatch rainbow trout were placed in individual wells of 24-well plates and exposed to 12 concentrations of test chemicals including a negative control (DMSO, culture water), for 24 hours. Test concentrations followed a tapered design based on the estimated LD_{50}; extending two orders of magnitude above this value (to capture acute mortality) and five orders of magnitude below this value (to capture the full transcriptomic response). To date, 12 chemicals (3,4-dichloroaniline, copper sulphate ($CuSO_4$), ethinylestradiol, permethrin, malathion, N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone), acetaldehyde, 4-fluoroaniline, glyphosate, ethanol, thiamethoxam, and allyl alcohol) have been tested using this design. LC_{50} values have been generated for 6PPD-quinone (5.6 ug·L^{-1}), malathion (0.605 mg·L^{-1}), $CuSO_4$ (0.31 mg·L^{-1}), 3,4-dichloroaniline (58.5 mg·L^{-1}), acetaldehyde (41.2 mg·L^{-1}), and 4-fluoroaniline (242.7 mg·L^{-1}). Repeated studies of $CuSO_4$ yielded consistent LC_{50} values (0.31 mg·L^{-1}). Work is underway to optimize transcriptomics assays from these samples using EcoToxChips and UPXome, with

the ultimate goal to be able to derive transcriptomic points of departure. Taken together these results provide a foundation towards establishing a novel testing platform for chemical and environmental risk assessment.

EcoToxChips as a new approach method in chemical toxicity assessment for amphibians (PL)

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While transcriptomics is increasingly being incorporated into chemical hazard assessment, more work is needed to support the development and adoption of these tools for amphibians. Chemical-induced transcriptomic responses that are generated using standardized protocols (e.g., early life stage exposures) are proposed as indicators of adverse outcomes in this sensitive taxonomic group while reducing animal use. Here, we describe the development of a novel standardized reduced transcriptome assay (EcoToxChip test system) for two amphibian species: the standard lab model *Xenopus laevis*, and the ecologically relevant northern leopard frog (*Lithobates pipiens*). For each species, a systematic design approach was used to select gene targets (370 for *X. laevis*, 294 for leopard frog) for inclusion on species-specific qPCR arrays, EcoToxChips, to evaluate chemical-induced modulations in gene expression. As part of the development and optimization of the arrays, we conducted short-term embryonic chemical exposures for 96 hours (starting post-hatch and sampled prior to independent feeding) to target non-protected stages as an animal alternative test method. For each chemical, whole-body larvae were pooled (3–5 per sample) and gene expression assessed using EcoToxChips (www.ecotoxchip.ca) followed by qPCR data analysis with EcoToxXplorer (www.ecotoxxplorer.ca). For some of the chemicals tested, several genes and pathways that were expected to be perturbed were dysregulated. For example, for lead, most of the dysregulated genes identified were related to glutathione and xenobiotic metabolism while for benzo[a]pyrene, many of the dysregulated genes were related to xenobiotic metabolism, especially cytochrome P450 enzymes. Analysis of additional chemical- and species-specific cases are in progress, but initial results indicate that these amphibian-specific EcoToxChips could be used as a rapid and economic tool for chemical toxicity assessment for amphibians.

In vitro screening of UV stabilizers and UV filters: Cytotoxicity, CYP1A activity, and mRNA expression in an immortalized embryonic double-crested cormorant cell line (PL)

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

Ultraviolet (UV) stabilizers and UV filters are added to industrial and personal care products to absorb UV rays and prevent photo oxidation. UV stabilizers and UV filters have been detected in a range of environmental matrices, including high trophic level bird species (e.g., double-crested cormorant). Little is known about the toxicological properties of these compounds, especially in birds. Previously, we established and characterized an immortalized embryonic double-crested cormorant hepatic cell line, DCH22, for chemical screening. In the present study, DCH22 cells, cultured as 3-dimensional spheroids, were exposed to three UV stabilizers (UV-328, UV-329, UV-9) and a UV filter (BP-3) at 0.1 to 1000µM to determine cell viability, CYP1A activity and changes in mRNA expression. Cell viability and CYP1A activity were measured using intracellular ATP concentration and ethoxyresorufin-O-deethylase activity. A customized species-specific PCR array, EcoToxChip, comprising 384 genes, was used to determine changes in mRNA expression. The UV stabilizers and UV filter elicited similar effects on cell viability with LC_{50} values ranging from 112 to 160µM. UV-9 and BP-3 exposure resulted in a concentration-dependent increase in CYP1A activity whereas UV-328 and UV-329 did not induce CYP1A activity. The four chemicals (10µM) altered genes associated with multiple pathways and the common pathways dysregulated were thyroid homoeostasis and signal transduction. Concentration-dependent (0.1 to 100µM) changes in mRNA expression will be evaluated for the four compounds and benchmark doses will be determined. Given the increased use and prevalence of these compounds, these findings will generate much needed toxicity data in a wildlife avian species.

Performance of Japanese quail EcoToxChips in a multi-lab ring test (PL)

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The field of chemical risk assessment is increasingly moving towards new approach methods (NAMs) that reduce animal use and incorporate 'omics endpoints. These approaches hold promise, but uptake of NAMs by the regulatory community will require demonstration of their reproducibility. Here, we use a multi-lab ring test to evaluate the reproducibility of transcriptomic responses in early life stage Japanese quail (*Coturnix japonica*). Three independent laboratories (McGill University, Environment and Climate Change Canada (ECCC) and United States Geological Survey Eastern Ecological Science Center (USGS)) participated in the ring test. At each lab, fertilized, unincubated quail eggs

were exposed via air cell injection to DMSO (solvent control) or 7 concentrations (0.04–40 $\mu g \cdot g^{-1}$ egg) of chlorpyrifos (CPF) on embryonic day 0 (ED0). Hepatic gene expression in ED9 livers was assessed using the 384-gene Japanese quail EcoToxChip (n=5 per dose group). All methods and reagents were common between the three laboratories, but the strain of Japanese quail used by the USGS lab was different. The transcriptomic points of departure (tPODs) calculated by each lab were similar (17.5, 16.5, and 16.1 $ug \cdot g^{-1}$ for McGill, ECCC, and USGS respectively), and corresponded to the tPOD previously calculated from RNASeq data (21.6 $ug \cdot g^{-1}$). All tPODs were below our previously published organismal lowest observable effect level of 41.1 $ug \cdot g^{-1}$ for CPF in Japanese quail embryos. Overall, our results suggest that transcriptomic dose-response analysis holds promise as a NAM that is reproducible and of potential interest to the regulatory community.

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

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New approach methods (NAMs) have the potential to inform ecological risk assessment and require uptake into regulatory frameworks. The objective of this case study is to evaluate an approach to use NAMs in ecological risk assessment in a way that looks and feels like traditional test data. Species sensitivity distributions (SSDs) can be used to estimate chemical exposure concentrations expected to have minimal to negligible effects on ecosystems (i.e., predicted no effects concentrations [PNECs]). To date, SSDs have typically included traditional (survival, growth, and reproduction) endpoints. This case study will compare PNECs derived from SSDs that include 1) only traditional endpoints, 2) only NAM endpoints (e.g., gene expression, enzyme activity, *in vitro* testing) and 3) both traditional and NAM endpoints. The hypothesis is that SSDs that contain NAM data are more sensitive than traditional SSDs and can estimate a PNEC that is more protective of chronic (i.e., sub-lethal) endpoints. Through automated extraction and filtering of both traditional and NAM data from the United States Environmental Protection Agency's (US EPA's) ECOTOX knowledgebase, SSDs will be derived for over 50 chemicals. Chemicals included in this case study were selected to ensure a broad coverage of log Kow, Henry's Law Constant values, modes of action, and acute *in vivo* and *in vitro* toxicity (LC_{50} and EC_{50}), while considering data availability within the ECOTOX knowledgebase. The approach developed in this case study offers an immediate application for the potential use of NAM data in ecological risk assessment, including transcriptomic points of departure and new tools developed through an ongoing Genome Canada funded EcoToxChip project. The potential benefits and impacts of incorporating NAM data into SSDs used in regulatory ecological risk assessment will be discussed. The results will be presented as a case study through Accelerating the Pace of Chemical Risk Assessment (APCRA), an international government-to-government initiative whose aim is to promote collaboration and dialogue

on the scientific and regulatory needs for the application and acceptance of NAMs in regulatory decision making. *The contents of this abstract neither constitute, nor necessarily reflect US EPA policy.*

Addressing endocrine disruption in freshwater: A glimpse into developing recommendations to implement new monitoring methods into policy (PL)

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Endocrine disrupting chemicals (EDCs) are contaminants that can affect the normal function of the endocrine system and its hormones. EDCs are found in various products (e.g., pesticides, pharmaceuticals, plastics) and can be release in freshwater by various paths (e.g., wastewater, landfills). Those aspects, among others, makes EDCs difficult to tackle by policymakers. Several countries are currently exploring policy options to address EDCs in freshwater. This presentation will give a glimpse into the development process of policy recommendations for the monitoring of EDCs in freshwater at an intergovernmental organization such as the Organisation for Economic Co-operation and Development (OECD), where the author was an intern in 2022–2023. Using examples, the presentation will give insight on how OECD develops guidance based on two main aspects: the current state of scientific knowledge and country practices. For EDC monitoring, comparing the state-of-the-art methods (e.g., bioassays, non-targeted analysis) is necessary to understand the best available approach; while looking at country practices can provide an insight on how to use the methods (e.g., thresholds of effect), the learnt lessons (e.g., usefulness of transition phase), and the remaining barriers for implementation (e.g., lack of large data set and method standardization). Based on the acquired knowledge, policy recommendations are developed for different needs and levels of ambition. Moreover, this process provides a comprehensive overview of what is done internationally by academia and government, but also identifies the future opportunities for method improvement, international cooperation, and harmonization of practices.

Method development and application of walleye as a test species to evaluate the toxicity of constituents of concern from Alberta (PO)

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As a culturally and ecologically important fish species in Alberta, limited information exists on the sensitivity of walleye (*Sander vitreus*) to constituents of concern (COCs) from industrial processes. It is of growing interest to include walleye in ecotoxicity assessments to determine their sensitivity to COCs, and to establish if their sensitivity differs from other well-studied fish used routinely in standardized toxicity tests, such as rainbow trout and

fathead minnows. However, walleye are challenging to culture and test under laboratory conditions due to constraints on organism availability, a fragile larval stage, and their piscivorous nature. Therefore, standardized toxicity test methods do not exist for this species. In collaboration with InnoTech Alberta and the Cold Lake Fish Hatchery, this study was undertaken to further develop test methods to successfully use walleye. Test method development has included a 96-hour static test using juvenile walleye (1.0–2.5 g) and a 7-day daily-renewal sublethal test using newly hatched larvae (<24-hour post-hatch). A reference toxicant warning chart is being developed to assess the consistency of the response of juvenile walleye in acute tests. Methods and results of tests performed using walleye exposed to field samples collected in Alberta, as well as exposures to sodium chloride as a reference toxicant, are presented here and highlight that tests with different life stages of this species are possible. Further work required to refine methods for early life stage tests, including the development of tests that incorporate the embryo stage, are described.

The influence of salinity on the acute lethality of select metals and other deleterious substances to two marine invertebrates (PO)

Marriah Grey[1], Pam Howes[1], Melissa Thompson[1]

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There continues to be interest in understanding the sensitivity of the *Acartia tonsa* acute lethality test method (STB 1/RM/60) to common deleterious substances under a range of saline conditions. Dischargers falling under the purview of the *Metal and Diamond Mining Effluent Regulations* (MDMER), whose effluent salinity exceeds 4‰, must employ this test method to demonstrate regulatory compliance. End of pipe salinity of discharge can vary widely, posing a challenge for data interpretation. Much of the published literature suggests metals toxicity abates as salinity increases, but there are examples where this trend does not hold true. To assess the potential difference in lethal response to a number of commonly detected deleterious substances, a series of acute *A. tonsa* and *A. bahia* exposures were conducted under both brackish (10‰) and marine (30‰) conditions using serially diluted natural seawater spiked with select reagent grade chemicals. LC_{50} data were derived for each test based on measured values in each test concentration.

A high throughput *in vitro* assay using Ultraplex RNA sequencing to derive transcriptomic points of departure (PO)

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Interest in developing cheaper and faster alternative non-animal testing strategies has grown due to ethical concerns and high costs associated with traditional animal-based

toxicity testing. Concurrently, advances in sequencing and 'omics technologies leading to reduced sequencing costs has made it more feasible to derive transcriptomic points of departure (tPODs) and integrate molecular data into risk assessment. The objective here was to establish a high-throughput *in vitro* transcriptomic-based toxicity test system that couples rainbow trout RTgill-W1 and human Caco-2 cell lines with QIAGEN's Ultraplex technology to derive tPODs. The library preparation workflow was optimized using unexposed RTgill-W1 cells, and libraries were checked for quality on a Bioanalyzer. Cell lines were then exposed for 24 hours to solvents (dimethyl sulfoxide and methanol) and positive controls (3,4-dichloroaniline–RTgill-W1 and hydrogen peroxide–Caco-2) based on the OECD fish cell line test guideline-249, from which libraries were prepared and sequenced using Illumina MiSeq. The advantages of this workflow are the microplate-based cell lysis and reverse transcription steps, and the incorporation of a unique sample index into the cDNA from each sample resulting in a simpler workflow, which enables processing of multiple 96-well plates in one workday. No differential gene expression was observed in solvent-exposed RTgill-W1 cells compared to controls. Preliminary analysis identified 40–82 differentially expressed genes in 3,4-dichloroaniline-exposed RTgill-W1 cells including stress-related and signaling genes such as hsp90ba, aldoa, cavin1b. Ongoing studies include evaluating the performance of this platform using Caco-2 cells and conducting further exposures with environmental samples and chemicals of regulatory concern in several case studies.

Prospective optimization, evaluation, and application of *in vitro* methods to study biotransformation of organic chemicals in birds (PO)

Matthew Schultz[1], Michelle Embry[2], Robert Letcher[3], Christy Morrisey[1], Markus Brinkmann[1]

[1]University of Saskatchewan, [2]Health and Environmental Sciences Institute, [3]Environment and Climate Change Canada

Avian species have a critical role in aquatic and terrestrial ecosystems but are understudied in the realm of bioaccumulation assessment and toxicokinetic studies. Regulatory frameworks require the provision of high-quality data from standardized bioaccumulation assays to inform their prospective risk assessment. Whole animal studies are limited in throughput and raise ethical and economic concerns. Thus, *in vitro* biotransformation assays in combination with *in vitro-in vivo* extrapolation (IVIVE) models have been proposed as an alternative. In fish, such assays have been recently standardized. However, the development and adoption of avian methodologies have been slow to date. The present study seeks to bring avian biotransformation assays to parity with fish by optimizing and expanding the use of *in vitro* substrate depletion assays, focusing on isolated hepatocytes and liver subcellular fractions. Initial optimization efforts are focused on methods of isolation and cryopreservation, as well as assay feasibility in mallard duck and herring gull. These optimized assays will then be applied to ten organic chemicals, including polycyclic aromatic hydrocarbons, polychlorinated biphenyls, bisphenols,

organophosphates, and neonicotinoids, to generate high-quality substrate depletion data. This work also provides an opportunity to identify metabolites and evaluate biotransformation pathways and enzyme metabolism activities. Finally, at least two additional species will be selected for the application of optimized assays. This selection will take into account ecological relevance, practicality, as well as trait-based selection to contribute to a robust database of high-quality data. The prospective goal of this study is to lay the groundwork for a future ring trial, furthering the adoption of avian *in vitro* biotransformation assessment.

High-throughput transcriptomic and phenotypic assessments to characterize hazards for per-and poly-fluoroalkyl substances (PFAS) in zebrafish embryos (PO)

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Per- and poly-fluoroalkyl substances (PFAS) are anthropogenic chemicals used in industrial and commercial products. The continuous entry of PFASs into the environment is of important global concern. Given the poor toxicity data with a variety of defined PFASs, a high-throughput new approach for toxicity testing is required to characterize their hazards in humans and living organisms. Zebrafish embryos <120 hours post-fertilization (120 hpf) are considered a powerful *in vitro* model to assess systematic biological effects covering crucial developmental stages. Using negative control zebrafish embryos exposed to only dimethyl sulfoxide, we first identified suitable embryo pool and sample sizes, and differentially expressed gene (DEG) analysis methods on the TempO-Seq platform. Afterward, zebrafish embryos were exposed to eight individual PFAS of differing chain lengths with sulfonic acid (i.e., PFBS, PFHxS, PFOS, and PFDS) and carboxylic acids (i.e., PFBA, PFHxA, PFOA, and PFDA) at 4–120 hpf. We screened phenotypic effects by evaluating developmental toxicity, behavioural movement, metabolic activity, and examined transcriptomic perturbations. Phenotypic and transcriptomic benchmark concentration modeling was processed with Rcurvep and BMDExpress v2.3, respectively. Our results showed the toxicity potencies of PFAS corresponding to chain length and functional groups, offering the optimal design for a dose-response study utilizing the zebrafish model and TempO-Seq platform. In the future, this study will be used to develop points of departure for use in ecotoxicological and human risk assessments of environmentally relevant chemicals.

Developing non-lethal techniques for identifying endocrine disruption in fishes (PO)

Emily Kennedy[1], David Janz[1], Markus Hecker[1]

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The release of endocrine disrupting chemicals (EDCs) into aquatic environments presents a considerable danger to the well being of fish populations. EDCs often target steroidogenic pathways, making the quantification of circulating steroid hormone concentrations a valuable tool for assessing their effects. However, traditional methods of hormone quantification in blood face challenges in species commonly used for studying EDCs, such as the fathead minnow, due to limited sample volumes. More importantly, ethical concerns arise from the sacrifice of large numbers of animals required for both traditional laboratory- and field-based studies. Therefore, our research aims to develop non-lethal techniques for quantifying steroid hormones in fish, applicable in both lab and field settings. First, a novel liquid chromatography tandem mass spectrometry method will be developed to quantify excreted steroid hormones extracted from tank water with the intent of providing a non-lethal/non-invasive alternative to blood sampling. Additionally, mucus and scales will be explored as media for non-lethal hormone measurement. The developed methods will then be employed in laboratory settings to identify and characterize the effects of select EDCs in fishes, contributing to our understanding of their impact on the endocrine system. Furthermore, these techniques will be applied in field-based biomonitoring efforts to assess the impact of EDCs on wild fish populations in their natural habitats. By prioritizing non-lethal approaches, this research emphasizes the importance of understanding the effects of EDCs in fishes while considering ethical implications aiding in the preservation of aquatic ecosystems and safeguarding the health of fish populations.

The effects of hypoxia on fathead minnow behaviour and ‘omics (PO)

Raina Hubley[1], Theresa Warriner[1], Aisha Pasha[1], Meghan Allen[1], Denina Simmons[1]

[1]*Ontario Tech University*

Behaviour is a useful parameter for monitoring the health of vertebrates in the context of environmental changes. Behavioural monitoring is non-invasive and could be utilised as a refinement for fish studies in both the lab and field environments. Traditional ‘omics methods can be utilized to determine changes in fish protein, metabolite, and lipid abundance due to toxic exposures. Dissolved oxygen fluctuations in water bodies have become more prevalent due to climate change and these fluctuations can lead to increased frequency and intensity of hypoxic conditions. We aimed to combine non-invasive methods with traditional methods to determine the effect of hypoxia on fathead minnow (*Pimephales promelas*) behaviour and ‘omics. We exposed 60 fathead minnows to hypoxic

(2.10–2.80 mg·mL^{-1} DO) and normoxic (5.80–7.00 mg·mL^{-1} DO) conditions over a 7-day period. Fish were video recorded on days 1, 3, 5, and 7. Three blind observers analysed the videos for fish tank location activity, foraging behaviour, and novel object behaviour. On day 8, we anaesthetized fish with 100 mg·L^{-1} MS-222 and collected mucus, plasma, gill, and brain samples, and also measured blood glucose. We found that hypoxia altered fathead minnow activity levels over the course of the 7-day treatment. In this presentation, we will share the integrated results of fish behaviour, brain 'omics, mucus 'omics, and blood glucose levels.

Impact of irradiated and non-irradiated very low sulphur fuel oils on the righting behaviour of common periwinkle snails (*Littorina littorea*) (PO)

Danielle Philibert[1]

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Very low sulphur fuel oils (VLSFOs) are a new generation of fuels that are physically, chemically, and toxicologically diverse and are less understood than their traditional counterparts. The impact of environmental processes on the toxicity of VLSFOs, especially the role of photooxidation by ultraviolet light, are poorly understood. The aim of this project is addressing this data gap using the common periwinkle snail (*Littorina littorea*) righting assay, and to determine the sensitivity of this gastropod to hydrocarbon exposure. Found throughout the North Atlantic, periwinkles snails are found in high numbers in intertidal zones, and field observations made during the Sea Empress and Amoco Cadiz oil spills suggest that they may be sensitive to hydrocarbon exposure. In this study, exposures were conducted by generating water accommodated fractions (WAFs) of petroleum products (n=12) and exposing select WAFs to 18 hours of UV light while mixing. Changes in WAF chemistry due to UV light exposure were quantified using fluorometry, total organic carbon (TOC), and biomimetic extraction via solid-phase microextraction (BE-SPME) measurements. Exposure to both irradiated and non-irradiated WAFs resulted in a reduction in the proportion of snails that righted themselves, however the inhibition rate varied between the different petroleum products tested (e.g., 5–85% inhibition). Photomodification decreased the toxicity of VLSFO WAF to periwinkle snails which is the opposite of what we observed with American lobster larvae (*Homarus americanus*) and Atlantic cod larvae (*Gadus morhua*) exposures. The data generated in this study will be used to improve our understanding of VLSFO spills and the impact they could have on intertidal gastropod species.

Dietary exposure of stormwater contaminants in biofilm to two freshwater macroinvertebrates (PO)

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Biofilms are known to bioconcentrate a variety of aquatic contaminants and are useful tools for characterizing stormwater pollution in urban ecosystems. However, as an important food source for many aquatic biota, concerns arise regarding the potential toxicological risks of contaminant accumulation to biofilm consumers. More, this accumulation may alter the quantity and nutritional quality of the available biofilm crop. To address this, we selected two freshwater invertebrate species as subjects in a series of dietary bioassays: *Neocloeon triangulifer* and *Planorbella pilsbryi*. These are sensitive, ecologically-relevant indicators of biological integrity and can be cultured in a laboratory environment. Treatment diets consisted of biofilm cultured on artificial substrates deployed in 15 stormwater pond sites in Brampton, Ontario, representing a gradient of urbanization. Three control diets included a starved control, culturing diet, and biofilm collected from a relatively pristine river in Ontario. Biofilm samples were analyzed for a suite of ~500 current-use and legacy pesticides, as well as 22 heavy metals. Ash-free dry weight and chlorophyll-a were used as proxies for biofilm nutritional value. Following a 7-day acute trial with *N. triangulifer* nymphs, survival and biomass production were measured. A 28-day chronic trial was used for *P. pilsbryi* juveniles, with endpoints consisting of survival, growth, biomass production, and behaviour. We tested whether treatments with higher pesticide and metal loads had lower nutritional quality, and resulted in differences in measured endpoints in the test subjects. The results of these studies will contribute to the limited knowledge of dietary exposures to environmentally-relevant contaminant mixtures.

Aquatic mesocosms for environmental research: Insights from three studies in six years (PO)

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The mesocosm facility at the InnoTech Alberta Vegreville site, designed and constructed in 2016, serves as a research tool to understand the impacts of stressors on the physical, chemical, biological, toxicological, and ecological parameters of model ecosystems. The facility consists of 30 open-top polypropylene tanks with an operational volume of ~14 000 L and 16 above-ground tanks with a volume of 4500 L, offering versatility for terrestrial and aquatic environments. The modular design allows for a wide range of sampling and monitoring options. These facilities are suited to studies that compare outcomes (a) between different types of treatment technologies, (b) multifactor studies to

examine treatment interactions, (c) of a range of concentration/intensity gradients of the same technology to find the optimal application rate, and/or (d) over a time series (acute vs chronic effects). Three studies have been conducted at the facility, expected to conclude by late 2023. The initial mesocosm studies have demonstrated that the presence of soil and vegetation significantly influences the response of biological and chemical variables. It also demonstrated the facility's unique capability to maintain communities over winter, enabling the investigation of ecological dynamics over multiple years. This presentation provides an overview of the facility's design and construction, highlighting its unique features and advantages for researchers. Lessons learned from these experiments have produced operational efficiencies, refined monitoring approaches, and improved data interpretation. The insights gained from these studies contribute to the refinement of environmental research and guide future experimental design, ultimately facilitating better informed decision making.

The effects of contaminants on the taxonomic composition of micro-meiofauna present in biofilms (PO)

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Rotifers, zooflagellates, heliozoans, ciliates, amoebas… do these ring a bell? These organisms, commonly known as micro-meiofauna, belong to various heterotrophic taxa and range in size from 2 µm to 2 mm. They can be found as free-living forms or associated with particles and benthic substrates. Micro-meiofauna communities are important components of aquatic ecosystems and play an important role in biofilms. However, most biofilm studies primarily focus on autotrophic and bacterial taxa, often overlooking the impact of contamination on biofilm micro-meiofauna. Considering that micro-meiofauna are widespread, easy to sample, and highly responsive to environmental changes, they may serve as bio-indicators of pollution or disturbances, yet their full potential in this regard remains largely untapped. In this work, we investigated the effects of the optical brightener (DAST) and the insecticide fenoxycarb (FNX) on the biodiversity of biofilm micro-meiofauna. These two contaminants were studied in mixtures with two ubiquitous contaminants: the herbicide glyphosate and its main metabolite aminomethylphosphonic acid (AMPA) at environmental concentrations. A microcosm experiment was conducted to examine the impacts of these contaminants at three exposure durations (1, 14, and 28 days) on a micro-meiobenthic community collected from a freshwater pond in Gazinet-Cestas (France). The results revealed that these contaminants modified the taxonomic composition of the micro-meiofauna, resulting in an increase in ciliates density and a decrease in amoebae density. Our study shows the sensitivity of micro-meiofauna to

contaminants in biofilms. Further work should focus on the impact of these changes in micro-meiofauna community composition on biofilm function and structure.

Refinement of soil nitrification assessment procedure using genomics tools (PO)

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Nitrification is a microbe-mediated key ecological process in nitrogen cycling. Traditionally, effects on soil nitrification are assessed using an OECD guideline (OECD, 2000), with dose-response relationships derived to calculate ECx values (i.e, EC_{50}, etc.); however, the test is time (e.g., ~12 weeks) and labour intensive. As an alternative, genomic-based methods may be used, based on gene expression data, to derive transcriptional points of departure (tPOD), using benchmark dose (BMD) modelling. This approach has the potential to identify nitrification inhibition, in a shorter duration, for application to environmental risk assessment (ERA). However, studies comparing the traditional assay with novel genomic approaches are scarce, especially for soils. Herein, we present preliminary results of such a comparison, wherein genomic tools were used to assess impacts to soil nitrification, relative to the traditional-based assay. A sandy loam soil mixed with alfalfa (to induce nitrification) was spiked with silver nanoparticles (AgNP) (0–1500 $mg \cdot kg^{-1}$), as a representative nitrification inhibitor. Test soils were incubated for 10–12 weeks, and samples were taken in 2–4 week intervals for RNA extraction, as well as traditional analyses of nitrate/nitrite (OECD, 2000). The changes in gene expression, in response to AgNP, were assessed using RNA Seq. Preliminary data, based on day-28 sampling, suggest that nitrification related genes were differentially expressed and corresponded to the significant inhibition of nitrification observed in the traditional-based assay at high AgNP concentrations. Results suggest the potential application of this novel method for detecting soil nitrification inhibition.

Transcriptomic points of departure using EcoToxChips: A case study with early-life stage rainbow trout exposed to ethinyl estradiol for 24 hours (PO)

Emily Boulanger[1], Niladri Basu[1], Aylish Marshall[1], Hugo Marchand[1], Krittika Mittal[1], Jessica Ewald[1], Markus Hecker[2], Jianguo Xia[1], Jessica Head[1]

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Transcriptomic points of departure (tPODs) are emerging as a promising approach for evaluating the toxicity of environmental contaminants while simultaneously obtaining mechanistic data. The objective of this study was to evaluate whether we could couple a rapid early life stage (ELS) fish embryo exposure study with EcoToxChip analysis to generate a tPOD. Rainbow trout (RT) hatchlings (1–2 days post-hatch) were exposed to a

solvent control (0.5% DMSO) or 11 concentrations of ethinyl estradiol (EE2): 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, and 100 ng·L^{-1} in 24-well plates for 24 hours. Gene expression was analyzed in three replicate samples of four pooled larvae per dose using the 384-gene RT EcoToxChip. Differential gene expression and transcriptomic dose-response analysis was performed using EcoToxXplorer (www.ecotoxxplorer.ca). No mortalities or deformities were observed following EE2 exposure. Common biomarkers of EE2 exposure including vitellogenin-like and estrogen receptor genes were dysregulated. tPOD values ranged from 0.8 to 8.3 ng·L^{-1} EE2 were calculated depending on input parameters. Similar tPOD values were previously derived using RNA Sequencing in a 4-day exposure of RT hatchlings (0.2–3.6 ng·L^{-1}), and microarray analysis of gonads from male fry exposed for 76 days (~3 ng·L^{-1}). Taken together, the findings from this case study lend support to the notion that 24-hour microplate-based bioassays with RT embryos (1–2 days post-hatch) coupled with EcoToxChips can yield molecular data that provide insights into mechanisms of action and are also conducive to benchmark dose modeling to derive quantitative tPODs.

The use of Japanese quail EcoToxChips to improve the understanding of the mechanism(s) of action of hexabromocyclododecane (HBCD) in early life stage embryos (PO)

Emily Boulanger[1], Niladri Basu[1], Doug Crump[2], Jessica Head[1]

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

Monitoring studies have documented the presence of hexabromocyclododecane (HBCD) in the eggs of avian species, but little is known about the molecular mechanisms by which HBCD may affect birds, particularly during early life stages. The objective of this study was to increase understanding of HBCD's mechanisms of action in an early life stage avian model using the EcoToxChip Test System. Briefly, fertilized unincubated Japanese quail (JQ) eggs were injected via air cell on embryonic day 0 with DMSO (solvent control), 0.7, or 11 ug·g^{-1} HBCD (35 eggs per dose group). Eggs were artificially incubated at 37.5 ± 0.5°C and 60 ± 5% humidity. At embryonic day 9 (mid-incubation for JQ), liver samples from five viable embryos were harvested per dose group. Hepatic gene expression was assessed using the 384-gene Japanese Quail EcoToxChip. The qPCR data generated from the JQ EcoToxChips were analysed with EcoToxXplorer (www.ecotoxxplorer.ca). The number of differentially expressed genes (DEGs) was 81 and 49 in the 0.7 and 11 ug·g^{-1} HBCD groups, respectively. Some of these genes were found in adverse outcome pathways associated with peroxisome proliferator-activated receptor (PPAR) and estrogen receptor (ER) antagonism. Pathway-level investigation of the gene expression data suggests that the main biological pathways affected by this exposure were those related to metabolism. These findings increase understanding of how avian models may be affected by early-life exposure to HBCD and demonstrate that the EcoToxChip Test System may serve as an affective new approach method.

The use of Japanese quail EcoToxChips to improve the understanding of the mechanism(s) of action of ethinyl estradiol (EE2) in early life stage embryos (PO)

Emily Boulanger[1], Niladri Basu[1], Doug Crump[2], Jessica Head[1]

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

While monitoring studies have documented that various avian species are exposed to ethinyl estradiol (EE2), much less is known about the molecular mechanisms by which EE2 (and more generally, estrogenic chemicals) affect birds, particularly during early life stages. The objective of this study was to increase understanding of EE2's mechanism(s) of action in an early life stage avian model using the EcoToxChip Test System. Briefly, fertilized unincubated Japanese quail (JQ) eggs were injected via air cell on embryonic day 0 with DMSO (solvent control), 6, or 54 ug·g^{-1} EE2 (35 eggs per dose group). Eggs were artificially incubated at 37.5 ± 0.5°C and 60 ± 5% humidity. At embryonic day 9 (mid-incubation), liver samples from five viable embryos were harvested per dose group. Hepatic gene expression was assessed using the 384-gene Japanese Quail EcoToxChip. The qPCR data generated from the JQ EcoToxChips were analysed with EcoToxXplorer (www.ecotoxxplorer.ca). The number of differentially expressed genes (DEGs) was 39 and 41 in the 6 and 54 ug·g^{-1} EE2 groups, respectively. Some of these genes were found in adverse outcome pathways associated with constitutive androstane receptor (CAR) and estrogen receptor (ER) antagonism. Pathway-level investigation of the gene expression data suggests that the main biological pathways affected by this exposure were related to metabolism. These findings increase understanding of how avian models may be affected by early-life exposure to EE2, and demonstrate that the EcoToxChip test system may serve as an effective new approach method.

Incorporating Indigenous Perspectives into Environmental Management: The Importance of Two-Eyed Seeing

Bridging Indigenous and western knowledge systems: exploring the importance of Two-Eyed Seeing in environmental management (PL)

Jessica Wakefield[1], Karoliina Munter[2], Shyla O'Donnell[2]

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In Canada, the legal requirement of the duty to consult and accommodate mandates federal and provincial governments to engage Indigenous communities in discussions regarding proposed projects that may impact their rights and interests. The inherent limitations of solely relying on western scientific methods to guide not only the management of environmental and ocean resources, but impacts on rights and interests, have led to the growing recognition of Two-eyed Seeing (2ES) as a practical approach to increase Indigenous Knowledge (IK) and perspectives within environmental management. 2ES refers to the ability to view the world through Indigenous and western perspectives, simultaneously valuing and integrating the strengths and knowledge of both systems. By emphasizing the importance of bridging the two knowledge systems during the earliest stages of environmental and ocean resource management, we are demonstrating a path towards an increasingly comprehensive understanding of complex issues that can lead to a more equitable decision-making process that considers the perspectives of rights holders being ultimately impacted by the project being reviewed. It is essential to accept IK as the intellectual property of Indigenous Nations. Moreover, it is necessary to differentiate between relationship building and the legal consultation and accommodation process. This distinction becomes clearer as we examine project examples at various stages of the journey towards 2ES. After providing an overview of the 2ES gold standard, various projects are discussed, considering common barriers, challenges and lessons learned. By exploring these projects, participants will gain insight into the preliminary positive outcomes of embracing this integrated approach, such as fostering a more inclusive, equitable, and sustainable future.

Human health and ecological risk assessments in Indigenous communities: A survey of practitioners in Canada (PL)

Katherine Chong[1], Gordon Hickey[1], Niladri Basu[1]

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Environmental pollution poses unique and complex risks to many Indigenous communities in Canada, often because of unique exposure pathways and impacts on

culture, spirituality, language, and traditional food systems. However, to date institutionalized human health and ecological risk assessment (RA) approaches have not been developed or implemented with these unique contexts in mind. Therefore, we conducted an online survey of those who are involved with RA in Indigenous communities in Canada, including community members and those they collaborate with, to: first, understand RA practice in Indigenous communities in Canada; second, explore challenges with conventional RA methods; and third, gather perspectives on the development of new approaches. The survey was launched on May 9 and has received 25 responses as of late May from which we draw preliminary results. For our first objective, RA practice professionals spanned community environment offices (25%), Indigenous governments (4%), federal and provincial governments (22%), and academia (52%), with most participants conducting research as their primary role (58%). For our second objective, 54% of respondents were not satisfied with current RAs. There was agreement amongst nearly all respondents that time (83%), cost (75%), and resource availability (83%) were moderate to severe problems. In response to our third objective, relatively few respondents (22%) had heard of new approach methodologies for RA. Participants emphasized consideration of socio-cultural factors, weighing risks and benefits of traditional foods, risk communication, ongoing monitoring, and assessing chemical mixtures as priorities for new RA methods. The survey's applications will be realized in collaboration with the Kanien'kehá:ka nation of Kanesatake, who lead a project on developing new approaches to assess risks from a nearby contaminated site.

Workshop on best practices for recognizing and including multiple ways of knowing in State of Environment reporting (PL)

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State of Environment (SOE) reports are now a common reporting requirement, supported by the United Nations, for tracking environmental condition. The reports should play an important role in providing baseline information for environmental assessments, but a review of >140 SOE reports identified real gaps in the consideration or inclusion of Indigenous perspectives. A workshop on best practices for honoring and co-presenting Indigenous Knowledge Systems in SOE reporting included 28 Indigenous community members from 14 First Nations, Métis, and Inuvialuit communities located within the Peace, Athabasca, Great Slave, and Mackenzie Great Bear River basins (four of the six Mackenzie River sub-basins) in the Northwest Territories and Alberta. The presentations and discussions at the workshop focused on the various challenges, solutions, and approaches for strengthening the inclusion of Indigenous perspectives and indicators in SOE reporting. Four priority aspirations were identified for future SOE reporting in the

Mackenzie River Basin: inclusion of Indigenous Language, Audio Recordings (stories), Case Studies to highlight the work being done by Indigenous-led Community-Based Monitoring programs, and Indigenous Expert Assessments on ecosystem health and environmental indicators. In addition to evaluating and prioritizing approaches, the workshop also generated several new ideas and recommendations, which may require more development. These included Interactive StoryMaps with connections to Audio Recordings and Indigenous Language, including mentorship programs for Indigenous Language revitalization, partnerships between Nations to share stories and data, including data management in accordance with ownership, control, access, and possession (OCAP®) principles, communication strategies specific to Indigenous communities, and curriculum development to engage high school students.

Development of a traditional foods study framework that prioritizes Indigenous community involvement (PL)

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CanNorth has been involved in a growing number of traditional foods studies, which has resulted in the development of a Traditional Foods Study Framework. This framework has evolved over time and has had proven success in many communities across Saskatchewan. Traditional foods studies involve collecting information on the quantity, type, and location of traditional foods consumed by community members and using this information to establish a sampling program to test the safety of traditional foods. The samples are chemically analyzed, and the data analyses often include a human health risk assessment (HHRA). These types of studies have become more common as we have observed a strong push by provincial regulators in Saskatchewan to undertake traditional foods studies as part of the environmental assessment. In addition, First Nations are proactively seeking out these studies to inform their communities and to aid in interactions with industrial developments. A key aspect to the success of the framework is the high level of community involvement. In CanNorth's framework, community members are trained to complete food frequency questionnaire interviews, and use ArcGIS. Community input is used to inform the sampling program, data analyses, and aspects of the HHRA. Additionally, the sampling is completed by, or with, residents. Finally, methods for communicating the results are driven by the communities and can include presentations, brochures, and information dissemination at Treaty Days. This presentation will discuss our Traditional Foods Study Framework with a focus on how CanNorth designs the program to be community led.

An academic's perspectives on science communication and teamwork when working with Moose Cree First Nation and Wildlife Conservation Society Canada (PL)

Denina Simmons[1], Keisha Deoraj[1], Claire Farrell[2], Gretchen Lescord[3], Constance O'Connor[2], Jacob Seguin[2], Jennifer Simard[4], Moose Cree First Nation*

*[1]Ontario Tech University, [2]Wildlife Conservation Society Canada, [3]University of Florida, [4]Moose Cree First Nation, *Co-principal Investigators, the need for this project and the communication of results were driven by Moose Cree First Nation*

Denina Simmons, speaking in the first person: In my first collaboration with a Canadian First Nation and a Canadian not-for-profit science organization, I am just learning how to build relationships outside of my training grounds (the academy and government). Learning from Lake Sturgeon is a co-created research effort between Wildlife Conservation Society (WCS) Canada and Moose Cree First Nation Resource Protection to improve stewardship of Lake Sturgeon and the rivers they call home within the Moose Cree Homeland. I joined the Learning from Lake Sturgeon project as a new collaborator in 2021, five years after the project was created. In this presentation, I will briefly describe the program, and my role within it, including our youth and science programming goals. Finally, I will share some learning materials and experiences from our interactive community booth at the 2023 annual Gathering of our People (a.k.a. G.O.O.P.) which took place in Moose Factory August 1-5, and gathered together the people in the traditional lands of the Moose Cree people. The goal of our booth and media was to engage youth, Elders, and everyone between, and to share our scientific results. This presentation will be mostly stories and examples to illustrate lessons learned with some interactive components to engage the audience, with examples of how we are starting to adopt a two-eyed approach when communicating our science with the Moose Cree community. It is our hope that the data we collect, which are co-owned by the Moose Cree First Nation, will aid in future environmental assessments for new hydro-electric projects, and will support community decision-making and data autonomy.

Wildlife Ecotoxicology: Exposure, Accumulation, and Effects

Target development for molybdenum using wildlife risk assessment techniques – A case study on the challenges associated with the use of uncertainty factors during guideline development (PL)

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The current British Columbia (BC) water quality guideline for molybdenum is unique because the guidelines for the protection of ruminant cattle and wildlife are substantially less than the guideline for protection of aquatic life. Although guideline development involved the use of conservative toxicity reference values and exposure assumptions, it also includes a 20-fold uncertainty factor, resulting in a recommended ruminant livestock and ruminant wildlife guideline of 16 $\mu g \cdot L^{-1}$ and 34 $\mu g \cdot L^{-1}$, respectively. A detailed wildlife risk assessment was conducted at the Copper Mountain Mine, located near Princeton, BC, to assess the applicability of the guideline relative to site-specific exposure conditions from abiotic (i.e., water, soil, and sediment) and biotic (i.e., plant tissue concentrations) media to evaluate potential molybdenum exposure to cattle and wild ungulates that may forage on, and downgradient of, the mine. The assessment involved identifying molybdenum sources, receptors, and key exposure pathways, leading to the development of detailed exposure models. The study also involved the collection of effects data, which included cattle blood, fecal matter, and hair samples, as well as wild ungulate hair samples. These data were used during risk characterization to propose a protective site-specific benchmark of 608 $\mu g \cdot L^{-1}$. Interestingly, 608 $\mu g \cdot L^{-1}$ is 38x the ruminant livestock guideline and 18x the ruminant wildlife guideline, suggesting that the 20-fold uncertainty factor used in guideline development is likely overly protective considering the other conservative approaches used in guideline development.

Mercury exposure in relation to foraging ecology in breeding razorbills (PL)

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Mercury concentrations vary widely across different spatial and temporal scales. Seabirds, being mobile marine predators, offer a unique opportunity to directly link variations in foraging strategies with mercury burdens. During the breeding season, razorbills (*Alca torda*) are constrained around the colony, exhibiting significant variation in environmental habitat, foraging area, and diet among individuals. Here, we aim to test the influence of foraging strategy and interannual variations on blood mercury concentrations in razorbills breeding in the Gulf of St. Lawrence. Over two breeding seasons, we equipped

23 razorbills with precise GPS and depth loggers. Foraging behaviours and blood stable isotope values ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) were then related to blood mercury levels. Our findings revealed high variability in total mercury concentrations both among individuals and between years. Trophic level (derived from $\delta^{15}N$) played an important role in determining blood mercury concentrations, while foraging habitat (derived from $\delta^{13}C$ and $\delta^{34}S$) had no significant impact. Preliminary findings indicated a significant positive correlation between an individual's proportion of foraging in coastal areas and contamination levels, whereas the utilization of different water column depths did not influence contamination. By investigating potential ecotoxicological risks associated with foraging-related contamination at fine scales, this study contributes to the development of robust management plans and conservation strategies.

Unveiling discrepancies in mercury biomagnification through compound-specific stable nitrogen isotope analysis and bulk methods (PL)

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Compound-specific stable isotope analysis of amino acids (CSIA-AA) is a valuable tool for estimating baseline $\delta^{15}N$ values in food webs, enabling baseline-corrected determination of trophic positions for organisms. Bulk stable isotope methods do not include baseline correction and thus are unadjusted. Improved accuracy in trophic position estimates can enhance the assessment of contaminant biomagnification. In this study, we investigated mercury biomagnification in the Gulf of St. Lawrence by analyzing four species of fish and northern gannets (*Morus bassanus*) within the food webs. We examined the effect of CSIA-AA-derived trophic positions on trophic magnification factors (TMF) and compared them with results obtained using the conventional bulk stable isotope approach. CSIA-AA-derived trophic positions yielded the lowest TMF (10), while the bulk stable isotope analysis resulted in one of the highest TMFs (43) recorded yet in the literature. These inconsistencies highlight the potential for undetected discrepancies in biomagnification assessments when relying solely on a single method. Our results suggest that previously published studies may have overestimated the biomagnification potential of contaminants in aquatic food webs.

Lead exposure of scavenging birds in Canada due to accidental ingestion of lead ammunition (PL)

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Multiple bird species, including raptors and corvids, accidentally ingest spent lead ammunition while scavenging on gut piles of hunted game, which can result in acute and chronic toxicological effects. This phenomenon has been reported worldwide and for some bird species is associated with population-level impacts. To evaluate lead poisoning of scavenging birds across Canada, we initiated a national scale study in 2018, with the specific objective of using a pan-Canadian database to (i) assess the extent of lead exposure of scavenging birds and (ii) identify bird species and regions at higher risk. Through a collaboration between Environment and Climate Change Canada, the Canadian Wildlife Health Cooperative, territorial governments, and other partners, a sample set of ~900 bird livers has been collected. Bald eagle was the most sampled species followed by common raven, red-tailed hawk, great horned owl, golden eagle, American crow, merlin, and turkey vulture. Measurements of lead burdens in the livers are currently in progress. Preliminary results will be presented on the prevalence of lead exposure and poisoning using documented concentration thresholds of lead in bird liver and pathology findings on post-mortem examinations. Preliminary species and spatial trends will be discussed. This dataset will provide a baseline to track long-term trends of avian exposure to lead in Canada.

Elements and essential fatty acids in fishes along a large, dammed river (PL)

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Dams can trap and elevate levels of sediment-bound elements and alter food web dynamics in created reservoirs. As such, fish upstream and downstream of these dams may differ in their levels of elements (toxic and beneficial) and other nutrients, like essential omega-3 fatty acids (n-3 FAs). This study examined the spatial and species differences in contaminants and nutrients in fish from the Wolastoq | Saint John River (New Brunswick, Canada), a river which supports both recreational fisheries and subsistence fishing by Indigenous communities and has a large hydroelectric dam (Mactaquac Generating Station;

MQGS). In 2020 and 2021, smallmouth bass, yellow perch, American eel, and striped bass were collected from locations upstream (reservoir and river) and downstream of the MQGS and analyzed for mercury (Hg) and 30 other trace elements, n-3 FAs, $\delta^{15}N$, and $\delta^{13}C$. Fish from the reservoir were highest in the beneficial elements phosphorus (P), sulphur (S), and potassium (K), but also had high levels of Hg, while fish upstream from the reservoir had lower levels of toxic elements, including Hg. The dam appeared to alter food web dynamics, and consequently contaminant and nutrient concentrations; fish from the reservoir and downstream of MQGS had higher $\delta^{15}N$, which was positively correlated with some n-3 FAs and Hg, and reservoir fish were depleted in $\delta^{13}C$, which was positively correlated with other n-3 FAs in smallmouth bass. Overall, this study suggests that the dam altered the uptake of contaminants and nutrients by fish, likely through changes in food web structure, and that location and species are important factors when examining the risks and benefits of consuming wild fish from a system impacted by a large dam.

Ecological factors driving differences in PCB bioaccumulation in two Laurentian Great Lakes birds (PL)

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Most studies of polychlorinated biphenyls (PCBs) in wildlife have focused on singular bioaccumulation mechanisms of organism physiology, food web effects, or environmental factors. We attempted to combine these multiple bioaccumulative pathways to predict the contaminant burdens of different species. We provide a synthesis of PCB bioaccumulation pathways in two Laurentian Great Lakes fish-eating birds, the herring gull (*Larus argentatus*) and the double-crested cormorant (*Nannopterum auritum*). We related PCB concentrations of bird eggs to their fatty acid and stable isotope signatures across environmental gradients and from nine jointly occupied colonies (2014–2018) in the Great Lakes. Differences in egg PCB burdens between species paralleled levels of parental investment, with semi-precocial gulls accumulating 70% more PCBs than altricial cormorants due to their higher lipid content in eggs. Approximately 84% and 47% of the variation in PCB concentrations was site-linked in gulls and cormorants, respectively. Based on ecological tracers, gulls switched to foraging on lower PCB terrestrial prey in less productive lakes, while cormorants bioaccumulated the least in more productive lakes. Lastly, we speculate that up to 23% of cormorant egg contamination may be driven by subsidies from contaminated southern wintering grounds, which were proportional to winter severity and migratory flyway used. Our findings illustrate that contaminant patterns in avian predators have both compound and complex, and at times, counter-intuitive origins, and must be understood in light of multiple life history, ecological and geographic mechanisms.

Long-term trends of organohalogenated contaminants and mercury in the great blue heron across the St. Lawrence River and Estuary (PL)

Raphaël Lavoie[1], Anaïs Kerric[1]

[1]*Environment and Climate Change Canada*

Great blue herons (*Ardea herodias*) are high trophic-level fish-eating birds and are ubiquitous across Canada, which makes them good bioindicators. As they accumulate contaminants through biomagnification along their food chain, herons have been selected as a sentinel species to monitor the contamination of the St. Lawrence River and Estuary. Since 1991, eggs have been collected every five years at four locations in the St. Lawrence along an anthropogenic gradient to assess spatio-temporal trends. Brominated and non-brominated flame retardants, organochlorine pesticides, polychlorinated biphenyls, mercury, as well as tracers of foraging ecology (stable carbon and nitrogen isotopes) were measured. Perfluorinated compounds were measured in eggs collected in 2022 only. Organohalogen contaminant concentrations decreased significantly throughout the studied area, while mercury declined at a slower rate in marine locations compared to freshwater ones. Declines were less pronounced in the upstream colonies closer to urban centres, and this was especially the case for flame retardants. Stable isotopes revealed changes in foraging ecology that partly explained the changes in contaminant concentrations. Furthermore, pentabromodiphenyl ethers diphenyl ethers exceeded toxicity threshold at the upstream site, suggesting possible adverse effects. Perfluorinated compounds were higher in upstream sites, but the highest concentration was measured in one egg at the most remote site and reached 7.8 $\mu g \cdot g^{-1}$, which is higher than reported toxicity thresholds. This study emphasizes that persistent contaminants are still ubiquitous in this vast ecosystem, despite their long-standing regulations indicating their persistence in aquatic environments and suggests a continued need for chemicals management efforts.

Multi-omic investigation of namew (lake sturgeon) health in an intact and an impacted watershed in the Moose River Basin (PL)

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Namew (nah-may-yo) or lake sturgeon (*Acipenser fulvescens*), are large, long-lived, benthivorous fish that are ecologically and culturally significant to many Indigenous communities. Historically, they were found throughout most of North America's Hudson Bay, St. Lawrence, and Mississippi drainage basins. Overexploitation and extensive habitat loss and alteration since the 1800s caused massive declines in lake sturgeon abundance over much of its range. The Moose Cree Homeland includes a diversity of rivers for namew, some of which are heavily impacted by hydroelectric development, forestry, mining, and

other impacts, while others are free-flowing and without any development. Therefore, these internationally endangered fish face impacts and threats at varying levels of severity within the Moose Cree Homeland. The People of the Moose River Basin have compiled over a century of knowledge regarding the impacts of hydroelectric development and activity on Moose Cree Peoples, including declines in namew in some areas of the Moose Cree Homeland. Here, our collaborative team examined fine-scale measures of namew health and condition in the Mattagami River, which has been impacted by hydropower, forestry, and other industrial development, and the free-flowing and unimpacted North French River, both tributaries of the Moose River system. We measured proteins, lipids, and amino acids in namew blood plasma using liquid chromatography and non-targeted tandem high-resolution mass spectrometry paired with chemoinformatics and bioinformatics software. We present results from one sampling season and consider potential health implications for namew in these two river systems.

Pollutants of concern in aquatic mammals of Western Canada, their interaction with parasitic infection, and effects on host energetic condition (PL)

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Studies investigating the populations of North American river otter (*Lontra canadensis*) and mink (*Neovison vison*) are limited, though they are integral parts of riparian ecosystems and are commercially trapped in Canada. Almost nothing is known about their parasitic communities, and there is limited knowledge about how parasites and anthropogenic stressors may be influencing population health and fitness. Previous reports have emphasized the danger of a variety of contaminants, including hydrocarbons around Albertan oil sands, and metals from mines in British Columbia, but our knowledge is still limited as to the prevalence and intensity of these threats. We investigated the effects of multiple pollutants and parasites in river otter (n=155), mink (n=106), and muskrat (n=41) submitted by licensed fur trappers in Alberta and British Columbia. We modeled the effect of anthropogenic geospatial moderators on pollutant accumulation in host livers and the interaction effect of a variety of pollutants and parasites on host energetic condition. Heightened levels of pollutants identified included polychlorinated biphenyls (PCBs) congeners of concern, polycyclic aromatic hydrocarbons (PAHs) (fluorene, phenanthrene, and naphthalene), and toxic levels of metals (aluminum (Al), cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni), selenium (Se), strontium(Sr), and zinc (Zn)). Both anthropogenic and biological moderators influenced pollutant concentrations and host energetic condition, and we identify specific regions of concern in Alberta and British Columbia. Continued monitoring of aquatic mammals, especially those of commercial and indigenous importance like river otter, mink, and muskrat is needed, as both parasites and pollutants

have serious implications for individual and population fitness, as well as the health of rural communities.

A first detailed meta-assessment of PCBs, OCs, and brominated flame retardants in killer whales across the North Atlantic Ocean indicates that diet explains most variation (PL)

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Recent studies highlighted the critical threat posed by consistently high concentrations of persistent organic pollutants (POPs) in killer whales (*Orcinus orca*) due to their high trophic position and limited biotransformation and elimination capacity. Although tissue concentrations of legacy POPs are well documented for Northeast Pacific killer whales, less is known for North Atlantic (NA) conspecifics or levels of new and emerging POPs. Further, POP concentrations are influenced by feeding habits, but quantitative estimates of killer whale diet composition were unavailable until recently. Here, we aimed to address these gaps by measuring legacy polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs), and legacy and emerging brominated flame retardants in ~150 NA killer whales. We also examined the relationship between diet composition inferred from quantitative fatty acid signature analyses carried out in the same individuals. PCBs ranged from ~ 100 mg·kg^{-1} lipid weight (lw) in the western NA, to ~50 mg·kg^{-1} lw in the mid NA, to ~10 ng·kg^{-1} lw in the eastern NA. We observed similar patterns for other POP classes. Based on a threshold for health risk at 10 mg·kg^{-1} lw, killer whales in the western NA face high risks of health effects due to PCBs alone. The composition of their diet, particularly the proportion of marine mammals consumed, explained most of the elevation in contaminant concentrations. Differences were also found within locations, which were associated with sex and individual feeding variation. These results highlight the critical importance of quantifying killer whale diets to understand the dynamics and threats of contaminant loads in these populations.

Tracking flame retardants in air from the back of a gull: The landfill effect (PL)

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Elevated levels of halogenated flame retardants (HFRs) have been reported in tissues of breeding ring-billed gulls from the Montréal area (Québec, Canada). Previous studies from

our lab demonstrated that atmospheric exposure to HFRs determined using miniature passive air samplers (PAS) carried on the back of these gulls was highest in individuals foraging predominantly in landfills. The present study aimed to investigate the distribution of various HFRs in air samples collected within one major landfill in the Montréal area that is frequently visited by ring-billed gulls, and the contribution of different HFR exposure pathways (diet, inhalation, and ingestion during preening) in these gulls. Miniaturized PASs along with active air samplers were deployed in six different areas within this landfill for 34 days. Gulls were also equipped on their back with a similar PAS along with a GPS datalogger for ten days. Tracking of gulls confirmed that landfills represent a major emission source for several HFRs for birds visiting a landfill at least once, with significantly greater concentrations of PBDE-47 (a polybrominated diphenyl ether) measured in their PASs. We further showed, using structural equation models, that atmospheric exposure of gulls to HFRs could occur through both inhalation (air) and ingestion (feather preening of HFR-laden particles and stomach content). Lung concentrations of PBDE-28 were directly influenced by air levels determined in PASs, and several PBDE concentrations in liver increased with those in lungs, feather surface, and stomach content. Atmospheric exposure to HFRs should not be underestimated compared to the traditional dietary exposure pathway in urban-adapted birds.

Assessing the lethal and sublethal effects of oil sands process-affected water in early life stage wood frogs (*Lithobates sylvaticus*) (PL)

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Oil sands process-affected water (OSPW) is a by-product resulting from the extraction process of bitumen in the Canadian oil sands industry and is currently stored in large tailings ponds on site. OSPW is comprised of a mixture of various organic and inorganic constituents. The composition and toxicity of OSPW is influenced by its age, source, and location within tailings ponds. Due to OSPW's complex composition and known acute toxicity to a variety of aquatic life, this experiment is part of a multi-year study aimed to evaluating biomimetic extraction via solid-phase microextraction (BE-SPME) analytical technique for toxicity screening of OSPW. BE-SPME has recently been developed as a cost-effective tool for hazard assessment of the bioavailable organic components in OSPW. We investigated the potential lethal and sub-lethal effects of a range of dilutions of OSPW (80, 40, 20, 10, 5, 2.5, and 1.25%) in wood frogs (*Lithobates sylvaticus*) native to Alberta, Canada from pre-hatching embryonic stages through to metamorphosis. Mortality at 80% of raw OSPW and developmental delays at concentrations of 40% and 80% are evident. As well, low concentrations have significant incidences of deformities (scoliosis and hind limb malformations). In addition, thyroid histopathological analyses are underway. The BE-SPME and naphthenic acid (NA) concentration data will be used to derive concentration-

effect relationships for OSPW exposure in an environmentally relevant amphibian model. The results will be beneficial for future screening assessments of raw and remediated OSPW in the Canadian oil sands.

Quantifying insecticide exposure risk to barn swallows of Saskatchewan (PL)

Christy Morrissey[1], Sonia Cabezas[1,2], Dan Garrett[1], Mercy Harris[1], John Headley[2]

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Declines in aerial insectivore populations across North America have prompted research into potential causal mechanisms affecting species such as the barn swallow. One hypothesis is that agricultural intensification and the rising use of toxic insecticides may be causing both direct and indirect effects through consumption of contaminated insects and reduction in insect biomass. We modelled the agricultural intensity risk to swallows across Southern Saskatchewan and mapped regions that are predicted to have high versus lower risk based on available metrics for pesticide use, landscape simplification, and water availability. In 2022 and 2023, we conducted surveillance sampling of barn swallow blood and insects to quantify the detection prevalence and concentrations of six systemic insecticides (imidacloprid, clothianidin, thiamethoxam, acetamiprid, thiacloprid, flonicamid). The neonicotinoids clothianidin, imidacloprid and thiamethoxan were detected in 12%, 12% and 18% of 2022 samples, respectively. Average concentrations and ranges for these 3 compounds were: 5.5 $\mu g \cdot L^{-1}$ (range: 1.025–13.583 $\mu g \cdot L^{-1}$, n=9) for clothianidin; 2.936 $\mu g \cdot L^{-1}$ (range: 1.964–4.95 $\mu g \cdot L^{-1}$, n=8) for imidacloprid; and 32.146 $\mu g \cdot L^{-1}$ (range: 1.583–155.0 $\mu g \cdot L^{-1}$, n=13) thiamethoxan. Insecticide concentrations in barn swallow blood declined over the season and based on the first year of data, concentrations and probability of detection were not strongly related to agricultural intensity suggesting further refinement of risk maps and consideration of the ubiquity of neonicotinoid insecticides in the region. We are also working on new methods to quantify these insecticides in the insects and other sample media to better inform and validate agricultural exposure risk models to aerial insectivores.

Exposure, repellency, and learned aversion to neonicotinoid-treated seeds in granivorous birds (PL)

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Neonicotinoid seed treatments are the most widely used insecticides and are known to induce lethal and sublethal toxicity to vertebrate wildlife, including granivorous birds that may inadvertently consume treated seeds. This study aims to evaluate 1) the exposure of granivorous songbirds to neonicotinoid seed treatments and 2) assess whether birds exhibit avoidance or learned aversion to treated seeds following exposure. Field

experiments were conducted in spring 2022 and 2023 on five crop fields, simulating clothianidin-treated seed spills and observing wildlife interaction via camera traps and radio-frequency identification passive integrated transponder (RFID PIT) tags. Wild birds were captured, blood sampled, tagged, and released at each field. Each field contained two stations baited with clothianidin-treated wheat seeds and two with untreated seeds and a camera trap and RFID reader hidden under the seed pile. The setup remained up for 7 days before treatments were reversed at each bait station for another 7 days. In 2022, the camera traps recorded 7 mammalian and 17 avian species visiting the simulated seed spills. A higher consumption duration was observed across all species at untreated seed pile than treated ones. Initial exposure to treated seeds resulted in an inconsistent subsequent untreated seed consumption rate. However, number of seeds consumed decreased when treated seeds were introduced after untreated ones. Blood plasma analysis revealed >60% detection frequency for imidacloprid across all species, and 100% detection for thiamethoxam and clothianidin in 10 out of 11 bird species. Additional data from spring 2023 are being analyzed to further assess detection and consumption across more sites, as well as an experiment to test bird preference for red versus blue seed treatments. Our findings will inform the regulation and application risks of neonicotinoid insecticides and contribute to understanding the risk of seed treatments to granivorous grassland birds.

Modulatory effects of nutrition on neonicotinoid-induced developmental toxicity in tree swallows (*Tachycineta bicolor*) (PL)

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The rising use of neonicotinoid insecticides in modern agriculture not only decreases non-target insect populations, which limits the availability of food for aerial insectivores, but also poses adverse effects on them due to toxicity. Neonicotinoids were detected from 100% of wild-captured tree swallows in agricultural landscapes of Saskatchewan. Therefore, neonicotinoids are suspected to cause steep declines in aerial insectivore populations. Besides, wetland loss due to agricultural intensification also threatens aerial insectivores with decreased habitats and food quality. We hypothesize that wetlands loss may exasperate toxicity of neonicotinoid exposure on aerial insectivores, as wetlands are critical habitats providing nutritious aquatic insect prey enriched with polyunsaturated fatty acids (PUFAs), especially omega-3 fatty acids. We examined the toxicity of neonicotinoids (imidacloprid and clothianidin) on the development of tree swallow nestlings and the potential mitigating effects of experimental supplemented PUFAs or higher wetland availability. In 2022 and 2023, we dosed nestlings in the field with neonicotinoids, and assessed their post-fledging survival and movement by tracking dosed and control chicks using the Motus telemetry network. Our results show that both insecticides significantly impaired growth and condition of nestlings in dose-depended manners. Nestlings at sites with high wetland availability had higher plasma PUFA levels.

However, the short-term beneficial effects of co-exposure to PUFA supplementation for reducing toxicity appear limited when wetland coverage is high. Together, this is the first study of neonicotinoids toxicity in an aerial insectivore, providing evidence of beneficial effects of aquatic resources in agricultural landscapes to reduce risks to aerial insectivores.

Quantifying responses of American toad tadpoles to microplastics in the environment using an *in-situ* assay (PL)

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Microplastics have varied effects on aquatic organisms but have been understudied in amphibians. Amphibians play critical roles in ecosystems but are in global decline due to anthropogenic impacts including pollution. Therefore, understanding how pollutants such as microplastics affect amphibians is of great importance for their conservation and protection. Currently, research has been limited to primarily laboratory studies at the organism scale, with a lack of environmentally relevant experiments that examine effects at higher levels of biological organization. Therefore, we are examining American toad (*Anaxyrus americanus*) tadpole responses to microplastic additions using an *in-situ* assay, as part of a whole-lake manipulation study at the International Institute for Sustainable Development-Experimental Lakes Area. For multiple years, a mix of microplastic polymers (polyethylene, polystyrene, and polyethylene terephthalate) containing additives have been added to a whole research lake at environmentally relevant concentrations to examine the fate and effect of microplastics in a freshwater lake. To examine effects on amphibians, tadpoles were collected and reared in flow-through enclosures within the microplastic-contaminated lake or a reference lake. We are quantitatively comparing organismal and population endpoints (e.g., growth, developmental timing, mortality) between tadpoles from the two lakes, and tadpoles reared in the microplastic-contaminated lake are also sampled for body microplastic accumulation. Altogether, this study looks at a widespread toad species in Canada as a representative for anurans (encompassing frogs and toads) to provide novel insight into how amphibians can respond to microplastics under natural conditions more relevant for informing management policies, regulations, and risk assessments.

Using transcriptomics and early life stage toxicity testing to assess the sensitivity of two avian species to UV absorbents (PL)

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UV absorbents (UVAs), including stabilizers and filters, are an emerging class of contaminants that protect objects from UV degradation. In fish, they are known ligands of the aryl hydrocarbon receptor, which mediates organism response to dioxin-like compounds; however, their effects in avian species are unknown. The objective of this study is to use transcriptomics to identify genes associated with toxicity of two UVAs, UV328 and BP3, in Japanese quail (JQ; *Coturnix japonica*) and double-crested cormorants (DCCO; *Nannopterum auritum*) and determine species differences in sensitivity. UVAs were injected into the air cell (n=20 per dose group) on embryonic day 0. The highest injection concentration reflected the upper solubility limit in safflower oil (5.25 $ug \cdot g^{-1}$ for UV328 and 12.59 $ug \cdot g^{-1}$ for BP3), while the lower was selected to reflect an environmentally relevant dose (1 $ug \cdot g^{-1}$ for both chemicals). Eggs were artificially incubated, and a subset was dissected at mid-incubation (n=5) and the remainder just prior to hatching (n=10–15). We collected liver tissue at both timepoints. There were no significant changes in lethality, embryo, liver mass or tarsus and head plus bill length in JQ or DCCO. We analyzed hepatic gene expression in JQ and DCCO using EcoToxChips, species-specific custom quantitative polymerase chain reaction (qPCR) arrays featuring genes that help predict apical outcomes. This analysis is ongoing for DCCO, and in JQ, we found differentially expressed genes involved in endocrine regulation and metabolism. This study introduces novel avian toxic and transcriptomic data, permitting species comparisons and determining effects of UVAs.

Nontarget screening reveals the presence of multiple plastic related compounds in blubber of polar bear, killer whale, narwhal, and pilot whale from East Greenland (PL)

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The monitoring of established contaminants in sentinel marine mammals often reveals some of the highest concentrations globally, particularly in species like polar bear and killer whale. However, investigations into the presence of chemicals of emerging Arctic concern (CEACs) or other less-known or unknown contaminants are rarely conducted, if at all. Here, we used a nontargeted approach using high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (HPLC-QTOF-MS) to screen the blubber/adipose of polar bear, killer whale, narwhal, and pilot whale in East Greenland for thousands of different chemicals, including many CEACs and plastic-related compounds

(PRCs). A total of 1,830 compounds were tentatively identified, and a clustering analysis of these features revealed distinct chemical profiles among species. Among the identified compounds, four PRCs was confirmed using available standards: di(2-ethylhexyl) phthalate (DEHP), diethyl phthalate (DEP), di(2-propylheptyl) phthalate (DPHP), and one antioxidant (Irganox 1010). Additionally, three other PRCs, namely 4-nonylphenol, 2,6-ditert-butylphenol, and dioctyl decanedioate, exhibited MS/MS fragmentation patterns matching those in preexisting library databases. While the phthalates were only detected above detection limits in some polar bear and narwhal samples, Irganox 1010, 4-nonylphenol, and 2,6-ditert-butylphenol were detected in >50% of all samples. From matrix-spiked samples of DEHP and DEP, semi-quantitative concentrations will be presented. To our knowledge, this study is the first to implement a nontargeted screening approach in killer whale, narwhal, and pilot whale, and to identify multiple different PRCs in their blubber. Considering the limited understanding of these PRCs, further investigation into their persistence and toxicity, especially at potentially elevated concentrations, is warranted.

Avian ecotoxicology effects assessment of resident and migratory breeding waterbirds at oil sands tailings ponds in Northern Alberta (PO)

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The Athabasca oil sands region of Northern Alberta is under a major migratory flyway where frequent bird landings occur on oil sands process-affected water (OSPW) in active tailings ponds. OSPW contains high levels of naphthenic acids, polycyclic aromatic hydrocarbons and heavy metals in complex mixtures. These sites may be an attractive migratory stopover or breeding site for migrant and resident waterbirds as they are often vegetated and the water is seasonally open longer due to the constant efflux of warm OSPW. The extent of toxicant exposure to water birds through ingestion of contaminated water, sediment, plants, and aquatic invertebrates is largely unknown and there is a lack of information on which species use the sites. We characterized 12 tailings ponds to determine if invertebrate and macrophytic plants occur as habitat and food resources. Avian species abundance and focal behavioural observation surveys were conducted from May to July weekly on each of the tailings ponds and nearby reference freshwater ponds. Chemical analysis of water, sediment, eggs and feces will be used to determine polycyclic aromatic hydrocarbon and naphthenic acids concentrations. Preliminary surveys in 2023 found Canada goose (*Branta canadensis*) and killdeer (*Charadrius vociferus*) to occur on the ponds among other waterbird species. Behaviour observations revealed birds were primarily using the ponds for resting and feeding. Previous work has focused on deterrent strategies but this comprehensive assessment will be the first to study pond use and exposure risk to waterbirds that differentially use tailings ponds to inform mitigation strategies for the energy sector.

Behavioural ecotoxicology of Canadian leeches: Species-specific reactions to UV light and to a pollutant (PO)

David Vergote[1], Matthieu Zolondek[1]

[1]*University of Alberta*

Polluted environments can alter the natural behaviour of exposed species. Although freshwater leeches have been marked as potential ecotoxicological tools, it is unclear whether all leech species native to Canada can be affected by pollutants. We here explored how the natural response of a subset of leech species indigenous to Alberta (*Piscicola punctata*, *Erpobdella obscura*, and *Myzobdella reducta*) to UV light exposure is altered by a pollutant. The behaviour of leeches was recorded in different experimental paradigms and videos were analyzed by noting and timing the occurrence of specific behaviours (immobility, movement, exploration, and swimming). Interestingly, the increased activity triggered by near-UV light (395nm) exposure in our three species manifests through exploration and movements in *P. punctata* and *M. reducta* and through swimming in *E. obscura*. Exposed to gasoline (1:5000 dilution) as our model pollutant, *P. punctata* and *M. reducta* were more active, while *E. obscura* showed a decrease in mobility. We also showed that a pre-exposure to gasoline prevented UV-induced changes in exploration in both *P. punctata* and *M. reducta*. Our study shows that the behaviour of Hirudinae found in Alberta can be measured, both qualitatively and quantitatively. Moreover, the described behaviour appeared to be altered in a species-specific manner with the similarity of response to UV light and to pollutants between *P. punctata* and *M. reducta* being in accordance with the close evolutionary relationship between them and with their identical feeding behaviour (sanguivorous in contrast to the carnivorous *E. obscura*).

Mercury concentrations in marine bird eggs in Lake Melville before and after flooding of the Muskrat Falls hydroelectric reservoir (PO)

Margaret Eng[1], Neil Burgess[1]

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Construction of the Lower Churchill Hydroelectric Project in Labrador began in 2013, and impoundment was completed in the fall of 2019. There is concern over the extent to which methylmercury will increase in food webs in Lake Melville following reservoir flooding. To establish baseline concentrations in avian wildlife, mercury (Hg) was measured in common tern and ring-billed gull eggs from western Lake Melville, and in common tern, ring-billed gull, herring gull, great black-backed gull, common eider, and red-breasted merganser eggs from eastern Lake Melville, between 2013 to 2017. Initial post-impoundment data from 2021 and 2022 in western Lake Melville show a decrease in Hg in common tern eggs (geometric mean: 0.20 to 0.12 $mg \cdot kg^{-1}$ wet weight), whereas Hg in ring-billed gull eggs increased 4.8x (0.17 to 0.81 $mg \cdot kg^{-1}$ wet weight). Further downstream, Hg

in common eider eggs increased (0.12 to 0.19 $mg \cdot kg^{-1}$ wet weight), but no other species in eastern Lake Melville showed significant changes in Hg. Preliminary stable isotope data from 2021 compared to baseline indicate that both common tern and ring-billed gull eggs in western Lake Melville had enriched $\delta^{15}N$, while $\delta^{13}C$ was enriched in common tern eggs, but depleted in ring-billed gull eggs. Hg was positively correlated with $\delta^{15}N$ and negatively correlated with $\delta^{13}C$. These results suggest shifting diet composition and subsequent effects on Hg accumulation. In contrast, stable isotope signatures in eastern Lake Melville eggs did not shift post-impoundment compared to baseline. Monitoring will continue long-term to track changes in Hg in avian wildlife in Lake Melville.

The importance of riparian vegetation in agricultural ditches for the development and survival of northern leopard frog (*Lithobates pipiens*) tadpoles (PO)

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Wetlands are disappearing worldwide, largely due to urbanization and modern intensive agriculture. Wetland species must adapt accordingly and find new suitable habitats. Agricultural drainage ditches are designed to receive excess water from precipitation to prevent flooding and erosion of crop fields. Amphibians using these ditches as habitat may be exposed to harmful agricultural contaminants through runoff. Riparian or edge vegetation bordering drainage ditches may help buffer the effects of contaminants by acting as natural filters. Current ditch management strategies involve the removal of vegetation and the dredging of soils surrounding these ditches to improve drainage. Consequently, the buffering capacity of these riparian strips may be significantly reduced. The goal of this study was to determine how riparian vegetation height and forest cover can affect amphibian development and survival in drainage ditches in southeastern Ontario. Nytex cages containing northern leopard frog (*Lithobates pipiens*) tadpoles were placed within nine ditches differing in their vegetation height and percentage of forest cover. Water was sampled for the presence of pesticides and nutrients. After an eight-week (May–July) exposure to ditch water, tadpoles were collected and assessed for their growth, development, and survival. Vegetation height and forest cover did not appear to have any significant effect on tadpole development or survival. However, tadpoles housed at sites with higher forest cover tended to have higher body mass. The results of this study may help in the development of land management strategies that will support biodiversity and species conservation in these ecosystems.

Assessing the health of whooping crane migratory wetlands in the Alberta oil sands region (PO)

Maureen Freemark[1], Mark Bidwell[1], Lukas Mundy[1], Bruce Pauli[1]

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Whooping cranes (*Grus americana*; WHCR) summer in Wood Buffalo National Park (WBNP) and adjacent areas of the Northwest Territories and migrate 4000 km every year to their wintering grounds on the Gulf Coast of Texas. Although the population increased from 16 to over 500 individuals since the 1940s, WHCR remains listed endangered under Canada's *Species at Risk Act* and the United States' *Endangered Species Act*. Exposure to environmental contaminants was listed as a critical threat to the long-term survival of WHCR by the Crane Specialist Group of the IUCN. Since 2010, satellite transmitters used to monitor WHCR migration demonstrated that approximately 75% of migrating cranes land in the Alberta oil sands region (AOSR). Previous work on wood frogs (*Lithobates sylvaticus*) in the AOSR detected oil sands-related contaminants such as heavy metals, polycyclic aromatic compounds and naphthenic acids in wood frog tissues and their wetland breeding habitats. To assess contaminant levels at known WHCR migratory wetlands in the AOSR and at WHCR nesting wetlands in and near WBNP, we deployed passive samplers (semi-permeable membrane devices and polar organic chemical integrative samplers) and collected water and sediment samples in spring 2023. Additionally, wood frog tadpoles, a potential crane prey item, were collected to assess exposure pathways and burdens. Results will contribute to a multi-year health assessment for WHCR including an evaluation of how migratory wetlands may be impacted by oil sands mining, and if those impacts have consequences for the health of individual cranes or resiliency of the population.

Assessing the health of wood frogs and boreal wetlands in the Athabasca oil sands region of Northern Alberta (PO)

Lukas Mundy[1], Danna Schock[2], Maureen Freemark[1], Bruce Pauli[1]

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Several recent studies have reported evidence that surface mining operations in the Athabasca oil sands region (AOSR) contribute to the deposition and accumulation of inorganic (metals) and organic contaminants (polycyclic aromatic compounds (PACs) and naphthenic acids (NAs)) within the vicinity of oil sands surface mines. As part of the Canada-Alberta Oil Sands Monitoring Program we attempt to characterize the risk of exposure for wildlife, specifically wood frogs (*Rana sylvatica*), to these particular contaminants of concern in boreal wetlands, with the goal to 1) assess the levels of contaminants of concern in wetlands, and 2) assess the toxicity and biological responses caused by exposure to oil sands-related contaminants of concern to investigate potential effects of exposure. Previously, we found detectable levels of contaminants of concern in

wetlands located in the AOSR and found detectable levels of metals and PACs in wood frog tissues. Data generated using passive sampling techniques demonstrated that PACs were highest in boreal wetlands located within 25 km of oil sands mines. Extracts generated from passive samplers were used to further characterize the toxicity of PAC mixtures using sensitive *in vitro* bioassays; a tool to potentially identify 'hot spots' and spatial trends of contaminants in wetlands in the AOSR. During the 2022 and 2023 field seasons, we targeted key wetlands representing a gradient of exposure to organic contaminants to assess the health of wood frog tadpoles, using morphometrics (size, weight, liver somatic index), biomarkers of exposure (EROD enzyme activity), and white blood cell profiles.

Emerging Contaminants in Wastewater Effluents: Exposure, Effects, and Possible Environmental Risk

Antibiotics and antimicrobials in wastewater treatment plants and environmental water samples (PL)

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The availability of cost-effective, reliable, and rapid analytical techniques for quantifying a wide range of antibiotics and antimicrobials in the aquatic environments is important to aid in environmental risk assessments, developing treatment mitigation strategies, and estimating removal efficiencies of antibiotics and antimicrobials in wastewater treatment plants over time. This research developed a unified method to quantify eight widely used antibiotics (ampicillin, cephalexin, ciprofloxacin, methicillin, meropenem, sulfamethoxazole, trimethoprim, and tetracycline) and ten universal antimicrobials (benzalkonium chloride (BAC), BAC-C10, BAC-C12, BAC-C14, BAC-C16, BAC-C18, benzethonium chloride, DDAC, triclosan, triclocarban, and chlorohexidine) by solid phase extraction (SPE) and ultra-high performance liquid chromatography-tandem mass spectrometry with polarity switching. In addition, carbamazepine was added to provide for a marker in the extraction, detection, and analysis efficiencies. SPE was performed using Oasis® HLB cartridges to optimize analyte recoveries and reduce matrix effects. Method validation was conducted by determining the minimum limit of quantification, dynamic linear working ranges, accuracy of internal standard recoveries, inter- and intra-day variability, presence of matrix effects, and method ruggedness/robustness. The method was applied to samples collected in collaboration with the City of Winnipeg North End Water Pollution Control Centre in the summer versus winter seasons of 2023 and these results will be presented.

Partitioning of micropollutants in biota and environmental samples collected in replicate artificial streams (PL)

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Although micropollutant occurrence has been extensively evaluated in wastewater and surface water, their partitioning in other aquatic environmental components and aquatic organisms remains underexplored. To obtain more information on their presence in these components, extraction procedures were first developed for the quantification of target

micropollutants in sediments, biofilms, amphipods (gammarids), and fish (spoonhead sculping and long nose dace). Solid phase extraction (SPE) was used as a sample clean-up method for aqueous samples (wastewater and streams) whereas QuEChERS salt mixtures of $MgSO_4$:NaCl, $MgSO_4$:NaOAc, and $MgSO_4$:NaCl:NaCitrate:DCS were utilized for biofilm, gammarids and sediments, and fish tissues, respectively. The QuEChERS extraction was then followed by a dispersed-SPE clean-up via primary secondary amine (PSA), C18, and $MgSO_4$ sorbents followed by Z-sep+ or graphitized carbon black (GCB) to remove lipids, colour, organic acids, and excess water. The extracts were then analyzed via liquid chromatography, triple quadrupole mass spectrometry (LC-QQQ). Preliminary results show that 18 of the 22 chemical substances were found in the streams, including analgesics, antidepressants, neuro-inhibitors, antibiotics, cardiovascular drugs, and personal care products. By contrast, fewer chemicals were observed in other matrices, suggesting that further sample cleanup is necessary for other substances. Of the target substances analyzed, carbamazepine (anti-epileptic) and venlafaxine (antidepressant) appear to be consistently present, albeit at low concentrations in biofilm, fish, and gammarids (<0.1– ~48 $ng \cdot g^{-1}$), while higher amounts were found in sediments (<0.1– ~114 $ng \cdot g^{-1}$).

Regional analysis of opioid consumption with wastewater-based epidemiology (PL)

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The opioid crisis, intensified by the COVID-19 pandemic, continues to be identified as a leading health concern in Canada, with Ontario witnessing a 60% increase in opioid-related deaths in 2020 compared to 2019. Preliminary 2021 data suggest a continuation of this upward trend. The Region of Durham specifically is experiencing a rise in opioid-related fatalities, with 130 deaths in 2021. The crisis demands urgent attention, and acquiring relevant information on opioid use is crucial for developing effective harm-reduction strategies. Traditional data analysis methods rely on clinical overdose data and are often expensive, cumbersome, and subject to biases. Conversely, municipal wastewater contains valuable information, making it an essential, cost-effective strategy for public health monitoring and forensic applications. The study of public health information in wastewater is termed wastewater-based epidemiology (WBE). Municipal wastewater contains numerous parent compounds and metabolites excreted by individuals after consuming licit and illicit substances. By pairing established metabolic pathways for illicit compounds with data on wastewater flow, biomarker concentrations can estimate collective drug consumption. WBE's main advantage is its objectivity and suitability for near real-time consumption pattern estimates. We propose a novel approach to explore WBE's utility in detecting and quantifying opioids and associated metabolites at multiple spatial scales within the Durham region. Our aim is to develop and validate a reliable method for

detecting and quantifying a suite of opioids and their major metabolites in wastewater using LC-HR-MS/MS. By identifying spatial patterns, temporal trends, and consumption estimates, our research will offer valuable information on the region's opioid usage. By leveraging WBE, our study aspires to significantly contribute to the development of more effective harm reduction strategies and mitigate the opioid epidemic's devastating impact on communities.

Monitoring endocrine disrupting chemicals in wastewater with bioassays: A proof-of-concept from Québec, Canada (PL)

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Endocrine disrupting chemicals (EDCs) are compounds which can interact with the hormonal system and generate adverse effects (e.g., reproduction, growth). Despite their impacts, EDCs are hardly monitored or regulated in wastewater due to their presence in many chemical classes (e.g., steroids, pharmaceuticals, plasticizers) and their low concentrations of effect. Those characteristics among others makes them difficult to monitor with a traditional substance-by-substance approach. To palliate those issues, one way is to use bioanalytical tools or bioassays. Bioassays have the advantage of integrating mixture effects which allows to have lower limits of detection and detect unknown chemicals. This project aims to explore the usefulness of a bioassay-based approach to monitor EDCs in wastewater. The designed bioassay-based approach is constituted of two-tier. The first tier of testing is completely *in vitro* and composed of the transactivation assay for the receptor of estrogen (ER) and androgen (AR). The second tier is *in vivo* with the fish short-term reproduction assay with *Pimephales promelas*, which aims to confirm effects if required. To validate the approach, two studies were conducted on a municipal and an industrial effluent. Both studies indicate that fish reproduction was impacted by the effluents and could be linked to activities detected by the *in vitro* bioassays. Moreover, other parts of this project indicate *in vitro* bioassays could be helpful to evaluate wastewater treatment efficiency and agricultural run-off. Overall, this project provides basis to a first proof-of-concept for the use of bioassays to monitor EDCs in wastewater in the province of Québec.

Progress and understanding of genotoxicity and mutagenicity testing of treated water for emerging contaminants (PL)

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Over the past few decades, progress of collaboration between Environmental Bio-Detection Products Incorporated (EBPI), research groups, and universities has played a vital role in assisting with the monitoring of mutagenicity and genotoxicity in water sources. Bioassays using engineered strains of bacteria can be incorporated to assist with evaluating and monitor water quality and assist with optimization of water treatment facilities. These bioassays can be used to detect the genotoxic and mutagenic effects of contaminants of emerging concern (CEC's) and disinfection by-products (DBPs) from different water treatment methods. Testing using various bioassays can be conducted in non-specialized laboratories helps provide insight into the biological effects of emerging contaminants in municipal wastewater. Genotoxic and mutagenic compounds in wastewater effluent can affect not only the genetics of aquatic ecosystems, but also human health in communities that depend on surface water for human consumption. By employing appropriate bioassays and implementing thorough data interpretation, the monitoring of wastewater effluent for genotoxicity and mutagenicity can enhance the assessment of treatment techniques. This approach has the potential to decrease the discharge of genotoxins and mutagens into the environment, ultimately contributing to the protection of both the ecosystem and human well-being.

Direct and indirect effects of flowback and produced water (FPW) from hydraulic fracturing on California blackworm (*Lumbriculus variegatus*) and fathead minnows (*Pimephales promelas*) (PL)

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Horizontal hydraulic fracturing in oil and gas extraction from deep shale pools results in the formation of large volumes of hydraulic fracturing flowback and produced water (FPW). Although stringent discharge regulations are in effect for FPW, it still enters aquatic ecosystems through unintentional spills. FPW toxicity has been studied in numerous species to evaluate the hazard of an FPW spill in aquatic environments. However, the effects of residual sediments from spills of FPW on benthic species have not been studied. We exposed California blackworm to 1% FPW-spiked sediment for 7 days and showed that there was a significantly higher (1.53-fold) oxidative stress response and higher (1.63-fold) EROD activity in exposed worms. Additionally, FPW exposed worms (1% FPW) were then fed to unexposed fathead minnows daily for 7 days to examine the effects of feeding on exposed worm on the physiology of the minnows. Fish EROD activity increased by 123%

and oxidative stress (TBARS) response increased by 72% compared to the control group. Overall, our data shows that prior FPW exposure in benthic organisms can have secondary effects on fish that feed on previously FPW-exposed organisms.

Comparison of the sensitivity of early life stages of three North American fish species to antimicrobial chemicals (PL)

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Antimicrobial agents in personal care and industrial products exhibit pseudo-persistence in freshwater systems due to their continuous discharge via wastewater effluent, which may negatively affect the health of aquatic organisms. Previous studies have focused on effects of antimicrobials in model fish species and there is limited data available to define thresholds for adverse effects in ecologically relevant species. Thus, it is imperative to assess the potential effects of these compounds on organisms in the receiving environments. This study aimed to characterize and compare the effects of triclosan (TCS) and chloroxylenol (PCMX) exposure on the early life stages of three species of commercial, recreational, and indigenous importance to Canadians, namely rainbow trout (RBT, *Oncorhynchus mykiss*), lake trout (LKT, *Salvelinus namaycush*), and white sucker (WS, *Catostomus commersonii*). Fish were exposed from hatch to one week post-swim-up to TCS or PCMX at nominal concentrations of 0.38–400 $\mu g \cdot L^{-1}$. Incidence of developmental abnormalities (edema and spinal deformities) were higher in LKT compared to RBT. There was no impact on time to swim-up. Mortalities at the highest concentrations were greater in LKT relative to RBT. Rainbow trout is more sensitive to PCMX with a median lethal concentration (LC_{50}) of 357.4 $\mu g \cdot L^{-1}$ compared to 734.1 $\mu g \cdot L^{-1}$ in LKT. Triclosan is overall more potent in LKT with an LC_{50} 77.19 $\mu g \cdot L^{-1}$ compared to 189.4 $\mu g \cdot L^{-1}$ in RBT. Results to date suggest differential sensitivity to antimicrobial compounds for the early life stages of two ecologically relevant salmonid species. Study results will aid in developing strategies to minimize ecological risks associated with these antimicrobials.

Impacts of antimicrobial exposure on the gut microbiome of early life stage fish: A chemical and species comparison (PL)

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Antimicrobials are contaminants of emerging concern as they are continuously discharged via wastewater effluent into aquatic environments where they may negatively

affect fish due to their pseudo-persistence. Antimicrobials may alter fish gut microbiomes which serve central roles in the overall health of fishes. This study assessed the impacts of two antimicrobials on early life stage rainbow trout (RBT) and lake trout (LT) gut microbiomes. Embryos were exposed from hatch to 28 days post-hatch to six graded concentrations of triclosan (TCS) and chloroxylenol (PCMX). Excised whole gut was collected and 16S rDNA amplicon sequencing of gut microbiome was applied to investigate responses to antimicrobials. Gut microbiome of RBT was dominated by phyla *Proteobacteria* and *Bacteroidota* and genera *Aeromonas* and *Acinetobacter*, whereas LT was dominated by phyla *Proteobacteria* and *Firmicutes* and genera *Aeromonas* and *Ahrensia*. Significant changes in Shannon diversity were observed in TCS-exposed RBT, with PCMX-exposed RBT having a marginal (trend but not significant) response. A marginal change in Shannon diversity was also detected for TCS-exposed LT. TCS caused significant changes in both RBT and LT community structure as detected by weighted Unifrac analysis. Three genera correlated with measured concentrations of TCS for RBT while no genera were significantly correlated for LT. Overall, TCS had the largest impact on gut microbiomes of exposed fish and the RBT gut microbiome had a greater response to antimicrobials than that of LT. This study provides insights into the potential effects of emerging antimicrobials on alevin salmonid gut microbiome and species-specific response.

Enantioselective effects of venlafaxine on the antioxidative defense system in rainbow darters (*Etheostoma caeruleum*) (PL)

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Over the last decade, wastewater effluent has been identified as a primary source in the introduction of contaminants of emerging concern (CECs) into aquatic environments. Antidepressants form a major class of CECs that is increasingly being detected in waterbodies worldwide and alters the physiology of organisms inhabiting them. Of these, venlafaxine is one that has been detected in appreciable quantities in the Grand River in Waterloo, Ontario. Since the drug is sold as a mixture of two enantiomers (R and S) which differ significantly in their pharmacological properties, this study aimed at evaluating the effects of each on fish physiology. To do so, males from a local sentinel species, i.e., rainbow darter (*Etheostoma caeruleum*), were exposed to environmentally-relevant concentrations (1 $\mu g \cdot L^{-1}$) of either the R- or S-enantiomer for 1, 4, or 14 days. The activities of free radical-scavenging enzymes that protect cells from oxidative damage including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX) were measured in liver and gill tissue. Preliminary analyses indicate that CAT activity is elevated only in the tissues of fish exposed to the R-enantiomer relative to controls at 1 and 4 days. Such a response was not detected at 14 days, suggesting the existence of mechanisms that buffer fish against this stressor in the long term. SOD and GPX activity will also be quantified to understand the effects of each enantiomer on the antioxidant defense system. This study underscores the

need to evaluate enantiomer-specific effects of chiral contaminants to accurately determine their impact.

The effect of 17β-estradiol on *Capitella teleta* adult behaviour (PL)

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Estrogenic compounds are found in the environment at low concentrations and their effects in vertebrates are well studied. Estrogen receptors (ERs) are present in lophotrochozoans, and *in vitro* studies suggest they are activated by estrogens (estradiol, estrone, and estriol) at low concentrations. *Capitella teleta* is a marine lophotrochozoan used as an indicator species for pollution due to its high growth rate in contaminated sediments. *In vivo* biological effects of estrogens have not been investigated in this species. *Capitella teleta* males and females were exposed to either a solvent control (0.01% DMSO) or 17β-estradiol (70, 700, or 7000 nM) for 4 hours prior to a behavioural assessment. Total distance moved, velocity, time to reach the edge of the arena, and the time to first movement were assessed after animals were placed centrally in an arena. For exposed males, but not females, total distance and velocity were decreased after exposure to 700 and 7000 nM 17β-estradiol. To investigate whether this effect occurred through the estrogen receptor, we exposed males to either 0.01% DMSO, fulvestrant (6400 nM), 17β-estradiol (700 nM), or a co-incubation of fulvestrant and 17β-estradiol. Co-incubation with the ER antagonist fulvestrant decreased the time to reach the edge of the arena compared to 17β-estradiol alone, suggesting that decreased locomotion with estrogen exposure is mediated through the estrogen receptor. These data will help elucidate the role that the estrogen receptor plays in *C. teleta* and suggests that environmental estrogens may impact polychaete locomotion.

Responses of freshwater invertebrate microbiomes to municipal wastewater effluents (PL)

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The host microbiome is vital to nutrient acquisition and immune defense. Freshwater ecosystems receive nutrients, bacteria, and pharmaceuticals from municipal wastewater treatment plants (WWTPs) that may alter the host microbiome. Aquatic insects provide critical energy linkages across the aquatic-terrestrial boundary; however, this linkage may expose/introduce effects to riparian insectivores from aquatic-derived contaminants. Our study examined the effects of WWTP effluents on the microbiome of downstream macroinvertebrates and spiders in the Bow River and in three mesocosm streams at the Advancing Canadian Wastewater Assets (ACWA) facility, Alberta, across two timepoints in

2022. DNA was extracted from whole-body macroinvertebrates and spiders for sequencing of the 16S bacterial rRNA gene (V3-V4 region). Stable nitrogen ($\delta^{15}N$) isotopes were analyzed to assess the use of wastewater-derived nutrients by macroinvertebrates and spiders. Lastly, animal-mediated nutrient recycling rates were assessed. Microbial communities within the ACWA mesocosms and across the Bow River sites differed between all invertebrate families; however, only the mesocosms showed spatial differences due to wastewater exposure. Indeed, the greatest change in relative abundance of major bacterial phyla and families in the Bow River was observed at an upstream, non-wastewater exposed site. While $\delta^{15}N$ values were enriched in most organisms and basal food sources collected at one of two effluent exposed sites in the Bow River, there was little influence of wastewater effluents on animal-mediated nutrient recycling rates. Overall, our study demonstrated some incorporation of wastewater-derived nutrients into invertebrates, but minimal effects of direct exposures of effluents to functions of nutrient recycling and microbial community compositions.

Introducing shinywqbench: An online tool for calculating aquatic life water quality benchmarks for emerging contaminants (PL)

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Since the 1970s the diversity and rate of production of synthetic chemicals in developed nations vastly increased. Given the degree of effort required to derive water quality guidelines (WQGs), the number of WQGs is dwarfed by the ever-increasing number of synthetic contaminants in production. British Columbia (BC) has less than 300 WQGs but the number of chemicals used in BC and released to the environment is likely at least an order of magnitude higher. Shinywqbench is an R package and associated shiny app that calculates chronic long-term benchmarks. It pulls data from the United States Environmental Protection Agency's ECOTOX database and applies a series of steps based on the BC WQG derivation protocol. Each step is transparent, and users can remove data if necessary. The final step adds assessment factors to account for interspecies extrapolation and species coverage. Although the application is designed for BC, users outside of BC can change the default factors and manually recalculate the benchmark. The data in the ECOTOX database do not undergo critical evaluation so the resultant aquatic life benchmark does not have the same reliability as WQGs. However, the purpose of shinywqbench is not to replace jurisdiction specific WQGs but rather to provide a tool for interpreting monitoring results of emerging contaminants.

Toxicological evaluation of emerging antibacterial compounds using the Microtox® test system (PO)

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With the surge of the SARS-CoV-2 virus, increased use of sanitizers with quaternary ammonium compounds (QACs) as the active antimicrobial chemicals led to their rise in wastewater treatment plant effluents and in surface waters. Currently, there is a lack of toxicological data concerning the effects of these emerging antimicrobials and their comparison to other classes of antimicrobials in aquatic biota. In this research, the acute toxicity of selected QACs and other antimicrobials, methylisothiazolinone (MIT), chloroxylenol (PCMX), and triclosan (TCS), was assessed using the Microtox® test system with the bacteria *Vibrio fischeri*. The QACs evaluated in this study were alkyl dimethyl benzyl ammonium chloride (ADBAC), dimethyl dioctadecyl ammonium chloride (DODMAC), didecyl dimethyl ammonium chloride (DDAC), decyl trimethyl ammonium bromide (C10TAB), tetradecyl trimethyl ammonium bromide (C14TAB), and hexadecyl trimethyl ammonium chloride (CTAC). The luminescence activity of *V. fischeri* was measured at 5- (T5) and 15-minute (T15) time points. For the QACs, IC_{50} values (inhibitory concentration for 50% luminescence elimination) were in the range of 1.46 to 11.77 $mg \cdot L^{-1}$ with DODMAC exhibiting the highest toxicity. However, the other antimicrobial compounds tested were marginally more toxic than QACs with TCS exposure eliciting the greatest inhibition on the bioluminescence activity with IC_{50} values of 0.99–1.04 $mg \cdot L^{-1}$. This study provides data support for the toxicological evaluation of antimicrobial compounds including emerging antimicrobials such as QACs.

Screening-level assessment of the ecological impacts pharmaceuticals and personal care product contamination to aquatic and terrestrial systems at Alaksen National Wildlife Area (PO)

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Pharmaceuticals and personal care products (PPCPs) have been termed contaminants of emerging concern, and assessing the risks of this large class of contaminants has been constrained by a general lack of toxicity data to inform risk assessments in the past decades. Consequently, an increased number of toxicity data sets characterizing the adverse effects of a greater number of PPCPs on non-target wildlife are now available. In this study, the risk of several PPCPs occurring in surface waters and sediments of Alaksen National Wildlife Area (ANWA) were screened using a deterministic assessment approach. Thirteen classes of PPCPs were detected including NSAIDs, beta-blockers, anti-depressants, stimulants, and illicit drugs. This preliminary assessment utilized passive sampling

techniques (polar organic integrative chemical samplers) as well as traditional sediment grab sampling to determine environmental concentrations of PPCPs. Preliminary results show that current surface water concentrations of PPCPs do not pose a threat to aquatic life, however PPCPs in sediments (DEET, erythromycin and BPA) could have potentially deleterious effects on lower trophic groups such as aquatic plants and the benthic community. DEET was detected in all sampled media and was detected at a maximum sediment concentration of 1400 $\mu g \cdot kg^{-1}$. Bioaccumulation and food web modelling were performed on potentially bioaccumulative PPCPs and were assessed using the Kow (based) bioaccumulation model (KABAM) to determine the risk to terrestrial wildlife and endangered species known to frequent ANWA. Current data suggest that the antifungals clotrimazole and miconazole may induce harmful acute effects on higher trophic groups such as birds of prey.

Are early life stage rainbow trout (*Oncorhynchus mykiss*) sensitive to benzalkonium chloride, an emerging antimicrobial compound of concern? (PO)

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In recent years, the use of disinfectants and personal care products increased to combat the SARS-CoV-2 pandemic. In particular, increased use of antimicrobial products containing quaternary ammonium compounds (QACs) has led to their ubiquitous nature in wastewater effluent and freshwater systems. Of the QACs, benzalkonium chloride (BAC) is a compound of emerging concern due to its high detection in freshwater systems and potential toxicity to aquatic organisms in the receiving environment. This study aims to understand the toxicity and biological effects of BAC on the early life stages of rainbow trout (RBT, *Oncorhynchus mykiss*), a salmonid species of ecological, commercial, recreational, and indigenous importance in Canada. A sub-chronic experiment is planned where RBT will be exposed from hatch until one week post-swim-up to a range of concentrations of BAC. Survival, incidence of abnormalities, and time to swim-up will be recorded through the exposure, while other endpoints will be assessed at the end of exposure (e.g., morphometrics, organ histopathology, microbiome profiles). In addition, individuals will be sub-sampled after the initial 96 hours of exposure to assess chemical-induced transcriptional responses using RBT-specific EcoToxChips. This study will address knowledge gaps on the potentially toxic effects of BAC in the environment, and results generated will support risk assessments and strategies to develop approaches to mitigate the ecological hazards of these antimicrobial substances.

Transcriptomic changes at the reproductive level of catfish (*Trichomycterus areolatus*) associated with sewage effluents in Greater Santiago, Chile (PO)

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Trichomycterus areolatus is a Chilean native freshwater catfish widely distributed between Central and Southern Chile, areas highly urbanized. In Santiago city, which is in the central zone of Chile, there are two wastewater treatment plants (WWTPs) responsible for handling nearly 90% of discharges, both of which flow into the Mapocho River. Worldwide, it has been observed that fish exposed to sewage discharges show changes at different levels; changes in the proportion of females and males, changes in individual size, and the presence of intersex have been observed. In addition, modifications in gene expression have been observed. In the case of catfish, information is scarce regarding transcriptomic changes in habitats subjected to sewage discharges. The purposes of the study are to quantify by quantitative polymerase chain reaction (qPCR) gene expression changes at the sex level that may be affected by sewage effluents. The collection sites were determined according to their anthropic pressure and presence of *T. areolatus*, two reference sites, two urban sites, and two sites downstream of the WWTPs. The collection of individuals was carried out during the Chilean springs of 2022. A total of 91 individuals (Males=37 and Females=54) were collected at five of six sites. The genes of interest are androgen receptor, estrogen receptor subtype 1, vitellogenin, aromatase, estrogen receptor beta, spermatogenesis associated protein 4, and as control genes, elongation factor 1 alpha and ribosomal protein S18. Laboratory analyses are in process. This study would be the first to include changes in gene expression of *T. areolatus* exposed to sewage discharges.

Transfer of aquatic PPCP contaminants to terrestrial food webs by emergent invertebrates exposed to WWTP effluent (PO)

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Wastewater treatment plant (WWTP) effluents contain complex mixtures of pharmaceuticals and personal care products (PPCPs) due to their incomplete removal or transformation during treatment. This leads to detection of many classes of PPCPs in surface waters, and potential accumulation in macroinvertebrates and transfer to riparian ecosystems through emergence of aquatic insects. This study will quantify the internal concentrations of select PPCPs and metabolites in benthic macroinvertebrates and riparian spiders to assess the fate of aquatic contaminants in downstream ecosystems impacted by WWTP effluents. Several taxa of aquatic larval and terrestrial adult invertebrates representing different feeding groups, and nearshore spiders that rely on emergent prey as

a food source, were collected upstream and downstream of four WWTPs along the Grand River. Collections occurred during the summer of 2022 when emergence events are more common. Internal concentrations of PPCPs are being determined by HPLC-ESI-MS/MS and paired with measures of relative trophic position ($\delta^{15}N$) and carbon source ($\delta^{13}C$) to assess food web structure and spider reliance on aquatic prey. Twenty one PPCPs and metabolites, which may exert comparable effects as the parent compound on non-target organisms, were selected for LC-MS/MS analysis of PPCPs in whole body invertebrates. Quantifying PPCPs in invertebrates and spiders will be used to determine accumulation in primary and secondary consumers and transfer of aquatic contaminants to terrestrial food webs through emergence. Based on previous research, larval invertebrates are predicted to contain elevated levels of ΣPPCPs downstream relative to upstream of WWTPs. Bioamplification occurs as mass is lost during metamorphosis and is expected to produce higher ΣPPCPs in emergent adults compared to larval stages, and similar or elevated profiles of PPCPs may be observed in riparian spiders. These data will support previous studies investigating the role of WWTP effluents on accumulation of PPCPs in aquatic organisms and the potential transfer to terrestrial food webs through emergent insects.

Quaternary ammonium compounds (QACs) in Canadian wastewaters and biosolids, and the potential impact of COVID-19 (PO)

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Quaternary ammonium compounds (QACs) are contaminants of emerging concern (CEC) given their high hazard to many organisms, their broad use in high quantities, e.g., disinfectants, sanitizers, personal care products, and detergents, as well as their increased use during COVID-19. In one of the first studies to quantify QACs in Canada, the concentrations of 13 QACs from 3 groups were measured at 11 representative wastewater treatment plants (WWTPs); namely, alkyldimethylbenzylammonium compounds (ADMBAC-C10, -C12, -C14, and -C16), alkyltrimethylammonium compounds (ATMAC-C10, -C14, -C16, and -C18) and dialkyldimethylammonium compounds (DADMAC-C10, -C12, -C14, -C16, C18). During sampling (2018–2021), the range of concentrations detected for each group were (i) influents: ADMBAC, <0.03–122 $\mu g \cdot L^{-1}$; ATMAC, <0.02–38 $\mu g \cdot L^{-1}$; DADMAC , <0.1–85 $\mu g \cdot L^{-1}$; (ii) effluents: ADMBAC, <0.03–1 $\mu g \cdot L^{-1}$; ATMAC, 0.01–10 $\mu g \cdot L^{-1}$, DADMAC, <0.1–43 $\mu g \cdot L^{-1}$; (iii) biosolids: ADMBAC, <0.005–150 $mg \cdot kg^{-1}$; ATMAC, 0.02–145 $mg \cdot kg^{-1}$, DADMAC, 0.06-80 $mg \cdot kg^{-1}$. The QACs with the highest contribution to these concentrations were ADMBAC-C14, ATMAC-C14, and DADMAC-C10 for influents, ADMBAC-C16, ATMAC-C14, and DADMAC-C18 for effluents, and ADMBAC-C14, ATMAC-C14, and DADMAC-C18 for biosolids. When comparing influent and effluent concentrations, WWTPs appear somewhat effective at removing these QACs via adsorption, resulting in accumulation of QACs in biosolids. Significant increases in 2021 influent concentrations

have been noted for ATMAC-C14 and -C16 in four WWTPs when compared to the 2019 pre-COVID-19 sampling, which may reflect a change in use patterns/quantities associated with the pandemic. Finally, a non-target analysis of wastewater in 2019 also allowed the detection of ADMBAC-C18 and ATMAC-C22 as major QACs. ECCC continues to investigate this group of CECs.

Development of the optimal extraction method of chiral pharmaceuticals from rainbow darter (*Etheostoma caeruleum*) (PO)

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Pharmaceuticals are ubiquitous in the aquatic environment and enter via wastewater effluent. These compounds have been found to bioaccumulate in small fish collected downstream from wastewater treatment plants, and impact biological and behavioural endpoints. More than 50% of commonly used pharmaceuticals are chiral. Historically, the chirality of pharmaceuticals has not been considered when completing risk assessments. The chirality of pharmaceuticals has been of specific interest and importance because of the potential enantioselectivity of their environmental distribution, fate, and ecotoxicity. One chiral compound of interest is the antidepressant venlafaxine since it is persistent through wastewater treatment processes and can be found at relatively high levels in effluents. It has also been shown to impact aquatic biota in receiving environments. A method was developed and validated for the extraction of whole, small-bodied fish that is unbiased in the extraction of the R- and S-enantiomers of venlafaxine. Ultrasonic extraction and accelerated solvent extraction have been tested for the extraction of venlafaxine from small-bodied fish, and high recoveries have been achieved with ultrasonic extraction. EMR-lipid SPE and commercial QuEChERS kits have been tested for the subsequent lipid cleanup steps, with EMR-Lipid SPE resulting in satisfactory recovery and reproducibility. The optimized and validated extraction method is being applied to wild small-bodied fish (rainbow darter; *Etheostoma caeruleum*) to determine whether there is an enantiomeric shift of chiral venlafaxine seen in the fish tissue relative to the water in controlled lab experiments, as well at sites associated with municipal wastewater treatment plant outfalls in the Grand River.

Estimation of the drugs of abuse consumption in Southern Ontario via wastewater-based surveillance (PO)

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Wastewater-Based Surveillance (WBS) is a powerful approach for assessing and monitoring drug consumption patterns in communities by analyzing drug presence and metabolites in wastewater, proven to be important in public health, particularly during the COVID-19 epidemic. However, implementing WBS programs faces challenges such as lack of reliable strategy to normalize the mass load per capita as well as the complexity of extracting these drugs from the wastewater. To overcome these issues, an analytical method combining solid phase extraction (SPE) with liquid chromatography tandem mass spectrometry (LC-MS/MS) was developed, revealing varied drug concentrations ranging from 20 to 5000 $ng \cdot L^{-1}$ across several WWTPs in Southern Ontario. These data are important for comparing per capita drug usage and developing effective approaches on the determination of consumption levels.

Mining and the Environment

Defining and quantifying stress/disturbance gradients for young wetlands forming in reclaimed oil sands landscapes (PL)

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Northeastern Alberta is dominated by a mosaic of wetlands and peatlands, reflecting hydrology, topography, and successional processes operating since the last glaciation. Mining companies have created reclaimed watersheds harbouring seemingly productive and biodiverse wetlands. However, methods of assessing the effectiveness or 'functionality' of wetland reclamation are lacking especially during early succession. To assess patterns of environmental stress – biological response relationships, we surveyed 120 newly formed or created wetlands (age 2–40 years) in oil sands mine lease reclaimed landscapes (n=60) or adjacent reference regions (n=60). We wished to assess and delineate the range of three principal classes of stress against which to assess the community composition of three forms of biota – aquatic invertebrates, vegetation, avifauna. We developed a structural equation model for each of the three classes of stress – "landscape disturbance" (drone-surveyed setting, local topography, vegetation, surrounding physical disturbances), "permanence" (inferred from interannual imagery of surface area, intra-annual water level logging, evaporation/transpiration, surface/groundwater ratios), and "water quality" (cations, anions, nutrients, organic compounds).

Developing monitoring triggers to detect water quality changes associated with unplanned releases from an oil sands tailings pond in the Athabasca oil sands region of Alberta (PL)

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Concerns over threats to the local environment in the Athabasca oil sands region were heightened following reports of uncontrolled wastewater releases from the Kearl Oil Sands Project mining site. A seepage of industrial wastewater from an external tailings pond was first detected in May 2022 followed by a second from an overflow drainage pond in February 2023. The closest Athabasca River tributary to the release sites is the Firebag River, and there are historical water quality data for the Firebag River from the oil sands monitoring program through Environment and Climate Change Canada (four sites from 2012 to 2022), Alberta Environment and Parks (two sites from 2017 to 2022), and from

the Regional Aquatics Monitoring Program (two sites from 2002 to 2014, and three sites from 2015 to 2017). The historical data provide a baseline to develop decision thresholds that could be used to detect the appearance of discharge associated with unplanned releases. The objective of this study is to characterize the variability of selected water quality parameters and quantify potential environmental baselines through upstream-downstream and temporal comparisons. The data were used to develop potential monitoring triggers for detecting this unplanned release of wastewater into the environment. The approach used also provides a template and approach to develop water quality state of the environment reporting approaches that include consideration of historical baselines, monitoring triggers to identify departures from baseline, and management triggers that include Canadian Council of Ministers of the Environment (CCME) water quality guidelines as well as Indigenous-developed criteria for the protection of Indigenous uses.

Modelling background conditions in the lower Athabasca River to support adaptive monitoring in the oil sands region (PL)

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To support adaptive monitoring in the Lower Athabasca River (LAR), Alberta Environment and Protected Areas (AEPA) completed three years (2018, 2019, 2021) of baseline monitoring of the mainstem of the LAR, under what was called the Enhanced Monitoring Program (EMP). This included a 60 km stretch of river from downstream of Fort McMurray to just upstream of Fort MacKay, where there may be potential future releases of treated oil sands processed mine waters. The EMP monitored conventional parameters including sentinel adult fish populations, benthic invertebrate communities, benthic algae communities, water and sediment quality variables, and body and tissue burdens of contaminants of potential concern in fish and benthic invertebrates. As such, the EMP provides baseline data on which to assess spatial and temporal variation in the mainstem of the LAR. Complimenting the EMP dataset is Canada's Oil Sands Monitoring Program (OSMP), which has also been collecting similar data in the mainstem, but from a broader spatial area, and for a longer period (since 2009). There are obvious synergies between the EMP and OSMP that have not been examined. Our work summarizes sources of variation in measured aquatic environment monitoring variables collected under both programs. We use generalized linear models to account for background variation in the data through the inclusion of a variety of influencing predictor variables related to climate and development-related pressures. The models developed, using both OSMP and EMP datasets, produce residuals that are used to estimate normal ranges of variation for multiple endpoints measured. Combined, the models and normal ranges can be used to

assess subsequent monitoring data and provide potential triggers for follow-up monitoring activities, and illustrate a potential methodology for characterizing sources of historical and baseline variability.

Toxicity assessment of sediment from an oil sands pilot pit lake containing permanent aquatic storage structure (PASS) treated tailings (PL)

Immanuela Ezugba[1], Lorne Doig[1], Banamali Panigrahi[1], Catherine Davila-Arenas[1], Karsten Liber[1]

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Lake Miwasin, a pilot-scale pit lake, was designed to evaluate the efficacy of PASS fluid tailings treatment and closure landform design, aiming to enhance overall reclamation planning and performance. This study assessed the toxicity of polymer-treated tailings making up the bottom substrates in Lake Miwasin. Sediment samples were collected in the Lake's littoral (deep and shallow areas) and limnetic zones in 2020–2022 to evaluate substrate toxicity. Sediment metal concentrations in both lake zones did not exceed the existing Canadian Council of Ministers of the Environment (CCME) Sediment Quality Guidelines for the Protection of Aquatic Life-Freshwater. Quantification of organics is in progress. *Chironomus dilutus* larvae were acclimated to reconstituted saline water (RSW), formulated to mimic Lake Miwasin bottom water. These larvae were subjected to 14-day static-renewal bulk sediment toxicity tests, where they were exposed to littoral and limnetic sediments in conjunction with either RSW or Lake Miwasin bottom water. Results from 2020 showed toxicity for bulk limnetic (≤5% survival) and deep littoral (≤15% survival) sediments. A whole-sediment Toxicity Identification Evaluation (TIE) was performed on limnetic sediment collected in 2021 to identify the class of toxicant(s) most likely responsible for the observed toxicity. However, no individual class of contaminants could be identified as the exclusive source of toxicity. Polymer toxicity testing was carried out across the concentration range measured in the surface and pore water using static-renewal 10-day exposures of *C. dilutus* larvae. The results showed no polymer toxicity. Shallow littoral sediment was collected in 2022 and, using similar 14-day exposures, were found to have no effect on animal survival. Two batches of limnetic sediment were also collected in 2022 and demonstrated mixed toxicity results, with survival ranging from ~40–80% between batches. A follow-up sediment toxicity test comparing core (top 2 cm) versus grab (top 10 cm) samples was conducted using limnetic sediment collected in 2022. Results showed a survival range of 86–100% for the top 2 cm sediment layer and 60–80% survival for the grabs consisting of the top 10 cm of sediment. These results suggest a vertical gradient of toxicity in the sediment profile. However, collectively, our data could also indicate a decrease in bulk sediment toxicity over time. Further studies will be carried out in 2023 to confirm vertical and possible temporal toxicity trends for limnetic sediment in Lake Miwasin.

Spatio-temporal analysis of water chemistry and ecotoxicological risk characterization of a pilot-scale pit lake in the Athabasca oil sands region (PL)

Banamali Panigrahi[1], Lorne Doig[1], Immanuela Ezugba[1], Catherine Davila-Arenas[1], Karsten Liber[1]

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Substantial quantities of fine tailings and oil sands process-affected water (OSPW) require reclamation in the Athabasca oil sands (AOS) region, Canada. Towards this end, Lake Miwasin was created as a pilot-scale pit lake containing treated fluid tailings (bottom sediment) capped with a blend of OSPW and surface water. Long-term monitoring is ongoing to determine the trajectory of the water quality of this test lake. The current study characterized spatial and temporal changes in surface water chemistry using a wireless sensor network (WSN); particularly to identify potential periods of sediment resuspension and contaminant release from the consolidating tailings. WSN technology was deployed to remotely measure water quality parameters at different depths of the water column. Field measurements and manual water sampling were carried out periodically to validate the sensor measurements and analyze additional variables requiring more complex laboratory instrumentation. A Tier 1 screening-level method employing hazard quotients (HQs) were calculated for individual metals using water quality benchmarks and published toxicity data. Total osmolarity was used to identify the risks associated with major ions. Finally, detailed ecotoxicological risks for selenium (Se) and arsenic (As) were estimated from sensor conductivity (EC) monitored data (2021 and 2022) using the linear model established (EC vs Se/As) based on manual sampling data from 2019–2022. Measurements indicate a seasonally stratified water column, with the epilimnion spanning 0 to ~3.5 m and the hypolimnion from ~3.5 to lake bottom (~4.3 m depth). Increased EC near the sediment-water interface during stratification suggests pore water expression, which has elevated salt content relative to the overlying water. Decreasing EC towards the end of the monitoring period suggests water input from the surrounding catchment and possibly also a decrease in porewater expression as the tailings continued to consolidate. Individual metal HQs are calculated, and the threshold HQs are classified based on the ranges from 1.0 (high risk) to 0.1 (negligible risk). Cumulative HQs for metals were >1 but decreased over time. The major ions risk was <1 for all years and seasons except in 2019 and winter 2020. Modeling results indicate that the 90th percentile HQs for Se and As were 1.6 and 0.44, respectively, suggesting that the Se element may represent a possible ecotoxicological risk.

Putting the puzzle pieces together: Metal modeling, toxicity testing, and tissue uptake to identify cause of effects for a gold mine EEM (PL)

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In accordance with the *Fisheries Act*, mines in Canada regulated under the *Metal and Diamond Mining Effluent Regulations* are required to conduct periodic environmental effects monitoring (EEM) studies as part of their authority to deposit effluent. During the current EEM program undertaken for a gold mine, biological studies focused on the final mine effluent discharged and took an Investigation of Cause (IOC) approach to examine the potential causes of effects that were previously observed in the fish and the benthic invertebrate community. The IOC study examined acute and sublethal toxicity testing (using *Oncorhynchus mykiss*, *Daphnia magna*, *Hyalella azteca*, *Pimephales promelas*, *Ceriodaphnia dubia*, *Lemna minor* and *Pseudokerchneriella subcapitata*) and chemistry results for final effluent from the mine, spanning the period 2016 to 2023. Potential roles of metals, ammonia, and cyanide in mine effluent toxicity were evaluated. The Windermere Humic Aqueous Model using the Toxicity Function (WHAM FTOX) model was used to assess the potential for metals or metal mixtures to cause toxicity to aquatic life based on the water chemistry. Metal accumulation in tissues of *H. azteca* and *L. minor* were also measured and compared to previously published endpoints for toxic responses. The weight of evidence indicates that copper was the most frequent cause of effluent toxicity or toxic potential in the mine effluent, with minor or occasional roles assigned to ammonia and cyanides. The results of the IOC study will be used to inform the study design for biological studies of the next EEM program.

Best practices for establishing exposure and reference sites for metal mining environmental effects monitoring (EEM) studies (PL)

Sarah Costantini[1], Jason Plamondon[1], Maria Story[1], Ken Korman[1]

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Environmental effects monitoring (EEM) studies are a requirement of metal and diamond mines that discharge effluent to the environment under Part 2, Section 7 of Environment and Climate Change Canada's *Metal and Diamond Mining Effluent Regulations* (MDMER) under the *Fisheries Act*. The MDMER requires mining companies to conduct EEM studies to determine if their effluent is adversely affecting the receiving body of water and its fish and benthic communities. For a control-impact design, reference and exposure areas must be established. As defined by the MDMER, the reference area is "water frequented by fish that is not exposed to effluent and that has fish habitat that, as far as practical, is most similar to that of the exposure area". Consequently, it is critical to

consider any confounding factors and the impact they may have on the similarity of the sites. Best practices can be followed to determine the most appropriate reference and exposure areas, as it is important to take proactive measures to verify the suitability of the area ahead of the study. This can be done by completing reconnaissance or using available information from other studies. Based on these findings, it may be necessary to change the reference area or incorporate multiple reference sites. By utilizing best practices, an EEM study has a higher chance of being successful and determining whether there are any adverse effects on water and the resident fish and benthic communities.

The characterization of natural variability within EEM and subsequent decision-making processes (PL)

Kevin Martens[1], Pierre Stecko[1]

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An 'effect' to fish or fish habitat within the *Metal and Diamond Mining Effluent Regulations* environmental effects monitoring (EEM) is any statistical difference between exposure and reference areas. If any result of two consecutive studies indicates the same type of 'effect' for a single endpoint for which the cause is not known, the mine is required to investigate the cause of that 'effect'. Multiple reference areas have been used in EEM studies to provide a measure of natural variability within aquatic environments comparable to the exposure area. However, the regulations stipulate that a difference between the exposed area and any reference is considered an effect, even if there is no difference from one of the references. Multiple reference areas have been used for fish health and benthic invertebrate community studies over multiple phases of EEM for a mine discharge on the middle Fraser River. Results have consistently shown no difference for benthic invertebrates and significant differences between reference fish populations but no difference for one exposed-reference comparison. Despite demonstrated natural variability among the populations, the consistent differences in length frequency distribution over two studies between the exposed population and one reference area for leopard dace (*Rhinichthys falcatus*) triggered an investigation. Through the investigation evaluating six sampling areas on the Fraser River using leopard dace and lake chub (*Couesius plumbeus*), natural variability among reference populations was characterized. The subsequent evaluation of 'effects' within EEM should consider the normal range of reference conditions rather than focusing on comparison to a single reference.

Elevated copper and shifts in aquatic and riparian invertebrate microbiomes at a site impacted by historic mining (PL)

Karen Kidd[1], Amy Marcarelli[2], Brittany Perrotta[1], David Walters[3], Gordon Paterson[2]

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Historic copper mining left a legacy of metal-rich tailings along and in Torch Lake, an Area of Concern in the Upper Peninsula of Michigan, USA. Given the toxicity of copper to invertebrates and microorganisms, this study assessed the influence of this legacy on present-day, nearshore aquatic and terrestrial ecosystems. Metal mixtures in sediment, pore water, surface water, larval and adult insects, and two riparian spider families at three highly impacted sites in Torch Lake were measured and compared to sites in a nearby lake with no historic mining activities. In addition, we collected aquatic and emergent insects and spiders to assess whether current-day metal exposures alter their microbiomes. Torch Lake had elevated metals in all sediments and some water samples, resulting in elevated predicted toxicity. However, copper was the only element in larval tissues from this lake that was close to predicted effects concentrations, and elevated copper was also found in riparian spiders demonstrating that the risk extended across the aquatic-terrestrial boundary. We found a significant alteration of the larval insect, adult insect and spider microbiome communities from Torch Lake when compared to the reference lake. Overall, these results highlight that copper remains a contaminant of concern in Torch Lake and that, coupled with the shifts in host microbiomes, may impact other processes as insects provide critical subsidies of energy and material fluxes across the land-water interface to riparian food webs.

Trace elements in fish from an industrial region located within the Traditional Territory of two First Nations near Sudbury, Ontario (PL)

Adam Lepage[1], Gretchen Lescord[1,2], Robert Paishegwon[3], Lori Richer[3], Curtis Assance[3], Thomas Johnston[4], John Gunn[1]

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Mining activities can introduce trace elements like arsenic (As) and selenium (Se) into aquatic environments. These elements can bioaccumulate in fish, which may pose a risk to both recreational and subsistence harvesters. Our project seeks to assess the bioaccumulation patterns of trace elements in highly valued fish species in 17 lakes, both on and off First Nation reserves, at varying distance from the metal smelters in Sudbury, Ontario. Mining-related environmental contamination and degradation is a major concern in the region, with early mining and smelting activities dating to the late 1800s. Although contaminant concentrations in local lakes have generally fallen below Provincial Water Quality Objectives over the past 50 years because of legislated pollution control, some fish

still exceed consumption advisory benchmarks for mercury (Hg), Se, As, and other elements. In addition to comparisons with benchmark values, drivers (e.g., distance from smelters, water quality, lake morphometry, food web structure, presence of other elements (e.g., Hg), etc.) of variability in contaminant levels between lakes and fish species, as well as among individual fish will also be assessed. For confidentiality reasons lakes are identified only with code numbers. These results will help inform local communities about the contaminant burdens of fish from local lakes and improve our understanding of trace element dynamics in chemically complex mining environments.

Linking the competition among three rare earth elements (lanthanum, cerium, and yttrium) with the bioavailability to *Chlamydomonas reinhardtii* using the biotic ligand model (PL)

Laurianne Pagé[1], Marie-Hélène Brunet[1], Kevin Wilkinson[1]

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Rare earth elements (REEs) are increasingly used because of their unique optical, magnetic, and catalytic properties. With increasing mining and production, it essential to determine the potential environmental impacts of the REEs. The biotic ligand model (BLM) can be used to predict metal bioaccumulation and toxicity. In theory, it can consider the impact of competition, which is an important point when studying environmental mixtures. The objective of this study is to link the bioaccumulation measured in carefully controlled laboratory conditions to those that are measured in a potentially contaminated natural environment (REEs mine effluent). We have thus measured the bioaccumulation of three cation REEs to determine their biouptake constants for potential use in a BLM (La^{3+}: K_{Me} = (0.13 ± 0.04) x10^7 M^{-1}; Ce^{3+}: K_{Me} = (0.10 ± 0.04) x10^7 M^{-1}, Y^{3+}: K_{Me} = (0.14 ± 0.02) x10^7 M^{-1}). The next steps will be to study the competition among these three REEs cations for uptake sites on the cell membrane of *Chlamydomonas reinhardtii* (*C. reinhardtii*). The first part of the project is being performed in a well-controlled environment (synthetic waters in the laboratory). In a second step, we will study real samples, taken from the first REE mine in Canada (Nechalacho, Northwest Territories; Cheetah Resources). This mine extracts bastnaesite, an ore containing mainly lanthanum (La), cerium (Ce), and Yttrium(Y). This work will help predict the impacts of this mine and similar projects on natural Canadian environments.

Environmental monitoring and remediation at Giant Mine – 'It takes a village' (PL)

Tamara Darwish[1], Collin Arens[1], Elaine Irving[1], Kate Sinclair[1]

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Giant Mine is one of Canada's largest former gold mines and one of the world's most contaminated sites. Owners abandoned the site in 1999, leaving governments with a legacy of environmental challenges. Approximately 273 000 tonnes of soluble arsenic trioxide is stored below ground, near where people live, where drinking water is sourced, and adjacent to a waterbody, Yellowknife Bay, that hosts fishing and recreational activities. Pre-mining, Baker Creek, a small watercourse draining through the site, was known for its fishing and berry picking, vital to local Indigenous groups. After the site was abandoned, studies found no fish and few benthic invertebrates downstream of Giant Mine. Water in Baker Creek was acutely toxic to fish. After improved environmental and water management, recent monitoring shows that fish in Baker Creek are reproducing and food for fish is available; however, slimy sculpin (*Cottus cognatus*) have larger livers. Closure objectives for remediation of Baker Creek include improving water and sediment quality, restoring the channel to encourage natural rehabilitation, and enhancing fish habitat. Monitoring of future conditions is a requirement to make sure these objectives are met. The lesson is that 'it takes a village' to get the monitoring right. Elders from Indigenous groups were vital in understanding pre-mining conditions. Multiple disciplines were needed to plan future monitoring. To facilitate this, the Government of Canada formed an Aquatic Advisory Committee (AAC) comprising Rights holders, stakeholders, and regulators. The AAC allows members to participate in meaningful conversations and exploration of concepts related to remediation and monitoring.

Monitoring to management: A framework for integrating and interpreting complex aquatic monitoring data (PO)

Daphne Lewarne[1], Giovanna Diaz[2], Emily-Jane Costa[1], Jennifer Ings[3], Cait Good[2], Mariah Arnold[2], Adrian de Bruyn[4], Todd Hatfield[5], Jason Ammerlaan[5]

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Mining proponents conduct routine monitoring that collects many types of data, including water quality, tissue chemistry, physical conditions, chronic toxicity, benthic invertebrate community composition, and fisheries assessment endpoints. Taken together, these data can provide an intensive (many types of data are measured) and extensive (at many sites) characterization of the state of the aquatic environment. However, in intensively monitored areas, it can become difficult to assemble monitoring information in a way that allows assessment of conditions at both local and regional scales. The Aquatic

Data Integration Tool (ADIT) was developed to provide a useful broad-scale overview and more detailed local-scale evaluation of monitoring data. The ADIT is a framework for integrating multiple types of stressors and biological response metrics, aligned to be as co-located and concurrent as possible, to support an integrated interpretation of the state of the aquatic environment throughout a study watershed. Stressor and biological response metrics are assigned scores ranging from zero (no effect) to three (strong evidence for effect), providing an integrated and easily visualized interpretation of data. This tool was developed in consultation with several stakeholders, which results in a shared understanding of the data collected and stimulates an effective dialogue for the interpretation of those data.

Application of novel approach to evaluating acute lethality limits for sulphate (PO)

Breda Rahmanian[1], Emily-Jane Costa[1], Kerrie Serben[1], Maíra Mendes[1], Gary Lawrence[1]

[1]*WSP Canada Inc.*

A legacy North American mine is undergoing remediation with a new water treatment plant proposed that will improve treated effluent quality. However, there is a concern that major ion composition of effluent source water may shift towards a more toxic condition, due to changing source terms and/or increased sulphate concentrations. A preliminary 2019 study provided confidence that acute toxicity was not expected for *Daphnia magna* or rainbow trout up to sulphate concentrations of 2860 $mg \cdot L^{-1}$. However, uncertainty remained in bounding the degree to which increased sulphate and/or a shift in major ion composition may affect sulphate toxicity. To support development of a sulphate limit for the former mine, a laboratory toxicity study was undertaken to identify acute toxicity thresholds of sulphate under future predicted site-relevant conditions. Due to the practical laboratory solubility limit of sulphate (of approximately 1500 $mg \cdot L^{-1}$) a novel approach was conducted to simulate pre-treatment of field-collected contaminated mine water to maintain >3000 $mg \cdot L^{-1}$ sulphate, while reducing all other constituents to below a level of concern for acute toxicity. Sulphate was tested over a wide range of exposure site conditions, providing a margin of safety and an opportunity to bound the threshold for acute toxicity. Three site water types were sampled, pre-treated, and tested for toxicity, each representing a different major ion composition profile. Fish survived in concentrations up to 10 010 $mg \cdot L^{-1}$ and *D. magna* survived in concentrations up to 5090 $mg \cdot L^{-1}$ sulphate under the tested range of site-relevant ionic compositions.

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

Confirmation bias and the proliferation of erroneous research and publication bias (PL)

Shane de Solla[1], Karen Kidd[1]

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Attempts to replicate previously published studies has revealed a "hidden" crisis in science. The successful replication rate (% occurrences where the replicating study has the same results as the original study), is roughly 50%; roughly the same likelihood as obtaining "heads" by randomly flipping a coin. Studies that fail to be replicated successfully appear to be biased towards false positives and have more citations, than studies that are successfully replicated. "Natural selection" may favour poor research designs and data analyses, as studies that have surprising results or large effects sizes are rewarded by a greater number of citations and therefore influence. Further, journals are often biased towards publishing significant results as opposed to "null results", again leading to overestimation of effect sizes, including overestimating toxicity. Confirmation bias may also contribute to the proliferation of false positives, as researchers may have cognitive biases towards hypotheses that support their world view, without due consideration of alternative hypotheses. We give examples in the ecotoxicology literature where the authors attributed significant results to the effects of contaminants, to the exclusion of alternative hypotheses that are also consistent with the results. Although "ancient history", Bradford Hill's seminal work on causality published in 1965 (e.g., strength of association, consistency, specificity, temporality, biological gradient, plausibility, coherence, experiment, and analogy), may aid ecotoxicologists in avoiding making strong inferences in the face of relatively weak evidence. Our inability to successfully replicate previous research, and tendency towards publishing false positives, corrodes the credibility of scientific method and practitioners, and weakens scientific practices. We must do better.

Trends in water quality for irregular time series data (PL)

Jeff Row[1], Patrick Schaefer[1], Jennifer Ings[1]

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A thorough and accurate understanding of temporal trends in water quality is essential for identifying areas in need of aquatic resource protection. Detecting trends in water quality data, however, can be challenging due to the inherit characteristics, such as: autocorrelation, censoring and seasonal variation, and the need to be able to detect different types of trends (e.g., monotonic, step-changes). There are different statistical methods that can evaluate trends in water quality, and the selected method should

maximize the power to detect trends when they exist (i.e., low false-negative error rate) while minimizing instances of incorrectly identifying a trend when there is not. These aspects of performance vary by statistical method and attributes of the data, but it is not always clear *a priori* which methods to use under different situations. Simulations are an effective approach to compare the performance of statistical methods because the true outcome is already known. Here, we use real water quality data to parameterize a simulated framework for the comparison of three approaches: non-parametric seasonal Kendall analysis, ANOVA analysis using month and year terms, and generalized additive models. We used empirical data to set sample variance, seasonality, level of censoring, and varied the rate and type (monotonic, seasonal, and/or step change) of trends underlying the data. Overall, we quantify and compare the power to detect simulated trends for each approach and develop a framework for comparing water quality trend methods using simulated data.

Establishing best practices in quantifying aquatic chemistry reference conditions through a simulated approach (PL)

Emily Dutton[1], Jeff Row[1], Patrick Schaefer[1], Jennifer Ings[1]

[1]*Minnow Aquatic Environmental Services*

Comparing areas exposed to disturbances with less impacted reference areas is a common approach to understanding exposure to impacts such as mining, forestry, and agriculture. Despite decades of scientific investigation, there remain outstanding questions related to the best methods for conducting these comparisons. To address some of these gaps, we asked two questions: 1) what is the relationship between the accuracy of the characterization of the reference range (i.e., difference between estimated and actual reference percentiles) and sample size, and 2) what is the relative importance of sampling more areas versus repeated sampling (i.e., emphasis on sampling more areas versus more replicates)? To answer these questions, we characterized reference distributions using an extensive dataset (103 areas) for tissue and sediment chemistry collected over >10 years and used the results to simulate sets of reference areas in a simulation study. We sampled the reference areas with varying numbers of total sample sizes while holding the number of replicates or total numbers of areas constant to compare the accuracy of prediction for different sampling strategies (more areas or more replicates per area). As a secondary goal, we also incorporated simulated detection limits (~5–20 % of values below the laboratory reporting limit) into our datasets and estimated percentiles using Maximum-Likelihood estimation and Kaplan-Meier percentiles and compared the accuracy of these approaches. Overall sampling strategy and approach influenced the accuracy of the characterization, and our results establish best sampling practices for deriving reference conditions for chemistry datasets.

Pairing wild rice and fish farming in a circular economy (PL)

Nicholas Blandford[1], Krista Robertson[1], Leah Dickenson[1], Richard Grosshans[2], Bruce Hardy[3], Nazim Cicek[1], Vince Palace[2]

[1]University of Manitoba, [2]International Institute for Sustainable Development-Experimental Lakes Area, [3]Myera Group

Aquaculture is a major food production sector that offers a sustainable alternative to conventional fisheries. However, inland aquaculture operations produce nutrient-rich wastewater that poses a risk of eutrophication and threatens the health of downstream aquatic ecosystems. Constructed wetlands (CWs) with aquatic macrophytes offer a potential solution for nutrient removal. The ability of northern wild rice (*Zizania palustris*) to assimilate nutrients in aquaculture wastewater (AWW) was assessed in mesocosms (2.56 m diameter; 1500 L) amended with AWW over 12 weeks. Phosphorus load removals from CW water were strongly correlated to phosphorus loading conditions, ranging from 1.21 ± 0.50 g·m^{-2}·yr^{-1} in reference treatments to 32.42 g·m^{-2}·yr^{-1} in the highest nutrient loading treatment studied (6.06 g·m^{-2}). Wild rice CWs effectively buffered the impact of nutrient loading, reducing total phosphorus concentrations below 0.5 mg·L^{-1} for ~2 months. However, eutrophic conditions developed in high loading treatments thereafter. Increases in periphyton and phytoplankton chlorophyll *a* indicated that excess phosphorus was available for algal growth. No relationships were observed between plant biomass or soil nutrient content and nutrient loading conditions. Our study reveals the potential of coupling land-based aquaculture systems with wild rice production while minimizing phosphorus inputs into receiving environments. This circular economy approach can be expanded further to include the production of algae, which can be incorporated into functional fish feeds. Through the implementation of CWs and integrating various biological components, aquaculture systems can more effectively manage high-nutrient wastewaters and benefit from resource recycling.

The response of zooplankton communities when exposed to aquaculture wastewater within wild rice planted mesocosms (PL)

Leah Dickenson[1], Krista Robertson[1], Nicholas Blandford[1], Mark Hanson[1], Bruce Hardy[3], Vince Palace[2]

[1]University of Manitoba, [2] International Institute for Sustainable Development-Experimental Lakes Area, [3]Myera Group

Inland aquaculture leads to a surplus of effluent that tends to be high in phosphorus and nitrogen, which can result in deleterious impacts to receiving aquatic ecosystems. Wild rice production from flooded paddy systems is a cultivation process that requires significant inputs of fertilizers to achieve sufficient growth. The nutrient demand of wild rice presents a sustainable way to manage aquaculture waste. However, there is concern

for possible effects on the ecology of the flooded paddies and the receiving aquatic ecosystem. We evaluated the possible effects on ecosystem health by the application of aquaculture wastewater to simulated flooded northern wild rice (*Zizania palustris*) paddy systems, using zooplankton community response as an indicator. An array of 16 mesocosms (~1400 L) planted with a commercial strain of wild rice, and 17 mesocosms planted with a native strain were colonized with aquatic invertebrates. Treated mesocosms received a gradient of aquaculture wastewater with total phosphorus loads ranging from 0.251–22.8 g, plus untreated controls. The zooplankton communities were sampled bi-weekly, enumerated, and taxonomically identified. Preliminary results indicate a positive trend of total abundance as the total phosphorus loadings increased. This trend is largely driven by the increases in rotifers and cyclopoid copepods within the higher treatments. Overall, aquaculture wastewater resulted in shifts to zooplankton community composition to species that are more tolerant to poor water quality. The outcomes of this study will help identify the maximum concentration of aquaculture fertilizer that could be applied to wild rice paddies to optimize growth while protecting freshwater ecosystems.

Eutrophication is a cumulative environmental impact problem for watersheds not waterbodies (PL)

Lynn McCarty[1], Dean Fitzgerald[2]

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We have evaluated federal and provincial regulatory documents associated with projects, largely mining related, near Wawa, Ontario, for Michipicoten First Nation. Eutrophication was not reported and nutrient-related (e.g., total phosphorus) regulatory problems were minimal. However, recent publications present a grave view of the past, present, and future trends in fish tissue methylmercury contamination in Ontario. There is a discrepancy between regulatory nutrient monitoring and increasingly elevated fish tissue methylmercury levels where both human and wildlife health are at risk. The discrepancy is related to Ontario's 1979 interim Provincial Water Quality Objective (PWQO) for total phosphorus, focusing on aesthetics issues: preventing algal blooms and excessive macrophyte growth. Since 1979, a better understanding of eutrophication and fish methylmercury levels has been developed. Although increased nutrient levels may not always increase methylmercury levels, the presence of substantial and increasing fish methylmercury levels in a watershed are diagnostic causal evidence of eutrophication from elevated watershed loadings of plant nutrients (e.g., phosphorus), particularly in historically nutrient poor watersheds in Northern Ontario. The current application of the interim PWQO for phosphorus does not directly address the methylmercury issue, but it does include a provision to "... prevent any undesirable changes in the aquatic ecosystem...". Additionally, PWQO Policy 2 provides broad general guidance: "Water Quality which presently does not meet the Provincial Water Quality Objectives shall not be degraded further and all practical measures shall be taken to upgrade the water quality to the

Objectives." An overview of both the problems and suitable measures to comply with PWQO Policy 2 will be presented.

Toxicity of oil sands groundwater naphthenic acid fractions in fathead minnow and walleye embryos and larvae (PL)

Joanne Parrott[1], Stacey Clarence[1], Christine Lavalle[1], Hufsa Khan[1], Kallie Shires[1], Kaiying Sally Ju[1], Blake Skanes[1], Maegan Rodrigues[1], Adrienne Bartlett[1], Patricia Gillis[1], Greg Bickerton[1], Jim Roy[1], Mark Hewitt[1], Richard Frank[1]

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Oil sands areas of Northern Alberta contain bitumen deposits that can comprise parts of the soil/substrate and river sediments. Groundwater flow allows some bitumen-derived compounds to enter rivers naturally. Groundwater near mining operations also may be influenced by mixtures of oil sands process-affected water (OSPW) that originate from oil sands tailings ponds. This project tests two types of groundwater, 'natural' bitumen-influenced groundwater and OSPW-affected groundwater collected 'near a tailings pond'. Each groundwater had the organics (including naphthenic acids) extracted, and extracts were divided into six fractions for acute toxicity testing in embryo-larval fish. Fathead minnows exposed to 100% extracts for 6 days showed that natural groundwater and groundwater near a tailings pond both contained naphthenic acids that were toxic to fathead minnow embryos and larvae. Survival was 32 and 43% for natural and tailings-pond groundwater extracts, respectively. Toxicity was associated solely with fraction 2 of each groundwater (the second most-polar fraction) and was not found in any of the 5 other fractions (fraction 1 the more polar fraction, nor in the less polar fractions 3, 4, 5, or 6). Chronic exposure of walleye embryos and larvae for 21 days (14 days as embryos, and 7 days as larval fish) to the near-tailings pond groundwater extract showed little toxicity at up to 50% groundwater extract. Work will continue assessing the chemicals in fraction 2 to isolate the most toxic naphthenic acids in fish, and to determine the relative sensitivity of the walleye compared to the fathead minnow.

Investigating the barriers to recovery in culturally and ecologically valuable clam populations impacted by an oil spill (PL)

Tyler Black[1], Kyle Artelle[2], Diana Chan[2], Benjamin de Jourdan[3], Conrad Pilditch[4], Ryan Prosser[1]

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In 2016, the Nathan E. Stewart articulated tug and barge ran aground, releasing over 100 000 litres of marine diesel oil and other petroleum products into Gale Creek, a site home to highly productive clam beds of ecological, economic, and cultural value. Since the spill, clam densities and juvenile clam recruitment have been low, lagging recovery. As part

of a project investigating barriers to recovery in intertidal bivalves exposed to this spill, we assessed the following: (a) presence of polycyclic aromatic compounds (PACs) within sediment 6 years following the spill, (b) reproductive capacity in adult clams at impacted and reference sites, (c) health of adult clams in impacted and reference sites determined via scope for growth (SFG) measurements and biochemical measures of stress, and (d) survival and behavioural endpoints in juvenile clams exposed to contaminated sediment from select sites impacted by the oil spill. The above analyses indicated no differences between reference and impacted sites. It seems unlikely that there are persisting effects related to health and reproduction on adult manila clam, or long-term direct toxicity from persisting petroleum products within the sediments. This talk will provide a high-level outline of our investigation into the barriers to recovery and will discuss next steps as we investigate other drivers that may be suppressing clam abundances within the area.

Characterizing the geochemical evolution and subsequent aquatic toxicity of a diluted bitumen spill within the saturated zone of a sand aquifer (PL)

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Prior to pipeline transport, bituminous crude oil often requires dilution with lighter oils (diluted bitumen or dilbit). The overall production and transportation of Canadian dilbit is expected to increase despite limited investigations into the behaviour and fate of dilbit spills within groundwater systems. To better understand this research gap, we utilized a large sand tank model (12 m^3) to conduct a controlled spill of 15 kg of dilbit (Cold Lake Blend) in 11 000 kg of glacial-fluvial sand receiving a 1000 $L \cdot day^{-1}$ unidirectional-flow of water to mimic a below-ground spill within the saturated zone. Water and soil samples were collected for a suite of geochemical measurements over 3.5 months from a series of piezometers at varying distances and depths from the dilbit source zone. Groundwater flow through the aquifer, including the dilbit impacted soil, was characterized with a tracer test. Water at the outflow of the system was collected for weekly 7-day fathead minnow (*Pimephales promelas*) embryologic teratogenicity assays. Concentrations of polycyclic aromatic hydrocarbons (PAHs) remained relatively low and consistent throughout all the exposures (<12 $\mu g \cdot L^{-1}$). Concentrations of benzene, toluene, ethylbenzene, and xylenes (BTEX) were initially high, reaching 1.7 $mg \cdot L^{-1}$ immediately following the spill, before decreasing to 0.2 $mg \cdot L^{-1}$ 70 days post-spill. Significant reductions in body length were observed throughout the 3.5 months, however no other toxic effects were observed. This experiment provides a temporal profile of an underground dilbit spill over time and identifies the risk that such spills pose to associated freshwater systems.

Assessing the effect of a minimally invasive oil spill remediation method on shoreline biofilm communities (PL)

Hakeem Omilowo[1], Madeline Stanley[1,2], Lauren Timlick[2], Lisa Peters[2], José Luis Rodríguez-Gil[2], Dave Findlay[3], Mark Hanson[1], Vince Palace[2]

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When transporting crude oil there is a probability of a spill that could damage aquatic ecosystems. While crude oil spill and remediation response effects are widely researched for marine ecosystems, there is a knowledge gap on the effects of remediation response efforts on freshwater environments. This study seeks to understand the effect of enhanced monitored natural recovery (eMNR), a non-invasive remediation method, on biofilm communities after an oil spill in freshwater shoreline environments. We used in lake shoreline enclosures and simulated a spill and the eMNR remediation method while comparing to reference enclosures. Chlorophyll *a*, ash free dry weight, species abundance and species richness were measured in treated enclosures and then compared to reference enclosures. All oiled+eMNR enclosure had no statistically significant change in chlorophyll *a*, ash free dry weight, species richness and abundance compared to the reference enclosures. These results shows that the eMNR remediation could be viable for use in oil spill remediation and cleanup as minimal environmental impact was observed.

Plant-enhanced phenanthrene reduction from freshwater microcosms (PL)

Madeline Stanley[1,3], Aidan Guttormson[1,2], Lisa Peters[2], Thor Halldorson[1], Gregg Tomy[1], Blake Cooney[1,2], Michael Opyr[1], Richard Grosshans[1,2], David Levin[1], Vince Palace[1,2]

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Since 2019, the IISD Experimental Lakes Area and collaborators have explored the use of engineered floating wetlands (EFWs) as a non-invasive alternative to conventional practices for the secondary remediation of oil spills in freshwater environments. Conventional recovery through the physical removal of oil can cause damage to sensitive environments and unfortunately can leave significant amounts of oil behind. EFWs may offer plant enhanced biodegradation of oil by providing surface area for microbial colonization and a suite of exudates that can stimulate the microbial community. To confirm that plants enhance the removal of polycyclic aromatic compounds, we exposed three emergent wetland plant species to phenanthrene in a microcosm scale experiment. Microcosms were established with 3 L of gently aerated lake water amended with 5–10% Hoagland nutrient solution and a stainless steel hydroponic pot containing either *Typha* sp., *Carex lasiocarpa*, or *Carex atherodes*. After five weeks of acclimation, experimental microcosms received phenanthrene standard at an initial nominal concentration of 1 $mg \cdot L^{-1}$. Over 21 days of exposure, microcosms were monitored for phenanthrene

concentration, basic water quality, plant development, and microbial activity. After 21 days, all treatments removed >94% of phenanthrene from water, however preliminary analyses suggest rates of removal may be higher for *Typha* and *C. atherodes*. Preliminary water quality (pH, conductivity, dissolved oxygen) analyses also varied between treatments, possibly due to differences in biomass, exudation patterns and products, and nutrient uptake. Differences between treatments and phenanthrene reduction potential will inform guidance on EFW use and design for remediation of oil spills.

Behaviour and brain size of larval zebrafish exposed to environmentally relevant concentrations of β-methylamino-L-alanine (BMAA) (PL)

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Harmful algal blooms (HABs) release toxic compounds in water and are increasing in frequency worldwide. The neurotoxin β-methylamino-L-alanine (BMAA) is released by HABs and is associated with human neurodegenerative disorders, but its effects on wildlife are still largely unknown. This study characterized the effects of chronic exposure to environmentally relevant concentrations of BMAA on the behaviour and brain size of developing zebrafish (*Danio rerio*). Zebrafish were continuously exposed to 0, 1, 10, or 100 $\mu g \cdot L^{-1}$ waterborne BMAA between 0 and 5 days post-fertilization (dpf) before the onset of exogenous feeding. At 5 dpf, locomotion and responses to vibrational and visual stimuli were assessed. Following behavioural testing, larval body and brain size were measured. Mortality between 0 and 5 dpf did not differ between treatments. Additionally, BMAA exposure did not affect most assessed behaviours (thigmotaxis, startle response magnitude, habituation to repeated presentation of vibrational startling stimuli) or relative brain size. A moderate increase in overall activity was observed in larvae exposed to 10 $ug \cdot L^{-1}$ BMAA under light, but this effect was not seen in dark conditions, indicating that chronic BMAA exposure may have modulated visual processing. Thus, passive continuous exposure to environmentally relevant concentrations of BMAA prior to first feeding in zebrafish did not affect survival or selected measures used to represent brain development, anxiety, and motor reflexes, but a limited light-dependent effect on locomotion suggests targeted neurotoxicity within the visual system. Funded by NSERC through the Canada First Research Excellence Fund and Discovery Grant programs.

Characterizing the environmental fate and behaviour of diluted bitumen within freshwater systems (PO)

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Increased extraction and transportation volumes of Canadian bitumen crude oil diluted with naphtha condensates (diluted bitumen; dilbit) are projected despite the limited understanding of environmental fate and behaviour, especially in freshwater systems. To this end, we conducted spills of dilbit and comparative conventional crude oils in both terrestrial and aquatic model systems to characterize chemical profiles over time and the subsequent embryotoxicity to fathead minnows. Terrestrial spills of Cold Lake Blend dilbit or Conventional Heavy Blend (CHB) were conducted with large soil columns to assess the chemical evolution of oil substituents within infiltrating water of an unsaturated zone. Malformations and expression of CYP1A in exposed fish were greatest up to 34 days post-spill, with no differences in toxicity between the two oil types, and an absence of correlation to the petroleum contaminants measured. Fresh surface water spills were conducted with Cold Lake Blend dilbit or a Medium Sweet Blend (MSW) within a large meso-scale spill tank. Concentrations of BTEX and PACs were higher in the MSW contaminated water, correlating with greater malformations, reduced growth and heart rate in exposed fish. However, significantly greater lethality was observed for up to 2 weeks post-spill for the fish exposed to the water contaminated with dilbit without correlation to the petroleum contaminants measured. In conclusion, our studies demonstrated variance in the fate and behaviour of dilbit and conventional oils reflective of the original chemical composition of each. Further characterization of the petroleum hydrocarbon classes causing toxic effects in the absence of characteristic crude oil toxicants.

Characterization of chloride exposure levels and ecological risk in the surface waters of Manitoba, Canada (PO)

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The concentrations of salts in freshwater systems have been increasing globally (from both natural and anthropogenic sources), making the ecological impacts of salinization a pressing concern. We are interested in characterizing the current state of salinity and salinization in the surface waters of Manitoba, as evaluated through the lens of a community-based monitoring (CBM) program. Volunteers gathered samples over three field seasons (2020–2022), from more than 130 sites across Manitoba, plus northwestern

Ontario, and North Dakota. The exposure concentrations were compared with freshwater chloride toxicity water quality guidelines to assess the risk. During the three field seasons, multiple sites across differing dominant land-uses exceeded the Long-Term Canadian Water Quality Guideline (CWQG) for the chloride Ion (120 mg $Cl^{-}\cdot L^{-1}$), with 12.3%, 17.3%, and 3.6% exceedance in 2020, 2021, and 2022, respectively. There was a low acute risk of exceedance across sites; however, two urban sites exceeded the Short-Term CWQG (640 mg $Cl^{-}\cdot L^{-1}$) multiple times in 2020 and 2021. The sites that experienced the greatest salinity levels were in the Red River Valley and near the city of Winnipeg, Manitoba, suggesting that anthropogenic factors may be driving exposure in these regions. Overall, this project will help to provide data that are useful for risk assessment, as well as identify a possible driver of ecological change in Manitoban surface waters, while also enhancing public participation in their protection.

The role for amphibians in monitoring ecosystem health and environmental change (PO)

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Research and monitoring programs assessing ecosystem health and environmental change often select organisms defined as a sentinel species, model organism, bioindicator or valued ecosystem components (VECs). Amphibians are often chosen as one of these, however, the use of amphibians to assess ecosystem health has been debated. We reviewed the literature to identify the main contributing factors that support amphibians as VECs or sentinel species and here discuss the advantages and limitations to their use in assessing ecosystem health. Key characteristics of amphibians as VECs became clear: their intrinsic value, contributions to ecosystem services, research and monitoring tractability, and ability to provide insight into the effects of anthropogenically-relevant stressors. Support for amphibians as VECs include their well-known ecology and physiology, small home ranges and unique life-history, multiple modes of research, multiple levels of biological organization, cost-effective researchability, sensitivity to change, measurable fitness-relevant responses, and large geographical span covering diverse habitats. On the other hand, our study identified differences among amphibian species, developmental-specific effects, compensatory growth, and the lack of repeatability depending on life-stage as potential but not insurmountable limitations. Therefore, in light of both the advantages and limitations, we argue that amphibians as VECs are useful to include in research and monitoring programs that are assessing ecosystem health and cumulative effects, but recommend caution when results from single-species, single-stressor scenarios are used to draw conclusions on whole ecosystem health.

Can analysis of sediments, vegetation, and waters provide differentiation between the sources of anthropogenic and natural contamination in the Tambo River Basin in Southern Peru? (PO)

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The Tambo River Basin in Southern Peru has been impacted by additions from a myriad of sources, both natural and anthropogenic, including volcanism, traditional and localized modern intensive agriculture, mining, and urban community effluents. The leaching of potentially toxic compounds and elements (PTEs) from these diverse sources has led to the contamination of this river basin, used by many communities as the sole source for potable water. This preliminary study has provided baseline elemental data from the analysis of acid digested sample materials from biota, sediments, and waters. This baseline data, specifically for elements such as arsenic, boron, lead, and phosphorus, will inform local communities on the levels and sources of contamination, and may be used to guide future watershed restoration efforts. The longitudinal distribution of PTEs in the various sample media collected from the Tambo River Basin from the river source regions in the Andean Foothills to the discharge into the Pacific Ocean is documented. Analysis of digested sediment samples by ICP-MS analysis showed elevated levels of arsenic, boron, lead, iron, phosphorus, and sulfur near the mining site. Near the sampled geysers, elevated sediment levels of chromium, nickel, sodium, and strontium were documented. Downstream, near the heavily populated regions nearer the Pacific Coast, elevated levels of cadmium, cobalt, copper, manganese, and zinc were measured. Evaluations of different digestion methods demonstrated the higher recovery levels with an HF digestion compared to reverse aqua regia for elements such as aluminum and lead. The observed differences in sediment load, vegetation, and dissolved water load for elements such as lead suggest that different remediation techniques should perhaps be applied for different environmental media. Future work will focus on an assessment of seasonal variability of different PTE's in the diverse media, especially as related to an assessment of potential faunal and human bioavailability and bioenrichment in organisms in the water, sediment, and plant food web.

Impacts of land use and ultraviolet radiation on floodplain zooplankton: An integrative ecotoxicity assay with *Daphnia magna* (PO)

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Floodplain habitats support high zooplankton production often crucial for fisheries. However, the expansion and intensification of agriculture in floodplains could impact zooplankton communities through habitat loss, soil erosion and consequent increased turbidity, and leaching of agrochemicals. Studying zooplankton communities in Lac Saint-Pierre (LSP), the largest floodplain complex along the St. Lawrence River, we found that natural wetlands such as flooded forests support a higher abundance of zooplankton compared to nearby (flooded) agricultural fields. We hypothesized that the higher turbidity and pesticide concentrations of flooded agriculture fields could explain why these habitats have lower zooplankton abundance than natural wetlands or fields with less intensive land use. To test this hypothesis, we exposed the model species *Daphnia magna* to soils collected along a land use gradient in the LSP floodplain (natural wetlands, old pastures/grasslands, and intensive monocultures/corn fields). Because the higher turbidity of agricultural fields could both impair zooplankton (e.g., through gill clogging) but also play a photoprotective role in shallow habitats with strong irradiance, we also crossed the soil treatment with a UV treatment in a fully factorial experiment. We tracked the survival, reproduction (number of gravid females and neonates produced), and performance indicators (movement, heart rate, and frequency of thoracic limb and post-abdominal claw movements) over 23 days. Our preliminary observations indicate no impacts of soil leachates or UV exposure on the survival, reproduction, and performance of *D. magna*. We conclude by suggesting that other mechanisms could account for lower zooplankton abundance observed in flooded agricultural fields of the LSP floodplain.

Using a passive dosing system to assess the toxicity of individual aromatic compounds to intertidal bivalves (PO)

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The Nathan E. Stewart sank on the coast of British Columbia resulting in 110 000 L of marine diesel oil (MDO) and other oil products to spill into waters within Haíłzaqv (Heiltsuk) First Nation territory. The lack of recovery of surrounding bivalve populations since has highlighted our limited knowledge of the impact of oil products. Understanding the biological effects of individual aromatic compounds (ACs) can allow for modelling of the overall effects of oil products to bivalves. This 7-day study investigated the acute toxicity of five ACs to juvenile *Venerupis philippinarum* and *Crassostrea gigas* using a

passive dosing system design. The ACs covered mono and polycyclic classes within a range of log octanol-water partition coefficient (Kow; 2.9–4.5) values. Polydimethylsiloxane (PDMS) O-rings partitioned ACs with uniform concentrations into media. The study examined three nominal concentrations relative to the sub-cooled water solubilities for each AC, and the analytically confirmed concentrations were used to analyze time-dependent median lethal/effect concentration (L/EC_{50}) values. Test species survival was monitored every 24 hours. Styrene, naphthalene, and 1-methylnaphthalene displayed significant effects on *V. philippinarum* and *C. gigas* survival. Mortality in *V. philippinarum* was higher than *C. gigas* for dibenzothiophene and phenanthrene.

Environmental risks of arsenic in irrigated agricultural areas with abnormal arsenic levels in waters from the Paracatu River (Minas Gerais), Brazil (PO)

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Supplying food security for humanity is one of the largest concerns in the twenty-first century. The use of contaminated water in irrigation can represent a source of contamination of the food chain with potentially toxic elements, such as arsenic (As). Brazil stands out in world agricultural production. The municipality of Paracatu is known for its large irrigated agricultural area and its As-rich soils. Recently, high levels of As were found in the waters of the Paracatu River. These waters are used for human consumption and for irrigation of agricultural areas. This work evaluated total and available As in agricultural soils cultivated under irrigation and in soils under native vegetation in Paracatu, Minas Gerais, Brazil. We also assessed As levels in plant shoots. Different depths of soil samples (0–5, 5–10, 10–20, and 20–40 cm) and plant samples were collected in four irrigated agricultural areas and four reference areas. In all areas, total As levels did not differ among soil depths, indicating that the source of As in agricultural soils is natural. This was evident when comparing arsenic and phosphorus (P) contents at different soil depths, as both accumulate on the surface of oxidic soils when added to agroecosystems by anthropogenic routes (e.g., phosphate fertilization for P and irrigation for As). Arsenic levels in plants were below detection limit. Our research showed that the irrigated soils are not As polluted due to the low enrichment and accumulation of As. However, periodic monitoring of As bioavailability in these areas is recommended.

Arsenic speciation in freshwater fish: A systematic review with implications for monitoring and research (PO)

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Arsenic can accumulate in fish, sometimes to levels of concern for subsistence and recreational harvesters. The toxicity of arsenic strongly depends on its chemical speciation. However, risk assessments are often based on total arsenic concentrations ([As]), with an adjustment applied assuming a small percentage of total [As] is the most harmful inorganic species. Fewer studies of arsenic speciation have been conducted on freshwater fish compared to marine, where the dominant form is commonly reported to be non-toxic arsenobetaine (AsB). To consolidate these findings, we conducted a systematic literature review on arsenic speciation in freshwater fish using Covidence® software. From the 1094 studies screened, 39 studies were selected for inclusion based on predefined criteria. These studies reported highly variable arsenic speciation patterns in freshwater fish, challenging the assumption that AsB is always dominant. Lakes and rivers with suspected or known arsenic contamination issues made up about 50% of data reviewed. Although AsB and other organic forms typically dominated, some fish had elevated concentrations of inorganic arsenic (>0.5 $mg \cdot kg^{-1}$ dry weight). Arsenic speciation results rarely accounted for all of the arsenic in fish; a considerable proportion of total [As] was not explained by the measured arsenic species. Given this variability, it appears that total [As] based risk assessment is unlikely to be accurate across diverse locations and taxa. More work is needed to characterize arsenic speciation in freshwater fish and assess the toxicity of various arsenic species to accurately assess the environmental and human health risks associated with arsenic in fish.

Evaluating the effect of wastewater treatment plant upgrades on contaminants of emerging concern (CECs) (PO)

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Contaminants of emerging concern (CECs) are known to affect aquatic organisms downstream of Municipal wastewater treatment plant effluent (MWWE) discharges. Previous studies conducted in the Grand River watershed on the small bodied, benthic rainbow darter (*Etheostoma caeruleum*), confirmed changes in gene expression, sex steroid levels, gonad size, and the occurrence of intersex (testis-ova) conditions associated with wastewater outfalls. In response to these observed biological effects, the municipal government has invested over $450 million in upgrading the two major wastewater treatment plants (WWTP) located in the Grand River watershed, specifically in Waterloo

and Kitchener. Although extensive research is performed in the Grand River to determine the biological impacts of WWTP upgrades on exposed fish and benthos, there was no comprehensive work being done on the chemistry of the effluent itself. The current study aimed to monitor the effect of process upgrades carried out at each WWTP between 2009 and 2019, on the concentration of select CECs, total estrogenicity, nutrients and hormones present in the effluent. Effluent samples were analyzed using LC-MS/MS for selected CECs and the yeast estrogen screen (YES) assay for total estrogenicity. Following the completion of the upgrades, a rapid decline in estrogenicity was observed in the effluent. The removal of specific CECs varied depending on their physiochemical properties. While the treatment process upgrades significantly reduced environmental exposure to many CECs like naproxen, some substances, such as carbamazepine, still recalcitrant at relatively high concentrations, posing ongoing risks to the environment.

Using narcotic and phototoxic target lipid models to develop water quality guidelines for polycyclic aromatic hydrocarbons (PAHs) (PO)

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Polycyclic aromatic hydrocarbons (PAHs) are a complex group of organic substances that are ubiquitous in the environment, including in surface waters, with thousands of individual non-substituted (parent) and alkylated PAHs possible. Due to their sources, PAHs do not occur as individual substances but rather as a mixture of PAHs in the environment. In addition, many PAHs demonstrate increased toxicity in the presence of ultraviolet light, a process called phototoxicity. Current Canadian Council of Ministers of the Environment (CCME) water quality guidelines are outdated, limited to eleven non-substituted PAHs, do not provide a means to consider the overall toxicity of PAHs as a mixture, and have limited consideration of their phototoxicity. Therefore, CCME has initiated a project to update or derive new water quality guidelines for PAHs for a large number of parent and alkylated PAHs using the narcotic target lipid model (NTLM) and phototoxic target lipid model (PTLM). This approach will be trialed as a method to address current challenges to PAH guideline development, including overcoming data limitations, phototoxicity, and mixtures of multiple PAHs. This presentation describes the proposed approach for deriving water quality guidelines for both low and high ultraviolet light conditions as well as how to apply them to mixtures in the environment.

nZVI in the field: Remediating mine soils contaminated with a metal(loid) mixture (PO)

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Globally, metal(loid)s released from anthropogenic processes are causing enrichment in surface soils, potentially leading to harmful concentrations. This poses negative risks to the environment and human health spotlighting the need for effective remediation technologies. Nano zerovalent iron (nZVI) is frequently used to reduce the toxicity of organic compounds in groundwater and is being investigated for the same functions in soils. The current study compares the effectiveness of 5% nZVI and 0% nZVI in remediating 16 soils with varying characteristics collected from the decommissioned Deloro Gold Mine, including contamination with arsenic (As), cobalt (Co), chromium (Cr), copper (Cu), nickel (Ni), and lead (Pb) in concentrations greater than regulatory guidelines. The objectives of this research are to determine how nZVI alters the soil including physicochemical properties and metal(loid) availability as well as assess how nZVI alters the plant's response to the soil including growth and uptake of metal(loid)s. The expected outcome that nZVI would reduce the extractable metals was true in some (As, Co) but not all cases (Cr, Cu, Ni, Pb). Growth indices (shoot length and root length) were determined for barley (*Hordeum vulgare* L.) and tomato (*Solanum lycopersicum* L.) after a growth study. The results showed an increase in root length in the 5% nZVI treatment, a decrease in soil pH and extractable potassium (K) and an increase in extractable sodium (Na). The results indicate that 5% nZVI may be able to aid in the remediation of field soils contaminated with a metal mixture.

Determining the impacts of an aquatic copper exposure to northwestern salamander larvae (PO)

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Metal contamination is a global concern, but few studies have examined the toxic effects of metals on salamanders. In this study we test the sensitivity of the Northwestern salamander (NWS), *Ambystoma gracile*, to copper (Cu). We conducted: 1) a 96-hour acute toxicity test at nominal Cu concentrations of 0*, 0.001, 0.01*, 0.1*, 1*, and 10 $mg \cdot L^{-1}$ (*indicate concentrations tested in hard (103 $mg \cdot L^{-1}$ $CaCO_3$) and soft water (10.8 $mg \cdot L^{-1}$ $CaCO_3$)); and, 2) a sub-chronic 35-day modified amphibian metamorphosis assay (OECD Test: 241) at nominal Cu concentrations of 0, 0.005, 0.01, 0.02, 0.03, 0.06 $mg \cdot L^{-1}$, and a thyroid hormone positive control. Effects on survival, growth, and development were measured in both experiments, and genes involved developmental and detoxification pathways were also examined in the acute experiment. In the acute 96-hour assay water

hardness was protective of larvae survival with an LC_{50} in soft water of 0.0330 ± SE 1.003 $mg \cdot L^{-1}$ Cu compared to an LC_{50} of 2.286 ± SE 2.121 $mg \cdot L^{-1}$ Cu in moderately hard water, and there was a significant increase in metallothionein-1 in the 0.01 $mg \cdot L^{-1}$ soft water compared to the hard water control, but not compared to other treatments. In the sub-chronic exposure, 100% mortality occurred in 0.06 $mg \cdot L^{-1}$ Cu treatment, but the 0.03 $mg \cdot L^{-1}$ Cu treatment was sub-lethal and significantly delayed development and decreased growth. Current Canadian water quality guidelines are expected to be protective of NWS in terms of both acute lethality and sub-lethal effects.

Derivation of a nickel benchmark for the protection of aquatic environments using species sensitivity distributions (PO)

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The current water quality guideline (WQG) for nickel (Ni) in Canada is hardness dependent and based on the information provided by the Canadian Council of Resource and Environment Ministers (CCREM) in 1987. For waters with a hardness of less than 60 $mg \cdot L^{-1}$ (as $CaCO_3$) and above 180 $mg \cdot L^{-1}$ (as $CaCO_3$), the WQG for Ni is 25 $\mu g \cdot L^{-1}$ and 150 $\mu g \cdot L^{-1}$, respectively. For hardness values between 60 and 180 $mg \cdot L^{-1}$ (as $CaCO_3$), the WQG is calculated following a hardness-corrected formula. This guideline does not reflect the latest available scientific knowledge regarding Ni toxicity. Furthermore, derived Ni concentrations obtained using the hardness-corrected formula may not be protective of major aquatic taxa including algae, invertebrates and fish. For example, a hardness value of 100 $mg \cdot L^{-1}$ (as $CaCO_3$) yields a Ni guideline of 96 $\mu g \cdot L^{-1}$, a concentration known to cause adverse effects to some aquatic biota groups (e.g., daphnids). Accordingly, a more defensible benchmark for Ni that considers current scientific literature is derived here using the Species Sensitive Distribution (SSD) method. Published studies on Ni toxicity for species relevant to Canadian waters were compiled and ranked according to their quality and reported toxicity endpoints. The resulting SSD analysis produced hazardous concentrations (HCs) for Ni affecting 20% of species of 14 $\mu g \cdot L^{-1}$ which is considered a conservative benchmark as its derivation doesn't include consideration of factors known to ameliorate Ni toxicity (e.g., hardness).

Tackling the Challenge of Understanding Ecological Effects of Per and Polyfluoroalkyl Substances

Potentiation of the PFOA uptake in daphnia (*Daphnia magna*) by TiO_2 nanoparticles (PL)

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Hydrophobic persistent organic pollutants (POPs) such as polycyclic aromatic hydrocarbons (PAHs) and perfluorinated organic acids (PFOAs) are near-ubiquitous in the environment. They are known to negatively affect living organisms even at very low concentrations. Recently, our lab has demonstrated that certain hydrophobic plastics can adsorb PAHs and potentiate uptake of these PAHs in the marine pacific oysters and zebrafish. We also hypothesized an exponential increase in organics uptake when adsorbed by other hydrophobic nanoparticles. We hypothesized that the similarly processes may be occurring in other species and that this potentiation would increase as particle size decreases. In this research we used ^{14}C- PFOA to explore the potential for binding of PFOA to titanium dioxide (TiO_2) nanoparticles and whether that association could potentiate the uptake of PFOA into daphnia (*Daphnia magna*). We developed a novel radiotracer-based method to track the uptake of radioactively labeled PFOA in the presence and absence of 5, 25, and 100 nm TiO_2 nanoparticles. Our study demonstrates that the presence of TiO_2 nanoparticles significantly increases the rate of uptake of PFOA and that 5 nm TiO_2 nanoparticles have higher rates of potentiation when compared to 25 and 100 nm TiO_2 nanoparticles. It is also demonstrated that presence of TiO_2 nanoparticles results in a significant increase in PFOA sub-chronic effects such as lipid peroxidization (LPO) using the standard test for thio-barbituric acid-reactive substances (TBARS). We also show that the presence of 5 nm TiO_2 nanoparticles increases lipid peroxidation PO compared to the 25 and 100 nm TiO_2 nanoparticles.

Exposure to perfluoroalkyl sulfonic acids (PFSAs) increases liver mass in northern leopard frog (*Rana pipiens*) tadpoles (PL)

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The chronic toxicity of perfluoroalkyl sulfonic acids (PFSAs) is relatively understudied despite the prevalence of these compounds in the environment. Therefore, we investigated the chronic toxicity and bioconcentration of perfluorobutane sulfonic acid (PFBS) and

perfluorohexane sulfonic acid (PFHxS) using northern leopard frog (*Rana pipiens*) tadpoles. We exposed Gosner stage (GS) 25 tadpoles to either PFBS or PFHxS at nominal concentrations of 0.1, 1, 10, 100, and 1000 $\mu g \cdot L^{-1}$ up to GS42. We measured tadpole growth and development metrics, blood glucose, PFSA concentrations and fatty acid composition in liver and whole-body tissues, and assessed leukocyte profiles. We found no effects of PFSA exposure on tadpole mass, snout-to-vent length, scaled-mass index, days-to-metamorphosis, sex, or blood glucose levels. Tadpoles exposed to 1000 $\mu g \cdot L^{-1}$ of PFBS and 1 and 1000 $\mu g \cdot L^{-1}$ of PFHxS had a significantly higher mean hepatosomatic index (HSI) relative to controls. These results suggest that exposure to PFSAs may interfere with lipid metabolism leading to enlarged livers. The measured bioconcentration factors for both compounds were <5 $L \cdot kg^{-1}$, wet weight. However, we observed preferential accumulation of both compounds in livers, which may explain the observed HSI increase. Analysis is ongoing for tadpole tissue fatty acid composition and leukocyte profiles. These analyses may provide insight into whether these compounds interfere with lipid metabolism and/or the stress response of amphibians. Currently, our results suggest that PFBS and PFHxS may influence tadpole hepatic health. Further research is required to determine the potential hepatotoxicity of PFSAs.

The impact of per and polyfluoroalkyl substances (PFAS) on oxylipin stress markers (PL)

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The oxidized lipid (oxylipin) metabolome refers to the lipid metabolites derived from polyunsaturated fatty acids (PUFAs). Oxylipins have far-reaching roles as regulators of inflammation, vascular tone, cardiac function, and development. LC-MS/MS based tools to analyze the oxylipin metabolome are gaining popularity in pharmacological based studies of drug development and mechanisms of disease. However, little is known about how the oxylipin metabolome is impacted from exposure to environmental contaminants. The purpose of this study was to determine relationships between levels of per and polyfluoroalkyl substances (PFAS), PUFAs, and oxylipins in *in situ* caged freshwater snails. An LC-MS/MS targeted metabolomic method was developed to include 4 PUFAs and 45 oxylipins derived from several PUFAs (e.g., the omega-6 PUFA arachidonic acid and the omega-3 PUFA docosahexaenoic acid), and quantified with 20 labelled oxylipin standards. Instrumental limits of detection ranged from 0.5–3 $ng \cdot g^{-1}$. To assess extraction efficiency, spike and recovery experiments with biotic tissue were carried out, yielding recovery values between 70–125%. Snails (n=25) were exposed to mixtures of PFAS by caging them downstream from a local airport, at locations where PFAS contamination has been reported. Samples were homogenized, extracted with acidified methanol and cleaned using HLB SPE prior to instrument analysis. Metabolite profiles were differentiated using a three component, partial least squares discriminant analysis model. We anticipate that total

PFAS levels will significantly correlate with total PUFAs and select downstream oxylipin metabolites. More advanced location-specific analyses on these freshwater organisms and insights into the altered biological mechanisms and toxicology are underway.

Are individual perfluoroalkyl acids associated with reproductive success of tree swallows? Beyond PFOS and PFOA (PL)

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Bioaccumulative perfluoroalkyl acids (PFAAs) have been detected in several wild bird species, yet to date, there has been limited study of the potential toxic effects of PFAAs on reproductive success of wild birds. As we have previously shown, tree swallows (*Tachycineta bicolor*) breeding near a wastewater treatment plant (WWTP) outfall in southwestern Ontario, had elevated concentrations of most measured PFAA compounds relative to those at a nearby reference site. The objectives of the present study were to 1) compare reproductive measures of tree swallows between two study sites, and 2) assess potential associations between concentrations of individual PFAAs and reproductive measures, including initial lay date, clutch size, and hatching and fledging success. In 2019–2022, reproductive data and tree swallow eggs and nestlings were collected and analyzed for 18 major PFAA compounds. There were significant differences in reproductive endpoints between sites, with tree swallows near the WWTP outfall requiring more time to lay their complete clutches and experiencing longer incubation periods, than reference birds. Overall, egg concentrations of two long-chain replacement PFAAs, perfluorodecanoic acid (PFDA) and perfluoroundecanoic acid (PFUdA), were positively correlated to clutch size and incubation period; as egg concentrations of PFDA and PFUdA increased, there were associated increases in clutch size of each pair, and longer incubation periods until eggs hatched. Moreover, PFDA concentrations in nestlings were significantly and negatively associated with their weight. These preliminary results will be presented, and as well as results from additional modelling to investigate potential influences of individual PFAAs on avian reproduction.

Sex differentiated biotransformation of 6:2 disubstituted polyfluoroalkyl phosphate in human fecal *in vitro* suspensions (PL)

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Polyfluoroalkyl phosphate esters (PAPs) have diverse commercial applications and are ubiquitous in modern society. This raises concern as PAPs have been shown to be biotransformed into fluorotelomer alcohols (FTOHs) and perfluorinated carboxylic acids (PFCAs) with known negative environmental and human health impacts. Our previous

research has shown that human intestinal microbes, found in fecal samples, are capable of biotransforming PAPs. In this study we investigated sex-based differences in intestinal microbe 6:2 diPAP biotransformation. *In vitro* testing was completed by incubating the fecal samples in an anaerobic chamber at 37°C with 6:2 diPAP (concentrations ranging from 1–5 ppm) up to 2 hours. The reactions were then terminated and the samples prepared for GC- and LC-MS analysis. Metabolites of interest include the immediate hydrolysis product of 6:2 diPAP, the 6:2 monoPAP and its subsequent hydrolysis product the 6:2 fluorotelomer alcohol (6:2 FTOH), and twelve additional metabolites shown to form from 6:2 FTOH in both oxic and anoxic conditions. Fecal samples also underwent 16S rRNA sequencing to compare the relative abundance of the various microbial phyla in males vs females. Based on preliminary data it is hypothesized that there are differences in the rate of biotransformation in male vs female gut microbiomes. This sex differentiated response shows the unequal additive impacts from the gut microbiome on host detoxification and/or activation of xenobiotics. This study is important for equitable and informed risk assessment of the continued and future use of PAPs in consumer products.

Potential impacts of climate change on the levels of perfluorooctane sulfonate in the Beaufort Sea Shelf food web and implications for human health risk (PO)

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Perfluorooctane sulfonate (PFOS) is a persistent organic pollutant (POP) of global concern due to its capacity to bioaccumulate, biomagnify, and cause adverse human health effects. Climate change can potentially alter the transport and fate of environmental contaminants. Studies across the Arctic have reported increased levels of methylmercury and POPs in marine mammals due to reduced sea ice and increased sea surface temperatures. PFOS is ubiquitous in the Arctic environment, and elevated concentrations have been reported in marine biota such as beluga and Arctic char. These can be a key source of exposure to PFOS, illustrated by higher blood PFOS levels among Inuit who consume marine country foods. This study aims to explore the potential impacts of climate change on PFOS levels in a Beaufort Sea Shelf marine food web, and subsequent potential changes in PFOS exposure for Inuvialuit via marine country food consumption. Ecological modelling software Ecopath with Ecosim will be used to predict PFOS concentrations in country foods under two projected climate scenarios. These concentrations along with dietary intake data will be used to calculate dietary exposure estimates and health risk indices. Here we present preliminary modelling work predicting potential PFOS levels under the two projected scenarios. This modelling approach has not yet been used for perfluoroalkyls; thus, this study will provide novel insight into the potential impacts of climate change on this class of compounds. The results may also provide guidance for Inuit

communities and regulating authorities to make informed public health decisions and climate change adaptation plans.

Proteomic analysis of short-chain perfluorinated alkyl substance (PFAS) exposure in fathead minnows (*Pimephales promelas*) (PO)

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Perfluoroalkyl substances are widely used anthropogenic compounds with many structures and applications. Over the years, there has been a great concern regarding their usage as exposure can result in liver toxicity, endocrine disruption, and bioaccumulation. As of recently, the presence of short-chain PFAS within commercial products and within the environment has risen. Current research focuses on legacy long-chained PFAS, with very little information available for the health and environmental risks of short-chain PFAS. Therefore, more information on short-chained PFAS toxicity is required as their prevalence increases. To determine the toxicity of short-chain PFAS in comparison to its' long-chained counterparts, fathead minnows (*Pimephales promelas*) were exposed to PFOS (5 ug·L^{-1}), PFHxS (5 ug·L^{-1}), PFBS(5 ug·L^{-1}) as well as an environmentally relevant mixture (PFOS: 110 ng·L^{-1}, PFHxS:10 ng·L^{-1}, PFBS: 20 ng·L^{-1}) for 28 days. Post-exposure mucus, blood, and brain were collected and stored at -80°C for future proteomic analysis. Muscle was also collected to determine bioaccumulation. Proteins from samples were digested with heat and formic acid, and analyzed with a LC-QTOF (Agilent 1260 LC and 6545 QTOF) using data-dependent acquisition (DDA) to create a spectral peptide library, and then MS1 filtering was performed using Skyline software and their DDA workflow. In this presentation, preliminary results from the mucus, blood, and brain proteomes will be presented.

Characterization of the replacement PFAS, perfluoroethylcyclohexane sulphonate (PFECHS) and perfluorobutane sulphamide (FBSA) *in vitro* individually and in mixture with perfluorooctane sulponate (PFOS) (PO)

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

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

The widespread application of poly and perfluoroalkyl substances (PFAS) has resulted in some substances being ubiquitous in environmental matrices with associated toxic effects. While certain substances of concern have been phased-out or banned, new substances continue to be produced. Two such substances are perfluoroethylcyclohexane sulphonate (PFECHS) and perfluorobutane sulphamide (FBSA), both of which are developed analogues and replacements of perfluorooctane sulphonic acid (PFOS).

However, there is little information on the toxic potency of PFECHS and FBSA relative to legacy substances and other known PFAS replacements. Initially, this research aimed to characterize PFECHS and elucidate its effects in the aquatic environment using *in vitro* techniques. It was determined PFECHS exposure did not result in apical adverse effects at environmentally relevant concentrations apart from decreasing plasma membrane polarity, however, molecular alterations were observed at exposure concentrations nearing those of environmental relevance. These results raised concerns about other replacement PFAS, and FSBA and PFECHS were further studied in response. While FSBA was determined to have less toxic potency than PFOS and PFECHS *in vitro* using the fish gill cell line RTgill-W1, concerns surrounded how they may interact in environmental mixtures containing multiple replacements and legacy substances. Therefore, the cytotoxic and phospholipidosis effect of PFECHS and FSBA was determined individually *in vitro* in relation to PFOS before their effect in binary and tertiary mixtures was assessed *in vitro* using the toxic unit, equipotency framework. Preliminary results have suggested that depending on the substances involved in the mixture, both super-additive and reductive effects are possible. Determining the toxic potency of emerging replacement PFAS is an important step that will help better inform the viability of replacements as a strategy for PFAS management in the future.

Derivation of Canadian environmental quality guidelines for perfluorooctanoic acid (PFOA) (PO)

Janet Cermak[1], Tamzin El-Fityani[1], William Martin[1]

[1]*Environment and Climate Change Canada*

The Canadian Council of Ministers of the Environment (CCME) is the federal, provincial and territorial interjurisdictional forum responsible for the development of Canadian environmental quality guidelines (CEQGs). Currently, CCME is deriving water, soil, and groundwater environmental quality guidelines for perfluorooctanoic acid (PFOA). PFOA is an anthropogenic substance that falls under the broader class of chemicals known as per and polyfluoroalkyl substances (PFAS) and is highly persistent and able to accumulate in organisms. The Government of Canada previously concluded that PFOA, its salts, and its precursors are toxic to the environment. As such, tools are needed to help assess and manage the risks of PFOA to environmental receptors in the ambient environment and at contaminated sites. One such set of tools is CEQGs, which are science-based benchmarks for environmental protection based on hazard. Environmental concentrations can be compared against the guideline values to inform site screening, risk assessment, and/or risk management; aid in interpreting monitoring data; or provide a scientific basis for control measures. CEQGs represent concentration thresholds below which there is low likelihood of adverse impacts from PFOA on ecological organisms or, in the case of soil, below which important ecological functions of the land will be maintained. The derivation process will be discussed and available draft guidelines will be presented.

Advancements in Biogeochemistry, Environmental Fate, Ecotoxicology, and Environmental Management of Selenium

Guidance for assessing potential impacts of selenium in freshwater aquatic ecosystems (PL)

Vince Palace[1], Stephanie Graves[2], Jessica Brandt[3]

[1]International Institute for Sustainable Development-Experimental Lakes Area, [2]Queen's University, [3]University of Connecticut

Despite decades of fate and effects studies, environmental selenium (Se) contamination and management remains an issue for many aquatic systems in North America. Several regulatory bodies have promulgated updated targets or management levels for Se, but additional guidance regarding best practices for monitoring Se to protect aquatic life is warranted. This review describes current approaches for assessing the ecological risks of Se in impaired aquatic systems and outlines recommended methods for collecting and analyzing biological and abiotic samples and interpreting data. Because reproductive impairment of fish populations is most commonly used to determine potential impacts of Se, several biological factors with the potential to affect Se toxicity are explored, including diet, trophic positions, body size and maturity, migratory movements and seasonal habitat use, and reproductive biology. Measuring Se concentrations in mature eggs is the most reliable metric for estimating potential reproductive impairment in fish populations, because the range of toxicity thresholds is relatively narrow for all but a few tolerant fish species. In situations where collecting mature eggs is not feasible, we review the use of alternative fish tissues for estimating potential effects. Factors affecting Se uptake from water are also considered with guidance provided for collecting abiotic (e.g., water and sediment) and biotic components of aquatic foodwebs (e.g., benthic macroinvertebrates, biofilm).

The implications of science communication in reporting of the risks posed by selenium in Canadian coal mine effluents* (PL)

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The future of the coal mining industry is receiving intense scrutiny in western Canada. Among the issues discussed by opponents of coal mining are environmental concerns about the significance of elevated selenium in surface water bodies near mine sites. This paper briefly reviews the current understanding of selenium ecotoxicology and risk to aquatic life, and the risk communication approach opponents and the news media have adopted with the public when addressing the selenium issue. Canadian coal mining policy has been

influenced by the public's misunderstanding of the threat of selenium in coal deposits, coal processing, and releases to the aquatic environment. We find that the claims made by opponents when reporting scientific information about selenium in western Canada have unnecessarily alarmed the public and raised fears unwarranted by the current scientific understanding of selenium. Most claims are not supported by scientific research and environmental monitoring studies. In the absence of accurate communication of selenium science and environmental risks, there is a high possibility that misinformation, intentional or otherwise, will unduly influence decisions about the future of Canadian mining policy. While several environmental concerns associated with coal mining, including selenium, need open and public debate, we advocate for the implementation of regulatory decisions informed by credible scientific evidence. In this paper, we review several key concerns raised about selenium releases from coal mining from a scientific viewpoint.

**An extended abstract/short paper was submitted and can be found after the abstracts.*

Mechanisms of waterborne selenite uptake in freshwater invertebrates and fish are not conserved: Effects of water chemistry on selenium toxicity (PL)

Chantelle Klaczek[1], Greg Goss[1], Chris Glover[1,2]

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Coal mining activity is one of the major sources of the toxicant selenium to aquatic systems. In the province of Alberta, the potential toxicological impacts of aquatic selenium are of renewed interested with the proposed development of 14 new mining permits being issued along the North Saskatchewan River. While the major route of selenium uptake to organisms is via the diet, the uptake of waterborne selenite ($HSeO_3^-$), the most prominent chemical form in waters at circumneutral pH, can be an important contributor to selenium body burden. We utilized radiolabelled selenite (Se^{75}) to characterize selenium uptake in the freshwater crustacean, *Daphnia magna*, and two trout species, *Oncorhynchus clarkii lewisi* (Westslope cutthroat trout) and *Oncorhynchus mykiss* (rainbow trout). Selenite uptake was initially measured in *D. magna* in artificial waters that independently varied in bicarbonate, chloride, sulphate, phosphate, and selenate concentrations. We identified that selenite uptake was phosphate-dependent, an outcome validated by studies with a phosphate transporter inhibitor that also reduced selenite uptake. A similar mechanism was subsequently identified for *O. clarkii lewisi*. However, selenite uptake in *O. mykiss* occurred independently of water phosphate concentration suggesting an alternate route of uptake. These findings suggest that uptake mechanisms of waterborne selenite may be species specific, and that effluent water chemistry in coal mining affected areas could potentially alter selenite toxicity to aquatic organisms.

The inverse relationship between ovary selenium concentrations and gonadosomatic index: Implications for sample collection and data interpretation (PL)

Nicole Zathey[1], Lucinda Tear[2], David DeForest[2], Katherine Raes[3], Markus Hecker[3], Marko Adzic[1], Kevin Brix[4,5]

[1]Teck Coal Limited, [2]Windward Environmental LLC, [3]University of Saskatchewan, [4]EcoTox LLC, [5]University of Miami

Selenium (Se) can pose an ecological risk to fishes through maternal transfer to eggs, which can result in developmental effects on offspring. Environmental guidelines for eggs and ripe ovaries range from 11 to 14.7 $\mu g \cdot g^{-1}$ dry weight in Canada. Egg selenium concentrations are the best predictor of developmental effects on early life stages. Practical limitations in collecting eggs from reproductively ripe fish means that ovary Se data are often used as surrogates. Ovary Se concentrations in northern pikeminnow have been monitored in Koocanusa Reservoir for over a decade with relatively high variability observed. To investigate this variability, a multivariate analysis evaluating candidate factors influencing ovary Se concentrations (sampling date, location, size, and gonadosomatic index (GSI)) was completed. GSI was an important factor with higher GSI associated with lower ovary Se concentrations. This finding contradicts the prevailing conceptual model that immature oocytes not having completed vitellogenesis will have lower Se concentrations, and has implications for regulatory monitoring. For example, spawning windows used for environmental effects monitoring (EEM) studies are often used to inform sampling activities but are not location specific and do not consider maternal Se transfer dynamics. Consequently, sampling within these large windows prior to oocyte maturation may not allow for meaningful comparisons to existing egg Se guidelines/criterion or species-specific thresholds. This analysis demonstrates the importance of sampling ovary Se within location- and species-specific spawning windows and documenting reproductive status (e.g., GSI) of sampled fish. When this is not logistically feasible, models can be used to correct ovary Se data biased by sampling immature oocytes.

State of practice: Selenium bioaccumulation models in Canadian environmental assessment (PL)

Kate Mill[1], Vince Palace[2], Liz Ashby[3]

[1]KCM Environmental, [2] International Institute for Sustainable Development-Experimental Lakes Area, [3]Environmental and Climate Change Canada

Environment and Climate Change Canada (ECCC) updated the Federal Environmental Quality Guidelines for selenium in 2022, which differ from other water quality guidelines in that they are tissue-specific, relating to fish egg/ovary, fish whole body, and bird eggs. The

Federal Environmental Quality Guidelines do not provide a water column concentration due to the site-specific complexities of predicting selenium uptake to biota from water. In order to assess the potential risks of selenium to aquatic life in projects undergoing environmental assessment, a selenium bioaccumulation model is required to relate predicted water quality concentrations to tissue concentrations. In Canadian environmental assessments, selenium bioaccumulation models developed for a different project or geographic area are often used in the absence of a site-specific selenium bioaccumulation model. Use of models that do not account for key ecological and biogeochemical factors that may change rates of selenium bioaccumulation at a specific site introduces uncertainty into the evaluation of project-related environmental effects from selenium. In order to understand best practices for selenium bioaccumulation modelling in environmental assessment, ECCC is compiling a state of practice report that will review available guidance documents and selenium bioaccumulation models developed for projects in Canada that have undergone provincial and/or federal environmental assessment in the past 10 years. ECCC will present the methods for its state of practice document and seek input on these methods.

Lessons learned in an uncertain climate: Refining a large monitoring program affected by unexpected weather (PL)

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[1]Teck Coal, [2]ADEPT Environmental Sciences Ltd.

Organoselenium species contribute disproportionately to the bioaccumulation of selenium in aquatic biota. One of the world's largest investigations into organoselenium generation is occurring in the Elk Valley in British Columbia, Canada. This presentation examines the impact of recent unexpected weather events on this monitoring program. We discuss how the atmospheric rivers that British Columbia experienced in 2021 affected our dataset by resetting accumulated aqueous organoselenium concentrations compared to typical mid-summer conditions, which decoupled selenium concentrations in biota from concurrent water chemistry. By adjusting our program, we were able to monitor potential decoupling events and has also provided the study team with insight into the behaviour of aqueous selenium species in lentic areas with variable flow. We have had to assume that 'unusual' weather events will continue to occur regularly. Things to consider when planning a monitoring program include extreme fluctuations in water temperature, precipitation patterns, and water chemistry resulting from flushing. We explore the ways in which these changes affected our aquatic monitoring program, including the need to change sampling strategies, to be flexible, and to allocate more time and resources to plan for and respond to unexpected weather events. We highlight the need for greater adaptability and flexibility in aquatic monitoring programs to ensure their continued effectiveness in the face of a changing climate.

Selenium bioaccumulation in lab-cultured and field-collected *Daphnia pulex* via dissolved and dietary exposure routes (PO)

Catherine Davila-Arenas[1], Lorne Doig[1], Xiaowen Ji[1], Banamali Panigrahi[1], Immanuela Ezugba[1], Xia Liu[1], Karsten Liber[1]

[1]*University of Saskatchewan*

Selenium (Se) is an essential micronutrient for aquatic organisms, but excessive levels can be toxic. This study assessed the bioaccumulation of Se by lab-cultured *Daphnia pulex* through both dissolved and dietary exposure routes for comparison to field-collected specimens from Lake Miwasin, a pit lake consisting of treated tailings capped with freshwater and oil sands process-affected water (OSPW). The mean dissolved Se concentration in Lake Miwasin water (LMW) was 1.74 $\mu g \cdot L^{-1}$ (2019–2021). In semi-static tests (Experiment 1) lab-cultured *D. pulex* were exposed for 12 days to water and algae grown in media spiked with selenate [Se (VI)], ranging from 1.76 ± 0.05 $\mu g \cdot L^{-1}$ to 5.41 ± 0.04 $\mu g \cdot L^{-1}$. Daphnia were also exposed to LMW and algae grown in LMW. In Experiment 2, *D. pulex* collected from Lake Miwasin were cultured in-house and exposed for 8 days to combinations of control water or LMW and algae grown in control water or LMW. Tissue concentrations from these exposures were then compared to tissue Se concentrations observed in *D. pulex* collected directly from Lake Miwasin. In Experiment 1, bioaccumulation in lab-cultured *D. pulex* increased in most exposure treatments from days 5 to 12, with the greatest Se bioaccumulation (3.08–3.47 $\mu g \cdot g^{-1}$ dry weight) observed at 2.12 and 2.59 $\mu g \cdot L^{-1}$ Se (VI) spiked waters. Tissue Se did not increase after day 8 for animals exposed to LMW. Interestingly, a lower Se bioaccumulation concentration (1.26 $\mu g \cdot g^{-1}$ dry weight) was observed in the highest Se treatment (5.41 $\mu g \cdot L^{-1}$ Se) on day 12. It is unclear why this occurred. In Experiment 2, field-collected *D. pulex* exposed under laboratory conditions had the greatest Se bioaccumulation concentrations (2.00–2.04 $\mu g \cdot g^{-1}$ dry weight) when exposed to algae grown in LMW, regardless of water source. This demonstrated that Se uptake was predominantly from diet. *Daphnia pulex* collected directly from Lake Miwasin had Se tissue concentrations ranging from 1.60 to 2.42 $\mu g \cdot g^{-1}$ dry weight, which were similar to tissue Se concentrations obtained in our laboratory exposures. The average Se concentrations in *D. pulex* for both laboratory exposures and those animals collected from Lake Miwasin all fell below the interim chronic dietary guideline (4 $\mu g \cdot g^{-1}$ Se dry weight) for invertebrates established by the British Columbia Ministry of Environment (2014). Based on this, the results of this study suggest that as food items, daphnia pose little risk to higher trophic level organisms in Lake Miwasin.

Characterization of lab-maintained periphyton from lotic systems in British Columbia using eDNA metabarcoding and selenium uptake kinetics (PL)

William Muzyka[1], Kerstin Bluhm[1], Esteban Gillio Meina[1], Milena Esser[1], Katherine Raes[1], Kevin Brix[2,3], Derek Green[4], Mariah Arnold[4], Karsten Liber[1], Som Niyogi[1], Markus Hecker[1]

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Selenium (Se) is a naturally occurring element essential to organismal health but can cause toxic effects when its concentrations rise above tolerable biological thresholds due to the release of excess Se through anthropogenic activities. The critical step of Se accumulation in aquatic food webs is its uptake via microbial and algal communities, in particular periphyton, which serve as the initial point of energy fixation, as well as the uptake point of nutrients and chemicals into food webs. Selenium is then transferred via macroinvertebrates to higher trophic level consumers such as fish that are particularly sensitive to dietary exposure of this element. To better understand the dynamics that drive Se uptake at the base of a food web in natural environments, it is essential to study how the initial uptake kinetics of Se are influenced by variations in the periphyton communities. In this context and to gain insight into the relationship between Se uptake kinetics and periphyton community composition, natural periphyton communities were collected from lotic systems in British Columbia. The samples were then maintained over a period of eight months in a temperature and light controlled environmental chamber, using closed systems with water circulation and periodic water renewal using water collected from the field locations, before transferring them into lab-prepared water that replicated the field water. Subsamples were analyzed using eDNA metabarcoding to quantify the shifts in periphyton community composition over time and were exposed to Se-spiked media to quantify Se uptake kinetics as a function of community composition.

Plastic Pollution in Terrestrial and Aquatic Environments – Fate, Impacts, and Management strategies

The effects of shape and size on microplastic atmospheric settling velocity and particle entrainment (PL)

Colette Preston[1], Cheryl McKenna Neuman[1], Julian Aherne[1]

[1]Trent University

Microplastics (MPs) have been found in all environments including remote regions, implying that atmospheric transport is an important pathway when considering the fate of MP sources. However, limited empirical data exist regarding atmospheric MP particle transport. Our research measured the atmospheric settling and horizontal drift velocities and threshold entrainment velocities of various sizes and shapes of MPs. The settling and drift velocities were measured in two specifically designed settling columns using a laser Doppler anemometer (LDA). The threshold entrainment velocities of the MPs were determined in the Trent Environmental Wind Tunnel, which is a suction-type boundary layer wind tunnel with a working length of 13.8 m long and a cross section of 70 cm wide by 77 cm high. Threshold velocities were measured under a variety of surface roughness and turbulence conditions. The turbulence conditions were created by introducing turbulence elements at the opening of the tunnel inducing wake and stagnation patterns in the airflow and were profiled with an LDA. The shear stress exerted on the surface by the airflow was measured with Irwin sensors. Surface roughness and MP entrainment were measured with a laser scanner and high-resolution photography. We found that the settling velocities of the MPs were generally lower than modelled values and that shape exerted the most significant influence on the rates of settling and particle drift. The results for settling velocity and particle entrainment thresholds provide empirical data to contribute to the development and parameterization of MP-specific atmospheric transport models.

Distribution of microplastics in a background headwater lake during the ice and ice-free period (PL)

Brittany Welsh[1], Erin Bennett[1], Andrew Paterson[2], Ken Drouillard[3], Julian Aherne[1]

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Microplastics are pervasive contaminants of concern but the influence of climatic conditions, specifically the influence of ice formation on microplastic distribution in freshwater lakes, is still relatively unknown. In this study, we examined the distribution of microplastics in a background headwater lake in Muskoka, Ontario, Canada during the ice-free and ice-covered periods to assess the role of atmospheric deposition as a pathway for

microplastic transport to freshwaters. Monthly lake samples were collected from the centre of the lake, 1 m below the surface during the ice-free period, with surface grab samples collected once. During the ice-covered period, under-ice water samples were collected from the surface and 1 m below the surface, in addition to snow and ice samples (divided into upper and lower layers) from the surface of the frozen lake. A sub-set of the snow, ice, and under-ice lake water samples were also collected for dissolved organic carbon content to assess the source contribution of atmospheric deposition. Preliminary results show that microplastic concentrations in both the lake surface and 1 m are two times greater during the ice-free period than the ice-covered period. Furthermore, microplastic concentrations appear to be slightly higher in the lake surface than at a depth of 1 m. It is expected that microplastic concentrations will be highest in the snow and ice, as they prevent microplastics in atmospheric deposition from reaching the lake, thereby acting as a temporary microplastic sink during the ice-covered period, and a microplastic source once melted during the ice-free period.

Spatial distribution and risk assessment of microplastic pollution in surface waters of the St. Lawrence Estuary (PL)

Noreen Kelly[1]

[1]*Fisheries and Oceans Canada*

This study quantified the distribution and abundance of microplastics (MPs) in surface waters of the St. Lawrence Estuary along a gradient of oceanographic properties and anthropogenic factors. Thirty-one stations were sampled along 500 km of the estuary, from Québec City to the northern Gulf of St. Lawrence in August 2020. MPs were identified and enumerated based on particle morphology, colour, and size class. Fourier transform infrared (FTIR) spectroscopy was used on a selection of particles to identify the different polymer types present. Finally, a risk assessment model, using data on concentrations and chemical hazards of polymers, was used to estimate the pollution risk from microplastics. Concentrations of MPs were highest at the head of the estuary, at 230 particles·m^{-3} and declined to 44 particles·m^{-3} towards the mouth. However, MPs were not distributed homogenously along the length of estuary, but rather showed distinct spatial patterns related to surface water currents and the volume of effluent released from the nearest wastewater outflows. Black, blue, and transparent, fibers and fragments <150 μm in size were the most common particles. Predominant polymer types included semi-synthetic celluloses, polyethylene, PET, alkyd-based paints, and polypropylene. While the overall pollution risk of MPs in the entire estuary was considered low, several stations, particularly near urban centres were at considerable, high, or very high risk. This study provides new insights into the environmental transport of MPs and first estimates of the risk of MP pollution to ecosystem and human health in the world's largest estuary.

Long-term fate and remediation of microplastics in subarctic lake sediments in the experimental lakes area (ELA), Canada (PL)

Hannah Vonberg[1], Tony Walker[1], Andrew Medeiros[1], Rebecca Rooney[2]

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

Microplastics (MPs) are increasingly found in natural environments, including freshwater ecosystems such as lakes. However, knowledge on how MPs impact these complex systems in terms of the life cycle of MPs and the subsequent recovery of lake ecosystems is limited, partially due to a lack of data from ecosystem-wide experiments. MP particles eventually settle in sediments, but where exactly they sink (deep versus shallow areas) and which subsequent interaction processes with sediments occur depends on particle size, polymer (varying densities), and transport characteristics of the ecosystem. Understanding the long-term fate of MPs in the benthic compartment via sediment interactions is crucial to inform risk assessment, management, and remediation. Employing a Before-After-Control-Impact (BACI) design, this study will dose lake #378 of the Experimental Lakes Area (ELA) bi-weekly throughout the ice-free season, resulting in an annual load of 330 billion MP particles (916 kg total) comprised of polyethylene (PE), polyethylene terephthalate (PET), and polystyrene (PS). Sampling will occur mid-season (July/August 2023) and end-of-season (October 2023), consisting of sediment cores across all depth gradients and substrates, and will be complemented by sediment trap data. The two MP-contaminated sampling batches will be analyzed using optical methods and a limited Fourier transformed infrared (FTIR) analysis before being cross-referenced with depth gradients and substrates to determine contamination 'hotspots'. Based on our MP dosing protocols and the literature on comparable systems, an overall abundance of 60 000 MP particles·kg^{-1} of dry sediment per year is expected. This study is part of the pELAstic project (https://rochmanlab.wordpress.com/the-pelastic-project/).

The influence of microplastics on leaf litter degradation in oligotrophic freshwater lakes (PL)

Rachel McNamee[1], Rebecca Rooney[1], Stephanie Graves[2], Émilie Montreuil Strub[1], Timothy Hoellein[3], Chelsea Rochman[4]

[1]University of Waterloo, [2]Queens University, [3]Loyola University, [4]University of Toronto

Microplastic pollution in lakes is widespread, but its fate and effects have not been fully elucidated. Using a whole ecosystem approach, at the International Institute for Sustainable Development-Experimental Lakes Area, we evaluated the effects of a mixture of microplastics on the rate of leaf litter decay in an experiment with a Before-After-Control-Impact design. We measured decay coefficients of local-sourced, deciduous leaf litter during two years of baseline monitoring in a reference lake and an experimental lake, using leaf litter bags. Bags were made of red polypropylene mesh (3 mm), allowing admittance to

invertebrates. The bags were filled with leaves that had been collected the previous fall and air dried. Bags were deployed annually in May and collected on a regular basis. In summer 2023, we added microplastics to the experimental lake on a bi-weekly basis, under the water surface, in equally interspersed transects throughout the lake. Baseline decay coefficients in 2021 for the reference lake: $0.0057 \cdot day^{-1}$ (d.f.=33, R^2=0.76) and at the experimental lake: $0.0046 \cdot day^{-1}$ (d.f.=34, R^2=0.66); additionally in 2022, both decay coefficients were lower in both lakes, at the reference site: $0.0034 \cdot day^{-1}$ (d.f.=21, R^2=0.79), and at the experimental lake: $0.0025 \cdot day^{-1}$ (d.f.=22, R^2=0.54). We will compare these baseline decay coefficients with rates of decay after 1 year of exposure to microplastics. These results reflect the dynamic effects of microplastics (an emerging contaminant of concern) on a whole ecosystem.

The effects of microplastics on freshwater phytoplankton and zooplankton communities in a boreal lake (PL)

Desiree Langenfeld[1,6], Kennedy Bucci[2], Rachel Cable[3], Cody Veneruzzo[4], Rachel McNamee[5], Chelsea Rochman[3], Michael Paterson[6]

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There is evidence that high microplastic (MP) concentrations can negatively impact phytoplankton and zooplankton under laboratory conditions, but community level effects under natural conditions are unknown. Two large scale *in-situ* mesocosm experiments were conducted at the International Institute for Sustainable Development-Experimental Lakes Area (IISD-ELA) in northwestern Ontario, Canada to assess the responses of phytoplankton and zooplankton communities to MP additions. In the first experiment, a mixture of common polymers (polyethylene, PE; polystyrene, PS; and polyethylene terephthalate, PET) were added in equal contributions to mesocosms (10 m diameter, 2 m deep) in a range of environmentally relevant nominal concentrations (0 to 29 240 $MP \cdot L^{-1}$ for all polymers together). In the second experiment, the same mixture of polymers was added to mesocosms at a total concentration of 29 240 $MP \cdot L^{-1}$ each with and without chemical additives and these were compared to controls with no MPs to distinguish if there was a physical or chemical mechanism for MP toxicity. Phytoplankton biomass was not affected in either experiment, but there may be some potential for MPs without additives to affect community composition over time. There was weak evidence that total zooplankton abundance and biomass were stimulated by MPs in both experiments, and copepod reproduction was slightly reduced. Overall, there was little evidence of significant negative impacts of MPs on the plankton communities.

Investigating the combined effects of microplastic fibers and temperature on *Daphnia pulex* (PL)

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Aquatic organisms almost never experience one stressor alone in nature. For example, the global emergence of microplastics as a contaminant of concern has coincided with the pervasive and ongoing warming of aquatic ecosystems. Beyond climate change induced temperature warming, organisms also experience different temperatures throughout the year due to seasonality. To evaluate the combined effects of temperature and microplastics, we exposed juvenile (<48 hours old) *Daphnia pulex* to three environmentally realistic microplastic scenarios (in regard to size, shape, polymer type, morphology, and concentrations) and three different temperatures: spring (12°C), ambient (20°C), and warm (climate change-24°C), with daily temperature fluctuations (±2°C). Microfibers (~100 µm in length) were produced from a polyester soccer jersey using a cryo-microtome and particles were weathered in pond water to facilitate biofilm growth prior to the experiment. The experiment lasted 21 days for the spring temperature and 17 days for the ambient and warm temperature treatments. We evaluated microplastics ingestion and quantified effects on survival, growth, and reproduction. Preliminary results show that the medium and high microfiber concentrations (1 and 10 microplastics per mL) significantly decreased survival in daphnia grown at ambient and warm temperatures, but this was not observed in the spring temperature treatment. Furthermore, decreases in growth were found to be proportional to increasing microplastic concentration in all temperature treatments. Our study demonstrates that even at environmentally relevant concentrations, warm temperatures exacerbated the negative effects of microplastic fibers on a widespread freshwater zooplankton.

The effects of microplastics on emerging insect and zooplankton communities in a limnocorral experiment (PL)

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Aquatic invertebrates have integral roles in the functioning of freshwater ecosystems but may experience high levels of exposure to microplastics, due to their feeding strategies and habitats. Although microplastic research has rapidly accelerated in the last few years, our understanding of the effects of these contaminants on freshwater invertebrates under environmentally relevant conditions remains limited. Using *in situ* limnocorrals at the

International Institute for Sustainable Development-Experimental Lakes Area (IISD-ELA), we investigated the effects of a microplastic mixture on zooplankton and emerging insect communities. We installed 12 open-bottom limnocorrals in the littoral zone of a boreal lake at IISD-ELA in June of 2022 and added an environmentally relevant range of microplastic concentrations (0 to 2 511 886 particles·kg^{-1} dry weight sediment) in a regression-based design. Our microplastic mixture consisted of distinctively coloured polystyrene, polyethylene, and polyethylene terephthalate fragments in equal parts, spanning an environmentally relevant size distribution. We sampled the emerging insect and zooplankton communities pre-addition and every week thereafter for the 8-week duration of the experiment. To evaluate possible longer-term effects, we sampled the emerging insect community again for 3 weeks in May of 2023 over the partially enclosed bases of the limnocorrals. We assessed abundance, diversity, community composition and biomass of insects and zooplankton on a concentration-response curve to elucidate community-level effects. Additionally, we compared the timing of peak insect emergence across concentrations to investigate potential effects on insect phenology. We anticipate our results will inform policy regarding ecological risk thresholds for microplastics in freshwater ecosystems.

Examining aquatic-terrestrial transfer of microplastics sourced from wastewater treatment facilities in the Grand River watershed, Ontario (PL)

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Microplastics are a persistent and widespread contaminant found in aquatic environments. Aquatic insects spend larval life stages in aquatic ecosystems where they may interact with microplastics, but few studies consider the impacts of microplastics on emergent insect communities and their capacity to carry microplastics from aquatic to terrestrial environments as emerged adults, and to their riparian predators, spiders. This study takes place over three sampling times, upstream and downstream of four municipal wastewater treatments plants in the Grand River watershed, to assess wastewater treatment facilities as a source of microplastics to aquatic ecosystems. To consider the aquatic compartment, microplastics were quantified in base resources of sediment and periphyton, as well in larval mayflies, caddisflies, and midges. In the terrestrial compartment, microplastics retained through metamorphosis were examined in the emerged adult form of these insects, and trophic transfer of microplastics was determined in riparian spiders. Microplastics were extracted from sediment with calcium chloride ($CaCl_2$) density separations, from periphyton using hydrogen peroxide (H_2O_2), and from insects and spiders using warmed H_2O_2. Early assessments of mid-summer data suggest no relationships in microplastics abundance in the various sample types between upstream and downstream ($p<0.05$). However, some sample types suggest differences in the amount of microplastics found at four locations studied. This research will address a key gap in our

understanding by providing novel and much-needed information on the sources, uptake, and movement of microplastics in nearshore food webs.

The proof is in the poop: Assessing microplastics bioaccumulation and toxicity in freshwater macroinvertebrates (PL)

Quinn Allamby[1], Karen Kidd[1], Ryan Prosser[2]

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Microplastics (MPs) pollution has become one of the most pressing environmental issues to date. However, freshwater macroinvertebrates are substantially underrepresented within the current MPs literature, despite their extensive use in aquatic biomonitoring. This study aimed to determine the risk of MPs to freshwater macroinvertebrates through bioaccumulation and toxicity assessments using ecologically realistic exposures. Three macroinvertebrate species, *Tubifex tubifex*, *Planorbella pilsbryi*, and larval *Hexagenia* spp., and two MPs types, polystyrene microbeads (6 and 45 µm) and polyester microfibers (100 µm) were used for this study. For each species, bioaccumulation and toxicity were assessed following exposure to each MPs type. MPs biofouling was also incorporated into the exposures to replicate the microbial attachment and biofilm formation on MPs within the environment, with pristine and biofouled MPs assessed separately. To assess toxicity, reproduction and survival were measured across all tests following MPs exposure. Additionally, the host microbiomes of *Tubifex tubifex* and *Planorbella pilsbryi* were examined to determine the potential for dysbiosis following MPs exposure. To date, no significant effects to reproduction or mortality have been observed for any of the three species, across any MPs type or condition. Microbiome analysis is still underway. Preliminary bioaccumulation analysis has determined that macroinvertebrates are capable of ingesting large amounts of MPs, but the rate of ingestion and subsequent egestion is still under investigation. This study will provide new insights regarding the potential impacts of MPs to freshwater macroinvertebrates to determine the risks of implications of MPs contamination in freshwater ecosystems.

You are what you eat: Microplastic accumulation in *Planorbella pilsbryi* (PL)

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While it is known that every level of the food web interacts with microplastics, the effects of these interactions remain under question. As Canada strives to establish a risk assessment and management framework for microplastics under the Great Lakes Water Quality Agreement, the importance of understanding the effect thresholds for microplastics is increasing. As bioindicators of ecosystem health, freshwater benthic invertebrates have

long been used to understand the toxicological effects of contaminants. They are particularly suited to the study of microplastics, often being exposed to high concentrations of microplastics that settle in freshwater sediments. We sought to determine the effects of microplastics consumption on a common freshwater benthic invertebrate species, *Planorbella pilsbryi*, the ramshorn snail. Using a range of environmentally relevant concentrations, spanning from zero to over two million particles·kg^{-1} dry sediment, we cultured microplastic-contaminated biofilm in mesocosm enclosures. We fed this biofilm to *P. pilsbryi* juveniles in a 4-week trial, measuring endpoints of survival, growth, and body burden. We found that microplastics introduced through a biofilm diet are retained in the bodies of *P. pilsbryi* juveniles beyond a 5-day depuration period. While we observed no significant effects on survival or growth of the snails, the accumulation of plastics in their bodies could lead to the trophic transfer of microplastics.

Measuring ingestion of microfibers in different tissues of a freshwater mussel (PL)

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Plastic debris polluting waterways has been a concern for decades. Recently increased attention has been placed on microplastics (MPs) in aquatic ecosystems. Small plastic particles (<5 mm) have been observed in marine and freshwater ecosystems globally. There is a need to assess environmental risk of MPs by determining the concentrations that cause adverse effects. Freshwater mussels are filter-feeding organisms that have experienced a decline due to habitat destruction and poor water quality, yet they are under-represented in MPs research. Microfibers are common MPs detected in freshwater environments due to textile shedding and discharge from wastewater treatment plants. In this study, 7-day exposures were conducted with polyester microfibers generated from a red fleece blanket at 150 000 MP·mL^{-1}. Adult *Megalonaias nervosa* (washboard) mussels were exposed through spiked algae food stocks, and tissue dissections occurred at days 1, 3, 7, and 2 days after exposure ceased. Digestive gland, gonad, foot, and hemolymph samples were collected at each time point to determine whether microfibers tend to concentrate in a particular tissue of the mussel anatomy. Since MPs are prone to biofouling when released in the environment, a test with both aged and pristine microfibers was conducted to assess whether biofilm coatings influence initial ingestion and tissue translocation once ingested. This study serves as a preliminary assessment of the fate of microfibers, an MP commonly detected in Canadian surface waters. Furthermore, this study investigates the potential of microfibers to accumulate in an organism that is a critical component of freshwater ecosystems.

Transcriptomic analysis of developing wood frog larvae following continuous exposure to a microplastic mixture (PL)

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To date, little is known about the effect of exposure to microplastics on amphibian immune function. To address this gap, North American wood frog (*Rana sylvatica*) embryos were exposed throughout development to each of 0, 50 000, or 500 000 particles·L^{-1} of a microplastics mixture consisting of equal parts polypropylene, polystyrene, and polyethylene terephthalate microplastics (40–1400 μm shortest dimension) in quadruplicate aerated outdoor mesocosms containing 200 L of filtered lake water at Queen's University Biological Station. RNA was isolated from dorsal skin tissues collected from one individual from each mesocosm after six weeks (Gosner stage 31–35) and eight weeks (Gosner stage 40–45) of exposure and as individuals reached the end of metamorphosis (Gosner stage 45). NEBNext Ultra II Directional polyA mRNA library preparation and 150 base paired-end sequencing on a NovaSeq 6000 were performed by The Centre for Applied Genomics (Toronto, Ontario), yielding over three billion read pairs. Performing genome-guided transcriptome assembly using StringTie, 94 741 transcripts were discovered, belonging to 44 661 genes. Differential expression analysis with DESeq2 revealed between 63 and 898 differentially expressed genes depending on the treatment and timepoint. The most differentially expressed genes were found after six weeks of exposure to 50 000 particles·L^{-1} of microplastics and the least found in animals at Gosner stage 45 exposed to 500 000 particles·L^{-1}. These data suggest that exposure to microplastics elicits a transcriptional response in the skin of developing wood frog tadpoles. Ongoing analysis will aid in identifying key physiological pathways affected by exposure to microplastics. Funded by ECCC-IKPP and NSERC DG grants.

The effects of a microplastic mixture on the growth and development of wood frogs (*Rana sylvatica*) in an outdoor mesocosm experiment (PL)

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Amphibians are one of the most threatened classes of vertebrates on the planet, yet our understanding of the effects of microplastic (<5 mm) pollution on their development is limited. We investigated how microplastic exposure affects hatching success, growth, survival, and development of wood frogs (*Rana sylvatica*) in a mesocosm experiment conducted at the Queen's University Biological Station. Wood frogs were exposed to an additive-containing microplastic mixture at nominal concentrations of 0 (negative control), 50 000, or 500 000 particles·L^{-1} (n = 4 per treatment). The microplastic mixture consisted

of equal parts polypropylene, polystyrene, and polyethylene terephthalate. We used photographic sampling to assess the size, developmental stage (Gosner stage), and body condition of the wood frogs over 96 days. Lethal subsampling occurred weekly during the larval phase and at Gosner stages 44 and 45 when wood frogs became terrestrial. In microplastic treatment groups, microplastics were detected in the tissues of tadpoles and metamorphs, at concentrations of up to 2600 particles per individual, after a 20% potassium hydroxide tissue digestion. Hatching success, time to hatch, and survival did not differ among treatments. However, we found evidence to suggest that microplastics cause increased growth (body length over time) in both treatment groups and developmental delays (time to metamorphic climax) at the highest concentration in comparison to the control. Our study provides new insights into the effects of microplastic pollution on amphibians throughout their development, from embryo to metamorph, thereby improving our understanding of possible effects on their populations.

Microplastics in a chronosequence of biosolid-amended agricultural soils in Southern Ontario (PL)

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Microplastics, small plastic particles less than 5 mm in size, are emerging environmental contaminants of concern due to their ubiquity in the environment. The contamination of agricultural soil with microplastics has raised public concern due to its proximity to food systems and potential impact on human health. Municipally sourced biosolids from wastewater treatment plants are commonly used as cost-effective fertilizers, diverting material from landfills, and contributing to the circular economy. However, biosolids contain high concentrations of microplastics and are a source of microplastic contamination to the environment. Despite this, there is a lack of environmentally relevant field studies. Our research investigates whether microplastics are accumulating in fine textured agricultural soil amended with biosolids, and how this microplastic soil pool changes over time. In 2022, soil samples were collected from seven agricultural fields in Southern Ontario, Canada, representing a chronological sequence of zero to nine years since last biosolid application. Soils were analyzed for microplastic content, particle size distribution, organic matter, and bulk density. Further, samples of biosolids applied in 2022 were analyzed to estimate microplastic loading to the fields. Microplastic particles were extracted from samples by density separation, characterized visually by stereomicroscope, and polymers identified using Fourier transform infrared spectroscopy. Here we report on the characteristics, abundance, and polymer type of microplastic particles in the study area to assess their transport and fate in biosolid-amended soils. Quantifying their fate in an agricultural context will help advance our understanding of the extent of microplastic contamination in agricultural soil.

Your #2 is my #1: Assessing the microplastic content of biosolids and agricultural fields (PL)

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Microplastics (<5 mm) are an emerging ecological concern. Primary microplastics are intentionally created for commercial use (e.g., microbeads for cosmetic products) while secondary microplastics are created via the degradation of larger plastic materials. Due to their persistence in the environment, the unknown effects of microplastics are a potential threat to the health of various ecosystems. To date, the overwhelming majority of microplastic research has focused on their impacts on aquatic ecosystems, creating a need for research to be performed on their effects on terrestrial ecosystems. One potential significant source of microplastics to agricultural soils are biosolids. Biosolids are nutrient-rich field amendments that are derived from the processing of wastewater. Biosolid samples were collected from fourteen wastewater treatment facilities. Additionally, soil samples were collected from thirteen biosolid-amended agricultural fields. Soil and biosolid samples were digested with Fenton's reagent and density separated with sodium bromide (NaBr) to isolate microplastic content. Microplastics were counted and categorized based on size, colour, and shape. Biosolids were found to contain 3.95×10^5 ($\pm 3.19 \times 10^4$) microplastics·kg^{-1} (dry weight), while biosolid-amended fields contain 1645 ($\pm$ 98) microplastics·kg^{-1} (dry weight). On average, 32.8% of microplastics found within biosolids are identified as microfibres. Furthermore, microfibres comprise 22.8% of microplastics found in biosolid-amended fields. Microplastic characterization of biosolid samples identified polyester and acrylic as the two most abundant types of microfibre. In addition, high-density polyethylene (HDPE), low-density polyethylene (LDPE), and polypropylene were identified as the most abundant microparticles. Quantifying and characterizing microplastic content of biosolids and biosolid-amended fields is crucial for developing an accurate risk assessment of microplastics in terrestrial ecosystems.

Unveiling nanoplastic biouptake in the environment: Advancing environmental analysis through label-free localization and identification (PL)

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With ever-growing concerns over plastic pollution, there is a need to understand the biological uptake and subsequent impacts of plastics in aquatic and terrestrial organisms. However, there is a lack of effective methodologies to detect and identify micrometer and nanometer sized polymer particles in whole organisms. This study introduces a label-free approach for the localization and imaging of plastic particles within organisms utilizing

optical photothermal infrared microscopy (O-PTIR) and cryotome techniques. The new methodology enables high-resolution visualization of plastic particles as small as 700 nm, without the need for plastic labelling. Through the integration of O-PTIR imaging with cryotome sample preparation, exceptional spatial resolution and sensitivity are achieved, enabling the detection and identification of nano-sized plastic fragments. The results demonstrate successful visualization of micro and nanoplastics at low exposure concentrations (0.1 ppm) in various organisms. The versatility of the technique is demonstrated using different aquatic and terrestrial organisms (e.g., *Daphnia magna*, *Drosophila melanogaster*, and *Eisenia andrei*). The new methodology enables the comprehensive analysis of environmental samples while eliminating the requirement for plastic labelling that limits our ability to study environmental samples. The combination of high-resolution imaging with vibrational microspectroscopy provides a powerful tool for understanding the distribution and potential impacts of microplastics and nanoplastics in natural systems.

Sorption of three pesticides to weathered and pristine LDPE and PLA microplastics (PO)

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More microplastics (MPs) are estimated to be added to European and North American farmlands annually than the current accumulated total of MPs present in ocean surface water globally. MPs can enter the environment through biosolid amendments, litter, plastic mulching, irrigation, flooding, tire wear, and atmospheric deposition. MPs present in soil can sorb organic contaminants, affecting their mobility and behaviour. Degradation of plastics by photo-oxidation lead to changes to the formation of oxygen-containing functional groups on the surface, increased surface area, and fragmentation into smaller particles. The changes in surface chemistry and physical properties of microplastics can affect the sorption of organic contaminants. Weathered microplastics could thus sorb hydrophilic contaminants more readily than pristine particles. Biodegradable plastics have greater degradation than conventional plastics, which could lead to a greater change in the sorption behaviour due to weathering. This research uses accelerated laboratory weathering by UV radiation on low-density polyethylene (LDPE) and polylactic acid (PLA) microplastics. The weathering is characterized with Fourier transform infrared (FTIR) spectroscopy for surface chemistry changes. Changes in surface area, roughness, and crystallinity are characterized with scanning electron microscopy (SEM), differential scanning calorimetry (DSC). The sorption of pristine and weathered LDPE and PLA microplastics is determined for three pesticides – glyphosate, atrazine, and 2,4-D – using sorption isotherms and kinetics experiments. Sorption is dependent on both the specific polymer and contaminants, and much is unclear about the effects weathering will have on

conventional and biodegradable plastics. Further research will examine the environmental factors affecting sorption, such as pH, ionic strength, and organic matter.

The role of terrestrial isopods in macroplastic breakdown: Microplastic formation and potential impacts on smaller organisms (PO)

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With concern growing regarding the potential impact of microplastics in terrestrial ecosystems, it is vital to assess the role invertebrates may play in the fate of microplastics within these ecosystems. Microplastics commonly enter these environments through improperly discarded waste or the application of biosolids on agricultural soils. Invertebrates such as isopods play an important role in terrestrial ecosystems by breaking down waste and unlocking nutrients for plants and other organisms. This study investigated whether three species of isopods (*Porcellio scaber*, *Porcellio laevis*, and *Porcellionides pruinosus*) would attempt to feed on plastic and generate microplastics. Treatment vessels were designed to expose mature isopods to plastic fragments weathered in soil. The size and number of microplastics generated by isopods was monitored over a 2-week duration. Isopods were sampled after 1, 3, 7, and 14 days of exposure. Isopods exposed to the weathered plastics generated microplastics with no adverse health effects or mortality observed. In the 2-week exposure experiment alone, *P. scaber* and *P. pruinosus* generated >1000 microplastics and *P. laevis* generated slightly less than 1000. This indicates that isopods may be capable of producing large amounts of microplastics in environments containing larger plastic fragments with little impact on their health.

Microplastic ingestion by aquatic-emergent insects and an aerial insectivore; observational and experimental field studies (PO)

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Microplastics (MPs), plastic particles between 0.1 um and 5 mm in diameter, are found in all environments, though the scope in which terrestrial and aquatic biota are ingesting it remains to be explored in detail. With little insight into MPs in freshwater and terrestrial biota, this research aims to explore MP fate at two different levels of the food web: first within an abundant prey group, aquatic-emergent insects, and secondly through tree swallows (*Tachycineta bicolor*), an aerial insectivore and top predator. Both larval and adult emergent insects, primarily belonging to the family Chironomidae, were collected from mesocosm and whole-lake experiments at the International Institute for Sustainable Development-Experimental Lakes Area as a part of a large-scale study assessing the fate and effects of polyethylene, polystyrene, and polyethylene terephthalate. Twelve littoral

mesocosms, with one control and 11 treatments, were dosed to achieve a range of concentrations from 251 to 2 511 886 MP particles·kg^{-1} dry weight sediment, and the whole-lake experiment had biweekly additions to achieve a nominal concentration of 1000 MP·L^{-1} over a six-month period. Insects were collected using submerged substrate and emergence traps and subsequently digested and analyzed for the presence of experimental MPs. Tree swallows can rely heavily on aquatic-emergent insects and are thus often used as bioindicators of their habitats. Fecal sacs from tree swallow chicks were collected in both terrestrial, agricultural areas as well as from freshwater impoundments and coastal wetlands. Samples will be analyzed for MPs to assess exposure levels across habitat types.

The impacts of microplastics on physiology, behaviour, and survival of a freshwater gastropod, *Planorbella pilsbryi* (PO)

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Microplastics are a pervasive environmental contaminant that can pose a serious threat to the health of aquatic ecosystems. The impacts of microplastics on freshwater organisms, such as gastropods, remain widely unknown. This study will be investigating the effects of microplastic exposure on a species of freshwater gastropod, *Planorbella pilsbryi*. Freshwater gastropods play an essential role in ecosystem function and are frequently used as bioindicators of ecosystem health. In this study, *Planorbella pilsbryi* were exposed to both aged and pristine microplastic fibers at three concentrations for a period of 14 days. Ageing of fibers is done to establish biofilm attachment, representing microplastics found in the environment. Two snail sizes (large and small) and two fiber lengths (short and long) were used to assess how microplastic size and snail size influence ingestion and toxicity. Ingestion, reproduction, movement behaviour, and mortality were assessed as endpoints. Behaviour was evaluated by measuring reaction time. In both snail size groups, no significant differences were found in reproduction, reaction time, mortality, or ingestion across treatments. A significant difference in reaction time was found between fiber lengths in large snails; snails that ingested long fibers had longer reaction times compared to those that ingested short fibers. Low ingestion counts and evidence of microplastics in feces indicate efficient egestion of fibers in *Planorbella pilsbryi*. These findings have shown that *Planorbella pilsbryi* do not experience increased mortality or decreased reproductive success from ingestion of these types of microplastics in the environment, but may experience alterations in behaviour.

Impact of microplastics to invertebrate survival and reproduction in a field soil: Direct amendment and biosolid application (PO)

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Microplastics (MP) in the environment have received a lot of attention in recent years, from sensational images in the media to governmental bans. It is estimated that a substantial amount of micro and nanoplastics will end up in the terrestrial environment through: aerial deposition, irrigation, tire wear, and agricultural practices, including biosolid application and the use of agro-plastics such as plastic mulch. However, the impact of plastics in soil environments is only just beginning to be examined. In this study, an experimental field site was used to look at the effects microplastics on the survival and reproduction of earthworms (*Eisenia andrei*) and springtails (*Folsomia candida*). The field site was treated with 13 tonnes per hectare biosolids, in the same manner as is common in agriculture across Canada. A wide range of plastic types and forms were identified in the applied biosolids. Two different exposures were studied (1) pre-biosolid application soil spiked with 0, 0.1, 0.25, 0.5, 1, and, 2% 20 µm fluorescent polyethylene (PE) MP beads (springtails only) and, (2) soils before, 1 month post- and 1 year post-biosolid application (earthworms and springtails). In all cases, no significant difference in invertebrate survival or reproduction was observed. Despite no effect in reproduction, springtail and earthworm tissues did show evidence of plastic uptake. The uptake of plastics into these organisms may lead to other physical or physiological effects, which will be looked at in upcoming studies.

Testing the effects of a blocking primer on 16S rRNA gene amplicon sequencing of North American wood frog skin swabs (PO)

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The skin-associated bacterial community is important to amphibian health and defense against pathogens, however, capturing an accurate community composition can pose challenges. To determine whether exposure to microplastics can alter the composition of the bacterial skin microbiome of amphibians, North American wood frog (*Rana sylvatica*) tadpoles were raised in aerated outdoor mesocosms containing 200 L of filtered lake water at the Queen's University Biological Station with continuous exposure to either 0, 50 000, or 500 000 particles·L^{-1} of a mixture of equal parts polypropylene, polystyrene, and polyethylene terephthalate microplastics (40–1400 µm). DNA was extracted from skin

swabs collected from Gosner stage 36–41 tadpoles and the microbial 16S rRNA gene V4 region was PCR amplified using the 515F (Parada)-806R (Apprill) primer set. However, amplification was inhibited due to high levels of host mitochondrial DNA, which has a high degree of similarity with the forward primer, in swab extractions. To overcome this, a blocking primer was designed to anneal to the wood frog 12S rRNA gene, blocking binding of the 515F primer to host DNA. However, sequencing with the blocking primer versus without resulted in a trend of reduced read count and the non-detection of 144 bacterial genera. The Shannon diversity α-diversity metric was not altered by sequencing with the blocking primer; however, the relative frequencies of taxa were altered. Although these biases disqualify this blocking primer from further use, this problem highlights that it is essential to validate even widely used assays in new study systems. Funded by ECCC-IKPP and NSERC DG grants.

Multi-level responses of benthic macroinvertebrates to experimental microplastic pollution in a boreal lake (PO)

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The effects of plastic pollution on freshwater benthic macroinvertebrates are not well understood. In the present study, we investigated the effects of microplastics on benthic macroinvertebrates under an environmentally relevant gradient of microplastic concentrations. Twelve open-bottom limnocorrals were deployed in the littoral zone of a boreal lake at the International Institute for Sustainable Development-Experimental Lakes Area in Ontario, Canada. In June 2022, a mixture of polymers (polystyrene, polyethylene terephthalate, and polyethylene) was added to the limnocorrals to achieve target sediment concentrations of 251 to 2 511 886 microplastic particles per kg of dry weight sediment. Naturally pre-colonized leaf litterbags were added to the limnocorrals and sampled after eight weeks of microplastic exposure to characterize the benthic macroinvertebrate community. We quantified the relationship between sediment microplastic concentrations and benthic macroinvertebrate responses at the organismal (head size of *Hyalella azteca*), population (sex ratio of *Hyalella azteca* and relative abundance of nine taxa), and community (total abundance, diversity, structure) levels. We found evidence of moderate effects of microplastic on the sex ratio of *Hyalella azteca*, and weak effects on total richness and community structure of benthic macroinvertebrates. Moreover, we found no evidence of effects of microplastics on size of *Hyalella azteca* nor on total abundance or diversity of benthic macroinvertebrates. The results of this study suggest that under ecologically relevant conditions, exposure to microplastics may only have minor effects on benthic macroinvertebrates, at least in the short term.

Fate and effect of microplastics on freshwater zooplankton in a whole-lake addition experiment (PO)

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Plastic is a widespread pollutant in the environment. Of particular concern are microplastics, particles 5 millimeters or less in size, which often originate from the breakdown of larger plastic items. Despite the ubiquity of microplastics in the environment, their fate and effects on aquatic ecosystems are largely unknown. At the International Institute for Sustainable Development-Experimental Lakes Area (IISD-ELA), we are adding microplastics to an entire lake to simulate environmentally relevant microplastic additions from stormwater. Zooplankton, organisms central to freshwater food webs, are being collected and analyzed for ingestion of microplastics at multiple timepoints to better understand the fate of microplastics in the food web. As well, zooplankton are being monitored for changes in community structure (abundance, biomass, and species composition) resulting from the microplastic additions. Following a before-after-control-impact (BACI) design, the data collected post-microplastic addition are being compared to baseline data from the same lake, as well as to a nearby unmanipulated lake. The first of its kind, this project will provide further understanding of the environmental impacts of microplastics at the scale of an entire ecosystem, and will inform industry and government level policies to mitigate plastic pollution.

Living Laboratories – Advancing Sustainable Production Practices in Canadian Agriculture

The Canadian agroecosystem Living Laboratories Network (PL)

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Agriculture and Agri-Food Canada's (AAFC) Living Laboratories Initiative used a new approach to agricultural innovation in Canada, bringing together farmers, scientists, and other collaborators to co-develop, test and monitor innovative practices and technologies to address agri-environmental issues. The goal is to accelerate the development and adoption of sustainable practices and technologies by Canadian farmers. Forming a nation-wide network of agroecosystem living labs (ALL), the initiative focused on innovative solutions to environmental issues related to agriculture, such as climate change, soil health, water quality, and biodiversity. The concept of agroecosystem living labs is relatively new in the innovation and research world; Canada is advancing the concept with various international collaborators. Core principles of living labs include user-centred innovation, working in partnership, and testing in the user's real-life context. The Living Lab-Ontario (LLON) project is one of 13 ALL across Canada. Through regional consultations, the Lake Erie Basin was chosen as the project geography. Agricultural partners wanted to study innovative practices to achieve continuous soil cover and reduced tillage. Conservation authorities and researchers studied if these practices reduced soil and nutrient runoff from agricultural land into Lake Erie, improved soil health, and increased biodiversity on agricultural land. This presentation will highlight what agroecosystem living labs are, using the LLON project as an example, and how they are being implemented across Canada, including through AAFC's new 10-year Agricultural Climate Solutions program.

Using aquatic ecological endpoints to assess agricultural practices in the Lake Erie Basin (PL)

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The Living Laboratories Initiative provided a forum to bring together farmers, scientists, and other partners to co-develop, test, and monitor agricultural best management practices (BMPs) and new technologies in a real-world context. The Lake Erie Basin was identified as a priority geography for Ontario, and common BMPs of interest

identified by agricultural partners were continuous soil cover and reduced tillage. One aspect of assessing the impact of BMPs at the watershed level was using aquatic ecological endpoints to evaluate water quality and the overall health of aquatic ecosystems. A paired study design was implemented to compare watersheds with a high adoption of BMPs to those with low adoption of BMPs in geographically similar regions. Two pairs of watersheds were selected based on geographical location and the ability to leverage established watershed monitoring sites/projects and historical data: Upper Medway Creek (high BMP) and East Medway Creek (low BMP) in the uplands, and Wigle Creek (high BMP) and Big Creek (low BMP) in the lowlands. Multiple lines of evidence with both field- and laboratory-based approaches were used to assess aquatic ecosystem health, including analysis of resident macroinvertebrate communities, exposure of aquatic invertebrates to stream water *in situ* and in lab tests, and evaluation of fish communities and fish habitat. In addition, an intensive sampling effort was conducted at Wigle Creek to evaluate the movement of pesticides from soil to creek during precipitation events. An overview of our research approach, lessons learned, and future opportunities will be presented.

Assessing the fish community in tributaries of Lake Erie adjacent to agricultural activity (PL)

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Significant research has been done on the topic of how agriculture is affecting fish habitat usage, yet previously performed research has not addressed the concern from an ecosystem level. In this study we utilize a fish community assessment approach to compare the biodiversity of aquatic biota in streams adjacent to fields where best management practices (BMPs) are being incorporated to that of conventional tillage. The Lake Erie water basin (Ontario, Canada) contains an abnormally high amount of agricultural land which has led to major changes in fish community structure. To evaluate how agricultural practices may affect fish habitat usage in the Lake Erie water basin from an ecosystem-level perspective, electrofishing data and data for 15 habitat variables were collected on three sampling dates at four experimental sites. The Upper Medway and Wigle Creek sites were adjacent to farms using several BMPs, while the Big Creek and East Medway sites were adjacent to farms using few BMPs. Species distribution models confirm that differences in habitat usage are occurring. Correlation-based analyses identified 28 statistically significant species-habitat-variable relationships and significant interspecific relationships. A biomass per volume analysis revealed that the capacity of the four sites was not statistically different suggesting that as the biomass of a particular species decreases due to agriculture changing habitat conditions, that of another species may increase. The results of the study suggest that differences in lotic habitat adjacent to agricultural practices results in the reorganization of fish communities and trophic structures.

Living labs in Southern Ontario: Stream invertebrate health and watershed agricultural best practices (PL)

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As part of an integrated multiple-lines-of-evidence approach to assessing impacts of agricultural practices on receiving waters health, two pairs of streams with contrasting levels of best management practices (BMPs) in their watersheds were evaluated for effects of continuous soil cover and/or reduced tillage (high BMP) compared to low BMP. Stream health in 2 years was characterized by (a) benthic invertebrate community composition and density, (b) stream water toxicity to four invertebrate species in laboratory tests, and (c) stream water toxicity to two invertebrate species exposed to *in situ* conditions. Endpoints of the analyses include taxonomic descriptors, as well as lethal and sublethal toxicity. Analyses will focus on joint responses of invertebrates to stream conditions and address lab versus field endpoints, coherence of responses, benthic community versus toxicity, and the influence of pesticide concentrations. Limits of the assessment, including treatment replication, will be discussed.

Edge-of-field water quality monitoring for Living Lab-Ontario (PL)

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The Living Lab-Ontario water quality team, led by water management engineers from Agriculture and Agri-Food Canada (AAFC), established three edge-of-field stations to monitor surface runoff and tile discharge on a farm in Essex County. The monitoring stations are located on three different fields, each in a separate crop of a corn-soybean-winter wheat crop rotation. The farmer uses a conservation management system which utilizes no-till and strip tillage to minimize soil disturbance, subsurface placed phosphorus fertilizer and cover crops, or a fourth crop buckwheat, after winter wheat. As a part of Living Lab-Ontario, there were a number of other researchers working at this site, collecting data looking at various parameters. A good example of the collaboration fostered at this site was the sharing of one of the monitoring stations with researchers from Environment and Climate Change Canada (ECCC), who were interested in collecting and analyzing water samples for pesticides. Together AAFC and ECCC collected samples generated by precipitation events from the fields (surface runoff and tile discharge) and from the small tributary into which the three fields drain. Samples were analyzed for nutrients (nitrogen, phosphorus, and carbon) and pesticide levels. The results will contribute to increasing knowledge on soil health measures as well as the impact of this farming system on water quality and aquatic organisms.

Pesticide occurrence and dynamics in surface waters of two distinct agricultural and ecological regions of Canada (PL)

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Pesticide contamination in water bodies is an ever-growing concern due to the increasing global pressures on food production. Climate change is shifting pest populations, resulting in heavier reliance on pesticides and an increasing need to better understand the environmental risks of pesticide use. We collaborated with two living labs in Canada to investigate pesticide levels in surface waters. Our research focused partly within the traditional territory of Swan Lake First Nation, who collaborated with Manitoba's Living Lab, and Prince Edward Island (PEI) Living Lab. Throughout the growing seasons of 2021 and 2022, bi-weekly to monthly water sampling was conducted at over 20 combined sites and analyzed for a suite of >100 pesticides. The Prairie and Atlantic locations represent distinct Canadian agricultural and ecological regions. In the Prairie sites, neonicotinoid transport was driven by snowmelt and rain events close to applications, whereas in the Atlantic region, detections were more consistent throughout the growing season, with higher concentrations closer to applications. Overall concentrations of neonicotinoids in surface waters were higher in Manitoba than PEI, except for clothianidin which was consistently higher in PEI. Frequent detections of the diamide chlorantraniliprole were observed in PEI, with concentrations up to 2548 $ng \cdot L^{-1}$. In contrast, few detections of chlorantraniliprole were observed in Manitoba, reflecting differences in crops, pests, and use. Our data demonstrates the variability of pesticide contamination in surface waters across the different climatic and agronomic regions of Canada, which pose challenges for water quality management and national approaches to mitigate risks to surface waters.

Soil health: Adapting soil ecotoxicity measures to soil health measures (PO)

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The Living Laboratories Initiative is an integrated approach to agriculture innovation through collaboration with farmers, scientists, and other partners to co-develop, test, and monitor new practices and technologies. Within Ontario, soil quality played an integral role in assessing sustainable practices, including determining methods of measuring and monitoring soil health. In our research, we used standard soil test species, normally used in the context of ecotoxicological testing, as biological indicators of soil health. The test species included earthworms (*Eisenia andrei*), springtails (*Folsomia candida*), and mites (*Oppia nitens*), all of which play key roles in soil nutrient cycling and organic matter decomposition. In using these species as biological indicators, soils were collected from five

different active farms from within the Lake Erie Basin, based on management regime (e.g., cover crop or none, organic and conventional practices), and over time. Soils were sieved, air-dried and remoistened and the test species incubated within, and endpoints of survival and reproduction assessed; in some cases, microbial activity was measured via bait-lamina tests. Species performance varied by site, given the variety of soil types, but did not vary by management practice, although performance varied across time. In some cases, microbial activity was affected by management regime. Adaptability of using soil ecotoxicological tests for monitoring soil health was feasible, and the results of the study will be presented and discussed accordingly.

From waste land to retention ecosystem: A success story of the Lake St. Pierre Living Lab, Québec (PO)

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The focus of agroecosystem living laboratories is to integrate three general components: (1) multidisciplinary approach, (2) co-development with stakeholders and participants, and (3) monitoring activities and on-site research. The project discussed here, which was the idea of the producer, exemplifies this new research paradigm. A hydro-agricultural ecosystem of three basins interconnected by weirs was constructed on waste land with the goal of protecting soil and water, as well as fostering biodiversity. Research activities were then carried out on the site, which quickly became a technological showcase promoting the circulation of knowledge between farmers. The objectives of this communication are (1) to present the process of ideation and co-development of this project, which became part of the Lake St. Pierre Living Laboratories Initiative in Québec and (2) to highlight the scientific and producer knowledge that guided the achievement of this beneficial management practice (BMP). The last objective aimed to better understand the transport of dissolved nutrients and contaminants, and to assess the toxicity of waters and sediments retained in the system, using a multidisciplinary approach. Preliminary results (2021–2022) suggest that this constructed ecosystem is effective in reducing nutrient and suspended sediment levels reaching the adjacent watercourse. Under certain hydrological conditions, it could also dilute pesticide concentration peaks, thereby improving water quality downstream. In the context of climate change, where the quantity and quality of water will become critical issues for large parts of agricultural land, retention ecosystems could be part of the solutions put forward to ensure the resilience of agroecosystems.

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

Canadian water quality guidelines for metals (PL)

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Environmental Quality Guidelines (EQGs) have a long history in Canada. The National Guidelines and Standards Office (NGSO) of Environment and Climate Change Canada (ECCC) contributes to both CCME (Canadian Council of Ministers of the Environment) and Federal EQGs. Water quality guidelines (WQGs) for the protection of aquatic life have been developed for many metals (e.g., aluminum, copper, chromium, iron, lead, manganese, and rare earth elements), using CCME methods. Because the aquatic toxicity of metals is strongly dependent on physical and chemical characteristics of the exposure water, a careful consideration is given to bioavailability and toxicity of specific metals to aquatic organisms under site-specific conditions. The species sensitivity distribution (SSD), multiple linear regression (MLR), and biotic ligand model (BLM) are among the approaches that we have used for guideline development and their applications to specific metals will be discussed. The WQGs for aquatic life provide benchmarks for the quality of the ambient environment. Where the WQG is met there is low likelihood of adverse effects to aquatic life. WQGs are regularly used to aid in environmental protection by providing targets for acceptable environmental quality, in evaluating the significance of concentrations of chemical substances currently found in the environment, and as performance measures of the effectiveness of risk management activities.

Evaluation of total aluminum guidelines using chronic toxicity tests with aluminum-spiked mine water (PL)

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The Canadian Federal Environmental Quality Guideline for total (T) aluminum (Al) was approved in 2022. British Columbia (BC) adopted this aquatic life guideline in 2023 with the application of a 3× assessment factor, replacing the former BC guideline for dissolved Al. Elevated T-Al concentrations occur in water bodies near many mine sites and in effluents. Oftentimes, elevated T-Al concentrations measured in these waters exceed guidelines but are driven by Al that is biologically unavailable. Therefore, T-Al guidelines may be overly-conservative for use as the basis of environmental assessments or water management decisions where compliance targets are based on guidelines. Using water collected downstream of a mine waste storage facility in BC, Al-spiked chronic toxicity tests

were conducted using *Ceriodaphnia dubia*, *Chironomus dilutus*, and *Pseudokirchneriella subcapitata*. The site has a history of ambient T-Al concentrations that exceed the BC guideline. Corresponding dissolved Al concentrations fall below the former BC guideline for dissolved Al. Toxicity test endpoints in Al-spiked site water were generally similar to or more sensitive than endpoints in other laboratory studies. However, historic toxicity tests conducted by the mine in site water containing T-Al at similar concentrations showed no effects. These results are consistent with other studies that suggest spiked laboratory toxicity tests using site water may yield endpoints that are overly sensitive for use as the basis of T-Al Site Performance Objectives or other benchmarks where ambient total Al concentrations exceed guidelines.

Development of water quality guidelines for the protection of aquatic life for nickel using the biotic ligand model and other approaches (PL)

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The Canadian Council of Ministers of the Environment (CCME) is in the process of developing short-term (acute) and long-term (chronic) water quality guidelines (WQGs) for the protection of aquatic life for nickel in freshwater and marine environments. The existing freshwater nickel WQG is over thirty-five years old, is outdated by new science, and is based solely on acute toxicity data. The CCME used this update as an opportunity to explore emerging approaches for WQG development, including assessing the potential applicability of the biotic ligand model (BLM). The BLM is a mechanistic model that takes into account the effects of toxicity modifying factors on metal bioavailability, and incorporates knowledge of chemical speciation and binding to biological membranes to predict toxicity to aquatic life. The ability of the BLM to normalize the toxicity data to site-specific water quality can be used to derive site-specific WQGs. Therefore, regulatory agencies around the world are using the BLM to develop WQGs (and effluent quality standards). The purpose of this presentation is to present the draft CCME WQGs for the protection of aquatic life for nickel, and to present tools available to guideline users for implementation. In addition, differences between the CCME and British Columbia nickel BLMs are explained. Furthermore, short-term and long-term water quality guidelines for protection of aquatic life in marine waters are presented, following a traditional single linear regression approach.

Characterizing an effect of bicarbonate on chronic nickel toxicity to *Ceriodaphnia dubia* (PL)

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Nickel toxicity to aquatic organisms depends on water chemistry, and consideration of exposure- and toxicity-modifying factors (ETMFs) is critical in predicting effects. Invertebrates are relatively sensitive to nickel, with cladocerans (notably *Ceriodaphnia dubia*) being one of the most sensitive and best studied invertebrate groups. Although models exist to characterize ETMF effects on nickel toxicity to *C. dubia*, the ranges of ETMFs previously studied did not fully capture the range of mine-influenced conditions in our study area. We conducted *C. dubia* toxicity testing over a range of site-relevant ETMF conditions, including very high hardness and bicarbonate, and combined our results with literature data to develop a site-specific model of nickel effects on *C. dubia* reproduction. Multiple linear regression (MLR) identified two candidate models that performed well on this combined dataset. Both models contained hardness and dissolved organic carbon as ETMFs, and the third ETMF was either bicarbonate or pH. We present these modelling results, plus supporting analysis used to evaluate available evidence for bicarbonate vs pH effects on nickel toxicity to multiple invertebrate species. Our analysis indicated stronger support for a bicarbonate effect than a pH effect for some species, leading to the selection of the bicarbonate MLR model as a basis for site-specific benchmarks for nickel in the Elk Valley.

Polar problem: Investigating the nickel sensitivity of Arctic aquatic invertebrates (PL)

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Nickel (Ni) is an essential trace metal of economic interest, but due to increased global demand, environmental contamination may arise. The Arctic is at risk for such contamination, with substantial Ni production in northern zones leading to contamination of surrounding environments. Global warming may lead to further contamination, with increasing access to reserves allowing for greater exploitation of these resources. Nickel toxicity is dictated by its bioavailability to an organism, with many Arctic freshwaters predicted to have high Ni bioavailability and thus toxic risk due to their abiotic composition (hardness, pH, DOC). While marine waters are more protective of Ni toxicity than freshwaters, heterogeneity in composition and resident sensitive organisms make Ni

contamination of concern. Nickel risk in Arctic marine waters is being evaluated using the Arctic green sea urchin (*Strongylocentrotus droebachiensis*). Green sea urchins are one of the best represented invertebrate species in Arctic marine waters, making evaluation of their sensitivity important to protecting Arctic ecosystems. Green sea urchin embryos are being assayed for reproductive and developmental impacts, as well as indicators of Ni mechanism of action under Arctic-relevant exposure scenarios. These results align with preliminary studies which showed *S. droebachiensis* embryos may be highly sensitive to Ni contamination, with a 96-hour effect concentration (EC_{50}) of 1.38 $\mu g \cdot L^{-1}$ for induction of developmental abnormalities. These results taken together will provide insight into the sensitivity of relevant organisms in Arctic-exposure scenarios, furthering our understanding of risks posed by Ni in these unique environments.

A predictive model created to understand how specific water quality characteristics govern the association of cadmium to microplastics in freshwater systems (PL)

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Speciation and partitioning of metals between water, sediment, and biotic ligand components are relatively well understood to be governed by environmental characteristics including water quality and sediment properties. Alternate ligands for metals, such as microplastics, are being introduced into aquatic systems through anthropogenic activity. Using laboratory experiments and machine learning, we have developed and tested a model that outlines which environmental characteristics predominantly govern the association of cadmium with microplastics. To increase accessibility and usability, we have formatted this tool into a dichotomous-style key where users determine whether an environment falls above or below the given thresholds for each water quality parameter, which collectively determine the proportion of cadmium that is bound to microplastic. Further, we coordinated our model with the biotic ligand model, another model of metal fate that determines the activity and toxicity of metals at the gill and other biotic ligands. Using fathead minnows, we assessed how water quality characteristics determined by our model (assessing cadmium-microplastic interactions) works in tandem with the biotic ligand model (assessing cadmium-gill interactions) to provide insight on how cadmium-microplastic ligand interactions relate to cadmium toxicity.

Applicability of partial ultrafiltration for determination of critical metals complexation with natural organic matter investigated by HPSEC-ICP-MS (PL)

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It has been widely demonstrated that the speciation of a dissolved metal, i.e., its distribution among the different chemical species, can be used to explain and even predict its effects on aquatic organisms. However, metal speciation can be difficult to estimate in the presence of natural organic matter (NOM) which is a complex heterogeneous polymeric ligand. Among the possible experimental methods, partial ultrafiltration (PUF) is a relatively fast and affordable technique which allows the determination of the complexation of a metal by NOM using a membrane with low molecular weight cut-offs. An important premise behind this method is that the ultrafiltrate is free of metal-binding NOM. To verify this assumption, four NOM samples from different origins (10 mg $C \cdot L^{-1}$) containing a mixture of gallium, lanthanum, and platinum (360 nM) were used. The contents of samples and the corresponding ultrafiltrates (with Vivaspin20: 3 kDa, and Vivaspin15R: 2 kDa, Sartorius) were investigated using high performance size exclusion chromatography (HPSEC) with real-time metal quantification with ICP-MS. Results show an important reduction of NOM in the ultrafiltrates compared to the original samples (absorbance at 254 nm) and a quasi-total absence of NOM-metal complexes (ICP-MS), supporting the applicability of PUF to determine metal speciation in natural waters.

Lanthanum speciation and bioavailability in aqueous media containing natural organic matter (PL)

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Rare-earth elements (REEs) are in high demand given their applications in the medical, green energy and high-tech industries. Canada has started to mine for REE, and it is important to ascertain the risk that such activities represent for our environment. Current bioavailability models such as the biotic ligand model (BLM) were designed for divalent cations and therefore need to be validated for REEs (mainly trivalent). The BLM considers that internalization and biological effects are directly proportional to free ion concentration. We studied the bioaccumulation of lanthanum (La), a REE, through the measurement of internalization fluxes for *Chlamydomonas reinhardtii*. Lanthanum bioaccumulation decreased in the presence of natural organic matter (NOM). However, this decrease is less significant than what is predicted by calculated La^{3+} concentration. Furthermore, impacts of NOM varied with their origin. Therefore, dissolved organic carbon concentration is not sufficient to predict the impact of NOM on La bioaccumulation. It is

also necessary to consider its physicochemical nature. NOM samples have been extensively characterized in order to adjust speciation calculations with respect to NOM quality. In addition, free ion concentrations were estimated using an ion exchange resin. Our goal is to determine if a modified BLM can be used to predict La bioavailability and if so, what parameters need to be measured in order to better protect our environment from potential REEs contamination.

Effects of neodymium, praseodymium, and yttrium: Bioaccumulation studies (PL)

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Neodymium (Nd), praseodymium (Pr), and yttrium (Y) are three rare earth elements (REEs) that occur in the mineral ore bastnaesite, the primary ore of Canada's first REE mine at Nechalacho, Northwest Territories (NWT). These elements are being increasingly used in modern technologies including green energy equipment (e.g., wind turbines and electric vehicle batteries) therefore, there is an increased concern for the potential environmental risk associated with anthropogenic contamination. There is very little data available for individual REEs and even less available on mixtures. The objective of this study was to investigate the bioaccumulation of Nd, Pr, and Y in *Daphnia magna*, alone and as ternary mixtures. Mixture exposures were designed using a toxic unit (TU) approach, based on converting the EC_{50} concentrations from previously conducted toxicity tests to TUs and applying a matrix isobologram approach. Daphnids were exposed to sublethal concentrations for 24 hours in an artificial soft water medium (hardness of 50 mg $CaCO_3 \cdot L^{-1}$, pH of 6.8, and no added bicarbonate). For example, at 0.2 TUs of each element (36 $\mu g \cdot L^{-1}$ Nd, 133 $\mu g \cdot L^{-1}$ Pr, and 190 $\mu g \cdot L^{-1}$ Y), similar levels of REE accumulation were observed whether it was in single-metal exposures or in mixture exposures indicating that accumulation was not influenced by other REEs. Bioaccumulation tests were also conducted using environmentally relevant conditions found in Thor Lake, NWT (the first fish bearing lake downstream of Nechalacho). This research is supported via an NSERC Alliance Grant with additional funding from Environment and Climate Change Canada, Stantec Inc., and Cheetah Resources.

Toxic effects of rare earth elements cerium, neodymium, and europium both as single and ternary mixture exposures on tomato and durum wheat (PL)

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Rare earth elements (REEs) are increasingly important in today's technologies. They co-occur naturally in mineral deposits and display similar physicochemical properties, with

bioaccessibility dependent on soil factors such as pH and organic matter. Despite increasing exploitation, REEs remain data-poor metals with no regulatory guidelines. At low concentrations in soil, some REEs appear to stimulate plant growth. However, at high concentrations REEs have been shown to be toxic to plants. For singular exposures, toxicity has been determined for some REEs, but quantitative understanding of mixture ecotoxicity and interactions of REEs is limited. Given the potential for extractive mining of REEs in Canada's north, this is an important knowledge gap to be filled. Thus, toxicity thresholds most conservative for risk assessment (EC_{10}, EC_{20}) were determined for tomato and durum wheat using three REEs, cerium (Ce), neodymium (Nd), and europium (Eu) in singular exposures and a ternary mixture exposure. Singular thresholds revealed Ce appears to be the most toxic element, with all REE thresholds (EC_{10}) ranging from 23–596 $mg \cdot kg^{-1}$ based on total soil concentrations. Internal tissue concentrations were more similar between all three REEs, and may serve as a better predictor of toxicity compared to external for both singular and mixture exposures. Assuming concentration addition (CA), endpoint responses to ternary mixture exposures were mostly antagonistic or additive depending on plant species and concentration descriptors (i.e., internal vs external dose); environmental protection from these REEs may be achieved using single-metal toxicity thresholds.

Assessing the potential risk of lithium mining environments using mineralogy and geochemistry (PL)

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Demand for lithium (Li) for use in Li-ion batteries is increasing as part of the global energy transition. Despite advances in Li exploration and mining, the potential environmental risk is still poorly understood. To address the paucity in data on element mobility and toxicity in Li mines and their waste products, samples from Li-bearing granites in the United Kingdom (UK), and two Li tailings management areas in Québec are being studied. Bulk geochemistry and solubility tests identify and quantify elements of concern and their respective environmental mobility. Characterization of the modal mineralogy, accessory, and secondary phases identifies the mineral hosts of the elements of concern, which control their release during weathering. This information is essential to informing mine waste management strategies that will minimize impacts to the environment. Initial results identified chromium (Cr), fluorine (F), Li, uranium (U), and tantalum (Ta) as elements of interest for further investigation. Bulk Li concentrations varied from 98 $mg \cdot kg^{-1}$ to 380 $mg \cdot kg^{-1}$ with the highest concentrations found at the historic tailings site. Cr concentrations exceeded 400 $mg \cdot kg^{-1}$ in some of the UK samples. While F levels are higher at the UK site due to the composition of the granites, Li and Ta were significantly higher in the Québec tailings. U-bearing secondary phases were identified at the historic tailings site and possibly formed during Li processing. Twenty-four-hour

solubility tests using pH 6 and 8.5 simulated surface water demonstrate the influence of pH on both major and trace element release. Work is ongoing to further characterize these materials including by sequential extractions and characterization of microbial communities.

Chronic lithium toxicity on the fingernail clam (*Pisidium dubium*) (PL)

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Lithium (Li) is a metal considered essential in the transition to a green economy. Increased use of Li in mining and extraction activities, as well as in the pharmaceutical industry, are leading to increased levels of Li in the environment. The development of an environmental guideline to identify concentrations below which Li has no effect on freshwater organisms is needed for use in environmental risk assessments and management. However, necessary chronic toxicity data are lacking on freshwater organisms for full guideline development. To fill critical data gaps, our study aims to assess the chronic toxicity of Li to the fingernail clam, *Pisidium dubium*. Chronic toxicity tests were conducted over a 28-day period with seven Li concentrations (0, 0.05, 0.10, 0.50, 1.00, 5.00, and 10.00 $mg \cdot L^{-1}$) to evaluate effects on survival, growth, and burrowing behaviour. A chronic EC_{50}, EC_{25}, and EC_{10} of 1.354, 1.095, and 0.9043 $mg \cdot L^{-1}$, respectively, were calculated for survival, with a 100% mortality rate observed at 5 and 10 $mg \cdot L^{-1}$ Li. The growth and burial results were not significant. It can be hypothesized that the bioconcentration and bioaccumulation of Li by mussels alters their energy, disturbing several physiological functions, leading to decrease in survival rate above 1 $mg \cdot L^{-1}$. This presentation will summarize the results from the fingernail clam study, providing new data to evaluate the chronic toxicity of lithium on freshwater organisms and offering insights into potential mechanisms and future data needs.

Can skin-associated mucous chemically protect against low-level mercury in teleost fish? (PL)

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Demethylation of organometallic mercury compounds is known to occur within the brain and the intestinal tract of humans as a mechanism of protection against toxicity, although this process is not yet fully understood. Similar protective mechanisms against organometallic mercury are known to occur in many animal species. Here, the potential for demethylation in the skin-associated mucosa of a teleost fish was examined. X-ray absorption spectroscopy (XAS) was used to analyze the speciation of mercury following the

titration of different concentrations of organometallic and inorganic mercury compounds with mucosa obtained from steelhead trout (*Oncorhynchus mykiss*) sourced from a local aquaculture facility. At the lowest concentration tested, demethylation of organometallic mercury was observed. Furthermore, the accumulation of mercury in a previously unexplored area, the outermost epithelial layer, was investigated using X-ray fluorescence imaging (XFI) and larval zebrafish that were exposed to either organometallic or inorganic mercury compounds. Accumulation of mercury in this potential target location was observed following exposure to all mercury compounds.

Metals impact fathead minnow's ability to regulate oxygen (PL)

Natalie Nykamp[1], Erin Leonard[1]

[1]*Wilfrid Laurier University*

Eutrophication from nutrient pollution can result in oxygen depleted waters leading to overall stress of aquatic ecosystems. Fish have adapted strategies to cope with these low oxygen or hypoxic waters such as increasing their ventilation rates (known as the hypoxic ventilatory response (HVR)) to bring in more oxygen to meet metabolic demands. Additionally, fish utilize behavioural changes such as reducing movement, seeking shelter, and aquatic surface respiration to cope with low oxygen environments. Metals have become an increasing concern in aquatic environments because of anthropogenic inputs as well as their persistence in the aquatic ecosystem. This study examines the effects of lead (Pb) on the oxygen regulation in fathead minnows (*Pimephales promelas*). The fish are exposed to Pb for 48 hours prior to observation, and the ventilation rates and behaviours of the fish are assessed in comparison to control groups. Gill tissue samples will be assessed to determine the amount of Pb accumulation in the gills and if it is positively correlated to a change in oxygen regulation in the fish. These responses will be compared to the blunt in the HVR that was observed when exposing fish to neodymium, a rare earth element critical for a low-carbon economy. Fish exposed to 94 $mg \cdot L^{-1}$ neodymium showed a 17% decrease and 31% decrease in the HVR under normoxic and hypoxic (40% dissolved oxygen) conditions, respectively. This blunting of the HVR by metals may impact a fish's survival in the wild.

Multiple stressors: How metals impact a fish's ability to regulate oxygen (PL)

Erin Leonard[1], Grant McClelland[2], Jim McGeer[1], Natalie Nykamp[1]

[1]*Wilfrid Laurier University,* [2]*McMaster University*

Control of breathing, whether gill ventilation or air-breathing, is influenced by receptors located in the peripheral and/or central nervous system. These receptors respond to changes in PO_2 and PCO_2/H^+, a trait which is essential for the survival of aerobic organisms. Oxygen-sensing neuroepithelial cells (NECs), which initiate the

cardiorespiratory reflexes in aquatic vertebrates, are the peripheral receptors involved in the control of breathing. There is building evidence that exposure to environmental contaminants, such as copper (Cu) and rare earth elements (REEs) affect the ability of these cells to respond appropriately to changes in PO_2 and PCO_2/H^+ in the environment. We examined the effects of Cu and REEs on the hypoxic ventilatory response (HVR) of killifish and fathead minnows at several levels of biological organization. Both Cu (100 $\mu g \cdot L^{-1}$) and neodymium (Nd; 85 $\mu g \cdot L^{-1}$) blunted the HVR by 15 and 31%, respectively, reducing the fishes' ability to respond to hypoxia. Using ratiometric calcium imaging, we investigated the potential of Cu to modulate intracellular calcium $[Ca^{2+}]i$ response to hypoxia. We produced direct evidence that Cu (100 $\mu g \cdot L^{-1}$; nominal) reduces the $[Ca^{2+}]i$ response to hypoxia in killifish NECs by blocking T-type low threshold Ca channels and not L-type (HVA) Ca channels. Overall, Cu and REEs prevent fish from mounting an appropriate physiological responses to low oxygen and this occurs upstream of the $[Ca^{2+}]i$ response to hypoxia by blocking T-type low threshold Ca channels.

Subchronic impacts of copper and a copper, cadmium, and zinc mixture on the liver and gill proteomes of developing rainbow trout (*Oncorhynchus mykiss*) (PL)

Michael McKay[1], Laura Baseler[2], Jason Rogalski[3], Renata Moravcova[3], Jordan Beblow[4], Mark Cleveland[4], Vicki Marlatt[1]

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Metal pollution is a global issue caused by anthropogenic activities, most notably associated with urbanization and industry, especially metal mining. The molecular mechanisms underlying the whole organism and tissue-level adverse effects (survival, growth, development, reproduction, and histopathology) are not well characterized for copper (Cu), cadmium (Cd), and zinc (Zn), yet these common metal pollutants pose increased risk as co-contaminants in fish. The objective of the present study was to examine the impacts of Cu and a Cu mixture containing Cd and Zn on the liver and gill proteomes during early growth and development of rainbow trout (*Oncorhynchus mykiss*). To achieve this, rainbow trout were exposed for 31 days from eyed embryos through the swim-up fry life stage to waterborne treatments of Cu only (31, 47, 70, and 104 $\mu g \cdot L^{-1}$) and a mixture of Cu (31, 47, 70, and 104 $\mu g \cdot L^{-1}$), Cd (4.1, 6.2, 9.3, and 14 $\mu g \cdot L^{-1}$), and Zn (385, 578, 867, and 1300 $\mu g \cdot L^{-1}$). Label-free LC-MS/MS was employed to evaluate differences in liver and gill protein expression (n = 8 individual tissue samples per treatment) and to identify biological pathways and processes associated with sub-chronic metal toxicity. A total of 45 liver proteins and 25 gill proteins were differentially expressed (10% false discovery rate (FDR)) in metal-exposed rainbow trout relative to unexposed controls. The patterns of protein expression and pathways associated with metal exposure were generally tissue- and treatment-specific. Overall, this study provides insights into tissue-

specific molecular modes of action and identifies novel candidate biomarkers of metal exposure in an early life stage salmonid.

Interactions of trace metals on heart mitochondrial reactive oxygen species metabolism (PL)

Pius Tetteh[1], Zahra Kalvani[1] , Don Stevens[1], Ravinder Sappal[2], Collins Kamunde[1]

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To sustain contractions and relaxations necessary to pump blood throughout the body, the heart has a high ATP demand which is met via energy conversion reactions in the mitochondria. This high energy demand predisposes the heart to production of reactive oxygen species (ROS) and a potential for oxidative stress. Although mitochondria are considered a unifying target for stressors, there is little knowledge of how combinations of stressors alter mitochondrial ROS metabolism. We probed the effects of copper (Cu), cadmium (Cd), and zinc (Zn), singly and in binary combinations, on ROS (as hydrogen peroxide, H_2O_2) emission and consumption in rainbow trout heart mitochondria. Isolated mitochondria were energized with glutamate-malate or succinate and exposed to a range of trace metal concentrations singly and in equimolar binary concentrations. The concentration-response patterns for Cu and Cd on H_2O_2 emission were biphasic or linear, depending on the substrate, whereas Zn induced a hyperbolic response pattern irrespective of the substrate. The Cu-Zn and Cu-Cd mixtures exhibited antagonistic interactions on H_2O_2 emission, in which Cu reduced the effects of both Cd and Zn, whereas the Cd-Zn mixture displayed additive or antagonistic action depending on the substrate. Notably, the inhibitory effect of Cu on H_2O_2 emission resulted from stimulation of consumption and this effect was reversed by Cd and Zn. In contrast, Cd and Zn stimulated H_2O_2 emission and consumption in tandem suggesting direct stimulation of ROS production. Overall, our study demonstrates that Cu can be used to mitigate mitochondrial oxidative stress caused by Cd or Zn.

Bridging the gap: Estimating heavy metals uptake into native vegetation consumed by Indigenous peoples and wildlife (PL)

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As industrial activities continue to expand across Canada, there is an increased need for risk assessors to provide more accurate estimates of how deposition of heavy metals to soil may affect the quality of native vegetation consumed by members of Indigenous communities and by terrestrial wildlife. Currently, published literature focusses on metal

uptake in market crops. Developing uptake factors (UFs) for native species that are relevant to Indigenous communities and wildlife would provide a better understanding of potential changes to human and wildlife exposure to metals through food consumption. Accordingly, we reviewed environmental impact assessments from the Canadian Impact Assessment Registry and the Alberta database for environmental impact assessments to identify mining projects where published baseline data for paired soil and vegetation concentrations of heavy metals were available. Our analyses focused on three metal contaminants of concern (arsenic, cadmium, and lead) and five vegetation groups (berries, berry leaves, Labrador tea leaves, cattail roots, and other leaves). Uptake factors were determined for each metal and vegetation group and the relationship between UFs and metal concentration in soil was estimated using regression analysis and coefficient of determination. Our findings showed differences between UFs in market crops and newly calculated UFs in the vegetation groups. Integrating these results in risk assessment could provide a greater understanding of potential human health and ecological risks associated with consumption of these vegetation groups. Challenges in the analysis and details of the estimations will be discussed.

Proteomic profiles of kidney and liver tissues of rainbow trout (*Oncorhynchus mykiss*) exposed to low concentrations of waterborne nickel (PO)

Kylee Ronnenberg[1], Urvi Pajankar[1], Denina Simmons[1]

[1]*Ontario Tech University*

The nickel rich ore of the Ontario Ring of Fire in Northern Ontario is presumed to be a lucrative mining site. It is crucial to understand environmentally relevant concentrations of metals that can negatively affect wildlife prior to the commencement of mining efforts. We examined changes in protein profiles of kidney and liver tissues of rainbow trout using untargeted proteomics to study the impacts of low-concentration nickel (1–46 $ug \cdot L^{-1}$). Findings suggest that the organs' proteomes showed fewer significant protein changes compared to non-lethal and non-invasive sample types (e.g., epidermal mucus and blood plasma) with regard to nickel toxicity. The proteome of the kidney as compared to the liver was more significantly affected by low-dose nickel. Additionally, preliminary findings suggest that proteins involved in the regulation of biological and cellular processes were impacted and that the kidney proteome of rainbow trout is sensitive to low concentrations of nickel.

Ecological risk classification of inorganic substances (PO)

Rachel Bouwhuis[1], Melissa Ma[1], John Prindiville[1], Michael Beking[1], Rebecca Dalton[1]

[1]*Environment and Climate Change Canada*

Metals and inorganics are of longstanding regulatory interest and play critical roles in technologies required for establishing a low-carbon economy. As information about these substances continues to increase, new approaches are needed to systematically review existing fate, hazard, and exposure information; highlight critical data gaps; and incorporate new science-based concepts to help inform regulatory risk assessment activities. This poster will provide an overview of the Ecological Risk Classification of Inorganic substances (version 2) (ERC-i-2), a prioritization approach developed under the Chemicals Management Plan, a Government of Canada initiative aimed at reducing the risks posed by chemicals to Canadians and the environment. ERC-i-2 is a risk-based approach for identifying the relative likelihood of inorganic substances to cause ecological harm by considering their hazard potentials and multiple lines of evidence for exposure. Site-based risk classifications are considered within a rule-based framework to produce a final prioritization score. ERC-i-2 uses extensive empirical and estimated exposure information for metals and inorganics and currently includes over 1000 exposure locations and over 1 million individual data points. ERC-i-2 is intended to be an evergreen approach and given the high number of data points, it is built using a computational framework to allow for future integration of new and updated datasets. This poster will describe key aspects of ERC-i-2 and its applications for prioritization, including identification of substances that may be of higher ecological concern, and informing research, monitoring, and surveillance needs.

Rare earth elements: Toxicological assessment of neodymium (sulfate salt and organometallic) in boreal soil (PO)

Patrick Boyd[1], Heather Lemieux[1], Dina Schwertfeger[1], Jessica Velicogna[1], Rick Scroggins[1], Juliska Princz[1]

[1]*Environment and Climate Change Canada*

Rare earth elements (REEs) are naturally present in soil, but with increased use in green technologies (e.g., batteries, wind turbines, electric vehicles), demand and therefore mining and processing have also increased, elevating the potential risk to surrounding soil environments. Through the application of standardized soil toxicity test methods, we captured the effects of REEs on different ecological niches occupied by soil invertebrate species (e.g., earthworms, collembolans, mites). Our current work explores the impact of neodymium (Nd) (e.g., as a chloride and sulfate salt), and a Nd-organometallic (Nd-2-ethylhexanoate), to examine the influence of metal moiety on toxicity, and whether toxicity changes over time. Results indicate that the organometallic is significantly more toxic to

soil invertebrates than the metal alone, suggestive that the organic component induces higher toxicity than the metal moiety within the complex. Similarly, with aging of this compound, toxicity has been reduced across species and suggests that this might be due to the degradation of the organic component with time, as the metal remained relatively stable. Therefore, efforts are ongoing to assess the organic component using the same experimental scheme. The research will fulfill toxicity data gaps for the soil compartment and contribute to read-across frameworks for inorganic and organic metal substances.

Iron spiked sediment 42-day survival, growth and reproduction toxicity testing with Twin Lakes *Hyalella azteca* (PO)

Francesca Farrow[1], Heather Rousselle[1], Grace Chen[1], Amy Festarini[1], Sarah Frye[1], Isabelle Gosselin[1], Dan Festarini[1], Hailly Degagne[1], Kiley Sims[1], Glen Bird[1], Marilyne Stuart[1]

[1]*Canadian Nuclear Laboratories*

The Chalk River Laboratories Toxicity Laboratory (CRLTL) at Canadian Nuclear Laboratories (CNL) performed a toxicity study on iron (III) oxide in low-ionic strength water using *Hyalella azteca*. The CRLTL maintains a culture of *H. azteca* that were harvested from a low-ionic strength water body at CNL, Twin Lakes. This culture enables the CRLTL to investigate the effects of toxicants in conditions reflective of the waterbodies at Chalk River Laboratories (CRL) and across the Canadian Shield. Iron is a naturally occurring element that is also involved in biological processes. It is found to have a high natural presence in the groundwater at Chalk River Laboratories. Iron became a focus of the CRLTL when toxic effects were observed in a wetland toxicity study. Following statistical comparison of the affected wetland versus the unaffected wetlands, iron was identified as a potential source of the observed toxic effects. Sediments were spiked using iron (III) oxide to complete a 42-day spiked sediment toxicity test with the iron concentration in sediment ranging from 7600 $\mu g \cdot g^{-1}$ dry weight to 200 000 $\mu g \cdot g^{-1}$ dry weight. No mean survival rates less than 50% were observed, with the highest treatment having a mean survival rate of 55.6%. However, the data produced displayed a highly linear relationship (R^2=0.995), enabling the calculation of the LC_{50} for iron in sediment to be 301 995 $\mu g \cdot g^{-1}$ dry weight, and the body burden LC_{50} to be 6025 $\mu g \cdot g^{-1}$ dry weight. This study was initiated to support the CRL environmental risk assessment (ERA) at the request of CNL's Environmental Protection Program. The CRL ERA will be issued later this year, and more on the context of this study and its findings will be discussed there.

Fate of gadolinium-based contrast agents in the environment: Stability of complexes in aqueous phase and bioavailability (PO)

Aissatou Sow[1], Huy Dang[2], Claude Fortin[1]

[1]Institut national de la recherche scientifique, [2]Trent University

The increasing use of the rare earth element gadolinium (Gd), in manufacturing and as contrast agents in medical imaging, is leading to the disruption of its natural distribution in aquatic systems in many regions across the world. Due to high stability of gadolinium-based contrast agents (GBCA) and the difficulties of conventional wastewater treatment methods to remove them, considerable amounts of Gd are introduced into the aquatic environment through wastewater treatment plant effluents. As emerging contaminants, there is a scarcity of information on the environmental fate of Gd species in the aquatic environment. To assess the toxicity and the bioavailability of Gd, parameters that influence the speciation of the metal in complex natural waters, such as the presence of dissolved organic matter (DOM) need to be taken into account. Therefore, this project aims to study the stability of GBCA in the presence of DOM. Whether DOM affects the bioavailability and the toxicity of Gd on unicellular green algae (*Chlamydomonas reinhardtii*) will also be investigated. By the means of partial-ultrafiltration, size exclusion chromatography, inductively coupled plasma mass spectrometry, and toxicity tests, this project will provide toxicity data (i.e., EC_{10}, EC_{50}) for different Gd species and insight on the fate of Gd-based contrast agents in the presence of DOM. As a first step, the toxicity of inorganic Gd toward *C. reinhardtii* was determined as a function of pH. Complexation experiments of Gd with DOM and toxicity tests with Gd-based contrast agents are being prepared and these results will be presented.

Development of a predictive model for metal accumulation (copper, nickel, and zinc) in biofilm (PO)

Amandine Greil[1], Claude Fortin[1], Isabelle Lavoie[1]

[1] Institut national de la recherche scientifique

In Canada, the mining sector is a major part of the economy but leads to an increase in metal mobility. The increase in metal concentrations in the environment can impact aquatic ecosystems and adequate monitoring is needed. Biofilm can be used as a bioindicator for metal contamination and reflect actual metal bioavailability as opposed to simple measurements of metal concentrations in water. Bioavailability depends on many parameters like pH, hardness, competition with other metals, and metal speciation. This study aims to develop a predictive model based on the premises of the biotic ligand model (BLM) for copper, nickel and zinc accumulation in biofilm. Different environmental conditions are tested in laboratory to establish the predictive model. We recovered epilithon with their substrate from the Cap Rouge River (Québec). This biofilm was placed

in reconstituted water spiked with metals. A single parameter was modified at a time for each experiment to determine its influence on metal accumulation. A first experiment was completed in which a range of nine nickel concentrations were tested (from 0 µM to 20 µM) at neutral pH and constant zinc and copper concentrations (0.1 µM). As expected, nickel accumulation increased in biofilm in proportion to the concentration present in water. Additional experiments are planned with zinc and copper as variables to feed the model calibration. This project will provide a predictive tool to estimate the impact of water quality on nickel, zinc, and copper bioavailability.

Federal Water Quality Guidelines for iron using a multiple linear regression approach (PO)

Tamzin El-Fityani[1], Sushil Dixit[1], Rachel Wideman[1]

[1]*Environment and Climate Change Canada*

Federal Environmental Quality Guidelines (FEQGs) are recommended chemical thresholds for substances in the environment below which there is a low likelihood of direct adverse effects from that chemical. FEQGs support various Canadian federal activities including risk assessment, risk management, and environmental quality monitoring. The Federal Water Quality Guideline (FWQG) for iron was developed following Canadian Council of Ministers of the Environment (CCME)'s 2007 Protocol for the Derivation of Water Quality Guidelines for the Protection of Aquatic Life. The draft FWQG for iron was updated with new toxicity data in 2023. The effect of toxicity modifying factors such as pH and dissolved organic carbon (DOC) on iron toxicity will be discussed, as well as their incorporation into the FWQG with the use of multiple linear regression (MLR) models. Additionally, a species sensitivity distribution (SSD) approach using model averaging was implemented for guideline development. This poster showcases the iron FWQG derivation process as well as how the effects of pH and DOC on iron toxicity were incorporated into the development of a look-up table for site-specific guideline values.

From Lab to Nature: Applying Environmental Relevance to Standardized Toxicity Testing

Influence of confounding variables in toxicity tests (PL)

Josh Baker[1], James Elphick[1]

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The toxicological assessment of specific compounds or effluents can be complicated by methodological characteristics of laboratory toxicity tests. The addition of food or nutrients, and sometimes the growth of the organism itself, can alter water chemistry; changes to water chemistry (e.g., dissolved organic carbon) can be particularly pronounced in sublethal toxicity tests that require addition of food or nutrients to support the test organism performance. The potential for changes occurring to water chemistry during the test often need to be addressed to ensure that the outcome of the test is applicable to real world conditions. Examples are provided of modifications which can be made to assess the toxicity of specific contaminants, including nitrogenous compounds, rare earth elements, and divalent trace metals. The role of food addition and growth rates is reviewed with respect to test variability and organism sensitivity in standardized and non-standardized tests with fish.

Flux your stressors! Including diurnal fluctuations in temperature for more realistic stressor exposures (PL)

Paul Craig[1], Jaycelyn Caklec[1], Sana Gavarikar[1], Allison Weber[1]

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The traditional approach towards understanding the impacts of environmental contaminants is to reduce the number of changing variables, such as temperature, oxygen, or pH in order to identify the mechanism of toxicity. While a valuable approach towards studying mechanisms of toxicity for a single contaminant or stressor, it certainly does not reflect how daily variation in abiotic factors influence contaminant uptake, animal metabolism, and detoxification pathways in a natural, multi-stressor setting. As stressors related to climate change add increasing pressure to aquatic systems, it is imperative that laboratory studies take a more realistic approach using multiple stressors and diurnal fluctuations in abiotic factors. Temperature is one of the most critical factors for poikilothermic ectotherms that dictate how they will respond to contaminant exposure. Our lab has begun taking multiple stressor approaches in effort to identify how daily temperature fluctuations can alter responses in molecular and physiological endpoints of contaminant exposures. Here, we'll examine how cycling temperature influences both endemic (darter; *Etheostoma* spp.) and model (zebrafish) fish species in relation to

venlafaxine exposure, which is one of the most abundant antidepressants detectable in surface waters of the Grand River in Southern Ontario. We have found that several molecular and physiological endpoints, including heat shock proteins, inflammatory cytokines, and whole animal respiration did not change in response to fluctuating temperature and contaminant exposure compared to static temperatures. This highlights that much of the contaminant work previously generated under static conditions may not reflect natural responses to environmental contaminants.

A critical evaluation of inherent toxicity and hazard paradigms in regulatory ecotoxicology (PL)

Lynn McCarty[1], Christopher Borgert[2], Lyle Burgoon[3]

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Inherent/Intrinsic chemical properties are consistently characteristic, irrespective of the number of molecules. Toxicity is an extensive/extrinsic biochemical property that depends on the number of molecules. As adverse effects are routinely related to dose metrics based on the number of molecules, toxicity cannot be an intrinsic/inherent biochemical property. Hazard-based approaches also treat toxicity as a fixed, constant property, yet toxicity testing results vary within and between chemicals, test organisms, and experimental conditions. Hence, toxicology's primary task, determining case-specific risks of adverse effects from interactions of toxic doses/exposures, toxic mechanisms, and modifying/influencing factors, is impeded with inherent- and hazard-based approaches. An alternative is the standard-risk–case-specific risk model. For standard-risk metrics the nature, magnitude, and adverse effects are standardized to the organism, chemical, and test conditions; effectively standard toxicity test results. Although standard test results are suitable for relative toxicity comparisons, interpolation and extrapolation risks for case-specific conditions requires a detailed understanding of complex interactions within and between differing species, conditions, and toxicity-modifying factors. Paracelsus's paradigm is better abbreviated as “dose–causality–response”, as an essential interpretive requirement is establishing toxicity causality by separating mode/mechanism/adverse outcome pathway of toxic action from modifying factor influences in organism effect endpoints. Regrettably, the current knowledge base is inadequate. Moving to a standard-risk–specific-risk paradigm would highlight the importance of improving the toxicity causality knowledge base. It would also provide a rationale for enhancing the design and interpretation of toxicity testing that is necessary for achieving advances in routine translation of standard-risk to specific-risk estimates—the raison d'être of regulatory risk decision making.

Unraveling the influence of metamorphosis on the fate and effects of contaminants in amphibians (PL)

Chantal Lanctôt[1], Danielle Hill[1,2], William Bennett[1], Tom Cresswell[2]

[1]Griffith University, [2]ANSTO

Amphibians are well-recognized as sensitive models for aquatic toxicity testing. During development, amphibians undergo complex morphological and physiological transformations involving the degeneration and remodeling of virtually all tissues and organs as they transition from aquatic stages to terrestrial adults. We conducted a meta-analysis to examine the concentration ratios of metal(loid)s in amphibians before and after metamorphosis. Results showed that while most elements were largely eliminated during metamorphosis, relatively high concentrations of cadmium (Cd), mercury (Hg), and selenium (Se) were retained or increased post-metamorphosis. Experiments using live animal radiotracing and imaging techniques were carried out to further investigate the fate of Cd, methylmercury and selenite throughout tadpole development and metamorphosis. Specifically, we investigated biokinetic changes over ontogeny and the role of metamorphic tissue degeneration and remodeling in influencing contaminant biodistribution. Striped marsh frogs (*Limnodynastes peronii*) were found to retain significantly more Hg (66%) compared to Cd and Se (10%) post-metamorphosis. We observed stage-dependent bioaccumulation kinetics of Cd, with pro-metamorphic tadpoles showing the greatest accumulation and tadpoles at metamorphic climax exhibiting the lowest. Biodistribution changes observed suggest tissue transference during metamorphic tissue remodeling, which varied based on the developmental stage at the time of exposure. Our study highlights the need to account for the non-linear growth rate of metamorphic animals when interpreting biokinetic data, as conventional growth rate constant subtraction methods applicable to fish cannot be directly applied to metamorphosing tadpoles. The influence of growth, development and metamorphosis and their implications for assessing the toxicity of bioaccumulative substances will be discussed.

Field and lab analysis on the toxicity of lead from recreational fishing gear (PL)

Sabrina St-Hilaire[1], Gerald Tetreault[2], Richard Frank[2], Ève Gilroy[2], Sheena Campbell[2], Jason Miller[2], Amy Sett[2], Erin Leonard[1]

[1]Wilfrid Laurier University, [2]Environment and Climate Change Canada

Using standardized lab toxicity testing in conjunction with field data, we investigated the impact of Pb-based recreational fishing gear to aquatic biota. Lead (Pb) is a highly toxic element with no known biological function. Although the toxicity of Pb salts is well studied, the toxicity of Pb from fishing gear remains unknown. Water, sediment, and fish tissue (brown bullhead (*Ameiurus nebulosus*), yellow perch (*Perca flavescens*), and walleye (*Sander vitreus*)) were collected from highly fished sites in Ontario. Sediment samples

exceeded the Canadian Sediment Quality Guidelines by three-fold and muscle Pb concentrations of yellow perch and walleye exceeded Tissue Residue Guidelines for Consumption by two-fold. To bridge the gap between field and lab studies, solutions were made by spinning Pb sinkers in water at different temperatures, and time periods. Concentrations of Pb in lab solutions exceeded Water Quality Guidelines by over 20-fold. Acute 48-hour toxicity of NaCl (LC_{50} = 5.2 $g \cdot L^{-1}$ (C.I. 3.4–7.7); to assess brood health) and $Pb(NO_3)_2$ (LC_{50} = 482 $\mu g \cdot L^{-1}$ (C.I. 116–2616); to compare LC_{50} values with previous studies) were conducted. Pb from sinkers was not acutely toxic to *D. magna* as concentrations did not exceed predicted 48-hour LC_{50} values. Isotope analysis of sediment and tissue was conducted to identify the source of Pb in environmental samples. Chronic toxicity testing was conducted to assess effects of Pb sinkers on growth and reproduction of *D. magna*. Understanding the toxicity of Pb from fishing gear is crucial for the protection of aquatic biota.

A laboratory-based chronic toxicity model is predictive of nickel effects on benthic invertebrates in the field (PL)

Adrian De Bruyn[1]

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We needed a way to understand and predict chronic effects of nickel on stream invertebrate communities in a mine-affected watershed in southeast British Columbia. Challenges included large spatial and temporal variation in exposure- and toxicity-modifying factors (ETMFs), a paucity of toxicity information for field-relevant taxa (mainly insects), and wide variation in benthic invertebrate community structure related to habitat and stressors other than nickel. We tackled these challenges by combining laboratory-based chronic toxicity data with field-based biological monitoring data to build a stressor-response relationship. A site-specific toxicity model for *Ceriodaphnia dubia* was used to translate nickel concentrations and ETMF conditions into predicted toxicity, and a quantile regression of field data from a wide range of sites was then used to describe the limiting effect of nickel toxicity on benthic invertebrate community metrics. The most sensitive metric was percent Ephemeroptera, with an EC_{10} occurring at 22% effect to *C. dubia* reproduction. These findings support previous estimates of 20% for a critical effect size in laboratory test organisms that could translate into effects on sensitive invertebrates in the field.

Twin Lakes *Hyalella azteca* 42-day survival, growth and reproduction toxicity testing of Chalk River Laboratories wetland sediment (PL)

Francesca Farrow[1], Samantha Jarron[1], Amy Festarini[1], Glen Bird[1], Marilyne Stuart[1]

[1]Canadian Nuclear Laboratories

Canadian Nuclear Laboratories (CNL) Chalk River Laboratories (CRL) is a nuclear research organization that has been in operation since the mid-1940s. The CRL site is approximately 40 km^2 in area, of which approximately 10% is wetlands. The Chalk River Laboratories Toxicology Laboratory (CRLTL) is capable of performing non-radiological and radiologically based toxicity tests both in lab and *in situ*. The CRLTL maintains a *Hyalella azteca* culture that thrives in low-ionic strength waters. The culture was established from collections from CRL's Twin Lakes (TL) and allows the CRLTL to perform toxicity studies that are reflective of the CRL site and other Canadian Shield lakes and wetlands. In this toxicity study, six CRL wetlands were selected, three of which are minimally impacted by activities at CRL: Upper Bass Lake, No Name Swamp, and Canadian Forces Base fence line; and three of which have been impacted by CRL operations: East Swamp Weir, South Swamp Weir, and West Swamp. Both radiological and non-radiological contaminants of potential concern were considered in this study, including radionuclides, metals, chloride, and phenols. This investigation included a 96-hour water-only acute toxicity test, followed by a 42-day sediment toxicity test following Environment and Climate Change Canada's Biological Test Method. The survival in all treatments of the 96-hour water-only acute toxicity test was greater than 50%. For the 42-day sediment toxicity test, toxicity was observed at the West Swamp wetland, where the average survival was only 34% and reproduction was also significantly lower than the reference sediments with only 0.1 young per surviving female. Findings from these toxicity tests spawned two further investigations, one into the effects of iron and one into the effects of strontium-90.

Ecotoxicity assessment of petroleum-coke-treated oil sands process-affected water, Part 1: Sublethal toxicity assessment (PL)

James Elphick[1], Martin Davies[2], Stephanie Schiffer[1], Connor Pattem[3]

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Dedicated to the memory of Warren Zubot, Syncrude Canada Ltd.

A novel technology was developed to treat oil sands process-affected water (OSPW) using fresh fluid petroleum coke as an adsorbent, to improve its quality with an overall goal of making treated OSPW safe for future, managed release to regional aquatic receiving environments. Relative to untreated OSPW, a pilot-scale facility operated in 2021 greatly reduced concentrations of organic constituents of OSPW and of several dissolved and total metals, and eliminated acute toxicity, with small or negligible changes in major ions and

total dissolved solids. Treated effluent in serial dilutions with Athabasca River water was subject to a broad ecotoxicity assessment, which collected and considered multiple lines of evidence to assess effects of full-strength and diluted treated OSPW on aquatic biota. Of 249 water quality analytes measured, only seven exceeded relevant aquatic-life guidelines in 100% treated OSPW, and only one (dissolved vanadium) exceeded its guideline in lower dilutions. Numerous sublethal toxicity endpoints were assessed for fish (fathead minnow, rainbow trout, walleye), invertebrates (*Ceriodaphnia*, *Hyallela*, freshwater clams, and mussels), and green algae. About half of survival/viability endpoints showed effects in 100% treated OSPW but not at lower dilutions. Sublethal effects were noted to fathead minnow growth at concentrations above 50% treated OSPW, but generally not to other fathead minnow sublethal endpoints. The most apparent sublethal effects were to growth of green algae, which was reduced at a mean IC_{25} of 13.3%, and *C. dubia* reproduction (mean IC_{25} = 20.1%). Walleye tests did not show sublethal effects of 100% treated OSPW.

Ecotoxicity assessment of petroleum-coke-treated oil sands process-affected water, Part 2: Mesocosm assessment (PL)

Chris Perrin[1], Martin Davies[2], Morgan Edwards[3], Zachary Mueller[2]

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Dedicated to the memory of Warren Zubot, Syncrude Canada Ltd.

A novel technology was developed to treat oil sands process-affected water (OSPW) using fresh fluid petroleum coke as an adsorbent, to improve its quality with an overall goal of making treated OSPW safe for future, managed release to regional aquatic receiving environments. Relative to untreated OSPW, a pilot-scale facility operated in 2021 greatly reduced concentrations of organic constituents of OSPW and of several dissolved and total metals, and eliminated acute toxicity, with small or negligible changes in major ions and total dissolved solids. As part of a broad assessment to test longer term ecotoxicity, a site-based mesocosm with 32 independent experimental streams was used to test a series of dilutions of treated OSPW in Athabasca River water on epilithic algae and benthic invertebrate assemblage patterns. Seven logarithmically separated dilutions ranging from 100% treated OSPW to a control of 0% treated OSPW plus an extra control were replicated four times and controlled using a precision pump system. Substrate and native biota from the Athabasca River were introduced to the streams. Periphyton response was additionally tested using measurement of growth on artificial substrata. The mesocosm operated for five weeks. Dose-response curves and assemblage patterns showed enrichment at lowest doses and inhibition of growth and abundance at doses greater than or equal to 3.2% treated OSPW. Assemblage patterns shifted at 10% or more of treated OSPW. The mesocosm findings coupled with sublethal toxicity testing provided powerful evidence to support decisions on potential discharge of OSPW.

Safety in numbers? Manipulating the social context in the standardized LC50 toxicological assessment (PL)

Sienna Overduin[1], Tamzin Blewett[1]

[1]*University of Alberta*

Many animals live in groups due to greater foraging opportunities, reduced likelihood of predation, and social buffering (i.e., reduced stress concurrent with exposure to members of the same species). Fluctuating environmental parameters due to climate change and anthropogenic pollutants are damaging aquatic ecosystems, and stressing fish populations and social structures within. Our current risk assessment practices rely on consistent social groupings, which may not incorporate true species sensitivity in real-world scenarios. To address this question, we performed a 96-hour copper (Cu) LC_{50} (lethal concentration at which 50% of the organisms' experience lethality) assessment in marine threespine stickleback (*Gasterosteus aculeatus*) whilst manipulating the social context. Specifically, we sought to understand whether the social stress of isolation would increase the fish's ventilation rate leading to greater brachial accumulation of Cu, resulting in a larger proportion of isolated fish experiencing lethality than fish exposed in groups to the same concentration of Cu. Wild-caught stickleback were exposed to one of eight Cu concentrations (nominally; 0 (control), 50, 100, 200, 400, 600, 1000, 1500 $\mu g \cdot L^{-1}$ of Cu) for 96 hours in either isolation (1 fish), pairs (2 fish), or groups (6 fish). Tissue-specific Cu and ion body burdens were collected on gill, intestine, liver, carcass, and whole-body samples and gill lamellae were also taken for histological samples. The importance of understanding whether fish sociality can alter the response to a given toxicant is rarely if ever investigated. This assessment will help to inform the importance of sociality in the field of toxicology.

Are laboratory organisms still relevant? A comprehensive assessment of *Daphnia pulex* reared in laboratory and wild environments and their responses to anthropogenic contaminants (PL)

Aaron Boyd[1], Connor Stewart[1], Jenelle McCuaig[1], Sidney Martin[1], Ally Legge[1], Dustin Doty[1], Tamzin Blewett[1]

[1]*University of Alberta*

The freshwater invertebrate, *Daphnia pulex*, has been a feature of ecotoxicology research for nearly 80 years due to their sensitivity to many contaminants, widespread distribution, rapid maturation rate, and genetic stability through parthenogenetic reproduction. *D. pulex* are easy to cultivate in laboratory environments, allowing for the establishment of a small number of well characterized subtypes that are widely shared amongst research groups. These standardized laboratory lineages have been raised for countless generations in stable, sterile conditions in an effort to minimize confounding

variables during research; this in stark contrast to their wild counterparts, which must endure the consequences of living in dynamic natural environments such as temperature fluctuations, predation, disease, resource competition and more. These differences in rearing conditions leave much potential for deviation in response to environmental contamination; with widespread consequences for the myriad of studies that seek to model environmental outcomes using lab-maintained organisms. We collected wild *D. pulex* from Driedmeat Lake, Alberta in June 2023. Cultures of lab (lineage from the USEPA Newtown culture) and wild reared *Daphnia* were maintained in both laboratory water and Driedmeat Lake water to test for deviation in toxicology outcomes, driven by differences in population and water source, over 48-hour and 21-day exposures to ultraviolet filters avobenzone, octocrylene, and oxybenzone, utilizing standard OECD test methods. This study highlights the challenges faced by ecotoxicologists who seek to model environmental outcomes using standardized laboratory-based tests, in an effort to improve risk assessment practices and better protect our valuable aquatic ecosystems from anthropogenic contamination.

Comparative acute toxicity of herding agents (ThickSlick 6535 and SilTech OP-40) to multiple aquatic species (PO)

Davide Asnicar[1], Tahereh Boloori[1], Jason Durante[1,2], Benjamin de Jourdan[1], Kenneth Lee[3]

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Oil spills pose significant environmental challenges, necessitating innovative strategies for containment, recovery and remediation. Herding agents are an alternate response measure for the containment of an oil slick. By decreasing the oil-water surface tension on the edge of the oil, they enhance the formation of a thicker oil slick over a smaller area. Little is known about their potential effect on wildlife as their toxicity is yet not adequately addressed in the literature. ThickSlick 6535 and SilTech OP-40, are listed as herding agents on the National Contingency Plan product schedule for consideration in US waters, however, in Canada they are not yet approved for use. In this study, the acute toxicity of SilTech and ThickSlick was investigated in different life stages of several economical and ecologically relevant species. The toxicity of SilTech was 15 to 59 times higher than ThickSlick in nearly every species tested; microalgae were the only test species that did not show sensitivity differences between the two products. Effect concentrations across endpoints and products ranged from 0.01 to 402.7 $mg \cdot L^{-1}$, with green sea urchin fertilization as the most sensitive endpoint for each product. These results provide insights into the relative toxicity of each product, which, when combined with herding efficacy data, will help to inform oil spill response decisions in Northern Atlantic Canada. The acute toxicity results shown here are at concentrations above what would be expected following *in situ* application as the highest observed aqueous concentrations was 0.02 ppm for Siltech and 0.01 ppm for ThickSlick.

Inspiring Science in the Capital – Investigation, Integration, and Implementation

Bridging science and policy through environmental quality guidelines (PL)

Allison Dunn[1], Janet Cermak[1], Sushil Dixit[1], Tamzin El-Fityani[1]

[1]*Environment and Climate Change Canada*

The National Guidelines and Standards Office (NGSO) within Environment and Climate Change Canada (ECCC) is a centre of excellence for environmental quality guideline (EQG) development, science advice and tool advancement. NGSO leads the development of EQGs through ECCC's Federal EQG program and contributes to collaborative development of Canadian Environmental Quality Guidelines (CEQGs) through the Canadian Council of Ministers of the Environment (CCME). EQGs are science-based thresholds developed in various media (freshwater, marine, tissue, wildlife diet, sediment, soil, groundwater) whose purpose is to protect aquatic environments or maintain important ecological functions and services of terrestrial ecosystems. Since science-based guidelines are used in policies and regulations for chemical risk management, NGSO's work successfully bridges the science and policy spheres. This presentation will provide an overview of NGSO's mandate, expertise, and role in innovative EQG development, and will demonstrate, through a case study of the polycyclic aromatic hydrocarbon guideline project, how this collaborative CCME work bridges science and policy and integrates multiple scientific disciplines.

Canada's Chemicals Management Plan – Ecological risk assessment perspectives (PL)

Rebecca Dalton[1], Kelly Potter[1], Parisa Ghahreman[1], Delaney Large[1]

[1]*Environment and Climate Change Canada*

In 1999, the *Canadian Environmental Protection Act* (CEPA) required categorization of the 23 000 substances already in commerce in Canada based on specific criteria related to their bioaccumulation, persistence, inherent toxicity, and the greatest potential for exposure. This process identified approximately 4300 substances as priorities that met one or more of these criteria. The Chemicals Management Plan (CMP), a Government of Canada initiative aimed at reducing the risks posed by chemical substances to Canadians and the environment, was subsequently launched with the goal of addressing the potential risks of these substances and taking risk management action on those found to be harmful. This presentation will provide an overview of the scientific approaches used to conduct ecological risk assessments under CEPA since the start of the CMP and how they have evolved over time. This presentation will also highlight the CMP's key achievements and

will outline how Canadian scientists have contributed to the CMP and informed efforts to protect the Canadian environment from harmful substances.

Validation and standardization are key design features for a new approach method: Lessons from the EcoToxChip Project (PL)

Jessica Head[1], Doug Crump[2], Markus Hecker[3], Niladri Basu[1]

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

Since 2016 our team has set out to develop, test, and commercialize toxicogenomic solutions (i.e., EcoToxChips, and a data evaluation tool www.EcoToxXplorer.ca) for the characterization, prioritization, and management of environmental chemicals and complex mixtures. EcoToxChips have been developed for early life stage species representing key vertebrate groups in ecological risk assessment (fish – fathead minnow and rainbow trout; bird – Japanese quail and double-crested cormorant; amphibian –*Xenopus laevis* and northern leopard frog). To date, more than 1800 EcoToxChips have been analyzed covering 28 distinct chemicals and 4 complex environmental samples. The objective of this presentation is to describe how key aspects of validation and standardization were built into the project and its deliverables. Foremost is that the project was driven by stakeholder needs (i.e., Environment and Climate Change Canada, and others) and centred around social sciences research in the areas of institutional entrepreneurship and design thinking. Validation of the EcoToxChip Test System focused on: a) technical performance; b) biological interpretation; and c) user experience. Standardization was built around three prioritized users (i.e., internal R&D by industry, environmental monitoring, chemical screening). Taken together, insights from diverse case studies have enabled us to rapidly iterate, prototype, test, and improve the design of the EcoToxChip Test System to yield a toxicogenomic new approach method that is accessible, standardized, and user-validated. This study was conducted as a part of a large-scale Genome Canada-funded project (EcoToxChip project – www.ecotoxchip.ca).

Protecting the environment with forethought in environmental impact assessment (EIA) (PL)

Carolyn Brown[1,2], Kelly Munkittrick[2,3]

[1]Wilfrid Laurier University, [2]Canadian Rivers Institute, [3]University of Calgary

There have been many criticisms of the integration of science in environmental impact assessment (EIA). These include inadequate baseline data to understand the current environment, which means that project effects cannot be predicted quantitatively. These shortfalls lead to knowledge gaps such that the actual effects of the project post-construction are difficult, if not impossible, to determine. This presentation will discuss some of the science gaps in EIA and provide encouragement and demonstration for

practitioners and policy makers on how to improve this process. Before collecting baseline data, consideration for what information needs to be collected pre-construction, that will help to determine if there are project-related effects post-construction, needs to become common practice.

Global Atmospheric Passive Sampling (GAPS) Network: Integration and cross-cutting issues (PL)

Tom Harner[1], Amandeep Saini[1], John Liggio[1], Jasmin Schuster[1], Egide Kalisa[1], Jacob Mastin[1], Cassandra Johannessen[2], Xianming Zhang[2], Pourya Shahpoury[3], Sabina Halappanavar[4], Anita Eng[1]

[1]Environment and Climate Change Canada, [2]Concordia University, [3]Trent University, [4]Health Canada

The Global Atmospheric Passive Sampling (GAPS) Network and GAPS-Megacities (GAPS-MC) Network, support Canadian and international chemical risk assessment and risk management. They are the only global-scale programs to support the Global Monitoring Plan (GMP) of the Stockholm Convention on persistent organic pollutants (POPs) and deliver unique spatial and temporal trend information for legacy POPs, newly listed POPs, and emerging chemicals. These data also help to form the basis of the effectiveness evaluation of the Stockholm Convention, which is conducted every six years, and to the process of listing new POP candidate chemicals under the Convention. The utility and impact of the GAPS and GAPS-MC monitoring platforms is being enhanced through new research and monitoring which integrates measurements of chemicals in air using polyurethane foam (PUF) disk passive samplers with cross-cutting issues that span policy-policy interfaces. Examples that will be discussed include: i.) Health: application of new approach methodologies e.g., toxicity assays and trace metal determinations, to assess air health; ii.) Biodiversity: characterizing and tracking bacteria and fungi in the aerobiome using passive air samplers and making linkages between chemicals and biodiversity; iii.) Climate: assessing black carbon in air using PUF disk samplers – a tool for assessing global scale deposition of this short-lived climate forcer; and iv.) Chemical Mixtures: conducting a more holistic assessment of the chemical burden in air, including chemical transformation products and other new and unknown chemicals, using non-target analysis.

Working towards a long-term water monitoring program for pesticides (PL)

Cassie McLean[1]

[1]Health Canada

Health Canada's Pest Management Regulatory Agency (PMRA) considers comprehensive water monitoring data to inform regulatory decisions for pesticides to protect human health and the environment. Abundant high quality monitoring data are required to estimate exposure concentrations of pesticides in aquatic habitats and drinking

water; however, for many pesticides, existing monitoring datasets from a variety of sources including federal departments, provinces, territories, registrants, and public literature may be low in quantity and have high variability and uncertainty making its interpretation challenging. A Canada-wide approach to monitoring of pesticides would ensure data are useful for pesticide regulation. PMRA is developing a framework for water monitoring of pesticides to provide guidance that will result in more high-quality, real-world data for regulatory decision-making by PMRA that could also be used by other parties. Additionally, the framework will assist other jurisdictions with their water monitoring programs and increase public understanding, awareness, and accessibility of information. PMRA aims to avoid duplication of work by other jurisdictions through expansion of partnerships and improved collaboration. To inform the development of the framework, the PMRA hosted a series of technical workshops in Spring 2022 to discuss various aspects including site selection, priority pesticides, sampling methods, analytical methodology and data management. A 2-year pilot water monitoring program was initiated at targeted sites across Canada to understand the feasibility of a long-term program and contribute to the development of the framework. The PMRA's long-term vision of how water monitoring for pesticides should be conducted across Canada will be discussed.

Future directions for ECCC's ecological risk assessment program (PO)

Kelly Potter[1], Rebecca Dalton[1], Parisa Ghahreman[1], Delaney Large[1]

[1]*Environment and Climate Change Canada*

Under the *Canadian Environmental Protection Act*, 1999 (CEPA), Environment and Climate Change Canada (ECCC) and Health Canada conduct assessments of substances to determine whether they pose a risk to the environment or to human health. For substances that are found to meet the CEPA definition of toxic, risk management instruments can then be implemented to prevent or reduce releases of those substances to the environment and exposure to Canadians. Since its inception 35 years ago, ECCC's ecological risk assessment program has been very effective, continuously evolving by studying and integrating scientific and technological advances. The program is now at an exciting juncture as it moves into a new phase of substance prioritization and risk assessment. Innovative prioritization tools that make use of computational approaches, *in silico* profiling, and automated workflows to integrate data from multiple lines of evidence are being developed to help inform future ecological risk assessment priorities. Efforts are also underway to explore going beyond a chemical-by-chemical approach and consider cumulative effects of substances, recognizing that organisms in the environment may experience exposure to multiple co-occurring chemicals. Further, the ongoing international effort to replace, reduce, or refine the use of whole-animal toxicity testing will increasingly generate data using new approach methods (NAMs), including *in silico*, *in chemico*, and *in vitro* approaches. Tools and frameworks are needed to facilitate continued integration of novel testing approaches and data into ecological risk assessments. This poster will highlight

areas which are being further explored for ecological risk assessments of existing substances in Canada.

DFO's National Contaminants Advisory Group – a ten-year retrospective on research priorities for investigating the biological effects of aquatic contaminants (PO)

Sarah Marteinson[1], Judith Leblanc[1], Sarah Sine[1], Trinity Berube[1], Remy Tadonleke[1], Cory Dubetz[1], Amanda Winegardner[1]

[1]*Fisheries and Oceans Canada*

Fisheries and Oceans Canada (DFO) uses science-based evidence to ensure the sustainability of aquatic ecosystems and to facilitate effective management of Canada's fisheries. Environmental contaminants are anthropogenic stressors that can negatively impact aquatic environments and fish health and populations. In 2013, the National Contaminants Advisory Group (NCAG) was formed within the Ecosystems and Ocean Science Sector (EOSS) at DFO to manage external partnerships responding to this issue, and to provide advice on contaminants to the Department. NCAG's priority research areas on the biological effects of aquatic contaminants are generated and guide external research proposals. Priorities fall within several theme areas, currently microplastics, pesticides and contaminants, and issues of emerging concern (formerly aquaculture therapeutants and oil and gas related chemicals, priorities now covered by other DFO groups); they are obtained from management sectors within DFO and other departments but also have been developed from identified data gaps in the field using evidence-based approaches and consultation with experts. NCAG's priorities are updated regularly to ensure current relevance. Herein, the progression of these priorities will be presented alongside measurements of their fulfillment.

Exposure, Effects, and Risk Assessment of Pesticides in the Environment

Multigenerational exposure of the earthworm *Eisenia andrei* to two insecticides (PL)

William Martin[1], Paul Sibley[1], Ryan Prosser[1]

[1]*University of Guelph*

Earthworms (subclass: Oligochaeta) are widespread and abundant members of the soil macrofaunal community and, as "ecosystem engineers", can have a significant influence on the structure and function of soil ecosystems. In the field of soil ecotoxicology, earthworms such as *Eisenia andrei* have been used extensively due to the availability of standardized test methods and their adaptability to laboratory culture and testing. Current standardized test methods generally consist of a 14- or 56-day exposure to assess acute and chronic effects, respectively. Multigenerational studies have gained popularity and attention in recent years, with a shift towards the use of long-term assays and lower concentrations of test chemicals. The use of environmentally relevant exposure concentrations presents a realistic exposure scenario that may not show significant effects in contemporary, shorter-term studies. In this research, the current standardized test methods were used as a basis for the development of multigenerational studies using *E. andrei*. The effects of exposure to a single application of the insecticides thiamethoxam and cyantraniliprole on the survival and reproduction of *E. andrei* were observed over multiple generations using consecutive 56-day reproduction tests. No significant impacts on adult survival were reported in any generation for either insecticide, while differences in juvenile production were identified between generations. These results indicate that continued exposure to these insecticides may result an increased magnitude of impact to subsequent generations of earthworms, a result that would not have been identified through the use of conventional biological test methods.

Water monitoring for pesticides pilot program (PL)

Cassie McLean[1]

[1]*Health Canada*

To support the Government of Canada's commitment to ensuring Health Canada's Pest Management Regulatory Agency (PMRA) makes timely, science-based decisions to support the safe and sustainable use of effective pesticide products in Canada, PMRA is working towards developing a long-term collaborative national-scale water monitoring program for pesticides. To investigate the logistics and feasibility of a long-term program, Health Canada is conducting a 2-year pilot program in collaboration with Environment and

Climate Change Canada, Agriculture and Agri-Food Canada and other sampling partners across Canada. For the first year (2022-2023) of the pilot program, 89 surface water sites were selected from eight provinces across Canada using knowledge from sampling partners of intensive agricultural activities (suggesting high pesticide use), past pesticide detections, and previous experience with sampling in these areas. Health Canada Pesticide Laboratory (HC-PL) successfully developed a multi-residue method to analyze 185 current-use pesticides and two transformation products. In the first year, 1294 samples were analysed by HC-PL with a subset analyzed by the University of Guelph for additional pesticides and transformation products. Overall, the top 10 most frequently detected pesticides, out of the 190 pesticides tested, were the following: fluopyram (75%), atrazine (71%), bentazone (69%), transformation product atrazine-desethyl (64%), chlorantraniliprole (62%), metolachlor (55%), metalaxyl (54%), clothianidin (47%), imazethapyr (47%) and thiamethoxam (37%). Further details about the pilot program, the data generated from year 1 of the pilot program, and specifics about how the results can be used by the PMRA for risk assessment purposes will be discussed during this presentation.

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

Fleur Issac[1]

[1]*University of Alberta*

Agricultural pesticides can potentially leach into the environment, causing detrimental effects on surrounding aquatic biota. Nanopesticides (e.g., copper nanoparticles (CuNPs)) are cost-effective, have improved dispersibility, and provide a greater surface area for application ensuring a more targeted pesticide delivery. While useful for reducing pests, nanoformulations exhibit novel properties with unknown effects compared to conventional counterparts. However, NPs tend to aggregate and fall out of suspension, raising concerns about the long-term effects on aquatic organisms and environmental deposition. Furthermore, CuNPs can dissolve over time. We aimed to compare and contrast the established impacts of acute and chronic toxicity of dissolved Cu to that of CuNPs in agricultural pesticides, as its toxicity is unclear. The 48-hour lethal median concentration (LC_{50}) of CuNPs was tested using the freshwater crustacean *Daphnia magna*, as it represents a critical species in many food webs. The observed acute LC_{50} concentration for ionic Cu was 38.5 $\mu g \cdot L^{-1}$ (95% CI 32.48–46.68), while only partial mortality was observed during acute CuNP exposures, suggesting CuNPs are less acutely toxic than ionic Cu. However, the toxicity effects of CuNPs change as the suspensions age. LC_{50}'s on aged (~2 weeks) CuNPs indicated that toxicity increases over time, likely as ionic Cu becomes more dominant in the solution. Understanding the toxicity of nanopesticides used in agriculture will help us understand the potential risks associated with NPs and allow for better regulatory limits on pesticides, protecting sensitive species. This project is funded by the Alberta Conservation Association.

An assessment of the toxicity of pesticide mixtures in periphyton from agricultural streams to the mayfly *Neocloeon triangulifer* (PL)

Moira Ijzerman[1], Melanie Raby[2], Gab Izma[3], Yaryna Kudla[1], Nicholas Letwin[1], Melanie Gallant[4], Stephanie Schiffer[4], Brian Atkinson[1], Rebecca Rooney[3], Paul Sibley[1], Ryan Prosser[1]

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Residual concentrations of pesticides are commonly found outside the intended area of application in Ontario's surface waters. Periphyton are a vital dietary component for grazing organisms in aquatic ecosystems but can also accumulate substantial levels of pesticides from the surrounding water. Consequently, grazing aquatic organisms are likely subjected to pesticide exposure through the consumption of pesticide-contaminated periphyton. The objectives of this study were to determine if pesticides partition into periphyton in riverine environments across Southern Ontario, and if so, to determine the toxicity of pesticides in periphyton when fed to the grazing mayfly *Neocloeon triangulifer*. Ten sites across Southern Ontario were sampled between May and September 2021. Artificial substrate samplers were utilized to colonize periphyton *in situ*, which was then analyzed for the presence of ~500 pesticides. Results from this study demonstrate that periphyton is capable of accumulating pesticides in agricultural streams. A novel 7-day toxicity test method was created to investigate the effects of pesticides partitioned into periphyton when fed to *N. triangulifer*. Periphyton collected from the field sites were fed to *N. triangulifer*, and survival and biomass production were recorded. Survival and biomass production significantly decreased when fed periphyton colonized in streams with catchments dominated by agricultural land use ($p<0.05$). However, the relationship between pesticide concentration and survival or biomass production was not consistent. Using field colonized periphyton allowed us to assess dietary toxicity of environmentally relevant concentrations of pesticide mixtures, however nutrition and taxonomic composition of the periphyton may vary between sites.

Bioaccumulation and physiological/behavioural effects of a common pesticide mix on pond snails (PL)

Jared Sparr[1], Patrick Bergeron[1], Isabelle Lavoie[2], Lounes Haroune[3]

[1]Bishop's University, [2]Institut national de la recherche scientifique, [3]Université de Sherbrooke

Modern agricultural practices heavily rely on agrochemicals which can accumulate in soil, water, and living organisms, leading to potential ecological risk. Many organisms receive exposure and accumulate chemicals through polluted water. Moreover, chemical mixtures resulting from agricultural runoff in natural waterways have complex interactive effects and increasing toxicity compared to individual chemicals. To assess chronic (28-day) toxicity of a pesticide mix commonly observed in water surrounding corn/soybean

farming of Québec, we propose focusing on simple trophic interactions utilizing well-studied model organisms, the great pond snail (*Lymnaea stagnalis*). The experimental design involves outdoor mesocosms containing pond snails and their food source (microbial/algal pond biofilm). The snails will be exposed to a mixture of seven pesticides (atrazine, glyphosate, S-metolachlor, chlorantraniliprole, thiamethoxam, clothianidin, and imidacloprid) at environmentally relevant concentrations. During summer 2023, bioaccumulation, snail physiology, and behaviour will be evaluated. Additionally, effects of the pesticides on the biofilm and *Lemna* sp. (duckweed) will be assessed. Combining mesocosm tests with laboratory tests on *L. stagnalis* will provide a comprehensive understanding of the range of mixture effects. The results of this study will contribute valuable data on the health of freshwater ecosystems in the context of evolving agricultural practices. Our findings will support evidence-based decision making for agronomists and will be disseminated through the Réseau Québécois de recherche en agriculture durable (https://rqrad.com/). This research project serves as an important step towards understanding the potential recovery of ecosystems, providing valuable insights into the effects of pesticide mixtures on freshwater organisms and their associated foods.

Assessing agricultural pesticides risks for aquatic life: Potential health effects of a glyphosate-based herbicide (GBH) on *Elliptio complanata* at environmental concentrations (PL)

Yannick Nombré[1], Maxime Gauthier[1,2], Monique Boily[1], Lise Parent[3]

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In 2021, pesticide sales in Québec exceeded 5.1 million kg of active ingredient (a.i.). Most pesticides are used in the largest agricultural region, the Montérégie, and pose a risk to farmers, the population, and the health of ecosystems. This study aims to identify the effects of the main agricultural pesticides on a sentinel species, through laboratory and *in situ* experiments, to develop a risk assessment tool based on weight of evidence and integrated biomarker response (IBR). We characterized selected tributaries of the St. Lawrence River in the Montérégie Ouest region to determine which pesticides were of greatest concern. A native mussel, *Elliptio complanata*, was used to assess the effects of a glyphosate-based herbicide (GBH), thiametoxams, and chorantraniliprole, individually and in mixture, in the laboratory and *in situ*. Biomarkers were adapted on the mussels to define the LOECs of each pesticide for oxidative stress, defense mechanisms, energy metabolism, neurotoxicity, reproduction, and DNA damage. The first results of the experiment were for a GBH, Credit® Xtreme (50, 100, and 150 µg a.i.·L^{-1}). The data were synthesized in an IBR to assess its risk to mussel health. Results showed that GBH caused oxidative damage in the gills, a marked decrease in acetylcholinesterase activity, and a slight decrease in vitellogenin, suggesting limited effects on reproduction, and that energy metabolism was modulated by an increase in triglycerides content and a decrease in total lipid content.

Finally, IBR values increased with glyphosate concentrations, indicating an increasing health risk for *E. complanata* at environmental concentrations of the herbicide.

Toxicity of individual and combined effect of mefenpyr diethyl safener and its co-herbicide, fenoxaprop-p-ethyl, to early life stages of zebrafish (PL)

Oluwabunmi Femi-Oloye[1,2], Femi Oloye[1], John Giesy[1]

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Safeners formulated with herbicides for crop protection are classified as inert for regulatory purposes. Despite their presence in surface water and their ability to move around, there are limited data regarding their toxicity to non-target organisms in aquatic environments. Thus, this study aimed to assess the toxicity of mefenpyr diethyl (MEF), a safener, and its co-herbicide fenoxaprop-p-ethyl (FEN), used in pre- and post-emergence of grains, on early life stages of zebrafish. These chemicals were assessed both individually and in combination using an *in vivo* method. This allows for the assessment of acute, sublethal, and chronic effects. Twenty embryos which are less than 2 hours post-fertilization (hpf), for each concentration and in triplicate, with the embryos exposed to the chemical solution (0, 0.1, 1, 3, 6, 9, 12, and 15 $mg \cdot L^{-1}$) in a 24-well plate and Petri dishes for acute and chronic studies, respectively. Four days of acute studies assessed impacts, with hatchability being the primary endpoint. Meanwhile, a 21-day exposure was observed for sublethal and chronic effects to consider the influence on growth and survival. Results from the study showed sublethal and lethal effects on the organisms exposed to MEF and FEN singly and in a mixture at environmentally relevant concentrations. A dose-dependent decrease in survival was observed as exposure increased. The results highlight the potential dangers of these substances in the environment. The analysis reveals significant deformations in embryos and larvae exposed to MEF, suggesting that MEF causes sublethal effects. Mixture studies showed intra-additive reactions on endpoints, including survival. The mixture effects provided relief from adverse effects, indicating protection for the exposed animals. While safeners help reduce the negative effects of herbicides on animal models, they still have sublethal effects that are cause for concern. Additionally, safeners previously deemed inert should be re-evaluated for their potential toxicity. This study helps to further advance the study of mixture toxicities.

Water temperature influences the sensitivity of larval sea lamprey (*Petromyzon marinus*) to lamprey-specific pesticides (PL)

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The persistence of organic aromatic phenols in the environment is a growing concern. Phenol toxicity leads to rapid and extensive compound accumulation in the tissues, attributed to its dual hydrophilic and lipophilic characteristics leading to eventual death. A lampricide (lamprey-specific pesticide) 3-trifluoromethyl-4'-nitrophenol (TFM) could be a potential model system for studying phenol toxicity due to its simple structure and well-defined toxicological effects in fishes. TFM is applied to streams of the Great Lakes to control parasitic sea lamprey populations. Compared to non-target fishes, larval sea lamprey are more sensitive to TFM due to their limited ability to detoxify and excrete the lampricide, impairing mitochondrial ATP production and leading to death. In the aquatic environment, TFM behaves like a typical phenol where environmental factors, such as pH and alkalinity, can significantly influence TFM's effectiveness by altering its bioavailability. Recent work suggests that water temperature may also influence TFM toxicity, which may explain why sea lamprey TFM tolerance increases in summer. LC-MS/MS was used to measure TFM within the liver and muscle of larval sea lamprey acclimated to one of three temperatures (6, 12, or 24°C) and exposed to 12-h LC_{25} TFM. Lower TFM accumulation was observed in the tissues of sea lamprey at warmer temperatures, supporting our hypothesis that sea lamprey's ability to survive TFM treatments at warmer temperatures was due to an enhanced capacity to detoxify TFM. These findings suggest that temperature could similarly affect the accumulation of other phenol compounds, which could have important implications for their ecological risk assessments.

Examining effects of neonicotinoids singly and in mixtures using salmonid cell lines (PL)

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Neonicotinoids (NNs) are a relatively new class of pesticides with chemical structures like nicotine, created to be selective neurotoxic insecticides. In the 30+ years since their introduction, NNs have become the most widely used insecticides in agriculture to protect both horticultural crops and livestock. NNs are efficient as they become systemic within the tissues of plants and animals, i.e., incorporated into the tissues and are long-lasting persistent chemicals. These properties, however, are also detrimental as NNs can affect non-target organism such as humans and wildlife. Fisheries and agriculture are major industries throughout Canada intrinsically linked to local ecology. These industries also contribute to the food supply and have implications for human health. The effects of NNs'

contamination on aquatic vertebrates have not been extensively researched. Using three NNs that have been identified at the highest concentrations in the Lower Mainland in British Columbia, Canada: imidacloprid (IMI), clothianidin (CLO), and thiamethoxam (THM), the impacts on cell cultures (continuous cell lines) derived from different tissues of three important salmonid species in Canada were examined: rainbow trout (*Oncorhynchus mykiss*), coho salmon (*Oncorhynchus kisutch*), and Atlantic salmon (*Salmo salar*). Cells were exposed to individual NNs using Health Canada's Pest Management Regulatory Agency's guidelines covering environmentally relevant doses. The cell lines were exposed for varying exposure times and monitored for effects on cell viability, cell proliferation, motility and phagocytic ability. Experiments are being performed to examine how the same NNs and/or NN mixtures may also affect cellular functions and how these may compromise the aquatic environments.

Characterizing the cytotoxic and molecular effects of environmentally relevant pesticides on human Caco-2 and HepG2 cell lines (PL)

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Pesticides play important roles in food security, yet many have the potential to pose threats to both ecosystem and human health. Traditional animal-based testing of these chemicals is resource-intensive, time-consuming, and has ethical concerns. As a result, there is growing interest in developing new approach methods (NAMs) to increase the efficiency of toxicity testing and help replace, refine, and reduce animal studies. The objectives of this study were to: (A) determine the cytotoxicity (LC_{50}) of 19 pesticides of concern to Canadian ecosystems on human Caco-2 (intestinal) and HepG2 (liver) cell lines; (B) identify molecular responses and derive transcriptomic points of departure (tPODs); and (C) compare the tPOD values with the *in vitro* LC_{50} values and regulatory thresholds to determine if the tPOD values are protective against adverse outcomes. Each pesticide was tested at concentrations ranging from 0.46 μM to 1 mM. Alamar Blue tests found that 42% of the pesticides were cytotoxic to Caco-2 cells with measurable LC_{50} values, and 26% were cytotoxic to HepG2 cells. Imazethapyr was the most toxic tested pesticide to Caco-2 cells (LC_{50} = 12.66 μM) while not being toxic to HepG2 cells (LC_{50} > 1000 μM). Chlorothalonil was observed with the lowest LC_{50} value in HepG2 cells, 9 times higher than that of Caco-2 cells. Ultraplex RNA sequencing technology will characterize the transcriptomic changes to calculate tPODs. This research is expected to help support the method development of *in vitro* studies as alternatives to animal testing strategies.

Cytotoxic and transcriptomic effects of environmentally relevant pesticides exposed to RTgill-W1 cells (PL)

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

The environmental health burden of pesticides in the United States (PL)

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While occupational exposure to agricultural pesticides has been found to be correlated with a variety of adverse health outcomes, non-occupational exposures remain poorly understood. One challenge is that pesticide exposure pathways for non-occupational community members are difficult to ascertain, particularly at large geographic scales (i.e., state-wide, regional, or national). Fortunately, annual pesticide use information for over 500 pesticides from every county in the United States is publicly available on a federally managed webpage. These data shed considerable light on the geographic variation of pesticide use. For example, pesticide profiles among the 11 states found in the western United States are divided into two distinct groups, those that are herbicide-dominated and those that are fumigant-dominated. States and counties with pesticide profiles dominated by fumigants also have statistically higher incidences of adverse human health outcomes,

most notably pediatric cancer. On a national scale, it is readily apparent that the pesticide profile changes regionally, creating different exposure regimes for US citizens across the nation. Assessing the pesticide load that US residents are exposed to is challenging, however annual pesticide-use data provide invaluable information that can be used to initiate the development of a national pesticide profile.

Toxicity of flupyradifurone and sulfoxaflor commercial formulations to non-target species in soil (PO)

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Neonicotinoids have been intensely used in agriculture, and due to persistence and non-target effects, are becoming replaced with other products, restricted in usage, or phased out entirely. In Canada, alternate products, containing flupyradifurone and sulfoxaflor, have been registered for use. Our previous research demonstrated that a commercial product containing clothianidin was toxic to earthworm and collembolan reproduction in soils at environmentally-relevant concentrations; the product was more toxic than that containing thiamethoxam. Our current research examines the toxicity of replacement products containing flupyradifurone and sulfoxaflor to earthworms, springtails, and oribatid mites. Flupyradifurone demonstrated comparable toxicity to the previously studied neonicotinoids (e.g., IC_{50}s ranging from 0.18 to 1.3 $\mu g \cdot g^{-1}$ for reproduction). Relative to clothianidin, flupyradifurone degraded in soil at a comparable rate, decreasing by 40% by the end of the 8-week earthworm reproduction test. To date, results suggest that the fate and effect of flupyradifurone are similar to that of clothianidin; testing of sulfoxaflor is currently ongoing. Results from both flupyradifurone and sulfoxaflor test will be presented and discussed relative to our previous research with clothianidin and thiamethoxam. This research will fill knowledge gaps for the soil compartment, contributing fate and effects data to risk assessment initiatives.

Pesticides in Québec streams: Mixture toxicity on freshwater organisms and environmental risk (PO)

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Biodiversity loss in fresh waters is a major environmental issue. Among different environmental disturbances, water contamination by pesticides poses an important risk to aquatic life. In Québec, portraits of pesticide concentrations show that watercourses contain mixtures of pesticides that often exceed water quality criteria for aquatic life protection. Given that pesticides risk to freshwater organisms is generally assessed through studies involving a single pesticide at a time, without taking into account possible

additive effects or synergistic interactions between compounds, this risk may be underestimated. Moreover, studies often carry out toxicity experiments with contamination occurring via water exposure only. However, food contamination is another possible exposure path and pesticides transfer through freshwater trophic networks may lead to increased bioaccumulation and therefore toxicity. To investigate these issues, we will conduct toxicity bioassays with pesticide mixtures representative of Québec's streams on freshwater organisms: the great pond snail *Lymnaea stagnalis* and the mussel *Elliptio complanata*. Mixtures effects will be compared with individual pesticides effects. Moreover, an algal biofilm grown under laboratory conditions will be contaminated with pesticide mixtures and used to feed the model organisms in order to assess the role of trophic network in bioaccumulation for these species. This study aims to help decision making in terms of pesticide use regulation in agriculture.

Species sensitivity distributions for two bath pesticides used in the finfish aquaculture industry (PO)

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Azamethiphos and hydrogen peroxide are bath pesticides used to treat sea lice infestations in farmed salmon held in net-pens. Once in the environment, bath pesticides remain in the aqueous phase due to their hydrophilic nature with differences in dispersion patterns depending on site locations and whether wellboats or tarps were used to treat fish. We have updated and reviewed available toxicity data for hydrogen peroxide, and azamethiphos (LC_{50}, EC_{50}, and NOEC/LOEC) and inferred new thresholds i.e., HC_5 values (the hazardous concentration for which 5% of species are affected or potentially affected) using species sensitivity distributions (SSDs) based on a model averaging approach. HC_5 values could be used as the basis for the establishment of environmental quality standards (EQS). Through the examination of literature data on the toxicity of both compounds including sublethal studies, we opted to use tests with limited exposure times to ensure environmental relevance in term of exposure. We also separated life stages for some of the sensitive taxa to account for differences in sensitivities and exposure risk. The resulting threshold concentrations in seawater are 0.10 $\mu g \cdot L^{-1}$ for azamethiphos and 0.15 $mg \cdot L^{-1}$ for hydrogen peroxide. The inclusion of a better understanding of risk in the selection of toxicity tests to be amalgamated for threshold generation is an important consideration to ensure the relevance of potential EQS values.

Screening-level assessment of pesticide and heavy metal contaminated sediments at the Alaksen National Wildlife Area (PO)

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The Alaksen National Wildlife Area (ANWA) in Delta, British Columbia, stands as a unique habitat, unparalleled along the Pacific Coast of North America. This area serves as a vital habitat for a number of important migrating and overwintering bird species. Due to its location on the Fraser River, various contaminants are present resulting from local agriculture, industrial activities, and wastewater treatment facilities. This study assessed the risk of heavy metals and pesticides in sediment samples within the ANWA. Comparison with environmental quality guidelines and conservative toxicity reference values demonstrated that six heavy metals (arsenic, chromium, copper, iron, manganese, and nickel) in sediment, three pesticides in sediment (DDT and its metabolites), and seven in porewater (alachlor, pendimethalin, DDT, Endosulphan (α + β), hexachlorobenzene, chlorpyrifos, and linuron) exceeded recommended levels, resulting in hazard quotients greater than one. To further understand the risks associated with these contaminants, a bioaccumulation food web model (KABAM) was employed. Based on the results of the KABAM model and the hazard quotients from environmental quality guidelines it is clear that pesticides at the ANWA are present at levels that can cause significant effects to organisms from benthic invertebrates in sediments to species occupying higher trophic positions such as the great blue heron. The ANWA is subjected to a number of contaminants and this screening-level assessment revealed that the pesticides and heavy metals present are at elevated concentrations that could pose risks to organisms that inhabit and frequent the area.

Building a biobed: Practical points for effective experiments (PO)

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Biobeds are an effective tool to reduce point-source pollution with biobed effluent showing 95–99% reductions in pesticide concentrations relatively to the biobed influent (tank rinsate). Biobed systems are highly diverse and most commonly used in farming operations in Latin America and Europe. In essence, biobed iterations can be endless depending on the needs and resources available to the user (e.g., farmers, municipalities) so a wide variety of biobed designs and operations exist. Built in 2020, the University of Manitoba's Ian N. Morrison Research Farm in Carman, Manitoba, Canada, has effectively utilized an above-ground two-cell biobed to treat the wide variety of pesticides, while also advancing biobed research. However, even though effective, issues regarding operation,

usability, statistical power and variation, analytical method development and validation, and supply chain have occurred over the last four years. We will discuss what to consider when designing a biobed to ensure robust and quantitative measures of biobed efficacy and to prevent any major issues, such as pressure, plumbing, electrical, or water quality, that can impact the ability to effectively quantitate biobed efficacy. We also advocate for all research farms in Canada to implement a biobed systems, and for more Canadian farms and municipalities to adopt biobed systems for reducing point-source pollution by pesticides.

Assessing biobed efficacy in the Prairies within Canada for processing pesticide rinsate (PO)

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The large area and landscapes of the Prairie Region within Canada as well as the reduced farming labour force has required the utilization of pesticides to manage crop pests. Although pesticides have increased crop yields across the Prairie region within Canada, current-use pesticides are detected at low concentration in both surface and groundwaters due to both non-point and point-source pathways. Point-source pollution by pesticides can be largely minimized by capturing rinsate disposal generated by the cleaning of sprayers post-pesticide application, and processing this rinsate in biobeds. Biobeds are below- or above- ground on-farm pesticide bioreactors that utilize biomixtures (50% wheat straw, 25% peat, 25% topsoil v:v:v) to degrade and retain pesticides in rinsate, effectively reducing 90–99% of many pesticide concentrations before releasing the processed rinsate in the environment. To further gauge the efficacy of biobeds in the Prairies within Canada, this field study focused on comparing concentrations of current-use pesticides in rinsate to the effluent in two sequential biobed cells by using ultra-high performance liquid chromatography-tandem mass spectrometry. In addition, this study developed a robust, validated, and quantitative method for biomixture extraction to quantify the persistence and distribution of current-use pesticides within the two sequential biobed cells.

Pollution's Power Play: Effects of Contaminants on Metabolism and Energetics

Venlafaxine alters mitochondrial respiration of zebrafish brains, in part due to mitochondrial microRNA regulation (PL)

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Freshwater ecosystems are a common destination for pharmaceuticals via wastewater treatment plant (WWTP) effluent. One pharmaceutical of interest, venlafaxine (VFX; SNRI antidepressant), is regularly measured downstream of the Waterloo, Ontario WWTP at levels that cause adverse behavioural and metabolic effects to fish; however, mechanisms regulating these effects are not fully understood. Thus, we hypothesized that VFX exposure may cause changes in mitochondrial function in zebrafish brains. *In vitro* exposure of zebrafish brain homogenate to below environmentally relevant concentrations of VFX (<1 $\mu g \cdot L^{-1}$) resulted in a decrease in mitochondrial oxygen consumption, though this was not driven by changes to NADH dehydrogenase nor succinate dehydrogenase function. To identify whether these effects also occur *in vivo*, zebrafish were exposed to 1 $\mu g \cdot L^{-1}$ VFX for 0, 1, 6, 12, 24, and 96 hours. *In vivo*, VFX exposure caused changes in brain oxygen consumption and succinate dehydrogenase function. In addition, preliminary evidence suggests that there are differences in night-time brain mitochondrial respiration when fish are exposed to VFX. We further hypothesized that these functional changes *in vivo* may be the result of microRNA regulation of mitochondrial genes. Previously, we demonstrated that microRNAs are present in the mitochondria of three darter fish species and predicted that they play a role in silencing mitochondrial protein expression. Thus, in the current study, mitochondrial microRNAs were quantified to identify their role in regulating mitochondrial genes after VFX exposure. This research will contribute to broader scientific understanding of how pharmaceuticals impact fish mitochondrial energetics both physiologically and molecularly.

Naphthenic acids contribute to oxidative stress by eliciting the emission of reactive oxygen species in mitochondria (PL)

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Oxidative stress is a major cellular mechanism of toxicity elicited by a plethora of toxicants. The mitochondrial oxidative phosphorylation pathway is a major cellular source of reactive oxygen species contributing to oxidative stress. Recent research shows that 3,5-

dimethyladamantane-1-carboxylic acid, a model quaternary naphthenic acid, increased reactive oxygen species (ROS) emissions in isolated mitochondria. The purpose of this study was to determine if a primary carboxylic acid, the moderately hydrophobic naphthenic acid, 3,5-dimethyladamantane-1-acetic acid, would cause the hydrogen peroxide (H_2O_2) production by mitochondria in multiple bioenergetic states. It was hypothesized specific mitochondrial electron transport complexes are the site of ROS emission. Mitochondria isolated from rainbow trout (*Oncorhynchus mykiss*) liver were exposed to commercially available 3,5-dimethyladamantane-1-acetic acid. The emission of ROS during States 3 and 4 respirations was quantified using a respirometer. Subsequently, each ROS emission site in the mitochondrial complexes was isolated using inhibitors, and the ROS emission of each site was measured in multi-well plates using the Amplex UltraRed-Horseradish Peroxidase (AUR-HRP) system. The compound 3,5-dimethyladamantane-1-acetic acid caused ROS emission in State 3 and State 4 with EC_{50} of 174.847 $mg \cdot L^{-1}$. The test compound increase ROS emission in a dose-dependent manner at all ROS production sites, OF, IF, IQ, IIF, IIIQo. However, some sites were more responsive to the production of ROS than others with IQ > IIIQo > IF > IIF > OF. Overall, the research shows that the mitochondrial H_2O_2 metabolism in rainbow trout liver is a possible source of oxidative stress in response to naphthenic acids.

The role of animal energetics in contaminant body burdens and biomarkers of health (PL)

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Assessments of wildlife health often use correlations between body burdens of contaminants with biochemical or physiological responses to infer toxicity. The scientific literature is replete with many examples of negative correlations between body condition (a proxy for energy reserves) and contaminants such as polychlorinated biphenyls (PCBs), and are often cited as evidence of toxicity of these chemicals. However, as the adage goes, "correlation is not causation"; further, for correlations, the direction of the cause-effect relationship is not necessarily obvious. A common consequence of changes in body condition is the redistribution of lipophilic contaminants among tissues following the loss or gain of lipid stores, which can also lead to negative correlations between burdens and body condition. In addition, many measures of animal health used to assess the effects of contaminants, such as immune function, stress response, and thyroid function, are driven by availability of energy or energy reserves. If body condition affects both contaminant burdens and biochemical measures of animal health, significant relationships may be found between contaminant levels and health measures without any toxicity. To illustrate this phenomenon, first we demonstrate the mechanics of how changes in body condition can alter body burdens, based on both laboratory and field experiments. Second, we show how thyroid hormones are regulated in vertebrates in relation to energy reserves, from

published literature as well as the putative mechanistic pathway driving this relationship. Hence, the simplistic assumption that negative correlations between body burdens and health measures are due to toxicity may lead to many "false positives" for inferring toxicity.

Understanding darter (*Etheostoma* spp.) interspecific energetic responses to climate-induced temperature changes (PL)

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Freshwater aquatic environments are predicted to experience increases in temperature variability as the occurrence and severity of heat waves continues to rise. Here, we examined the impact of extreme temperature fluctuations on the metabolic function of three darter species: fantail (*Etheostoma flabellare*; FTD), rainbow (*Etheostoma caeruleum*: RBD), and johnny darter (*Etheostoma nigrum*; JD) native to the Grand River of Southern Ontario. Brain and heart enzymatic activity and expression of pyruvate kinase, lactate dehydrogenase, malate dehydrogenase, citrate synthase, and cytochrome c oxidase were characterized for each species at 15°C baseline and following critical thermal maximum (CTmax) thermal challenge. For brain tissue, FTD exhibited higher baseline enzymatic activity compared to JD and RBD, however at CTmax, this difference was lost as JD had significantly higher activity compared to baseline. Heart tissue displayed no interspecific differences in activity levels at baseline. At CTmax, however, JD had increased activity than RBD for all enzymes, although neither JD or RBD were different from FTD. These results suggest that FTD may be better equipped at responding to temperature-induced elevated metabolic demand due to their higher baseline enzymatic activity, and that JD, although often having the lowest baseline activity, may possess a plastic ability to increase enzyme activity on relatively acute scales, potentially improving their handling of thermal stress. Collectively, this work provides insight on how climate change may impact local fish species and lays the groundwork for future predictions on their responses to environmental temperature changes in combination with other anthropogenic stressors, such as contaminants of emerging concern.

Rearing temperature influences the development and function of the eye and the behavioural performance of yellow perch larvae (PL)

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Due to anthropogenic impacts, water temperatures are projected to rise in the Great Lakes region nearly 3°C over the next 2 decades. These elevations in temperature may be of concern for the yellow perch (*Perca flavescens*), a cool-water fish of great cultural and economic importance to Ontario. Studies have shown that increases in ambient temperature may lead to improved development of eye function in fish, and our previous work has shown that yellow perch reared at elevated temperatures during embryogenesis possess larger eyes at post-hatch stages. However, whether this specifically alters eye function in this species is unknown. In this study, we tested the hypothesis that eye function is altered by rearing temperature in the yellow perch. We reared yellow perch at 12, 15 (optimal), and 18°C during embryogenesis, with hatched fish raised at common garden conditions (18°C). We assessed basic morphology of the eye and performed electroretinograms (ERGs) at 0, 0.01, 0.1, 1, 10, and 90 $cd \cdot s^{-1} \cdot m^{-2}$ to determine sensitivities to light in both light- and dark- adapted conditions at 20 days post-hatch. To tie eye function to animal performance, we used both established and novel light-dark behavioural assessments. The results indicate that yellow perch reared at a higher temperature (18°C) are more sensitive to light following dark adaptation, capable of seeing 0.1 $cd \cdot s^{-1} \cdot m^{-2}$, compared to perch reared at colder temperatures (12°C and 15°C), which only responded to levels closer to 1 $cd \cdot s^{-1} \cdot m^{-2}$. Interestingly, when light adapted, 18°C fish are less responsive to light compared to their conspecifics. In the context of behavioural performance, colder fish appear to be more responsive to light, exhibiting more movement when pulsed with light. Together, these results demonstrate that eye performance is significantly altered by early rearing environment, which may influence animal responses to environmental signals.

The effect of rearing temperature on the cardiometabolic development of yellow perch (*Perca flavescens*) (PL)

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Increasing water temperatures due to anthropogenic activities may pose a significant risk to aquatic species. The yellow perch (*Perca flavescens*) is of great importance to Ontario, however, this species has recently experienced drops in populations. It is unclear

what the impacts of elevations in rearing temperature will have on the subsequent cardiometabolic development of the yellow perch. This study hypothesizes that elevated embryonic temperatures will alter cardiovascular development and metabolism in the yellow perch. To test this, we reared yellow perch at 12°C, 15°C (optimal), and 18°C until hatch, following which, they were raised at the common garden temperature of 18°C. We assessed larval heart rate, metabolism, and whole-animal performance throughout development. Prior to hatch, higher heart rates accompany perch reared at elevated temperatures. However, at 20 days post-hatch (DPH), perch reared at the optimal temperature exhibited lower heart rates. Interestingly, perch reared at the highest temperature exhibited the lowest metabolism at hatch, which increases throughout development. At 20 DPH, 18°C display the highest metabolic rate when compared to fish reared at the optimal temperature (15°C), matching the output seen with heart rate. Fish reared at colder temperatures appear to be more active during a general swimming assay. Quantitative-PCR further identified potential differences in the expression of genes critical for cardio-vasculature, metabolism, and muscle development. Together, these results demonstrate the impacts of elevated temperature on the cardiometabolic development of yellow perch, suggesting that elevations during embryogenesis may have long-term impacts on yellow perch performance.

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

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Early life stages of fish are sensitive to diluted bitumen (dilbit), a Canadian crude oil product; however, no work has characterized how the timing of the exposure during embryonic development affects the response. The goal of this study was to compare the activation of the Phase I biotransformation pathway and key fitness metrics in sockeye salmon (*Oncorhynchus nerka*) embryos exposed to realistic concentrations of the water-soluble fraction of dilbit during discrete developmental windows: fertilization to eyed stage, eyed to hatch, and hatch to swim-up. Fitness metrics included cardiorespiratory performance, growth, and mortality. Cardiorespiratory performance was impaired in sockeye exposed to dilbit (6 $\mu g \cdot L^{-1}$ total initial polycyclic aromatic compounds), however the response depended on the developmental window in which they were exposed. Specifically, sockeye exposed from fertilization to eyed stage and then reared in clean water to swim-up showed no difference in performance relative to unexposed controls; however, sockeye exposed from eyed to hatch or from hatch to swim-up experienced a 14% and 30% reduction in cardiorespiratory performance, respectively, when measured at swim-up. This suggests that either sockeye are more sensitive to dilbit when exposed from hatch to swim-up, or that a period of depuration can help to ameliorate the negative effects

of dilbit on cardiorespiratory performance. These data are important for making evidence-based decisions in the event of a spill, and for improving the accuracy of risk assessments that consider impacts to salmon stocks.

The impact of methylene blue on energy metabolism on early zebrafish development (PO)

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Methylene blue (MB) is a widely used antifungal agent in zebrafish care and maintenance; however, limited information is available on the impact of this chemical on zebrafish development. Since the early life stages of zebrafish are increasingly used as a new approach method (NAM) for assessing the risk of environmental contaminants, it is important to know how rearing conditions influence the overall growth and performance of this model. The aim of the present study was to investigate the metabolic and behavioural effects associated with commonly recommended concentrations of MB in larval zebrafish rearing protocols. Our results demonstrated that exposure to MB for 18 hours resulted in a significant increase in mitochondrial oxygen consumption rate in 1-day post-fertilization (dpf) zebrafish embryos, this was also accompanied by an overall decrease in non-mitochondrial oxygen consumption rate. Ongoing experiments are investigating the effects of MB on energy expenditure, glucose uptake and behaviour in larval zebrafish. In summary, our preliminary results suggest that MB exposure has the potential to alter zebrafish energy metabolism by enhancing mitochondrial oxygen consumption. These findings underscore the importance of exercising caution and warrant careful consideration when utilizing this compound in zebrafish research.

Investigating quantitative links between polycyclic aromatic compound (PAC) content and cardiorespiratory performance in sockeye salmon (*Oncorhynchus nerka*) exposed to sublethal concentrations of diluted bitumen (PO)

Derin Calik[1], Amanda Reside[1], Geoffry Su[2], Christopher Kennedy[2], Sarah Alderman[1], Todd Gillis[1]

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Diluted bitumen (dilbit) from Canada's oil sands is transported by pipelines across North America, including the Transmountain pipeline which crosses through the lower Fraser River watershed en route to tidewater. With the current expansion of this pipeline there is greater risk of a spill into critical habitat of juvenile sockeye salmon (*Oncorhynchus nerka*). This study is part of a project aiming to establish quantitative markers of dilbit exposure that link biological responses and adverse outcomes across a range of exposure scenarios, using tissues conducive to nonlethal sampling in the field. Sockeye salmon fry

were exposed to either clean water (control) or to environmentally relevant concentrations of the water-soluble fraction of dilbit (nominal total polycyclic aromatic compounds (PAC) of 1 or 10 μg·L^{-1}) for 48 hours or 4 weeks. A subset of each exposure group underwent a 1-week depuration in clean water. At each time point, total body burden of PACs and water PAC concentration were measured, and cardiorespiratory performance of exposed fish was assessed by quantifying time to loss of equilibrium in a hypoxia challenge test. A key goal of this work is to determine whether water and/or tissue PAC content predicts organismal effects, which would benefit post-spill impact assessment of salmon stocks. Funded by Fisheries and Oceans Canada.

Exposure to environmental levels of metformin and guanylurea does not impair the metabolism and energetic stores of zebrafish larvae (PO)

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Metformin is a widely used oral therapeutic agent used in the treatment of type-2 diabetes in humans. Because of its widespread use, metformin, and its transformation product, guanylurea, have been detected at levels of 0.1–40 μg·L^{-1} in aquatic habitats. Previous literature remains scant on the effects of metformin and guanylurea on animals that live in aquatic environments, such as fish, particularly at early life stages. Given that this compound is often used to treat energetic imbalances in humans, we tested the hypothesis that exposure to metformin and guanylurea will disrupt the early development, metabolism, and performance of larval zebrafish. To test this, we exposed zebrafish embryos at 3 hours post-fertilization (hpf) to environmentally relevant (0.4, 4, and 40 μg·L^{-1}) and supra-environmental (400 and 4000 μg·L^{-1}) concentrations of metformin and guanylurea until first feed (120 hpf). The data collected suggest that supra-environmental levels of both compounds can lead to increases in mortality and an elevated incidence of spinal curvature. There were no measurable impacts on metabolic endpoints (ATP, glucose and glycogen), and oxygen consumption remained unchanged. The metabolic findings agree with the results of behavioural assessments, which suggest no changes in startle response, general swimming, and visual motor cues. Interestingly, despite no changes in overt movement, metformin and guanylurea appear to disturb anxiety-like behaviour (thigmotaxis). Our data suggest that exposure to metformin and guanylurea at environmentally relevant and supra-environmental concentrations moderately affects larval zebrafish.

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

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Naphthenic acids (NAs) are thought to be a primary contributor to the toxicity of oil sands process-affected water. Despite this, few studies have examined the mechanisms of the toxic action of NAs. Recent findings indicate that the model quaternary naphthenic acid, 3,5-dimethyladamantane-1-carboxylic acid, reduced mitochondrial membrane potential and oxygen consumption and resulted in increased emissions of reactive oxygen species (ROS) in isolated mitochondria in an identical fashion to extracted NAs. The purpose of the present study was to determine if a primary carboxylic acid, the moderately hydrophobic naphthenic acid, 3,5-dimethyladamantane-1-acetic acid, would also affect mitochondrial oxidative phosphorylation. It was hypothesized that this effect occurred through the inhibition of specific complexes in the electron transport chain. Mitochondria isolated from rainbow trout (*Oncorhynchus mykiss*) liver were exposed to commercially available 3,5-dimethyladamantane-1-acetic acid. The effects of these compounds on States 3 and 4 respiration and mitochondrial membrane potential were quantified using respirometry. Subsequently, each mitochondrial complex was isolated using inhibitors, and the activity of each complex was measured in response to graded doses of the substance using spectrophotometric methods. The compound 3,5-dimethyladamantane-1-acetic acid inhibited States 3 and 4 respiration and uncoupled mitochondrial membrane potential with an EC_{50} of 174.847 $mg \cdot L^{-1}$. The activity of all complexes was impaired by the naphthenic acid but there was a 10-fold range in sensitivity. Complex CIV was more sensitive to naphthenic acids than CI and CII; CIII was the least sensitive. These results suggest that toxicity and mitochondrial oxidative phosphorylation are influenced by adamantane 3,5-dimethyladamantane-1-acetic acid NAs via protein complexes in the electron transport chain.

Responses of longnose dace and fathead minnow to stormwater exposure in the Nose Creek watershed in Calgary, Alberta (PO)

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The Nose Creek watershed is under cumulative human impacts as it flows over a variety of lands uses including rural, agriculture, industrial, and various forms of residential urban development. Considering the contribution of Nose Creek to the Bow River and the cumulative effects of various human activities on the watershed, the current study is aiming at identifying whether fish in the Nose Creek watershed show response to chronic stormwater condition in the Nose Creek compared to the nearby sites in Bow River.

Longnose dace (*Rhinichthys cataractae*) were collected at six different locations in the Nose Creek watershed and in the Bow River, and fathead minnow (*Pimephales promelas*) were collected at 7 sites in Nose Creek. Fish were measured for length, weight, and organ weights, and gonads and livers were preserved for future molecular and physiological analyses. Longnose dace show clear effects in Nose Creek, with signs of fin erosion and having significantly higher condition, liver sizes, and gonad sizes than have been detected at other sites in the Bow River Basin. Fathead minnow showed few whole organism differences across sites within Nose Creek.

‘Omics in Ecotoxicology: Predictive and Diagnostic Applications

Characterizing transcriptional responses to three antimicrobial compounds in early life stages of rainbow trout using the EcoToxChip (PL)

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Antimicrobial compounds are chemicals that prevent or reduce the growth and spread of microbes. As aquatic contaminants, however, they have the potential to induce adverse effects in developing fish through diverse mechanisms of action. Comparing transcriptomic responses in fish to different chemicals can support mechanistic-based hazard assessment and provide an early indication of adverse outcomes across chemicals. In this study, we evaluated the transcriptional effects of three antimicrobials, triclosan (TCS), chloroxylenol (PCMX) and methylisothiazolinone (MIT), on early life stage (ELS) rainbow trout (*Oncorhynchus mykiss*). Embryos were exposed, from hatch to 4 days post-hatch, to graded concentrations (nominal: water/solvent control, 0.38–400 $\mu g \cdot L^{-1}$) of each antimicrobial compound. Individuals were sampled at 4 days post-hatch (dph) and whole-body larvae were pooled (3 per sample) to assess early transcriptional responses using a novel standardized reduced transcriptome qPCR array (EcoToxChip; www.ecotoxchip.ca) specific to rainbow trout. Analysis of EcoToxChip data (using EcoToxXplorer.ca) showed dose-related increase in the number of differentially expressed genes (DEGs) with 55 dysregulated genes in TCS, 25 for PCMX, and 3 for MIT at 400 $\mu g \cdot L^{-1}$. The majority of DEGs affected by TCS and PCMX were related to reproduction, ovarian maturation, and metabolism, notably vitellogenin, vitelline envelope protein alpha, zona pellucida glycoprotein, heat shock protein 90-alpha 1, arginase-2, and estrogen receptor 1. Our results support that TCS is a putative endocrine toxicant in fish; similarly common dysregulation patterns (directionality, magnitude) for several DEGs suggest that PCMX may have similar mechanisms of action.

Beyond the prescription: Unveiling the disturbing effects of venlafaxine on aquatic life (PL)

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Many wastewater treatment plants (WWTPs) lack the capability to eliminate pharmaceutically active compounds (PhACs) like antidepressants. These bioactive

compounds are a growing concern due to their detectable concentrations in surface waters, which chronically expose aquatic species. PhAC exposure can lead to oxidative stress through reactive oxygen species (ROS), causing cellular damage. This can reduce fitness, negatively impacting population dynamics and species diversity. The objective of my thesis is to leverage stress tolerance variability of four darter species (rainbow darter, johnny darter, fantail darter, and greenside darter) through comparative transcriptomics, and tissue specific metabolic analysis, in an attempt to understand the impacts of long-term exposure to PhACs. Venlafaxine (VFX) was identified as the most concentrated PhAC downstream of the Waterloo Municipal WWTP effluent outfall site. Hepatic transcriptomes were sequenced from both upstream and downstream populations, followed by bioinformatic analysis. The findings indicate that chronic exposure to effluent has multiple detrimental effects, including metabolic dysregulation, immunotoxicity, and dysregulation of lipid transport and remodeling. Rainbow darters exposed to VFX exhibited a varied enzymatic response related to ROS scavengers in the brain. However, stronger evidence of brain damage associated with ROS generation was found through increased lipid peroxidation and DNA damage. This study highlights the use of transcriptomics for a comprehensive understanding of contaminant impacts on biological systems. We emphasize the necessity for direct biomarkers to accurately quantify cellular damage rather than less definitive proxies. Additionally, we provide evidence that VFX exposure can negatively impact aquatic ecosystems and advocate for improved wastewater treatment measures.

Comparison of *Daphnia magna* and Japanese medaka exposure to pharmaceuticals via targeted mass spectrometry (MS)-based metabolomics (PL)

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Pharmaceuticals are a class of pollutants commonly found in aquatic ecosystems. As a result of their widespread presence and the inefficiency of wastewater treatment removal processes, pharmaceuticals have been reported to harm aquatic organisms, such as provoking gill alterations and delayed hatching in fish, and decreasing heart rates in crustaceans. To better understand how pharmaceuticals impact the health of aquatic organisms beyond traditional apical toxicity endpoints, more molecular-level information is needed. This study compared changes in the metabolite profiles of two model organisms: water flea (*Daphnia magna*) and Japanese medaka (*Oryzias latipes*), exposed to acetaminophen (ACT), diclofenac (DCF), and ibuprofen (IBU). Both organisms were exposed to sub-lethal concentrations of ACT, DCT, or IBU for 48 hours. Metabolomic analysis was performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and targeted 52 metabolites, from which 45 were detected above the limit of quantification. Partial Least Square-Discriminant Analysis (PLS-DA) revealed significant separation ($p \leq 0.05$) from the control group for all pharmaceuticals in both organisms,

except for Japanese medaka exposed to IBU. Exposure to pharmaceuticals with *D. magna* resulted in more significant disruptions ($p \leq 0.05$) to metabolite concentrations than the medaka exposure. Pathway analysis identified perturbations to protein synthesis and amino acid metabolism for all three pharmaceuticals in *D. magna* and DCF in Japanese medaka. No pathway disruptions were observed in Japanese medaka exposed to ACT and IBU. Our findings suggest that although ACT and DCF elicited metabolic perturbations in both organisms, the extent of the perturbations was more pronounced in *D. magna*.

Mucus as a non-lethal alternative: Proteomic evaluation of fathead minnows exposed to nutrient loadings (PL)

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The growing demand for nutritious and low-fat foods has led to the expansion of both the wild rice and finfish aquaculture industries. Northern wild rice (*Zizania palustris*) requires large quantities of phosphorus and ammonia-based fertilizers to achieve high yields. Meanwhile, finfish aquaculture produces wastewater high in phosphorus from unconsumed feed, and ammonia from nitrogenous excretions. Therefore, using aquaculture wastewater to fertilize wild rice offers a sustainable approach to recycle wastewater while promoting optimal plant growth. Yet, adding excess nutrients to an ecosystem presents ecological risks, including the addition of ammonia, which is toxic at nominal concentrations, and phosphorus loadings which can lead to eutrophication and consequently, oxygen depletion in aquatic ecosystems. We assessed the potential ecotoxicological risks of adding aquaculture wastewater to an aquatic ecosystem by measuring proteins in mucus of fathead minnows (*Pimephales promelas*) chronically exposed to nitrogen and phosphorus for 30 days in laboratory aquaria. The exposure concentrations used were selected to be comparable with a preceding field study which determined the appropriate nutrient loadings to fertilize flooded wild rice paddy systems with aquaculture wastewater effluent. The objective was to establish optimal nutrient loadings for maximizing wild rice production, while ensuring minimal stress to fish populations within the ecosystem. Furthermore, we aim to advance minimally invasive techniques to evaluate mRNA transcript and protein patterns in fish using epidermal mucus, while validating the efficacy of mucus as a non-lethal alternative to liver tissues for proteomic analysis. This study may further develop the use of mucus in omic analyses and limit lethal sampling in ecotoxicological research.

Understanding the knowledge gaps in using toxicogenomic approaches for assessing aquatic ecosystem health (PL)

Nefertiti Roldán-Wong[1], Laurie Chan[1]

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Omics technologies, such as genomics, transcriptomics, proteomics, and metabolomics, have revolutionized our understanding of biological systems. In ecotoxicology, these technologies, collectively known as toxicogenomics, have emerged as promising tools to investigate biological responses induced by chemical exposure and environmental stressors. However, several information gaps must be addressed to fully exploit their potential in ecological health assessment, particularly in aquatic ecosystems research, including marine ecosystems. Also, the application of toxicogenomics in One Health needs to integrate human and ecological health assessment. This presentation explores the latest advancements accomplished by omics technologies in the ecotoxicology of aquatic ecosystems and the remaining challenges that require attention for future research. Key discussion topics include the assessment of complex mixtures, trophic chain level studies, cross-species comparisons, research on non-mammalian pathways and network interactions, field-based multi-omics investigations, and the long-term consequences for food security for the coastal populations, all within the context of aquatic ecosystem health. Furthermore, this presentation emphasizes the urgency of adopting more comprehensive approaches. It proposes alternatives to bridge the existing knowledge gaps, which is crucial to formulate evidence-based strategies for effective environmental management and decision-making. The presented work serves as the foundation for an ongoing Ph.D. project, aiming to develop evaluation tools for assessing the impact of toxic elements from mining activities on the health of marine ecosystems. The project utilizes a case study in the Gulf of California and includes multiple omics datasets from non-model marine organisms.

How does tertiary treated municipal effluent affect hepatic transcriptome in longnose dace (*Rhinichthys cataractae*) caged under semi-controlled conditions in artificial raceways? (PL)

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Environmental substances of concern (ESOCs) are detected in the Bow River in Calgary, Alberta, and these substances can induce changes in the hepatic transcriptome of longnose dace (*Rhinichthys cataractae*; LND). With projections of population increase, developmental pressure, and climate change over the next several decades, the Bow River

is expected to be at heightened risk, compromising its ability to naturally assimilate ESOCs. In this study, we utilized semi-controlled raceways at the Advancing Canadian Water Assets (ACWA) at the Pine Creek wastewater treatment plant to address questions about the impacts of increasing concentration of ESOCs on fish. We conducted caging experiments with LND under two scenarios. LND were exposed at (1) current effluent percentage in the Bow River (~5–6%), and (2) predicted percentage increase scenario (~15%). The LND were collected from a reference site in the Bow River (REF) and were caged in the ACWA raceways containing 5% or 15% Pine Creek effluent (PC) or Bow River water (BR; control) over 28 days. In addition, LND were also caged at the REF site and wild LND were collected concurrently to account for confinement effects. Liver transcriptomes were analyzed in male and females sampled over time (14 and 28 days) from BR and PC and compared to wild and caged REF fish sampled on days 0, 14 and 28. Samples for selected ESOCs analysis were collected from BR and PC. RNAseq revealed that 5% effluent did not cause significant changes in the liver transcriptome. The 15% experiment data analysis is currently underway.

Role of toxicokinetics in determining species sensitivity to polycyclic aromatic hydrocarbons in birds (PO)

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

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants. In birds, the mechanisms underlying differential species sensitivity to PAH exposure are poorly understood. Previous results from our group showed a 30-fold difference in sensitivity (LD_{50}) and discordant gene expression profiles in early life stage Japanese quail (JQ; *Coturnix japonica*) and double-crested cormorant (DCCO; *Phalacrocorax auritus*) following exposure to benzo(a)pyrene (BaP) via egg injection. This contrasts with their expected sensitivity based on their shared aryl hydrocarbon receptor 1 (AHR1) genotype. The goal of this project is to link transcriptomic responses to quantifiable measures of metabolism, and to assess how these responses vary between species. Fertilized, unincubated eggs of both species were injected with 2 doses of BaP (100 and 500 $ng \cdot g^{-1}$ egg for JQ and 10 and 100 $ng \cdot g^{-1}$ egg for DCCO, the more sensitive species). RNA was isolated from tissues at different embryonic days, culminating at mid-incubation for both species. Whole body homogenates, chorioallantoic membrane and liver were sampled. BaP will be determined in homogenized embryos and egg contents using gas-chromatography/mass spectrometry. Preliminary quantitative polymerase chain reaction (qPCR) data indicates that phase I biotransformation genes (CYP1A4/5) peak early in liver formation and return to baseline levels of expression by mid-incubation. Expanded gene expression profiles will be analyzed using avian EcoToxChips.

Non-targeted multi-omic analyses of blood plasma for health exploration of namew (lake sturgeon) in an intact and an impacted watershed in the Moose River Basin (PO)

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Namew (nah-may-yo) or lake sturgeon (*Acipenser fulvescens*), are large, long-lived, benthivorous fish that are ecologically and culturally significant to many Indigenous communities. Historically, they were found throughout most of North America's Hudson Bay, St. Lawrence, and Mississippi drainage basins. Overexploitation and extensive habitat loss and alteration since the 1800s caused massive declines in lake sturgeon abundance over much of its range. The Moose Cree Homeland includes a diversity of rivers for namew, some of which are heavily impacted by hydroelectric development, forestry, mining, and other impacts, while others are free-flowing and without any development. Therefore, these internationally endangered fish face impacts and threats at varying levels of severity within the Moose Cree Homeland. The People of the Moose River Basin have compiled over a century of knowledge regarding the impacts of hydroelectric development and activity on Moose Cree Peoples, including declines in namew in some areas of the Moose Cree Homeland. Our collaborative team examined fine-scale measures of namew health and condition in the Mattagami River, which has been impacted by hydropower, forestry, and other industrial development, and the free-flowing and unimpacted North French River, both tributaries of the Moose River system. We used non-targeted metabolomics, lipidomics, and proteomics to profile and investigate location-specific variation among detected biomolecules, before functionally characterizing biomolecules to identify possible health effects for namew in these two river systems. We present our findings from one sampling season and discuss our future non-targeted multi-omic approaches.

Proteomic analysis of perfluorooctane sulfonate (PFOS) on the freshwater gastropod (*Planorbella pilsbryi*) (PO)

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Per- and polyfluoroalkyl substances (PFAS) are anthropogenic chemicals that are widely used in consumer and industrial applications, including non-stick cookware, personal care products, disposable food packaging, stain resistant coatings, and firefighting formulations. These compounds are notoriously environmentally persistent due to the chemical stability of their fluorine-carbon bonds. Specifically, perfluorooctane sulfonate (PFOS) has been under scrutiny due to its environmental persistence, toxicity,

bioaccumulation potential, and long-range transport. PFOS can enter waterways and have adverse impacts on aquatic life through a broad range of toxic effects that can impair growth, development, reproduction, mobility, and survival. Thus, PFOS has become a health concern worldwide. The present study focuses on a 28-day chronic laboratory waterborne exposure investigating the effects of PFOS on freshwater pulmonate snails (*Planorbella pilsbryi*). The toxicity endpoints assessed include survival, growth, and fertility. In addition, whole-snail tissues were subjected to PFOS-burden analysis and were analyzed using non-targeted proteomic analysis. Preliminary proteome results indicate that PFOS disrupts fertility, promotes acute lung injury, and is linked to carcinogenesis. This study will build upon the current knowledge of PFOS effects, and further explore which proteins are affected by PFOS exposure. Currently, very little is known about the molecular effects of PFAS on freshwater snails in response to exposure. Understanding molecular-level responses in snails will help deduce the mechanisms causing whole-organism effects such as survival, growth, and development.

Use of transcriptomic points of departure to assess the toxicity of pesticides in early life stage fish (PO)

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Transcriptomic points of departure (tPODs) are increasingly put forward as an efficient and informative method for assessing chemical hazard. However, the potential for this tool to characterize species difference in sensitivity to contaminants has never been assessed. Here, we use tPODs to compare the early-life sensitivity of two laboratory fish species (rainbow trout [*Oncorhynchus mykiss*] and zebrafish [*Danio rerio*]) and two listed fish species native to the Richelieu River Basin (copper redhorse [*Moxostoma hubbsi*] and river redhorse [*Moxostoma carinatum*]) to agricultural pesticides. In preliminary studies, we developed an exposure method using rainbow trout larvae in 24-well plates. Rainbow trout embryos were raised until hatching and then exposed to solvent control or 10 exponentially increasing concentrations of pesticides for 24 hours. RNA was isolated from 3 pooled larvae per dose group (3 replicates) for RNA sequencing followed by tPOD analysis. The same method is currently being used to evaluate sensitivity to six agricultural pesticides (atrazine, metolachlor, thiamethoxam, chlorantraniliprole, glyphosate, and clothianidin) in copper redhorse and river redhorse. Experiments with zebrafish will follow. This work will provide transcriptomic data on listed species and may provide insights on species sensitivity to agricultural pesticides that can contribute to our understanding of the limited recruitment success in two listed species native to Canada.

Use of transcriptomic dose-response analysis to assess species sensitivity: A case study using 6PPD-quinone, metformin, and guanylurea in a 24-hr microplate-based fish bioassay (PO)

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Transcriptomic dose response analysis (TDRA) is emerging as a powerful tool to determine safe levels of exposure to contaminants. Here, we use TDRA in combination with a 24-hour microplate-based larval bioassay to assess sensitivity to environmental contaminants in a commonly used lab fish (rainbow trout, *Oncorhynchus mykiss*) and two listed fishes, (the threatened river redhorse – *Moxostoma carinatum* and the endangered copper redhorse – *Moxostoma hubbsi*). Newly hatched fish (24–48 hour) from each species were exposed to 12 concentrations (including a control) of one of the following contaminants of concern – N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone), metformin, or guanylurea – in 24-well plates. The concentration range for each chemical/species was based on the estimated LC_{50}, extending two orders of magnitude above this value (to capture acute mortality) and five orders of magnitude below this value (to capture the full transcriptomic response). Due to difference in species sensitivity to 6PPD-quinone, rainbow trout were exposed to 0.00001–100 ug·L^{-1} and river and copper redhorse to 0.0003–3000 ug·L^{-1}. All species were exposed to 0.001–10000 mg·L^{-1} of metformin or guanylurea. The LC_{50} for 6PPD-quinone was calculated at 5 ng·L^{-1} in rainbow trout, similar to previously determined LC_{50} of 1 ng·L^{-1} in trout using a 72- to 96-hour exposure time. Transcriptomic results are pending. The results obtained will provide potentially meaningful differences in species sensitivity that can help provide specific protective threshold values. Coupling all of this together results in a bioassay that is fast, ethical, resource efficient, and more informative than traditional testing.

Development, optimization and application of toxicogenomic endpoints for soil invertebrates (PO)

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Current soil ecotoxicity methods encompass a suite of key ecological receptors (e.g., earthworms, springtails, and mites) that have been standardized as biological indicators for soil pollution. These receptors represent significant contributors to key soil processes (e.g., nutrient cycling), and are adapted to the assessment of multiple stressors in soils. Although toxicological endpoints have been standardized for longer-term chronic

exposures, toxicogenomic profiles are generally lacking, limiting our mechanistic understanding between soil pollution and organism and ecosystem health. Efforts to this regard have yielded a high-quality genome for the springtail, *Folsomia candida*, with similar profiles developed for the earthworm, *Eisenia andrei*, and through our research, the mite, *Oppia nitens*. Using *F. candida* as a model species, we evaluated the toxicity of neodymium (Nd) (inorganic sulphate and organometallic forms) using transcriptomics following a short-term exposure, as well as through the standard definitive test for effects on reproduction. Herein, we present the methodologies used to optimize species exposure and collection from soil from short-term (e.g., 48-hour) exposures, as well as optimization of RNA extraction and rRNA depletion, including the development of custom probes, which were applied to both *F. candida* and *O. nitens* specimens. The results from the short-term toxicogenomic and long-term sublethal exposures with *F. candida* will be presented and compared, based on the application of dose-response modelling and gene expression analyses. The development and use of genomic expression profiles for soil species will advance current test methods for soil species and enable the inclusion of novel endpoints to risk assessment.

Impacts of erythromycin and an antibiotic-mixture on juvenile rainbow trout gut microbiome (PO)

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Erythromycin (ERY) is a commonly used antibiotic that can be found in wastewater effluent globally. Due to their mechanism of killing and preventing bacterial growth, antibiotics may have significant unwanted impacts on fish gut microbiome. The project was split into two experiments to assess gut microbiome in response to exposure with ERY alone or in mixture with other common antibiotics. The objectives of experiment 1 were to understand uptake and depuration of ERY in juvenile rainbow trout (RBT) and profile gut microbiome response to ERY. In brief, RBT were exposed via diet to three concentrations of ERY and one solvent control in six replicate tanks. The exposure period lasted 7 days followed by a 7-day depuration period with mid-gut, bile, and plasma being collected for downstream analysis. In experiment 2, a follow-up study was conducted to assess the impacts of an antibiotic-mixture (ERY, ampicillin, metronidazole, and ciprofloxacin at 100 $\mu g \cdot g^{-1}$ each) using the identical experimental design as described for experiment 1. Here, three matrices were analyzed, with guts collected for 16s metabarcoding, plasma for metabolomics, and brain for mRNA-seq. For experiment 1, ERY was removed rapidly from the fish, and an elevated amount of ERY metabolite was detected in the plasma at 7 days. ERY-treated fish had an overall increase in Proteobacteria in the 1000 $\mu g \cdot g^{-1}$ treatment. For

experiment 2, remodeling of the gut microbiome and plasma metabolome during growth was detected. Several metabolites and genes were found to be dysregulated, with larger impacts due to ERY (1000 $\mu g \cdot g^{-1}$). The results of these studies will help inform aquaculture and risk assessors when assessing the potential impacts of antibiotics in fish feed and the environment, with implications for host health.

Using transcriptomic dose-response analysis (TDRA) to assess the toxicity of oils in Atlantic cod (*Gadus morhua*) larvae (PO)

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Transcriptomics dose-response analysis (TDRA) is a promising approach for predicting the organismal level toxicity of environmental contaminants from transcriptomics data. Here, we apply TDRA to early life stage (ELS) Atlantic cod (*Gadus morhua*) exposed to two different types of oil; ultra-low sulfur fuel oil (ULSFO) and cold lake blend diluted bitumen (CLB). In an initial experiment, cod larvae (~200 degree days) were exposed to seawater control or water accommodated fractions (WAFs) made using variable loadings (0.01, 0.1, 1, 10, 25 $g \cdot L^{-1}$) of each oil for 24 hours. Whole larvae were flash-frozen in pools of 5 and preserved for RNA sequencing. Analytical determination of polycyclic aromatic hydrocarbons (PAHs) and other organic chemicals in the WAF revealed that the ULSFO and CLB oils each contained a distinct mixture of PAHs that increased with oil loading. At equal loadings, ULSFO had higher concentrations of PAHs and was associated with a higher level of mortality in exposed cod. Concentration-dependent increases in the number of differentially expressed genes (DEGs) were apparent in fish exposed to both oil types with a maximum of 298 and 1011 DEGs for CLB and ULFSO respectively. The strong transcriptomic response across all doses precluded TDRA, so we repeated the experiment using lower oil concentrations. These results are pending. Overall, *in vivo* endpoints, chemical analysis, and number of DEGs all suggested that the ULSFO oil was more acutely toxic to ELS fish. Our ongoing analysis will evaluate benefits of the TDRA approach and evaluate its potential for assessing the toxicity of environmental mixtures.

Using omics approaches to identify sublethal, chronic effects of artificial sweeteners on rainbow trout (PO)

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Artificial sweeteners are sugar substitutes added to foods, beverages, and various pharmaceutical and personal hygiene products. Sucralose and saccharin – two highly used artificial sweeteners – are not metabolized by humans and are poorly removed during wastewater treatment, resulting in their widespread release to environmental

waterbodies. Constant discharge, increasing usage, and environmental persistence of artificial sweeteners yield worsening chronic exposure conditions for aquatic organisms. While not acutely toxic, artificial sweetener chronic toxicity is largely unstudied and a potential threat to aquatic life. Stressors, including contaminant exposure, often cause rapid perturbations in metabolite abundance. Thus, metabolomics – the comprehensive study of an organism's metabolites at a specific time – is a sensitive approach to detect sublethal health effects. Following chronic (28-day) sucralose and saccharin exposure, we will identify metabolite alterations in blood plasma and tissues of rainbow trout (*Oncorhynchus mykiss*) using liquid chromatography and tandem mass spectrometry. We will then map metabolite alterations to affected biological pathways using bioinformatics tools and present our resulting preliminary findings on the environmental health threat of artificial sweeteners.

Assessing the sub-lethal metabolomic perturbations of selected carrier solvents used in pollutant exposure studies on *Daphnia magna* (PO)

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According to the Organisation for Economic Co-operation and Development, studies have shown that using carrier solvents in aquatic toxicity testing to help dissolve lipophilic contaminants without exceeding the 0.01% (v/v) of the total exposure media is recommended as it avoids complications related to mortality and morphological abnormalities. However, to our knowledge, no studies have explored the metabolic perturbations after applying carrier solvents using a sensitive environmental metabolomics approach. We addressed this by exposing *Daphnia magna* to four carrier solvents used in exposure studies: acetone, acetonitrile, methanol, and dimethyl sulfoxide at three different levels (0.01%, 0.05%, and 0.1% (v/v)). Based on the multivariate, univariate and pathway analysis, dimethyl sulfoxide and methanol were more disruptive in comparison to acetone and acetonitrile. Due to the synergistic or antagonistic nature that arises when combining carrier solvents with the tested pollutant, we decided to test phenanthridine in water, and in 0.01% (v/v) of acetone and acetonitrile. Statistical and pathway analysis revealed that phenanthridine dissolved in acetonitrile triggered an antagonistic impact on the daphnids' metabolic profile when compared to water and 0.01% (v/v) acetone. Hence, we conclude that acetone applied at 0.01% (v/v) was the most biocompatible solvent in *Daphnia magna* sub-lethal exposure studies since using carrier solvents is inevitable in aquatic ecotoxicology, especially when dealing with hydrophobic chemicals. Furthermore, environmental metabolomic methods were able to provide more insight at the biomolecular level for recommended carrier solvents than apical endpoints.

The Effects of Contaminants in a Changing Climate

Acute thermal stress impacts cardiac function in diploid and triploid Chinook salmon (*Oncorhynchus tshawytscha*) (PL)

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Climate change-associated pressures are predicted to negatively impact wild fish populations; therefore, preserving genetic diversity in these populations will increase the potential for their successful adaptation to an altered environment. Farming of sterile triploid fish is more sustainable since any aquaculture escapees are unable to breed, preventing reduction of wild stock genetic diversity. However, triploid fishes respond poorly to stressful conditions and will be negatively affected by climate change as well. The objective of this study is to characterize cardiac performance of diploid and triploid Chinook salmon (*Oncorhynchus tshawytscha*) during and after recovery from an acute temperature challenge. Diploid and triploid fish were surgically implanted with heart rate loggers (Star-Oddi) to assess how both ploidies responded during a critical thermal maximum (CT_{max}) experiment. Fish were then allowed to recover for 1 hour after reaching CT_{max} to assess whether increased ploidy influences cardiac recovery at a molecular level. Although both ploidies had similar CT_{max} temperatures, triploids became agitated ~1°C earlier than diploids. Heart rate data were analyzed to assess whether there are differences when triploids reach peak heart rate and whether their hearts start to fail (arrhythmia) at lower temperatures compared to diploids. Finally, cardiac expression of genes associated with thermal protection (e.g., HSPs) and cardiorespiratory function were measured to identify whether a hindered recovery response explains why triploids are less resilient to elevated temperatures. This study provides insight into the cardiac performance of triploid fishes and how climate change could negatively affect sustainable aquaculture practices.

Degrees of change: Warmer water exacerbates the immediate and latent effects of crude oil exposure on developing salmon (PL)

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Diluted bitumen (dilbit) is toxic to developing fish, including Pacific salmon that are at risk of exposure from pipeline spills. Since temperature alters the physiochemical properties of oil as well as the physiology of ectotherms, rising environmental temperatures may compound the adverse outcomes of crude oil exposure in fish. We used two environmentally relevant concentrations of dilbit (~0.3 $\mu g \cdot L^{-1}$ and ~1 $\mu g \cdot L^{-1}$) and a

suite of molecular and whole-animal endpoints to demonstrate that a modest +3°C increase in ambient water temperature (T_a) exacerbates dilbit toxicity in coho salmon (*Oncorhynchus kisutch*) when exposed from fertilization to swim-up. The 10–20% embryonic mortality and 25% reduction in cardiorespiratory performance with dilbit exposure at T_a increased by 5% and 20%, respectively, in warmer water. Effects of dilbit exposure on fitness metrics persisted after 6 weeks of rearing in clean T_a water, including a 10% reduction in body mass and 10% post-exposure mortality. Again, despite 6 weeks in clean T_a water, embryonic exposure to dilbit at elevated temperature exacerbated latent effects on fitness, including an added 10% decrease in body mass and 8% mortality, and a residual 20% reduction in cardiorespiratory performance not seen with dilbit alone. The concentration-dependent increase in mRNA expression of the Phase I detoxification enzyme CYP1A with dilbit exposure at T_a was significantly suppressed in fish exposed in warmer water, suggesting a mechanism for the observed potentiation of dilbit toxicity. We conclude that the thermal stability of the receiving environment must be factored into risk assessments and spill management decisions.

The impacts of carbamazepine and temperature on the diurnal and nocturnal behaviour of yellow perch larvae (PL)

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The yellow perch (*Perca flavescens*) is a freshwater perciform that breeds in cooler-waters. In recent years perch have experienced population declines, however, the cause of this decline is unknown. Elevations in temperature may pose a substantial risk to this native fish species, as freshwater environments are expected to increase nearly 3°C in the coming decades. Concomitantly, the presence of contaminants, such as the anticonvulsant carbamazepine, may also lead to perturbations in function. We have previously shown that rearing temperature can lead to substantial changes in behaviour at post-hatch stages in the yellow perch, but whether contaminants further perturb behaviour is unknown. Preliminary data demonstrated that larvae exhibit increased movements at night, prompting us to measure diurnal and nocturnal behaviour in this animal. We tested the hypothesis that exposure to carbamazepine will influence behaviour of yellow perch larvae exposed to different temperatures during embryogenesis. Yellow perch were reared at 12°C, 15°C (optimal), and 18°C until hatch, and then raised to a common garden temperature (18°C). We assessed fish behaviour at 1-, 5-, and 10-day post-hatch (DPH). These timepoints were chosen as they represent the onset of movement, the transition to light-preference, and an age they are at equivalent temperature, respectively. Perch were exposed to either 0 (control), 0.5 (environmental level), or 10 $\mu g \cdot L^{-1}$ (environmental extreme) carbamazepine, and immediately assessed for behaviour during peak activity periods. The data suggest that carbamazepine induces hyperactivity in 1 DPH larvae, but

only at night, suggesting a time-based sensitivity to contaminants. At 5 DPH, only fish reared in 15°C are susceptible to exposure, moving more compared to their conspecifics. Overall, yellow perch larvae are more active at night, and temperature appears to alter sensitivity to carbamazepine exposure.

Effects of elevated temperatures and exposure to atrazine on the health, immunity, and locomotor performance of larval northern leopard frogs (*Lithobates pipiens*) (PL)

Melody Gavel[1], Mark Forbes[1], Stacey Robinson[1,2]

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Amphibians are experiencing global declines. These declines have been attributed to multiple causative agents, including climate change, pollution, and emerging diseases. Importantly, these stressors can interact to have exacerbated effects on amphibian health. Amphibians are excellent bioindicators due to their sensitivity to contaminants. Furthermore, as ectotherms, amphibian physiology is highly influenced by changes in temperature. Surprisingly little research has investigated the effects of increasing temperatures, individually, or in combination with contaminants on amphibian immunity. Our project aims to address this gap in the literature by conducting a 2x2 factorial mesocosm experiment. We expose northern leopard frog (*Lithobates pipiens*) larvae to elevated or ambient temperatures, with a targeted temperature differential of 3°C. These temperatures are combined with exposure to dechloraminated City of Ottawa tap water, or environmentally relevant concentrations of atrazine (2 $\mu g \cdot L^{-1}$), a commonly used pesticide. After two weeks of exposure, we assess the larvae for their stage of development, body length, blood glucose as an indicator of stress, and blood cell profiles as an indicator of immunity. Finally, we assess the larvae's startle response and locomotor performance. Our findings will contribute to the re-evaluation of atrazine use in Canada and will help us understand contaminants' effects on non-target wildlife in a warming climate. This knowledge will help to conserve biodiversity. Furthermore, our project will help to determine whether current toxicity testing, which often uses a single temperature, may underestimate the effects of contaminants on non-target wildlife.

Combined effects of manganese and thermal stress on the metabolic capacities of Arctic charr (*Salvelinus alpinus*) (PL)

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In Northern Québec, Arctic charr (*Salvelinus alpinus*) is exposed to both rising temperatures resulting from climate change and to manganese contamination attributed to

past and present mining activities, and these are expected to increase in this region. While previous research has suggested that fish are less resistant to contaminants when subjected to heat stress, few of these studies have evaluated the combined effects of these two stressors on northern species. Therefore, in this study we investigated the combined effects of manganese contamination and thermal stress on the metabolic capacities of Arctic charr. Juvenile Arctic charr (10–15 g) were exposed for 8 weeks to either 0 $\mu g \cdot L^{-1}$ or 1000 $\mu g \cdot L^{-1}$ of manganese at 7°C or 16°C, after which manganese concentrations in tissues were measured. To estimate the metabolic capacities of the four experimental groups, the activities of five enzymes were measured in liver and muscle. Mitochondrial respiration was represented through the evaluation of the activities of cytochrome c oxidase (CCO) and citrate synthase (CS). The activity of β-hydroxyacyl-coenzyme A dehydrogenase (HOAD) informed on lipid metabolism. The activity of phosphofructokinase (PFK) was used to estimate glycolytic capacity and lactate dehydrogenase (LDH) as an indicator of anaerobic capacity. Our first results suggest that mitochondrial respiration was impaired in fish exposed to the two stressors. This study will allow us to deepen our knowledge on the combined effects of metal contamination and heat stress on the metabolic capacities of a northern species rarely used in ecotoxicological studies and its impacts on their energetic metabolism.

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

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Metal pollution impacts aquatic environments across Canada and the globe. Salmonid species in particular are sensitive to metals such as cadmium (Cd) and copper (Cu), as well as changes in water temperature. The goals of this study are to determine the relative sensitivity of northern fish species to their temperate counterparts, and to understand the influence of climate change on the impacts of pollution. Our initial chronic Cd or Cu range-finder experiment demonstrated that Arctic charr (northern distribution) and lake whitefish (temperate to northern distribution) both experienced impacts on olfactory-mediated behaviours at environmentally realistic metal concentrations. The next experiments examined whether temperature modified the response to metals. Fish were exposed to Cd (0.25 and 2.5 $mg \cdot L^{-1}$) or Cu (10 and 40 $mg \cdot L^{-1}$) for 6 to 8 weeks; rainbow trout (temperate species) and lake whitefish were exposed at 14°C or 17°C, while Arctic charr were exposed at 8°C or 11.5°C. Fish behaviours were then monitored in a Y-maze that contained a food odour in one arm. There were species- and metal-specific impacts on behaviour. For example, rainbow trout showed decreased activity (total distance travelled during the 15-minute trial) with Cd exposure and hyperactivity with Cu exposure. In contrast, Arctic charr showed changes in exploratory behaviours following metal exposure,

with lake whitefish less sensitive to metals. Finally, temperature did not appear to greatly modify the impact of metals on behaviour.

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

A validated and optimized environmental DNA detection assay for Arctic grayling – A Species of Special Concern (PL)

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We have developed and validated an environmental DNA (eDNA) detection assay for the freshwater fish Arctic grayling (*Thymallus arcticus*). This species, which has a largely northern distribution, is considered to be a Species of Special Concern in Alberta, and more effective tools are required to monitor the current distribution of these fish, and to provide a baseline against which future population changes can be gauged. We developed an eDNA assay based on cytochrome oxidase I sequences of Artic grayling from all known lineages. Thereafter, we ensured species specificity, determined the best filter size and the best DNA extraction method, tested for field sensitivity, and ensured best practices for limiting contamination. To gain a better understanding of the spatiotemporal acuity of eDNA, we assessed detection limits in laboratory settings under various flow rates, and we modelled shedding and decay rates, comparing these data to similar measures for environmental RNA (eRNA). This eDNA assay can be used to support creel census data by government agencies in Alberta, and more broadly, this research will be useful in understanding the best methods and applications for studying environmental nucleic acids and how they can be used for monitoring organism and ecosystem health. Our assay has been applied to field settings and will be used to help establish a baseline for Arctic grayling distributions, and how these will change over time under anthropogenic pressures such as climate change.

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

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Ongoing monitoring is vital for conservation of amphibian species and is conducted through conventional auditory and visual surveys. An emerging method, termed environmental DNA (eDNA) barcoding, may offer a more sensitive method of species detection that negates the need for direct species observation. Our research aims to conduct a comparative analysis of eDNA barcoding versus conventional (audio/visual)

species detection methods for six amphibian species in Southern Ontario. We hypothesize that eDNA barcoding will offer equal or greater species presence detections compared to the conventional methods. Conventional surveys and eDNA collections were conducted in three vernal pools from April–July 2019 in collaboration with *rare* Charitable Research Reserve (Cambridge, Ontario). Conventional methods included collection of daily audio files from acoustic song meters and weekly/biweekly visual encounter surveys. Audio data were analyzed using Kaleidoscope Pro. Alongside conventional surveys, duplicate water samples containing eDNA were collected at multiple sampling locations around three vernal pools. After water collection, eDNA was concentrated by filtration, extracted, and quality controlled. eDNA samples were processed using optimized eDNA barcoding assays using quantitative polymerase chain reaction (qPCR). Data analysis is currently underway and preliminary results indicate that amphibian species detection is affected by the method employed as well as vernal pool, sampling site, and time of year. Results will provide evidence needed to evaluate the ability of eDNA detection methods to enhance ongoing amphibian monitoring by end users in academia, government, and private industry. [Funding: CFREF-GWF]

Application of novel eDNA methods to determine distribution and estimate biomass of oolichan (*Thaleichthys pacificus*) (PL)

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Protection of aquatic species relies on timely, reliable, and detailed monitoring data, which can be challenging for species whose life histories and distributions are not well described. Oolichan (*Thaleichthys pacificus*), a culturally and ecologically important anadromous smelt, develop in the ocean and return to fresh water along the Pacific Coast of North America as adults to spawn in the region, but not necessarily the specific river, in which they hatched. Their populations have steeply declined since the 1990s, and supporting their recovery relies on accurate knowledge of spawning locations and abundance. Here we assess spawning oolichan populations in Haisla First Nations territory near Kitimat, British Columbia, a region with significant recent development including a liquefied natural gas processing plant and increased marine traffic. We developed a highly specific and sensitive oolichan environmental DNA (eDNA) assay with a limit of detection of 0.6 copies per sample, limit of quantitation of 2.2 copies per sample, and efficiency of 90%. Using eDNA we have detected oolichan in over ten streams in the study region, corroborating Haisla traditional knowledge and demonstrating greater sensitivity than paired conventional monitoring methods. Daily variation in the eDNA signal during oolichan spawning is correlated to egg drift and can be used to estimate stock strength and spawn timing. Additional analyses are planned to integrate water quality and flow as well as monitoring eDNA signals during the larval outmigration period to support estimation of

oolichan stock biomass using eDNA. This project presents a unique opportunity to protect a vital endangered species while advancing eDNA method capabilities.

Investigating key validation steps to optimize eDNA metabarcoding assays targeting rRNA genes of amphibians found in the Grand River watershed (PL)

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Home to 19 amphibian species, the Grand River watershed is the largest in Southern Ontario. As the human population of the watershed is expected to increase rapidly in the next 20 years, targeted conservation efforts will be essential in preserving habitats for amphibians. The use of environmental DNA (eDNA) to detect species DNA in occupied habitats to inform conservation management is increasing, but acceptance of eDNA metabarcoding assays has lagged behind eDNA barcoding. The objective of this research was to investigate key steps for developing eDNA metabarcoding assays targeting amphibians of the Grand River. Selected assays minimized primer-template mismatches and discriminated between all mitochondrial rRNA sequences of recorded amphibian species. Mock communities were created by use of gene-blocks, based on the sequences of mitochondrial 12S and 16S rRNA genes of local amphibian species and containing a genetic signature discernable from true detections upon sequencing. Assay performance was measured using a standard curve via quantitative polymerase chain reaction (qPCR) and further assessed by calculating the level of detection (LOD) of the mock community. Standard curves of primers compatible with various metabarcoding workflows were compared. While untagged metabarcoding assays had a LOD of <10 copies per sample achievable with technical replication, longer primer additions reduced the efficiency of the qPCR reaction based on shifted cycle threshold (Ct) values and produced primer-dimers with melt curves indistinguishable from true amplifications. Results suggest that careful *in silico* validation, qPCR evaluation and optimization using mock communities, and an assessment of primer tagging strategies are essential to optimizing the performance of eDNA metabarcoding assays.

Targeted qPCR-based workflow for detecting fish sedimentary DNA in aquatic systems (PL)

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The recovery of DNA preserved in the sediment of aquatic systems (sedDNA) has provided short-term and long-term data on biological groups (e.g., bacteria, phytoplankton,

zooplankton, and fishes) and has advanced our understanding of how environmental changes have affected aquatic communities. For monitoring freshwater communities, the use of sedDNA provided greater temporal resolution into the historical faunal records, bridging the knowledge gap that spanned from 100- to 150-year-old data. However, substantial challenges remain for recovering the genetic material in sediments from macro-organisms such as fish. Herein, we present a comprehensive quantitative polymerase chain reaction (qPCR)-based environmental DNA (eDNA) workflow for the detection of fish sedDNA from lake sediment cores. Results show that combining column- and precipitation-based PCR inhibitor clean-up, nucleic acid concentration steps, and the use of endogenous chloroplast DNA as a sample integrity control help avoid false-negative detection of target fish DNA. The present study also highlights the importance of using highly sensitive species-targeted qPCR assays with known limits of detection (LOD) and quantification (LOQ) to increase confidence in the reported results. Lastly, increasing the number of technical replicates for qPCR analysis aids in improving the detection of less abundant fish DNA preserved in sediment layers. Overall, this study addresses common methodological concerns in processing lake sediment samples for fish sedDNA detection. This approach can be used to better understand the influence of human-mediated introductions on fish diversity in lakes and refine the native freshwater fish ranges, providing essential baseline data for environmental impact assessments.

The use of eDNA in concert with conventional electrofishing in streams adjacent to agricultural activities in the Lake Erin Basin (PL)

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Environmental DNA (eDNA) metabarcoding is becoming increasingly common in aquatic ecological studies. Limited research has been performed on the topic of eDNA data collection techniques being utilized as a tool in collaboration with electrofishing, rather than eDNA data collection techniques being the primary mode of data collection. To determine the strengths and differences of eDNA data collection techniques, eDNA and electrofishing data were collected on three sampling periods at four experimental sites adjacent to agricultural activities in the Lake Erin Basin. Duplicate eDNA samples, and a single blank sample, were collected just prior to backpack electrofishing during each campaign. On site, fish were collected, identified to species, and enumerated prior to returning to the stream. Filtered eDNA samples were extracted and analyzed targeting both the 12S and COI markers for metabarcoding. Analysis confirmed that the data collected using eDNA and electrofishing are statistically different, and revealed that eDNA detects more higher-trophic-level species than electrofishing; species which were not collected using electrofishing. The results of the study suggest that eDNA data collection techniques identified more species than conventional data collection techniques, many of which were not geographically plausible. However, there were also differences in fish species identified

between the two molecular markers. Results from the 12S marker using the MiFish database were more similar to electrofishing as they both identified more cyprinids species, whereas COI identified more sucker species. This study demonstrates that taxonomic annotation is required for eDNA to be used as a reliable surrogate for conventional community assessments.

A data management solution for eDNA research, from field to lab (PL)

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Modern environmental survey operations are diverse, often complicated and technology-intensive. Yet they follow a similar general pattern, including field collection, transfer and storage of environmental samples, their processing, laboratory analyses, and results interpretation. The quality of research outcomes depends on the robustness of material tracking and the ability to integrate analytical data with sample provenance information. We present a conceptual data architecture for environmental research design that was implemented as a data management system (MS Access user interface linked to SharePoint backend tables) built around the needs of a small environmental DNA (eDNA) research operation and integrated with its standard workflows. This system facilitates real-time digitization of field sample provenance data and associated environmental characteristics. It further tracks the chain of custody and analytical history for collected biological materials and all their derivatives (e.g., DNA extracts), connecting each environmental sample with its analytical results. Structured database records are linked to digital objects in a searchable online repository of images, documents, spreadsheets, and DNA sequence files, providing rich context on the origin of samples, sampling conditions, protocols used, and raw data generated. Data integrity is validated using a set of geocoding, nomenclatural, and molecular taxonomy references. Additional external connectivity tools are being developed to aid with data digitization, conversion, and exchange with other systems and to allow publication and archival with global data aggregators. Although designed primarily for eDNA research, the proposed data structure and operational framework could be adopted for other activities involving the collection, storage, and laboratory analyses of environmental samples.

Inhibition of RT-qPCR and RT-dPCR of SARS-CoV-2 RNA and fecal indicators in wastewater-based surveillance (PL)

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Implementation of wastewater-based surveillance (WBS) during the current COVID-19 pandemic has been a crucial tool in understanding the prevalence of infections with SARS-CoV-2 in the population. However, there are technical challenges with accurate quantification of viral RNA fragments via reverse transcription quantitative polymerase chain reaction (RT-qPCR). One of the challenges is the presence of substances in wastewater (WW), which inhibit RT-qPCR, lessening the measured amount of viral RNA from the actual abundance. Samples from the Galt wastewater treatment plant (WWTP) in Cambridge, Ontario, Canada, often contained unknown substances that inhibited quantification of SARS-CoV-2 via RT-qPCR. The wastewater samples from Galt also contained approximately 3% of the Rotary Drum Thickener (RDT) filtrate from a down-stream process. Using the component that caused this inhibition, the Rotary Drum Thickener (RDT) filtrate or known environmental inhibitors, including tannic and humic acids, the objective of this study was to understand where inhibition was occurring (i.e., reverse transcription (RT) or PCR amplification), and how this affects quantification of viral RNA in wastewater samples. This study also determined if RT-dPCR (digital polymerase chain reaction) can mitigate effects of inhibition observed when compared to RT-qPCR by use of two environmental relevant inhibitors. Results suggest that inhibition causes a significant underestimation of viral RNA in wastewater and RT phase is affected. Results of this study demonstrated that when comparing RT-qPCR and RT-dPCR, RT-dPCR is similarly affected by inhibition of RT and does not rectify inhibition of RT observed in wastewater. Understanding of how inhibitory substances can affect accurate quantification or detection of viruses or other RNA targets is crucial to implementing WBS and environmental RNA (eRNA) studies, and use of various technologies should also be examined and compared.

Wastewater-based surveillance of SARS-CoV-2: Lessons learned in sewersheds that can be applied to watersheds (PL)

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Wastewater-based surveillance (WBS) of SARS-CoV-2, the virus which causes COVID-19, has been effectively employed to support public health decisions and actions. Over the

past three years, we have applied our knowledge from the field of environmental DNA (eDNA), where we characterized fish communities in the Grand River, to the field of WBS. Methods were developed to measure SARS-CoV-2 viral fragments in wastewater using polyethylene glycol (PEG) precipitation with centrifugation, RNA extraction, and reverse transcription quantitative polymerase chain reaction (RT-qPCR) . In addition, the pepper mild mottle virus (PMMoV), which is commonly found in feces, was measured and applied to normalize the SARS-CoV-2 signal. Extensive QA/QC has been applied, including testing for RT-qPCR inhibition. In collaboration with the Ontario Ministry of the Environment Conservation and Parks, the Region of Waterloo, Peel Region, and York Region, a surveillance program was established in several communities in Southern Ontario. Sampling has occurred three to six days per week since the summer of 2020 and reported directly and rapidly to the public health teams and made available on several public dashboards. We used our foundation in the field of eDNA as our starting point and have furthered our understanding of how to accurately quantify specific, low-level nucleic acid targets in complex matrices. Furthermore, though there are techniques and theories that are unique to the fields of WBS and eDNA, we believe that there are similar goals and challenges in both fields and that both fields can benefit each other.

Rapid design, validation, and use of RT-qPCR for quantification of SARS-CoV-2 variants in wastewater (PO)

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Wastewater-based surveillance (WBS) has been widely used for detection of environmental DNA (eDNA) and chemicals in the ecosystem. During the COVID-19 pandemic, WBS was also applied to the indirect monitoring of SARS-CoV-2. Our group has been continuously developing variant-specific reverse transcription quantitative polymerase chain reaction (RT-qPCR) assays to track the frequency of multiple variants of concern (VOCs) in wastewater in near real time. By designing tailor-made probes for target mutations and an intra-amplicon universal probe, we were able to develop and validate novel multiplex RT-qPCR assays to accurately and rapidly estimate VOC frequencies in wastewater collected from several municipalities in Ontario. The assays not only trace different waves caused by Alpha, Delta, and Omicron variants, but also show similar trends when compared with clinical and wastewater whole genome sequencing (WGS) data from the communities. As the virus continues to mutate, these reliable RT-qPCR assays are effective tools for rapid and accurate estimation of VOC frequency, with the advantages of shorter turnaround time, covering larger portion of the population at a lower cost, and being more readily accessible than WGS. The concept of designing these assays can be

easily applied to develop other new assays to support research in the field of eDNA and ecotoxicity.

Comparing the partitioning behaviour of SARS-CoV-2, PMMoV, and surrogates in liquid and solid fractions of wastewater (PO)

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Surveillance of concentrations of SARS-CoV-2 in wastewater has become a vital resource for tracking the spread of COVID-19 in and among communities. In the absence of standardized methods, there continues to be a need to characterize how SARS-CoV-2 or its RNA fragments partition in wastewater as partitioning can affect the interpretation of data for public health efforts. To address this, the partitioning of SARS-CoV-2 was investigated in a series of experiments that were conducted using centrifugation with varied durations of spin and centrifugal force, polyethylene glycol (PEG) precipitation followed by centrifugation, and ultrafiltration of wastewater. The partitioning of the endogenous pepper mild mottled virus (PMMoV), used to normalize the SARS-CoV-2 signal for fecal load in trend analysis, was also examined. In addition, two surrogates for coronavirus, human coronavirus 229E and murine hepatitis virus, were analyzed as process controls. With a moderately hard centrifugation condition (12 000 g, 1.5 hours, no brake), the partitioning of SARS-CoV-2 was evenly split between the liquid and solid fractions of wastewater. Moreover, the surrogates were more reflective of SARS-CoV-2 under the same centrifugation condition. Unlike SARS-CoV-2 and the surrogates, PMMoV remained predominantly in the liquid fraction irrespective of the centrifugation conditions. Interestingly, ultrafiltration devices were inconsistent in estimating RNA copies in wastewater, which can influence the interpretation of partitioning. Overall, it is important to develop a better understanding of the fate of viruses in wastewater and to create a foundation of best practices to carry forward to prepare for future potential infectious diseases.

Wastewater surveillance of influenza (A, B) and respiratory syncytial virus (RSV) (PO)

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With the onset of the COVID-19 pandemic, wastewater-based surveillance (WBS) emerged as a potential technique to monitor the incidence and spread of the virus in

communities, as a complementary public health surveillance tool. Viral SARS-CoV-2 RNA fragments were successfully detected in wastewater in several countries and were found to strongly correlate with clinical cases. As wastewater surveillance of COVID-19 was demonstrated to be highly effective and valuable, it begs the question of its applicability in monitoring other detrimental respiratory viruses, such as influenza (A, B) virus and respiratory syncytial virus (RSV). The current testing program in Ontario for influenza and especially for RSV, may be severely underestimating the true number of cases. Just as with traditional detection of COVID-19, there is a lag between clinical detection and community spread. WBS is a tool independent of clinical testing that could provide valuable information on the spread of these viruses in communities. The goal of this study is to assess the capability of monitoring influenza (A, B) virus and RSV in wastewater, using similar methodology applied in the surveillance of SARS-CoV-2. This will be assessed by running quantitative polymerase chain reaction (qPCR) assays on weekly influent wastewater samples from September 2022 to June 2023, from the Kitchener and Waterloo wastewater treatment plants. The trends of influenza and RSV in wastewater will then be assessed and correlated with the clinical cases and/or hospitalizations reported by the Region of Waterloo Public Health. This data will also be regularly communicated to the Region of Waterloo public health team and could be used as a supplementary public health tool in management of respiratory diseases.

Enumeration potential of environmental DNA for Pacific salmon stock assessments (PO)

Geoffrey Su[1], Michael Allison[2], Jordan Beblow[3], Kevin Koch[3], Jeffrey Anderson[4], Leithen M'Gonigle[1], Mark Cleveland[3], Caren Helbing[2], Vicki Marlatt[1]

[1]Simon Fraser University, [2]University of Victoria, [3]Gitanyow Fisheries Authority, [4]Geomorphic Environmental Services

The field of environmental DNA (eDNA) has advanced over the past decade, with multiple approaches available for a variety of sampling media and species. While using eDNA for the purpose of simply detecting species is becoming a routine process, the utility of eDNA to estimate species abundance is poorly understood. Here, we quantify salmon environmental DNA along with river velocity, and together, use these values to determine the correlation between salmon numbers passing by a fish enumeration fence daily with daily eDNA rates before, during, and after the salmon spawning season for four Pacific salmonids (*Oncorhynchus gorbuscha*, *O. kisutch*, *O. tshawytscha*, *O. nerka*; pink, coho, Chinook, and sockeye respectively). Throughout the spawning season approximately 182 000 salmon were counted passing through the fence, of which >98% were pink salmon. Pink salmon exhibited a strong correlation between human counts (effect size = 0.65, SE = 0.040) and eDNA rates, and exhibited day-to day-variation and a unimodal profile rising and falling with human fish counts. The other three salmon species, observed in much lower numbers, exhibited weaker correlations. Thus, for salmon spawning runs with less

than ~1000 adults and daily counts less than ~100, the juvenile and/or prior seasons eDNA signal appears to be indistinguishable from the adult spawning eDNA signal in our river system. However, for the large pink salmon run, eDNA rates appeared to reflect a local signal of salmon in space and time, essentially tracking these fish within days of passing through the eDNA sampling site.

Ecotoxicology of Tire and Road Wear Particles and Related Contaminants – Where Are We Three Years After the Identification of 6PPD-quinone?

Toxicity of 6PPD-quinone across fishes of commercial, cultural, and ecological importance (PL)

Markus Hecker[1], Catherine Roberts[1], David Montgomery[1], Summer Selinger[1]

[1]*University of Saskatchewan*

Stormwater runoff from urban landscapes has recently been linked to mass mortalities of coho salmon in the U.S., also dubbed urban runoff mortality syndrome (UMRS). The chemical responsible has been identified as N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine-quinone (6PPD-Q), a transformation product of the rubber tire antioxidant 6PPD. This presentation summarizes a series of studies that assessed the acute toxicity of 6PPD-Q across eight other fishes of commercial, cultural, and ecological importance in North America, and to characterize the specific mechanisms that drive toxicity using acute and sub-chronic experiments with select fishes. Acute toxicity differed greatly among species: No mortality occurred for Arctic char (AC), brown trout, bull trout, westslope cutthroat trout, and white sturgeon even at the highest measured concentrations (>13 $\mu g \cdot L^{-1}$), while lake (LT), brook, and rainbow (RBT) trout were sensitivity to 6PPD-Q exposure with LC_{50} values between 0.33 and 0.59 $\mu g \cdot L^{-1}$. Furthermore, sub-chronic exposures of RBT and LT demonstrated teratogenic effects in both larval RBT and LT. Initial experiments with primary cultures of RBT gill cells suggest that the mechanism of toxicity may be related to uncoupling the mitochondrial electron transport chain. Comparative cardiac ultrasound, electrocardiography and blood gas analysis revealed significant decreases in hemoglobin oxygenation and sympathetic stimulation in sensitive RBT but not insensitive AC, further supporting this hypothesis. Whole transcriptome analysis in RBT identified several molecular toxicity pathways that may explain the apical effects described above. Further research supporting development of a comprehensive toxicity pathway model supporting risk assessment of 6PPD-Q across fishes is underway.

Comparison of the toxicity of 6PPD-quinone to early life stage rainbow trout and lake trout (PL)

Catherine Roberts[1], Evan Kohlman[1], Niteesh Jain[1], Junyi Lin[1], Mawuli Amekor[1], Alper James Alcaraz[1], Natacha Hogan[1], Markus Hecker[1], Markus Brinkmann[1]

[1]University of Saskatchewan

N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone) is a rubber- tire derivative which leaches into roadway runoff from tire particles. This compound has been identified as a possible driver of urban runoff mortality syndrome, wherein coho salmon experience, gasping, loss of coordination, and pre-spawn mortality. Sensitivity to this toxicant is highly variable across fish species, and with environmental concentrations meeting or exceeding toxicity thresholds, the potential for ecologically relevant effects on aquatic species is significant. Rainbow trout (*Oncorhynchus mykiss*, RBT) represent a model species which are known to be sensitive to 6PPD-quinone, and there are currently no data regarding the sensitivity of lake trout (*Salvelinus namaycush*, LT). As early life stages of fishes are typically more sensitive than adult, the goal of these studies was to evaluate the toxicity of 6PPD-quinone to early-life stage rainbow trout and lake trout. Alevins were exposed beginning at hatch, with RBT exposed to time-weighted average concentrations ranging from 0.06–2.35 $\mu g \cdot L^{-1}$ 6PPD-quinone, and LT to 0.22–13.5 $\mu g \cdot L^{-1}$ 6PPD-quinone. RBT exhibited a 28-day median lethal dose (LC_{50}) of 0.56 $\mu g \cdot L^{-1}$, and in LT, a 45-day LC_{50} of 0.33 $\mu g \cdot L^{-1}$. Subsequent acute studies with exogenously-feeding RBT and LT fry determined 96-hr LC_{50}s of 0.56 and 0.50 $\mu g \cdot L^{-1}$, respectively. Deformities throughout growth were observed in both species, with a unique caudal fin pathology of particular note. From this study, we can conclude that early-life stage RBT and LT alevins are sensitive to 6PPD-q, with older RBT and LT fry exhibiting greater acute sensitivity than sub-adult RBT.

Changes in the blood chemistry of brook trout (*Salvelinus fontinalis*) and Atlantic salmon (*Salmo salar*) after acute exposures to 6PPD-quinone (PL)

Danielle Philibert[1], Tillmann Benfey[2], Benjamin de Jourdan[1]

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The tire wear leachate, N-(1,3-dimethylbutyl)-N’-phenyl-p-phenylenediamine-quinone (6PPD-quinone) has been recently implicated as the contaminant responsible for the mass mortality of salmonids exposed to urban stormwater runoff. Of the species that have been tested, only a few salmonids were sensitive, and the mechanism of action deriving these differences in sensitivity are not well understood. To address this data gap, we conducted 24-hour exposures to a range of concentrations of 6PPD-quinone with juvenile brook trout and Atlantic salmon pre-smolts and compared changes in blood chemistry and gill tissue morphology. We also examined changes in Atlantic salmon blood parameters in smolting

fish exposed to 6PPD-quinone for 3 hours, and then moved to seawater. Blood parameters were measured using an i-STAT blood analyzer CHEM8+ and CG4+ cartridges, covering a suite of analytes used to measure ion dysregulation. Hematocrit was measured in a larger subset of fish with glass capillary tubes, and gill structure changes were examined using histology. Exposure concentrations were measured at the start and end of each exposure. Histological examination of the gill filaments revealed morphological changes consistent with mounting a response to osmotic dysregulation. Ion dysregulation and gill inflammation appear to be contributing factors in 6PPD-quinone toxicity to juvenile brook trout. No ion dysregulation effects were observed in the pre-smolt salmon, however in the smolting trial there were increased oxygen concentrations in the blood of exposed smolts. The changes in blood chemistry and gill morphology we observed support ion dysregulation as a contributing mechanism of action of 6PPD-quinone in sensitive salmonid species.

Comparative analysis of cardiometabolic effects of 6PPD-quinone on juvenile salmonids (PL)

Summer Selinger[1], David Montgomery[1], Markus Hecker[1], Steve Wiseman[2], Lynn Weber[1], David Janz[1], Markus Brinkmann[1]

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

N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone) is a transformation product of the most widely used rubber tire antioxidant, 6PPD. Commonly found in road-way runoff, this compound has been reported to cause acute lethality at roughly ≤1 ug·L^{-1} in a variety of salmonid species. However, additional studies have shown other salmonid species to be insensitive, even at significantly greater concentrations (20 ug·L^{-1}). Sensitive species show distinctive symptoms including gasping, spiraling, increased ventilation, and loss of equilibrium, suggesting a possible impact on cardiorespiratory physiology. Here, we investigated the acute cardiorespiratory effects of 6PPD-quinone to two salmonids of differing sensitivity: a sensitive species, rainbow trout, and a tolerant species, Arctic char. Fish were exposed to 1 or 10 ug·L^{-1} 6PPD-quinone in respirometry chambers for 48 hours to assess changes in resting oxygen consumption. Following exposure, cardiac ultrasound, electrocardiography, and blood gas analyses were used to analyze changes to the cardiovascular system. Data show that 6PPD-quinone exposure does not significantly impact oxygen consumption rates in either species. In rainbow trout, an increase in passive ventricular filling and cardiac output and a decrease in end systolic volume and PR interval length were observed, providing evidence of sympathetic stimulation. Symptoms observed following rainbow trout exposure might partly be driven by a significant increase in methemoglobin, resulting in an impaired ability to oxygenate the tissues. This is the first study to analyze the toxicity of 6PPD-quinone to the cardiorespiratory system of fishes at environmentally relevant concentrations, and provides information invaluable to understanding the mechanism of toxicity.

Unraveling the molecular mechanism of 6PPD-quinone toxicity using whole transcriptome analysis in lake trout (PL)

Junyi Lin[1], Catherine Roberts[1], Evan Kohlman[1], Mawuli Amekor[1], Niteesh Jain[1], Alper James Alcaraz[1], Natacha Hogan[1], Markus Brinkmann[1], Markus Hecker[1]

[1]*University of Saskatchewan*

Conventional chemical hazard assessment heavily relies on costly, time-consuming, and ethically questionable live-animal testing. However, these methods fail to catch up with the global need to test the growing number of contaminants of emerging concern. For instance, N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone, commonly known as 6PPD-quinone, has recently been identified as a cause of acute mortality in coho salmon exposed to urban runoff. Interestingly, 6PPD-quinone exhibits a wide range of species sensitivities among fishes, and the underlying mechanism of toxicity remains unknown. This study utilizes whole transcriptome analysis to investigate the toxicity of 6PPD-quinone in early life stages of lake trout (*Salvelinus namaycush*), a sensitive salmonid for which the molecular effects of 6PPD-quinone are still unknown. The use of early life stages of fish prior to independent exogenous feeding, which are not considered live animals under current legislations, has the advantage of being the most sensitive stage in the fish life cycle. This approach aims to identify key gene expression patterns and to derive transcriptomics points of departures (tPODs) associated with 6PPD-quinone toxicity in lake trout. Furthermore, we will construct a putative mechanistic pathway of 6PPD-quinone toxicity in lake trout by linking apical and physiological responses in a parallel study with the transcriptomic responses. Through this research, we aim to identify molecular processes driving species sensitivity differences caused by 6PPD-quinone.

6PPD-quinone poses a low risk to freshwater invertebrate populations (PL)

Jack Salole[1], Ryan Prosser[1]

[1]*University of Guelph*

Tire wear particles are a common source of microplastic pollution to surface waters. 6PPD-quinone [N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone] is a chemical found in tire wear particles that has recently been recognized as the cause of urban runoff mortality syndrome. This phenomenon results in the mass mortality of coho salmon in urban watersheds. As 6PPD-quinone has since been detected in several waterbodies at sufficient levels to cause these effects, assessing the toxicity and exposure to other species is an important step in determining the risk this compound may pose to aquatic ecosystems. In this study, *Daphnia magna* and *Hexagenia* spp. were exposed to 6PPD-quinone for 21-day and 96-hour exposures, respectively. Road runoff samples were also collected around Guelph, Ontario to assess potential exposure to aquatic ecosystems in Southern Ontario. Neither organism showed any sign of toxicity at relatively high

concentrations of 6PPD-quinone (55 $\mu g \cdot L^{-1}$ and 306 $\mu g \cdot L^{-1}$, respectively). The concentration of 6PPD-quinone in road runoff in the Guelph area ranged from 0.0603 to 0.262 $\mu g \cdot L^{-1}$, which is considerably lower than concentrations reported in the literature. The relatively low exposure measured in road runoff around Guelph combined with the relatively low toxicity of the two test invertebrate species indicates that 6PPD-quinone likely poses a low risk to freshwater invertebrate populations.

Acute toxicity of 6PPD-quinone on *Planorbella pilsbryi* and *Megalonaias nervosa* (PL)

Spencer Hang[1], Jack Salole[1], Ryan Prosser[1]

[1]*University of Guelph*

Past studies have shown that N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone) is acutely toxic to coho salmon at extremely low concentrations and is the cause of mortality during runoff events on the west coast of the United States. Other studies have generated toxicity data for this chemical but they have been limited to fish species. The goal of this study was to fill the gap in our knowledge on toxicity to aquatic invertebrates. In this study, acute toxicity tests were conducted on *Planorbella pilsbryi* (file ramshorn snail) embryos and adult *Megalonaias nervosa* (washboard mussel). Snail egg masses were exposed to various concentration of 6PPD-quinone for 24 hours and mortality was measured over 10 days. Adult washboard mussels were exposed for 24 hours, observed daily over a period of 8 days for mortality, and then kept under observation for 4 months post-acute testing to determine any chronic toxicity effects. No mortalities occurred in any treatment during either the acute or chronic toxicity tests. These results suggest that freshwater mollusks have a lower sensitivity to 6PPD-quinone than the fish species. Road runoff was also sampled during rainstorm events across Guelph, Ontario and analyzed for 6PPD-quinone content. 6PPD-quinone was detected in all samples but the concentration was considerably lower than the concentrations used to assess toxicity to freshwater mollusks.

Identifying and characterizing tire-related chemical (6PPD-quinone) toxic hotspots in salmon habitat in British Columbia, Canada (PL)

Tanya Brown[1,2], Sofya Reger[1], Xiangjun Liao[1], Carys Gallilee[1]

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

N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone) has been found to be an important cause of toxic injury and death of adult coho salmon returning to urban and semi-urban waterways along the west coast of North America. Over the past year, we have been working to characterize the presence of this highly toxic breakdown product from vehicular tires at 40 sites in salmon-bearing creeks in the greater

Vancouver area, Squamish, and Vancouver Island before, during, and after rain events across four seasons. The sample collection has been carried out with help from World Fisheries Trust, Peninsula Streams Society, Pacific Streamkeepers, Tsolum River Restoration Society, Redd Fish, BC Conservation Foundation, and the Musqueam, Tsleil-Waututh, Tsawwassen, and Cowichan First Nations. Preliminary data from the Vancouver area in 2022 showed significantly higher levels of 6PPD-quinone in creeks during rainfall events, with levels approaching or exceeding concentrations known to be lethal in coho salmon. A hotspot map has been generated to provide initial targeted recommendations for the implementation of green infrastructure that may serve to protect salmon in urban waterways. This project is delivering new data on 6PPD-quinone levels in fish habitat in coastal British Columbia, and is informing the protection and recovery of Pacific salmon populations.

Interlaboratory study on analytical methods for aqueous 6PPD-quinone – on the path towards comparability (PL)

Sarah Marteinson[1], Markus Brinkmann[2], Tanya Brown[1], Richard Grace[3], Xiangjun Liao[1], Harold Malle[4], Danielle Philibert[5], Sarah Pomfret[4], Remy Tadonleke[1], Amanda Winegardner[1], Million Woudeneh[3]

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N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone) was recently identified as the contaminant responsible for road runoff mortality syndrome in coho salmon, which had been observed unexplained for 20 years. To respond to this discovery and urgent environmental issue, research to determine aquatic concentrations globally has accelerated and many distinct projects are underway to evaluate its toxicity to multiple species including other salmonids, and to characterize its mechanisms of action. As part of these projects, analytical methods are simultaneously being developed or refined to measure 6PPD-quinone and there is now a need to determine how they compare. This is critical in the immediate for the interpretation of currently available evidence, but is also an important first step towards the development of comparable methods that will ultimately ensure compatibility of results between studies. The objective of this project is to evaluate the proficiency of multiple laboratories for the analysis of 6PPD-quinone in natural water through standard proficiency testing methodology employed by Information and Quality Management (Environment and Climate Change Canada), which will respond to a priority of the National Contaminants Advisory Group (Fisheries and Oceans Canada). Initially, water will be collected from multiple locations in Canada, timed to follow rain events and capture the highest possible environmentally relevant concentrations ($<2 ug \cdot L^{-1}$). A series of test solutions will be generated over a range of concentrations and stored under optimal conditions. These will be shipped to voluntary participants who will submit the resulting data for analysis. The purpose of this presentation is to outline the

planned methodology but also to promote awareness for the project as increased participation will strengthen the meaningfulness and interpretability of results for all laboratories.

Use of toxicity identification techniques to isolate causes of toxicity in stormwaters in British Columbia: Case studies with zinc and 6PPD-Q (PO)

Josh Baker[1], Ian Cronshaw[1], Joseph Monaghan[2], Erik Krogh[2]

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As prolonged droughts and extreme rain events are expected to increase as a result of climate change, the monitoring of stormwaters during rain events directly following dry periods becomes of heightened concern. Of particular concern is concentrated road runoff, which has been found to be acutely lethal to salmonids due to the environmental transformation of a tire preservative to N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-Q). In this study, stormwaters were collected in the Greater Vancouver area of British Columbia, Canada and assessed for acute lethality to rainbow trout (*Oncorhynchus mykiss*). The cause of toxicity in the samples that exhibited acute lethality was characterized using Toxicity Identification Evaluation (TIE) techniques. Zinc was determined to be the cause of acute lethality in one of the stormwaters while 6PPD-Q was suspected to be the cause in another; supporting analytical and toxicity testing was subsequently conducted with zinc and 6PPD-Q. TIE techniques were applied to a solution containing 6PPD-Q to provide a toxicity signature for 6PPD-Q under this framework.

Investigating environmental sources of the toxic tire-derived chemical 6PPD-Q (PO)

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[1]University of Saskatchewan

In 2021, mass mortalities of coho salmon were linked to N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-Q), a transformation product formed from an antioxidant in rubber tires. Since then, the focus of scientific efforts concerning contamination pathways has been on paved roads. However, about half of the road network in Canada consists of dirt roads. Additionally, recycled tire products are becoming common with end uses including landscaping and sports fields. We seek to understand (1) the potential for unpaved road networks to act as a source for 6PPD-Q and (2) the potential for recycled tire products to leach 6PPD-Q under environmental conditions. To investigate this, we collected dirt from the surface of unpaved roads around Saskatoon. Chemical extractions and 72-hour leachate studies will be performed on the collected road surface to detect and quantify potential 6PPD-Q concentrations in leachate. We also performed a

leaching study to detect the potential for recycled tire products to leach 6PPD-Q under environmental conditions. To this end, we are collecting rainwater and meltwater that has infiltrated through a layer of rubber crumb or rubber mulch, then quantifying the 6PPD-Q concentrations in the leachate. During these studies, we expect to quantify environmentally relevant concentrations of 6PPD-Q in the collected leachate and road dirt. Preliminary results from the recycled tire product study suggests that the concentrations found in the leachate exceed the median lethal concentration for various salmonids. The results of these novel studies will fill a critical gap in our knowledge and understanding of environmental sources of 6PPD-Q.

A histological assessment of 6PPD-quinone exposed juvenile coho and Chinook salmon (PO)

Bonnie Lo[1,2], Vicki Marlatt[1], Gary Marty[3], Tanya Brown[2]

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N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone), an ozonation product of the tire rubber antioxidant 6PPD, has been identified as an important driver of urban stormwater runoff mortality syndrome. This phenomenon affects adult coho salmon (*Oncorhynchus kisutch*) upon entry into urban streams and results in high rates of pre-spawn mortality. Studies of 6PPD-quinone toxicity have revealed a significant range in species sensitivity among fish, including Chinook (*Oncorhynchus tshawytscha*) and other Pacific salmon. Both etiology and sublethal effects of 6PPD-quinone are not well understood, although cardiorespiratory and neurological effects have been suggested in response to the documented physiological and behavioural symptoms. Additionally, disruption of the blood–brain barrier in coho and mitochondrial respiration in rainbow trout gill cell lines have been reported. To further explore the sublethal effects of 6PPD-quinone, juvenile coho and Chinook salmon were exposed to a range of concentrations for 24 hours and individuals were histologically assessed. The presence/absence of tissue and cell anomalies were observed and scored for severity. Results from this research will provide insight into organ level effects of 6PPD-quinone as well as enable a deeper exploration of the sublethal impacts across species.

Exposure, Accumulation and Effects of Radionuclides

Comparison of radium-226 separation methods based on chromatographic and extraction resins for its determination by ICP-MS in environmental matrices (PL)

Marine Roulier[1], Laurie Martin[1], Dominic Larivière[1]

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The longer-lived radium isotopes ^{226}Ra ($t_{1/2}$ = 1600 years) and ^{228}Ra ($t_{1/2}$ = 5.75 years) are ubiquitous in the environment. Because ingested radium can substitute for calcium ions in biological systems, ^{226}Ra and ^{228}Ra radiotoxicity is of concern for health safety. The WHO has defined a limit of 1 $Bq \cdot L^{-1}$ for drinking water. At these low levels (ultra-trace), its analysis by inductively coupled plasma mass spectrometry (ICP-MS) requires separation and preconcentration of ^{226}Ra prior to its analysis. Several methodologies have been developed for this purpose, based on resin separations. While their analytical performances have been reported individually, their direct and accurate comparison based only on the literature data is quite impossible due to various sample conditions (matrix, range of ^{226}Ra concentrations) and different analytical instruments or parameters (ICP-MS, ICP-MS/MS, SF-ICP-MS, Apex-Q-ACM) used. Therefore, we selected five methodologies based on various resins (AG50W-X8, AnaLig Ra-01, Sr-Spec), loading (samples volume and pH, flow-rate), rinse and elution (acid/basic) conditions. We applied these methodologies from the same certified waters and analyzed ^{226}Ra concentrations in the final samples by ICP-MS according to the same instrumental parameters. Thereby, we were able to adequately compare them in terms of analytical figures of merit (recovery yield, enrichment factor, method limits, sensitivity, accuracy, turnaround time, cost). This work provides information for selecting the appropriate methodology according to the individual requirements of performance and cost.

Accumulation of radium-226 in three microalgal species and its effect of growth (PL)

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Canada is one of the world's leading producers of uranium, a radioisotope that can disintegrate into radium-226 (Ra-226). Uranium is also naturally present in many residues and tailings from other mining activities which can result in the release of Ra-226 into surface waters. However, there is little information on the toxicity of Ra-226 to aquatic organisms. In order to evaluate the potential risks of radium transfer in aquatic food webs, it is crucial to study its bioaccumulation in phytoplankton. The toxicity and bioaccumulation of radium was therefore determined in three species of microalgae

(*Chlamydomonas reinhardtii*, *Chlorella fusca*, *Raphidocelis subcapitata*). To determine the effects of Ra-226, microalgae were grown for 72 hours in MHSM-1 medium with a pH adjusted to 7 with different doses of Ra-226 (0.5, 5, 10, 50, and 100 $Bq \cdot L^{-1}$). Furthermore, for *Chlamydomonas reinhardtii*, 50 $Bq \cdot L^{-1}$ exposures were carried out at different calcium concentrations (0.05, 1, 1.5, and 2 mM), to determine whether calcium can have an effect on Ra-226 bioaccumulation. The results obtained highlight differences in radium accumulation and effects between the three algae species. Subsequently, the fate of radium in the cell and its impact on genes will be studied in *Chlamydomonas reinhardtii*, in order to gain a better understanding of the effects of Ra-226 on a molecular scale.

Chronic radium-226 toxicity to and bioaccumulation in the aquatic invertebrate *Daphnia magna* (PL)

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

[1]*University of Saskatchewan*

Mining and milling of uranium ore generates by-products that contain radionuclides such as radium-226 (^{226}Ra), for which Canada does not have a water quality guideline for protection of aquatic life. This project aimed to generate data on ^{226}Ra toxicity to the aquatic invertebrate *Daphnia magna*. First, two 21-day chronic toxicity tests were conducted using an OECD protocol and nominal treatments of 5, 16, and 50 $Bq \cdot L^{-1}$, and 16, 24, and 36 $Bq \cdot L^{-1}$. Results showed that 50 $Bq \cdot L^{-1}$ caused 100% mortality within 2 days of exposure, while the other activity concentrations had no significant effects on survival or reproduction, suggesting a toxicity threshold between nominal activities of 36 and 50 $Bq \cdot L^{-1}$ of ^{226}Ra. Subsequently, a transgenerational study was conducted following the same methods repeated over two consecutive generations, with treatments of 24, 36, and 48 $Bq \cdot L^{-1}$. Results revealed no transgenerational effects and no significant effects on reproduction, but generated nominal LC_{50} values of 35.1 and 33.7 $Bq \cdot L^{-1}$ for the F0 and F1 generations, respectively. Finally, a study on ^{226}Ra bioaccumulation was similarly conducted over 21 days using a single treatment of 24 $Bq \cdot L^{-1}$ and reconstituted water at a hardness of 80 $mg \cdot L^{-1}$ as $CaCO_3$. Results yielded a bioaccumulation factor of 72.8 for ^{226}Ra in *D. magna*. These results show that ^{226}Ra is bioaccumulative and can have significant effects on survival of *D. magna* at high activity concentrations. However, the MDMER effluent limit is 0.37 $Bq \cdot L^{-1}$, suggesting that adverse effects on zooplankton survival and reproduction seem unlikely downstream of uranium operations.

Radium-226 water quality guidelines 14-day water-only toxicity test with Twin Lakes *Hyalella azteca* (PL)

Francesca Farrow[1], Amy Festarini[1], Alex Hancock[1], Marilyne Stuart[1]

[1]*Canadian Nuclear Laboratories*

Scientifically-based water quality criteria for radium-226 are lacking. This absence of recognized defensible criteria and the resultant reliance on radiological dose modelling have been perceived by the public and some governmental appointed panels as evidence of a significant knowledge gap threatening safe regulation. Canadian Nuclear Laboratories' Chalk River Laboratories Toxicity Laboratory (CRLTL) studied the effects of radium-226 on *Hyalella azteca* inhabiting low-ionic strength waters. The culturing water spiked to create the test water had a conductivity of approximately 65 $\mu S \cdot cm^{-1}$, once spiked and neutralized the conductivity of the test water was approximately 200 $\mu S \cdot cm^{-1}$. The CRLTL is able to test with low-ionic strength water without causing added stress to the organisms because it initiated a culture of *H. azteca* by harvesting the amphipods from a local low-ionic strength waterbody, Twin Lakes, at Chalk River Laboratories. This enables the CRLTL to perform toxicity tests reflective of waterbodies across the Canadian Shield. The CRLTL performed a 14-day water-only toxicity test according to Environment and Climate Change Canada's biological test method to assess the effects of radium-226 concentrations ranging from 1 $Bq \cdot L^{-1}$ to 1750 $Bq \cdot L^{-1}$ on survival and growth. No detrimental effects on survival or growth were observed within this radium-226 activity concentration range. Reported environmental Ra-226 levels are typically <10 $Bq \cdot L^{-1}$.

Chronic toxicity of radium-226 to fingernail clams (PL)

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

[1]*University of Ottawa,* [2]*Kilgour & Associates Ltd.,* [3]*Natural Resources Canada*

In Canadian surface waters, background activity concentrations of Radium-226 (Ra-226) are low and relatively narrow. However, several industrial activities such as the mining and milling of uranium can expose or concentrate Ra-226, enhancing radionuclide levels. The objective of this present study was to generate Ra-226 sublethal toxicity and bioaccumulation data using fingernail clams. We conducted a 28-day chronic exposure experiment in which *Sphaerium striatinum* were exposed to a control and seven concentrations of Ra-226 (0.1, 0.5, 1.0, 5, 10, 25, and 50 $Bq \cdot L^{-1}$). Toxicity endpoints assessed at the end of the experiment included survival, growth, burrowing behaviour, and reproduction. Survival did not differ significantly across treatments, with negligible mortalities occurring across all replicates (< 12%). Growth, assessed using total shell length, differed significantly between the control treatment and both the 25 $Bq \cdot L^{-1}$ and 50 $Bq \cdot L^{-1}$ treatments. Reproduction, assessed using brood size, differed significantly between the control treatment and the 5, 10, and 50 $Bq \cdot L^{-1}$ treatments. Both growth and

reproduction were reduced with Ra-226 exposure. Burrowing behaviour also seemed to decrease with increasing Ra-226 concentrations, along with the day of the chronic test. Fingernail clam tissue and shell samples were collected to assess the bioaccumulation of Ra-226 in tissues. Ra-226 speciation in water and sorption to substrate will be modeled using the Windermere Humic Aqueous Model (WHAM). The results of this study provide new insights into the toxic effects of Ra-226 to freshwater organisms and will help derive a federal water quality guideline for the protection of aquatic life.

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

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The Anthropocene is associated with profound changes in ecosystems, including an increase in the presence of radioactive elements in natural waters. Among these elements, there is radium-226 (^{226}Ra), which is released during activities such as mining and milling of uranium ores, or extraction of coal, oil, and gas. To date, no Canadian water quality guideline for the protection of aquatic life exists to guide environmental managers regarding the presence of this element in fresh waters. To address this gap, it is necessary to collect ^{226}Ra toxicity data with Canadian freshwater species. In this study, we are testing ^{226}Ra toxicity using the great pond snail (*Lymnaea stagnalis*), a pulmonate snail that is widespread in Canadian fresh waters and sensitive to trace metals. It is expected that this calciphilic organism will take up ^{226}Ra in large amounts, since ^{226}Ra is a calcium analog. To verify our hypothesis, we exposed two early life stages of *L. stagnalis* (embryos and juveniles) to increasing aqueous doses of ^{226}Ra, and measured ^{226}Ra bioaccumulation in tissues, as well as snail growth, hatching success, and survival. The embryonic toxicity test shows that ^{226}Ra reduces hatching rate without affecting growth. At the juvenile stage, we expect ^{226}Ra-exposed snails to have reduced growth with large amounts of ^{226}Ra accumulated in the shell. This research project aims to better understand the impact of ^{226}Ra on *L. stagnalis* and to improve water quality standards for the protection of aquatic life in Canada.

Low radium-226 doses increase growth and condition in rainbow trout (*Oncorhynchus mykiss*) fry at swim-up (PL)

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While industry complies with strict discharge limits for radium (^{226}Ra) in mining effluents, a Canadian federal water quality guideline that protects aquatic biota from hazardous ^{226}Ra exposure is not in place, bringing into question the condition of current

fish populations. To understand ^{226}Ra toxicity in fish, we performed chronic early life stage exposures with rainbow trout using a modified Environment and Climate Change Canada biological test method (EPS 1/RM/28) and a series of ^{226}Ra activities (0, 0.1, 0.5, 2.5, 12.5, or 62.5 $Bq \cdot L^{-1}$). Under a static renewal design, embryos were exposed to ^{226}Ra in reconstituted very soft water (10–13 $mg \cdot L^{-1}$ as $CaCO_3$), representing high bioavailability conditions, with the aim of identifying sensitive toxicity endpoints. To date, the results indicate that exposure to ^{226}Ra did not affect rainbow trout survival or the cumulative time (in degree-days) that it took embryos to develop to the eyed stage, hatch, or swim-up. However, a significant interaction ($p<0.05$) was detected between ^{226}Ra activity and time to swim-up, where increases in mass, length, and the scaled mass index (SMI) were detected at lower activities of 0.1 and 0.5 $Bq \cdot L^{-1}$ as exposure time increased. Overall, the no observed effect concentration (NOEC) for mortality and developmental endpoints was 62.5 $Bq \cdot L^{-1}$, while the lowest observed effect concentrations (LOEC) for length and mass was 0.1 $Bq \cdot L^{-1}$ and SMI, 0.5 $Bq \cdot L^{-1}$. At present, these data have potential implications for the current mining effluent discharge limit for ^{226}Ra in Canada, which is set at 0.37 $Bq \cdot L^{-1}$ total ^{226}Ra as the maximum authorized monthly mean concentration.

Baseline environmental monitoring program for a deep geological repository (PL)

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The Nuclear Waste Management Organization (NWMO) is responsible for the long-term management of Canada's nuclear waste. The Adaptive Phased Management (APM) Project includes a siting process for the Deep Geological Repository (DGR) and associated infrastructure. There are two remaining sites being considered as informed and willing hosts for the Project. An Environmental Media Baseline Program (EMBP) was initiated at both of these sites to collect baseline environmental data to understand the current conditions. The first step was to develop a Conceptual Site Model of the biophysical environment that outlined how the Project and the environment could interact. A scientifically-robust EMBP was then designed that included engagement with rights-holders and local stakeholders, input from academic advisors, and innovative approaches. A wide range of parameters are included in the EMBP, including general chemistry, metals and trace elements (an expanded list that includes rhodium, ruthenium, samarium), and organic compounds, as well as radionuclides. Samples were analyzed for radionuclides that may be associated with the project (e.g., H-3, C-14) as well as natural radionuclides (e.g., K-40, U-238), and some limited data on artificial radionuclides (e.g., Np-237, Pu-238). The program includes different media and is designed to extend over multiple years in order to understand natural variability. Results of the first year for surface water are available and these will be presented to the community. The program is meant to be reviewed

periodically and adjusted to respond to input from the local community and meet the needs of the Project.

Extended Abstract/Short Paper

Preface: As is customary for the Canadian Ecotoxicity Workshop, authors are invited to submit extended abstracts or short papers for inclusion in the Proceedings. These extended abstracts or short papers are accepted as-is and are not peer reviewed.

The implications of science communication in reporting the risks posed by selenium in Canadian coal mine effluents

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ABSTRACT

The future of the coal mining industry is receiving intense scrutiny in western Canada. Among the issues discussed by opponents of coal mining are environmental concerns about the significance of elevated selenium in surface water bodies near mine sites. This paper briefly reviews the current understanding of selenium ecotoxicology and risk to aquatic life and the communication on risk that the news media and opponents have adopted with the public when addressing the selenium issue. Canadian coal mining policy has been influenced by the public's misunderstanding of the threat of selenium in coal deposits, coal processing, and releases to the aquatic environment. We find that the claims made by opponents when reporting scientific information about selenium in western Canada have unnecessarily alarmed the public and raised fears unwarranted by the current scientific understanding of selenium. Most claims are not supported by scientific research and environmental monitoring studies. In the absence of accurate communication of selenium science and environmental risks, there is a high possibility that misinformation, intentional or otherwise, will unduly influence decisions about the future of Canadian mining policy. While several environmental concerns associated with coal mining, including selenium, need open and public debate, we advocate for the implementation of regulatory decisions informed by credible scientific evidence. In this paper, we review several key concerns raised about selenium releases from coal mining from a scientific viewpoint.

KEYWORDS: Aquatic Effects, Coal Mining, Effluents, Public Opinion, Selenium.

INTRODUCTION

Selenium is a naturally-occurring metalloid in the Earth's crust and is found in sedimentary bedrock worldwide (Sobolev et al. 2020); it has long been recognized as particularly concentrated in the soils of arid regions where the soil tends to be more alkaline. In North

America, selenium has been reported in high concentrations across the Great Plains, the Prairie Provinces of Canada, westward to the Pacific, and south into Mexico (NRC 1983). It is especially concentrated in the soils of Wyoming and South Dakota, and the western provinces of Canada. Selenium naturally finds its way into the soil and surface water ecosystems, then into plants and animals, and eventually into the people who eat those plants and animals. Selenium is a trace mineral widely considered by public health organizations as an essential dietary supplement at trace levels for human and animal health (Rayman 2000; Pecoraro et al. 2022). Selenium treatment of crops and grazing lands is encouraged in some agricultural regions in which selenium is naturally low to guard against selenium deficiency (Gupta and Gupta 2000).

Selenium occurs in coal in small amounts (Coleman et al. 1993; Yudovich and Ketris 2006). The potential for selenium to enter the environment principally occurs when coal-bearing strata, associated ore, and waste rock are exposed to air and water during mining and processing (Gearson et al. 2022). As a result, coal mines typically generate leachates from ore bodies and the waste rock that can mobilize selenium. These waste streams currently require treatment prior to release. The fate and effects of selenium in aquatic environments have been well studied following historical releases from agricultural drainage and fly ash that resulted in bird and fish kills (Ohlendorf et al., 2011; Cumbie and Van Horn, 1978). Comprehensive reviews of selenium physical and chemical properties, environmental behaviour, ecotoxicity, and treatment technologies are available elsewhere (Hamilton 2004; Yudovich and Ketris 2006; Tan et al. 2016; Saha et al. 2017).

Coal mining has a long history in Canada, beginning with the excavation of seaside cliffs in Nova Scotia in the 1600s (Muise and Macintosh 1996). Alberta's first commercial coal mine opened in 1864 on the Oldman River near present-day Lethbridge and by the early 1900's, coal mining was a major industry in Alberta and British Columbia (Piper and Green 2017). The first government policies addressing environmental and land use considerations applicable to coal mining were developed by the western provinces and the federal government in the 1970s (e.g., Alberta's 1976 Coal Policy).

Canada's coal mining industry is under intense scrutiny. P*olicy* pronouncements by the *Canadian* government in 2021 indicate a reluctance to approve permits for new thermal *coal mines (ECCC 2021)*. Alberta's recommendations for the future management of coal resources emphasize the need for public *consultation* on coal mining along the province's Eastern Slopes and other regions in which mining significantly impacts local communities and the economy (Coal Policy Committee 2021). Tens of thousands of Western Canadians spoke up when the Alberta Government rescinded the *1976* Coal Policy in May of 2020. In February of 2021, when its long-standing policy was reinstated, the provincial government announced the formation of a committee representing a cross-section of stakeholders charged with engaging Albertans on the future of coal mining in the province (Coal Policy Committee 2021). The committee's final Coal Policy Consultation Report released in December 2021 contained eight principal recommendations for the long-term management of Alberta's coal resources (Coal Policy Committee 2021). Throughout the Committee's public engagement process and deliberations, the public's attention has been repeatedly drawn to the environmental and cultural issues that led to the provincial government's

rejection in 2021 of an Environmental Certificate supporting the Grassy Mountain Metallurgical Coal Project. Opponents cited concerns for surface water quality (Lemly 2019), the impacts on key aquatic and terrestrial species such as the westslope cutthroat trout, whitebark pine, and little brown bat, as well as the loss of lands used for traditional activities by First Nations. Similar environmental concerns related to elevated selenium levels in surface waters downstream of coal mines, have led to the rejection of the environmental assessment certificate for the Sukunka Metallurgical Coal Project in 2023, and have impeded the progress on approvals of other coal mine development projects.

During this same period, selenium and other environmental issues involving coal mining operations have been the focus of the Canadian federal government's *proposed Coal Mining Effluent Regulations* (*CMERs*) – the first of their kind in Canada (ECCC 2022). The CMERs will specify national effluent quality limits for selenium, nitrate and total suspended solids, in addition to requirements for environmental effects monitoring. Once promulgated, the regulations will apply to the 28 existing coal mines in Canada and all future mine expansions and new coal mine projects.

The Alberta and British Columbia governments' rejections of the Grassy Mountain and Sukunka metallurgical coal mining projects and the federal government's deliberations on national *CMER*s have prompted considerable news reporting about selenium, which have had a significant impact on Canadian environmental policy and regulations affecting the future of coal mining in Canada. Among the issues receiving a high level of attention was the renewed focus on the environmental significance of elevated selenium in surface water bodies near coal mine sites. Selenium was only one of many issues associated with these rejections. This issue was studied extensively in the early 2000s when concerns were first raised by both the provincial and federal governments (Chapman et al. 2009).

This paper briefly reviews the current understanding of selenium aquatic ecotoxicology and the risk communication approach that coal mine opponents, including the news media, have adopted in their reporting. The accuracy of science communications in the news reporting about selenium and its environmental risks has the potential, intentional or otherwise, to influence decisions about the future of Canadian mining policy. This brief review concludes that credible and accurate scientific reporting is needed to inform the regulatory decision-making process.

SELENIUM MANAGEMENT AT COAL MINING SITES

The main issue associated with coal mining and selenium is the oxidation of selenium in rock piles to soluble forms (primarily SeO_3 and SeO_4) and leaching into nearby surface waters (Ziemkiewicz et al., 2011). Most coal mining practices, including those conducted in Alberta and British Columbia, involve the collection and treatment of leachate and wastewater containing elevated selenium (Golder 2020). Several technologies for controlling metal leaching and acid rock drainage apply to selenium management at coal mines (Naidau et al. 2019; Skousen et al. 2019). Collection systems to capture surface water runoff and leachate from historical waste rock piles are common, and new approaches involve landform design to manage mining rock piles and improve the collection and

treatment of water, and, in some cases, can entirely avoid or minimize runoff (McKenna 2009; McKenna and Gilron 2021).

Potential adverse effects can be avoided if adequate steps are taken to prevent or reduce hazards by controlling or treating the source. The approach that Lemly (2007) has described for assessing selenium hazards and developing environmental management plans for coal mining in the US is comparable to the approach followed more recently in Canada (Lemly 2014, 2019). This involves conducting a detailed environmental baseline assessment at proposed coal mining sites, preparing a comprehensive water management plan, and then matching the mine's operational parameters with environmental protection measures.

SELENIUM IN THE ENVIRONMENT

Much is known about selenium in soils, surface waters, and plants in aquatic and terrestrial ecosystems surrounding coal mines in Canada and the US (Chapman et al. 2010; Etteieb et al. 2020). A large body of knowledge and considerable progress has been made in mine management and selenium control and treatment technologies (Tan et al. 2016).

Two incidents of selenium contamination have received the most attention in news reporting and opposition analysis of coal mining. Selenium in freshwater fish at Belews Lake in North Carolina represents one of the most extensive and prolonged incidents of contamination associated with the release of coal-fired power plant wastewater (Lemly 2002; Browne and Lutz 2010). The second incident, at Kesterson Reservoir and Kesterson National Wildlife Refuge in California, involved high levels of selenium that leached from irrigated soils and accumulated in agricultural drainage water and evaporation ponds as the primary cause of the high rates of mortality and deformities among aquatic birds (Ohlendorf et al. 1989; Ohlendorf et al. 2020).

In both cases, selenium concentrations exceeded 100 μg/L in surface water, resulting in high concentrations in aquatic plants and prey consumed by fish and wildlife, and elevated concentrations in fish and aquatic bird eggs. The primary route of exposure determined from field studies was dietary consumption of prey in the food chain, rather than direct exposure to the surface water (Chapman et al. 2010). This is consistent with research reported by Presser and Luoma (2010) and Adams et al. (2000), concluding that diet is the main route of exposure to selenium in aquatic ecosystems.

NEWS REPORTING ON SELENIUM

During the past ten years, the coal mining industry has been the focus of nearly 1,000 news stories published in Canada. Of those news stories, nearly one-third (i.e., 225) have focused on the presence of selenium in the Canadian environment and its relationship to coal mining. The remaining two-thirds of news reports have focused on the impacts of the industry on regional and national economies, environmental quality, social and cultural issues, climate change, and energy consumption. Examples of news stories addressing selenium in the coal mining industry in western Canada are presented in Table 1.

Table 1. Examples of recent media articles citing issues regarding selenium that are not adequately supported by scientific information.

Article Title	Reference	Statements/Assertions	Issue
How pollution from Canadian coal mines threatens the fish at the heart of communities from B.C. to Idaho	Cruickshank (2022)	"For fish, the effect of selenium on reproduction is one of its most insidious threats. When female fish transfer the contaminant through their eggs, their larvae may be born with deformities - curved spines, misshapen skulls, abnormal gills - or fail to hatch at all. These impacts have already been observed in the Fording River."	**"These impacts have already been observed in the Fording River."** While these effects have been shown to occur in controlled multi-generational laboratory studies, evidence of these effects in fish populations in the Fording River has not been reported.
Alberta government knew bighorn sheep contaminated with coal-mine selenium: scientist	Weber (2021)	" 'They're getting it from the coal mines,' Kneteman said. "We found no evidence of elevated selenium in Alberta in any of the other populations that we sampled."	**" 'They're getting it from the coal mines……'"** There is no specific evidence that Alberta bighorn sheep are exposed to selenium downstream of coal mines. **"We found no evidence of elevated selenium in Alberta in any of the other populations that we sampled."** Kneteman (2016) only sampled one other bighorn sheep herd in Nevada.
Recent data spell trouble for Koocanusa fish	McLaughlin (2022)	"Data over the past nearly four decades show a steady uptick of selenium concentrations, initially breaching Canada's current 2 µg/L guideline during the early 1990s, in the Elk River near its confluence with Koocanusa waters, according to the DEQ[1]."	**"…..past nearly four decades show a steady uptick of selenium concentrations….."** **"……breaching Canada's current 2 µg/L guideline during the early 1990s……"** The data do not show an upward trend (only over two decades), but rather, steady concentrations that approximate 1 µg/L (see Figure 1 below), which is 50% of the British Columbia guideline of 2 µg/L, and 66% of the USEPA lentic criterion of 1.5 µg/L.
Selenium from open-pit coal mining may pose downstream risk to ag	Whelan (2021)	"In studying the impacts of open-pit coal mining in B.C.'s Elk Valley …… devastated fish populations before flowing south into Montana and Idaho with levels of selenium significantly higher than state regulations allow."	**"……devastated fish populations…."** Despite elevated aqueous concentrations of selenium, there is no evidence of devastated fish population or even population-level impacts in the Elk Valley. "**levels of selenium significantly higher than state regulations allow**." Selenium are below all state and federal criteria in the United States.

[1]Montana Department of Environmental Quality.

Article Title	Reference	Statements/Assertions	Issue
The Case Against Coal Mining in Alberta	Atwood (2021)	"So even if there is a fairly low concentration of selenium up the water if it's above that threshold, it can seriously impede the development of different aquatic species and then that obviously can also make it way up the food chain," they said.	**".....seriously impede the development of different aquatic species and then that obviously can also make it way up the food chain."** There is not always a correlation between aqueous and tissue concentrations.

Relative to the economic and climate-related issues confronting both Canada and the coal mining industry, the current state of the science regarding environmental impacts and technologies for controlling and treating selenium at coal mines would suggest that the focus of nearly one-third of news reporting during the past ten years has been excessive and misplaced. While stakeholder concerns are certainly crucial in any deliberations considering environmental protection, such concerns should always be informed by credible scientific evidence. News stories reporting on selenium and coal mining have tended to focus on five environmental concerns. These are discussed below.

Concern # 1 – "Historical data describing elevated selenium conditions at a coal mine are relevant to other coal mining sites."

This argument is most common in arguments opposing new coal mining in western Canada. At five active coal mines in the province, coal mine effluents have been discharged to the Elk Valley watershed for over 120 years. Historically, high volumes of exposed rock piles, some used in cross-valley fills, have weathered over several decades, and have contributed substantially to selenium loadings into the Elk River watershed. However, Teck Resources Ltd.'s (Teck) implementation of the Elk Valley Water Quality Plan (Teck, 2014) during the past decade has reduced selenium concentrations in surface water and biota. New leachate and surface water bodies downstream of the mines. This situation has led to advances in the understanding of selenium behaviour and environmental management, and the implementation of the Elk Valley Water Quality Plan (https://www.teck.com/media/2015-Water-elk_valley_water_quality_plan_T3.2.3.2.pdf), and Teck's recent focus on mitigation measures and treatment technologies, including Saturated Rock Fill (SRF) technology (Mackie et al. 2022), have contributed to substantial declines in selenium concentrations.

Thus, historical mining practices are no longer indicative of current and future mining practices and environmental consequences. Modifications to operations at active coal mines and mine management plans at proposed/new coal mines throughout western Canada reflect the current state-of-practice involving the implementation of mine design features, mitigation opportunities, treatment methods, monitoring and adaptive management measures and strategies to minimize and control selenium in mine wastewaters (McKenna and Gilron 2021).

Concern # 2 – "Selenium impacts on aquatic life can be extrapolated to humans, livestock, and terrestrial wildlife."

The use of scientific studies relevant to determining the potential for adverse effects of selenium on aquatic organisms should not be used in news reporting to describe the

potential impacts to humans, wildlife (Kneteman, 2016) and livestock (Whelan, 2021). There are fundamental differences in the biological mechanisms of action associated with selenium exposure to fish, birds and mammals (Chapman et al., 2010; Gilron et al., 2021). Studies used to derive health benchmarks for selenium in drinking water applicable to livestock and human consumption support 50 ppb (parts-per-billion) as the relevant drinking water guideline, nearly 25 times higher than the surface water guideline applicable to aquatic life in Canada (i.e., 1-2 ppb) (CCME, 2009; Health Canada 2014). Concerning wildlife, Kneteman's (2016) work has prompted claims in Canadian news reporting that selenium has adverse effects/impacts to Bighorn sheep. These concerns rely on data indicating that selenium concentrations in blood serum near Alberta coal mines are higher than in Bighorn sheep in another part of Alberta and in the US state of Nevada. An increase in blood serum is an indicator of exposure, but not necessarily effects (Ohlendorf et al. 2020).

Concern # 3 – "Selenium is adversely impacting fish populations in western Canada."
News reports describing that elevated selenium concentrations in surface water near coal mines adversely affects fish populations throughout Canada have often been attributed to publications by Lemly (2014, 2019). Statements cited in news reports attributed to Lemly's work, are based on the author's opinions about selenium impacts originating from different industries, not coal mines. Further, the statements refer to several decades-old environmental data and observations from the U.S. and are not based on data relevant to surface waters adjacent to developing coal mines in Canada.

Several studies have shown that fish species differ in their sensitivity to selenium (DeForest et al., 2012; USEPA, 2016). A recent critical review of selenium in fish indicates there is significant variability in fish tissue concentrations and consequences to fish populations (Gilron et al. 2021). Bioaccumulation and effects of selenium have been demonstrated to vary significantly in different geology and geographic areas and different fish species (Presser and Luoma 2010; Chapman et al. 2010). It is most appropriate to match exposure data with the toxicity data for resident species.

Concern # 4 – "Transboundary selenium pollution is evident in Lake Koocanusa, situated downstream of the Elk Valley watershed."
Numerous news reports on both sides of the Canada-US border suggest that selenium pollution has migrated from BC's Elk Valley watershed to Lake Koocanusa. For many years, the state of Montana and the province of British Columbia have monitored the lake's aquatic environment. The available monitoring data do not support news media reports. Lake Koocanusa selenium concentrations have been stable for the past decade at a mean concentration of approximately 1 μg/L, which is below the British Columbia guideline value of 2 μg/L (Beatty & Russo, 2014), and below the USEPA (2016) criterion for slow-flowing (lentic) waters (1.5 μg/L) (see Figure 1). Fish egg and ovary tissue data collected from Lake Koocanusa indicates that the selenium concentrations are below the USEPA (2016) egg/ovary threshold of 15.1 mg/kg, which is established to protect the most sensitive aquatic species.

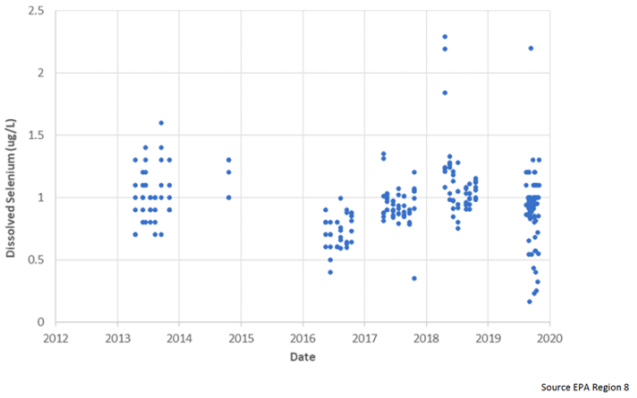


Figure 1. **Dissolved selenium concentrations in Lake Koocanusa, 2013-2020. Source: Montana Department of Environmental Quality (via e-mail; August 27, 2021).**

Concern # 5 – "Selenium treatment technologies are unproven."

Assertions have been made that current selenium treatment methods are unproven technologies and have not been demonstrated to be workable in operating coal mines and for post-closure mine reclamation. However, technology reviews conducted by Golder (2020) and Gilron and McKenna (2021) do not concur with this viewpoint; these authors describe the efficacy and readiness of several available treatment technologies used at active coal mining sites and proposed for use in future mining operations. Several technologies are already currently in full-scale operation (Golder, 2020).

SUMMARY

Claims in news media reports and by stakeholders during recent environmental policy reviews and assessments related to coal mining in western Canada have unnecessarily caused public alarm about the potential impacts of selenium in the aquatic environment. The science is well understood. Moreover, several environmental management strategies developed collaboratively by governmental agencies and the mining industry have the capability of limiting the release of mine leachate and liquid wastes containing selenium to the aquatic environment. The results of extensive monitoring of stream, river, and lake surface waters, fish populations and tissues, and ecosystems indicate these strategies can, and do, protect Canadian (and US) aquatic environments. Because Canada's environmental policy with respect to coal mining has significant ramifications to the national economy, it is paramount that decisions regarding the future of coal mining be informed by an accurate understanding and communication of the science and technology associated with selenium management at coal mining sites and the need to protect the environment. We strongly advocate for the application of scientific evidence to inform regulatory decisions.

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Author Index

A

Abu Farah, J. 193, 195
Adams, W. 94, 212
Adedokun, A. 179
Adzic, M. 96
Aguilar, L. 81
Ahabchane, H-E. 126
Ahad, J. 76, 79
Aherne, J. 100, 109
Alcaraz, A.J. 51, 172, 199, 201
Alderman, S. 167, 168, 183
Allamby, Q. 106, 113
Allen, M. 19
Allison, M. 189, 196
Alsop, D. 186
Amekor, M. 51, 172, 199, 201
Ammerlaan, J. 69
Amyot, M. 31
Anderson, J. 81, 196
Ankley, P. 7, 51, 180, 190
Apaza Viza, A. 81
Arens, C. 69
Arias Torres, G. 81
Arlos, M. 47, 175
Armstrong, O. 110
Arnold, M. 69, 99, 124
Artelle, K. 75, 82
Ashby, L. 96
Asnicar, D. 8, 145
Assance, C. 67
Atkinson, B. 153
Atlas, E. 18
Aucoin, M. 195
Azizishirazi, A. 54, 123

B

Badesab, S. 82
Badlani, Y. 193
Bahamonde, P. 57
Baillergeau, M. 22
Baker, J. 138, 204
Barrett, G. 33
Barst, B. 31
Bartlett, A. 5, 75, 88, 117, 118, 119, 121, 177
Baseler, L. 131
Basu, N. 11, 23, 24, 25, 26, 147, 157, 179
Baulch, H. 7
Bayen, S. 41
Beaudette, L. 6
Beblow, J. 131, 196
Bekele, A. 37
Beking, M. 134
Benfey, T. 199
Bennett, E. 100
Bennett, W. 140
Bergeron, P. 153
Bernier, N. 78
Berryman, E. 128
Bertolo, A. 82
Berube, T. 150
Besil, N. 161, 162
Bickerton, G. 75
Bidwell, M. 45
Birceanu, O. 169
Bird, G. 135, 142
Birks, J. 61
Black, T. 75
Blancho, F. 110
Blandford, N. 73, 174
Blewett, T. 124, 144
Blier, P. 185
Bluhm, K. 99
Boegel, S. 195
Bogard, M. 125
Boily, M. 154
Boloori, T. 145, 181
Bonk, E. 118, 191
Bonnineau, C. 22
Borgert, C. 139
Borisenko, A. 192
Boulanger, E. 11, 12, 13, 23, 24, 25, 178, 179, 181
Bourgeois, Z. 4
Bourque, L. 32
Bouwhuis, R. 58, 134
Bowyer, E. 86
Boyd, A. 144
Boyd, P. 120, 134, 159, 179
Boyer, L. 61
Bragg, L. 47, 52, 59, 60, 84, 193, 194, 195
Brandt, E. 13
Brandt, J. 94
Breadner, P. 193, 195

Brinkmann, M. ... 1, 4, 7, 9, 17, 51, 56, 92, 172, 180, 199, 200, 201, 203, 204
Brinovcar, C. ... 88
Britz-McKibbin, P. ... 32
Brix, K. ... 96, 99
Brown, A. ... 47, 161, 162
Brown, C. ... 147
Brown, T. ... 202, 203, 205
Brunet, M-H. ... 68, 126
Bryshun, L. ... 204
Bucci, K. ... 103
Bui-Marinos, M. ... 188
Bula, A. ... 156
Burgess, N. ... 43
Burgoon, L. ... 139
Burton, A. ... 123
Burton, E. ... 52

C

Cabezas, S. ... 38, 39
Cable, R. ... 103
Cadonic, I. ... 183
Caklec, J. ... 138
Calik, D. ... 167, 168, 183
Campbell, S. ... 140
Cantin, J. ... 4
Caplette, P. ... 121
Cárdenas-Soracá, D. ... 59, 60
Carpenter, D. ... 88
Celmer-Repin, D. ... 84, 195
Cermak, J. ... 85, 93, 146
Cesio, V. ... 161, 162
Challis, J. ... 4, 120, 180
Chan, C. ... 170
Chan, D. ... 75
Chan, H.M. ... 10
Chan, L. ... 91, 175
Chanes, V. ... 4
Chen, G. ... 135
Chen, M. ... 114
Chen, Z. ... 21
Chételat, J. ... 32
Cheung, W. ... 91
Chiang, G. ... 57
Choh, V. ... 166
Chong, C. ... 60
Chong, K. ... 26
Chow, E. ... 166
Ciborowski, J. ... 61
Cicek, N. ... 73
Clarence, S. ... 75
Clark, R. ... 132
Cleveland, M. ... 131, 196
Cochrane, P. ... 4
Coleman, B. ... 114
Colville, C. ... 12
Comfort, J. ... 4
Cook, D. ... 10
Cooney, B. ... 77
Costa, E-J. ... 69, 70, 124
Costantini, S. ... 4, 65
Couture, P. ... 185
Craig, P. ... 52, 138, 163, 165, 172, 183, 188, 190, 195
Crémazy, A. ... 124, 159, 206, 209
Cresswell, T. ... 140
Creusot, N. ... 22
Cronshaw, I. ... 204
Crump, D. ... 13, 24, 25, 33, 41, 147, 176
Curry, J. ... 8, 18
Curry, R.A. ... 32

D

D'Aurora, S. ... 132
Daley, J. ... 124
Dalton, R. ... 14, 134, 146, 149
Dang, H. ... 136
Dang, M. ... 105
Danis, B. ... 37, 86
Darwish, T. ... 69
Davies, J. ... 21
Davies, M. ... 30, 142, 143
Davila-Arenas, C. ... 63, 64, 98
Dawe, R. ... 194
de Bie, F. ... 32
de Bruyn, A. ... 69, 97, 124, 141
de Jourdan, B. ... 8, 75, 82, 145, 199
De Lange, C. ... 1
de Oliveira Lima, G.J.E. ... 83
De Silva, A. ... 88, 177
de Solla, S. ... 32, 33, 71, 164
Decelles, N. ... 178, 179
DeForest, D. ... 96
Degagne, H. ... 135
Deoraj, K. ... 29, 34, 177
DesJardins, M. ... 30
Desreac, F. ... 206
Desrosiers, M. ... 49
Dettman, H. ... 79
Devoy, C. ... 1, 9
Dhiyebi, H. ... 84, 193, 194, 195
Diaz, G. ... 69
Dickenson, L. ... 73, 174
Diesbourg, E. ... 53
Dietz, R. ... 36, 41
Dixit, S. ... 122, 137, 146

Dixon, B. 183
Do, C. 127
Doane, E. 132
Doering, J. 9
Doig, L. 63, 64, 98
Dolgova, N. 129
Doty, D. 144
Dow, T. 48
Doxey, A. 188, 190
Drew, S. 195
Drouillard, J. 100
Dubetz, C. 150
Dubiel, J. 9
Dunn, A. 85, 146
Durante, J. 145
Dutton, E. 72

E

Easwaramoorthy, M. 166, 184
Eaton, B. 21
Edgar, M. 183
Edwards, J. 115
Edwards, M. 143
El-Fityani, T. 93, 137, 146
Elliott, J. 32
Elliott, K. 30, 31
Elphick, J. 15, 124, 138, 142
Emberley-Korkmaz, S. 158
Embry, M. 17
Eng, A. 148
Eng, M. 43
Esser, M. 7, 99, 180
Ewald, J. 12, 23
Ezugba, I. 63, 64, 98

F

Farajizadeh, A. 50, 88
Farenhorst, A. 47, 161, 162
Farrell, A. 183
Farrell, C. 29, 34, 177
Farrow, F. 135, 142, 208
Fast, J. 57
Fattahi, A. 84, 195
Faure, A. 159
Feaver, A. 156
Femi-Oloye, O. 155
Fenton, H. 32
Ferguson, S. 36, 41
Fernandes, S. 210
Fernie, K. 90
Ferns, A. 3
Festarini, A. 135, 142, 208
Festarini, D. 135
Findlay, D. 77
Fischer, M. 27
Fitzgerald, D. 74
Fitzgerald, G. 32
Flores, A. 81
Flores, J. 55
Folkema, A. 195
Forbes, M. 88, 185
Fortin, C. 126, 136, 206, 209
Frank, R. 75, 140
Fransen, S. 117, 119
Fraz, S. 166
Freemark, M. 45
Frye, S. 135
Fugère, V. 82
Fuzzen, M. 193, 194

G

Gallant, M. 4, 124, 153
Gallilee, C. 202
Gardebled, E. 22
Garman, E. 124
Garnier, C. 185
Garrett, D. 38
Gasque-Belz, L. 10
Gauthier, M. 154
Gavarikar, S. 52, 78, 138
Gavel, M. 185
Gendron, A. 117, 121
Gene, S. 108, 114
George, G. 129
George, T. 6
Gere, A. 193
Gewurtz, S. 58
Ghahreman, P. 146, 149
Giang, A. 91
Giesy, J. 7, 51, 92, 155, 180, 188, 190, 193, 194, 195
Gillespie, A. 110, 111
Gillies, E. 91
Gillio Meina, E. 99
Gillis, E. 61
Gillis, P. 75, 107
Gillis, T. 167, 168, 183
Gilron, G. 94, 212
Gilroy, È. 3, 88, 117, 140, 177
Girard, L. 81
Giroux, J-F. 36
Glasner Regis, C. 4
Glover, C. 95, 188
Gobas, F. 37
Goertzen, M. 87, 122
Goldblatt, R. 122

Goldsmith, D. 32
Gong, Y. 180
Good, C. 69
Goss, G. 50, 88, 95, 188
Gosselin, I. 135
Goulet, R. 128, 129, 208
Grace, R. 203
Grapentine, L. 117, 119
Graves, S. 94, 102
Green, D. 99
Green, S. 35
Greil, A. 136
Grey, M. 16
Griffin, R. 124
Grosshans, R. 73, 77
Guay, I. 49
Guillemette, M. 31
Guimarães Guilherme, L.R. 83
Guimard, L. 209
Gunn, J. 67, 84
Gurney, K. 32
Gutierrez-Villagomez, J.M. 76, 79
Guttormson, A. 77
Gutzman, D. 58

H

Hajir, S. 182
Halappanavar, S. 148
Hale, B. 86, 127
Halldorson, T. 77
Hammond, M. 33
Hamoutene, D. 8, 160
Han, B. 39
Hancock, A. 208
Handyside, P. 117, 119
Hang, S. 202
Hanner, R. 118, 191, 192
Hanson, M. 73, 77, 79
Hardy, B. 73, 174
Harms, J. 32
Harner, T. 148
Haroune, L. 153
Harper, N. 188, 190, 194
Harris, M. 38
Hartenstein, P. 166, 184
Hatfield, T. 69
Hay, B. 156
Hayat, S. 193, 194, 195
Head, J. 11, 13, 23, 24, 25, 41, 147, 176, 178, 179, 181
Headley, J. 38, 39
Heath, J. 183
Hebert, C. 33
Hecker, M. 7, 10, 19, 23, 51, 55, 56, 96, 99, 147, 172, 174, 175, 180, 198, 199, 200, 201
Heddle, C. 97
Hedges, A. 5
Heinzen, H. 161, 162
Helbing, C. 189, 190, 196
Henaff, M. 32
Henriques, C. 92
Hepditch, S. 76, 79
Heshka, N. 79
Hewitt, M. 75
Hickey, G. 26
Hicks, K. 62
Higgins, K. 132
Hill, D. 140
Hobbs, M. 81
Hocking, M. 189
Hoellein, T. 102
Hoffman, M. 40, 104, 115, 116
Hogan, N. 1, 12, 51, 55, 56, 172, 199, 201
Hoggarth, C. 7
Hoover, C. 91
Hopkins, K. 90
Horton, M. 55, 161
Hourtané, O. 126
Howes, P. 16
Huancco Bravo, R. 81
Hubley, R. 19
Hughes, C. 30
Hughes, K. 33
Humeniuk, B. 79
Hunter, B. 27
Huntsman, P. 128

I

Ijzerman, M. 21, 153
Ikert, H. 193, 194, 195
Ings, J. 69, 71, 72
Irving, E. 69
Issac, F. 152
Izma, G. 21, 153

J

Jacyk, J. 210
Jagla, M. 58
Jain, N. 51, 172, 199, 201
Jakovljevic, D. 9
Jamal, S. 59
James, A. 129
Janz, D. 19, 200, 207, 209
Jarron, S. 142
Jeffries, K. 174
Ji, X. 98

Johannessen, C. 148
Johnston, E. 183
Johnston, S-E. 125
Johnston, T. 67
Jonah, L. 8
Jones, M. 32
Jones, P. 9
Joosse, P. 117
José Vilela, F. 83
Joseph, N. 158
Ju, K.S. 75
Juanes, F. 189

K

Kalisa, E. 148
Kalvani, Z. 132, 163, 170
Kamunde, C. 132, 163, 170
Karouna, N. 13
Katzenback, B. 40, 108, 114, 188, 190
Kelly, B. 37
Kelly, N. 101
Kennedy, C. 167, 168, 183
Kennedy, E. 19, 180
Kennedy, L. 6
Kennedy, R. 132
Kerric, A. 34, 36
Kettemer, L. 36
Khan, A. 177
Khan, H. 5, 75
Kidd, K. 32, 53, 57, 67, 71, 105, 106, 107, 113, 164
Kilgour, B. 62
Kim, J. 40
Kingsbury, M. 160
Kirk, B. 128
Kiszka, J. 36
Klaczek, C. 95
Klasios, N. 104
Kleiner, M. 9
Kleywegt, S. 84
Knight, R. 65
Knopper, L. 132
Ko, C. 10
Koch, K. 196
Kohlman, E. 51, 56, 172, 199, 201
Kolok, A. 158
Korman, K. 65
Koumrouyan, R. 13, 41
Kowalczyk, S. 52, 59
Kramer, S. 121
Krause, S. 4
Krogh, E. 204
Kudla, Y. 107, 153
Kumar, A. 47
Kvas, S. 6, 179

L

Laberge, F. 78
Lacombe, R. 30, 31
Lacroix-Durand, C. 207, 209
Laengle, T. 120
Lafond, J. 121
Laframboise, L. 53, 166, 184
Lagrue, C. 35
Lair, S. 32
Laird, B. 84
Lakanen, M. 81
Lam, J. 55, 161
Lanctôt, C. 140
Langenfeld, D. 103
Langlois, V. 49, 76, 79
Lapen, D. 44, 114
Larbi-Youcef, Y. 121
Large, D. 146, 149
Lari, E. 50
Larivière, D. 206
Larocque, È. 76
Lavalle, C. 75
Lavoie, I. 136, 153, 159
Lavoie, R. 30, 31, 34
Law, P. 84
Lawrence, G. 70
LeBlanc, H. 48
Leblanc, J. 150
Lee, H. 18
Lee, K. 145
Lee, L. 156
Lee, S. 32
Legg, A. 144
Legrand, E. 65, 132, 181
Lemieux, H. 120, 134, 159, 179
Leon, L. 210
Leonard, E. 130, 140
Lepage, A. 67, 84
Lescord, G. 29, 67, 84
Lesperance, M. 189
Letcher, R. 17, 36, 90
Letwin, N. 110, 153
Leung, J. W-H. 10
Levin, D. 77
Lewarne, D. 69
Lewis, Y. 104
Li, F. 110
Li, M-L. 91
Liao, X. 202, 203
Liber, K. 63, 64, 98, 99, 207, 209
Liggio, J. 148

Lima, A.C. 61
Lin, F. 183
Lin, J. 51, 55, 199, 201
Little, C. 117
Liu, L. 41
Liu, P. 23
Liu, X. 98
Lo, B. 205
Loan, A. 10
Locke, S. 58
Longstaffe, J. 111
Loor, V. 82
Lopez, M.L. 190
Lowes, H. 124
Lozier, T. 117
Lucas, B. 30
Lush, W. 50
Lynch, M. 190

M

M'Gonigle, L. 196
Ma, M. 134
Macairan, J-R.. 110
MacDonald, T. 129
MacKay, D. 3
MacKinnon, T. 128
Magnusson, K. 32
Mahaffey, A. 27
Mahoney, H. 1, 92
Maisonneuve, F. 159
Malika Ouedraogo, A.M. 129
Malle, H. 203
Mallory, M. 112
Mamani Luis, A. 81
Manzon, R. 166, 184
Marcarelli, A. 67
Marchand, H. 11, 23, 178, 179
Marchand, J. 44
Marjan, P. 47, 170, 175
Marlatt, V. 37, 55, 86, 131, 161, 196, 205
Marshall, A. 11, 23, 179
Marteinson, S. 150, 160, 203
Martel, R. 76, 79
Martens, K. 66
Martin, A. 87
Martin, L. 206
Martin, S. 144
Martin, W. 93, 151
Martinko, C. 4
Marty, G. 205
Martyniuk, C. 175
Masood, N. 166, 169
Mastin, J. 148
Matthews, C. 36
Mazerolle, M. 36
Mazzocco, P. 65
McCann, K. 84
McCarty, L. 74, 139
McClelland, C. 32
McClelland, G. 130
McCuaig, J. 144
McCulloch, D. 166
McGeer, J. 130
McKay, M. 131
McKenna Newman, C. 100
McKinney, M. 36, 41, 90
McLean, C. 148, 151
McLeod, J. 32
McMaster, M. 62
McNabney, D. 3, 177
McNamee, R. 102, 103, 106
McPhedran, K. 204
McPhee, C. 117
McTavish, K. 160
Medeiros, A. 102
Melnichuk, R. 21
Melo, Y. 115
Mendes, M. 70
Mennigen, J. 8, 18, 168
Merma Velasco, E.V. 81
Mertens, E. 125
Métivier, M. 49
Middleton, E. 124
Mill, K. 96
Miller, J. 118, 140, 191
Miller, J.G.P. 9
Milne, M. 116
Misutka, M. 188
Mitrovic, D. 156
Mittal, K. 11, 16, 23, 157, 179
Moffatt, E. 32
Mombourquette, A. 61
Monaghan, J. 204
Monteil-Rivera, F. 58
Monteiro Feitosa, M. 83
Montgomery, D. 198, 200
Montreuil Strub, É. 102, 106
Moose Cree First Nation 29
Morales Murillo, A. 104
Moravcova, R. 131
Morrissey, C. 17, 38, 39, 42
Mueller, Z. 143
Mukytczuk, N. 81
Mundy, L. 45
Munkittrick, K. 27, 32, 47, 57, 61, 62, 147, 170, 175

Munter, K. 26
Murdoch, M. 65
Murillo, A. 53
Muscatello, J. 87
Musgrave, C. 112
Muzyka, W. 99

N

Nakagawa, P. 161, 162
Nearing, M. 210
Neves, N. 115
Ng, C.K.A. 193, 194
Nguyen, T. 8
Nikel, K. 118, 191
Nipu, N. 168
Niyogi, S. 99
Noble, T. 132
Nombré, Y. 154
Nykamp, N. 130

O

O'Brien, J. 8, 18
O'Connor, C. 29, 34, 177
O'Hara, L. 111
O'Donnell, S. 26
Okonski, A. 58
Oliveira Pereira, E. 173
Oloye, F. 155
Omilowo, H. 77
Opyr, M. 77
Orihel, D. 40, 104, 108, 114, 115, 116
Ouellet, F. 117, 119
Overduin, S. 144

P

Page, E. 117, 119
Pagé, L. 68, 126
Pagé-Larivière, F. 14
Paishegwon, R. 67
Pajankar, U. 133
Pajnic, N. 4
Palace, V. 73, 77, 94, 96, 174
Palomino Gutierrez, M. 81
Panigrahi, B. 63, 64, 98
Parent, L. 154, 159
Park, B. 10
Parker, W. 84
Parrott, J. 75
Parsons, C. 117
Pasha, A. 19
Paterson, A. 100
Paterson, G. 67
Paterson, M. 103, 104, 115, 116
Pattem, C. 142
Pauli, B. 45, 80
Pearson, A. 54
Pedersen, A. 41
Pelletier, D. 31
Pérez, E. 207, 209
Perrin, C. 143
Perrotta, B. 53, 67
Peskett, S. 90
Petalas, C. 30
Peters, L. 77
Pfeifer, L. 172
Pham-Ho, V. 3
Philibert, D. 20, 199, 203
Pick, F. 44, 129
Pickering, I. 129
Pilditch, C. 75
Plamondon, J. 65
Pochardt, M. 189
Point, A. 181
Poisson, G. 121
Pomfret, S. 203
Porter, H. 61
Potter, K. 146, 149
Prabhakar, G. 129
Preston, C. 100
Princz, J. 6, 23, 110, 114, 120, 134, 159, 179
Prindiville, J. 134
Prosser, R. 5, 21, 75, 82, 105, 106, 107, 110, 112, 113, 151, 153, 201, 202
Proulx, C. 62, 208
Provencher, J. 40, 104, 108, 112, 114, 115, 116
Pulgarin-Zapata, D. 47
Pyle, G. 125

R

Raby, M. 21, 153
Rae, K. 32
Raes, K. 96, 99, 180
Rahman, M.M. 61
Rahmanian, B. 70
Ramcharan, C. 81
Ramos Farfán, W. 81
Ramos Ramos, M. 81
Rand, A. 89, 90
Ravary, S. 3
Raza, Y. 9
Reger, S. 202
Remili, A. 36
Ren, S. 38
Rennie, M. 81
Rennie, M.D. 40, 104, 116
Resende, T. 83

Reside, A. 78, 167, 168
Rezende, S. 161, 162
Richards-Blukacz, A. 117, 119
Richer, L. 67
Rickwood, C. 128, 129, 208
Rikardsen, A. 36
Robert, T. 76
Roberts, C. 51, 172, 180, 198, 199, 201
Robertson, K. 73, 174
Robichaud, K. 3, 163
Robinson, S. 44, 80, 88, 177, 185
Robitaille, J. 15, 49
Rochman, C. 40, 102, 103, 104, 106, 108, 114, 115, 116
Rodrigues, M. 75
Rodríguez, D. 57
Rodríguez-Gil, J.L. 77, 79
Rogalski, J. 131
Rogan, E. 158
Rohonczy, J. 80, 88
Rojas Villafuerte, R.A. 81
Roldán-Wong, N. 175
Ronnenberg, K. 133
Rooney, R. 21, 102, 104, 106, 115, 153
Rosing-Asvid, A. 36, 41
Roulier, M. 206
Rousselle, H. 135
Row, J. 71, 72
Roy, J. 75
Ruecker, N. 175
Rupasinghe, P. 61
Ryan, T. 117

S

Sacko, A. 82
Saherwala, A. 110
Saini, A. 148
Salic, S. 60
Salole, J. 201, 202
Samarajeewa, A. 6, 23
Samarra, F. 36
Sanchez, R. 81
Sangiovanni, J. 176
Santana Alvarenga, I.F. 83
Sappal, R. 132
Sarhan, M. 50
Sayles, B. 27
Schaefer, P. 71, 72
Schiffer, S. 15, 142, 153
Schlekat, C. 124
Schock, D. 45
Schultz, M. 4, 17
Schuster, J. 148
Schwertfeger, D. 134
Scrimgeour, G. 61, 62
Scroggins, R. 6, 23, 134
Seguin, J. 29, 34, 177
Séguin, J. 62
Selinger, S. 198, 200
Serben, K. 70
Servos, M. 47, 52, 59, 60, 84, 175, 188, 190, 193, 194, 195
Sett, A. 118, 140, 191
Shahpoury, P. 148
Shanebeck, K. 35
Shanmuganathan, M. 32
Sharin, T. 13, 41
Sharron, S. 189
Shires, K. 3, 75
Shirose, L. 32
Sibley, P. 151, 153
Siciliano, S. 10, 179
Simard, J. 29, 34, 177
Simmons, D. 19, 29, 34, 48, 92, 133, 173, 174, 177, 181
Simpson, M. 173, 182
Sims, K. 135
Sinclair, K. 69
Sine, S. 150
Sing-Judge, C. 193, 194, 195
Siu, L. 50
Sivarajah, B. 114
Siwik, P. 27
Skanes, B. 75
Skinner, K. 84
Smith, D. 23, 179
Smith, S. 126
Smyth, S.A. 58
Sobaszek, A. 4
Somers, C. 166, 184
Sonne, C. 36, 41
Sora, K. 91
Sorais, M. 36
Sow, A. 136
Sparr, J. 153
Spiers, G. 81
Srikanthan, N. 84, 193, 194, 195
Stalwick, J. 32
Stammler, K. 117
Stanley, M. 77
Stead, J. 18
Stecko, P. 66
Stenner, K. 37
Stephenson, G. 110
Stephenson, M. 65
Stevack, K. 6

Stevens, D. 132, 163, 170
Stewart, C. 124, 144
St-Hilaire, S. 140
St-Laurent- Guérin, J. 177
Story, M. 65
Stoyanovich, S. 62
Strbac, A. 113
Stuart, M. 135, 142, 208
Su, G. 86, 167, 168, 196
Subasinghe, R. 23, 179
Suek, K. 28
Sullivan, K. 58
Suominen, E. 128, 129
Swan Lake First Nation Land Governance Office 120

T

Tadonleke, R. 150, 203
Taiba, J. 158
Tanna, N. 37
Taranu, Z. 117
Taridashti, F. 170
Taylor, L. 4
Tear, L. 96
Tetreault, G. 117, 118, 140, 191
Tetteh, P. 132
Thériault, G. 121
Thomas, J. 168
Thomas, P. 35
Thompson, A. 53, 166, 169, 184, 186
Thompson, J.D. 40, 108, 114
Thompson, M. 16
Thorarinson, M. 28
Tillmanns, A. 54
Timlick, L. 77
To, T.A. 76, 79
Todd, M. 35
Tomy, G. 77
Tourret, A. 22
Triffault-Bouchet, G. 76, 79
Tseng, M. 104
Tufenkji, N. 110

U

Ulmer, H. 42

V

Vachon, N. 178, 179
Vakharia, R. 60
van den Heuvel, M. 163, 170
Van der Vliet, L. 4
Van Geest, J. 124
Vanderhyden, B. 10
Vanderyagt, T. 127
VanEssen, D. 9
Vanrobaeys, J. 120
Vasiluk, L. 86, 127
Vedrenne, J. 22
Veilleux, É. 49, 121
Veilleux, H. 188
Velichka, J. 32
Velicogna, J. 114, 134
Veneruzzo, C. 103
Vergara, E. 57
Vergote, D. 43
Vermaire, J. 114
Verreault, J. 36
Villella, M. 3, 177
Villeneuve, D. 14
Vonberg, H. 102

W

Wakefield, J. 26
Walker, H. 109
Walker, T. 102
Wallace, E. 91
Walters, D. 67
Wang, J. 10
Wardlaw, C. 105
Warketin, L. 60
Warriner, T. 19, 92, 173, 181
Washington, S. 115
Watt, C. 120
Watt, C.A. 36
Weber, A. 138, 165
Weber, L. 200
Wei, L. 168
Wells, K. 210
Welsh, B. 100
Wendlandt, M. 61
Wenning, R. 94, 212
Wicks, B. 65
Wideman, R. 137
Wilkinson, K. 68, 126
Williams, A. 18
Williams, K. 33
Williams, S. 169
Wilson, J. 112
Wilson, J.Y. 53, 166, 169, 184, 186
Winegardner, A. 150, 203
Wiseman, S. 9, 125, 200
Wish, J. 47
Wolfe, K. 28
Woolhouse, K. 28
Woudeneh, M. 203
Wrona, F. 61

X

Xia, J. 23
Xia, P. 51, 180
Xie, Y. 180, 190, 195
Xin, Q. 79
Xu, I. 157

Y

Yarmey, N. 35
Yauk, C. 18
Young, S. 80

Z

Zamora, C. 188
Zathey, N. 96
Zhang, X. 148
Zhou, X. 125
Zhou, Y. 51, 180
Zimmer, M. 32
Zink, L. 125
Zolondek, M. 43
Zou, S. 114
Zvomuya, F. 161