Proceedings of the 45th Annual Canadian Ecotoxicity Workshop: September 30 – October 3, 2018, Vancouver, British Columbia

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PROCEEDINGS OF THE 45TH ANNUAL CANADIAN ECOTOXICITY WORKSHOP: SEPTEMBER 30 – OCTOBER 3, 2018, VANCOUVER, BRITISH COLUMBIA

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ABSTRACT

Bonnie P. Lo, Vicki L. Marlatt, Curtis V. Eickhoff, Travers R. Pretorius and Lisa N. Taylor (Editors). 2020. Proceedings of the 45th Annual Canadian Ecotoxicity Workshop: September 30 – October 3, 2018, Vancouver, British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. 3360: xxii + 131 p.

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

The 45th annual CEW was held at the Sheraton Wall Centre Hotel in Vancouver, British Columbia, from September 30 to October 3, 2018. The workshop included 131 platform presentations and 49 poster presentations. Total attendance was 327.

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

RÉSUMÉ

Bonnie P. Lo, Vicki L. Marlatt, Curtis V. Eickhoff, Travers R. Pretorius and Lisa N. Taylor (Editors). 2020. Proceedings of the 45th Annual Canadian Ecotoxicity Workshop: September 30 – October 3, 2018, Vancouver, British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. 3360: xxii + 131 p.

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

Le 45ième Atelier canadien annuel sur l'écotoxicité a eu lieu à l'Hôtel Sheraton Wall Centre à Vancouver (Colombie britannique), du 30 septembre au 3 octobre 2018. L'atelier a donné lieu aux 131 présentations orales et 49 présentations par affiche. Trois-cents vingt- sept personnes ont assisté à l'atelier.

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

Editors' comments

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

The editors would like to thank Dr. Jill Watson for her assistance in preparing these proceedings.

Remarques des éditeurs

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

Les rédacteurs voudraient remercier Dre. Jill Watson dans la préparation de ces comptes rendus.

45th Canadian Ecotoxicity Workshop Organizing Committee / Comité organisateur du 45e atelier canadien sur l'écotoxicité

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

Does inflammation facilitate permeation of organic chemicals through gill epithelia?

U. Fuchylo[1], M. Brinkmann[1], H. Alharbi[1], M. Hecker[1]

[1]*University of Saskatchewan*

In this study we attempted to elucidate whether inflammation could induce disruption of tight junctions in fish gill epithelia, thereby increasing passive transport of chemicals across the epithelia. Inflammation was experimentally induced in a permanent rainbow trout gill cell line (RTgill-W1) through exposure to non-cytotoxic concentrations of lipopolysaccharide (LPS). Transepithelial electrical resistance (TEER) was used to indicate epithelial tight junction integrity. Cells were also co-exposed to LPS and oil sands process-affected water (OSPW) to determine if the hypothesized reduction in tight junction integrity resulted in greater transport of OSPW from apical to basal side of a permeable cell insert membrane. Cells exposed to LPS showed significant reduction in TEER after 24 hours of exposure. Quantitative real-time PCR (qPCR) data showed that abundance of transcripts of genes coding for tight junction proteins (i.e., Claudin 28b and 10e) was significantly decreased in cells exposed to 20, 50, and 100 $mg \cdot L^{-1}$ LPS. Chemical analyses of OSPW content of basal chamber media showed significant increase in transport of OSPW from apical to basal chamber of permeable cell inserts at all concentrations of LPS. An *in vivo* exposure of fingerling rainbow trout to dietary LPS and waterborne OSPW was conducted to confirm *in vitro* results. Chemical analyses showed increase in OSPW content in fish exposed to both LPS and OSPW for 48 hours, compared to fish exposed to OSPW alone. These results indicate that fish living in environments high in pathogens would be at risk of greater uptake of contaminants than previously thought.

Cardiovascular and metabolic effects of dietary selenomethionine exposure in fishes

Connor Pettem[1], David Janz[1], Lynn Weber[1]

[1]*University of Saskatchewan*

Selenium (Se) is an essential micronutrient involved in important metabolic functions for all vertebrate species. As Se is reported to have a narrow margin between essentiality and toxicity, there is growing concern surrounding the adverse effects of elevated Se exposure caused by anthropogenic activities. Recent studies have reported that elevated dietary exposure of fish to selenomethionine (Se-Met) can alter aerobic metabolic capacity, energetics and swimming performance. This thesis aimed to further investigate mechanisms of sublethal Se-Met toxicity, particularly potential cardiovascular implications of chronic exposure to environmentally relevant concentrations of dietary Se-Met in adult zebrafish (*Danio rerio*) and rainbow trout (*Oncorhynchus mykiss*). In my first experiment, adult zebrafish were fed either control food (1.1 $\mu g\ Se \cdot g^{-1}$ dry matter [dm]) or Se-Met spiked food (10.3 or 28.8 $\mu g\ Se \cdot g^{-1}$ dm) for 90 days at 5% body weight per day. In the

second experiment, adult rainbow trout were fed either control food (1.3 μg Se·g^{-1} dry mass) or Se-Met spiked food (6.4, 15.8 or 47.8 μg Se·g^{-1} dm) for 60 days at 5% body weight per day. Following exposure, ultrahigh resolution B-mode and Doppler ultrasound was used to characterize cardiac function. Chronic dietary exposure to elevated Se-Met had opposing results in zebrafish when compared to the rainbow trout. Zebrafish exposed to the highest dietary concentrations of Se-Met (28.8 μg Se·g dm) had significantly reduced ventricular contractile rate, stroke volume, and cardiac output, while Se-Met exposed rainbow trout had significantly greater stroke volume, ejection fraction, and cardiac output. Following ultrasonography, energy homeostasis and storage concentrations were measured via whole body (zebrafish) and liver, heart and muscle (rainbow trout) glycogen and triglyceride concentrations. Zebrafish in the highest exposure group were observed to have greater whole body glycogen concentrations when compared to the control, while rainbow trout exposed to Se-Met concentrations greater than 15.8 showed significant increases in both glycogen and triglycerides in liver storage relative to the controls. In addition, rainbow trout in the highest exposure group had significantly reduced capability of managing blood glucose levels, as was evident after 48 hours in the glucose tolerance test. Exposure to Se-Met significantly decreased mRNA expression of a key cardiac remodelling enzyme, matrix metalloproteinase 2 (MMP2), in adult zebrafish heart, but significantly increased it (MMP9) in rainbow trout hearts. Se-Met significantly increased echodensity at the junction between atrium and ventricle, and these results combined with increased MMP2 expression are consistent with cardiac remodelling and fibrosis. Rainbow trout, however, didn't show any fibrosis and also had a significant decrease in SERPINH mRNA abundance, a molecular chaperone essential for the post-translational folding of fibril-forming collagens. This, taken together with the increase in MMP9, begins to show an anti-fibrotic response in the rainbow trout heart compared with the fibrosis seen in the zebrafish, which could help explain why opposing cardiovascular results were seen. As a result of the anti-fibrotic response in the rainbow trout, the heart was able to pump blood more effectively and led to the observed increase in stroke volume, ejection fraction, and cardiac output. The results of this study suggest that chronic exposure to dietary Se-Met can impact cardiac function and energy homeostasis, and can cause cellular perturbations; such physiological consequences could reduce the aerobic capacity and survivability of fish. The varying results seen could be attributed to species sensitivity differences or perhaps to the cold- vs warm-water fish selenium sensitivity. Further studies are clearly needed.

Behavioural Toxicology

The neuroprotective role of P-glycoprotein in teleost fish: A behaviour time-analysis (PL)

Vinicius Cavicchioli Azevedo[1], Chris Kennedy[1]

[1]*Simon Fraser University*

The importance of the blood brain barrier (BBB) and the role of P-glycoprotein (P-gp) located in the BBB in protecting aquatic organisms, particularly the brain, from potential central nervous system (CNS) neurotoxicants, has been examined in mammals, but little is known of its role in protecting fish. The role of the CNS in animal behavior is well known and several studies have demonstrated that environmental neurotoxins can cause behavioral and sensory disruption, affecting survival and fitness. The objective of this study was to assess the neuroprotective role of P-gp in fish through measurement of behavioural endpoints. Zebrafish (*Danio rerio*) were intraperitoneally injected with a CNS neurotoxin (ivermectin [IVM]) or a combination of IVM and a known P-gp inhibitor (Cyclosporin a [CysA]), and were separated into seven treatment groups: saline only, DMSO (0.01%) as carrier, IVM (2.24 $\mu mol \cdot kg^{-1}$), CysA (5 $\mu mol \cdot kg^{-1}$), IVM (2.24 $\mu mol \cdot kg^{-1}$) + CysA (1 $\mu mol \cdot kg^{-1}$), IVM (2.24 $\mu mol \cdot kg^{-1}$) + CysA (3 $\mu mol \cdot kg^{-1}$) and IVM (2.24 $\mu mol \cdot kg^{-1}$) + CysA (5 $\mu mol \cdot kg^{-1}$). Following injections, zebrafish were individually placed into an observation tank that consisted of two chambers separated by a shallow trough that allows fish to move freely between the chambers. Video tape was analyzed for mean speed, maximum swimming speed, and number of passages between chambers every 30 minutes for 3 hours. Overall, the results show that IVM causes behavioural dysfunction in most of the endpoints measured, and an increase in the onset and intensity of effects in groups injected with ivermectin combined with the P-glycoprotein inhibitor Cyclosporin a. These results suggest that P-gp plays an important role protecting the teleost brain from potential deleterious effects caused by environmental neurotoxins.

The toxicity of sediment-bound dilbit to freshwater amphipods (*Hyalella azteca*) (PL)

Sean Everitt[1], Steve Wiseman[1], Gregory Pyle[1]

[1]*University of Lethbridge*

The Alberta oil sands are among the largest fossil fuel deposits globally and contain bitumen, a heavy form of crude oil. Bitumen is more viscous and dense than conventional oils and must be diluted with natural gas condensates to form diluted bitumen or 'dilbit' before it can be transported through pipelines. Pipelines that transport dilbit are in close proximity to thousands of freshwater ecosystems, but currently the toxicity of dilbit to freshwater organisms is less understood than is toxicity of conventional oils. Dilbit behaves uniquely after a spill because the added diluents will evaporate rapidly, increasing the density and viscosity of the dilbit. The weathered dilbit that remains can combine with fine sediments in the water column, causing the dilbit to sink in freshwater. Weathered sediment-bound dilbit (WSD) can affect freshwater organisms by physically coating them

or by releasing organic compounds such as polycyclic aromatic hydrocarbons and their alkyl-derivatives into the water column. To date, research has focused on the water-soluble and water-accommodated fractions of fresh dilbit and its effects on aquatic organisms. Here, we present the first study where the toxicity of WSD is assessed in a freshwater organism. Adult freshwater amphipods (*Hyalella azteca*) were exposed for 96 hours to sediments that were 100, 50, 25, 12.5 and 6.25% WSD by volume. In all concentrations of WSD, there was 100% mortality. When exposed to the water-soluble fraction of WSD, no significant mortality was observed. Ongoing research is addressing the sublethal effects of the water-soluble fraction of WSD by measuring acetylcholinesterase inhibition, oxidative stress, and oxygen consumption after acute exposure. Behavioural experiments are also being conducted to determine if the water-soluble fraction of WSD can change the overall activity of amphipods as a result of these physiological changes. Ultimately, this is an important step towards better understanding the impacts that spills of dilbit may have on freshwater organisms and developing spill management guidelines that can be implemented in future dilbit spills.

Early lifestage zebrafish behaviour as high-throughput tools in ecotoxicology (PL)

Patrick Gauthier[1], Matt Vijayan[1]

[1]*University of Calgary*

The zebrafish (*Danio rerio*) embryo has already proven to be an excellent behavioural model for high-throughput pharmaceutical screening applications. As early as 30 hours post fertilization (hpf), zebrafish embryo photomotor responses (PMRs) can be recorded in 96-well plates, and rapid neuromodulatory effects of chemical exposure can be detected. At 96 hpf, larval locomotor activity can also be recorded in 96-well plates and changes in activity profiles in light and dark environments can be used as a model for anxiety. These robust and reliable early lifestage behaviours are highly plastic to chemical exposure and produce highly repeatable results, so we propose they can be applied in high-throughput bioassays for ecotoxicological purposes. We have developed two behavioural screening platforms to assess the neuromodulatory effect of contaminants and their mixtures on embryonic and larval zebrafish behaviour. Both phenotypes are exceptionally sensitive to neuroactive chemicals and can detect effects at $ng \cdot L^{-1}$ concentrations almost immediately following exposure. We demonstrate the application of these tools and showcase data from numerous toxicological studies involving pharmaceuticals, pharmaceutical mixtures, and whole municipal wastewater effluents.

Exposure to oil sands process-affected water (OSPW) alters shoaling behaviour in zebrafish (*Danio rerio*) (PL)

Trevor Hamilton[1], Matthew Ross[1], Erica Ingraham[1], Nathan Nadolski[1], Courtney Bailey[1]

[1]*MacEwan University*

The extraction of oil from surface-mined bitumen in Northern Alberta produces large amounts of oil sands process-affected water (OSPW), which contains a complex mixture of

toxic chemical compounds such as metals and organic acids. OSPW cannot be returned directly to its source due to its potential toxicity to aquatic and terrestrial life, but efforts are being made to reclaim OSPW so that it can be returned safely to the ecosystem. Reproductive endpoints have been used to understand the toxicity of OSPW on fish, but behavioural measures have not yet been fully investigated. In the present study, we exposed zebrafish (*Danio rerio*) to dilute OSPW (10% OSPW) for 30 days then returned them to normal freshwater. We studied their group shoaling behaviour using a motion-tracking software system on exposure days 10 and 30, then 25 and 60 days following return to freshwater. We found a prolonged and significant impact of OSPW on certain aspects of social behaviour in these fish.

Something smells funny: thyroid hormone disruption and the olfactory system in bullfrog tadpoles (PL)

Caren Helbing[1], Kevin Jackman[1], Jody Heerema[2], Nik Veldhoen[1], Rachel Miliano[3], Bonnie Robert[1], Linda Li[4], Azadeh Khojasteh[5], Xiaoyu Zheng[5], Tristan Zaborniak[1], Graham Van Aggelen[3], Mary Lesperance[1], Wayne Parker[4], Eric Hall[5], Greg Pyle[2]

[1]University of Victoria, [2]University of Lethbridge, [3]Environment and Climate Change Canada, [4]University of Waterloo, [5]University of British Columbia

The olfactory system is used to sense information-containing chemicals in the environment. Sensory cues link to behavioural responses. For example, food cues evoke foraging responses and predator cues evoke anti-predator responses such as freezing, hiding, or avoiding. Olfaction is an essential sensory function for the survival and fitness of frogs throughout their life. Olfactory requirements develop and change as the animal transitions from the herbivorous aquatic lifestyle of the larval tadpole into the carnivorous, terrestrial (in most) lifestyle of the juvenile frog. While the fundamentals of the olfactory system remain the same—receptors on the surface of the olfactory epithelium bind odorants and propagate a signal to the olfactory bulb of the brain, which elicits a behavioral response to the odorant—what constitutes an appropriate response to different odorants and is essential for survival differs depending upon life phase. Metamorphosis of the tadpole into a frog is completely dependent upon thyroid hormones (THs) and the frog olfactory system substantially remodels during this time. We are investigating the relationship between TH-responsiveness of olfactory system components and behavioral outcomes in the North American bullfrog (*Rana [Lithobates] catesbeiana*) tadpole in order to identify biomarker candidates for screening complex mixtures such as municipal wastewater for TH-disrupting activity. We have generated the first high-quality Ranid genome resource that enabled gene expression analyses. Using a combination of classic TH-response gene transcripts and novel ones identified by RNA-seq, we queried TH-disrupting activity in municipal wastewater effluent from two different treatment systems: anaerobic membrane bioreactor (AnMBR) and membrane-enhanced biological phosphorous removal (MEBPR). While we observed physical EDC removal in both systems, they retained some TH disruption activity. This work lays an important foundation for linking TH activity with olfactory system function in amphibians.

The effects of salmonid aquaculture anti-sea lice chemotherapeutants on non-target benthic organism growth and behaviour (PL)

Samantha Lundquist[1], Chris Kennedy[1]

[1]*Simon Fraser University*

The aquaculture industry along the North Pacific coast is continuously expanding to meet consumer demand. Sea lice (*Lepeophtheirus salmonis*) are ecto-parasites of fish and are a significant problem in salmonid aquaculture. The control of these parasites is an essential element in the protection of cultured fish as well as wild stocks and often involves the strategic use of chemotherapeutic agents. The release of these compounds into the environment from use in open-net pen aquaculture, causing potential impacts on non-target marine organisms, has emerged as a significant concern. Two chemicals used in sea lice control include the anti-parasitic chemotherapeutants ivermectin and SLICE®. Since these chemicals are persistent and have a high affinity for marine sediments, they pose a risk to benthic communities. This study assesses sublethal effects of both ivermectin and SLICE® (and a combination of both) on marine benthic species including the polychaete, *Nereis virens*. Polychaetes were exposed to sediment spiked with three environmentally relevant concentrations of the chemicals and two higher concentrations, based on previous literature. Growth was measured over the 60-day exposure period. Organism behaviour patterns were videotaped on day 14 and day 28, and locomotion, burrowing speed, and response to food stimulants were observed. Daily burrowing was recorded for the duration of the exposures. This research specifically addresses information gaps that need to be filled to properly manage any risks associated with the use of these chemotherapeutants. The data obtained from this research will ensure their proper and safe use, and the appropriate regulation of these aquaculture chemicals in Canada.

Maternal exposure to dietary selenomethionine led to dopaminergic hyperfunction and learning impairment in zebrafish offspring (PL)

Mohammad Naderi[1], Som Niyogi[1], Doug Chivers[1]

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Maternal exposure to environmental contaminants is a predisposing factor for neurodevelopmental disorders with associated cognitive deficits in offspring. Selenium (Se) is a contaminant of potential concern in aquatic ecosystems. In oviparous animals including fish, dietary form of Se (i.e., selenomethionine) can be maternally transferred to the eggs and affect the developing nervous system in animals, mainly through induction of oxidative stress. Dopamine is one of the major neurotransmitters in the brain which plays an important role in the development and function of neural circuits involved in learning and memory and social behaviours in fish. However, the dopaminergic system is highly susceptible to oxidative stress, and the oxidative insult during critical windows of development may disrupt this system and lead to neurodevelopmental abnormalities. In this study, we investigated the effects of maternal transfer of Se on learning and memory in F1-generation of adult fish, with a particular focus on changes in dopaminergic signaling. To this end, female fish (F0 population) were fed either a control food (1.2 μg $Se \cdot g^{-1}$ dry

weight) or food spiked with increasing concentrations of Se (as selenomethionone, 3.5, 11.1, 27.4 $\mu g \cdot g^{-1}$ dry weight) for 60 days. Following exposure, fish were bred and offspring were hatched and raised to adulthood (6 months old). Subsequently, cognitive performance of fish (F1 generation) was tested using a latent learning paradigm. Our results showed a marked learning impairment in all Se treatment groups compared to the control. This behavioural impairment was associated with an elevated level of dopamine in zebrafish brain along with increased mRNA expression of genes involved in the synthesis, storage, re-uptake, and degradation of dopamine. In addition, the mRNA abundance of different dopamine receptors also showed a significant increase in the zebrafish brain. This hyperfunction of the dopaminergic system led to the induction of oxidative stress in the zebrafish brain, as indicated by the depletion of reduced glutathione and increased lipid peroxidation. Collectively, our results suggest that maternal exposure to dietary Se leads to persistent neurobehavioural deficits in F1-generation adult zebrafish, via alterations in the dopaminergic signaling in the brain.

Rainbow trout (*Oncorhynchus mykiss*) chemosensory detection of and reactions to copper nanoparticles and copper ions (PL)

Parastoo Razmara[1], Justin Sharpe[1], Greg Pyle[1]

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Olfaction is crucial for fish survival because fish rely on olfaction to find food, reproduce, and avoid predation. However, copper (particularly Cu^{2+}) is a potent disruptor of the fish olfactory sense. Although the chemosensory detection and olfactory toxicity of Cu^{2+} in fish has drawn much research attention, the effects of copper nanoparticles (CuNPs) on the olfactory system are not well characterized. Copper nanoparticles have widespread application in different industries. Due to the global production of and applications for CuNPs, aquatic ecosystems will likely experience increasing exposure to CuNPs through effluent discharge. This study investigated the interaction of rainbow trout olfactory system to equitoxic concentrations of CuNPs or Cu^{2+}, using electro-olfactography (EOG, a neurophysiological technique) and olfactory-mediated behavioural assay (by a choice maze). To test chemosensory function of rainbow trout, the concentration of contaminants known to impair olfaction by 20% over 24 hours(EOG-based 24-hour IC20s of 220 and 3.5 $\mu g \cdot L^{-1}$ for CuNPs and Cu^{2+}, respectively) were tested as olfactory stimuli using both neurophysiological and behavioural assays. To determine whether the presence of CuNPs or Cu^{2+} can affect the ability of fish to perceive a social cue (taurocholic acid (TCA)), fish were exposed to Cu-contaminants for a short period (approximately 15 min), and the olfactory acuity and olfactory-mediated behaviour were measured in response to TCA. Results of both neurophysiological and behavioural experiments demonstrate rainbow trout can detect and avoid CuNPs. However, the 24-hour IC20 of Cu^{2+} was not enough to affect fish olfactory-mediated behaviour. Additionally, the brief exposure to CuNPs caused a significant reduction in olfactory acuity relative to the control and Cu^{2+} treatment. Results of the behavioural study also demonstrated that fish could not detect and respond to TCA after the short exposure period to CuNPs. The observed reduction in fish olfactory acuity during the brief exposure to CuNPs might be a result of either olfactory fatigue or changes

in extracellular surface potential of olfactory epithelium, as CuNPs have negative zeta potentials. Further investigations are required to identify the mechanism of effects caused by CuNPs.

The lasting effects of developmental crude oil exposure on ecologically relevant behaviours in the sheepshead minnow (PL)

Keith Tierney[1], Danielle Philibert[1], Danielle Lyons[1]

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Over the course of 87 days, the Deepwater Horizon oil spill released 3.19 million barrels of crude oil into the Gulf of Mexico, which then spread to the surrounding tributaries and wetland habitats. Dispersant was applied both at the surface and at the wellhead in an attempt to reduce the effects of the oil slicks on coastal habitat. Historically, studies focused on lethality and cardiotoxicity; complex behaviours have been overlooked, for the most part, despite the merits of including these endpoints in toxicological studies. Behavioural development coincides with the development of the nervous system in fishes, and behavioral abnormalities that occur as the result of a developmental exposure are referred to as "behavioral teratology". In our study, we examined the behavioral teratology of crude oil exposure from 1-10 days post-fertilization using ecologically relevant behaviours: juvenile prey capture, adult male aggression, and adult novel object responses. Exposure to the various water accommodated fractions (WAFs) had no direct impact on the response efficiency but caused a shift in the bold and shy behavioral phenotypes normally found. Complex behaviours are sensitive sublethal endpoints that could be used in the risk assessment of contaminant mixtures. The inclusion of complex behaviours in toxicological studies brings ecological relevance to a biomarker-dominated field.

Avoidance response of the benthic marine polychaete *Nereis virens* to aquaculture chemotherapeutant-contaminated sediment (PL)

Lindsay Woof[1], Chris Kennedy[1]

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The quality of near-shore coastal waters is of great concern to Canadians, particularly as these ecosystems become increasingly threatened by pollution. Due to the significant implications of sea lice infestation for both farmed and wild salmonid populations, the aquaculture industry in Canada utilises anti-sea lice chemotherapeutant treatment, resulting in the release of these compounds into marine ecosystems. Gaps in data have made it difficult to predict the persistence and toxicity of these chemicals, and the potential risks posed to non-target organisms. Benthic organisms are at risk of exposure to two chemotherapeutants, ivermectin (IVM) and SLICE® (0.2 % emamectin benzoate [EB]) that tend to partition to sediments. This study investigated the sublethal effects of chronic sediment exposures of these chemicals to the most potentially susceptible marine organisms. Sediment avoidance is considered a relevant and sensitive endpoint for assessing sediment contamination by xenobiotics. Sediment avoidance assays were

performed on the marine polychaete *Nereis virens*, in which animals were placed in test vessels that allowed congruent placement of two sediments (one contaminated) and measurement of the movement of organisms over a 7-day period. In the two-chamber system, sediment was spiked with SLICE®, IVM, or a combination of both and a control sediment. Prior to the avoidance assay, animals were unexposed or previously exposed to environmentally relevant concentrations of either 5 $\mu g \cdot kg^{-1}$ of IVM, EB prepared from SLICE®, or both for 30 days. Unexposed animals displayed both behavioural toxicity and avoidance to IVM, but no effects were observed for SLICE®. Preliminary work indicates a synergistic effect when organisms were exposed to both IVM and SLICE®. Preexposure to IVM for 30 days resulted in impaired burrowing behaviour and locomotion with avoidance behaviour at the highest concentration of spiked sediment. Preexposure to SLICE® resulted in no behavioural toxicity or avoidance. The data obtained from this research will help assess the environmental consequences of using sea lice chemotherapeutants in open-net pens in salmon aquaculture and inform their safe use and appropriate regulations in Canada.

Behavior characterization and tracing of an endocrine disruptors mixture (PO)

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A lot of resources are dedicated to characterizing the effect of trace chemicals on human health. But how do sublethal exposures to chemical mixtures affect natural ecosystems and population dynamics? It is known that climate change has disrupted the emergence of organisms (flowers, insects, etc.) and the resources on which they depend, which leads to a slow and constant decrease of the overall populations over decades. In this poster we address the development of endpoints to measure the effects on a small lab trophic chain of sublethal long-term exposure to contaminants mixtures. Our results suggest that such exposure may accelerate the life cycle, maturation, fertility and behaviors such has flight and feeding. These results may be the basis for new hypotheses about the long-term evolution of populations subjected to chronic exposure of mixtures of contaminants.

Directing the Future of Pesticide Research

Applying the Bradford Hill Criteria to the risk assessment of pesticides: A case study with thiamethoxam (PL)

Mark Hanson[1]

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Correlation is not causation. This concept is simple enough in principle, but difficult to distinguish in practice sometimes. Thankfully, we have guidance in the form of the Bradford Hill Criteria to keep us from going astray. Hill's 'aspects of association' (i.e., specificity, temporality, strength of association, consistency, biological gradient, plausibility, experiment, coherence, and analogy) form the foundation of epidemiology, and are wholly applicable to ecological risk assessment for pesticides. When these criteria are coupled with quantitative weight of evidence (especially evaluation of the strength of methods) and the adverse outcome pathway framework, you have a powerful tool to illuminate the real from the merely hypothetical. To demonstrate the utility of the approach, a case study with the neonicotinoid thiamethoxam (TMX) will be discussed. Specifically, we tested the hypothesis that TMX, or its metabolites, are responsible for significant impacts on aquatic insects and consequent cascading impairment on terrestrial biota in Canada. For context, current environmental concentrations of TMX in Canadian surface waters are typically less than a few hundred $ng \cdot L^{-1}$ when detected. Each 'aspect of association' was evaluated and data quality assessed when possible. Overall, the evidence for each aspect was either weak or non-existent, with inconsistent evidence for effects at the ecosystem-level a priori. This leads to the conclusion that TMX is not likely to be impairing ecosystems under most current use applications. It is recommended that risk assessors apply the Bradford Hill Criteria when trying to assign causation for observed effects to pesticides in the future.

Does citation bias exist in the pesticide ecotoxicology literature? (PL)

Ryan Prosser[1], Mark Hanson[2], Lorna Deeth[1]

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As scientists, we are tasked with letting evidence guide our conclusions. In the world of pesticides, this takes on added importance as the data can influence ecological and human health outcomes and regulations, and even the manner in which we grow food. Yet there seems to be a reluctance to engage with the totality of pesticide ecotoxicology literature, especially papers that report few or no effects or low risk to non-target organisms. We suspected that these studies would have fewer citations than studies that report significant effects or risk for the same compound, and this would be unrelated to the strength of the study, e.g., high-quality studies with few or no effects would be cited less frequently than studies of lesser quality that reported effects. To investigate this, we examined a subset of literature around the herbicide atrazine. We found that papers reporting an effect had significantly more citations per year than those that did not ($p<0.05$). There was no significant relationship between the strength of the study and number of citations, but a

general trend for weaker studies to have a greater number of citations. The impact factor of journals was not positively correlated with the strength of the study methods, but studies that reported effects were published in journals with a greater mean impact factor than those that reported no effects ($p<0.05$). This analysis reveals evidence of citation bias within the pesticide ecotoxicology literature, as well as bias by journals to publish studies that report effects, regardless of study quality. This study is currently being expanded to examine all research articles studying the effects of pesticides that have been published in the journal Environmental Toxicology and Chemistry since its inaugural issue in 1982.

Neonicotinoids alter leukocyte profiles and measures of oxidative stress in a native amphibian, *Lithobates pipiens* (PL)

Stacey Robinson[1], Sarah Richardson[1], Ryan Chlebak[2], Rebecca Dalton[1], Melody Gavel[2], Ryan Prosser[3], Celine Boutin[1], Adrienne Bartlett[1], Shane de Solla[1], Vance Trudeau[4], Frances Pick[4]

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Neonicotinoids are being recognized for their toxicity to aquatic invertebrates, as evidenced by the special review being conducted by Health Canada's Pest Management Regulatory Agency, which has a specific focus on the toxicity of clothianidin and thiamethoxam to aquatic invertebrates. However, relatively little is known about the effects of neonicotinoid insecticides on vertebrate wildlife. Amphibians are excellent vertebrate bioindicators due to their sensitivity to environmental stressors, and they may be vulnerable to neonicotinoid exposure because of their dual aquatic/terrestrial life cycle. The objective of our study was to characterize the sublethal effect(s) of exposure to clothianidin on northern leopard frogs (*Lithobates pipiens*, formerly *Rana pipiens*) during their aquatic life-cycle stage using a laboratory exposure. Our previous work (2015) exposed northern leopard frog tadpoles to formulated neonicotinoid products containing thiamethoxam or clothianidin as the active ingredient in outdoor mesocosms. The exposure resulted in no effects on survival, growth or development time, but did result in significant shifts in leukocyte profiles. Here we followed up on our previous work via laboratory static-renewal exposures with the technical product clothianidin to separate potential indirect and direct effects of neonicotinoids on tadpoles. In May 2016, adult northern leopard frogs were bred in outdoor mesocosms and egg clutches were allowed to develop. Tadpoles starting at Gosner stage 25 were exposed to clothianidin concentrations (0, 0.23, 1, 10 and 100 $\mu g \cdot L^{-1}$) or diquat (1.1 $\mu g \cdot L^{-1}$; positive control for oxidative stress parameters) until they reached Gosner stage 42 (an 8-week exposure period). We assessed the effects of chronic exposure to clothianidin or diquat on tadpole leukocyte profiles and measures of oxidative stress, as these more subtle alterations could affect amphibian fitness. We found changes in several types of leukocytes at 0.23 and 1 $\mu g \cdot L^{-1}$ compared to controls, which suggest these frogs were stressed and might have altered immune function. We also found that clothianidin induced an oxidative stress response at 0.23, 1 and 100 $\mu g \cdot L^{-1}$ compared to controls, with an effect comparable to that of diquat (a well-known oxidative stressor). Similar to our mesocosm work in 2015, we found no differences in survival, growth or development time. The results of this study will help in establishing water quality

guidelines and regulations for neonicotinoid use in Canada. However, more information is required on monitoring environmental concentrations of neonicotinoids and sublethal effects, including susceptibility to disease or parasites, on chronically exposed non-target organisms to fully understand the risk of neonicotinoids on ecosystems.

Breaking the cycle: What the neonicotinoid issue should be teaching us about the need for a more rational management of pesticides in agriculture (PL)

Paul Sibley[1], Ryan Prosser[1]

[1]*University of Guelph*

Concerns over the real or perceived environmental impacts of neonicotinoid insecticides have spurred public and scientific debate on the use of pesticides rivalling that which ensued from the publication of Rachel Carson's *Silent Spring*. Use of neonicotinoid insecticides has increased extensively in the past two decades because for farmers they represent a highly efficacious pest control tool that maximizes yields and ensures high quality marketable products. However, relatively unrestricted prophylactic use of these compounds has led to routine detections in the environment, occasionally at concentrations potentially associated with effects on aquatic and terrestrial non-target organisms. This has led to proposals to ban these chemicals; however, it is difficult to tease apart the effects directly attributable to neonicotinoids from those of other factors (e.g., homogeneous landscapes, habitat loss) also known to explain some of the observed impacts. Banning will inevitably lead to the introduction of new compounds or a resurrection of old compounds which, in the absence of a more structured approach to pesticide management, will only lead to a perpetuation of the cycle of "ramping up" use, as observed with the neonicotinoids, potentially leading to the same environmental problems we currently face and the inevitable public outcry for bans and/or stricter regulation. In this presentation, I draw from the literature and from work conducted in our lab on neonicotinoid insecticides to examine a number of approaches that I believe can be used to develop a more structured and rational approach to the use and management of pesticides. Among the aspects I will consider are the need to adopt more universal application of best management and technology practices, enhanced monitoring programs, promotion of ecologically diverse landscapes, and changes to the process in which pesticides are evaluated and registered. I will present a conceptual framework to argue that it is possible—indeed essential—to break the cycle by developing more holistic and responsible management practices for pesticide use in agriculture.

On the toxicity of the lampricide TFM in fish: the role of life stage, water chemistry and the gill microenvironment (PL)

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[1]*Wilfrid Laurier University*

Invasive sea lamprey populations in the Great Lakes are controlled by regularly applying the lampricide TFM (3-trifluoromethyl-4-nitrophenol) to streams infested with

larval lamprey. However, non-target fishes such as juvenile lake sturgeon may be exposed to TFM. This threatened species is also more sensitive to TFM than most non-target fishes. The goal of the present study was to determine what role life stage, water chemistry and the gill microenvironment had on lake sturgeon susceptibility to TFM. Acute toxicity tests demonstrated that young-of-the-year (YOY) lake sturgeon were much more susceptible to TFM than one year old (1+) sturgeon. Subsequent analysis of TFM detoxification products revealed that the YOY fish had a lower capacity to detoxify TFM than their one-year-old counterparts, in which TFM accumulation was 50% lower. Water chemistry also affected TFM toxicity, with fish at lower alkalinity most susceptible to TFM all dying within 6 hours. As alkalinity increased, sturgeon survival increased, with 100% survival after TFM exposure at high alkalinity. Given that TFM causes a mismatch between ATP supply and demand in fishes, we hypothesized that metabolites and fuel stores in lake sturgeon would also be affected by the exposure. Immediately following death, tissues (brain, liver, muscle, carcass) were taken to quantify metabolites/energy stores and white muscle intracellular pH (pHi). To determine if the gill microenvironment influenced TFM toxicity, divided chambers were designed to monitor how water pH at the gill surface influenced TFM speciation and uptake by rainbow trout and lake sturgeon during TFM exposure. Preliminary work with rainbow trout revealed that at neutral pH water expired from the gills was 0.2 units lower than the inspired bulk water, suggesting that changes in water pH in the gill microenvironment could alter TFM speciation, leading to changes in TFM uptake by the fish. Our work demonstrates the benefits of focusing future research on how the adverse effects of piscicides such as TFM are influenced by life stage, water chemistry and the chemistry of the gill microenvironment in potentially vulnerable non-target species such as the lake sturgeon.

The effects of triclopyr application on habitat quality in northern Saskatchewan transmission rights-of-way (PL)

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Managing vegetation along power line rights-of-way is challenging in remote boreal forests across Canada, where vegetation can be dense and the terrain unsuitable for mechanized vegetation removal. Limited data on the effects of herbicides in northern climates and on boreal vegetation creates difficulties in communicating potential risks and benefits to concerned local stakeholders and Indigenous communities. Working in partnership with SaskPower and Lac La Ronge Indian Band, we examine the impact of two herbicides, Garlon RTU and XRT (a.i. triclopyr) in northern Saskatchewan. Direct toxic effects in boreal forest soils due to herbicide application appear to be limited. Previous work demonstrated that the maximum herbicide application rates for Garlon XRT were below the effective concentration at 25% (EC_{25}) for common boreal soil invertebrates (*Folsomia candida, Oppia nitens*, and *Enchytraeus crypticus*). However, questions remain regarding the indirect effects of these herbicides and their potential impact on habitat quality. Herbicides can directly enter the soil ecosystem through application deposition or following translocation from stems into foliage, and indirectly through leaf abscission.

Herbicide residues in leaves treated by basal bark and low-volume foliar applications were examined under field conditions. Determining dissipation rates of herbicide residues within foliage is important for evaluating risk to browsing wildlife. To assess the indirect effects of herbicide application on litter and soil habitat quality, we compare treated and untreated leaf litter quality (C:N) and decomposition rates, using a buried bag technique. Herbicide-induced alterations to habitat quality could adversely influence boreal foliage litter degraders and consequently litter degradation rates. It is expected that treated leaves will have lower C:N ratios and increased decomposition rates compared with untreated litter. Laboratory microcosms will also be used to examine the influence of herbicide application on leaf litter decomposition and quality, as well as direct measures of mortality and reproduction of *F. candida* and *O. nitens* over a four-month period. Since these important mesofaunal decomposers could have an aversion to contaminated litter, standard avoidance tests are also conducted. The avoidance response of organisms able to use chemoreceptors to detect and avoid substances in the environment is an important behavioral endpoint being utilized in soil ecotoxicology. Current data indicate that both *F. candida* and *O. nitens* avoid contaminated litter, but EC50 values are expected to fall above the maximum herbicide application rates for Garlon XRT. Litter decomposition is essential for soil nutrient cycling and maintenance of ecosystem health. In addition, herbicide-treated foliage may be a vector of contamination to browsing wildlife. Therefore, the results of this study will provide important information to assist utilities and local stakeholders in making decisions regarding herbicide use on power line rights-of-way.

The role of vegetated buffers in agriculture, and variation in their regulation across Canada and the United States: A need for rationalization (PO)

Mark Hanson[1], Paul Hoekstra[2], Samantha Gene[3], Carol Hannam[4], Ryan Prosser[3]

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A vegetated buffer, barrier, or filter strip is a parcel of land that is designated to separate land used for agriculture from aquatic habitats. It exists partly with the intent to diffuse surface water runoff and to intercept soil, nutrients, pesticides, etc. from reaching surface waters. Mandatory buffer implementation is regulated at various levels of government in North America, from the federal to the state and provincial levels, and even by municipalities and counties. To better understand the degree and breadth of oversight, we undertook a comprehensive search and review of vegetative buffer regulations across North America. We determined the width of buffer required, under what habitat or field conditions, for which pesticides, and for which application types, amongst others. For ground application, margins could range from 1 m to upwards of greater than 4000 m depending on protection goals, with some being compound-specific and others being for all registered pesticides/compounds. Buffers tended to be used most often to protect surface water, groundwater (e.g., drinking water wells), and nearby sensitive crops, but the required distances showed no consistency between jurisdictions, regardless of stated protection goals. The same lack of consistency holds true for aerial applications. We recommend that a thorough science-based review take place to harmonize vegetated buffer

distances for surface water protection where ecological and agricultural conditions are similar with input from all stakeholders.

The effects of anti-sea lice drugs and pesticides on marine zooplankton (PO)

Jenna Keen[1], Chris Kennedy[1]

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Concern for the quality of near-shore coastal waters and estuaries is growing, particularly as these ecosystems become increasingly threatened by anthropogenic chemical inputs. An improved understanding of chemical impacts on near-shore ecosystems and their components is essential to responsible stewardship of these coastal areas. In recent years, the aquaculture industry has become a major contributor to the Canadian economy, however, this industry's use of chemicals, including those used in disinfectants, anti-fouling paints, and feed additives has resulted in the contamination of local net pen areas. Contamination associated with the use of chemotherapeutants to treat sea lice has emerged as a potential risk to non-target organisms. This study specifically addresses information gaps that need to be filled in order to understand the environmental consequences of exposure to two chemical therapeutants used for sea lice treatments: Salmosan® and Paramove 50®. Zooplankton play a key role in marine food-web dynamics, biogeochemical cycling, and fish recruitment; however, despite their importance in marine environments, our knowledge of the interactions between zooplankton and aquaculture chemotherapeutants is extremely limited. Studies on the lethal and sublethal effects of Salmosan® and Paramove 50® exposure in representative marine zooplankton species under realistic exposure scenarios were undertaken. The data obtained from this research will be useful to ensure the proper and safe use, and appropriate regulation of these aquaculture chemicals in Canada.

Comparing the toxicity of neonicotinoid pesticides to two collembolan species (PO)

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

[1]*University of Guelph*

Investigations into the effects of neonicotinoid insecticides on non-target organisms have become increasingly prevalent due to evidence of impacts on pollinators such as honeybees, as well as aquatic and terrestrial invertebrates. Neonicotinoids enter agricultural soils through foliar spraying and through seed treatment applications, and have half-lives of up to hundreds of days in soil systems. Springtails (Subclass *Collembola*) are an ecologically important native soil invertebrate in Canada that have been found to be sensitive to neonicotinoid exposure, but less so than other commonly studied soil invertebrates. These studies compared the effects of two second-generation neonicotinoid insecticides, thiamethoxam and clothianidin on the reproduction of two springtail species: the soil-dwelling *Folsomia candida* and the leaf litter-dwelling *Arrhopalites caecus. Folsomia candida* is considered relatively insensitive to contaminant exposure, while there is evidence that *A. caecus* is a more sensitive test organism. These studies aimed to determine

the variation in effects of neonicotinoid exposure to these species. Variation in the sensitivity of springtails to pesticides or other chemicals is currently not incorporated into assessments of risk to terrestrial ecosystems. This study has also investigated the potential for *A. caecus* as an effective test organism in soil toxicology.

Multi-Generational Ecotoxicology and Epigenetics

Response to AHR ligands in liver slices cultured from naïve and pre-treated chicken embryos: Does DNA methylation play a role? (PL)

Jessica Head[1], Krittika Mittal[1], Jonas Brandenburg[1]

[1]*McGill University*

Lipophilic environmental contaminants such as dioxin-like compounds (DLCs) and polycyclic aromatic hydrocarbons (PAHs) can be found in high concentrations in the eggs of wild birds. We are interested in how this early life exposure to contaminants affects sensitivity to subsequent exposures later in life, and whether sensitivity to re-exposure is regulated by epigenetic mechanisms. In the current study, the DLC, tetrachlorodibenzo-p-dioxin (TCDD), or the PAH, benzo[k]fluoranthene (BkF) was injected into fertilized chicken eggs prior to incubation. At embryonic day 19, livers were harvested and slices of the tissue were grown in culture. Levels of mRNA expression of the metabolic enzyme, cytochrome P4501A (CYP1A), were assessed in each tissue slice. We observed a 50-fold induction of CYP1A mRNA expression in tissues cultured from TCDD- but not BkF-treated embryos. The absence of a sustained response in tissues cultured from embryonic day 19 BkF-treated embryos was likely due to rapid metabolism of the PAH *in vivo*. Liver slices were also re-exposed to graded concentrations of TCDD or BkF in culture for 24 hours, resulting in dose-dependent increases in CYP1A expression for both test chemicals. Interestingly, liver slices exposed to BkF *in vivo* appeared to be more sensitive to CYP1A induction *in vitro*. We are continuing to study this phenomenon in the context of our previous findings relating to PAH-dependent methylation of the CYP1A promoter. DNA methylation of genes involved in xenobiotic metabolism may be useful as biomarkers describing an association between early life exposures to environmental contaminants and sensitivities to subsequent exposures later in life.

Mechanistic underpinnings of epigenetic effects of selenium using rainbow trout (*Oncorhynchus mykiss*) hepatocytes in primary culture (PL)

Ankur Jamwal[1], Justin Miller[1], Igor Kovalchuk[1], Alice Hontela[1], Steve Wiseman[1]

[1]*University Of Lethbridge*

Selenoprotein synthesis, selenium (Se) metabolism, and oxidative stress from supranutritional concentrations of Se can deplete the cellular pool of GSH, which can be replenished by diverting homocysteine from one-carbon cycle for GSH synthesis. The one-carbon cycle synthesizes methyl donors, and its disruption can reduce cellular methylation potential, which can result in DNA hypomethylation. Against this backdrop, it was hypothesized that exposure to a high sublethal concentration of Se will cause oxidative stress, resulting in a concomitant reduction in the concentration of cellular methyl donors, which will cause cells to demethylate DNA to reprocess methyl groups for housekeeping

and metabolic functions. To test this hypothesis, primary cultures of rainbow trout (*Oncorhynchus mykiss*) hepatocytes were exposed to a toxic but non-lethal concentration (500 μM) of selenomethionine (SeMet) for 48 h. Gene expression analysis revealed an oxidative stress response to SeMet exposure and a concomitant upregulation in expression of DNA methyl transferase (DNMT3a), responsible for *de novo* DNA methylation. This indicated a possible relationship between DNA methylation and oxidative stress. Estimation of cellular redox potential, cytosine extension assay for quantification of global DNA methylation and reduced representation bisulfite sequencing for determination of promoter specific DNA methylation status will be used to understand the mechanistic underpinnings of Se-induced epigenetic modulation.

Long-term programming shift: Venlafaxine impacts larval behaviour in multiple generations of zebrafish (PL)

Andrew Thompson[1], Matt Vijayan[1]

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Venlafaxine is a serotonin and norepinephrine reuptake inhibitor (SNRI) that is commonly prescribed to treat conditions such as depression, obsessive compulsive disorder, and anxiety. The consumption of this drug has increased globally and this has led to venlafaxine appearing in municipal wastewater effluent at $ng \cdot L^{-1}$ to $\mu g \cdot L^{-1}$ concentrations. However, very little is known about the impact of this drug on non-target organisms, particularly fish. We previously reported that venlafaxine deposition in embryos disrupts developmental programming in zebrafish (*Danio rerio*). Here we tested the hypothesis that venlafaxine-mediated developmental programming dysfunction impacts the behavioural phenotype of zebrafish larvae for multiple generations. Zebrafish embryos immediately after fertilization were injected with either 0, 1, or 10 ng venlafaxine to mimic a maternal transfer scenario. The fish were assessed for stress-related behavioural phenotypes, as well as neurogenesis in the F0 (exposure), F1, and F2 generations. Venlafaxine dose-dependently affects acute larval activity to a light and dark behavioural paradigm in the F0 generation. Impacts to behaviour were evident in subsequent generations, demonstrating long-term effects of antidepressant exposure in parental and ancestral generations. Combining these behavioural changes with disruptions to neurogenesis suggests that venlafaxine alters neurodevelopment in zebrafish. In all, venlafaxine in eggs may shift early programming related to neurodevelopment and behaviour, which is evident even in multiple generations. We propose epigenome modification by venlafaxine as a possible mechanism for the multigenerational effects, and this hypothesis is currently being tested.

Diluted bitumen vs. conventional crude oil: effects of developmental exposure on progeny of exposed zebrafish (PL)

Keith Tierney[1], Danielle Philibert[1], Danielle Lyons[1]

[1]University of Alberta

The viscosity of the bitumen produced in the oil sands poses unique challenges to pipeline transport, as it does not naturally flow and requires dilution. Dilbit, the dilution of bitumen with natural gas condensates, is used to increase fluidity of the product to make it more conducive to pipeline transport. The impacts of dilbit exposure on aquatic animals have not been well studied to date, despite the possibilities of accidental spills. In this study we examined the effects of crude oil and dilbit exposure on the breeding success of adult zebrafish (*Danio rerio*) and the basal gene expression of their progeny. Embryos were exposed to water accommodated fractions (WAFs) of crude oil for a 7-day period and then were raised to adulthood in uncontaminated water. Adult fish were bred to monitor breeding success and then the progeny were reared in clean water (unexposed second-generation embryos), raised to 7 days post fertilization (dpf), and then collected for qPCR analysis. Breeding success of the first-generation developmentally exposed fish was determined by measuring the number of pairs that spawned, number of eggs spawned, fertilization rate, and survival of unexposed offspring. Gene expression and DNA methylation were also measured in 7dpf offspring. Developmental exposure in the first generation did not affect the survival of embryos and also did not affect breeding success when compared to control, but differed among exposure groups. Some target genes were differentially expressed in the unexposed second-generation embryos when compared to control, indicating a heritable change in basal gene expression. This change in gene expression could potentially be due to changes in DNA methylation caused by the developmental exposure in the first-generation. Understanding what changes in DNA methylation mean for fish survival will require further study. Though second-generation endpoints are often overlooked, they are important to consider when evaluating the overall risk of oil exposure.

Modulation of DNA methylation and mRNA expression in early life stage zebrafish (*Danio rerio*) exposed to benzo[a]pyrene (PO)

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Early-life exposure to polycyclic aromatic hydrocarbons (PAHs) has previously been shown to cause persistent changes to patterns of DNA methylation in zebrafish (*Danio rerio*). We are interested in how these epigenetic changes affect the response to PAHs through the aryl hydrocarbon receptor (AHR) pathway. Zebrafish embryos were exposed to the model PAH, benzo[a]pyrene (BaP) at concentrations of 1, 10 and 100 $\mu g \cdot L^{-1}$ from ≤4 hours post fertilization (hpf) to 120 hpf. Water spiked with BaP was renewed every 24 hours. The survival, hatch success, and incidence of malformations were monitored daily, and larvae were sacrificed at 120 hpf. The treatment did not significantly induce mortality,

deformities, or changes in time to hatch relative to controls. We used reduced representation bisulfite sequencing (RRBS) to identify differentially methylated regions within the genome of control and 100 $\mu g \cdot L^{-1}$ BaP-exposed larvae. An overall pattern of hypermethylation was observed across the genome. Regions within promoters of several genes previously shown to be transcriptionally dysregulated by AHR ligands (e.g., serf2, gng7, and agt), were differentially methylated by >20% between control and treated samples. None of these genes were differentially transcribed in response to BaP in our experiment. Ongoing work is focused on using the RRBS data to examine the methylation status of the promoters of genes involved in the canonical AHR pathway, and investigating the role of expression of DNA methylatransferases (DNMTs) in the global hypermethylation that we observed. These data may add to our understanding of the role of DNA methylation in regulating the AHR-mediated response to PAHs in fish.

Effects of an early life-stage exposure to tebuconazole on the reproductive physiology of adult zebrafish (*Danio rerio*) (PO)

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Tebuconazole is a fungicide used to control pathogenic fungi common to a wide variety of fruits, vegetables and cereals. Its use is widespread, with current applications on every continent. Within Alberta, tebuconazole sales increased by 681.2% between 2008 and 2013, and it ranked 11th in pesticide sales by active ingredient. Tebuconazole has a moderate water solubility and has the potential for what are known as pulse inputs, where shortly after application a rain event occurs and mobilizes the compound, resulting in an elevated concentration within nearby waterways. This is often a more realistic exposure scenario for pesticides. Due to a slow degradation via sunlight and hydrolysis, there is the potential for tebuconazole to persist in these aquatic environments. Previous research has shown tebuconazole to disrupt sex hormone synthesis in nontarget species through inhibition of the cytochrome P450 (CYP) enzymes, CYP17 (17α-hydroxylyase/17, 20-lyase; converts pregnenolone/progesterone to androgen precursors) and CYP19 (aromatase; converts testosterone to 17β-estradiol). If pulse inputs of tebuconazole are occurring during the time of breeding, there is potential for endocrine disrupting effects in both the reproducing adult and resulting embryos. During the early life-stages, hormone levels are critical for proper embryonic growth and development. Successful reproduction by sexually mature adults is dependent on the normal ontogenesis of the gonads. An additional mechanism of persistence is through the epigenome: this research will specifically examine DNA methylation, as there are sensitive processes that occur during the early life-stages. This has been shown to be susceptible to environmental influence, with the greatest effect between 1-4 hours post-fertilization (hpf). This research aims to determine the effects of tebuconazole exposure on the early life-stages of the model teleost, zebrafish (*Danio rerio*), as well as any persistent effects at sexual maturity. Embryos are collected and divided evenly into one of the following treatments: 0 $\mu g \cdot L^{-1}$ (control), 10 $\mu g \cdot L^{-1}$ (low), or 1000 $\mu g \cdot L^{-1}$ (high). The low treatment represents an environmentally relevant concentration, as tebuconazole has been measured as high as 175-200 $\mu g \cdot L^{-1}$ in surface waters. The early life-

stage is exposed throughout the entire period of embryogenesis, then reared in clean water until sexual maturity. At that time, assays will examine persistent effects as a result of the embryonic exposure. Preliminary results indicate the concentrations chosen are not acutely toxic, based on lethality, time-to-hatch, and malformation data. The low concentration-exposed embryos displayed a significant increase in expression of aromatase compared to controls; however, this was not observed in the high concentration treatment. There were no significant changes in expression of DNA-methyltransferases in either treatment. Currently, sexually mature zebrafish are being assessed for changes in endocrine-related gene expression. Preliminary results show a significant increase in expression of aromatase in female fish. Mechanisms investigated here will contribute to the knowledge gap in persistent effects from environmentally relevant endocrine-disrupting compounds.

Latest Advances in Metal Bioavailability and Toxicity to Aquatic Organisms

Competition between rare earth elements and other ubiquitous trivalent metals for uptake and toxicity in algae: Lanthanum and iron as example (PL)

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Rare earth elements (REE) are 17 elements, including yttrium, scandium and the lanthanide series, that occur naturally together and have similar properties. These elements are used in a variety of recent industries, and interest in their extraction from mineral ores has increased recently in Canada. Although anthropogenic inputs of REE are expected in the future, only a few data are available on their biogeochemistry. The scarcity of data on REE interaction at biological interfaces limits the ability to predict their bioavailability and assess their environmental risks and hazards. Because of their chemical nature (generally trivalent and hard metals), REE can potentially compete with other ubiquitous trivalent metals (e.g., Fe(III) and Al(III)) for binding sites. Considering that living organisms require iron, this study aims to investigate the role of Fe(III)) on REE uptake and toxicity. Generated uptake and toxicity data will be integrated into toxicity prediction models (e.g., Biotic Ligand Model). Using lanthanum (La) as a model REE and the unicellular green alga *Chlamydomonas reinhardtii*, uptake (1 hour) and toxicity (120 hour) experiments were conducted using environmentally realistic conditions. First, short-term La uptake processes were characterized to confirm linearity over time. Fluxes were then probed as a function of concentration to determine the half-saturation constant and maximal uptake flux. Finally, the impact of Fe (pFe = 15.4 to 17.8) on La uptake was tested using a fixed La concentration of 100 nM, which proved to have a significant effect on La uptake. Long-term toxicity experiments are currently ongoing and further results will be presented.

Mystery of the naturally selenized fish (PL)

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Baseline studies in a pristine oligotrophic lake in northern British Columbia found that resident rainbow trout (*Oncorhynchus mykiss*) had tissue selenium concentrations exceeding health-based consumption guidelines, despite aqueous selenium concentrations less than 1 $\mu g \cdot L^{-1}$. We conducted a study to identify the source of selenium to these fish. Selenium speciation analysis of sediment cores and dialysis array ('peeper') water samples across the sediment-water interface indicated that lake sediments were a net sink, not source, of selenium to the lake. Organoselenides were present in all tested surface sediment samples. Gut content and stable isotope analysis revealed that rainbow trout (the only fish species present in the lake) were broadly omnivorous, feeding predominantly on

planktonic, benthic, and littoral invertebrates. Selenium analysis of potential prey in several areas of the lake identified few taxa with sufficiently high concentrations to explain concentrations in rainbow trout. Most invertebrates, including those that predominated in fish guts, had tissue selenium concentrations less than 5 $mg \cdot kg^{-1}$ dry weight (dw), whereas rainbow trout had muscle selenium concentrations ranging from 7 to 28 $mg \cdot kg^{-1}$ dw and egg selenium concentrations up to 48 $mg \cdot kg^{-1}$ dw. Benthic chironomids and littoral caddisflies had distinctly higher concentrations than other taxa, up to 17 $mg \cdot kg^{-1}$ dw. However, approximately 20% of rainbow trout captured had tissue selenium concentrations too high to be explained by the invertebrate prey sampled. A possible explanation is cannibalism. One fish gut contained a juvenile rainbow trout and the highest-selenium fish also had elevated d15N, consistent with feeding on juveniles. Our data suggest that the high baseline fish tissue selenium concentrations may have resulted from preferential feeding of juveniles on high-selenium invertebrate prey and cannibalistic feeding of adult rainbow trout on those juveniles.

Acute and chronic toxicity of technology critical elements to aquatic biota (PL)

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The growing use of rare earth elements and other technology critical element such as In and Ga in personal electronic devices, green technologies and medical applications results in a developing concern for impacts in aquatic environments. However, there are no water quality guidelines/criteria for REEs and few studies available. The overall objective of this research is to contribute data towards the establishment of assessment tools for the effects of REEs. We have studied the toxicity of Ce, Sm, Dy Tm, In and Ga to fish and sensitive invertebrates (e.g., *Hyalella azteca* and *Daphnia*). The toxicity modifying influences of cationic competition (Ca, Mg and Na) and dissolved organic matter (DOM) was assessed with the goal of developing toxicity prediction models that account for site-specific conditions. Standard methods (Environment Canada) were used for culture and testing which was done in intermediate hardness waters (60 $mg \cdot L^{-1}$ CaCO3, pH 7.2, Ca 0.5 mM, Mg 0.15 mM). With some exceptions, Ca and DOM provide protection against toxicity but the incorporation of these effects into toxicity prediction models was inhibited by a lack of understanding of solution geochemistry. The pH of test solutions had a significant influence on toxicity with, for example, low pH resulting in reduced Dy toxicity.

Geochemical speciation and toxicity of Ni in seawater and development of a biotic ligand model (PL)

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Nickel (Ni) toxicity in seawater is of increasing concern because of coastal Ni mining and processing activities. Determining Ni speciation is vital to understanding and predicting Ni

toxicity, and ultimately for bioavailability-based nickel risk-assessment. Application of existing freshwater bioavailability-based approaches for nickel in salt water predicts negligible binding of Ni to dissolved organic matter, but there are several examples of toxicity tests in natural seawater that are protective compared to artificial seawater control samples. The objectives of this project were to determine: (1) the source of this protective effect, (2) how geographically widespread protective saltwater sources are, and (3) to be able to predict Ni speciation and toxicity. As a test of Ni toxicity protection by ligand complexation in salt water media, defined solutions of artificial seawater (ASW) containing different model compounds (i.e., citric acid, EDTA, L-tryptophan, glutamic acid, and histidine) were titrated with Ni to determine speciation. In addition, Ni speciation was determined in real saltwater samples of diverse geographic origin from the east coast of the United States and Canada. The divalent Ni free ion in these synthetic and real seawater samples was quantified using Ion Exchange Technique (IET) with Ni measured by Graphite Furnace Atomic Absorption (GFAA). The measured Ni^{2+} values were compared with model predictions (using Visual Minteq) for evaluating the feasibility and applicability of the IET method for Ni in seawater. For the most part IET-measured [Ni^{2+}] agreed very closely with model predictions. In the same defined solutions, 96-hour Ni embryo toxicity tests were performed for a sea urchin (*S. purpuratus*). The dose response curves were expressed both as total dissolved Ni concentration ([NiD]) and free Ni concentrations from IET ([Ni^{2+}]). If the Ni toxicity is explained by [Ni^{2+}], all the toxicity response curves of different model ligands will overlap and this was in fact observed for the majority of samples. The results of this research contribute to the development of bioavailability-based prediction models for estimating the impacts of Ni in marine water.

Does metal-metal competition for binding to *Daphnia magna* explain less-than-additive acute toxicity in Cd-Ni mixtures? (PL)

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Previously we demonstrated that the acute lethality of binary mixtures of cadmium (Cd) and nickel (Ni) is strongly less than additive to *Daphnia magna* neonates when predicted by dissolved metal concentrations or by metal-ion activities. In those tests, sublethal concentrations of Ni decreased or eliminated Cd toxicity, which led us to speculate that either (1) competition between Cd and Ni for binding to the organism accounts for the protective effect of Ni, and/or (2) physiological interactions of the two metals inside the organism influence the metal-mixture toxicity. Here we present results of geochemical speciation modeling with the Windermere Humic Aqueous Model (WHAM) to test if geochemical partitioning in the bulk exposure water and at the organismal binding sites can explain that less-than-additive toxicity. All lethality tests were conducted for 48 hours according to U.S. Environmental Protection Agency recommended procedures, in moderately hard reconstituted water to which 3 $mg \cdot L^{-1}$ of dissolved organic carbon (DOC) was added as Suwannee River fulvic acid. In the speciation calculations, all of the measured DOC concentration was assigned to WHAM fulvic acid; but we also included a trace amount of WHAM humic acid (WHAM-HA) to act as a surrogate for organism binding of metals, as

previously suggested by other authors. Then we used both the concentration-addition (CA) and independent-action (IA) models of mixture toxicity in combination with WHAM-predicted concentrations of "organism-bound" metal to predict the lethality of all tested Cd-Ni combinations. With the default constants for binding of Cd and Ni to WHAM-HA, almost no protective effect of Ni was predicted at sublethal Ni concentrations. But because the default parameterization for binding of Cd and/or Ni to WHAM-HA might not accurately represent binding at *D. magna* sites of toxic action, we then varied the binding constants to try to improve the predictions of mixture toxicity. Although decreasing the strength of Cd binding or increasing the strength of Ni binding to WHAM-HA increased the predicted protective effect of Ni, neither CA-predicted or IA-predicted mortality decreased by as much as the observed mortality decreased. We demonstrate these results using cumulative density functions in which cumulative probability of occurrence is plotted *versus* extent of underprediction or overprediction of toxicity. These graphs provide a visual complement to the traditional 1:1 plots of predicted versus observed mortality. As a result of this modeling, we conclude that modified parameterization of WHAM-HA as a surrogate for binding of metals to *D. magna* partially improves predictions of the observed protective effect of Ni against Cd lethality; however, physiological interactions of Cd and Ni might also have to be invoked to explain the less-than-additive observed toxicity.

Toxicity and bioaccumulation of rare earth metals in *Daphnia magna* (PL)

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The Canadian Rare Earth Element (REE) Network aim to produce and process 20% of the global supply of critical REEs in Canada by 2018 may lead to increased REE emissions into the environment. The potential hazard of elevated concentrations of REEs to biota is still insufficiently investigated and is fraught with potential methodological problems. In the current presentation, three aspects of REE toxicity to *Daphnia magna* will be discussed: 1) effect of test medium on acute toxicity of REE; 2) chronic toxicity of REE; 3) toxicity and bioaccumulation of REE rich rock leachates. *Daphnia magna* is a particle-feeding zooplankton species with high potential of accumulating REEs. Acute and chronic toxicity of La, Ce, Pr, Nd and Gd nitrates to *D. magna* was evaluated according to OECD 202 and OECD 211 guidelines. Acute toxicity was tested in OECD 202 artificial freshwater (AFW) and natural lake water, and chronic toxicity was tested in natural lake water. To evaluate sedimentation, REEs' content in supernatants was measured by total reflection X-ray fluorescence spectroscopy (TXRF). Acute toxicity (48-hour EC50 in $mg \cdot L^{-1}$ REE) to *D. magna*, calculated based on nominal metal concentrations, was 19-31 in AFW and >50 in lake water. 48-hour EC50 ($mg \cdot L^{-1}$ REE), based on measured concentrations, was 0.2-1.5 in AFW and >0.2 in lake water. Chronic toxicity to *D. magna*, evaluated in lake water, was 0.3-0.5 $mg \cdot L^{-1}$ REE. TXRF analyses showed that sedimentation of REEs was higher in AFW (at low concentrations) and at high test concentrations (in both test media). Bioavailability of REE from rock leachates was measured from REE rich monazite (MON) or allanite (ALA) bearing pegmatite rock material from Quebec, Canada. The materials were leached with

artificial rainwater according to ISO/TS 21268-2 guideline. New leachate was produced at 6 leaching cycles performed during 28 days and analyzed with ICP-MS and ICP-OES. Filtered eluates from days 1 and 28 were tested for acute toxicity (OECD 202) to *D. magna,* and those from day 14 to measure REE bioaccumulation in *D. magna*. Toxicity was seen only for MON eluates with significantly lower REE concentration compared to ALA. The toxicity did not decrease with weathering despite the decreasing REE content in the leachates. Thus, the toxicity of MON rock eluent was probably not solely induced by REE content. REE concentration in the eluates was low (<0.2 $mg \cdot L^{-1}$) compared to the concentration in the rocks (7000−12000 $mg \cdot kg^{-1}$). Almost no accumulation of REE from rock eluates was seen. In conclusion, in OECD 202 and 211 test settings, REE solutions were more stable at low concentrations in organics-containing natural water (environmentally relevant conditions) compared to mineral artificial OECD 202 test medium. The release of REEs from rock material in the leaching tests was low and decreased in time. Toxicity of rock eluents to *Daphnia magna* was not only related to REE content but also to additional factors that are yet to be specified.

Investigation of metal mixture (Zn, Cu and Cd) interactions in the rainbow trout gill using synchrotron-based techniques (PL)

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There is a current emphasis on developing Biotic Ligand Models that are capable of predicting metal mixture toxicity in aquatic organisms including fish. This requires a better understanding of the interactive effects of metals in the fish gill (the biotic ligand), which can lead to additive, more than additive, or less than additive toxic effects in fish. In the present study, we used synchrotron-based micro X-ray fluorescence imaging (μ-XFI) and micro X-ray absorption near-edge spectroscopy (μ-XANES) techniques to examine the spatial distribution and speciation of Zn and how it is influenced by Cu or Cd during co-exposure. Juvenile rainbow trout (~100 g) were exposed to acutely toxic levels of waterborne Zn, alone and in combination with waterborne Cd or Cu for 24 hours (each at ~96 hours LC50). Gill sections were prepared and analyzed at the VESPERS beamline of the Canadian Light Source. Zn was found to be localized predominantly in the areas of the fish gill that corresponded to the regions with high density of mitochondria-rich cells, supporting the putative roles of these cells in Zn uptake. Zn distribution in the fish gill was markedly reduced during co-exposure to Cd, but not to Cu, suggesting a competitive interaction between Zn and Cd for uptake. These observations were consistent with the measurement of total Zn concentrations in the fish gill, which also showed a significant reduction during co-exposure to Zn and Cd, but not to Zn and Cu, in comparison to exposure to Zn alone. The speciation of Zn in the fish gill was dominated by Zn-phosphate, Zn-histidine and Zn-cysteine species; however, the interactions of Zn with Cd or Cu resulted in the loss of Zn-cysteine. In addition, we also used synchrotron-based Fourier transform infrared microspectroscopy (FTIRM) to examine, *in situ*, the interactive effects of Zn, Cu and Cd on the integrity of major lipid and protein constituents of the rainbow trout gill. The FTIRM analysis revealed that acute exposure to metals, both individually and in binary

mixture, resulted in the degradations of various components of proteins and lipids in the gill tissue. Generally, when comparing the effects of individual metals, Cu was found to induce the maximum adverse effects followed by Cd and Zn, respectively. Among the binary metal-mixture combinations, Cu and Cd produced additive effects on the degradation of major proteins and lipid moieties, whereas the co-exposure of Zn with Cd or Cu elicited ameliorative effects, indicating less than additive interactions between Zn and Cd or Cu in the rainbow trout gill. Overall, our study provides novel mechanistic insights into the interactive effects of metals in the fish gill, which may help to explain the toxicity of metal mixtures in fish.

The single and combined effects of forest fire runoff and sediment-bound copper on survival and growth in the freshwater amphipod, *Hyalella azteca* (PL)

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[1]*University of Lethbridge*

The frequency of forest fires is expected to increase with time as a function of climate change. Recent studies in our lab have demonstrated that some pyrogenic polycyclic aromatic hydrocarbons (PAHs) can cause greater-than-additive effects in *Hyalella azteca* in the presence of relatively low concentrations of copper. We hypothesized that freshwater animals inhabiting Cu-contaminated sites, such as those in the vicinity of Cu mines, may be vulnerable to potentiated toxicity from forest fire runoff (FFR). To investigate the possible interaction of copper and FFR we exposed *Hyalella azteca* for 14 days to binary mixtures of 225 mg•kg^{-1} Cu-spiked artificial sediment and a FFR dilution series (12.5%, 25%, 50%, 75%, and 100%). The sediment Cu concentration (225 mg•kg^{-1}) was sufficient to inhibit growth, but not sufficient to induce mortality. The combination of Cu-enriched sediment with 12.5% FFR had a more-than-additive effect on survival but there was no significant reduction in growth compared to the 225 mg•kg^{-1} Cu-contaminated sediment control. All other binary mixtures of Cu-contaminated sediment and FFR resulted in complete mortality. Results from this study suggest that Cu-contaminated water bodies that receive runoff from nearby forest fires are at risk of potentiated toxicity. Studies to confirm this effect in the field are ongoing.

Influence of water quality on copper and cadmium toxicity to fish olfaction (PL)

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Metals are well-known olfactory neurotoxicants to fishes and other aquatic animals. Very low concentrations—typical of concentrations likely to be encountered in nature—are sufficient to impair fish olfaction. This metal-induced olfactory impairment may have serious implications to fish, given that they rely on olfactory cues to migrate, avoid predators, find food, and reproduce. There have been a few recent attempts to develop

olfactory-based biotic ligand models (BLMs) for fish. However, very little research has characterized the effects of water quality on metal-impaired olfaction in fish. In this study, we exposed rainbow trout to copper or cadmium under variable water quality conditions to understand how they affect metal-impaired fish olfaction. Water hardness has little protective effect against Cu-induced olfactory impairment. However, water hardness was protective against Cd-induced olfactory toxicity. Dietary sodium can protect fish against waterborne copper olfactory impairment, but waterborne sodium cannot. Water pH between 6.5 and 9 has little to no effect on copper-induced olfactory impairment. However, Cd is more toxic to fish olfaction at higher pH than lower. These results suggest that water quality affects Cu and Cd olfactory toxicity, but not necessarily in the same way that they affect gill-based toxicity. Development of olfactory-based BLMs must consider the unique characteristics of the olfactory epithelium and how they are affected by variable water quality conditions.

Investigating climate change impacts on metal-contaminated sediments (PL)

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[1]*Natural Resources Canada*

Sediments play an important role as sinks for sequestering contaminants from local ecosystems. However, under future climate change scenarios, more frequent and intense precipitation events may lead to remobilization of sediments and an increase in the flux of contaminants to the local ecosystem. Sudbury, Ontario, has been exposed to decades of smelter emissions that have released metal(loid)-bearing particulate matter, which has accumulated in lake bottom sediments. The present study aimed to investigate the influence of resuspension on the fate and effect of metals in Sudbury-area lake sediments. A number of tests were performed on sediments collected from five different Sudbury-area lakes (Kelly, Long, Clearwater, Richard and Hannah) and Junction Creek. Shaker flask tests were conducted to investigate resuspension over a two-week time period with a resuspension event occurring at the beginning of each week. Results indicated that sediment disturbances, such as would occur during intense storm events, produce a range of geochemical changes and element behavior that differs from that of control samples that experienced no resuspension. For the toxicity tests, lethality and bioavailability of metals were assessed by exposing five- to six-day old *Daphnia magna* to four test conditions: suspended field water, suspended field sediments in control water, suspended field sediments in field water and settled field sediments in control water. Results suggest that sediment resuspensions can affect metal bioavailability, resulting in increased bioaccumulation and toxicity in aquatic primary consumers.

Understanding the fate and effects of metal-bound dusts at abandoned mine sites (PL)

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Dust pollution from extreme droughts is a serious concern to both environmental and human health. Recent studies in some mine sites have shown a significant contribution of metals associated with particulate matter entering watersheds due to localised wind erosion of waste piles, dust from roads, and atmospheric deposition during snowfall or rain fall events. The metals associated with these dust particles have the potential for remobilization in the environment, leading to impacts on ecosystems. CanmetMINING and Queens University are investigating the fate and effect of metals associated with dust particles from abandoned mine tailings. This presentation outlines the results of sampling at two sites in Nova Scotia, the Stirling Zn/Cu/Pb mine and the Goldenville Au mine. Results from a number of field and lab techniques will be presented, including passive air sampling, lichen sampling and toxicity assessments. This research aims to better understand the contribution of dust as a source of metals into local environments and provide valuable guidance for conducting similar assessments at other mine sites in Canada.

DGT-Labile copper monitoring above lake sediments impacted by the 2014 Mount Polley Mine Tailings Storage Facility breach (PL)

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In 2014, a foundational failure of the perimeter embankment of the Mount Polley Tailings Storage Facility resulted in a breach that released approximately 25 million cubic meters of debris (water and solids that consisted of tailings, construction materials, and scoured sediment and soil). The debris flowed into Polley Lake, along the length of Hazeltine Creek, and into the west arm of Quesnel Lake. This resulted in the enrichment of a number of contaminants of potential concern (COPC) in impacted sediments, of which copper was identified as being the primary COPC. Geochemical predictions suggested that the flux of potentially bioavailable copper out of impacted sediments should be minimal due to the limited solubility of copper minerals within the tailings. As part of an on-going monitoring program, diffusive gradients in thin-films devices (DGTs) were deployed just above the sediment-water interface during the summer or fall, 1-, 2-, and 3-years post-breach in order to monitor kinetically labile and potentially bioavailable dissolved copper. Although the relative DGT-labile copper concentrations (i.e., percent of dissolved copper) were slightly elevated at impacted compared to reference areas, the absolute concentrations were well below those associated with chronic toxicity to *Ceriodaphnia dubia*. Temporal analysis indicated that, over the study period, DGT-labile copper concentration at an impacted area in Quesnel Lake did not change more than would be expected based on changes at the reference area. However, they may have increased slightly at Polley Lake. Thus, bioavailable copper in water overlying sediments impacted by the tailings storage facility breach was limited. Monitoring of DGT-labile copper over time

will provide a cost-effective method for monitoring the release of potentially bioavailable forms of copper from impacted sediments in these valued water bodies.

Environmental Monitoring of Biota and Water in the Athabasca Oil Sands

Effects of diluted bitumen on the intertidal vascular plant, *Zostera marina* (PL)

Jessica Banning[1], Chris Kennedy[1]

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Canada has the third largest oil reserves in the world, most of which exist in the form of bitumen in the oil sands of the Western Canada Sedimentary Basin. Plans are underway to increase the export of petroleum products including diluted bitumen (dilbit) and crude oil to overseas markets, highlighting the potential risk of a spill into the Canadian marine environment. Understanding and evaluating risk in order to develop chemical management plans requires information on the toxicity of dilbit to key marine species. Little information exists regarding the toxic effects of most petroleum products to intertidal vascular plants. The objective of this project was to determine the lethal and sublethal toxicity of environmentally relevant concentrations of dilbit to eelgrass (*Zostera marina*), an intertidal vascular plant and keystone species in the Pacific Northwest. Eelgrass was collected from the intertidal zone of an uncontaminated site in the Strait of Georgia, near Boundary Bay, British Columbia. A short-term, 9-day exposure and a long-term 28-day exposure of shoots to multiple concentrations of a water-accommodated fraction (WAF) of dilbit were performed. Endpoints assessed in shoots from the short-term exposure included: electrolyte leakage, reactive oxygen species (ROS), activity of catalase and superoxide dismutase, and protein oxidation. Shoots from the long-term exposure were assessed for biological endpoints including plant growth, chlorophyll-a content, and the effective quantum yield of Photosystem II. Plant tissue was also assessed for gross discoloration and infection.

The effects of diluted bitumen on the developmental stages of the intertidal brown alga, rockweed (*Fucus distichus*) (PL)

Ranah Chavoshi[1], Sherryl Bisgrove[1], Christopher Kennedy[1]

[1]*Simon Fraser University*

Canada is the fourth largest exporter of oil in the world and major industrial projects have been proposed that will increase the transport of diluted bitumen (dilbit) to overseas markets. Scientific uncertainty exists regarding the risks associated with a dilbit spill in marine environments, in particular for habitat-forming algal species. The effects of diluted bitumen exposure were examined on a keystone brown alga, *Fucus distichus*, a foundational species in many intertidal ecosystems. Acute exposures using environmentally-relevant concentrations of diluted bitumen (as water accommodated fractions [WAFs]) were completed using two populations of *Fucus*. One population was located in a polycyclic aromatic hydrocarbon (PAH)-contaminated environment near an oil shipping terminal in

Burrard Inlet, British Columbia. The other population was located on Savary Island, BC, with no prior history of petroleum exposure. Reproductive fronds were sampled from both populations and stimulated to release zygotes which were exposed to diluted bitumen at several concentrations in a controlled laboratory setting. Effects on early developmental stages were examined and the responses to dilbit between these two different populations were compared. Diluted bitumen altered algal development in both populations, with the Savary Island population (uncontaminated source of algae) being more sensitive than those collected from the contaminated site. Continuing work is ongoing in the assessment of the effects of diluted bitumen on reproductive stages of other macroalgal species including kelp forest-forming species and *Porphyra* spp.

Using community-based methodologies linking traditional knowledge and western science knowledge systems to monitor freshwater clams in Alberta's Athabasca Oil Sands (PL)

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Indigenous communities in the northeastern Oil Sands Region of Alberta, in particular the McMurray Métis, have expressed interest in the current status of freshwater clams. Oral history shared by traditional knowledge holders indicates that the gathering and eating of freshwater clams have historically been part of traditional cultural practices, but the clams seem to have disappeared from the region over the past 20 years. This project is grounded in the idea of 'Community First', using a community-based action research approach that is driven by Métis Traditional Knowledge/wisdom/methodology with the aim to co-design an approach with Alberta Environment and Parks to answer community-based questions in a way that is meaningful to the community. This Project is about more than just "Where are the freshwater clams?'; it encompasses who the community is as a Métis people, which is in part reflected by their spiritual, cultural, and physical connection to the land and their social well-being.

Developing triggers to adapt fish environmental effects monitoring programs in the Lower Athabasca River (PL)

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Oil sands companies currently do not release process-affected water to receiving environments, but it is anticipated that there will be a need to do so in the near future. There are concerns about the potential effects of those discharges on the ecology of the Athabasca River. Environmental Effects Monitoring (EEM) provides a mechanism to quantify ecological effects. EEM programs currently in place for metal mining and pulp and paper sectors in Canada have demonstrable efficacy at quantifying effects and identifying

potential effluent-related causes of effects. Trout perch (*Percopsis omiscomaycus*) populations have been used as a sentinel species in the mainstem of the Athabasca River as part of the historical Regional Aquatics Monitoring Program (RAMP) and the Joint Oil Sands Monitoring Program (JOSMP). Variations in energy use (growth, gonad size) and energy storage (condition, liver size) were characterized between 2009 and 2015 in nine reaches on the Athabasca River: two reaches upstream of the City of Fort McMurray and seven reaches variously situated downstream of Fort McMurray, and upstream and downstream of existing oil sands operations. Generalized linear models (GLM) were used to construct models that related variations in standardized performance measures (size at age, condition, liver size, gonad size) and natural covariables including river discharge and air temperature. Prior to use of GLM, fish were standardized to a common age (i.e., 4+ years to assess growth), to a common length (i.e., 7.5 cm to assess condition), and to a common body weight (i.e., 4.6 g, to assess liver and gonad weight) in order to remove the size covariable and simplify analyses. The analyses reflect that fork length, gonad size and liver size decreased, while body weight increased in relation to flow volume, for both male and female trout perch. Air temperature varied positively with liver size but negatively with gonad size and body weight for females, and only positively with gonad weight for males. These models explained upwards of about 20 to 25% of the variation in adjusted body size, and upwards of 80% of the variation in adjusted body weight, liver weight and gonad weight. The residuals from these models can be used to estimate normal ranges of variation for individual fish performance and to develop triggers to signify change in monitoring programs.

Effects of oil sands chemicals of concern on early life stages of fish and amphibians: Results from outdoor aquatic mesocosm experiments (PL)

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Whereas the spatial extent of pollution from Canadian Oil Sands mines has received considerable attention, the ecological implications on aquatic biota remain poorly understood. Environmental monitoring of aquatic ecosystems is critical in this regard, but needs to be coupled with experimentation to elucidate cause-and-effect relationships and relevant ecotoxicological endpoints. Our team is investigating the chronic effects of airborne and waterborne oil sands chemicals of concern on early life stages of fish and amphibians using controlled outdoor mesocosm experiments. Here, we present the findings from three such experiments conducted at the Queen's University Biological Station using environmental samples collected in Alberta. In Experiment 1, we exposed embryos of wood frog (*Lithobates sylvaticus*) to melt waters from snowpack collected from impacted sites in close proximity (<50 km) to major bitumen upgrading facilities or from reference sites. Contaminants in snow near the Canadian Oil Sands are likely derived from atmospheric emissions from bitumen upgraders as well as fugitive dust from petroleum coke piles. In

another set of experiments, we exposed wood frog (Experiment 2) or fathead minnow (*Pimephales promelas*)(Experiment 3) embryos to environmentally-relevant concentrations of naphthenic acids extracted from oil sands process affected water—that is, the waste water currently stored in large tailings ponds in northern Alberta. In all three experiments, we documented the immediate (and in some cases, latent) effects of exposure to oil sands chemicals of concern on the growth, development, and survival of our model organisms. These results will inform effective risk assessment and design of environmental monitoring programs, particularly as Canadian Oil Sands operators and regulators plan ahead for the eventual discharge of treated waste waters to the environment.

Evaluating the co-dispersion of mercury sources and wildlife exposures in the Athabasca Oil Sands region (PL)

Bruce Pauli[1], Kristin Eccles[2], Craig Hebert[1], Danna Schock[3], Fardausi (Shathi) Akhter[1], Lukas Mundy[1], Philippe Thomas[1]

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Mercury (Hg) is a global pollutant of concern to human and wildlife health, given the neurotoxic and bioaccumulative properties associated with its methylated form (MeHg). While Hg has both natural and anthropogenic sources, industrial operations such as oil and gas development can be a source of anthropogenic Hg to the environment. To monitor chemical exposure of wildlife in the Alberta Oil Sands region (AOSR), the governments of Canada and Alberta initiated the Joint Oil Sands Monitoring (JOSM) program; under JOSM collection of data regarding the exposure of wildlife to oil sands chemicals of concern started in earnest in 2012. Integrating biotic and abiotic data with the wildlife contaminants biomonitoring program is an essential step in understanding the spatial relationships and landscape-level patterns between sources of Hg and exposures of biota to Hg. In this study we compile and integrate Hg data sources collected under the JOSM program including data from the JOSM wildlife contaminants monitoring (mammals, amphibians, and gull and tern eggs) program. Spatial methods, including hot spot analysis (Getis and Ord's Gi*) and co-dispersion analysis, are used to quantify patterns of Hg in the AOSR and investigate spatial relationships between these patterns. Results of the local analyses demonstrate a hot spot of mercury deposition in the mineable Oil Sands Region surrounding bitumen upgraders, while global analyses also indicate a trend of increased mercury levels north of the AOSR in the Peace-Athabasca Delta. The results will be used for making evidence-based decisions regarding the refinement and adaptive management of ongoing mercury monitoring programs in the Oil Sands.

Methylmercury in amphibians and their breeding ponds in northeast Alberta and the Northwest Territories (PL)

Danna Schock[1], Fardausi Akhter[2], Lukas Mundy[2], Catherine Soos[2], Bruce Pauli[2]

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We examined total mercury (THg) and methylmercury (meHg) levels in wood frogs (*Rana sylvatica* = *Lithobates sylvaticus*) and their breeding ponds from 2012 to 2017 in northeast Alberta and the South Slave region of the Northwest Territories as part of larger programs examining amphibian and wetland health. Grab samples of wetland water were collected in the immediate vicinity of tadpoles. Levels of THg in water samples (n = 147) varied geographically, ranging from less than 0.3 $ng \cdot L^{-1}$ to 19 $ng \cdot L^{-1}$. Levels of meHg ranged from less than 0.1 $ng \cdot L^{-1}$ to 2.34 $ng \cdot L^{-1}$ (n = 52 samples), except for a single sample at a repeatedly sampled site that indicated 5.52 $ng \cdot L^{-1}$. For the 52 water samples for which it was possible to compare THg and meHg levels, meHg accounted for more than 30% of the mercury detected in some samples and there was a modest but statistically significant positive correlation between the two ($R^2 = 0.58$, $P = 0.001$). Although THg was highest in tadpoles, meHg levels did not vary among life stages (0.067 $\mu \cdot g^{-1}$; $F_{2,52} = 1.028$, $P = 0.365$). There were no strong correlations between water and tissue levels for either THg or meHg. For a subset of well-studied wetlands in the Fort McMurray area, we compared THg and meHg levels in water and tadpole tissues before and after the 2016 wildfires. Overall, THg and meHg levels remained low (2015 to 2017). Wetlands that were most severely affected by the fires had higher THg levels in water in 2017 than in 2015 or 2016, although there was no discernible pattern with respect to meHg. No clear pattern emerged for THg or meHg in tissues, although it should be noted that the most severely impacted sites did not support tadpoles through to metamorphosis in 2016 or 2017. Finally, as part of our investigations, we assessed the feasibility of diffusive gradients in thin films (DGT) passive samplers for monitoring bioavailable mercury at our wetlands. Our ongoing research is contributing new knowledge about priority contaminants in boreal wetlands and the wildlife associated with them. This information is, in turn, informing long-term monitoring schemes across the region.

The effects of diluted bitumen and the dispersant Corexit® 9500A on eelgrass (*Zostera marina*) (PL)

Camelia Tavakoli[1], Jessica Banning[1], Chris Kennedy[1], Ian Chen[1], Cathy Tran[1]

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Canada is a large oil producing country and recent proposals by petroleum interests include increases in tanker exports of diluted bitumen (dilbit) and crude oil to overseas markets, highlighting the potential risk of a spill into the marine environment. There exists little or no information on the toxic effects of most petroleum products to intertidal vascular plants which provide a wide range of essential ecological functions in the marine environment. The purpose of the present research was to examine the effect of diluted bitumen (dilbit) alone or in combination with the dispersant Corexit® 9500A on a marine eelgrass species (*Zostera marina*). *Zostera marina* was collected from the intertidal zone of

an uncontaminated site in the Strait of Georgia, near Boundary Bay, British Columbia. Individual plants were planted in tall glass vessels and kept in growth chamber at a consistent temperature of 13.0°C, 60% humidity and a 24-hour light cycle where lights turned off from 21:00 to 5:30 and turned back on for the rest of the cycle. Plants were exposed to a logarithmic dilution series of a water-accommodated fraction of dilbit alone or in combination with Corexit® 9500A (100, 32 and 3.2, 0%) in triplicate. Exposures were for 30 days, following a 14-day acclimation period. The endpoints that were measured include effects on root and internode growth, the presence of infection, chlorophyll a content, quantum efficiency of photosystem II, enzyme activities (e.g., catalase and superoxide dismutase), as well as protein oxidation. The results presented here will aid in the development of plans for managing marine organisms in the event of a potential diluted bitumen spill on Canadian coasts.

Evaluating the toxicity of dissolved and particulate fractions of eroded oil sands: Differences in the sensitivity of *Hyalella azteca* between age 2 and 9 days old (PO)

Andrea Farwell[1], D. George Dixon[1], Che Lu[1], Jean Birks[2], Alberto dos Santos Pereira[2]

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In the tributaries and mainstem of the Athabasca River in the Oil Sands Region, inputs of bitumen-derived constituents from natural oil sands as well as potential contaminants associated with mining activities will affect water quality, thus raising concerns of cumulative impacts on aquatic organisms. The partitioning of bitumen-derived constituents into air, water and sediment during natural erosion influences the exposure to and effects of constituents on aquatic organisms. There is a need to better understand the environmental impacts associated with bitumen-derived constituents as a function of erosional processes in order to address the issue of potential cumulative impacts. The objective of this study was to isolate the dissolved and particulate fractions of bitumen ore mixed with water and evaluate the toxicity of the individual and combined fractions on the survival of *Hyalella azteca*. Two bitumen ore samples were evaluated; 1) bitumen ore collected from a McMurray Formation exposure in the MacKay River valley and 2) bitumen ore collected from an open pit mine and stored at the InnoTech Sample Bank. Bitumen ore was mechanically mixed for 24 hours in water to simulate erosion, and then filtered to separate the dissolved and particulate fractions. Toxicity tests were conducted using a 96-hour *H. azteca* survival test at bitumen concentrations of 0-2.5 dry wt $g \cdot L^{-1}$ for four different treatments: bitumen ore (unmixed), bitumen ore (mixed), dissolved fraction and particulate fraction. The results for both bitumen ore samples indicate significantly higher toxicity for all treatments that contain particulates relative to the dissolved fraction. Water chemistry analyses of metals and polycyclic aromatic compounds (PAC) were used to estimate threshold concentrations. Threshold concentrations were compared with available data on environmental concentrations of metals and PACs in suspended sediments collected from the Athabasca River. Interestingly, within the age range (2- to 9-day old) of neonates recommended for testing (Environment Canada, 2013), it appears that younger neonates (2- to 4-day) are more sensitive to fine particulates than older neonates (7- to 9-day old) in 96-hour water exposures. The findings of this study are important to

establishing a comprehensive environmental monitoring program and developing an effective watershed management strategy in the oil sand region.

Patterns in riverine benthic communities from reference and potentially impacted areas in the Athabasca oil sands area, Alberta, Canada (PO)

Allison Ritcey[1], Nancy Glozier[1]

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Benthic invertebrate sampling in tributaries of the Lower Athabasca River (LAR) in the Athabasca oil sands area has been ongoing since the 1970s, and over the decades invertebrates have been collected using several different sampling approaches. Recent scientific reviews of sampling approaches in the LAR made recommendations to improve the assessment of environmental impacts, including the implementation of a standardized sampling protocol carried out in clearly defined habitat. Such improvements would allow more robust statistical assessments of biological differences between reference and potentially impacted stream reaches. In 2012, the governments of Canada and Alberta launched the Implementation Plan for Oil Sands Monitoring, which set out phased monitoring plans for aquatic ecosystem health, water and air quality, terrestrial biodiversity, and wildlife toxicology. A key objective of the aquatic ecosystem health component was to improve the understanding of benthic invertebrate community structure in LAR tributaries by incorporating the recommended study design. From 2011 to 2014, samples were collected at approximately 50 reference and 30 potentially impacted sites using the Canadian Aquatic Biomonitoring Network standardized protocols for wadeable streams, targeting erosional habitat. Within reference sites, invertebrate communities were diverse and consisted of typical erosional benthic taxa (i.e., *Ephemeroptera*, *Plecoptera*, and *Trichoptera* [EPT]). Communities from reference sites were more similar within the same river, and community differences between rivers were linked to differences in substrate, flow, and algal primary production. Invertebrate communities in potentially impacted sites, i.e., those near active oil sands surface mining, had lower proportions of sensitive taxa (i.e., EPT) than corresponding reference sites, and multivariate analyses revealed that these communities shifted further away from reference condition as the proportion of disturbed area in the catchment increased. Shifts in community structure may be indicative of environmental stress. Further analyses are underway to include additional years (2015–2017) and sites, and preliminary results indicate that patterns among sites with all years included are consistent with those identified during the first 4 years. Next steps will include a thorough investigation of potential drivers to determine the effects of natural exposure to oil sands geology, natural landscape gradients, and/or oil sands surface mining activities on benthic community structure.

A review of the impacts of petroleum toxicity on marine mammals, reptiles, and fish (PO)

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The potential for increased shipment of both conventional crude oil and unconventional petroleum such as diluted bitumen out of the port of Vancouver means there is a need to assess the toxicological consequences of chronic or catastrophic petroleum spillage on the marine wildlife of Canada's Pacific coast. Currently there is a lack of peer-reviewed literature on the effects of diluted bitumen on marine wildlife. Additionally, a current and comprehensive literature review of petroleum toxicity on marine wildlife is required for future research. The purpose of our review is to use information on petroleum toxicity to inform research needs for diluted bitumen toxicity. The focus will be on marine vertebrates, specifically mammals and reptiles, and on updating a 2015 review on fish. Effects considered consisted of lethal and sublethal endpoints, including gene expression, physiology, and fitness. A literature search was conducted on the Web of Science database for papers from 1900 to 2018, with no filters applied. Papers examining a single component of petroleum (e.g., pyrene, cadmium) were excluded from the search. Papers included were reviews on the ecological effects of oil spills, as well as laboratory studies determining effects of polycyclic aromatic hydrocarbons, components of petroleum, or petroleum products on marine vertebrates. The results of this literature review will help inform academics, researchers, and policy makers on how to better protect British Columbia's marine wildlife from oil spills.

The R's of Contaminated Soils: Remediation, Reclamation and Risk Assessment

Life history and cellular energy reserve to understand *Oppia nitens* (a soil oribatid mite) responses to chemical stress in the soil (PL)

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The soil acts as habitat by providing habitable spaces and resources for soil invertebrates to perform optimally. The soil also protects the invertebrates from environmental stressors. However, there is significant exposure of soil invertebrates to anthropogenic stress arising from toxicants. Soil invertebrates' response to toxicants is reflected in changes at all levels of biological organization, from sub-organismal to population-level effects. Therefore, this study aims to determine how an organophosphate pesticide (dimethoate) and a polycyclic aromatic hydrocarbon (PAH; naphthalene) modulate the energy reserve and life history (juvenile production, egg-laying, and hatching success) responses of the soil oribatid mite *Oppia nitens* after a full life-cycle exposure in a standard OECD artificial soil. The expected outcome/result is to show the effect of sublethal levels of pesticides and PAHs on the cellular and physiological performance of soil mites and consequently affect risk assessment of these toxicants in the soil.

Soil invertebrate avoidance identifies petroleum hydrocarbon-contaminated soils toxic to sensitive plant species (PL)

Amy Gainer[1], Steven Siciliano[1]

[1]*University of Saskatchewan*

In recent years, laboratory soil toxicity testing has advanced with the introduction of ecologically relevant boreal forest soil invertebrate and plant species, as well as increased adoption of avoidance toxicity tests. Plant toxicity tests produce a wealth of information but their long duration (e.g., maximum 42 days) and high soil mass inputs deter site managers seeking inexpensive and short turn-around solutions. In this study, we assess toxicity of a binary petroleum hydrocarbon (PHC) mixture to six agronomic and boreal forest plant species (*Elymus lanceolatus, Lactuca sativa, Medicago sativa, Raphanus sativus, Pinus banksiana* and *Picea glauca*) and the avoidance response of five varying soil invertebrate species (*Eisenia fetida, Enchytraeus crypticus, Folsomia candida, Oppia nitens* and *Hypoaspis aculeifer*). *Lactuca sativa* and *M. sativa* displayed the greatest germination tolerance to PHC-contaminated soils: however, these species had the most sensitive growth endpoints. The growth endpoints of conifer species, *P. banksiana* and *P. glauca*, were most tolerant to PHC contaminated soils. Our key finding was that soil invertebrate avoidance of PHC-contaminated soil was in the similar range of toxicity values for growth endpoints of plant species sensitive to PHC-contaminated soils. Our study further supports the inclusion of

avoidance tests in toxicity test batteries for assessing PHC toxicity, as avoidance response appears to be linked to plant response and informative of plant habitat quality.

Effects-driven assessment and management of complex operating sites (PL)

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Contaminated sites with a complex industrial history and operations require a nuanced approach in their environmental management. This is particularly true when the original products that were used and produced on site are no longer detected, but potential ecological and human health risks remain due to persistent breakdown products. There is little toxicological information and few reliable risk assessment approaches for sites where complex mixtures consisting of metabolites and by-products are present. Yet, regulators and industry need to be able to reliably answer the question "Should we be concerned about potential impacts on human and environmental health?" To answer this question, a group of academic researchers, industrial partners, and the Provincial regulatory agency have partnered to develop an effects-driven assessment to estimate the distribution and risk of ill-defined contaminants of concern at a 70-year-old crop protection chemical blending and packaging facility in Western Canada. This presentation will describe our overall research approach, which integrates spatial characterization and modeling of the contaminant plume, systemic toxicological analysis (mammalian cell bioassays and rat exposures), effect-directed adverse outcome analysis (aquatic whole animal model), and a site risk assessment. Based on initial assessment work completed between 2010 and 2016, and toxicity analysis completed in 2016, 19 boreholes were drilled and 10 monitor wells were installed at the site in 2017. Soil cores (n=20) were collected and extracted using an acidified methanol method and reconstituted in solvent dimethyl sulfoxide (DMSO). Cytotoxicity of the extracts was assessed *in vitro* using the WST assay and a liver-derived human cell line (HepG2). Three soil samples were identified as demonstrating the highest toxicity over a 72-hour exposure and were subsequently used in a 30-day oral dose toxicity study in rats to identify the primary adverse outcomes associated with exposure to the unresolved mixture present on site. Specific organ and toxicity end-points were examined, including body and organ weights, food and water consumption, organ histopathology, blood chemistry and cell counts, and cardiorespiratory measurements. Preliminary results show a decrease in food intake and relative liver weight, an increase in white blood cell count (specifically lymphocytes), and atrial enlargement with exposure to one of the extracts. Histopathology of heart, lung and liver tissues is ongoing. Based on this rodent bioassay, we will focus our *in vitro* work on the organs that demonstrate the greatest sensitivity to the unresolved mixture and ultimately will be able to determine whether *in vivo* toxicological responses could be predicted using *in vitro* assays. Overall, it is anticipated that this project will apply and validate a state-of-the-art, efficient and economical approach for the unbiased characterization of complex exposure scenarios

while at the same time provide information for subsequent remediation, risk assessment and risk management activities.

Does zinc matter? Habitat quality's influence on the toxicodynamics and toxicokinetics of zinc to the mite, *Oppia nitens* (PL)

Olukayode Jegede[1], Steven Siciliano[1]

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The responses of organisms in soil ecotoxicity tests are often determined by the bioavailable or bioaccessible concentrations of contaminants they are exposed to. However, the direct effect of habitat quality on the performance or response of organisms in different contaminated soils is often neglected. Habitat quality is a measure of the extent to which habitat promotes individual and population fitness. This study assessed the effect of habitat quality on mite, *Oppia nitens,* exposed to different contaminated soils. Forty-seven (47) soils were ranked as habitat qualities by summing up the scores of enchytraeid and collembola survival and reproduction with the plant biomass in each of the soils. The soil physicochemical properties were determined. Using an ordered logistic regression, cation exchange capacity (CEC) was the only soil property that was significant to habitat quality ranking of the soils. From the 47 soils, eighteen soils comprised of six soils representing each habitat quality, were dosed with low to high concentrations of zinc. The mites were exposed to the soils for 28 days. Mite survival and reproduction were assessed after 28 days and bioaccessible metal concentrations in the soils were determined. Zinc body burden, biochemical responses in the mites, and bioavailability of the metals was determined. The results showed that mites in high habitat-quality soils were able to tolerate higher zinc body burdens than those in the other habitat qualities. At the highest concentration of zinc, lactate dehydrogenase activity was lower in the high habitat-quality compared to the medium and low habitat-qualities, indicative of less stress in the high habitat-quality mites. Although bioaccessible metal concentrations were the lowest in the high habitat-quality soils, bioavailability in the mites was higher in the high habitat-quality soils. Our study showed that CEC determines the fitness of mites in soils, which leads to higher resilience and less toxicity of metals on the mites.

Terrestrial ecotoxicology of selenium and influence of soil sulphate concentrations (PL)

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Selenium is a naturally occurring trace element that has been associated with toxicity (selenosis) in humans and other animals. While an essential element, it has a narrow range between deficiency and toxicity. Selenium is also toxic to plants, and similarly has a narrow range between deficiency and toxicity. The existing Canadian Council of Ministers of the Environment (CCME) soil quality guideline (SQG) is based on a limited number of available published studies with restrictions in terms of concentration range as well as number of species for which data are available. The CCME guideline was derived using the lowest

observable effect method. Selenium is frequently encountered in the Western Sedimentary Basin (WSB) at concentrations greater than the CCME SQG in areas absent of anthropogenic input, suggesting soils can be naturally enriched in selenium. This can lead to complications when remediating contaminated sites where a potential anthropogenic source of selenium may be introduced to the environment at a level similar to that observed in other areas of the WSB. Examples of impact sources include specific mine effluents in addition to drill sumps associated with conventional oil and gas production. As a result of the potential for low environmental stewardship performance associated with the accidental excavation of naturally occurring selenium at elevated concentrations near conventional oil and gas sites, the Petroleum Technology Alliance Canada (PTAC) sponsored work completed by an independent toxicology laboratory (InnoTech Alberta), with work scope and direction managed by Equilibrium. The purpose of this work was to: 1) increase the size of the toxicological dataset for guideline derivation for use in the more rigorous weight-of-evidence approach, including species relevant to the WSB as well as other areas of Canada; and, 2) conduct research and develop quantitative measures of interactions between sulphate and a soluble form of selenium frequently encountered in the environment (i.e., selenate), for a number of plant and invertebrate species. Results indicate that plants can 1) experience selenium deficiency associated with elevated sulphate concentrations; 2) experience selenium toxicity at low sulphate and high selenium concentrations; and,3) be protected from selenium toxicity at high sulphate concentrations. Invertebrates similarly demonstrated a reduction in selenium toxicity at elevated sulphate concentrations. A species sensitivity distribution (SSD) family of curves was developed using newly generated toxicological data for plants and invertebrates for derivation of a soil quality guideline (SQG). The curves were based on variable sulphate concentrations, and a final curve was derived showing selenium SRGs as a function of increasing soil sulphate. Data were also generated for plant bioconcentration of selenium based on an extractable soil paste technique in addition to a total soil selenium basis (via acid digestion), including a detailed evaluation of published literature. These data were critical to guideline development, given that high accumulation of selenium into plant tissue can pose a risk to livestock and other terrestrial wildlife receptors. This is augmented under alkali wetland conditions where a greater proportion of selenium is made available for absorption, which is why livestock selenosis has been correlated with grazing in alkali slough areas since the late 1800s.

Brownfield risk assessment in Canada: A comparison of provincial guidance for contaminated soils (PL)

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The environmental risk assessment processes in Ontario, Alberta, and British Columbia are quite advanced in comparison to those of other provinces for which less detailed guidance exists. The Ontario Record of Site Condition Regulation 153/04 has evolved since 2004, with updates in 2011, and the release of, and subsequent updates to the Tier 2 Modified Generic Risk Assessment (MGRA) model. The resulting process is relatively scripted, however, and can be implemented with little or no knowledge of toxicology. Risk-

adverse provincial policies and the booming condominium industry have driven out the opportunity for chemical or bioremediation of contaminated soils, or ecotoxicity testing, and the majority of risk assessments conclude with capping or a "dig and dump" approach to remediation, which does little to protect ecological health. Alberta brownfield regulators have recently released a number of new guidance publications, which facilitate clarity in the site management process post-risk assessment and place it on par with Ontario practices. However, remediation to the Tier 1 or 2 guidelines is still preferred and, with exposure control, the risk assessment process is relatively ambiguous in terms of site closure. While these two jurisdictions appear very similar in their directives, the approach to execution and regulatory review of risk assessments in British Columbia is somewhat different. The real estate market and regulatory guidance are more conducive to giving consideration to toxicity, bioavailability or other forms of ecological testing. With below grade construction being almost non-existent, "dig-and-dump" is a less appetizing option for dealing with brownfield soils. Risk assessment in other provinces is still largely unregulated. This presentation will outline the similarities and differences between risk assessment processes in various provinces, with a focus on the management of contaminated soils.

Is juvenile soil invertebrate avoidance of contaminated soil more sensitive than that of adults? (PO)

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Avoidance tests are simple toxicity tests with field- and ecology-relevant findings. Currently, the majority of soil invertebrate avoidance testing is conducted on adults, with no literature on juvenile avoidance responses. Existing toxicity literature in aquatic and soil environments agree that juveniles are more sensitive to toxicants than adults. In this study, our objective was to determine if juvenile avoidance response was more or less sensitive than that of adults. We tested the dual avoidance response of adult and juveniles of three standardized soil invertebrates (*Folsomia candida, Enchytreaus crypticus* and *Eisenia fetida*) to three soil contaminants (phenanthrene, copper and sodium chloride) in artificial soil. Interestingly, we found the juvenile avoidance response was both more and less sensitive than that of adults. In addition, non-avoidance was observed in both juvenile and adult *Folsomia candida* and *Enchytreaus crypticus* when exposed to phenanthrene-contaminated soils.

Mixtures: Dosing, Results and Risk Assessment

Soil enzymatic responses to metal mixture species (PL)

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In soil laboratory experiments, metal mixture studies are usually carried out with metals dosed as salts, followed by leaching with artificial rainwater to remove excess salts. In the leaching process, metals are lost unevenly, which affects the ratio of the mixtures in the soil. An efficient way of carrying out metal mixture experiments is by using the fixed ratio ray design. This design reduces the amount of experimental effort and allows the estimation of both additivity and interactions. With use of this design, metal concentrations should be fixed in specific ratios, but this is compromised when soils are leached. Hence, an alternative method of dosing that allowed fixed-ratio testing had to be determined. Two proposed alternatives were metal oxides and spinel minerals which were both abundantly found in aged metal salt-spiked soils and field metal-contaminated soils. The toxicity of the oxides and minerals to soil enzymes was tested and compared to that of salts. The experiment was conducted with three Canadian soils (pH: 3,5,7), three metal species, five fixed metal mixture rays, and five metals (Pb, Cu, Co, Ni, Zn) at one dose. The activity of the soil enzymes ammonia monooxygenases, beta-glucosidases, acid-phosphatases and arylsulphatases was determined colorimetrically. Results showed that leaching alone significantly inhibits the enzyme ammonia monooxygenases in all three soils. The response of acid phosphatases to the metal mixture rays followed known paradigms of bioaccessible concentrations defining toxicity. However, the response of ammonia monooxygenases followed a pH-dependent hormetic toxicity across the three soils. Here, ray toxicity was highest in the soil with a pH value of 5 and vice versa for pH 3 and pH 7. Generally, metal salts were the most toxic form, and the spinel minerals were the least toxic. Metal oxides were chosen as a replacement for carrying out metal mixture studies in soils because no leaching was required, and it was more toxic than the minerals.

Toxicity of gasoline and diesel-related petroleum hydrocarbons to freshwater and marine organisms (PL)

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The purpose of this study was to determine environmental effects-based concentrations of total petroleum hydrocarbons (TPH) for freshwater and marine aquatic organisms. The results of this study could be useful for assessing the impacts of fresh or recent gasoline and diesel spills in the freshwater and marine environments. The study was conducted by the Washington State Department of Ecology (Ecology) and Nautilus Environmental to

determine the no-observed effects concentration (NOEC) and lowest-observed effects concentration (LOEC) of gasoline (C7-C12) and diesel (C10-C24) in addition to IC_{25} and IC_{50} endpoints using aquatic toxicity bioassays. Currently, there are no environmental effects-based concentrations under either Washington State or Federal Regulations for TPH that are based on dose-response relationships or effects-based concentrations. In separate experiments, hydrocarbons within either the diesel or gasoline range were spiked into toxicity test solutions and tested with two freshwater and two marine organisms. Freshwater organisms were the fathead minnow (*Pimephales promelas*) and a cladoceran (*Ceriodaphnia dubia*). Topsmelt (*Atherinops affinis*) and the echinoderm, purple sea urchin (*Strongylocentrotus purpuratus*) were the marine species used. Tests were conducted according to the United States Environmental Protection Agency (US EPA) test methods and Ecology's whole effluent toxicity (WET) guidance document or "Canary Book" (Marshall, R., 2016. Washington State Department of Ecology, Olympia, WA. Publication No. WQ-R-95-80). Aquatic toxicity tests were conducted at Nautilus Environmental in Burnaby, British Columbia. Hydrocarbon concentrations in toxicity test solutions were measured using Northwest Total Petroleum Hydrocarbon−Gasoline/Diesel (NWTPH-Gx and NWTPH-Dx) Methods at Ecology's Manchester Environmental Laboratory in Port Orchard, Washington. Diesel was generally more toxic than gasoline to all test organisms. The order of toxicity from most to least for diesel are as follows: echinoderm ≈ cladoceran > topsmelt > fathead minnow. The most sensitive endpoints for diesel were: the cladoceran IC_{25} reproduction, 0.17 (0.16 – 0.19) mg diesel·L^{-1} with NOEC 0.15, and LOEC 0.22 mg diesel·L^{-1}. For gasoline, toxicity was similar between topsmelt, fathead minnow and the cladoceran, which were all more sensitive than the echinoderm. The most sensitive endpoint for gasoline was fathead minnow biomass IC_{25}, 1.5 (1.2 – 1.7) mg gasoline·L^{-1} with NOEC 1.0 and LOEC 2.1 mg gasoline·L^{-1}.

Utilization of metal bioaccumulation to assess metal mixture impacts: Site-specific assessment and toxic identification evaluation (PL)

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Bioaccumulation of metals in the benthic invertebrate *Hyalella azteca* is used to identify the cause of toxicity in exposures to sediments and waters collected from contaminated sites across Canada. Critical body concentrations are used to identify metals of greatest concern contributing to observed effects. A metal effects addition model (MEAM) is used to predict the cumulative impact of metal mixtures contaminating the sites as a result of the bioaccumulation of those metals. This presentation will demonstrate how to identify (1) metals of concern, (2) acute and chronic toxicity, (3) possible causes of the toxicity, and (4) the combined impacts of metal mixtures.

General Ecotoxicology

Online monitoring of copper toxicity using a microbial fuel-cell-based biosensor (PL)

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Elevated copper concentrations in water bodies due to natural resources exploitation such as mining pose a significant risk to biological life. Currently, traditional biological methods used to assess copper concentrations or copper-induced mortality in water bodies are lengthy and labor intensive. As such, the need to improve on these unfavorable attributes has been met through explorations of online monitoring methods and techniques. This study describes the performance of a microbial fuel cell (MFC) biosensor for real-time detection of elevated copper concentrations. The MFC biosensor was able to detect in real-time, changes in copper concentrations from 10 – 35 $\mu g \cdot L^{-1}$ in an aqueous environment. Furthermore, results comparison of MFC biosensor outputs with those of a standard toxicity assay with *Daphnia magna* showed a linear dependence of *Daphnia* survival rates (%) with sensor output at a 0.94 coefficient of determination. Finally, results of a field test of the MFC biosensor in two lakes that receive discharges at different rates from a mining site are shown. The biosensor used in this study is low cost, autonomous, and has the potential for further development and widespread field utilization.

Effects of the aquaculture chemotherapeutant formulation SLICE® (emamectin benzoate) on the spot prawn (*Pandalus platyceros*) (PL)

Steven Barrett[1], Chris Kennedy[1]

[1]Simon Fraser University

Atlantic salmon aquaculture has been used as a means of meeting demands for landed salmon in the context of volatile wild fisheries stocks in British Columbia. Although open net-pen fish production has become a major economic driver in many coastal communities, it is not without challenges. One of the greatest threats to salmon farm productivity is infestation with sea lice, naturally-occurring parasitic copepods. To combat this problem, the aquaculture industry relies on chemical and physical treatments to control sea lice. The coincidence of spot prawn habitat and aquaculture facilities on British Columbia's coast and the long half-life of emamectin benzoate in marine sediment may represent risk to spot prawn populations and the spot prawn fishery. This study investigated the effect of the chemotherapeutant formulation Slice® (active ingredient: emamectin benzoate) to the non-target native spot prawn (*Pandalus platyceros*) in long-term spiked-sediment exposures. The toxicity of these treatments was assessed using the endpoints of mortality, condition factor, moulting, olfaction, and locomotion.

Acute and sub-chronic effects of neonicotinoids on Northwestern salamander larvae (PL)

Blake Danis[1], Tiffany Ly[1], Amy Zheng[1], Vicki Marlatt[1]

[1]Simon Fraser University

Environmental concentrations of neonicotinoids in surface waters have been detected at levels as high as 320 $\mu g \cdot L^{-1}$ in Canada, and no studies have examined the toxicity of this group of pesticides in North American salamander species. This research investigated the adverse effects of neonicotinoids on the aquatic larval stages of the Northwestern salamander (*Ambystoma gracile*; NWS) after acute and sub-chronic exposures during early stages of development via whole organism- (i.e., growth, development) and molecular- (i.e., gene expression changes) level endpoints. In an acute exposure, NWS were exposed to four imidacloprid concentrations (250, 750, 2250, and 6,750 $\mu g \cdot L^{-1}$) and a water control treatment for 96 hours. There was no evidence of adverse effects on survival, body weight, snout vent length (SVL) and total body length. In subsequent experiments, NWS were exposed sub-chronically to imidacloprid alone and a mixture of neonicotinoids (imidacloprid, clothianidin, and thiamethoxam (TIC)) at 10, 100, and 1,000 $\mu g \cdot L^{-1}$ and a water control treatment for 35 days. In the imidacloprid-only exposure there was no evidence of an effect on NWS larval survival, body weight, SVL, and total body length at the concentrations tested. In the TIC mixture exposure experiment, there was no evidence of an effect on survival, body weight, SVL, and total body length in NWS larvae at the concentrations tested. These results demonstrate that imidacloprid is not acutely toxic with respect to survival and growth endpoints up to 6,750 $\mu g \cdot L^{-1}$ and sub-chronically up to 1,000 $\mu g \cdot L^{-1}$, nor was a mixture of neonicotinoids up to 1,000 $\mu g \cdot L^{-1}$. However, analyses measuring the sublethal effects on development and on molecular endpoints are currently underway and will further elucidate any adverse effects of neonicotinoids on this poorly studied Order, Caudata (salamanders and newts).

Giant mine and the tale of two fishes: A long-term study from one of Canada's largest legacy sites (PL)

Tamara Darwish[1], Hilary Machtans[1], Katherine Harris[2]

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Giant Mine is one of Canada's largest former gold mines, situated within the city limits of Yellowknife, Northwest Territories, along the shores of Great Slave Lake. The mine operated between 1948 and 2004, at times without stringent health or environmental controls. The owners abandoned the site in 1999, leaving a legacy for government, universities, engineers, scientists, and consultants to find a solution to officially close and remediate the site. Baker Creek, a small intermittent stream, has been heavily impacted by physical and chemical activities at Giant Mine. Historical studies indicated that aquatic ecosystem of Baker Creek was severely damaged; these studies found no fish inhabiting the area of Baker Creek downstream of Giant Mine. In more recent years, a portion of Baker Creek was re-aligned. The intent was to provide suitable spawning and rearing habitat for Arctic grayling (*Thymallus arcticus*). Now, Arctic grayling, a sensitive fish spawns and rears

its young in the creek in the middle of the site. Historical mine tailings are present in the stream and fish absorb metals such as arsenic from this material. Arctic grayling captured in the contaminated area had higher arsenic tissue concentrations relative to grayling captured in uncontaminated areas, particularly in juvenile fish. Another fish found in recent years in Baker Creek, slimy sculpin (*Cottus cognatus*), have consistently had higher arsenic tissue concentrations relative to those found in uncontaminated areas, indicating that arsenic in Baker Creek is bioavailable. Extensive sediment studies concluded that concentrations of arsenic in the sediment were elevated above applicable guidelines and were toxic in some areas. A multiple-lines-of-evidence approach was used for a recent an Investigation of Cause study into the causes of effects observed in fish during aquatic monitoring programs. Overall, studies indicate that despite elevated arsenic concentrations in water, sediment, and in slimy sculpin fish tissue, no obvious response in the health, survival, or reproduction is evident. Remediation options are currently being developed and will likely have to account for the dichotomous nature of the creek: highly contaminated but with resilient aquatic species such as the Arctic grayling and slimy sculpin.

Effects of Greater Victoria municipal sewage discharges on marine finfish and shellfish health and tissue quality (PL)

Martin Davies[1], Ben Beall[1], Ryan Stevenson[1], Shirley Lyons[2], Chris Lowe[2]

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Identifying and measuring the potential effects on organisms from the operation of municipal wastewater treatment plants is a necessary part of managing and mitigating human impacts on marine environments. A fish health monitoring program is underway in the marine environment adjacent to Victoria, British Columbia, Canada, to assess the effects of municipal wastewater treatment plant effluent on marine organisms. Multiple fish health indicators are measured in English sole (*Parophrys vetulus*), Irish lord (*Hemilepidotus* spp.), and Dungeness crab (*Cancer magister*) collected from exposure and reference locations. The program is assessing the potential effects to fish health and potential risks to ecological and human health using multiple lines of evidence. We will be presenting preliminary results and insights from the design and execution of the program.

Investigation of the toxicity of novel fire suppression gels to *Daphnia magna*, *Hexagenia* spp., and *Onchorynchus mykiss* (PL)

Sarah Graetz[1], Paul Sibley[1], Ryan Prosser[1]

[1]University of Guelph

Previous research has shown that fire suppression additives containing perfluorinated compounds negatively affect the environment through persistent contamination and bioaccumulation. Manufacturers have introduced 'environmentally-friendly' alternatives, but limited studies on their fate and effect in the environment have been completed. A study will be completed to investigate the toxicity of six fire suppression gels: Eco-Gel™, Thermo

Gel 200L™, Fire Aid 2000™, Solberg Fire Foam™, Novacool Foam™, and F-500™. Aquatic organisms can be indirectly affected by fire management efforts, as fire suppression gels can enter water systems through run-off or aerial application. Toxicity to *Daphnia magna*, a freshwater crustacean, was investigated through static acute tests and semi-static chronic tests. Toxicity to *Hexagenia* spp., a larval mayfly, was investigated through static acute tests. Toxicity to the freshwater fish *Oncorhynchus mykiss* was evaluated using a semi-static acute toxicity test. Survival and/or immobility was documented after each test, and the concentration-response relationship was modelled, which allowed for $EC50 \cdot L^{-1}$ values to be estimated. With the knowledge gained from this study, a better understanding of the potential fate and effect of novel fire suppression gels in the environment can be developed.

Effects of Faro Mine discharge on a downstream aquatic community (PL)

Dave Huebert[1]

[1]*AECOM*

The Faro Mine Complex is located within the Rose Creek watershed, approximately 20 road kilometers north of the Town of Faro, YT, and is currently in the midst of an extensive and comprehensive mine-site remediation. The mine was operational between 1969 and 1998, producing lead and zinc concentrates that were extracted for lead, zinc, silver, and gold. The mine also produced approximately $3.2x10^8$ tonnes of waste rock, and $0.7x10^8$ tonnes of tailings material. Both the waste rock and tailings material are potentially acid-generating, and the groundwater beneath these areas is heavily contaminated with sulfate and a number of metals. This contaminated groundwater is now entering Rose Creek from a variety of locations, which has resulted in measurable deterioration of surface water quality. At the site immediately downstream of the final discharge point into Rose Creek, the median zinc concentration for the low-flow period increased from 45 $\mu g \cdot L^{-1}$ in 2013 to approximately 200 $\mu g \cdot L^{-1}$ in 2017, iron increased from 360 $\mu g \cdot L^{-1}$ to almost 3000 $\mu g \cdot L^{-1}$, and sulfate increased from 250 $\mu g \cdot L^{-1}$ to almost 500 $\mu g \cdot L^{-1}$. Comparable increases were also observed at other exposure sites. Despite the poor quality of surface waters in the receiving environment, an Aquatic Effects Monitoring Program, completed in 2017, indicated that the aquatic community in the receiving environment continued to persist relatively unaffected. The periphyton species composition at reference and exposure sites was comparable, the median relative abundance of EPT (*Ephemeroptera* (mayflies), *Plecoptera* (stoneflies), and *Trichoptera* (caddisflies)) species was 55% for exposure sites and 46% for reference sites, and the fish community contained both slimy sculpin and Arctic grayling, although it is possible that the abundance of these fish had declined. It is postulated that the relatively healthy aquatic community that has persisted in the downstream receiving environment in Rose Creek is a result of the elevated hardness in surface waters, particularly during low-flow periods when metal concentrations are also high. During this seasonal period, the median hardness has been measured at almost 500 $mg \cdot L^{-1}$, with maximum values approaching 1000 $mg \cdot L^{-1}$. These results indicate the importance of calculating Site-Specific Water Quality Objectives for development of an understanding of environmental risk.

The sublethal effects of the anti-sea lice pesticides ivermectin and SLICE® on the swim performance, camouflage and avoidance behaviour of the starry flounder (*Platichthys stellatus*) following exposure to contaminated sediments (PL)

Daniel King[1], Christopher Kennedy[1], Michelle Young[1], Annie Cai[1]

[1]*Simon Fraser University*

Aquaculture is an important part of Canada's economy, and open net-pen Atlantic salmon (*Salmo salar*) aquaculture is a key facet of this industry. Chemotherapeutants, including anthelminthics, are often used in salmon aquaculture to prevent the loss of stock due to various pathogens. The formulation SLICE® (active ingredient: emamectin benzoate) and ivermectin (IVM) are two chemicals used in salmon aquaculture in Canada to treat and prevent sea lice infestations. SLICE® and IVM have low water solubilities and long half-lives in sediment (225 days and >100 days, respectively). Due to the persistence of these pesticides and their tendency to accumulate in marine sediments, it is crucial to understand the long-term effects of these compounds on wild benthic fauna, including fish. The sublethal effects of these chemotherapeutants on Pacific benthic teleosts (starry flounder [*Platichthys stellatus*]) using a sediment exposure system were examined. Ecologically relevant endpoints included swim performance, growth, oxygen consumption, as well as their camouflage ability and avoidance behaviour and ability to perceive and avoid these pesticides. This work will not only develop several new and novel methods for assessing the effects of chemicals on benthic marine species exposed in sediments, but will also contribute to estimating the risk of these chemotherapeutants to benthic Pacific fauna, including fish. These findings aid in the development of policies, regulations, and guidelines associated with the use of these chemotherapeutants, in order to minimize the sea lice infection rates on the farmed Atlantic salmon and increase yield, while also minimizing the risk to benthic species present below the open-net pens.

Let's go where we can breathe: *Gammarus* spp. (Amphipoda: Gammaridae) abundance responses to habitat and oxygen variability in eutrophic eastern Canadian estuaries (PL)

Kyle Knysh[1], Simon Courtenay[2], Christina Pater[1], Carissa Grove[1], Jerrica Cormier[1], Michael van den Heuvel[1]

[1]*University of Prince Edward Island,* [2]*University of Waterloo*

High nitrogen loads from agricultural sources have resulted in massive blooms of benthic macroalgae in temperate estuaries, with the resulting decomposing biomass reducing dissolved oxygen to hypoxic and anoxic levels. This issue is prevalent in Prince Edward Island (PEI) estuaries with 42% of the island's drainage basins under agricultural production. Additionally, estuaries form a dramatic gradient in salinity as this habitat is at the interface between river and ocean systems. Many euryhaline species persist throughout estuary gradients, with *Gammarus* spp. amphipods among the most abundant in PEI estuaries and a dominant prey item for estuary mesopredators. Declines in the abundance of amphipods in upper sections of local estuaries and poor understanding of habitat

requirements for PEI species has led to the question: does oxygen concentration predict survival and abundance of *Gammarus* spp. in the face of extreme habitat variability? In 2016, three eutrophic estuaries were regularly sampled over the agricultural production season in three locations along the estuary salinity gradient. Amphipods were captured with funnel traps and abundances compared to macrophyte biomass, sediment organic content, oxygen levels and other water quality parameters. In 2017, ten estuaries along a gradient of watershed agricultural intensity were sampled once in the upper estuary using funnel traps; captures were compared using sediment organic content, oxygen levels, and salinity. Oxygenated sediment assays compared survival of *Gammarus lawrencianus* (Bousfield, 1956) to the 2017 field-collected sediment over fourteen days. Both surveys found four species of *Gammarus*, *G. lawrencianus*, *G. mucronatus* (Say, 1818), *G. oceanicus* (Segerstråle, 1947) and *G. tigrinus* (Sexton, 1939). In 2016, abundances of *G. lawrencianus* and *G. oceanicus* correlated with oxygen variability and organic matter, whereas *G. mucronatus* and *G. tigrinus* abundances were negatively correlated with salinity, thus primarily restricted to brackish zones. However, brackish zones also had high levels of benthic macroalgae. Peak abundances of all species were highest in samples with high organic content, and high ranges of daily dissolved oxygen. In the 2017 survey, pooled *Gammarus* abundances were best predicted by high oxygen ranges (80% variation), with salinity variability being a secondary predictor (10% variation). Removing low oxygen stress using a laboratory sediment assay resulted in high survival in all exposed sediments, with dissolved oxygen being the best predictor of survival; however, the correlation was weaker than field oxygen metrics (16% variation). Amphipod species can occupy different parts of the estuary; however, organic matter relates to higher overall abundances with periods of high oxygen. Sustained depleted oxygen levels result in reduced *Gammarus* spp. abundances, through direct mortality or movement to oxygenated regions of the estuary. Sustained hypoxia may cause further stress on higher trophic levels from reduced prey availability.

The effects of diluted bitumen on the survival, swimming performance, and hepatic gene expression of juvenile sockeye salmon (*Oncorhynchus nerka*) during embryonic development (PL)

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The existing and recently proposed expansions in the transportation of diluted bitumen (dilbit) products via pipeline, railway, and marine terminals in coastal regions of British Columbia may potentially increase the risks of exposure to early life stage (ELS) Pacific salmon. The objective of the current study was to investigate the effects of dilbit exposure (from the embryonic to post swim-up fry) on sockeye survival, swimming performance, and hepatic gene expression at each life stage. Fertilized sockeye embryos were exposed to 4 concentrations (0, 3.5, 16.4, and 66.7 $\mu g \cdot L^{-1}$ initial total polycyclic aromatic hydrocarbon [TPAH]) of the water-soluble fractions (WSFs) of Cold Lake Summer Blend dilbit in a flow-through exposure system until 3 months post swim-up fry stage (3M fry). The mortality of individuals was recorded throughout the exposure period. Subsets of fish from each

treatment group were tested for the critical and burst swimming speed (Ucrit and Uburst) at both the swim-up and 3M fry stage. Whole body [total lipid], [total soluble protein], [glycogen], and [triglyceride] contents were also measured in non-swum fish. The expression of genes related to Phase I biotransformation, endocrine system regulating hormones, energy metabolism, and inflammation were measured using livers from 50% yolk sac abruption alevin, swim-up fry, and 3M fry. Mortality increased in individuals exposed to dilbit during embryonic development. Over 20% reduction in Ucrit and Uburst was observed in the two higher concentration groups in swim-up fry, while only the high exposure group had lower Ucrit and Uburst values at the 3M fry stage. Significant alterations were found in body biochemical measurements (whole body [total lipid], [glycogen], and [triglyceride] content) in exposed individuals at both stages, which may underlie the reductions in physiological performance and energy metabolism. Up-regulation of *CYP1a* and alterations of endocrine system genes (*ERα1*, *ERα2, ARβ,* and *GHR2*) were observed in exposed fish at various life stages. These results indicate that embryonic exposures of sockeye salmon to dilbit have the potential to affect their survival and future success.

Baseline toxicity of surface waters from proposed metal mine site in standardized aquatic toxicity tests (PL)

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[1]Simon Fraser University, [2]Gitanyow Fisheries Authority on behalf of Gitanyow Huwilp

Extensive baseline studies at proposed metal mine sites are required by regulators to characterize the biota and natural environmental concentrations of metals prior to future mining activities, in addition to ongoing environmental monitoring during the life of a mine. The purpose of this study was to examine potential background toxicity of surface waters collected from a proposed gold, copper, silver, and molybdenum mine in Northern British Columbia on indicator aquatic species in field and laboratory experiments. This study was intended to complement several baseline studies previously performed that indicated some surface waters in this area exhibit relatively high naturally-occurring background concentrations of metals, which in some instances are higher than BC water quality guidelines. The overall approach entailed the collection of additional baseline data using some of the standardized tests in the Metal Mining Environmental Effects Monitoring (EEM) program and the Metal Mining Effluent Regulations (MMER). Specifically, this study included water- and sediment-quality monitoring and laboratory sublethal toxicity tests of site water over two years. In addition, although not a requirement of the MMER, simultaneous fish growth and development field-based experiments in surface waters were also performed. The goal of the field experiments was to further characterize any baseline/background toxicity of surface waters within this mine site on fish, under realistic environmental scenarios. The study area focused on two creeks, Treaty and Teigen, located in the future Process Tailings and Management Area (PTMA). Year one toxicity tests indicated water flea (*Ceriodaphnia dubia*) reproduction was inhibited at two of the three Treaty Creek sites, yielding a 7-day IC_{25} of 33.4% (95% confidence limit 19.2-36.5) site water and 48.4% (95% CL 28.0-60.8) site water. However, in year one there were no effects

on water flea reproduction after exposure to site water from Teigen Creek. Site water from Treaty and Teigen Creeks in year one also had no effect on survival of water fleas, 7-day rainbow trout (*Oncorhynchus mykiss*) embryo viability, or on 72-hour green algae (*Pseudokirchneriella subcapitata*) growth inhibition. Year one of the field based *in situ* caging experiments demonstrated decreased survival in both Treaty and Teigen Creeks compared to the lab-reared control fish, but mortality was greatest in Treaty Creek. Several metals exceeded federal and provincial water and sediment guidelines at both Teigen and Treaty Creeks, and year two of this study is currently underway to examine the temporal variation in background metal concentrations and toxicity of these surface waters in these standardized lab and field aquatic bioassays.

Molt stage sensitivity of the Pacific spot prawn, *Pandalus platyceros*, to an anti-sea lice formulation, Salmosan® (PL)

Katherine Mill[1], Megan Ludlam[1], Chris Kennedy[1]

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Sea lice outbreaks in salmonid aquaculture cause economic and ecological impacts worldwide. Several of the chemotherapeutants used to treat these outbreaks are applied in multiple pulses and subsequently released into surrounding waters. As these formulations are designed to be effective against parasitic crustaceans, there is significant concern regarding their impact on proximal non-target crustaceans. One such chemotherapeutant, Salmosan® (active ingredient: azamethiphos), has recently been registered for use in British Columbia. Research demonstrates that low concentrations of Salmosan® can result in both lethal and sublethal impacts on non-target aquatic organisms. However, few studies have utilized experimental designs mimicking environmentally relevant scenarios such as multiple pulse exposures and multiple stressors (e.g., different temperatures). Furthermore, while behavioural effects have been observed, they have rarely been used qualitatively. In response to filling data gaps on non-target species (particularly Pacific coast species), Pacific spot prawns, *Pandalus platyceros*, in both the postmolt and intermolt life stages were subjected to multiple pulse exposures of Salmosan®. Postmolt prawns were found to be more sensitive than intermolt prawns. Following three 1-hour exposures, the [3x1h]-LC50 values were estimated to be 39.8, 27.1 and 17.1 $\mu g \cdot L^{-1}$ azamethiphos for postmolt prawns at 5, 11 and 17°C, respectively. The [3x1h]-LC50 values for intermolt prawns at 5, 11 and 17°C are estimated to be >100 $\mu g \cdot L^{-1}$, which is the target application concentration for Salmosan®. There was no evidence of an effect on olfactory, locomotory or grooming behavior in intermolt prawns and no evidence of an effect on mean time to molt, molting frequency, or mortality upon molt frequency. The results predict that environmental exposures to Salmosan® present negligible risk to intermolt prawns and low risk to postmolt prawns.

Assessment of radiological dose rates to aquatic organisms in the vicinity of Canadian nuclear power plants located near two Great Lakes (PL)

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Data collected over a decade on the concentrations of radionuclides in the water, sediment, and fish of two Great Lakes (Lake Ontario and Lake Huron) in the vicinity of Canadian nuclear power plants, and in reference areas, were analyzed. Concentrations of radionuclides in these media were converted into dose rates using the ERICA Tool and RESRAD-Biota models with conservative assumptions and model parameters. Results of this modeling exercise demonstrate that average dose rates to aquatic organisms for exposure areas were comparable to reference areas, and in both cases, were below the ERICA (10 $\mu Gy \cdot h^{-1}$) dose threshold as well as several orders of magnitude below the RESRAD (10 $mGy \cdot day^{-1}$) dose threshold (comparable to UNSCEAR 400 $\mu Gy \cdot h^{-1}$ dose threshold). Therefore, it can be concluded that radionuclides in the Great Lakes potentially impart negligible dose to aquatic organisms and do not pose a risk to the aquatic ecosystem.

Effects of bisphenol A and its analogs bisphenol F and S on life parameters, antioxidant system, and response of defensome in the marine rotifer *Brachionus koreanus* (PL)

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To understand the adverse outcome in response to bisphenol A and its analogs bisphenol F and S (BPA, BPF, and BPS), we examined acute toxicity, life parameter, and defensome in the marine rotifer *Brachionus koreanus*. Among the bisphenol analogs, BPA showed the highest acute toxicity and then BPF and BPS, in descending magnitude of toxicity. In life parameters including life span and reproduction, BPA, BPF, and BPS were found to cause adverse effects. Both intracellular reactive oxygen species (ROS) level and glutathione S-transferase (GST) activity were significantly increased ($P<0.05$) in response to each dosage of bisphenol analogs exposures. In response to bisphenol analogs, defensomes of phases I, II, and III detoxification mechanisms demonstrated an inverse relationship between the lipophilicity of bisphenol analogs and the expression patterns of defensomes. BPA and BPF were found to have significant modulation ($P<0.05$) in the expression of cytochrome P450 (CYP) and GST genes. In phase III, BPS with comparatively lower lipophilicity demonstrated a highly diversified expressional pattern, suggesting that BPS likely caused less toxicity compared to BPA and BPF. In this study, via phase I, II, and III detoxification mechanisms, bisphenol A and its analogs F and S demonstrated specific detoxification mechanisms in the rotifer. The toxicity effects of bisphenol analogs were examined using the monogonont rotifer *B. koreanus*. As BPA, BPF, and BPS are all structural analogs, they are expected to cause similar toxic effects within similar ranges. However, BPS demonstrated much lower toxicity compared to the other two bisphenol analogs. From phase I and II detoxification mechanisms, BPA and BPF were found to have a significant increase in the expression of CYP and GST genes. In phase III, the preprocessed metabolites of BPS are highly excreted in the hydrophilic state, with the effect that the threshold

concentration of BPS was significantly higher compared to BPA and BPF. Overall, this study could provide a better understanding of and insight into chemical-specific detoxification mechanisms and how each phase systematically functions in xenobiotic stress exposure at the molecular level.

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

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

Antidepressant drugs have been detected in municipal wastewater effluents (MWWEs) at ng·L^{-1} to low µg·L^{-1} concentrations. To assess the potential of these compounds to affect the survival, development and reproductive capacity of fish, we exposed fathead minnow (*Pimephales promelas*) over a full lifecycle in a flow-through system to a mixture of five antidepressants at nominal concentrations that have been detected in an Ontario MWWE: that is, venlafaxine at 2,400 ng·L^{-1} , citalopram at 240 ng·L^{-1} , fluoxetine at 90 ng·L^{-1} , sertraline at 20 ng·L^{-1} , and bupropion at 90 ng·L^{-1} . In addition to exposure to this 1x AntiD Mix, we also exposed minnows to 10x concentrations of these MWWE antidepressants (i.e., 10x AntiD Mix). Mean measured concentrations of venlafaxine, citalopram, fluoxetine, sertraline, and bupropion were 2,300, 160, 110, 7 ng·L^{-1} , and below detection limits, respectively, for the 5 compounds in the 1x AntiD Mix, and 33,000, 2,900, 1,000, 210, and 100 ng·L^{-1} , respectively, for the compounds in the 10x AntiD Mix. During the full life-cycle exposure period of 167-168 days , no significant changes were observed in survival of fathead minnows. When male fish from the exposed treatments reached maturity, their somatic weights were increased and secondary sex characteristics were enhanced compared to those of control males. There were no significant differences relative to controls in condition factor, gonadosomatic index, or liver-somatic index in the exposed fish. Exposed fathead minnows produced similar numbers of eggs as control fish. There were no changes in nest-defense behaviours of male minnows that were exposed to the antidepressant mixtures. Egg quality, % fertilization, and % hatching in F1 fry were unaffected by exposure to the antidepressants. Eggs hatched 0.5 day earlier, deformities in fry were 50% lower, and there were transient decreases in length of F1 larvae at 8 days post hatch (dph) in offspring from the treatment with the 10x AntiD Mix in comparison to controls. Overall, exposure to the antidepressant mixture at environmentally relevant concentrations (i.e., 1x AntiD Mix) caused no adverse effects in fathead minnows. Exposure to the 10x AntiD Mix increased the weight of adult male minnows and caused subtle effects in F1 offspring. This study is the first to assess sublethal effects in fish exposed to mixtures of antidepressants over a full lifecycle.

Investigations into the mechanism of life-stage and species-specific differences in the sensitivity of rainbow trout and white sturgeon to copper (PL)

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Fishes are the most diverse group of vertebrates. This diversity is also reflected by huge differences in the sensitivity among fishes to environmental pollutants, including metals. The high diversity found in fish species makes it challenging to test every metal and fish combination to estimate the differences in sensitivity. Therefore, alternative approaches for more efficiently estimating differences in sensitivity among fishes to toxic chemicals are needed. Studies addressing the physiological and/or molecular basis of the species-specific differences in sensitivity can be helpful in designing predictive models for predicting differences in sensitivity to metals among fishes. In the present study, we demonstrated that rainbow trout (*Oncorhynchus mykiss*) and white sturgeon (*Acipenser transmontanus*) are two disparate fish species with substantial species and life-stage (larval, swim up and juvenile) specific differences in acute waterborne Cu sensitivity, with sturgeon being more sensitive than trout. The life-stage specific acute toxicity (96h LC50) of Cu varied between 32-45 $\mu g \cdot L^{-1}$ Cu in white sturgeon, whereas it was in the range of 67-135 $\mu g \cdot L^{-1}$ Cu for rainbow trout. Short-term exposure (4.5 hours) to Cu (50 $\mu g \cdot L^{-1}$) caused a significant reduction in whole body sodium uptake in both species but the magnitude of effect was consistently higher in white sturgeon across all three life stages (70-80%) compared to that in rainbow trout (25-35%). Similarly, exposure to Cu (50% of respective 96-hour LC50) for 96 hours led to increased lipid peroxidation in both species, and the effect was significantly higher in white sturgeon (2-4 fold increase) compared to that in rainbow trout (approximately 2 fold increase) for all life stages. Acute exposure to Cu also decreased thiol balance (GSH/GSSG ratio) in both species by similar magnitude (2-3 fold) irrespective of life stages. In general, the differences in the degree of Cu-induced oxidative damage between the two fish species was less pronounced than that observed with Cu-induced reduction in sodium uptake. In addition, our study also revealed that the mRNA expression of heat shock protein 70 was significantly upregulated following acute exposure to Cu, but no significant differences between the two species was recorded for any life-stages. In contrast, rainbow trout demonstrated a greater capacity to induce the mRNA expression of metallothionein, especially in swim-up and juvenile stages (4-5 fold increase) relative to the similar life stages of rainbow trout (approx 2 fold increase). Overall, our findings suggest that the differential effects of Cu on sodium uptake and metallothionein induction could be more reliable indicators of species-specific differences in acute sensitivity to Cu in fish.

Assessing Toronto Harbour sediment quality using a tetrad approach (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. The Toronto and Region was designated as an AOC in 1986 due to degradation of water quality from centuries of

agriculture and urban development. Initiated in 1994 to address this, the Remedial Action Plan (RAP) has implemented numerous restoration actions aimed at the original 11 Beneficial Use Impairments (BUIs) identified by the RAP. This has led to significant improvements in water quality and sediment, such as decreasing concentrations of metals and organic compounds in sediments. Regarding Beneficial Use Impairments (BUIs) tied to sediment contamination, the Degradation of Benthos BUI was re-designated as "not impaired" in 2013. In this study, a sediment quality tetrad (SQTet) was applied in a weight-of-evidence assessment to evaluate current sediment condition within the AOC after the re-designation of the Benthos BUI. The SQTet includes assessment of bulk sediment chemistry, toxicity testing, benthic community analysis, and bioaccumulation. Samples were collected in summers of 2014 and 2015 from five monitoring stations within the Inner Harbour (Index, West End Gap, Keating Channel) and Humber Bay (Index, STP Outfall). Sediments were analyzed for contaminants of concern both years and used for laboratory toxicity and bioaccumulation exposures. In acute laboratory sediment bioassays using *Hyalella azteca* and *Chironomus dilutus*, survival was high and not significantly different among sites. In contrast, there was significant growth impairment in sediments near the outflow of the Humber STP. In life-cycle exposures with *C. dilutus* and *H. azteca*, there was significant growth and reproductive impairment present only in the Humber STP sediment, which is attributable to the effects of combined sewer overflow. Laboratory bioaccumulation exposures using *Hexagenia* spp., *Lumbriculus variegatus*, and fathead minnows were conducted in support of the Restrictions on Fish Consumption BUI to determine if there was evidence for mobilization of contaminants from the sediment into the food web. Compounds of continued concern include polychlorinated biphenyls (PCBs), which were found in fathead minnows at levels consistent across sample locations and with lake-wide levels. Bioaccumulation exposures conducted in 2015 resulted in elevated tissue concentrations of perfluorooctane sulfonate (PFOS) from the Humber STP sediments, which were not evident in the 2014 exposures. Such emerging contaminants pose an interesting question for the delisting process, and may need to be addressed under other programs. Within the benthic community, disturbance-tolerant taxa such as oligochaetes and chironomids dominated the Inner Harbour, as did dreissenid mussels in Humber Bay. The driving factor in the redesignation of the benthos BUI in 2013 was increased invertebrate diversity over time, and this continued trend was evident in the 2015 samples; however, the communities present are representative of those typically found in highly developed areas. Samples taken in 2015 highlight the effects of combined sewer overflow events, the dynamic impacts of which present a challenge to addressing sites in such urbanized environments. A return to pristine conditions is unattainable, but the goal of achieving a functional ecosystem has been reached through the concerted efforts of all those involved in the Remedial Action Plan Team.

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

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Pharmaceuticals and personal care products (PPCPs) have been studied for their toxicity to non-target organisms in the aquatic environment. This has been an important area of research due to the continual low-level release of PPCPs through wastewater treatment plants (WWTPs). A significant portion of this research has been attributed to understanding toxicity of 17α-ethinylestradiol (EE2), a highly potent estrogen mimic. In fish, EE2 toxicity has been primarily linked to the activation of nuclear estrogen receptors, known as classical estrogen signaling, typically resulting in alterations in gene transcription. Recent evidence has indicated that EE2 can also activate membrane estrogen receptors, specifically the G protein-coupled estrogen receptor (GPER). Activation of GPER presents a novel mode of action for EE2 in fish. It has been demonstrated that specific activation of GPER by EE2 could alter the heart rate of embryonic zebrafish *(Danio rerio*). The first aim of this study was to determine if EE2 could alter the heart rate of embryonic Japanese medaka (*Oryzias latipes*) at environmentally relevant concentrations of EE2. The second aim was to identify if the deodourizer/solubilizer hydroxyproply-β-cyclodextrin (HPβCD) could alter the observed toxicity of EE2 though guest inclusion. Three sets of 4-hour post fertilization (hpf) Japanese medaka embryos were exposed to either 0 $ng \cdot L^{-1}$ – 1000 $ng \cdot L^{-1}$ EE2 alone, 0 $ng \cdot L^{-1}$ – 17000 $ng \cdot L^{-1}$ HPβCD alone, or a range of mixtures of EE2 and HPβCD with 15 embryos per concentration. The heart rate of each embryo was monitored once every 24 hours for 15 seconds and converted to beats per minute. Percent change in heart rate from control was then determined and used for data analysis. A significant decrease in heart rate was observed in embryos exposed to all EE2 alone concentrations (0.1 $ng \cdot L^{-1}$ – 1000 $ng \cdot L^{-1}$ EE2) at 120 and 144 hpf in a dose-dependent manner ($p \leq 0.05$). Embryos exposed to a combination of EE2 and HPβCD experienced a significantly mitigated effect compared to EE2 alone ($p \leq 0.05$) at 120 and 144 hpf, at 144 hpf there was no significant difference between the control and EE2 + HPβCD at all concentrations tested (0.1 + 1.7 $ng \cdot L^{-1}$ – 1000 + 17000 $ng \cdot L^{-1}$ EE2 + HPβCD) ($p \leq 0.05$). There was no significant change in heart rate of embryos exposed to HPβCD alone (1.7 $ng \cdot L^{-1}$ – 17000 $ng \cdot L^{-1}$) compared to the control. This study has demonstrated that EE2 alone can cause a significant decrease in the heart rate of embryonic Japanese medaka at environmentally relevant concentrations and the presence of HPβCD can mitigate this effect.

Statistical methods for analyzing environmental monitoring data containing non-detects (PO)

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Analytical laboratory data are frequently left-censored (i.e., contain values reported below one more laboratory reporting limits). These data present challenges for conducting statistical analyses because the true values of the censored observations are known only to lie between zero and the reporting limit. Environmental practitioners generally replace the censored values with the reporting limit and proceed to analyze their data as if the values were detected. Replacement with the reporting limit creates biases in statistical analyses and is not recommended. We review statistical methods for three types of comparisons: 1) summary statistics, 2) group comparisons, and 3) trend analyses. These methods are presented for special cases that are typically observed in environmental monitoring that includes multiple laboratory reporting limits and small sample sizes.

Digging into the toxicity of benzotriazoles and benzothiazoles to benthic invertebrates (PO)

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In order to regulate chemicals detrimental to human health and the environment, the Chemicals Management Plan (CMP) was implemented by Canada's federal government in 2006. Few toxicological data are available for numerous industrial chemicals encompassed by the CMP. Benzotriazoles and benzothiazoles are among the Existing Substances Priority Groupings identified by the CMP. Both are high production-volume chemicals: benzotriazoles are primarily used as ultraviolet stabilizing additives and anticorrosive protection, and benzothiazoles are used as vulcanization accelerators for rubber, biocides, and corrosion inhibitors. These compounds are environmentally persistent, have the potential to bioaccumulate, and have been detected in environmental samples; however, the available toxicity information for aquatic invertebrates is scarce. Therefore, our objective was to conduct spiked-sediment exposures to assess the toxicity of three benzotriazoles and one benzothiazole to three species of aquatic invertebrates: *Hyalella azteca* (amphipod), *Hexagenia spp.* (mayfly), and *Tubifex tubifex* (oligochaete). Test compounds were UV234 (phenol, 2-(2H-benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl)-; CAS RN 70321-86-7), UV326 (phenol, 2-(5-chloro-2H-benzotriazol-2-yl)-6-(1,1-dimethylethyl)-4-methyl-; CAS RN 3896-11-5), UV329 (phenol, 2-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)-; CAS RN 3147-75-9), and MBTS (2-mercaptobenzothiazole disulfide; CAS RN 120-78-5). Three-week tests were conducted on *Hyalella* and *Hexagenia* with survival and growth as endpoints. Four-week tests were conducted on *Tubifex* with adult survival, number of cocoons produced per adult, percent of cocoons hatched, and number of juveniles produced per adult as endpoints. Nominal test concentrations were 1, 10, and 100 $mg \cdot kg^{-1}$ (dry weight sediment). Chemical analysis of water and sediment is

ongoing; therefore, results are currently based on nominal sediment concentrations. Survival of *Hyalella, Hexagenia*, and *Tubifex* was not significantly different between controls and treatments for any of the four compounds tested. Growth of *Hyalella* was also not affected; however, growth of *Hexagenia* was significantly reduced (by 8-10%) compared to controls at all test concentrations of MBTS. Cocoon production of *Tubifex* was unaffected, as was hatching success of cocoons with the exception of UV326 at 1 $mg \cdot kg^{-1}$, which was significantly lower than controls. Juvenile production of *Tubifex* was significantly reduced compared to controls for UV329 at 10 and 100 $mg \cdot kg^{-1}$ (by 14 and 18%, respectively), and for MBTS at 100 $mg \cdot kg^{-1}$ (by 18%). The results of this study will be compared to benzotriazoles and benzothiazoles measured in Canadian environmental samples, and will support environmental risk assessments to determine the impacts of these compounds on aquatic organisms.

Sublethal effects of clothianidin on early life-stage sockeye salmon (*Oncorhynchus nerka*) (PO)

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Pesticides are frequently detected in all environmental compartments and particularly in areas located near intensive agricultural activities. Neonicotinoid insecticides are one of the most widely used pesticide groups globally, and their low level, environmentally relevant adverse effects on fish are not well studied. Clothianidin is commonly used in agricultural practices in the Fraser Valley. Thus, in this study, the effects of clothianidin on embryonic, alevin and early swim-up fry developmental stages of sockeye salmon were investigated. Four genetically distinct pairs of wild sockeye salmon were collected and fertilized in clean water, and were subsequently exposed to 0.15, 1.5, 15, 150 $\mu g \cdot L^{-1}$ clothianidin from 1-hour post-fertilization through to the swim-up fry developmental stage in glass tanks and gravel-bed incubators for four months. No significant effect of clothianidin on survival, hatching, condition factor, or deformity was observed; however, there was a significant mean 4.7-fold increase in whole body 17þ-estradiol levels in swim-up fry after exposure to 0.15 $\mu g \cdot L^{-1}$. No effects were observed on testosterone levels. Individual swim-up fry livers from all genetic crosses and each treatment were also collected to investigate hepatic gene expression, and various genes of interest were quantified using quantitative PCR. The genes of interest evaluated were estrogen receptor alpha and beta 2, cytochrome P450 1A, suppressor of cytokine signaling 3, and glucocorticoid receptor 2. The glucocorticoid receptor 2 showed a significant 4-fold downregulation at 150 $\mu g \cdot L^{-1}$ compared to the control treated fish ($p<0.05$). Although beyond the scope of the present study, these results indicate the utility of quantitative PCR in these early life-stage studies, and additional examination of clothianidin and its potential impacts on salmonid gonad development, the reproductive endocrine axis, and the stress axis after prolonged exposure to clothianidin is warranted. In addition, the significant differences observed in growth and development of four unique genetic crosses of a wild-caught salmonid species in the controls in this study underscore the influence of genetics in variation in apical endpoints in this wild salmonid.

Toxicity of flotation reagent AERO 6493 to invertebrate *Hyalella azteca* and *Daphnia magna* (PO)

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Rare earth elements (REEs) are a group of metals on the periodic table that consist of scandium, yttrium and the 15 lanthanides. There is a growing environmental concern with the development of REE mining and processing. Although there are some recent studies on the toxicity of individual REEs, little is known about the environmental issues associated with REE processing. The goal of this study was to evaluate the toxicity of flotation reagents used in REE processing. The invertebrates *Hyalella azteca* and *Daphnia magna* were used. Testing procedures followed the Environment Canada standard test method for *Hyalella* and *Daphnia*. Serial dilutions of AERO 6493 stock solution were used to create test solutions. Preliminary testing suggests that AERO 6493 is very toxic. Dilutions of 10^{-22} % to 10^{-16} % showed an increase in mortality as flotation reagent concentration increased and tests conducted with dilutions at and above 10^{-15} % resulted in 100% mortality with *Hyalella*. This study will contribute to developing a better understanding of the potential environmental issues associated with REE mining. This research is supported by Natural Resources Canada, Environment and Climate Change Canada, NSERC Strategic Grants Program with contributions from the Ontario Ministry of Environment and Climate Change, Avalon Rare Metals, Inc. and International Zinc Association.

A possible approach for dredging sediments in the Pelagos Sanctuary (PO)

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The Mediterranean Sea is mostly highly anthropized, and marine biota, including marine mammals, are threatened by acoustic and chemical pollution, fishery and ship traffic. In 2002 the Pelagos Sanctuary for the protection of marine mammals was established in the north-western Mediterranean Sea, extending between south-eastern France, Monaco, north-western Italy and northern Sardinia, and surrounding Corsica and the Tuscan Archipelago. The Sanctuary is a pelagic Marine Protected Area (MPA) of 87,500 km^2. The area is characterized by increased primary productivity caused by a mixture of phenomena including the upwelling of deep water rich in nutrients. These conditions promote a great biodiversity, in particular regarding the number of predators on top of the food chain, such as tuna, sharks and marine mammals. Nine species of marine mammals are found in the Pelagos Sanctuary on a relatively consistent basis. Other species are rare but occasionally observed. Nevertheless, within the Italian borders of the Sanctuary, there are located many touristic and commercials harbours. Along the coast of Liguria, Toscana and Sardinia ports as well as Genoa, La Spezia, Massa Carrara, Leghorn and Olbia needed to be dredged periodically in order to ensure maneuverability. In Italy the authorization for dumping at sea must follow procedures according to the legislative decree "Decreto 15 Luglio 2016,

N.173". The law provides methods for the characterization of dredging sediments, establishing different sediment management according to the pollutants level and their ecotoxicological effects. The requirements for the characterization and monitoring of marine dumping areas are also included. However, further efforts must be made in order to better protect a sensitive area such as the Pelagos sanctuary, including additional specific measures. While problems related to acute and chronic noise due to military sonar tests, geophysical exploration and maritime traffic are known, there is a gap in direct and indirect evidence of the impacts on marine mammals caused by dumping operations. Furthermore, upwelling phenomenon can potentially cause chemical re-suspension with significant mobility from the bottom along the water column. With the aim of providing better protection of the sea environment and limiting the repercussions of economic activities on the harbours, some measures already adopted in similar challenges at international level are examined.

Effects of *in vivo* exposure to tritium: a multi-biomarker approach using the fathead minnow, *Pimephales promelas* (PO)

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Tritium (3H) is a radioactive isotope of hydrogen. In the environment, the most common form of tritium is tritiated water (HTO). However, tritium can also be incorporated into organic molecules, forming organically bound tritium (OBT). The present study characterized the effects of tritium on the health of the fathead minnow, *Pimephales promelas*. Fish were exposed to a gradient of HTO (activity concentrations of 12,000, 25,000 and 180,000 $Bq \cdot L^{-1}$) and OBT using food spiked with tritiated amino acids (OBT only, with an activity concentration of about 21,000 $Bq \cdot L^{-1}$). A combined exposure condition where fish were placed in 25,000 $Bq \cdot L^{-1}$ water and received OBT through feed was also studied. Fish were exposed for 60 days, followed by a 60-day depuration period. A battery of health biomarkers were measured in fish tissues at seven time-points throughout the 120 days required to complete the exposure and depuration phases. HTO and OBT were also measured in fish tissues at the same time-points. Results showed effects of increasing tritium activity concentrations in water after 60 days of exposure. The internal dose rates of tritium, estimated from the tissue-free water tritium (TFWT) and OBT activity concentrations, were consistently low (up to 118,731±1,141 and 2,377±99 $Bq \cdot L^{-1}$, respectively, for a maximum dose rate of 0.65 $\mu Gy \cdot h^{-1}$) compared to levels at which effects on population may be expected (>100 $\mu Gy \cdot h^{-1}$), and no effects were observed on survival, fish condition, gonado somatic, hepato somatic, spleno-somatic and metabolic indices (RNA/DNA and proteins/DNA ratios). Using multivariate analyses, it was observed that several biomarkers (DNA damage, MN frequency, brain AChE, lysosomal membrane integrity, phagocytosis activity and ROS production) were exclusively correlated with fish tritium internal dose rate, showing that tritium induced genotoxicity, as well as neural and

immune responses. The results were compared with another study on the same fish species where fish were exposed to tritium and other contaminants in natural environments. Similar results were obtained for overall health indicators as well as for genotoxicity and immune markers. However, biomarkers for oxidative stress were specific for each study. Together with the field study, the present work provides useful data to identify biomarkers for tritium exposure and better understand modes of action of tritium on the fathead minnow.

Set up a method for measuring mercury in foodstuffs with reference materials by Flow Injection Hydride Generation Atomic Absorption Spectrometry (FI-HG-AAS) (PO)

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The hydride generation atomic absorption technique was used for determination of hydride-forming elements since the 1970s. These elements, which include arsenic, bismuth, and selenium, form volatile hydrogen compounds in the presence of a suitable reducing agent in acid solution. The gaseous compounds are transferred from the sample solution to a nitrogen or argon gas phase, and swept into a heated tube for atomisation and determination. Steps of the method were optimized, and analytical as well as statistical parameters of the method were determined (detection limit 1 $\mu g \cdot kg^{-1}$, quantification limit 3 $\mu g \cdot kg^{-1}$). Accuracy of the method was evaluated using GBW10043 (Rice), GBW100052 (Green tea), T0770 (soya flour), NCS-ZC 73013(spinach), T0766 (milk powder) reference materials. The results obtained from the samples were within the range stated in the analysis sheet. Depending on the results, the digested method (1ml deionized water, 2ml HNO3 65% (Merck), 1ml HClO4 70% (Merck) and 4ml H2SO4 98%) is approved.

Aquatic toxicity of the rare-earth element yttrium on Columbia spotted frog (*Rana luteiventris*) tadpoles (PO)

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Rare-earth elements (REEs) have seen a sizeable escalation in demand in recent years due to their many applications in today's modern technology. Yttrium (Y), for example, is one of the more abundant REEs in the earth's crust and is used in areas from display monitors to radar to medical and manufacturing lasers. However, the environmental effects from the mining, industrial and consumer waste of these emerging contaminants have not been widely explored, especially in the aquatic environment. The early stages of development in amphibians can be highly sensitive indicators of environmental pollution. The aquatic toxicity of Y was investigated on a local amphibian species, the Columbia spotted frog (*Rana luteiventris*). Tadpoles at Gosner stage 28/29 were exposed for 21 days to five concentrations of Y (as YCl3.6H2O) ranging from 10 to 1000 $\mu g \cdot L^{-1}$ and assessed for survival, development and growth at test termination. Testing was conducted under static-renewal conditions. Results were used to calculate mortality and growth point estimates

(LC and IC). The sensitivity of *R. luteiventris* to Y was evaluated relative to the findings from previous studies, such as on the cladoceran *Ceriodaphnia dubia*, amphipod *Hyallela azteca* and coho salmon *Oncorhynchus kisutch*.

The impacts of wastewater on fish communities in Hamilton Harbour (PO)

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Environments that receive a constant supply of municipal wastewater treatment plant (WWTP) effluent typically suffer from various biotic and abiotic stressors, including but not limited to nutrient enrichment, depletion of dissolved oxygen, thermal enhancement, and direct and indirect contamination. Such stressors have the potential to affect fish and other wildlife residing in such environments across all levels of biological organization. The objective of this study was to assess the impacts of WWTP effluent discharges on fish community structures downstream of two WWTPs in Hamilton Harbour. Hamilton Harbour is one of 43 locations around the Great Lakes that is identified as an "Area of Concern". While there are numerous ongoing projects aimed at remediating Hamilton Harbour, wastewater toxicity remains a major issue, with approximately 50% of the water flowing into Hamilton Harbour originating in WWTP effluent. This work will aid in the general understanding of how wastewater influences fish abundance, biomass, diversity, and richness. This study will also be instrumental to understanding how fish community responses to wastewater inputs vary across seasons, a subject that is poorly understood in the realm of ecotoxicology. Municipal WWTP effluents are one of the largest sources of pollution in Canada; consequently, their impacts on the environment must be carefully assessed, especially in light of increasing urban population growth.

An investigation of dicamba using hepatocytes of rainbow trout (*Oncorhynchus mykiss*) in primary culture (PO)

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Herbicides are used for selective eradication of weeds in agriculture. Currently, dicamba is the second most-used benzoic acid herbicide in agriculture, and usage extends to industrial, domestic, and municipal applications to control woody and broadleaf weeds. Because of its widespread use, dicamba is frequently detected in surface waters. For example, in Alberta, dicamba is found at concentrations of 0.3 $\mu g \cdot L^{-1}$ in irrigation canals and 2.6 $\mu g \cdot L^{-1}$ in storm water ponds. However, very little is known about the effects of dicamba on freshwater aquatic vertebrates. In the present study, toxicological effects of environmentally relevant concentrations of dicamba were investigated using primary cultures of rainbow trout (*Oncorhynchus mykiss*) hepatocytes, which are the main sites of the xenobiotic metabolism. Exposure for 48 hours to 22.00, 2.20, and 0.22 $\mu g \cdot L^{-1}$ of dicamba caused 10.12%, 9.06% and 4.03% mortality, respectively, relative to the control. It was hypothesized that dicamba exerts its toxic effects via disruption of redox homeostasis.

Therefore, lipid peroxidation and mRNA expression of the anti-oxidative enzymes catalase, superoxide dismutase, glutathione-s-transferase, and glutathione peroxidase are being quantified to determine if dicamba causes toxicity via oxidative stress. In addition, it was hypothesised that induction of oxidative stress by dicamba might impact DNA methylation. Therefore, effects of dicamba on mRNA expression of DNA methyltransferase 1 and 3a are being quantified and compared to global changes in DNA. This research work will provide novel information regarding potential mechanisms of adverse effects of dicamba on freshwater aquatic vertebrates.

Fish behaviour and physiology across two wastewater effluent gradients in Cootes Paradise and Hamilton Harbour (PO)

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Chemical inputs from wastewater treatment plants (WWTPs) pose serious environmental stressors for urban watersheds. Hamilton Harbour is one of 43 locations around the Great Lakes identified as an Area of Concern due to environmental damage and poor ecosystem health. While habitat remediation has reversed some ecosystem degradation, treated wastewater continues to enter the harbour, hindering restoration efforts. In this study, we examined the effects of WWTP effluent on multiple levels of biological organization in wild fishes. We studied two native species—green sunfish (*Lepomis cyanellus*) and bluegill sunfish (*Lepomis macrochirus*)—and one invasive species, the round goby (*Neogobius melanostomus*), along an effluent gradient near two WWTPs entering Hamilton Harbour. We used field-based behavioural tests and scored activity levels, boldness, and predator responses. We also assessed morphological and physiological markers: body mass, Fulton's body condition, gonadosomatic index (GSI), hepatosomatic index (HSI), haematocrit (Hct), and critical thermal tolerance (CTmax). We found that activity, boldness, and predator responses were not affected by proximity to WWTP effluent. Body mass and Hct were higher in fish caught closer to WWTPS, but body condition was not different between sites, and we saw varying responses in liver and gonad investment. These findings may suggest that fish living near wastewater have a greater metabolic need for detoxification. While CTmax did not vary with proximity to WWTP effluent, the three species showed strong differences in thermal tolerance: green sunfish had the highest CTmax and round goby had the lowest. Understanding the ecological thresholds for various fish species and how environmental stressors like wastewater effluent affect fish behaviour and physiology is vital for shaping resource management decisions and conservation policy.

Identification of causes of toxicity in a pulp mill effluent (PO)

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Following the Fisheries Act of 1992, pulp and paper mills are regulated for toxicity, where rainbow trout (*Oncorhynchus mykiss*) and *Daphnia magna* bioassays are completed following Environment and Climate Change Canada regulatory protocols. An effluent sample from a local pulp and paper mill was tested at the Nautilus Environmental laboratory using the 96-hour rainbow trout LC50 and 48-hour *Daphnia magna* LC50 toxicity tests. The full-strength untreated sample exhibited 75% mortality in the rainbow trout, whereas the *D. magna* test showed no toxicity, with a 48-hour LC50 of >100% (v/v). When tests exhibit toxicity like these and do not pass the regulatory standards, a toxicity identification evaluation (TIE) is employed. TIEs are typically conducted to determine specific causes of toxicity in order to separate naturally occurring toxicants (e.g., ammonia, hydrogen sulfide) from anthropogenic contaminants; to characterize appropriate remediation targets and strategies for specific contaminants; and to identify toxicant sources and responsible parties. The purpose of this investigation was to determine the cause of toxicity observed in the rainbow trout test. TIE procedures involve physico-chemical manipulations of the sample, followed by toxicity tests on the treated and untreated samples, to provide information on the type of contaminant responsible for toxicity. Each of the treatments alters the toxicity of a subset of contaminants; thus, a change in toxicity as a result of the treatment provides an indication of the identity of the toxicant. Follow-up treatments and analytical chemistry were then used to identify the actual contaminant responsible for toxicity. This presentation describes the approach undertaken for working through the TIE process with a pulp and paper effluent that exhibited unknown toxicity. Following phase three of the TIE process, it was hypothesized that a large concentration of terpenes were responsible for the toxicity observed.

Modeling the response of fish to major infrastructure upgrades in wastewater treatment plants (PO)

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Municipal wastewater represents one of the largest sources of effluent to Canadian aquatic ecosystems. These effluents contain a diversity of contaminants, including those that can alter endocrine function and reduce reproductive performance of fish. Recent studies in the central Grand River, Ontario, Canada, have demonstrated that a wide variety of endocrine active compounds (e.g., natural estrogens, ethinylestradiol, triclosan) are released into the environment from municipal wastewater outfalls. Fish populations, including rainbow darter (*Etheostoma caeruleum*), associated with these outfalls have shown a variety of biological changes, including altered gene expression (i.e., transcriptomes), physiology (e.g., steroid production), energy storage and reproductive success (e.g., egg survival). Of particular note has been the extremely high incidence and

severity of intersex (developing eggs in testes tissue) below the outfalls. The local municipality is currently investing hundreds of millions of dollars to upgrade several treatment plants in the watershed. More efficient aeration of secondary clarifiers, increased retention time, and a switch from chlorine to UV disinfection in the Kitchener treatment plant in 2012 led to an increase in nitrification and decreased estrogenicity of the effluent. In the three years since these changes, a recovery of several biological endpoints in fish, including *in vitro* steroid production and intersex, has been observed. Some effects, such as gene expression and low incidence of intersex, remain evident and are likely due to residual chemicals, upstream urbanization and other wastewater effluent outfalls. A mechanistic fate and effects model was developed and used to predict the distribution of estrogens in the wastewaters and the river, which predicts that until the upstream treatment plant is fully upgraded there will be detectable intersex in fish downstream of both outfalls. Further model development is planned to better predict changes in fish health related to wastewater and how these complex mixtures interact with multiple stressors in the watershed (i.e., cumulative effects).

Acute toxicity of thiocyanate and cyanate to rainbow trout across a range of conditions (PO)

Jordana Van Geest[1], Liz Ashby[1], Adrian deBruyn[1], James Elphick[2]

[1]Golder Associates Ltd, [2]Nautilus Environmental

Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) was observed in effluent associated with cyanide detoxification and acid rock drainage discharges from a gold mine in South America. A review of effluent chemistry indicated that effluent concentrations of nitrogenous compounds (ammonia, thiocyanates, and cyanates) sometimes exceeded published LC50 for rainbow trout. While the toxicity of ammonia and associated exposure and toxicity modifying factors (ETMFs) is well characterized, more limited data exist to characterize the toxicity and ETMF relationships for thiocyanates and cyanates. To address these data gaps, we conducted a laboratory toxicity study of the acute toxicity of thiocyanates and cyanates to rainbow trout across a site-relevant range of temperature, pH, and hardness. Relationships between toxicity and ETMF conditions were evaluated and described with regression analysis. There was no significant effect of temperature, pH, or hardness on thiocyanate toxicity under the conditions tested. Temperature and pH effects were inconsistent and generally small, while the effect of hardness was not monotonic. Cyanate toxicity could not be characterized at low pH of 6.0 due to rapid degradation of the compound under these conditions, which precluded characterization of pH effects in this study. At higher pH of 8.5, where cyanate concentrations were stable, there was no significant effect of temperature or hardness on cyanate toxicity.

A sulphate and total dissolved solids (TDS) toxicity interaction study for coal mine influenced waters in British Columbia (PO)

Jordana Van Geest[1], Gary Lawrence[1], James Elphick[2], Adrian deBruyn[1]

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The toxicity of sulphate to many freshwater organisms decreases with increasing water hardness, which is accounted for in a hardness-dependent British Columbia water quality guideline (BC WQG) for sulphate. However, the BC WQG recommends site-specific evaluation for very high hardness conditions due to uncertainty about cumulative effects of component ions in the mixture. Hardness in mine-influenced receiving waters of many coal mines in BC is currently or is predicted to increase to very high hardness. We conducted a sulphate and total dissolved solids (TDS) interaction study to address these uncertainties. The overall objective was to evaluate whether sulphate, along with related ions in a site-relevant mixture, is of potential concern with respect to toxicity in mine-influenced waters under current and modelled future conditions. A related objective was to assess how the toxicity of sulphate is expected to change as modifying factors such as TDS and hardness increase over time, based on predictions of future operations. Site-specific, chronic toxicity tests were conducted following standardized laboratory protocols using *Ceriodaphnia dubia, Hyalella azteca, Centroptilum trangulifer,* and rainbow trout *(Oncorhynchus mykiss)*. Mine-affected water samples were spiked with a series of sulphate concentrations added as calcium and magnesium salts in an ion ratio consistent with site waters. The range of tested sulphate concentrations encompassed current site conditions, predicted increases under future conditions, and concentrations anticipated to yield significant effects. A 25% effect on *H. azteca* growth (560 $mg \cdot L^{-1}$) and *C. triangulifer* emergence (840 $mg \cdot L^{-1}$) were observed in one test water, whereas effects concentrations were higher in the other test water. Apart from these results, the invertebrate species exhibited no effects on survival and sublethal endpoints at sulphate concentrations up to 1,400 $mg \cdot L^{-1}$. Rainbow trout exhibited variable responses, but on average showed no effects on embryo-alevin survival and development at sulphate concentrations up to 1,100 $mg \cdot L^{-1}$. For all four species, increasing hardness and associated concentrations of major ions did not appear to increase sulphate toxicity under the conditions tested. This study demonstrated that testing of site-relevant ion mixtures (i.e., calcium and magnesium increasing with sulphate) is more relevant to coal mines than the sodium-sulphate toxicity testing upon which the BC WQG for sulphate is based.

Advances in Environmental Quality Guidelines, Objectives, and Benchmarks

The relevance of amphibians in water quality guideline derivation: a case study using copper (PL)

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Amphibian populations are facing a global decline and several species have been extirpated from water bodies during the past few decades around the world. While a few studies suggested that contaminants can be responsible or co-responsible for global population decline of amphibians, a few others rejected this idea by arguing that generally amphibians are relatively less sensitive to contaminants. However, due to the distinct mechanism of toxicity of various contaminants, it is necessary to study contaminants individually. Nonetheless, amphibian toxicity data is not included in the minimum data requirement for derivation of water quality guidelines (WQGs) as the main tools in managing water quality. Copper (Cu) is one of the most studied contaminants globally and the abundance of data on Cu toxicity provides an opportunity to compare relative sensitivity of amphibians to other aquatic organisms. We conducted a thorough review of Cu toxicity studies with an emphasis on amphibians. In general, amphibians are understudied compared to other taxa and the limited toxicity studies suffer from a lack of associated water quality data (WQD) necessary to accurately understand Cu sensitivity. Amphibians display a very wide range of inter-species sensitivity to both acute and chronic Cu exposures. Amphibians are relatively tolerant of acute exposures; however, recent studies using ecologically-relevant chronic endpoints demonstrated extreme sensitivity to Cu at certain life stages, resulting in a greater acute to chronic ratio compared to other taxa (e.g., fish). Our review demonstrates that amphibians are at least as sensitive as other taxa to a globally important contaminant (i.e., Cu), while other contaminants of concern remain to be studied. Considering the current situation of amphibians, special attention to amphibians is essential in water quality management. Inclusion of amphibian toxicity data in the minimum data requirement for derivation of WQGs may be an effective approach which encourages conducting amphibian toxicity studies and managing amphibian populations simultaneously.

Chronic toxicity of rare earth elements to amphipods (*Hyalella azteca*), cladocerans (*Ceriodaphnia dubia*) and coho salmon (*Oncorhynchus kisutch*) (PL)

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Interest in the extraction of rare earth elements (REEs) has recently increased and with it a necessity to generate environmental benchmarks for long-term exposures to these

elements. REEs are used in a variety of manufactured products yet little is known of their ecotoxicological properties. Chronic toxicity tests, involving 35-day early life-stage tests with coho salmon (*Oncorhynchus kisutch*), 7-day reproduction and survival tests with cladocerans (*Ceriodaphnia dubia*) and 14-day water-only amphipod tests (*Hyalella azteca*) were conducted with lanthanum (La), neodymium (Nd), yttrium (Y) and cerium (Ce). Solubility studies were performed to determine appropriate test media for the invertebrate toxicity tests. Results of the toxicity tests indicated that the amphipod was the least sensitive to the REE exposures, with 25% inhibition of amphipod dry weight (IC_{25} values) ranging from 167 µg $Ce \cdot L^{-1}$ to 503 µg $Nd \cdot L^{-1}$. The IC_{25} values for reproduction in the 7-day *C. dubia* tests were determined to be 33.6, 13.6, 31.6, and 35.2 $\mu g \cdot L^{-1}$ for dissolved Ce, La, Nd and Y, respectively. REE toxicity to the coho salmon was intermediate of the three organisms tested, and was lower than that of toxicity observed historically with other salmonids; increased salmonid sensitivity was observed in exposures to cerium in comparison to the other REEs tested. Follow-up work with cerium-exposed salmonids indicated a unique mode of toxic action.

Investigating a percent change from background condition approach to potentially derive a water quality guideline for water hardness (PL)

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Salinization of freshwaters is increasing on a global scale. While an abundance of research has shown that an increase in the total ionic content of water can be toxic to freshwater aquatic life, more recent investigations have shown that shifts in the balance of major ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, HCO_3^-, SO_4^{2-}, Cl^-) can also be toxic. Despite this, and although a water quality guideline (WQG) exists for Ca for the protection of agricultural water uses, few WQGs for the protection of aquatic life exist for major ions (i.e., only for Cl^- and SO_4^{2-}). Nevertheless, increases in water hardness alone (Ca^{2+} + Mg^{2+} content) can be toxic to freshwater organisms. Moreover, Mg^{2+} has been shown to be more toxic than SO_4^{2-}. Anthropogenic sources have the potential to increase water hardness in receiving waters to potentially toxic concentrations (e.g., effluent from coal mining, produced waters from oil and gas extraction). Thus, a WQG for water hardness is warranted. While using Canadian protocols for WQG development, we attempted to derive a WQG for water hardness. We have determined that deriving a water hardness based WQG is not supported by application of an uncertainty factor to the lowest effect concentration. Here we present an alternative option using the percent change from background condition approach for both water hardness and the Ca:Mg ratio (a 2-component WQG). We discuss the applicability of the resultant (preliminary) criteria to the exemplar region of interest and challenges associated with their implementation.

Comparing empirical and mechanistic approaches to bioavailability modeling (PL)

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

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Mechanistic and empirical models have both been used to consider how various water quality characteristics affect the bioavailability of toxicants. These two model types have different data needs in terms of the kinds and amount of data needed to develop them. Empirical models are generally computationally simpler, but the development and calibration of these models requires data specific to the relationship being modeled, in this case toxicity data over a range of a chemical parameter. These same data are often the only data that can be used to evaluate the model, meaning that the dataset must be split into validation and calibration datasets in order to effectively evaluate model performance. Mechanistic models can be more computationally intensive, but the mechanistic nature of these models allows some relationships to be determined by physical and chemical constants, rather than calibration data. When calibration data are needed, the mechanistic nature of the modeling approach may allow for different types of data that are not direct measurements of toxicity to be used. The different structure of these two modeling approaches may also lead to different recommendations about the type of data that would be useful for refining model development. Complex inter-relationships between variables may be easier to represent in mechanistic models, while empirical models may require more data to investigate relationships between parameters. The requirements, structure, strengths, and weaknesses of these two kinds of models need to be considered when designing approaches for development, calibration, and evaluation. Thoughtful selection of calibration and validation datasets and handling of the data is needed in order to ensure that the comparison is not biased towards one model, and to evaluate the limits and usefulness of each model.

Multiple linear regression models for predicting aluminum and iron toxicity to freshwater aquatic life (PL)

David DeForest[1], Lucinda Tear[1], William Adams[2], Kevin Brix[3]

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The bioavailability of both aluminum (Al) and iron (Fe) to freshwater aquatic organisms varies as a function of several water chemistry parameters, including pH, dissolved organic carbon (DOC), and water hardness. Each of these parameters may individually affect Al or Fe bioavailability or interact such that the relative influence of one parameter is diminished or enhanced depending on the magnitude of another parameter. For example, the influence of DOC on Al bioavailability is greatest at approximately pH 6 and then reduced as pH increases. Multiple linear regression (MLR) models for predicting Al and Fe toxicity to a green alga (*Pseudokirchneriella subcapitata*), a cladoceran (*Ceriodaphnia dubia*), and a fish (*Pimephales promelas*) were developed as a function of multiple combinations of pH, DOC, and hardness conditions. These effects were tested by including individual water chemistry terms (pH, ln[DOC], ln[hardness]), as well as interaction terms (pH×ln[DOC], pH×ln[hardness], ln[DOC]×ln[hardness], pH^2) as independent variables in the analysis;

ln(EC_{10}) values were used as the dependent variable. The Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) were used to inform which combination of terms resulted in the best models for predicting Al and Fe EC_{10}s for each species and the models were evaluated using a variety of performance metrics (e.g., adjusted R^2, predicted R^2, residual plots) to assess model robustness. The MLR models were then applied to species sensitive distributions (SSDs) to develop possible water quality guidelines following Canadian protocols.

Update on federal Environmental Quality Guidelines: What's new (PL)

Tamzin El-Fityani[1], Sushil Dixit[1], Allison Dunn[1], Philippa Cureton[1], Doug Spry[1], Kathleen McTavish[1]

[1]Environment and Climate Change Canada

Federal Environmental Quality Guidelines (FEQGs) are recommendations that support various federal activities including risk assessment, risk management, and environmental quality monitoring. They are developed under the Canadian Environmental Protection Act, 1999, and support various federal programs including the Chemicals Management Plan and the Federal Contaminated Sites Action Plan. FEQGs are based on toxicity data and are set at a concentration below which there is low likelihood of adverse effects from a chemical on the ecological receptor. To date, 246 FEQGs for 12 substances have been developed for water, sediment, fish tissue, wildlife diet, and bird egg, and are available online on the Government of Canada website. This presentation will provide an update on the next series of FEQGs underway including lead, iron, strontium, and quinoline. Guidelines for lead and iron address bioavailability by incorporating multiple toxicity modifying factors using a multiple linear regression approach. Quinoline represents the first FEQG that includes soil and groundwater media, and will support risk management of contaminated sites. An overview of these draft FEQGs will be provided as well as other upcoming work in the FEQG program.

Managing non-toxic contaminants with non-toxic endpoints: Total phosphorus (PL)

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While the approaches to deriving water quality objectives for toxic substances are advanced and still advancing, there has been little progress for substances which are not only non-toxic, but are also required elements for aquatic productivity, such as total phosphorus. Phosphorus guideline derivation is compounded by the overriding influence of geology and watershed characteristics on phosphorus concentrations and the influence of lake characteristics and other elements, such as iron, on ecological responses to phosphorus enrichment. Various approaches to deriving phosphorus water quality objectives will be illustrated, including examples of generic, regional, and site-specific objectives based on numeric and proportional approaches. Adaptation of a generic PWQO for total phosphorus into a site-specific objective in support of an environmental assessment will illustrate the necessary balance between a) treatment requirements and

associated costs, b) protection of a sensitive receiver, and c) maintenance of future and downstream assimilative capacity. I will provide examples showing the role of ecology as a factor modifying ecological responses of cyanobacteria and nuisance filamentous algae to phosphorus enrichment, and the coupling of phosphorus and dissolved oxygen models for protection of sensitive fish species to illustrate the need to look in the right place when setting objectives.

Influence of water hardness and cation/anion mixture on chloride toxicity to aquatic life (PL)

Anthony Knafla[1]

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The Canadian Council of Ministers of the Environment (CCME) in 2011 released a revised Water Quality Guideline for chloride (120 $mg \cdot L^{-1}$ for chronic exposure), applicable to freshwater aquatic life receptors. The revision was based in part on toxicology data for sensitive species that have demonstrated effect concentrations lower than 100 mg $chloride \cdot L^{-1}$. As part of this work, CCME determined that water hardness and cation/anion mix may influence the toxicological response of some aquatic organisms, but further study is required. In order to further an understanding of these two factors on chloride toxicity, the Petroleum Technology Alliance Canada (PTAC) initiated laboratory toxicology work incorporating experts in the field at toxicology labs in Canada and the USA. An analysis of recently published literature along with commissioned work at three academic and commercial labs determined that hardness clearly influences chloride toxicity and this effect occurs in multiple sensitive and moderately-sensitive species. Based on this information and published data, a chloride guideline was developed that includes an adjustment for hardness. Analyses of recently published literature and commissioned supplementary toxicology work on mixtures of different anions/cations has demonstrated that there are multiple factors affecting the toxicological response of organisms and these factors are not consistent between species. However, in many cases, there are clear biologically plausible mechanisms explaining differences in toxic response. Furthermore, data analyses have discerned a difference in response for wild-collected species as a function of their source water of collection and a relatively consistent difference between laboratory and field water experiments (with laboratory tests tending to having greater sensitivity) in terms of toxicological response with increasing chloride concentration. This is of importance from a policy implementation point of view if guidelines are based on laboratory work, excluding field experiments (as per CCME methods), in that an additional layer of protection may be afforded by excluding field studies. Understanding the magnitude of difference between lab and field response can be of benefit during stakeholder communications, as demonstrated herein.

Current status and future directions for Canadian Environmental Quality Guidelines (PL)

Joanne Little[1], Doug Spry[2], Tamzin El-Fityani[2], Kathleen McTavish[2], Tim Fletcher[3], Monica Nowierski[3], Burton Andrew[4]

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The Canadian Council of Ministers of the Environment (CCME) is the primary interjurisdictional forum for development of Canadian environmental quality guidelines (CEQG). CEQGs for the Protection of Aquatic Life are currently developed under the guidance of the Guidelines Project Team of the Water Management Committee. This presentation will provide an overview of recently completed and current guidelines under development, including carbamazepine, nickel, zinc and manganese, and will report on the results of a recent project, the Alternate Water Quality Parameter Assessment, through which a consultant developed a framework for assessing guidelines from alternate jurisdictions for their applicability to Canadian waters.

Characterization of sediment chemistry, toxicity and ecological condition in wadeable streams of the Southeastern United States (PL)

Patrick Moran[1], Nile Kemble[1], Ian Waite[1], Barbara Mahler[1], Michael Meador[1], Pete Van Metre[1]

[1]US Geological Survey

As part of the US Geological Survey's (USGS) National Water-Quality Assessment (NAWQA) project, sediment samples were collected from streams representing an urban gradient in the southeastern United States (AL, GA, NC, SC, VA) in the summer of 2014. The study evaluated relations between sediment toxicity, sediment chemistry (metals, pesticides, organochlorines, and polycyclic aromatic hydrocarbons) and macroinvertebrate and fish communities at 75 sites. Sediment toxicity was evaluated by conducting whole-sediment laboratory toxicity testing with the amphipod *Hyalella azteca* (28-day exposure), the midge *Chironomus dilutus* (10-day exposure), and the mussel *Lampsilis siliquoidea* (fatmucket; 28-day exposure) in accordance with ASTM and US Environmental Protection Agency (EPA) methods. Although concentrations of metals and a few organochlorines and current use pesticides in sediments infrequently exceeded single chemical benchmarks (i.e., Probable Effect Concentrations (PEC) and Likely Effect Benchmarks (LEB) for pesticides), roughly one half of the sediment samples were identified as toxic in the lab; with at least one test species-endpoint significantly reduced relative to control. Evaluation of concentrations relative to their benchmarks, both individually and as summed PEC or LEB quotients, was explored on a carbon-normalized and a dry-weight basis; with carbon-normalizing generally giving better predictions of toxicity. The amphipod *H. azteca* was the most sensitive of the laboratory tested species. While a general tendency for lower scores with more urbanization was observed, toxicity test endpoints were more consistent in their response to urbanization than were the invertebrate and fish metrics from field collections. This study highlights the need to be explicit in selection of ecological endpoints and that

current single chemical sediment benchmarks may underestimate toxicity of sediment mixtures in the field.

Site-specific acute toxicity of chloride associated with effluent discharge to northern surface water (PL)

Breda Muldoon[1], Gary Lawrence[1], Jordana Van Geest[1]

[1]Golder Associates

Con Mine (the Mine), owned and operated by Miramar Northern Mining Ltd. (MNML), closed in 2003 and is currently undergoing decommissioning, demolition, and clean-up. A new water treatment plant was commissioned in 2015 and markedly improved the quality of treated effluent. However, chloride concentrations in the treated effluent may still be of interest for effluent management and could fluctuate in future, based on site water quality and annual treatment. Monitoring of acute toxicity under the existing effluent monitoring program provides confidence that acute toxicity is not expected to occur under existing conditions, but the degree to which additional chloride concentrations could be tolerated was unknown. Accordingly, as part of an effort to develop a chloride limit for the Mine, a laboratory toxicity study was undertaken to identify acute toxicity thresholds of chloride under current and future predicted site-relevant conditions. Several scenarios reflecting current and predicted ionic composition of treated effluent and receiving water were reviewed. Three mixtures were selected that represented combinations of variable chloride and sulphate proportions to total dissolved solids (TDS); these constituents were the dominant potential toxicants, and were also the ions that varied most among potential future exposure scenarios. Rainbow trout (*Oncorhynchus mykiss*) and *Daphnia magna* were exposed for 96 and 48 hours (respectively) to site water amended with chloride and relevant ions to reflect the three mixture scenarios at target chloride concentrations of 1,500, 2,500, 3,500, and 5,000 $mg \cdot L^{-1}$. Sulphate was capped at a maximum concentration of approximately 1,500 $mg \cdot L^{-1}$ in recognition of solubility constraints and plausible ranges of discharge conditions. The 48-hour LC50 thresholds for *D. magna* toxicity to chloride under site-relevant exposure conditions ranged between 2,900 and 3,080 $mg \cdot L^{-1}$. Rainbow trout were less sensitive to chloride toxicity, with a 96-hour LC50 threshold of 4,975 to greater than 5,000 $mg \cdot L^{-1}$. Both of these results were consistent with literature compilations for these species. Furthermore, sulphate concentrations did not contribute meaningfully to the variance in observed toxicity to the most sensitive species (*D. magna*), indicating that potential for acute toxicity will not be sensitive to the specifics of ionic composition within site-relevant mixtures. The results for both species are broadly consistent with literature studies. Given the results of the present study, there is reasonable confidence that chloride concentrations less than or equal to 2,500 $mg \cdot L^{-1}$ will not result in acute toxicity to *D. magna* or rainbow trout under current or predicted future conditions at Con Mine. It is possible that the threshold for chloride toxicity to daphnids is somewhat greater than 2,500 $mg \cdot L^{-1}$, but the NOEC from these studies was adopted as a basis for an acute threshold. Additional analysis would be required to evaluate the concentration-response relationship between 2,500 and 3,500 $mg \cdot L^{-1}$.

Field testing and monitoring of soil invertebrates: recent developments (PL)

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In the past, most work in soil ecotoxicology has been done in the laboratory. However, recently the focus seems to shift towards higher tiers, i.e., to the field level, at least in Europe. This trend is best visible for chemical risk assessment (e.g., for plant protection products (PPPs)), as exemplified in a Scientific Opinion published recently by the European Food and Safety Authority (EFSA). This document states that soil risk assessment should be based on the ecosystem service approach, meaning that services such as nutrient cycling or soil structure maintenance have to be protected. In this context long-term monitoring could be an important tool for recovery evaluation and risk mitigation measures. Especially for the re-registration of existing products, where concern exists for in-soil organisms, monitoring data can provide useful information about the effects on the actual community in the field situation. However, almost no methodological recommendations are provided on how to assess such data. One exception is the hint that suitable baseline scenarios should be defined (i.e., information on what "healthy" soil organism communities should look like in terms of diversity and abundance could be used in order to evaluate effects). Similar ideas have been proposed in the context of the authorization of veterinary medical products (VMPs); i.e., those with PBT (persistent, bio-accumulative, toxic) properties. The implementation of such an approach, however, is hindered by the lack of baseline data too (in this case, mainly of dung organisms). In parallel, a discussion started in Europe on how the biological quality (i.e., maintenance of the structural (biodiversity) and functional (services provided by the organisms) properties of soils could be protected most efficiently. Unfortunately, there is no Soil Protection Act in place in the European Union, but some countries (including Germany, France and The Netherlands) became active, setting-up their own biological monitoring programs. This contribution will focus on whether we have the appropriate (e.g., scientifically sound, robust, standardized) methods to perform such biological monitoring programs in the context of chemical risk assessment and the evaluation of potentially contaminated land. In particular, are there enough baseline or reference data available in order to identify those soils which are impacted with regard to their biological structure and functions? In this context the use of soil biodiversity databases can provide examples of this approach when evaluating the biological soil quality of Bavarian grassland and crop sites, focusing on the diversity and abundance of earthworms and enchytraeids communities.

Evaluation of statistical methods for comparing two groups with single and multiple detection limits (PL)

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Analytical laboratory data collected for environmental monitoring and assessment are frequently left-censored (i.e., contain values reported below one more laboratory reporting limits [LRLs]). These data present challenges for conducting statistical comparisons

between groups because the exact true value of the censored observation is unknown. Our study uses a simulated approach to evaluate the performance of a variety of substitution, non-parametric, and maximum likelihood estimation (MLE) methods designed to test for differences between two groups containing censored data when sample sizes are small. Multiple censoring scenarios were assessed (single LRL, multiple LRLs, and censoring at the bottom of the distribution or randomly in the distribution). Consistent with previous studies, we find that the best approach often varied with sample size, percent censoring, and the type of censoring. MLE generally minimized the average probabilities of Type I and Type II error. The exception was for high percentages of censoring at the bottom of the distribution when sample sizes are small. In these situations (typical for environmental monitoring and assessment studies), non-parametric methods are best. Our study is the first to compare all the common approaches for two group comparisons with multiple LRLs. The results of this study can be used to establish best practices for conducting statistical comparisons that contain censored data.

Determining permissible discharges when water quality guidelines vary over time: or, time variable WQG: What's the number? (PL)

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

[1]*Windward Environmental*

Several approaches for deriving water quality guidelines (WQGs) use functional relationships to account for water quality effects on bioavailability. Examples include hardness-equation based guidelines for metals and other substances, temperature and pH relationships for ammonia guidelines, and the biotic ligand model (BLM) which is a mechanistic modeling framework for predicting metal bioavailability and toxicity. Since water quality typically changes over time, it follows that if WQGs are determined by water quality characteristics then the guideline value will also change over time. This issue has become especially important for WQGs that have been derived using the BLM since as many as ten individual water quality parameters are used to estimate metal bioavailability. Seasonal variability in water quality can result in significant changes in WQG values. While environmental managers and decision makers may understand why WQGs change over time, they still need to make use of this information in setting discharge limits. In this context, time-variable guidelines may seem like continually moving goal posts without a clear answer as to what discharge would be allowable and protective of aquatic life. In effect, they may be wondering “What’s the number?” We have developed a fixed monitoring benchmark (FMB) approach that can be used to simplify time-variable guideline information into a single number that can be used to determine allowable discharges that will be in compliance with the time-variable guideline. The approach is based on a probabilistic analysis of water quality characteristics conditions, including the time-variable guideline values, the pollutant concentrations, and the allowable exceedance frequency. This approach is straightforward and offers a method to effectively make use of time variable guidelines in regulatory contexts where a single value is consistent with past practices and established methods. We will discuss the FMB methodology in the context of

BLM-based guidelines for copper, but the approach can easily be adapted to any guideline that would vary over time.

A global perspective on determining bioavailability-based Water Quality Guidelines for nickel (PL)

Chris Schlekat[1], Jenny Stauber[2], Adam Peters[3], Graham Merrington[3], Adam Ryan[4], Robert Santore[4], Emily Garman[1]

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The toxicity of metals like nickel to aquatic organisms is influenced by water chemistry. This influence creates situations where regulatory thresholds such as Water Quality Guidelines can be both under- and over-protective, depending on how local water chemistry compares with that used in tests to determine the threshold. Nickel toxicity is influenced by pH, hardness (Ca and Mg) and dissolved organic carbon (DOC). Nickel is classified as a Priority Substance in the European Union (EU), meaning that all 27 EU Member States have to comply with a single Environmental Quality Standard (EQS) under the EU Water Framework Directive (WFD). The Ni EQS under the WFD is bioavailability-based. It was determined by normalizing the extensive chronic ecotoxicity dataset (n=31 species) to a common combination of water chemistry parameters using nickel bioavailability models that were developed for four species (two invertebrates, one algae, and one fish). A reference EQS was calculated by determining the HC5 from a Species Sensitivity Distribution (SSD) of EC_{10} values that were normalized using the pH, hardness, and DOC from the most sensitive ecoregion occurring in Europe, which for Ni is the Alpine region of Austria (pH =8.2 , hardness = 120 mg $CaCO3 \cdot L^{-1}$, DOC = 2 mg·L). The resulting HC5 for conditions of high bioavailability is 4 µg $Ni \cdot L^{-1}$. Compliance with this bioavailability-based EQS is determined through a tiered approach, where measured ambient dissolved Ni concentrations are first compared with the reference EQS of 4 µg $Ni \cdot L^{-1}$. If the measured concentrations are greater than the reference EQS, at the second tier site-specific water chemistry is used to calculate the bioavailability of nickel at the site and this is compared to the EQS. If measured Ni concentrations are less than the bioavailable HC5, then compliance with the EQS is achieved. Applying the general approach established for the EU Ni EQS in other jurisdictions may result in different threshold concentrations. The reasons for variability are largely policy related: While the chemical and biological principles governing bioavailability are constant, other factors (e.g., protection goals, guidance for determining threshold concentrations, choice of bioavailability models, etc.) vary considerably among jurisdictions. We will present the processes for determining bioavailability-based thresholds for Ni in two jurisdictions, Australia and the USA, and compare these approaches with the current Ni EQS under the EU WFD. These jurisdictions differ in terms of ecotoxicity data selection processes, protection goals, bioavailability models used, and distributions of pH, hardness, and DOC. This comparison demonstrates that bioavailability principles can be incorporated into threshold concentrations for different jurisdictions, and this experience can be applied to other metals for which similar ecotoxicity data, bioavailability information, and models are available.

Toxicity tests in support of the derivation of non-radiological acceptance criteria for ruthenium and rhodium in water and soil (PL)

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A recent literature review indicated that available data for platinum group elements (specifically for rhodium (Rh) and ruthenium (Ru)) are insufficient to derive water and soil quality guidelines according to the methodology specified by the Canadian Council of Ministers of Environment (CCME). Chronic toxicity tests with Ru and Rh were therefore undertaken to support the derivation of environmental quality guidelines. Multi-concentration toxicity tests were conducted with spiked water or spiked soil, measuring lethal and sublethal responses of exposed organisms. Test organisms included: a salmonid fish (rainbow trout; *Oncorhynchus mykiss*), a non-salmonid fish (fathead minnow; *Pimephales promelas*), an epibenthic amphipod (*Hyalella azteca*), a cladoceran (*Ceriodaphnia dubia*), a monocotyledon terrestrial plant (barley), a dicotyledon terrestrial plant (alfalfa) and an earthworm species (*Eisenia andrei*). Exposure concentrations were confirmed analytically at the start and end of the tests and also at points during the tests. The frequency of sampling was streamlined and depended upon the test duration (i.e., tests ranged from 7 to 56 days) and the results of a preliminary aging and stability study. This presentation discusses the toxicity test results, sensitivity rankings and lessons learned. This work was funded by the Nuclear Waste Management Organization (NWMO) as part of their safety assessment activities associated with Adaptive Phased Management, Canada's plan for the long-term management of Canada's used nuclear fuel.

SSDCA: An R package and web page to calculate species sensitivity distributions (PL)

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Species sensitivity distributions (SSDs) allow water quality guidelines to be estimated with confidence intervals. SSDCA is an open source R package to fit SSDs using maximum likelihood (ML). Unlike non-linear least squares which relies on ad-hoc fitting procedures, ML allows an information-theoretic based approach (Akaike's Information Criterion corrected for small sample size) to model selection as well as model averaging. Confidence intervals are produced by bootstrapping. By default the log-normal, log-logistic, gompertz, log-gumbel, gamma, and weibull distributions are fitted to the data. A web page is available to allow non-R users to fit SSDs to their data using SSDCA. The web page allows users to save the resultant plots and tables and provides the R code required to rerun the analysis locally. The SSDCA package is available from https://github.com/bcgov/ssdca.

A statistical evaluation of the safety factor and species sensitivity distribution approaches to deriving environmental quality guidelines (PL)

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The species sensitivity distribution (SSD) approach to estimating water quality guidelines (WQGs) is the preferred method in all jurisdictions reviewed (Australia, Canada, New Zealand, Organisation for Economic Co-operation and Development members, South Africa, United States) and is one of the recommended methods for European Commission members for 33 priority and priority-hazardous substances. In the event that jurisdiction-specific criteria for data quality, quantity, and taxonomic representation are not met, all of these jurisdictions endorse the use of additional safety factors (SFs) applied to either the SSD-based WQG or the lowest suitable toxicity test endpoint. In Canada, the British Columbia Ministry of Environment endorses this latter approach as the preferred approach in the belief that so-derived WQGs are more protective than SSD-based WQGs. The level of protection afforded by the latter SF approach was evaluated by statistically sampling minima from random samples of the following distributions: normal, Gumbel, logistic, and Weibull, using a range of coefficients of variation (CVs) and applying the SFs of 2 or 10 used in British Columbia. The simulations indicate that the potentially affected fraction of species (PAF) can be as high as 20%, or approach 0%. The PAF varies with sample size and CV. Because CVs can vary systematically with mode of toxic action, the PAF using SF-based WQGs can also vary systematically with analyte class. The varying levels of protection afforded by SF-based WQGs are generally inconsistent with the common water quality management goal that allows for a small degree of change under long-term exposure. The findings suggest that further efforts be made to develop high-quality WQGs that support informed decision-making and are consistent with the environmental management goal instead of using SFs in the hope of achieving an acceptable, but unknown, degree of environmental protection.

Derivation of site-specific water quality objectives for uranium in northern Canada (PO)

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A proposed gold mine in northern Canada is located in an area with naturally elevated concentrations of metals and metalloids in groundwater and surface water. In particular, baseline uranium concentrations in surface water within the project area are naturally above the Canadian Council of Ministers of Environment (CCME) surface water quality guideline (WQG) of 15 $\mu g \cdot L^{-1}$ for the protection of aquatic life. In cases where background concentrations exceed WQGs, water quality objectives (WQOs) for these parameters may be developed based on site-specific conditions. Following the background concentration approach, a site-specific WQO of 86 $\mu g \cdot L^{-1}$ for uranium was calculated for the primary receiving environment of the project. A suite of toxicity tests that included the calculation of acute and chronic endpoints was performed to support the derived uranium WQO for the

project. The toxicity tests evaluated the effects of uranium-spiked site water on the survival and reproduction of the most sensitive aquatic organism identified in toxicological literature, the water flea *Ceriodaphnia dubia*. In addition, algae (*Pseudokirchneriella subcapitata), C. dubia* and rainbow trout (*Oncorhynchus mykiss*) were exposed to metal mixtures containing uranium to evaluate the potential for additive or synergistic effects on uranium toxicity. The results suggest uranium concentrations eliciting toxic responses were significantly higher than the proposed WQO for uranium and well above the generic uranium water quality guideline of 15 $\mu g \cdot L^{-1}$. The project site waters have naturally ameliorating properties that limit the toxicity of uranium to sensitive aquatic receptors.

Science and Decision Making

Estimating background concentrations of inorganics in surface water to inform ecological risk assessment under the Canadian Environmental Protection Act, 1999 (PL)

Rachel Bouwhuis[1], Catherine Proulx[2], Bruce Kilgour[2], Jonathan Hill[1]

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Many inorganic substances occur naturally in the environment and flow through environmental reservoirs (e.g., sediment, water, soil, air, etc.) via geochemical and biogeochemical cycling as well as originating from anthropogenic sources. It is therefore of interest to consider background concentrations when conducting ecological risk assessments of naturally occurring inorganic substances. Comparison to background concentrations can help identify elevated environmental concentrations resulting from anthropogenic activities. Background concentrations can also improve the realism of predictive exposure scenarios in the absence of measured concentrations in exposure areas of interest. This presentation describes a statistical methodology for quantifying background concentration ranges of various metals, metalloids and non-metals in surface water, which is proposed for use in ecological risk assessments conducted by Environment and Climate Change Canada as a part of the Chemicals Management Plan (CMP). Data from approximately 40,500 samples collected from over 1,200 monitoring locations, covering ten ecozones over the period of 2005-2015, were collected from multiple federal and joint federal-provincial/territorial monitoring programs. Samples were sorted as reference (i.e., background) or non-reference (i.e., contaminated) based on the underlying natural relationship between conductivity and alkalinity, as described in Proulx et al. 2018. Following this, normal concentration ranges in surface water were estimated for inorganic moieties of interest under the CMP to describe background ranges for Canada's ecozones. The estimated upper limits of the normal ranges for each ecozone were then compared to current Canadian Environmental Quality Guidelines developed by the Canadian Council of Ministers of the Environment. The applications of normal ranges or background concentrations in ecological risk assessments are discussed.

Five phases of aquatic monitoring at the Giant Mine: Are we making better decisions when science is used? (PL)

Elaine Irving[1], Hilary Machtans[1], Tamara Darwish[1], Peter Chapman[2], Katherine Harris[3]

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Giant Mine is one of Canada's largest former gold mines, situated within the city limits of Yellowknife, Northwest Territories, along the shores of Great Slave Lake. The mine operated between 1948 and 2004, largely under a very different regulatory environment than we have in place today. The owners abandoned the site in the 1999, leaving the challenges of how to officially close and remediate the site up to the Canadian government.

One particular challenge has been the remediation of Baker Creek, a creek historically influenced by the mine and more recently exposed to treated mine effluent for a number of years. Giant Mine has moved through five phases of aquatic environmental effects monitoring, culminating in an Investigation of Cause (IOC) Study. The main objective of the IOC study was to determine the cause(s) of observed effects in benthos and fish in Baker Creek. Four possible hypotheses to explain the observed effects were proposed, and then evaluated using a multiple lines of evidence approach. Causal criteria were reviewed, and support for and against each hypothesis was evaluated in a retrospective Weight of Evidence (WOE) synthesis, to determine conclusions with respect to causation. Overall, the study supported one of the hypotheses, and identified other contributing and/or confounding hypotheses in the determination of cause. Surprisingly, adverse effects documented in recent years were relatively minor to those documented historically where fish and benthos were virtually eliminated. The Giant Mine is actively planning remediation efforts that will improve treated effluent quality and remove a substantial volume of sediments from locations within Baker Creek, as well as the eventual relocation of the treated effluent discharge from Baker Creek to Yellowknife Bay in upcoming years. The findings from the IOC study was one of the main drivers in the decision to remove sediments from locations within Baker Creek, thus, providing scientific input to the decision making process. This case study provides an example where the outcome of aquatic monitoring studies, in addition to community input, influenced the direction of mine remediation.

Dissolved oxygen relationships of under-ice water column and pore water habitat: Implications for environmental guidelines (PL)

Eric Luiker[1], Joseph Culp[1] Nancy Glozier, Daryl Halliwell, Marianne Medina.

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Substantial reductions in dissolved oxygen concentration in freshwaters can negatively affect aquatic biota. Thus, existing regulatory criteria are designed to avoid environmental conditions that cause acute lethality, thereby reducing the likelihood of biological impairment. In North America, dissolved oxygen (DO) guidelines for protecting aquatic life assume that pore water and water column DO are correlated, with pore water values expected to be on average ≤3 $mg \cdot L^{-1}$ below water column values. Our study assessed the validity of this assumption during the winter period of ice cover in a large northern river ecosystem (Wapiti River, Alberta, Canada). We investigated the relationship between water column and pore water DO concentrations and examined whether this relationship was affected by industrial and municipal effluents. Our results indicate that under-ice DO concentrations of river pore water could not be accurately predicted from water column DO alone. Risk factors that may increase the potential for pore water DO to be more than 3 $mg \cdot L^{-1}$ lower than water column values include the input of oxygen-poor groundwater, infilling of the streambed with small inorganic and organic particles, water exchange rates between the water column and the streambed, and effluent discharges that raise nutrient concentrations and biochemical oxygen demand. Given that low pore water DO was evident

even in undeveloped reference sites, future work must establish the ecological relevance of chronic exposure to low pore water DO and its impact on river biota.

pH Stabilization during trout acute lethality testing of pulp and paper effluent: A government/industry success story (PL)

Rick Scroggins[1], Leana Van der Vliet[1], Lisa Taylor[2], Lesley Novak[2], Brian O'Connor[3], Sharon Gibbons[3]

[1]Environment and Climate Change Canada [2]Aquatox Testing and Consulting, [3]FP Innovations

The acute lethality test method using rainbow trout (EPS 1/RM/13) is commonly used to determine if an effluent is deleterious under s. 36(3) of the Fisheries Act and regulations (e.g., Pulp and Paper Effluent Regulations). The standard rainbow trout (*Oncorhynchus mykiss*) reference method requires continuous aeration of the effluent but does not include maintaining the initial pH as a condition of the test. In some cases, aeration of pulp and paper effluents during acute lethality testing can cause the pH to rise from the equilibration of carbon dioxide (CO2) partial pressure in the effluent with that in the atmosphere. The loss of CO2 due to aeration causes a shift in the carbonate buffering system of an effluent, and this leads to the upward shift of pH in the test sample. In these effluent samples, the CO2 content may be artificially elevated as a result of high biological activity. Any change in pulp and paper effluent pH during an acute lethality test may affect mortality if there are significant concentrations of pH-sensitive substances in the sample. Ammonia, which could be of concern in pulp and paper effluent, would be one such example of a pH-sensitive substance. As this issue was of high priority to member companies of the Forest Products Association of Canada (FPAC), a six-year effort was launched in late 2012 to develop a pH stabilization procedure that could be used as an add-on technique with the standard rainbow trout test during the acute lethality testing of pulp and paper mill effluent. Working with the FPAC Environment Committee, FP Innovations was mandated to demonstrate and validate the pH stabilization procedure and science staff from the ECCC's Method Development and Applications Section (MDAU) agreed to act as project advisers. The objectives of the three phases of the method validation project were to verify that: 1) pulp mill effluent could be pH stabilized during acute lethality testing; 2) the acute toxicity of effluent spiked with ammonia could be controlled through the addition of CO2; and 3) the technique was reproducible across multiple laboratories testing the same ammonia-spiked effluent samples from four representative mill process types. The FP Innovation toxicology laboratory demonstrated that a pH controller technique could successfully be applied to pulp mill effluents during acute lethality testing and effectively controlled the conversion of ammonia to the more toxic un-ionized form. For the inter-laboratory testing phase, five private sector laboratories located across Canada participated. During eight testing rounds involving ammonia-spiked effluents reflecting different pulping processes, each laboratory gained experience and the test methodology was improved until all participating laboratories were able to consistently meet the performance criteria established for the inter-lab validation study. The standardized method writing was prepared by Aquatox Testing and Consulting Inc. under contract to ECCC and was publicly distributed in June 2018 as Report STB 1/RM/59: Procedure for pH stabilization during the testing of acute

lethality of pulp and paper effluent to rainbow trout. During the presentation, we will cover some of the challenges faced during the inter-laboratory testing phase and summarize the key methodology elements including the four mandatory criteria to be met before using the procedure.

Development of a new standardized test using a marine invertebrate (PL)

Leana Van der Vliet[1], Ryan Hennessy[1], Paula Jackman[1], Craig Buday[1], Lesley Novak[2], Shawna Kirkpatrick[2], Paula Antunes[2], Rick Scroggins[1]

[1]Environment and Climate Change Canada, [2]AquaTox Inc.

Rainbow trout (*Oncorhynchus mykiss*) and *Daphnia magna* have been used to measure acute lethality for decades, but in the context of Canadian effluent regulations, there is currently no salt water equivalent to these freshwater test species. During the 10 year review of the Metal Mining Effluent Regulations, the importance of this gap was recognized, as there are some mines in the far North which are expected to discharge a saline effluent (mine pit waters, considered a treated effluent) into marine waters. To address this gap, the Methods Development and Applications Unit of Environment and Climate Change Canada began developing standardized test methods for a marine fish (the three-spine stickleback, *Gasterosteus aculeatus*) and a marine invertebrate (the copepod *Acartia tonsa*). Like *D. magna*, the *A. tonsa* test method under development is a 48-hour acute lethality test where lethality is expressed as a combination of egg hatching success and mobility of young. Challenges faced in developing the standardized test method for *A. tonsa* included a lack of Canadian experience in culturing and testing with this species and short timelines for method development. We faced these challenges by building collaborations and running some aspects of method development in parallel. Building on experience with *A. tonsa* in Europe, three toxicology labs (both government and private sector) began culturing *A. tonsa* and the feeder algae (*Rhodomonas salina*), and each lab addressed a different piece of method development. After about 18 months of experience, we began the inter-laboratory study, which involved six labs across Canada. This presentation will review some of the key aspects of method development as well as the results of the inter-laboratory study, and will also describe the final steps in developing this new standardized test method.

Expanded approaches for prioritization and assessment of flame retardants under the Canadian Environmental Protection Act, 1999 (PO)

Barbara Elliott[1], John Pasternak[1], Jenny Marie Ferone[1], Mark Bonnell[1]

[1]Environment and Climate Change Canada

Approaches for prioritizing and assessing chemicals under the Canadian Environmental Protection Act, 1999 (CEPA), evolve as the science supporting chemical risk assessment advances. Under CEPA, Environment and Climate Change Canada and Health Canada assess and manage, where appropriate, risks of chemical substances to the environment and to human health. The Chemicals Management Plan (CMP) is a Government of Canada initiative that addresses approximately 4300 substances identified as priorities for assessment under

CEPA. Many existing substances used as flame retardants have been subject to assessment under CEPA. To date, approximately 40 assessments have been completed or are underway on substances with flame retardant usage (including single substance assessments and chemical group/class assessments). Substances prioritized for assessment in the first two phases of CMP (2006-2016) have largely been determined based on categorization criteria for persistence (P) or bioaccumulation (B) and inherent toxicity (iT) to human or non-human organisms. In addition, substances may have been prioritized based on other sources of ecological concern (e.g., new substance assessments), the greatest potential for exposure to humans, or health effects of concern based on classifications by other national or international agencies for carcinogenicity, genotoxicity, developmental toxicity or reproductive toxicity. The categorization exercise for ecological concerns is referred to as the PBiT approach. To date, with the focus on PBiT prioritization, many flame retardant substances have been found to present a risk to the environment and meet criteria for persistence and bioaccumulation as defined in the Persistence and Bioaccumulation Regulations under CEPA. For the third phase of the CMP, expanded consideration involving hazard and exposure profiling was implemented through the ecological risk classification of organic substances (ERC). This approach has prioritized six organophosphate substances and one brominated substance requiring greater scrutiny through detailed assessments. The ERC process and approach are presented, as well as the overall ERC outcomes for the flame retardants. In addition to expanded profiling for prioritization, there is consideration given to new approach methodologies for hazard characterization.

Deriving predicted no-effect concentrations for ecological risk assessments (ERAs) conducted under the Canadian Environmental Protection Act using a new assessment factor (AF) approach (PO)

Alexander Okonski[1], Drew MacDonald[1], Tariq Francis[1], Howard Swerdfeger[2], Lesley Lander[1]

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Quantitative risk assessments include the derivation of predicted no-effect concentrations (PNECs), which represent the concentration of a substance in an environmental medium that is unlikely to cause adverse effects to populations in that medium, typically following chronic or long-term exposure. The PNEC is compared with a predicted environmental concentration (PEC) to calculate a risk quotient (RQ). For small toxicity datasets (e.g., data from less than seven species) that do not meet the requirements for a species sensitivity distribution, an assessment factor (AF) approach is used, where a PNEC is calculated by dividing the critical toxicity value (CTV) by an AF. The CTV is typically the lowest concentration of a substance, from the acceptable available data, at which an adverse effect was observed in a given environmental medium. AF approaches are not new, and many—if not all—regulatory jurisdictions have been using such approaches in environmental risk assessments for years. However, existing methods to derive AFs are prone to inconsistent application and have not incorporated advances in risk assessment methods, such as using analogue and read-across approaches, Quantitative Structure-Activity Relationship (QSAR) modeling, and consideration of specific modes of action. Consequently, the Ecological Assessment Division of Environment and Climate Change

Canada has developed an AF approach that accommodates alternative data described above and strives for increased consistency in application. This new AF approach will be presented.

Developments in Bioaccumulation Science

A Framework for the risk assessment of bioaccumulative substances at non-high risk sites in British Columbia (PL)

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Environmental contaminants can accumulate in human and ecological receptors through non-dietary uptake from site media as well as by ingestion of food items in which the contaminants have bioconcentrated. Some contaminants also biomagnify (i.e., tissue concentrations increase at higher trophic levels) as the contaminants are passed through the food chain. The degree of bioaccumulation and biomagnification for a given chemical is determined by the characteristics of each food web as well as by the characteristics of the chemical. Ecological benchmarks are most often protective of risks associated with direct exposure and are not necessarily protective of bioaccumulation risks. Furthermore, identification of bioaccumulative substances is not consistently outlined in guidance. Therefore, a risk assessment framework for the evaluation of bioaccumulation/biomagnification substances is needed to meet the expectations of the British Columbia Ministry of Environment and Climate Change Strategy (BC ENV) and to assist in the Contaminated Sites Approved Professional (CSAP) submissions on non-high-risk sites. This research project included meetings with BC ENV to frame working definitions of bioaccumulation processes and an approach for evaluating bioaccumulation. SLR also reviewed literature from provincial, federal, and other jurisdictional sources for definitions and approaches to evaluating these substances. This presentation will outline the current working definitions to be used in risk assessment, a summary of the literature reviewed, and a proposed approach to evaluating bioaccumulative substances.

Maternal transfer of selenium and the relative sensitivity of embryonic and juvenile birds (PL)

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[1]*Golder Associates*

Maternal transfer of dietary selenium into the eggs of birds can result in embryotoxicity and teratogenicity. These are widely recognized as among the most sensitive ecological endpoints for selenium toxicity, and have been incorporated into regulatory criteria in some jurisdictions. Laboratory studies have also shown direct effects of dietary selenium on juvenile birds, but juvenile effects have not previously been considered in setting regulatory values. We evaluated the relative sensitivity of embryonic and juvenile birds using toxicity data for mallards. To enable direct comparison between life stages, embryotoxicity data were converted from units of egg selenium to dietary selenium concentrations using laboratory-based trophic transfer factors and field-based regression equations to characterize maternal transfer. Our analysis indicates that mallard juveniles may be as

sensitive as embryos, or more sensitive when maternal transfer is relatively low. Lower maternal transfer occurs when dietary selenium concentrations are higher, suggesting that juveniles would become the more sensitive life stage as selenium exposure increases. Our analysis suggests that selenium regulatory values based on reproductive toxicity data may not be protective of juveniles in all cases.

Effects of selenium speciation on bioaccumulation downstream of a biological water treatment plant (PL)

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Biological treatment of selenium acts via microbial transformation of aqueous selenate (VI) to reduced species such as selenite (IV) and organoselenides (II). This process lowers aqueous total selenium concentrations, but can alter the bioavailability of the remaining selenium. We found increases in selenium bioaccumulation downstream of an active water treatment plant, despite reductions in total aqueous selenium concentrations. We combined laboratory algal uptake tests, field measurements of bioaccumulation, and selenium speciation analysis of treated effluent and receiving waters to develop a model of selenium bioaccumulation that explicitly accounts for speciation. We quantitatively attributed the observed increase in bioaccumulation to reduced species, including selenite, dimethylselenoxide, and methylseleninic acid. We then applied this model to predict how bioaccumulation would be affected by a post-treatment oxidation step that returns effluent speciation to a selenate-dominated condition.

Development and application of an *in vivo* test for estimating biotransformation rate constants and bioconcentration factors of hydrophobic organic chemicals in fish (PL)

Marianna DiMauro[1,2], Frank Gobas[2], Christopher Kennedy[2]

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Bioconcentration factors (BCFs) are amongst the most common metrics used by regulatory agencies to assess the bioaccumulation of chemicals in fish. However, due to various constraints of BCF testing—including high costs, effort, and animal use—there there are limited empirical BCF data. In addition, despite evidence that biotransformation rates of hydrophobic organic chemicals (HOCs) can impact the bioaccumulation potential of a chemical, no *in vivo* methods exist to measure these rates and current estimation techniques such as *in vitro* assays and extrapolation modelling require evaluation. In this study, we developed and investigated a method of measuring *in vivo* biotransformation rate constants of HOCs using an aqueous bioconcentration test based on guidelines outlined by the OECD test No. 305 aqueous test for fish. Furthermore, the methodology in this study is designed to reduce costs, animal use, and level of effort as compared with existing OECD 305 test guidelines. The test chemicals methoxychlor, pyrene, cyclohexyl salicylate and 4-n-

nonylphenol were chosen based on their varying rates of biotransformation and hydrophobicity (Log K_{OW} 4.68 to 5.76). Four BCF tests were conducted on rainbow trout (*Oncorhynchus mykiss*) with a 7-day uptake phase via a passive dosing technique that allows the constant partitioning of the dissolved portion of hydrophobic chemicals into water and eliminates the need to replenish chemicals during the exposure. Fish were collected during the 14-day depuration phase and carcasses were extracted and analyzed by GCMS for chemical concentrations. During the uptake phase, fish were simultaneously exposed to non-biotransformed reference chemicals. The reference chemicals were chosen to encompass the range of hydrophobicity of the test chemicals. Depuration rate constants (kT) of test chemicals were compared to those of the reference chemicals to obtain whole organism *in vivo* biotransformation rate constants (kM) and corresponding BCFs for each test chemical. The measured biotransformation rate constants and BCFs will be compared to existing estimates and used to evaluate *in vitro* testing methods and *in vivo* to *in vitro* extrapolation modelling techniques.

Trophic magnification of perfluorinated compounds within a terrestrial food-web of an avian top predator, the Cooper's hawk (*Accipiter Cooperii*) (PL)

Katharine Fremlin[1], John Elliott[2], Ken Droulliard[3], Frank Gobas[1], David Green[1]

[1]Simon Fraser University, [2]Environment and Climate Change Canada, [3]University of Windsor

Several types of legacy persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), dichlorodiphenyldichloroethylene (DDE), and emerging POPs like perfluorinated compounds (PFCs) are released from multiple sources into the ambient environment and are known to negatively impact endocrine and physiological functions within exposed wildlife. Protocols to assess bioaccumulation of these persistent chemicals within terrestrial systems are far less developed than those within aquatic systems. Presently, regulatory agencies in Canada, the USA, and the EU primarily use bioaccumulation information for fish to assess the bioaccumulation potential of chemicals. However, recent studies have shown that some chemicals that are not bioaccumulative in aquatic food-webs do biomagnify in terrestrial food-webs. To better understand the bioaccumulation behaviour of chemicals in terrestrial systems, we assessed the biomagnification of POPs and PFCs in a terrestrial food-web that included an apex avian predator, the Cooper's hawk. Over 100 samples were collected from various trophic levels of the food-web including hawk eggs, songbirds, invertebrates, and berries. We estimated the trophic position of each organism using stable isotope analysis of δ13C and δ15N signatures of the hawks, songbirds, invertebrates, and berries. We analyzed 18 PFCs within all of the biota samples collected. We expressed all sample concentrations in terms of protein equivalent concentrations to account for variability in the fractions of protein and non-lipid organic matter measured in each sample. We used censored regression by maximum likelihood estimation to assess the relationship between the natural logarithm of each PFC protein equivalent concentration and trophic position. Trophic magnification factors (TMFs) were determined as the antilog of the regression slope. We determined TMFs for PFCs that were detected at appreciable levels in all the biota samples (i.e., had 50% or greater detection frequency) and compared these terrestrial TMFs to those

observed in aquatic systems. TMFs of PFCs ranged from 10.8 (5.7 SE) to 55.1 (49.5 SE), indicating that PFCs are biomagnifying in this terrestrial system. Overall, the terrestrial TMF values for PFCs were considerably higher than TMF values found in aquatic systems.

Ecosystem-level characterization of selenium exposure and trophic transfer in a representative boreal lake food web (PL)

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Human activities such as coal and metal mining have increased the release of selenium (Se) to aquatic environments, but information about the trophic transfer dynamics of Se in Canadian boreal lake systems is lacking. The goal of the present study was to characterize the uptake and trophic transfer of Se after addition to *in situ* enclosures located in Lake 114 at the International Institute for Sustainable Development – Experimental Lakes Area (IISD-ELA) in northwestern Ontario. In June 2017, Se was added to 3000 L littoral enclosures as sodium selenite to reach nominal concentrations of 1 $\mu g \cdot L^{-1}$ (actual concentration (mean ± SD) = 1.01 ± 0.15 $\mu g \cdot L^{-1}$) and 10 $\mu g \cdot L^{-1}$ (actual concentration = 9.58 ± 2.63 $\mu g \cdot L^{-1}$) in triplicates for 77 days , and three additional enclosures were controls with no Se added (background concentration = 0.28 ± 0.04 $\mu g \cdot L^{-1}$ after 77 days). Ecosystem-level characterization of exposure and trophic transfer were determined by measuring total Se (TSe) concentrations in water, sediment, periphyton, benthic macroinvertebrates, and female fathead minnows (*Pimephales promelas*; added on day 33) collected at the end of the exposure period. Mean (± SD) periphyton Se concentrations after 77 days were 2.95 ± 0.71, 11.92 ± 2.52, and 71.36 ± 18.06 $\mu g \cdot g^{-1}$ dry weight (dw) in control, 1 $\mu g \cdot L^{-1}$ and 10 $\mu g \cdot L^{-1}$ treatments, resulting in enrichment functions (EFs) of 11891 ± 1754, 12142 ± 3208, and 7431 ± 2292, respectively. Se in surface sediments was higher in the 10 $\mu g \cdot L^{-1}$ treatment (21.90 ± 4.74 µg·g-1 dw) than in the control and 1 $\mu g \cdot L^{-1}$ treatment (2.58 ± 0.58 and 3.68 ± 1.74 µg·g-1 dw, respectively). Trophic transfer factors (TTFs) for benthic invertebrate taxa ranged from 0.6 for Gammaridae to 3.5 for Chironomidae over all treatments. Fathead minnow ovary Se concentrations were 6.91 ± 0.45, 10.87 ± 1.04, and 45.51 ± 2.20 µg·g-1 dw in control, 1 $\mu g \cdot L^{-1}$ and 10 $\mu g \cdot L^{-1}$ treatments, respectively. Se was enriched by up to four orders of magnitude by periphyton, and Se in fish ovaries accumulated to concentrations near or above the current US EPA criterion (15.1 $\mu g \cdot g^{-1}$ dw for fish ovary/egg) at 1 and 10 $\mu g \cdot L^{-1}$ exposure levels, suggesting that Se has the potential to accumulate to levels of concern and could pose a toxicity risk to sensitive organisms (i.e., fish and birds) in colder water ecosystems like Canadian boreal lakes. In addition, differences in Se concentrations among potential prey items of fish may influence Se accumulation in lake food webs and warrant further study.

Bioaccumulation of polychlorinated biphenyls in the southern resident killer whale food-web (PL)

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Chemical contaminants, including polychlorinated biphenyls (PCBs), have been identified as a threat to the health of the endangered southern resident killer whales (SRKW). The critical habitat identified for the SRKW population is located within the Salish Sea in British Columbia and the state of Washington. PCBs continue to be the most prominent contaminant in sediment in coastal BC and a priority for monitoring due to the biological risk, despite being legacy contaminants. PCBs have been linked to adverse health effects in marine mammals. Recent monitoring has shown that the concentration of PCBs in SRKW tissue has not significantly declined from 1996 to 2015, yet such a decline would be expected for a chemical that was banned in the 1970s. This observation suggests that there continue to be inputs of PCBs into the marine environment that supports the SRKW food web. It is critical to identify whether local environmental sources of PCBs have the potential to bioaccumulate to the PCB concentrations observed in SRKW samples. If local environmental sources can be identified, then it may be possible to take management actions that will reduce the exposure of SRKW to PCBs. This study investigated the contribution of PCBs stored in local sediment in the Salish Sea to PCB bioaccumulation in Chinook salmon and SRKW. PCB concentration data were obtained from government environmental monitoring programs and published literature. A previously developed food-web model was used to estimate the half-life of PCBs in Chinook salmon based on the PCB congener concentration in sediment. The estimated half-life for PCB congeners in Chinook salmon ranged from 0.5 to 1.5 years. These results suggest that it is possible for Chinook salmon to retain some PCB congeners taken up from elsewhere during their migratory route when they return to SRKW foraging areas in the Salish Sea. However, the half-lives of some PCB congeners are short enough that the PCB body burden could also be influenced by PCB concentrations in the local environment and food web. In addition, the same PCB congeners (PCB 153 and PCB 129) had the highest fraction of total PCBs in Chinook salmon and SRKW. To investigate the role of spatial variability in PCB sediment concentration in the potential bioaccumulation of PCBs in SRKW, the sampling locations and total PCB concentrations were mapped. The Salish Sea was divided into four smaller regions (Strait of Georgia, BC SRKW critical habitat, Juan de Fuca Strait and Puget Sound). Spatial analysis tools in ArcMap were used to estimate continuous surfaces of PCB concentrations for each region based on concentrations of PCBs in sediments measured at multiple sample points. These spatially-averaged PCB sediment concentrations were used with previously developed Biota Sediment Accumulation Factors (BSAF) to estimate the PCB concentrations in adult SRKW. The results indicated that the local PCB concentrations in sediment in all regions of the Salish Sea were sufficient to produce the PCB concentrations that have been observed in SRKW. The lines of evidence pursued in this study suggest that local environmental sources of PCBs in the Salish Sea could significantly contribute to the PCBs observed in SRKW. However, environmental monitoring targeted to measure the PCB congener concentrations in Chinook salmon and linking these

concentrations to specific environmental sources in their habitat would be needed to identify management actions.

Quantitative *in vitro* to *in vivo* extrapolation of biotransformation rates for bioaccumulation assessment: Focus on organic sunscreen agents in trout (PL)

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An improved understanding of chemical biotransformation in fish has been identified as a critical requirement in the environmental assessment of commercial chemicals. Recent studies demonstrate the utility of *in vitro* to *in vivo* extrapolation (IVIVE) approaches for estimating the impact of biotransformation on chemical bioaccumulation in fish. However, rigorous evaluation of the IVIVE approach requires studies that generate well-matched *in vitro* and *in vivo* data from the same species. Here we present results from both *in vitro* and *in vivo* experiments that measured biotransformation rates of two organic sunscreen agents (ultraviolet filters; UVFs), ethylhexyl trimethoxycinnamate (EHMC) and octocrylene (OCT) (logKow of 5.8 and 6.9, respectively) in rainbow trout (*Oncorhynchus mykiss*). For the *in vivo* studies, trout were exposed to three dietary concentrations of each UVF to investigate the relationship between dietary exposure concentration and observed accumulation and elimination. EHMC and OCT were significantly metabolized, resulting in mean *in vivo* biotransformation rate constants (kmet) of 0.35 ± 0.05 and 0.09 ± 0.01 day^{-1}, respectively. *In vivo* kmet values did not differ between dietary exposure concentrations, indicating that UVF concentrations in the fish were not high enough to saturate biotransformation enzymes. *In vitro* biotransformation rates were measured in hepatic and intestinal S9 fractions under demonstrated first-order conditions. These values were then extrapolated to the whole animal to estimate kmet. The kmet values determined for EHMC and OCT using *in vitro* data (hepatic plus intestinal) exhibited good agreement with measured values. Importantly, however, these comparisons suggest that biotransformation of EHMC occurs largely in the liver, while intestinal biotransformation plays a major role in elimination of OCT. Finally, the fraction unbound (fu) was measured in S9 fractions and blood plasma for EHMC, OCT, and several other hydrophobic test chemicals representing a range in logKow (4-8). Measured fu values were then compared to predictions generated using available binding algorithms. We conclude that IVIVE methods for bioaccumulation assessment require greater consideration of extrahepatic metabolism and improved algorithms for estimating unbound chemical fractions *in vitro* and in blood.

Bioaccumulation of very hydrophobic compounds in rainbow trout (PO)

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The main objective of these studies will be to determine *in vivo* and *in vitro* biotransformation rates of various siloxanes in rainbow trout (*Oncorhynchus mykiss*) fish. Alongside these, concentration-dependent biotransformation will be assessed by reviewing

the depuration rate constant over time at varying concentrations to determine if kT is dependent on the concentration of siloxanes within the organism. Given the wide use of hydrophobic siloxane compounds, more care is being taken by legislators around to globe to regulate any potential bioaccumulative and toxic substances in the environment. Given the abundance of the production of siloxanes, globally these studies seem to be of great importance in aiding in the addition of the knowledge of how these compounds interact in the environment. Also, with the current guidelines set by government bodies, only one chemical is tested at a time. The present proposal aims to help prove the importance of chemical mixture studies in the hopes of showing the complex nature of a cocktail of chemicals within the environment and any given organism that comes in to contact with said chemicals. Specific plans for these studies are as follows: (1) run individual siloxane exposures to juvenile rainbow trout fish (D4, D5, D6, L3, L4, L5) at a concentration of 4.5mM siloxane·g^{-1} food for and update duration lasting 21 days. Since the depuration rates for the cyclical siloxanes are very slow, and through previous work done in our lab, this high concentration should suffice to represent a depuration rate value, which will also save time and cost for the project. Also, the depuration phase will be extended further than that of the linear siloxanes. (2) After analysis of linear samples is completed, subsequent studies will be run at lower and varying concentration to determine if depletion rates are concentration-dependent. (3) The final *in vivo* experiment will be run as a mixture of all six siloxane compounds at various concentrations to determine if the presence of multiple compounds will alter the depletion rate of other compounds. Juvenile rainbow trout will be used for the *in vivo* studies. Test chemicals for both *in vivo* studies will include D4, D5, D6, L3, L4, L5, labeled 13C-L3/L4/L5, labeled 13C-D4, D5, D6 for internal standards; reference chemicals used will include 1,3,5-trichlorobenzene, 1,2,4,5-tetrachlorobenzene, pentachlorobenzene, hexachlorobenzene, PCB 52, PCB 153, and PCB 209 with pyrene used as an external standard.

Polychlorinated Dibenzo-p-dioxins/furans Human Health Risk Assessment in the north coast, British Columbia (PO)

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Core6 was engaged to complete a Human Health Risk Assessment (HHRA) as part of an environmental assessment for a north-coast British Columbia industrial development project. The project occurs in an area where a pulp and paper mill once operated and released polychlorinated dibenzo-p-dioxins/furans (PCDD/Fs) into the marine environment. There were concerns that disturbing the contaminated sediment during construction could increase the health risks to First Nations. A decision process was developed to address the sediment contamination and potential mitigation options. As part of the HHRA, the project incorporated a simulated sediment disturbance event to evaluate uptake of PCDD/Fs by aquatic invertebrates *Macoma nasuta* and *Neries virens*. Test organisms were exposed to slurries generated from overnight mixing (900 rpm) of sediment with seawater that was renewed weekly over an exposure period of 56 days. Uptake comparisons were made to a standard static (baseline) test exposure with bulk

sediment and filtered overlying water. An increase in PCDD/Fs uptake was not observed in resuspended sediment exposure as compared to the baseline test. The results supported the HHRA and the decision that confined disposal of contaminated sediment was not necessary.

Bioaccumulation of per- and poly-fluoroalkyl substances (PFAS) in the terrestrial and aquatic environments of an airport (PO)

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A terrestrial and aquatic tissue sampling and analysis program for per- and poly-fluoroalkyl substances (PFAS) was completed for the fire-fighting training area of an airport (hereinafter referred to as the Site). PFAS (e.g., perfluorooctane sulfonic acid [PFOS]) are persistent and can bioaccumulate in some organisms. In a detailed quantitative ecological risk assessment (DQERA) completed previously for the Site, potential risks to terrestrial and aquatic receptors from PFAS were identified based on the use of generic bioaccumulation factors (BAFs) and bioaccumulation regression models. The objective of the sampling and analysis program was to collect site-specific tissue data to assess the potential for bioaccumulation and food-chain transfer of PFAS. The following terrestrial tissue types were sampled and analyzed: plants (grasses and berries), soil invertebrates (earthworms) and small mammals. Terrestrial tissue samples were collected from areas representative of various levels of PFAS contamination (from low to highly contaminated areas) and from reference locations. The following aquatic tissue types were sampled and analyzed: plants, benthic invertebrates and small- and large-bodied fish. Aquatic tissue samples were collected from a river and creek from locations downstream of the fire-fighting training area (i.e., exposure areas) and locations upstream of the fire-fighting training area (i.e., reference areas). Concentrations of PFAS in grass, berry, earthworm and small mammal tissues collected from reference locations ranged from non-detectable to low-level detectable concentrations. PFAS were detected in grass, berry, earthworm and small mammal tissues collected from the fire-fighting training area. In general, concentrations of PFAS in tissues collected from highly contaminated areas in the fire-fighting training area were higher than those collected from areas with low contamination and those collected from reference locations. For some PFAS (e.g., PFOS), tissue concentrations were highest in small mammals, intermediate in earthworms and lowest in plants. This can be expected given that PFOS is known to bioaccumulate and biomagnify in wildlife. Sampling and analysis of aquatic plants, benthic invertebrates and fish from areas upstream and downstream of the fire-fighting training area in the river and creek suggested that these biota had accumulated higher concentrations of PFAS downstream of the fire-fighting training area. These biota represent different trophic levels in the aquatic food chain, and where detected, concentrations of PFAS tended to increase by trophic level, with the highest concentrations generally measured in fish. PFOS accumulated in aquatic tissues in both the river and creek to concentrations above available federal environmental quality guidelines protective of fish, and mammalian and avian consumers of aquatic biota. The

results of the terrestrial and aquatic tissue sampling program will be used in an updated evaluation of the potential risks to terrestrial and aquatic receptors for the Site.

Advances in Omics for Ecotox: Methods and Application

Metabolomic responses in tree swallows (*Tachycineta bicolor*) to local contaminant exposure within the Great Lakes (PL)

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Since 2010, tree swallows (*Tachycineta bicolor*) have been used to monitor contaminant levels as part of the Great Lakes Restoration Initiative (GLRI). Because tree swallows have a localized feeding radius and a diet consisting mainly of insects that spend their juvenile stages in nearby lake or river sediment, their tissue and egg contaminant loads are good indicators of levels of bioavailable contaminants in local sediment. In addition to providing information on contaminant levels, tree swallow tissue is also amenable to targeted metabolomics. This ability to compare endogenous metabolite levels from tree swallows across areas with differing contaminant loads allows us to investigate the potential physiological impacts of contaminant levels on local tree swallow populations. Identifying altered biological pathways via metabolite disturbances can potentially reveal insights into the effects of these complex, ambient exposures. In this study, 172 tree swallow nestlings were sampled across seventeen distinct sites in the Great Lakes basin to assess variations in their metabolite levels. Livers from tree swallows were sampled from Areas of Concern (AOCs) and non-AOCs during the summer months from 2013 to 2016 in Illinois (Waukegan), Michigan (L. Erie Metro Park), Ohio (Maumee River and Ottawa River) and Wisconsin (Sayner, Milwaukee and Sheboygan). Targeted metabolomics were performed on the liver tissues to quantitate levels of 235 metabolites including amino acids, biogenic amines, fatty acids, bile acids, hexose, lipids and metabolites associated with energy pathways. Contaminant concentration data for a variety of persistent organic pollutants and contaminants of emerging concern were also available for a number of these sites. Using multivariate statistical approaches, samples from different sites could be distinguished by their metabolomic profiles. Metabolite levels in tree swallows collected from the Wisconsin reference sites, Plum Lake and Star Lake, were highly similar and differed substantially in their lipid profiles from samples collected at Three Bridges and Lakeshore State Park, both in the Milwaukee Estuary AOC. In samples collected from Waukegan Harbor, an AOC that had recently undergone dredging to remove contaminated sediment, concentrations of several metabolites changed in a consistent direction over the consecutive collection years.

Selenomethionine-induced molecular toxicity in the fathead minnow (*Pimephales promelas*) (PL)

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Selenium is a naturally occurring trace element anthropogenically released to the aquatic environment from activities such as mining and fertilizer application. Within the aquatic environment, primary producers convert aqueous phase selenium oxyanions to organic species, including selenomethionine (SeMet), which can bioaccumulate and cause ecological harm, typically at the top of aquatic food webs. Excess SeMet exposure is known to cause several physiological alterations, including oxidative stress and altered stress response. Ingestion of elevated concentrations of SeMet can cause direct toxicity to exposed animals, as well as aggregate in the yolks of oviparous animals and potentially lead to complete recruitment failure through embryotoxicity. However, there is still uncertainty regarding the specific mechanisms by which SeMet causes these pathologies. Therefore, the goal of this study was to conduct an in-depth characterization of the molecular mechanisms underlying SeMet toxicities with the aim of identifying molecular toxicity pathways that enable prediction of pathologies before they occur. This study exposed adult fathead minnows (*Pimephales promelas*) to graded concentrations of dietary SeMet (1.18 $\mu g \cdot g^{-1}$ dry weight control, and 3.88, 8.75, and 29.58 $\mu g \cdot g^{-1}$ dry weight treatments) for 28 days in order to determine its effects on biochemical and molecular processes in this species. Thiobarbituric acid reactive substances, reduced to oxidized glutathione ratios, superoxide dismutase, and catalase assays are being employed on liver tissues to determine the extent of oxidative damage, while liver whole transcriptome and proteome analyses are being conducted to determine molecular toxicity pathways characteristic of SeMet exposure in these fish. These results are then intended to validate and inform the development of critical molecular endpoints of SeMet toxicity in fish that will be used to inform the generation of the EcotoxChip (@ecotoxchip), a qPCR microarray screening tool ultimately intended to expedite the risk assessment of chemicals and mixtures of potential environmental concern.

Using metagenomics to evaluate ecosystem health and recovery: Sediments from Quesnel Lake impacted by a major mine tailings spill as a case study (PL)

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Human activities such as mining can create environmental disturbances that necessitate monitoring to assess resistance and resilience of the ecosystem and planning of reclamation efforts, if required. Such instances include reclamation of inactive mine sites or remediation of areas impacted by mining-related disasters such as a mine tailings spill. In most cases, measurable indices for reclamation success include plant type and cover, and the presence or absence of certain key invertebrate species. However, the appearance of plants and/or other biota is dependent on an already functioning ecosystem at the microbial level, as

microbial metabolism plays a key role in all biogeochemical cycles essential to sustain the development of the macro component. Furthermore, due to their high diversity, fast metabolic rate, and short generation time, changes to microbial populations might occur faster than to populations of macro organisms. Despite this, the microbial component of the ecosystem is seldom utilized as a marker for its recovery or the progress of reclamation. This is mainly the result of the complexity of surveying microbial population using classic, culture-dependent methods. However, with the lowering cost and increased access to high throughput sequencing it has become simpler to study microbial communities in a culture-independent manner. Therefore, instead of focusing on comparing specific culturable microbial taxa between disturbed and pristine environments, one can compare whole microbial communities to inspect for trends and shifts that might be telling of the habitability of the ecosystem to larger biota. Here, we present a case study comparing the microbial communities of sediments within Quesnel Lake, located in the interior of British Columbia, that suffered a catastrophic tailings spill from an adjacent copper mine, two years post spill. Our study shows that the microbial communities from disturbed and undisturbed sites significantly differ in composition. Furthermore, we were able to generate a model based on random forest algorithm, which accurately classified the samples as belonging to disturbed or undisturbed sites. These results show that microbial communities can serve as markers for ecosystem health and recovery.

EcoToxChip: A toxicogenomics tool for chemical prioritization and environmental management (PL)

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Chemical contamination of our natural ecosystems is regarded as one of the planet's greatest threats. Legislation in North America and Europe mandates the assessment and reduction of risk for thousands of chemical substances used by society and released into the environment. However, current regulatory processes are challenged in that they rely on extensive animal testing that is prohibitively time- and resource-intensive, and raises significant ethical concerns due to the millions of animals required to fulfill current testing mandates. This Large-Scale Applied Research Program (LSARP) grant from Genome Canada aims to develop, test, validate, and commercialize a novel transcriptomics-based screening and testing tool (EcoToxChips) and a data evaluation platform (EcoToxXplorer.ca) for the characterization, prioritization, and management of environmental chemicals of regulatory concern that aims to overcome these challenges. EcoToxChips will be developed for laboratory model species representing the most important vertebrate groups in ecological risk assessment (fish: fathead minnow (*Pimephales promelas*); bird: Japanese quail (*Coturnix japonica*); amphibian: *Xenopus laevis*). Adults and early-life stages (ELS) of these species will be exposed via standardized tests to eight environmental chemicals covering a wide chemical and biological effect space (ethinylestradiol , chlorpyrifos, benzo(a)pyrene, lead, fluoxetine, selenomethionine, trenbolone, hexabromocyclododecane). An integrative systems approach based on functional 'omics (combined global transcriptomic and

proteomic profiling, targeted metabolomics) and physiological analyses across levels of biological organization will then be applied to characterize relevant toxicity pathways including adverse outcome pathways (AOPs); from this and other resources, species-specific EcoToxChips consisting of 384 environmentally-responsive genes of regulatory concern will be informed, built, tested, and optimized. EcoToxChip performance will be validated (and further optimized) through an inter-lab study with our collaborators. Knowledge from Phase 1 will then be translated to three native species (i.e., rainbow trout (*Oncorhynchus mykiss*), double-crested cormorant (*Phalacrocorax auritus*), wood frog (*Rana sylvatica*)). In parallel, a Cloud-based data evaluation platform (EcoToxXplorer.ca) will be developed to provide intuitive bioinformatics support. To position the team advantageously with regard to the commercialization and institutionalization of the deliverables, our GE3LS (Genomics and its Ethical, Environmental, Economic, Legal and Social Implications) research will produce and leverage social science knowledge about the phenomenon of "institutional entrepreneurship". The anticipated socioeconomic benefits associated with the adoption of our deliverables include significantly reduced and more focused animal testing, improved regulatory decision-making, and cost-efficiencies. (www.ecotoxchip.ca)

Transcriptomics as an early warning indicator of neonicotinoid insecticide exposure of the mayfly (*Hexagenia* spp.) (PL)

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Mayfly nymphs (*Hexagenia* spp.) are common test organisms for evaluating pesticide toxicity in freshwater aquatic environments. The project objective was to determine whether molecular endpoints (such as transcriptomics) can be used to predict phenotypic outcomes (behavioural changes) in *Hexagenia* spp. exposed to the neonicotinoid imidacloprid. Effects on gene expression can provide rapid insight into mode of action and targeted biomarkers that serve as surrogates for apical toxicity test endpoints (survival, growth) and as indicators of sublethal, deleterious effects (e.g., behaviour). We present the first steps in investigating whether changes in gene expression can be used to indicate effects of neonicotinoid insecticides. RNA was isolated from a limited number of *Hexagenia* spp. nymphs (n=5 per condition) that were held in water only or water containing 5 $\mu g \cdot L^{-1}$ imidacloprid for 96 hours. This concentration of pesticide elicited significant effects on mobility. RNA from each individual was subjected to RNA-seq analyses using our established *de novo* assembly pipeline to generate a *Hexagenia* spp. reference transcriptome comprising 720,123 total contigs. We were able to annotate 69% with the National Center for Biotechnology Information “nr” database. This new resource is a substantial augmentation of the 60 transcript sequences currently available in this public repository. Analysis of the transcriptome data with respect to imidacloprid effects revealed that the experimental animals were apparently a mixture of *H. limbata* and *H. rigida*. It was necessary to analyze each subgroup separately for pesticide effects. Thus statistical power was reduced to n=2-3 per group. Nevertheless, we found that imidacloprid significantly affected the abundance of 1,490 contigs (611 are annotated) in the presumed *H. limbata* group and 654 significant contigs (232 are annotated) in the presumed *H. rigida* group.

While the gene ontology profiles of imidacloprid-affected nymphs are distinct between groups, proteolysis and cell adhesion pathways were common to both. The transcriptomics data form the basis for the future development of targeted quantitative real-time polymerase chain reaction assays. While the data suggest that the presumed *H. limbata* may be more sensitive to imidacloprid than *H. rigida*, a higher n for targeted evaluation with a single, defined species and shorter time points (24-48 hours) to maximize capturing transcriptomic effects is warranted.

Alteration of secreted miRNA from stressed rainbow trout identified via high throughput sequencing (PL)

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Measurements of waterborne environmental DNA are currently being employed to monitor both invasive and endangered fish species in Canadian waterways. However, little research has examined environmental RNA within the water as a marker of the health status of a local community. MicroRNA (miRNA) are stable, targeted, post-transcriptional regulators of mRNA, and are therefore useful markers that can be linked to phenotypic responses. Changes in miRNA levels in tissues and circulation have previously been measured in fish in response to acute and chronic stress. Mir-21, a highly expressed miRNA in rainbow trout (*Oncorhynchus mykiss*) was measured in trout gill secretions, epidermal mucous, and in water samples. This study examines all miRNA present, from the potential sources of waterborne miRNA excreted by rainbow trout following an acute stressor, to the water, to identify miRNA to be used as an environmental marker of stress. Following a three-minute air exposure, adult rainbow trout epithelial mucus and gills were collected. Gills were emptied of blood followed by immersion in saline, to collect miRNA that would be released into water. From these samples, all microRNA were sequenced and characterized. Differential expression between the tissues and treatments were analysed. This approach was also used in water samples from a trout confinement stressor to characterize the miRNA within the water column. Ultimately, measurement of miRNA, secreted from gills and skin mucosa, and measured in the water will lead to the use of waterborne miRNA as novel, non-invasive biomarkers of stress in fish.

Hepatic proteome and toxic response of early-life stage rainbow trout (*Oncorhynchus mykiss*) to the aquatic herbicide, Reward® (PL)

Lisa McCuaig[1], Christoper Martyniuk[2], Vicki Marlatt[1]

[1]Simon Fraser University, [2]University of Florida

The objective of this study was to examine the acute toxicity and sublethal effects of the commercial formulation Reward®(diquat dibromide) on early life stages (embryo, alevin, fry, juvenile) of rainbow trout (*Oncorhynchus mykiss*) exposed to environmentally relevant concentrations. The continuous exposure 96hours LC50 derived for juvenile feeding fry

aged 85 days post-hatch was 9.8 mg·L^{-1}. In order to mimic the manufacturer's instructions for direct applications to water bodies, rainbow trout eyed embryos and juvenile feeding fry were also exposed to concentrations ranging from 0.12 to 10 mg·L^{-1}, during two 24-hour pulse exposures separated by 14 days of rearing in fresh water. Effects on growth and development were evident at 9.25 mg·L^{-1} during the embryo/alevin exposures, but not in feeding juveniles, indicating a higher sensitivity of the early life stage fish. Quantitative proteomic assessment and subnetwork enrichment analyses were conducted on hepatic proteins for both life stages to evaluate protein expression changes after 0.37 mg·L^{-1} exposure. Unique cellular process expression profiles for pre-feeding swim-up fry and for feeding juvenile fish were observed, reflecting differences between the two life stages in sub-cellular responses after diquat dibromide exposure. Hepatic proteome effects were more dramatic in the pre-feeding swim-up fry with 315 proteins significantly different between the control and fish exposed to diquat dibromide, while in the later life stage feeding fry, only 84 proteins were significantly different after Reward® exposure. This study is the first to report the sub-cellular and whole organism level effects of diquat dibromide in the commercial formulation Reward® Landscape and Aquatic Herbicide, and demonstrate that changes at the protein level occur at environmentally relevant concentrations based on manufacturer's recommended aquatic application rates.

Toxicity of Reward® herbicide to *Pimephales promelas*: Pulsed application with proteomic profile (PL)

Michael Moreton[1], Bonnie Lo[2], Denina Simmons[3], Vicki Marlatt[1]

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The objectives of this study were to assess the lethal and sublethal effects of the herbicidal commercial formulation, Reward® (373 g·L^{-1} diquat dibromide; DB), using application scenarios prescribed by the manufacturer. Specifically, a 14-day period between applications of Reward® in a water body undergoing treatment is required, yet the effects of these 'pulse' exposure scenarios on aquatic wildlife such as fish are unknown. In the first experiment, early life stage fathead minnow (*Pimephales promelas*; FHM) were exposed continuously to Reward® at DB concentrations from 0.12 to 12.1 mg·L^{-1} and yielded a larval 7-day LC50 of 2.04 mg·L^{-1} as well as a significant decrease in body mass (25.0 ± 11.6 %) in the 1.18 mg·L^{-1} Reward® concentration. In a second experiment, FHM larvae were exposed for a 24-hour pulse and then reared in clean water for 14 days followed by a second 24-hour pulse exposure to Reward® which produced a 16-day LC50 of 4.19 mg·L^{-1}, but no effects on body weight. Whole body proteomic analysis of this 0.37 mg·L^{-1} DB pulse exposure revealed few significant differences compared to the control larvae. In a third experiment, adult FHM were exposed in a pulse/discontinuous manner as described above to Reward® at DB concentrations ranging from 0.102 to 12.6 to mg·L^{-1}, and a 21-day LC50 of 6.71 mg·L^{-1} was derived. No significant changes were observed in gonadosomatic index or fecundity in the adult FHM pulse exposure experiment. A fourth experiment assessed the F1 generation's hatch success by rearing eggs spawned in the adult Reward® pulse exposure, but no significant effects in any of the treatments compared to the control were observed. These findings suggest that concentrations causing adverse effects on FHM

during acute exposures occur above the maximum concentration predicted by the manufacturer when applied according to the label for aquatic pest plant applications (i.e., >0.37 $mg \cdot L^{-1}$), with a LOEC of 1.18 $mg \cdot L^{-1}$ for larval growth during sub-chronic continuous exposure. No monitoring studies reporting environmental concentrations of DB have been performed in Canada. Due to the absence of studies reporting environmental concentrations of DB, it is unclear which exposure scenarios (whether aquatic pest plant treatments, run-off events from terrestrial applications, or if this herbicide persists in natural aquatic systems in concentrations that translate into acute or sub-chronic toxicity exposure) should be the focus of future studies.

The effects of waterborne chromate (Cr^{6+}) on mRNA expression patterns in lake trout (*Salvelinus namaycush*) (PL)

Jim Sherry[1], Tannis Neheli[1], Jason Miller[1], Dave Poirier[2], Trudy Watson-Leung[2], Richard Chung Kit[2], Stefanie Maedler[2], Vasile Furdui[2], Myrna Simpson[3], Eric Reiner[2], Denina Simmons[4]

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In anticipation of the development of chromite mining in the Ring of Fire in Northern Ontario, we assessed the effects of Cr^{6+} on the liver transcriptome of lake trout (*Salvelinus namaycush*), which is a native species in that area. Juvenile fish were exposed in the laboratory to waterborne Cr^{6+} (0.2 $\mu g \cdot L^{-1}$ and 3 $mg \cdot L^{-1}$) for 21 days (N=6 flow-through tanks of three fish per tank). The high treatment was truncated at seven days due to mortalities beyond that time. The liver transcriptomes were sequenced via a TruSeq/NextSeq 500 workflow and assembled *de novo* via Trinity. The Illumina reads of 12-fish from each treatment were then aligned to the transcriptome assembly and counted. At a false discovery rate (FDR) of 0.01, about 4,600 mRNA transcripts were differentially expressed (DE). The greatest effects were seen in fish from the 3 $mg \cdot L^{-1}$ Cr^{6+} treatment: ~2,200 transcripts were DE. The 0.2 $\mu g \cdot L^{-1}$ Cr^{6+} and control treatments also differed from the other treatments: ~1,200 and ~1,000 transcripts respectively were DE. The DE transcripts were identified, curated, and functionally analyzed. Our results suggested that waterborne Cr^{6+} can affect lake trout at an environmentally realistic concentration of 0.2 $\mu g \cdot L^{-1}$.

The effects of waterborne chromate (Cr^{6+}) on protein abundance patterns in lake trout (*Salvelinus namaycush*) (PL)

Denina Simmons[1], James Sherry[2], Tannis Neheli[2], Jason Miller[2], Dave Poirier[3], Trudy Watson-Leung[3], Richard Chong Kit[3], Stefanie Maedler[3], Vasile Furdui[3], Eric Reiner[3], Myrna Simpson[4]

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In anticipation of the development of the Ring of Fire in Northern Ontario for the mining of Chromium, we assessed the effects of Cr^{6+} on the plasma proteome of lake trout, a species which is native to that region. Juvenile lake trout were exposed in the laboratory to

waterborne Cr^{6+} (0, 0.2 ppb and 3 ppm) for 21 days (6 flow-through tanks (1 to 1.5 $L \cdot g^{-1}$ fish/day) of five fish per tank for each treatment). The 3 ppm treatment was halted after seven days due to mortalities. Plasma proteins were digested into peptides using formic acid, and then identified using an Agilent reverse phase liquid-chromatography tandem quadrupole time-of-flight mass spectrometry system with data-dependent full scan acquisition. Spectral files were subsequently searched using Spectrum Mill Software. Each injection was performed in duplicate for each individual plasma sample. Results from liver transcriptome analyses from these same fish indicate there was significant differential gene expression in both the 0.2 ppb and 3 ppm treatments. Plasma protein abundance will be compared to these results to detect where there is an agreement between the two datasets and we will discuss the potential health effects caused by exposure to Cr^{6+} which are implied by observable changes in the plasma proteome.

Protein profile analysis of the diatom *Chaetoceros calictrans* upon exposure to triclosan (PO)

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Personal care products (PCPs) which are biologically active, persistent, and considered to be emerging pollutants have raised major concerns because of their constant release into the aquatic environment. Within the PCPs, triclosan (TCS) is an antibacterial agent extensively used in household products such as toothpaste, shampoo, deodorant, etc. It has been the one of the most detected PCPs in aquatic ecosystems (concentrations ranging from 0.0014 – 40 $\mu g \cdot L^{-1}$). This study investigated the toxicological impacts of TCS in the estuarine diatom *Chaetoceros calcitrans* by analyzing differentially-regulated proteins through a proteomics approach. After exposing the diatoms to different concentrations of TCS for 96 hours, total protein extracted from both treated and untreated groups was subjected to SDS PAGE followed by 2D gel electrophoresis, and consequently identified the significantly regulated peptides through MALDI-TOF/TOF-MS. In SDS PAGE, protein polypeptide bands ranged between 10 – 75 kDa. Further, in 2-D gel electrophoresis, separation of highly resolved proteins spots (~206) were visualized per gel. The control and T1 (6.25$\mu g \cdot L^{-1}$ of TCS) group, when compared, exhibited up-regulation in eight protein spots while 14 spots showed down-regulation. Similarly, between control and T3 (25$\mu g \cdot L^{-1}$ TCS), four spots were up-regulated and 11 were down-regulated. Between control and T5 (100 $\mu g \cdot L^{-1}$ TCS), three protein spots were up-regulated and eight were down-regulated. Of all groups analysed, two protein spots 5.5pI/114MW and 4.75pI/17MW were chosen for identification using PMF and each was identified as phage tail protein of *Escherichia coli* ($p<0.05$) and Chain-B Porcine Epsilon-Trypsin ($p<0.05$) respectively.

Predicting adverse outcomes of selenomethionine exposure to embryonic white sturgeon (*Acipenser transmontanus*) using in-ovo microinjection (PO)

Derek Green[1], David Janz[1], Karsten Liber[1], Natacha Hogan[1], James Alcaraz[1], Taylor Lane[1], Kerstin Bluhm[1], Markus Brinkmann[1], Markus Hecker[1]

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Selenium (Se) is a trace element and nutrient for almost every form of life. However, it has a narrow margin between essentiality and toxicity that can be surpassed when anthropogenic activities cause elevated Se concentrations in aquatic environments. This toxicity occurs when primary producers biotransform the excess environmental Se to selenomethionine (SeMet), a bioaccumulative methionine analogue that can be maternally transferred to the eggs of oviparous species, leading to impairment or complete failure of recruitment. The primary mechanism identified for SeMet toxicity is redox cycling of the metabolite methylselenol, yet not all species exposed to SeMet show signs of oxidative stress. While SeMet exposure poses potential risk to many oviparous vertebrates, the white sturgeon (*Acipenser transmontanus*) is at particular risk due to its SeMet sensitivity, life history, and endangered status. Though tissue specific (eg., egg, ovary) Se concentration guidelines have been employed by the United States Environmental Protection Agency, these thresholds are only capable of indicating imminent or present risk. Protection efforts could therefore be enhanced through the development of more sensitive tools capable of predicting the risks that have yet to manifest. The purpose of this study is to identify the molecular initiating event of and possible key events leading to SeMet toxicity in the white sturgeon. In order to characterize this pathway, 1488 white sturgeon embryos were exposed to either negative or process control conditions, or graded SeMet injection (nominal: 8.8, 13.3 and 20 $\mu g \cdot g^{-1}$ dry mass) conditions in ovo in four replicates to determine the potential effects of SeMet exposure in early life stage white sturgeon. Embryos were sampled five days pre- and post-hatch for transcriptomic analyses, and at swim-up for biochemical, histological, and deformity analyses in order to delineate the causal chain of toxic events across levels of biological complexity. The results of this experiment will aid in developing a highly sensitive predictive model for SeMet toxicity and hopefully will enhance the assessment of Se risks posed to white sturgeon.

Evaluation of the effects of ethinylestradiol and chlorpyrifos using an early-life stage Japanese quail toxicity test and omics technologies (PO)

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Birds are sensitive indicators of ecosystem health, but avian toxicity data for many chemicals of environmental relevance are limited. The overall goal of our research project is to develop early-life stage (ELS) avian toxicity tests for rapidly screening chemicals of ecological and regulatory concern. In this study, we use a recently developed standardized egg injection protocol to assess the effects of two endocrine disrupters—ethinylestradiol [EE2] and chlorpyrifos [CPF] —on organismal and molecular level end points in ELS

Japanese quail (*Coturnix japonica*; JQ). The chemicals were dissolved in dimethyl sulfoxide and injected into the air cell of JQ embryos prior to incubation at 0, 0.33, 3.33, and 33.3 $\mu g \cdot g^{-1}$ egg for EE2, and 0, 0.4, 4, and 40 $\mu g \cdot g^{-1}$ egg for CPF. The highest concentration chosen for each test compound was predicted to result in ≤20% mortality. Liver tissue was collected from a sub-set (n=5/dose group) of embryos at mid-incubation (day 9) for subsequent 'omics and analytical chemistry analyses. The remaining embryos were examined on day 16 (1-2 days prior to hatch) for deformities, growth and health metrics. Mortality was low across all treatments, ranging from 3% for the vehicle group, to 14% and 9% for the high doses of EE2 and CPF, respectively. Exposure to both chemicals led to a significant reduction of embryonic growth and CPF caused an increase in gallbladder size and incidence of deformities of the feet and spinal cord. Analysis of RNAseq and metabolomics data from mid-incubation embryos is currently underway. Samples will also be subjected to proteomics and histological analyses. The use of avian embryos in toxicity testing helps address the need to replace live animals with alternative approaches for chemical screening. The co-determination of apical and 'omics end points will greatly contribute to ecological risk assessments and the development of adverse outcome pathways. This study is part of the EcoToxChip project (@ecotoxchip).

Relating molecular toxicity pathways to apical outcomes of chronic ethinyl estradiol exposure in *Xenopus Laevis* (PO)

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Current chemical testing for toxicological risks relies on extensive live-animal testing and focus on apical outcomes of regulatory relevance. This current approach is being challenged due to the ethical concerns over extensive animal testing that is also extremely time and resource intensive. Phenotypic adverse outcomes of chemical exposure are often preceded by changes in key molecular pathways, which can give early indications of subsequent physiological changes and their modes of action. Toxicogenomics therefore shows great promise as an early screening tool to prioritize chemicals with potential risk for adverse effects, without the need for long-term, animal-intensive exposures. The objectives of this study were to (1) evaluate apical outcomes of chronic exposure to ethinyl estradiol (EE2) on the model amphibian *Xenopus laevis*, (2) identify key molecular response patterns that may be altered during exposure to EE2, and (3) establish critical toxicity pathways for EE2 exposure in amphibians by linking molecular response patterns to apical outcomes of regulatory relevance. Tadpoles (*Xenopus laevis*) were exposed to EE2 (0.04, 0.2, 1 $\mu g \cdot L^{-1}$, nominal) from early life stage (immediately post-hatch) through to metamorphosis. Individuals were sampled after 96hours and assessed for whole body transcriptome effects (RNASeq). A subset of tadpoles was then transferred to a flow-through diluter system for exposure to metamorphosis (~ 50 days), at which stage they were assessed for developmental stage, morphometrics, and organ histopathology. Exposure to EE2 (1 $\mu g \cdot L^{-1}$) significantly increased total length, wet weight, and relative liver weight as compared to water-only control group. Apical effects will be assessed

through histopathology of key organs as well as measures of oxidative stress, immune and apoptotic markers. We anticipate that this work will provide new and valuable amphibian EE2 toxicity data to compare to well-studied fish species. The ultimate goal of this study is to identify critical toxicity pathways for early life stages that enable prediction of apical outcomes of ecological and regulatory relevance in amphibians, providing an alternative approach in chemical screening. This study is part of the EcoToxChip project (@ecotoxchip).

Multi-omic approach to the analysis of organism responses to Great Lakes sediment, effluent and surface water exposures (PO)

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Sediment, effluent and surface water samples were collected from various sites across Hamilton Harbour, Toronto Harbour, Humber Bay and Lake Erie between 2014 and 2015. Larval *Hexagenia* sp. and sexually immature rainbow trout (*Oncorhynchus mykiss*) were exposed to these samples in the laboratory for 48 hours. Liver, plasma, and fin tissue were collected from exposed and control rainbow trout while exposed and control *Hexagenia* were collected whole. Levels of 11 transcripts in rainbow trout and seven in *Hexagenia* were measured. Shotgun proteomics data were generated for rainbow trout and *Hexagenia.* Two hundred and nineteen metabolites including amino acids, lipids, bile acids, and fatty acids were quantified. A total of over 500 individual contaminants and water quality indicators were measured in the effluent, surface water, and sediment samples. Results showed distinct signatures by exposure type. Metabolomics data in *Hexagenia* exposed to sediment from Hamilton Harbour sites with high persistent organic pollutant concentrations showed 60 metabolites differing significantly between sites and correlating with contaminant levels. Two transcripts also showed statistically significant differences between these sites. One hundred and one *Hexagenia* proteins had significantly different levels following exposure to Hamilton Harbour effluent samples. It is expected that the combination of 'omic results will have higher differentiating power than data from a single endpoint measurement; however, this may not be so for rainbow trout, which were generally less responsive than *Hexagenia*—possibly due to differences between these two species and their relative levels of exposure.

Macro and Microplastics in the Pacific Northeast: The True Extent of the Problem

Canaries of the sea: Are microplastics the final challenge to shellfish? (PL)

Leah Bendell[1]

[1]Simon Fraser University

The west coast of British Columbia has been home to a healthy shellfish aquaculture industry since the turn of the century. However, recent years have seen the industry facing the challenges presented by 70 years of human activity, the era of the Anthropocene. As shellfish both wild and farmed rely on clean cool waters rich in algae, any impact that alters these conditions will have consequences on shellfish health. And it is not one singular event, but rather the ocean environment serves to integrate all that we have done and are doing to our planet. Ocean acidification, a warming ocean, increased incidences of harmful algae blooms (HABS) and their associated shellfish-borne diseases, increased incidences of viral infections, increased incidences of severe storm events, and now, the presence of microplastics which are being ingested by shellfish could combine to overcome an already struggling industry. Perhaps of greater concern, all that occurs for farmed shellfish will also be experienced by wild shellfish, a key species in marine ecosystems. Hence, shellfish serve as our canaries, showing us ahead of time what might possibly be in store for our ocean ecosystems.

Microplastic accumulation in British Columbia blue mussels (*Mytilus edulis*) (PL)

Julie Dimitrijevic[1], Leah Bendell[2], Marie Noel[1], Peter Ross[1]

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Microplastics are plastic polymers <5mm and are a global concern for marine ecosystems. Recorded in a multitude of taxa, microplastic ingestion reaches all levels of the marine food web, from marine mammals, sea turtles and sea birds, to bivalves, echinoderms and zooplankton. Microplastic abundances and accumulation were recorded for blue mussels (*Mytilus edulis*) within the Strait of Georgia, British Columbia. Individuals of the same genetic stock were deployed in cages within the Strait between January and March, 2017. A total of 11 survey sites of varying anthropogenic disturbance were chosen. Mussels and water quality data were collected on day 0, day 30 and day 60 at each sampling location. Using rigorous contamination control techniques and enzymatic digestion (Corolase 7090), mussels were processed over an 18-hour period at 60°C. The resultant solution was filtered through a 20µm polycarbonate filter for microplastic quantification using light microscopy (length, width, colour and shape of each particle was characterized). Polymer type was confirmed and identified using Fourier-Transform Infrared Spectroscopy (FTIR) and a comprehensive data set of polymer composition and abundances was established. Results are being compared to determine temporal and spatial differences between sites to determine if microplastics accumulate in these filter-feeding organisms.

Preliminary numbers are low, indicating that blue mussels are unlikely to accumulate microplastics within the body cavity. These findings may be a result of low environmental contamination, or the ability of this species to eliminate microplastics post ingestion.

Microplastic pathways: Secondary wastewater treatment plants as a source of microplastics in the environment (PL)

Anna Posacka[1], Stephanie Wang[1], Marie Noel[1], Peter Ross[1]

[1]*Ocean Wise*

We recently showed that 1.8-trillion microplastic particles enter the largest secondary wastewater treatment plants (WWTPs) in Vancouver annually; 99% of these are retained in biosolids, while 30 billion flow through into the receiving environment. Seventy-one percent of the microplastic particles consisted of fibers, of which the majority were polyester or modified cellulose (e.g., rayon). In order to provide additional insight into the source, transport and fate of microplastics in domestic wastewater, we are characterising the daily, weekly and seasonal variations of microplastics in domestic wastewater. A novel closed-containment sampling system was designed, enabling efficient and automated collection of microplastics suspended in wastewater while preventing airborne contamination. Polymeric composition of microplastics extracted from wastewater is determined using Fourier Transform Infrared Spectroscopy (FTIR). Results from this research provide insight into the importance of domestic waste as a source of microplastics in coastal waters, the fate of microplastics in the wastewater stream, and the effectiveness of different levels of treatment at removing microplastics.

Microplastic source identification: FTIR library development and material degradation during the controlled weathering study (PL)

Katerina Vassilenko[1], Mathew Watkins[1], Peter Ross[1]

[1]*Ocean Wise*

Identification of polymeric composition of microplastic particles is critical for determining microplastic sources and potentially for developing mitigation measures. Polymeric composition of microplastics can be analysed using Fourier Transform Infrared Spectroscopy (FTIR), but material identification is complicated by changes to the original materials due to exposure to ultraviolet (UV), oxygen and biofouling. To address this issue, we performed three controlled weathering experiments of 110 fabric samples provided by local outdoor retailors, our industrial partners. Ambient air, ocean and wastewater at different treatment stages were chosen to represent the most relevant weathering environments. Here, we discuss the results of our weathering study, report on the development of an FTIR library for parental (as made) and weathered materials, and also discuss observed changes to and degradation of synthetic versus natural fabrics under different weathering conditions. The results of this study allow for reliable and consistent identification of microplastic materials found in the environment and wastewater, therefore

supporting an overall understanding of microplastics sources, pathways and fate in the environment.

Identifying the sources of microfibres through a comparison of microfibre shed rates from home laundering of textiles of various types and introducing lint traps as a potential means of mitigation (PO)

Mathew Watkins[1], Katerina Vassilenko[1], Stephen Chastain[1], Peter Ross[1]

[1]*Ocean Wise*

Microplastics (MP, particles ranging from a few microns to 5 mm) play a key role in global marine pollution and are an emerging concern worldwide. Moreover, there is increasing evidence that the majority of MP particles in the environment are in fact microfibres (MFs). It was shown that, in the nearshore zone, up to 95% of total MP particles are microfibres, which raises questions regarding sources and transport pathways of MFs. Textile fabrics were named among the main sources of MFs, calling for the need to understand how MFs are formed and transported to the environment. To study textile fabrics as a source of MFs, we collaborated with outdoor garment retailers to analyse shed rates from the laundering of common textiles. Fabric characteristics such as material type, textile architecture, and mechanical and chemical finishing were analysed in relation to the shed rates. Also, we are testing lint traps and other devices meant to remove MFs from the washing machine wastewater before they go down the drain. Here we present our newly developed sampling and lint-quantification methodology and also discuss fabric characteristics affecting the shed rates, as well as some devices for mitigating the release of MFs into wastewater.

Alternative Approaches to Adult Fish Survey in Environmental Effects Monitoring Programs

If historic marine pollution ceases, will the natural intertidal community return? How exposure to and release from pollution disturbance shapes rocky intertidal communities in British Columbia (PL)

Shannon Bard[1], Julia Baum[2]

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Marine ecosystems in British Columbia are subject to a variety of pollution-based disturbances that restructure the composition of the vegetative and animal communities. A prime historical example of this is the effluent discharged from pulp mills across the province, which have been shown to have substantial negative impacts on coastal biodiversity. However, as environmental regulations have become more stringently enforced and the economic drivers of pulp mills have slowed down, in some areas much of this pollution has abated. The diminishment and, in some cases, elimination of such disturbances provides a unique opportunity to assess the recovery of communities once affected by the mill discharge. This also allows an opportunity to better understand how to develop successful marine habitat reclamation strategies for coastal sites when pollution inputs may also be present. To develop a marine ecosystem assessment model, we used a long-term monitoring dataset spanning three decades to study rocky intertidal communities situated around pulp mills in Prince Rupert, Powell River, and Howe Sound. Using this dataset, we demonstrate how proximity to the pollution source negatively influenced the intertidal community at these sites. Next, we examine how the community composition shifts and recovers once the pollution disturbance is alleviated. Lastly, we take a specific look at which species and which life history traits are best able to survive in a disturbed environment and which species are best able to recolonize those once-disturbed locations. This research provides insight into classic ecological theory, applied understanding of the impacts and implications of anthropogenic activity in marine ecosystems via ecological risk assessment, and novel methods by which to assess those industrial impacts and recommend successful reclamation and restoration strategies.

Estimated fish mortality caused by Metal Mining Environmental Effects Monitoring Lethal Fish Population Surveys, and an evaluation of the non-lethal alternative (PL)

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The Environmental Effects Monitoring (EEM) program, federally mandated to provide technical guidance for metal mines and mills in Canada, aims to ensure that fish and their environment are sufficiently protected by the Metal and Diamond Mining Effluent Regulations. Concerns have been raised regarding lethal fish sampling conducted for the program and the potential long-term risk to local fish populations, particularly in low-

productivity ecosystems. To estimate the extent of fish sacrificed for this program, Saskatchewan metal mining EEM data were compiled and extrapolated to a national level. It is estimated that 60,640 ± 19,978 fish have been sacrificed for the program across Canada over the past 16 years. Lethal fish population surveys are the standard sampling design for the program, with non-lethal surveys included in the EEM guidance document as an alternative when lethal sampling is not feasible. This study assessed concerns with the conventional effect endpoints and with EEM guidance for non-lethal fish population surveys. Additionally, a case study is used to illustrate that non-lethal and lethal fish survey effect endpoints often provide different information. Results from the same case study also indicated that hypothesized fishing pressure effects related to repeated lethal sampling are not supported by a weight of evidence approach for the specific fish species and reference lakes examined. Even though an environmental cost of monitoring was not detected in this case study, it is recommended that the use of non-lethal sampling of fish within the Canadian metal mining EEM program be re-evaluated to promote more sustainable monitoring practices across Canada.

Abatement of inhibitory effects on fathead minnow egg production by increased solids retention time in an activated sludge effluent treatment (PL)

Pierre Martel[1], Allan Elliott[1], Mark Hewitt[2], Brian O'Connor[1]

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Documented reproductive effects of pulp and paper mill effluents on fish include decreased egg production, altered production of sex steroids and decreased secondary sexual characteristics. The chemicals and mechanisms causing these effects are not clearly understood but effluent bio treatment, resulting in a reduction of effluent biochemical oxygen demand (BOD5), has been shown to reduce the inhibition of egg production in laboratory studies with adult fathead minnows (*Pimephales promelas*). However, exceptions were noted in the relationship between BOD5 and effects on fish egg production where final effluents from different mechanical pulp mills with similar BOD5 caused contradictory responses. A review of the activated sludge treatment systems suggested that a longer solids retention time (SRT) may be a factor in the removal of recalcitrant or non-organic species from the effluents. The objective of this study was to investigate if the SRT is a factor in abating effects on egg production. By using mixed liquor suspended solids (MLSS) of different ages in experimental treatments, we determined that final effluent BOD5 removal was not significantly different after treatment by systems operating at SRTs of 5 or 10 days. However, effects on egg production were abated by systems operating at >10 days SRT. Abatement of effects was accompanied by a >90% reduction in dissolved manganese (Mn) in effluents. The dissolved Mn was found to accumulate in the MLSS. This study confirms that in addition to achieving low residual BOD5 concentrations, operating an activated sludge treatment system at >10 days SRT is an additional parameter effective in eliminating effects of effluents on the egg production of fathead minnows in laboratory exposures.

Complex receiving environments: Prospects for a mummichog (*Fundulus heteroclitus*) development and reproductive bioassay as a scientifically-defensible alternative to adult fish surveys in environmental effects monitoring (PL)

Tim Vickers[1], Renée Morais[2]

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Federal Environmental Effects Monitoring (EEM) programs require the evaluation of effects to fish in the receiving environment of pulp and paper mills, and metal and diamond mines. Unfortunately, standard adult fish surveys (AFS) are often difficult or impractical to implement and results are often difficult to interpret due to questions about exposure to effluent or ecology of the fish. Our presentation outlines why three sites in New Brunswick are investing in research to develop a new fish bioassay as an alternative to AFS to address their EEM requirements. The Pulp and Paper Effluent Regulations (PPER) prescribe a cyclical, three-year EEM program for mills that includes an AFS to determine if there are changes in fish population characteristics (i.e., growth, reproduction, condition, and survival) across an effluent concentration gradient, or between exposure and reference areas. Where a standard AFS is not effective or practical, a scientifically-defensible alternative can be proposed for approval by the regulator. A consortium of three mills (Irving Pulp and Paper, Limited, Irving Paper Limited, and AV Atholville), Stantec Consulting Ltd., and Wilfrid Laurier University have proposed the use of a mummichog (*Fundulus heteroclitus*) Development and Reproductive Bioassay (MDRB) as a scientifically-defensible alternative to standard AFS. The MDRB would include an existing standardized mummichog adult reproductive bioassay (ARB) (Bosker et al. 2010) that provides a means to evaluate the EEM endpoints of condition factor and reproductive status but does not evaluate the potential effects on growth, development, or survival. As such, the development of a mummichog Early Life-Stage Bioassay (ELSB) is proposed to augment the ARB by evaluating growth, development, and mortality rates (i.e., survival) of newly hatched larvae. The use of a MDRB to interpret responses of effluent-exposed fish in the lab will be less confounded than results from field-based surveys in complex receiving environments, and shows promise as a potential new, scientifically-defensible alternative to a standard AFS. This presentation will describe the challenges at the three sites, and the rationale and plans for development of the MDRB.

Watershed-Based Monitoring & Assessment

Aquatic site characterization and monitoring using passive sampling technology in Point Pelee National Park, Ontario, Canada (PL)

Tara Bortoluzzi[1], Michael Ryan[1]

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Point Pelee National Park (PPNP) is located on the most southern tip of Ontario, a 15 km peninsula marsh and woodland habitat bordered by Lake Erie. The park provides habitat critical for hundreds of species of migrating birds and insects, including over 53 species at risk, but it is also designated a contaminated site on the Federal Contaminated Sites Inventory (FCSI). The contaminants of potential concern in PPNP are associated with pesticides (mainly DDT, dieldrin and lead arsenate) historically applied from the 1940s to 1970s. As of 2016, PPNP had minimal environmental contaminant data for the aquatic marsh areas of the park. With an objective to maintain the lowest disturbance to the park ecosystem, long-term monitoring and assessment of DDT was carried out using passive sampling devices (PSDs). PSDs bind chemicals from environmental media and provide assessments of the biologically available contaminants. The use of PSDs for monitoring concentrations of contaminants in surface waters may offer a number of advantages over conventional point/grab sampling as they measure contaminants over extended periods, providing time-integrated concentrations (smoother average of exposures), and measure contaminants in very low concentrations compared to typical small-volume sample analysis. PSDs can also act as biological surrogates, reducing the need for lethal sampling of living organisms and determining the bioavailability of contaminants that may fluctuate over time, which is critical in accurately estimating exposure for risk assessments. This research aimed to assess the potential value of passive sampling approaches to provide high-quality contaminant data to effectively and efficiently assess and monitor ecologically sensitive contaminated sites. PSDs were suspended in the water column for 30 days at 10 sites across the marsh in October of 2016 and 2017 to collect surface water data on biopharmaceuticals, metals, and pesticides. Surface water and sediment grab samples, as well as fish, were also collected for comparison to PSD data. Results indicate that the PSDs were more effective than conventional surface water grab samples at detecting DDT concentrations in surface waters of the marsh. DDT concentrations in whole fish samples were significantly correlated with DDT water concentrations as measured by PSDs. Conversely, DDT concentrations in sediments were not significantly correlated with DDT in whole fish samples. Aside from some disadvantages of PSD deployment, the PSDs are a reasonably viable, minimally invasive method for monitoring and assessing DDT in the aquatic environment at PPNP or other ecologically sensitive sites.

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

Andrew Burton[1]

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Pharmaceuticals and estrogenic compounds (estrogens) are contaminants of emerging concern as the risks associated with environmental exposure continue to be a relatively new area of study. Over the past few years, detections of various pharmaceuticals and estrogens in aquatic ecosystems have received international attention due to their potential to cause adverse effects to aquatic life, wildlife and human health. The primary sources of pharmaceuticals and estrogens in surface waters include industrial and municipal wastewater treatment plants which discharge effluent directly to receiving waters. The current state of knowledge on pharmaceuticals and estrogens suggests a variety of factors may govern their concentration, bioaccumulative nature, toxicity, and environmental fate such as the physical and chemical properties of the compounds and characteristics of the receiving water body (e.g., pH, dissolved organic carbon). Despite known effects to aquatic organisms, very few water quality guidelines for the protection of aquatic life have been developed for pharmaceuticals or estrogens. The primary objective of this study was to continue the long-term monitoring of pharmaceuticals (n = 40) and estrogens (n = 17) to determine background concentrations and variability in Manitoba rivers. In addition, the study aimed to characterize these compounds in the influent and effluent samples from a wastewater treatment plant (WWTP) and measure these compounds in the receiving environment. The preliminary results of the study are presented.

Pollution Tracker: British Columbia's coast-wide pollution monitoring network (PL)

Kelsey Delisle[1], Marie Noel[1], Peter Ross[1]

[1]*Ocean Wise*

PollutionTracker is the first long-term, integrated, marine pollution monitoring program in Canada. Blue mussels *(Mytilus sp.)* and nearshore subtidal sediment are being used to monitor spatial and temporal trends of 14 general contaminant classes, including over 400 chemicals, and microplastics along the British Columbia (BC) coast. The collection of high quality baseline data for a wide range of contaminants will inform source identification, emerging risks to sea life, and the effectiveness of regulations and best practices. Partnerships formed with government agencies, port authorities, First Nations, and community groups along the BC coast enabled the successful launch of PollutionTracker in 2015 and the completion of Phase 1 in 2017 (pollutiontracker.org). Phase 1 included the collection and analysis of 51 sediment and 33 mussel samples coast-wide. All 14 contaminant classes were detected in samples, with the highest levels typically found in industrialized and port areas. During Phase 2 (2018-2020), existing PollutionTracker sites will be re-sampled, and new sites will be established to fill spatial gaps along the coast. With the creation of a coast-wide network of partners during Phase 1 and growing interest from coastal communities and industry, PollutionTracker is well-positioned to enable long-term, integrated marine pollution monitoring along BC's coastline.

An adaptive environmental effects monitoring framework for assessing the influences of liquid effluents on benthos, water and sediments in aquatic receiving environments (PL)

Keith Somers[1], Bruce Kilgour[1], Kelly Munkittrick[2], Tim Arciszewski[3]

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Environmental effects monitoring (EEM) has been traditionally used to evaluate the effects of existing facilities discharging liquid effluents into natural receiving waters in Canada. EEM also has the potential to provide feedback to an ongoing project in an adaptive management context, and can inform the design of future projects. Additionally, EEM can and should be used to test the predictions of environmental effects related to new projects. Herein we describe a framework for designing and implementing an adaptive EEM program that utilizes triggers to move between the steps of a six-tiered monitoring program. Movement between tiers is based on the exceedance of Baseline Monitoring Triggers, Forecast Triggers and Management Triggers at various stages in the EEM process. For new facilities, the framework utilizes triggers based on forecasts from the project-planning and pre-development data collection stages that are typically produced in environmental impact assessments. This framework illustrates how traditional EEM can be extended to include the results from environmental impact assessments and the principles of adaptive management.

Cyanolichens on conifers as indicators of air quality in the Kitimat Valley (PL)

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Lichens that grow on conifers, particularly lichens with cyanobacteria as a photobiont (cyanolichens), are among the most sensitive species to acidifying air emissions such as SO_2. Few cyanolichens are reported growing on conifers in regions with elevated SO_2 exposure such as northern Europe and eastern North America. In contrast, the submaritime portions of the Coastal Western Hemlock Biogeoclimatic Zone of British Columbia have very low exposures to acidifying air emissions and support among the world's most diverse and abundant examples of cyanolichens on conifers. The Kitimat Valley is a regional exception as it receives approximately 30 tonnes/month of SO_2 air emissions from a recently modernized aluminium smelter. This study aims to understand if the distribution and diversity of cyanolichens on conifers in the Kitimat Valley are related to SO_2 exposure. We recorded cyanolichen richness at 32 sites throughout the Kitimat Valley at varying distance from the aluminium smelter. We then analyzed sulphur concentrations within cyanolichen samples as comparative measure of exposure. Cyanolichen tissue concentrations were compared with results from IVL passive diffuse samplers distributed throughout the valley to estimate exposure concentrations. We mapped the distribution of sensitive cyanolichens growing on conifers in the Kitimat Valley and compared this distribution with sulphur concentrations in cyanolichen tissue using linear regression. We found that spatial patterns of species richness closely followed exposure to SO_2. We estimated the critical levels

(concentrations) of SO_2 for particularly sensitive genera of cyanolichens. Critical levels of sensitive cyanolichens derived in British Columbia can be used to support the management of lichen diversity and the protection of rare and endangered species. This may also have applications in regions such as Europe where lowering exposures to acidifying emissions present opportunities for the recovery of sensitive species.

Toxicity and benthic alteration in the St. Mary's River Area of Concern: Changes in assessment techniques to determine the need for sediment management (PO)

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Sediment of the St. Mary's River Area of Concern (AOC), located in Sault Ste. Marie, Ontario, has elevated levels of contaminants such as petroleum hydrocarbons, polycyclic aromatic hydrocarbons, and oils and grease. In a well-defined depositional area of the river known as East Bellevue Marine Park (EBMP), sediment toxicity and altered benthic invertebrate communities are evident from past studies and are the focus of potential sediment management. Recently, the benthic invertebrate community from this area has been re-evaluated using revised statistical methods, which involved changes in selection of reference sites for comparison with test conditions. This newer approach, which included the development of new models by multiple linear regression to explain as much variation as possible in benthic community descriptors by habitat variables, was considered superior to previous assessments for the following reasons: 1) the previous assessments used data that were old for comparing to test conditions at the time, and 2) the assessments included data from lower lakes which are no longer considered good references for the upper Great Lakes sites. This revised approach has resulted in changes to overall assessment outcomes for 50% of the sites in EBMP, which has implications for management decisions. The assessment of sediment toxicity remains consistent where results are integrated from laboratory bioassays conducted with four species of benthic invertebrates: *Chironomus riparius, Hexagenia* spp., *Hyalella azteca* and *Tubifex tubifex*. However, this approach does not sufficiently address whether newer deposits are showing improvements in toxicity over time due to the sampling depth of sediment (0-10 cm) and is in contrast with other toxicity studies conducted using sediment from shallower depths (0-5 cm). To address the data gap and advance the decision process for this AOC, toxicity will be assessed from two separate sediment depths concurrently, comparing survival, growth, and reproduction endpoints. Coupled with recent information on the community composition in EBMP, this will provide a more complete picture as to next steps for this AOC. Aspects of the sampling design and survey, due to be carried out this fall, will be discussed.

Molecular Methods in Environmental Monitoring and Impact Assessment

Environmental DNA From a consultant's perspective (PL)

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Effective environmental protection, stewardship, and restoration require timely and accurate information about the status of a given ecosystem and the species that occupy it. Animals shed deoxyribonucleic acid (DNA) as they complete their life processes, and this environmental DNA (eDNA) can be detected via analysis of samples collected from occupied habitats. Studies of eDNA have gained scientific and regulatory acceptance in recent years, especially for surveying at-risk aquatic and semi-aquatic species. eDNA methods are gaining increasing consideration as an attractive (alternative) option for project proponents who are interested in more cost-effective, rigorous, accurate and sensitive methods to inform environmental management considerations. The field effort associated with sample collection for studies involving aquatic taxa is often greatly reduced relative to traditional baited trapping, electro-shocking and/or physical searches, thus enabling more efficient data acquisition over space and time. In addition, this method is non-invasive to the target species and its habitat; reduces the risk of pathogen transfer between sites; is highly accurate and very sensitive to detection of aquatic species; is able to detect the presence of pathogens; and generally is more cost-effective for species that are difficult to detect using traditional methods. Credibility of eDNA survey data, however, depends on adequate methodological validation and verification; accurate results require rigour during field sampling, sample processing, laboratory analysis, and primer design and/or verification. Working in collaboration with Dr. C. Helbing (University of Victoria, Genetics Department) Hemmera has developed accepted standards for collection of eDNA for the British Columbia Ministry of Environment and pioneered the advance of eDNA methods for application by qualified environmental professionals (QEPs) in Canada. Clear evidence of the utility of this approach is demonstrated in the completion of more than 40 eDNA projects in BC and Yukon since 2014 for 18 aquatic taxa, including fish, amphibians, water shrews, and pathogens. This presentation discusses the strengths and limitations of eDNA as a tool for addressing baseline data and monitoring requirements and discusses challenges and solutions for successful adoption of eDNA methods in the environmental sector.

Critical considerations in detecting environmental DNA (eDNA): A lab perspective (PL)

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In toxicological paradigms such as the Adverse Outcome Pathway (AOP), the ultimate desire is to link molecular and organismal indicators to population effects. Evaluation of populations has largely relied upon traditional time-constrained searches that are

particularly challenging for species that are cryptic and/or at low densities. The use of environmental DNA (eDNA) detection as a means to assess population distribution and dynamics is becoming increasingly popular as it is more sensitive and cost-and time-effective than traditional methods. eDNA is any trace fragment of exogenous DNA that is released by an organism into the environment. By obtaining a water sample, information about the presence or absence of a species can be determined by measuring the DNA contained within. Targeted species eDNA detection relies upon the use of primers and probes within the context of quantitative real-time polymerase chain reaction (qPCR). While considerable thought has been placed on eDNA field collection methods, comparatively little scrutiny has been directed to the qPCR-based tests for which eDNA detection poses unique challenges. Critical considerations include proper primer/probe design and validation for specificity and sensitivity, assessment of DNA sample integrity, and the incorporation of appropriate field- and lab-based controls. Based upon experience from over 40 projects and thousands of samples, these factors will be discussed with solutions for enhancing confidence in eDNA testing methods used in environmental assessment and monitoring.

The caudal fin and transcriptome analysis as a non-lethal means of determining biological effects of oil spills in Pacific salmon (PL)

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Low sulfur marine diesel (LSMD) is frequently involved in marine coastal spills, but monitoring ecosystem damage and the effectiveness of cleanup methods remains a challenge. The present study investigates the composition of polycyclic aromatic hydrocarbons (PAHs) dispersed in LSMD seawater accommodated fractions (WAF), the impact of exposure on juvenile hepatic Coho salmon (*Onchorhynchus kisutch*) liver, and the utility of a non-lethal sampling method involving the caudal fin combined with gene expression endpoints for evaluating PAH impact on juvenile Coho salmon. Three WAFs (low, medium, and high concentration) were prepared by adding 52, 165, and 517 g of LSMD to 155 L seawater. The sum of 50 known PAHs and alkylated-PAHs (tPAH50), measured by gas chromatography/triple quadrupole mass spectrometry, showed saturation at ~90 $\mu g \cdot L^{-1}$ at all concentrations. Fish were exposed to 30% diluted WAFs (100-1,000 $mg \cdot L^{-1}$ LSMD) or seawater control for 96-hours. Quantitative real-time polymerase chain reaction (qPCR) analyses were performed on liver and caudal fin from the same genotypically-sexed individuals to evaluate (1) PAH exposure: aryl hydrocarbon receptor α (ahr) and 3-methylcholanthrene responsive cytochrome P450 CYP1A (cyp1a); (2) oxidative stress: superoxide dismutase (sod); (3) general stress: heat shock protein 70 (hsp70); (4) sex hormone activity: vitellogenin A (vtg), vitelline envelope protein gamma (vepg), and cytochrome p450 family 19 (cyp19); and (5) defense against metal exposure: metallothionein A (mta). Sublethal toxic effects of LSMD WAF exposure are shown through significant increase in general and oxidative stress indicators in the liver and alteration of estrogenic activity in both tissues. PAH exposure markers were significantly increased in

both tissues and sexes relative to controls. Transcript abundance of mta was unaffected. The results demonstrate the utility of the caudal fin as a viable, non-lethal source of animal response status upon LSMD WAF exposure.

eDNA studies: A statistician's perspective (PL)

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Measurement of environmental DNA (eDNA) is a powerful method for species detection. eDNA study interpretation requires careful consideration of both field and laboratory components as there are many influencing factors. The inherent complexity of an eDNA study provides unique statistical challenges that must be identified and addressed. This includes defining the study purpose, establishing a sampling plan, choosing appropriate methodology by identifying statistical assumptions, and incorporating model/test validation. Each of these components will be critically examined with a statistician's lens for improving eDNA study design.

Temporal changes in brook trout (*Salvelinus fontinalis*) environmental DNA (eDNA) detection rates in a Grand River sub-watershed, Ontario: Washington Creek case study (PL)

Patricija Marjan[1], Barb Katzenback[1], Andrew Doxey[1], Paul Craig[1], John Giesy[2], Hadi Dhiyebi[1], Mark Servos[1]

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

Monitoring fish populations in freshwater ecosystems is of great importance for drawing conclusions about the systems' health and integrity. Nevertheless, conventional biomonitoring techniques such as electrofishing or seining may put on additional pressure, cause mortalities, and overall may not be adequate for the systems with rare and low-density species. Brook trout (*Salvelinus fontinalis*) is a fish species that inhabits cold streams in the Grand River sub-watersheds, and in Southern Ontario this species is particularly sensitive to the changes in the environment. This is related to climate change, urbanization, and intense agriculture activities in this area that threaten to increase the habitat loss for this species. Environmental DNA (eDNA) is a sensitive non-invasive method that uses genetic material to indirectly infer the presence or absence of species. In this study, we compared the relative sensitivity and efficiency of traditional capture-based and eDNA-based sampling to detect brook trout and to determine temporal changes in detection probabilities depending on fish density. A first order water stream was selected as the study site that was surveyed on monthly bases from March to September, combining traditional electrofishing, eDNA sampling, and stream hydrology measurements. The goal was to determine temporal variations in eDNA signal intensity and relate this to the brook trout population seasonal dynamics. Our preliminary results indicate that the eDNA approach can be successfully used as an alternative to the conventionally used capture-based methods for brook trout biomonitoring in the Grand River tributaries as it can detect

their presence in the stream. An increase in fish density in the summer months correlates with the increased eDNA signal. Although, these early findings are very promising caution should be taken when interpreting these results because physical parameters of the stream, such as high temperatures and low flows, can affect eDNA shedding rates and degradation. Considering that eDNA is a relatively new tool and that there is no general consensus over its methodology as indicated by the availability of various protocols, studying its dynamics over time and in conjunction with quantitative methods can further evaluate its reliability and also help to determine the most suitable seasonal sampling time for eDNA-based brook trout biomonitoring. Moreover, it can help us understand whether this method can be used on its own or to compliment the traditional methods.

Validating environmental DNA metabarcoding methods via conventional taxonomic identification in Ontario's Grand River watershed (PO)

Nathanel Harper[1], Patricija Marjan[1], Michael Lynch[1], Andrew Doxey[1], Mark Servos[1], Paul Craig[1], John Giesy[2], Barbara Katzenback[1]

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Metabarcoding of environmental DNA (eDNA) from water samples is a rapidly developing technique that has been shown to deliver high-resolution information on aquatic communities in Canadian waterways. eDNA shed into the environment from organisms can be non-invasively collected and analyzed as a proxy for organism presence. Metabarcoding strategies, where probable taxonomic identity can be assigned to sequence reads without a priori knowledge of the species present, can be applied to eDNA samples to provide community biodiversity information. The Grand River watershed is the largest in Southern Ontario and the resident population that depends on it is expected to increase by more than 50% by 2050, amplifying the existing anthropogenic pressures on the Grand River and the aquatic organisms that reside within. Despite the socioeconomic and ecological importance of the Grand River and its tributaries, few studies have focused on developing and validating eDNA metabarcoding as a method of non-invasive biomonitoring. This study examines the detection rate of various aquatic organisms such as fish via eDNA metabarcoding and compares them to the detection rates of conventional taxonomic identification methods, allowing for comparisons of the two methods at a species and site level. Immediately prior to conventional surveys, multiple water samples were taken from each reach. Samples were filtered, DNA extracted, and gene barcodes amplified via PCR and sequenced. Sequenced reads were quality checked and then compared against a reference library to assign a probable taxonomic identity. The assigned taxonomic identities were compared to the species detected during conventional taxonomic surveys. Further analysis of the relationship between environmental DNA concentration and organism biomass or abundance is underway. Ultimately, validation of eDNA metabarcoding methodologies in the Grand River watershed will allow the development of non-invasive, widespread aquatic community monitoring and conservation programs based on this technique.

From challenges to recommendations for brook trout (*Salvelinus fontinalis*) environmental DNA (eDNA) method development (PO)

Patricija Marjan[1], Barb Katzenback[1], Andrew Doxey[1], Paul Craig[1], John Giesy[2], Mark Servos[1]

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

Environmental DNA (eDNA) is a relatively new method for determining presence or absence of targeted species based on the extracted genetic material from an environmental sample that does not require handling or sacrificing the species of interest. This method has proven to be useful for monitoring rare but also invasive species in environments that are not easily accessible and especially in those environments that are remote and difficult to survey using traditional observation-based approaches. Although eDNA is rapidly gaining in popularity, even when coupled with more sophisticated next-generation sequencing technologies, there are still some unaddressed method development questions. In general, there is a lack of concisely synthesized studies concerning some of the challenges researchers face when developing a field-based eDNA protocol for targeted fish species biomonitoring. In this study we used brook trout (*Salvelinus fontinalis*) mitochondrial cytochrome b gene to develop a field-based protocol for brook trout eDNA biomonitoring and addressed various steps of the method development. First, we compared the efficiency of eDNA extraction using two commercially available kits (DNeasy PowerWater Kit and DNeasy Blood and Tissue Kit, QIAGEN) from various sample volumes of both environmentally- and lab-derived samples. The results indicated that there is a dose response when the DNeasy Blood and Tissue Kit was used. One of the challenges when working with environmental samples is dealing with untargeted material that is extracted along with the (e)DNA from samples that can inhibit the polymerase chain reaction, resulting in delayed amplification. The current protocol is designed to address this issue and it offers two methods that test for inhibitor presence. The first one is based on a simple placement of the targeted species in a small volume of the aqueous field sample (as little as $\sim 7\ g \cdot L^{-1}$), whereas the later one requires DNA material of the targeted species for developing a quantitative internal standard (e.g., 100 DNA copies$\cdot \mu L^{-1}$). This is a simple and inexpensive way to confirm if the eDNA extraction procedure is working and if there are inhibitors present in the eDNA extract. The protocol that was developed in our lab offers efficient procedures that ensure that the samples extracted and processed are of good quality and provide reliable results. Research reports that discuss technical challenges involved in the eDNA methods are rare but provide insights into the needs for method validation and strict protocols to avoid misleading results.

Late-Breaking Science

Chronic exposure to selenomethionine disrupts social behaviours and alters arginine vasotocin and isotocin gene expression in zebrafish (*Danio rerio*) (PO)

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For many species, social behaviours are critical for finding food or mates, defending territories, and avoiding predators. Many species of fishes live in groups that often shoal or school together to increase their probability of predator detection and escape. Selenium is an essential micronutrient that plays crucial roles in maintaining physiological homeostasis in all vertebrates, including humans. There is a narrow margin between Se essentiality and toxicity, as over-exposure causes bioaccumulation and subsequent toxicity. Here, we investigated the effects of different concentrations of Se (as selenomethionine) on shoaling and antipredator behaviours of zebrafish. While control and exposed fish demonstrated a significant increase in shoal cohesion in response to injured conspecific cues (i.e., alarm cues) at lower concentrations of Se (2.1 and 11.6 $\mu g \cdot g^{-1}$), those exposed to the highest dose (31.5 $\mu g \cdot g^{-1}$) failed to respond properly. Our results also showed that Se disrupts the expression of two important genes in the brain (avt and itnp) that are involved in regulation of social behaviour.

Evaluation of environmental DNA analyses as a tool in identifying biota in cooling water (PO)

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Many industrial and power generating facilities draw large volumes of water to serve as cooling water. Biota in the area of the cooling water intake can be entrained into the cooling water system and potentially impinge on the screens that are used to prevent large foreign material from entering the plant. The loss of these individuals as the result of entrainment and/or impingement has potential impacts on their populations, especially for species at risk. Currently in the nuclear industry, entrainment and impingement studies are required to estimate the number of biota drawn into the facility. For estimating entrainment, methods often involve using a pump or suspended plankton net to filter known volumes of water within the facility's forebay. The action of the pump and/or the velocity of the water occasionally damages fish larva and eggs, making identification difficult. In addition, the entrainment samples comprise a very small proportion of the total volume of cooling water that is used by the facility, and therefore species that are less abundant or rare may not be identified as elements of the entrained community. Environmental DNA (eDNA) offers a non-intrusive method to both assist in identification of collected samples, as well as to identify species that may be in the vicinity of the cooling water intake. In this study, samples were collected from a power plant forebay, as well as a controlled mesocosm using rainbow

trout (*Oncorhynchus mykiss*) fry. A qPCR primer and probe set were used to react with potential alewife (*Alosa pseudoharengus*) from the forebay and rainbow trout from the mesocosm in the sampled water. Species were successfully identified in both situations even with a large difference in densities. The results of this study demonstrate the feasibility of using eDNA in entrainment sampling. Future work will include further examination of temporal and spatial variability, as well as the relationship between eDNA and abundance.

Effects of 17β-trenbolone on adult fathead minnow (*Pimephales promelas*) (PO)

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Metabolites of synthetic steroids that are used in livestock production as growth promoters often are released into surface waters, inadvertently exposing and potentially causing adverse effects to aquatic wildlife. One such steroid is trenbolone acetate, an anabolic steroid used as a growth promoter in beef cattle. Trenbolone acetate is hydrolyzed in the blood into its active form 17β-trenbolone, which acts as an agonist to the androgen receptor. Significant concentrations of 17β-trenbolone are excreted through manure, which can result in exposure of aquatic life such as fish through runoff from feedlots or during field fertilization using manure. Current research suggests 17β-trenbolone impacts reproduction by acting as a potent androgen, thereby decreasing fecundity, altering plasma steroids and changing phenotype. The purpose of this study is to further our knowledge of the specific toxicity pathways of trenbolone by identifying key molecular response patterns that may be altered during exposure to 17β-trenbolone in adult fathead minnows (*Pimephales promelas*), and to link these with apical outcomes of regulatory relevance. Specifically, this study will expose male and female adult fathead minnows to 17β-trenbolone for 21 days in a flow-through system. Samples will be collected after 4 and 21 days, and will be subjected to 'omics (transcriptomics, metabolomics, proteomics) and apical outcomes (histology, meristic parameters, growth, fecundity, secondary sex characteristics, behaviour) analyses, respectively. We predict this research will identify critical biological endpoints and pathways, which could be utilized to predict apical outcomes across different species and chemicals acting through a similar mechanism of action (i.e., androgens). This study is part of the EcoToxChip project (@ecotoxchip).

Sublethal effects of thiamethoxam and a mixture of clothianidin, imidacloprid, and thiamethoxam on early life stages of sockeye salmon (*Oncorhynchus nerka*) (PO)

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Neonicotinoids are neurotoxic insecticides used worldwide on agricultural crops. Few studies have examined the sublethal adverse effects of neonicotinoids at environmentally

relevant concentrations, particularly in fish. Neonicotinoids are water soluble and can inadvertently enter water bodies via agricultural runoff, leaching, and/or spray drift, resulting in exposure to fish and other aquatic wildlife. In order to investigate the sublethal effects of neonicotinoids on wild early life stage sockeye salmon (*Oncorhynchus nerka*), multiple genetic crosses were exposed from one hour post-fertilization through to the swim-up fry developmental stage to the following: 1) 0, 0.15, 1.5, 15, 150 $\mu g \cdot L^{-1}$ thiamethoxam; and, 2) to a mixture of equivalent concentrations of imidacloprid, clothianidin, and thiamethoxam (0, 0.045, 0.45, 4.5, 45, 450 $\mu g \cdot L^{-1}$). In addition to apical endpoints (body length, weight, development, survival, and hatching success), whole body 17β-estradiol (E2) and testosterone (T) concentrations were measured in a subset of fish, in the mixture exposure, reared for an additional 2 weeks into the feeding fry developmental stage. There were no statistical differences in any apical endpoints due to neonicotinoid exposures; however, there were several differences due to genetic cross for these endpoints that warrant further investigation. While gene expression analyses are currently underway, no significant differences were observed in mean whole body E2 and T concentrations in the feeding fry exposed to the mixture of neonicotinoids. These data will provide insights into the risks posed by environmentally relevant concentrations of neonicotinoids in a wild salmon species.

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