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PLATFORM

Using fish to identify areas of concern in the Bow River system

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Distinguishing the effects of point-source or diffuse stressors in urban aquatic environments can be challenging. Primary stressors by-volume in the Bow River through the city of Calgary include stormwater (400 outfalls) and wastewater from 3 major tertiary treatment plants. Operators of the associated infrastructure and industrial facilities are directed by relevant regulatory agencies to characterize specific water chemistry analytes and/or, biological effects data, or both. In an effort to distinguish between and understand acute and chronic responses of resident fish populations to stormwater and wastewater effluent, we have collected longnose dace (Rhinichthys cataractae) at a series of locations at and upstream of the City of Calgary. Additionally, a series of caged exposures and elimination experiments were carried out to support disambiguation between wastewater and stormwater. Key fish performance indicators vary across the City, including condition (>25%), relative liver size (>70%) and relative gonad size (>100%) confirming responsiveness to both stormwater and municipal wastewater inputs. In proceeding to characterize the molecular initiating events that are occurring within wild fish and those subject to controlled experiments, the resulting data is produced to enable an assessment of the reliability of “omics” as an early warning tool of degradation in freshwater ecosystems receiving urban effluents and accordingly their utility in identifying areas of concern.

Keywords: wastewater, stormwater, fish biomonitoring

PLATFORM

Does the stormwater contaminant load in Nose Creek play a role in fish responses in the Bow River in Calgary, AB

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The Nose Creek Watershed is under cumulative human impacts as it flows over a variety of lands uses including rural, agriculture, industrial, and various forms of residential urban development. Previous studies conducted on the Bow River mainstem have identified physiological impacts in fish collected or caged in Bow River downstream of the confluence with Nose Creek. Considering the contribution of Nose Creek to the Bow River and the cumulative effects of various human activities on the watershed, the current study is looking to identify the relative importance of the Nose Creek loading to effects seen in the Bow River. Longnose dace, Rhinichthys cataractae has been collected at six different locations in the Nose Creek watershed and in the Bow River. Fish were measured for length, weight and organ weights, and gonads and livers were preserved for future molecular and physiological analyses. The fish responses show clear effects in Nose Creek, with longnose dace having significant higher condition, liver sizes and gonad sizes than have been detected at other sites in the Bow River basin.

Keywords: Nose Creek, anthropogenic impact, longnose dace, stormwater

PLATFORM

Reproduction, growth, and survival of Hyalella exposed to tertiary-treated municipal wastewater

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Wastewater treatment is becoming an ever-present necessity in modern society with rising demands for fresh, viable water. Therefore, its impact on the natural environment needs to be better understood to set adequate safety standards that ensure the sustainability of water use for both people and the environment. This study evaluates the impacts of tertiary-treated municipal wastewater effluent on the survival, growth, and reproduction of Hyalella azteca, a common indicator species due to their sensitivity to toxic substances and ease of culturing. Resident amphipods were sampled from replicate artificial streams within the Advancing Canadian Water Assets facilities at the Pine Creek Wastewater Treatment Plant in Calgary, Canada, at the end of August 2022 and the start of November 2022. Streams received either 5 or 15% final Pine Creek effluent or Bow River water. There were no significant differences in survival, reproduction, and growth in adult Hyalella compared to the Bow River control treatment when effluent exposure was 5 or 15%. However, neonates collected from adults exposed to effluent during winter and reared in effluent showed significant reductions in survival and growth, and a shift in sex ratio when exposed to 5% effluent. There was also a reduction in reproduction in effluent-exposed amphipods, but reproductive output was delayed and low in general in amphipods collected during winter. These results indicate possible second-generation effects of effluent exposure on Hyalella caused by shifts in reproductive traits or by increased sensitivity to exposure of overwintering amphipods.

Keywords: municipal wastewater, Hyalella, toxicity, sex ratio

PLATFORM

Comparing the sensitivity of early-life stage rainbow trout (Oncorhynchus mykiss) and lake trout (Salvelinus namaycush) to antimicrobial chemicals

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The use of antimicrobial agents in consumer and industrial products can contaminate freshwater systems via wastewater effluent, which may threaten the health of aquatic organisms. There is a need to identify and compare the potential effects of these compounds on Canadian fish species that may be exposed to these compounds in aquatic environments. Therefore, two independent studies were conducted to compare the effects of triclosan (TCS) and chloroxylenol (PCMX) exposure on the sensitive early life stages of two salmonid species, rainbow trout (RBT, Oncorhynchus mykiss) and lake trout (LKT, Salvelinus namaycush). Embryos of RBT and LKT was exposed to TCS and PCMX separately at nominal concentrations of 0.38-400 µg/L for one-week post-swim-up. Mortality and sublethal developmental responses, such as edema, and spinal curvature, were recorded. Overall, we saw the highest incidence of developmental abnormalities in RBT than in LKT. Mortality was highest in both RBT and LKT, with the highest concentration of TCS tested (400 µg/L) resulting in 100% mortality in RBT and 86% mortality in LKT. In the LKT exposure, the highest concentration of PCMX (400 µg/L) resulted in 66% mortality in RBT and 24% mortality in LKT. The median lethal concentration (LC50) for TCS in RBT and LKT was 189.4 µg/L and 77.19 µg/L, respectively. For PCMX, the LC50 in RBT 357.4 µg/L and 734.1 µg/L for LKT. These results suggest that there is differential sensitivity to antimicrobial compounds for the early life stages of two ecologically-relevant salmonid species. This information will aid in developing strategies to minimize ecological risks associated with exposure to these antimicrobials.

Keywords: Antimicrobial Chemicals, Species Comparison, Early Life Stage, Ecological Risks

PLATFORM

Partial life-cycle toxicity of radium-226 to early life stages of rainbow trout (Oncorhynchus mykiss) under chronic exposure conditions

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The chronic toxicity of 226Ra to rainbow trout early life stages was assessed using a modified Environment Canada protocol (EPS 1/RM/28), and a gradient of 226Ra activities (0, 0.1, 0.5, 2.5, 12.5, or 62.5 Bq/L). Using a static renewal design, embryos were exposed to 226Ra in very soft water (10-13 mg/L as CaCO3) representing high bioavailability conditions, with the objective of identifying sensitive toxicity endpoints. The results obtained to date indicated that there were no effects of 226Ra exposure on mortality or on the cumulative time (in degree-days) that it took rainbow trout embryos to develop to the eyed stage, hatch, or swim-up. However, for fry length, mass, and the scaled mass index (SMI), a significant interaction ($p<0.05$) was detected between 226Ra activity and time to swim-up. These results suggest that the fixed effect of activity on lengths, mass, and SMI (a measure of fitness) depended on the cumulative time that the organisms were exposed to a certain 226Ra activity. Significant enhancements ($p<0.05$) were detected for lengths, mass, and SMI at lower 226Ra activities of 0.1 and 0.5 Bq/L as the exposure time increased. Overall, the no observed effect concentration (NOEC) for mortality and developmental endpoints was 62.5 Bq/L, while the lowest observed effect concentrations (LOEC) for lengths and mass was 0.1 Bq/L, and SMI 0.5 Bq/L. Ongoing analyses include bioaccumulation of 226Ra in embryos and fry, deformity analyses, and targeted transcriptomics. To date, these data suggest potential implications for the current mining effluent discharge limit for 226Ra in Canada, which is set at 0.37 Bq/L total 226Ra as the maximum authorized monthly mean activity.

Keywords: Radium-226, fish early life stages, sensitive toxicity endpoints, enhancements

PLATFORM

Developmental and behavioural effects of early life stage exposure to arsenic in zebrafish (*Danio rerio*)

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The current study is designed to investigate the effects of sub-lethal embryonic exposure to arsenic (As) on the early development and behaviour of zebrafish. To this end, zebrafish embryos were exposed to increasing concentrations of arsenic via culture media [0 (control), 5, 10, 50, and 100 µg/L; as arsenite]. Survival, hatching success, and reactive oxygen species (ROS) production and apoptosis were evaluated following embryonic exposure to arsenic from 1 – 120 hour post fertilization (hpf). In addition, to examine the effects of As exposure on larval behaviours, fish were exposed to As from 1 hpf - 26 dpf (days post fertilization). We evaluated the photo-motor response (an indicator of reflexive behaviour) at 5 dpf, thigmotaxis at 15 dpf, social preference at 21 dpf, and novel object recognition at 26 dpf, respectively. Our results indicate that survival rate and hatching success were not affected by embryonic As exposure. However, developmental As exposure was found to increase ROS production and apoptosis in 120 hpf zebrafish embryos, as revealed by DCFDA and acridine orange staining methods, respectively. Moreover, developmental As exposure was also found to impair the neurobehavioural performance of larval zebrafish by increasing anxiety-like behaviour, decreasing locomotion, and disrupting object recognition memory.

Keywords:Arsenic, Behaviour, Zebrafish, Embryos

PLATFORM

Developmental Health Effects of Metformin and Guanylurea on Larval Zebrafish (*Danio rerio*)

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Metformin is the most common first-line oral therapeutic agent used in the treatment of type-2 diabetes, one of the most prevalent chronic diseases in North America. Post excretion, the compound enters wastewater treatment plants where it is partially bio-transformed by bacteria into guanylurea. Both metformin and guanylurea enter freshwater environments as wastewater effluent where they are frequently may encounter aquatic biota. Studies on the effects of these compounds and aquatic fauna are limited especially with respect to early-life stage fish. The aim of this research was to understand the potential influence of metformin and guanylurea on developmental, cardiometabolic and behavioral responses in zebrafish embryos, from the 4-cell stage (3 hours post fertilization, hpf) to first feed (120 hpf). To this end, embryos were exposed to environmentally relevant (0.4, 4, 40 µg·L−1) and supra-environmental (400 and 4000 µg·L−1) concentrations of the two chemicals. Metformin caused an increased mortality and spinal abnormalities in all concentrations compared to controls. and increased pericardial and yolk sac edema at the highest tested concentration. Metformin did not cause alterations in hatch or heart rate over the examined developmental stages. In addition, metformin did not cause alterations in general swimming, light-dark movement, startle response or thigmotaxis, irrespective of exposure concentration. Exposure to guanylurea over the same developmental stages caused a significant difference in mortality at 40 µg·L−1 only. The results suggest that these compounds impact survival and spinal abnormalities but sublethal effects are limited.

Keywords: Metformin, Guanylurea, Aquatic toxicology, Developmental Biology

PLATFORM

A Potential Toxicokinetic Mechanism of Action for 6PPD-quinone Toxicity: Interspecific Differences in Detoxification

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6PPD-quinone is a novel contaminant derived from oxidation of 6PPD in tires and other rubber products. 6PPD-quinone is acutely toxic to select salmonids while other species of salmonids (and non-salmonids) are tolerant to high concentrations in the aquatic environment. The objective of this research was to distinguish species sensitivity using toxicokinetics. Rainbow trout, brook trout and coho salmon displayed respectively increased sensitivity to in vivo acute 6PPD-Q exposure at environmentally relevant concentrations, while westslope cutthroat trout, white sturgeon, chinook salmon and Atlantic salmon were tolerant to high concentrations. Most exposure concentrations and durations varied, however, some similarities were present. Bile was extracted after exposure, frozen, thawed and subsequently diluted in methanol and assessed for metabolites. Semi-quantification of metabolites followed expected trends in most cases, aside from the results for rainbow trout. Ex situ liver perfusions of 6PPD-quinone in rainbow trout livers portrays a rapid extent of biotransformation. Rapid biotransformation and prolonged duration of exposure may explain bile semi-quantification results. Acute toxicity thresholds and metabolite semi-quantifications suggests sensitive species are insufficient detoxifiers of 6PPD-quinone. Semi-quantification of 6PPD-quinone metabolites in bile extracted after in vivo exposures at the same low concentration of 6PPD-quinone for a similar duration could potentially be used for refinement of mortality in vivo assays to distinguish sensitive from tolerant species for risk assessment.

Keywords: 6PPD-quinone, Mechanism of Action, Detoxification, Interspecific Differences

PLATFORM

Characterization of Chloride Exposure Levels and Ecological Risk in the Lake Winnipeg Watershed

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The concentrations of salts in freshwater systems have been increasing globally (from both natural and anthropogenic sources), making the ecological impacts of salinization a pressing concern. We are interested in characterizing the current state of salinity and salinization in the Lake Winnipeg watershed, as evaluated through the lens of a community-based monitoring (CBM) program. Volunteers gathered samples over three field seasons (2020-2022), from more than 130 sites across Manitoba, northwestern Ontario, and North Dakota. The exposure concentrations were compared with freshwater chloride toxicity water quality guidelines to assess the risk to sensitive species. During the three field seasons, multiple sites exceeded the Long-Term Canadian Water Quality Guideline for the Chloride Ion and the United States Environmental Protection Agency's National Aquatic Life Criteria for Chloride (>500 mg/L of chloride). Sites that experienced the greatest salinity levels were in the Red River Valley and near the city of Winnipeg, Manitoba, suggesting that anthropogenic factors may be driving exposure in these regions. Overall, this project will help to provide data that is useful for risk assessment, as well as identify a possible driver of ecological change in the Lake Winnipeg watershed, while also enhancing public participation in its protection.

Keywords: Freshwater Salinization, Chloride, Road Salts, Community-Based Monitoring

PLATFORM

Characterization of the emerging perfluoroalkyl substance replacement, perfluoroethylcyclohexane sulphonate (PFECHS) in vitro and its preliminary investigation in vivo individually and in mixture with other perfluoroalkyl substances

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The widespread application of poly- and per-fluoroalkyl substances (PFAS) has resulted in some substances being ubiquitous in environmental matrices with associated toxic effects. While certain substances of concern have been phased-out or banned, new substances continue to be produced. One such substance is perfluoroethylcyclohexane sulphonate (PFECHS), an analogue of perfluoroctanesulphonic acid (PFOS) which has recently been detected in multiple environmental media around the globe. However, there is little information on the toxic potency of PFECHS and other cyclic-PFAS in general. Initially, this research aimed to characterize PFECHS and elucidate its effects in the aquatic environment using in vitro techniques. The liver cell line RTL-W1 was used to predict the exposure response of PFECHS in rainbow trout, and further analyses focused on membrane effects were completed with rainbow trout leukocytes. PFECHS exposure did not result in apical adverse effects at environmentally relevant concentrations apart from decreasing plasma membrane polarity. However, molecular alterations were observed at exposure concentrations nearing those of environmental relevance, regardless of the chemical analyses that supported PFECHS is not as acutely potent, nor as potentially bioaccumulative as legacy congeners. These results raised concerns about other replacement PFAS, and how they may interact in environmental mixtures containing multiple replacements and legacy substances. Determining the toxic potency of emerging replacement PFAS is an important step that will help better inform the viability of replacements as a strategy for PFAS management in the future.

Keywords: Replacement PFAS, Aquatic Toxicology

PLATFORM

Using zooplankton community response as a measure of ecosystem health within wild rice planted mesocosms fertilized with aquaculture wastewater

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Aquaculture is currently the fastest growing food production system globally. Due to the high density of cultivated organisms and the intensive feed inputs within many of the inland aquaculture systems, the associated waste effluent is typically high in concentrations of phosphorus and nitrogen. There is potential for aquaculture effluent to be used as a sustainable alternative to conventional fertilizers for wild rice cultivation. However, the environmental implications to the receiving aquatic systems must be considered. This study examined the community response of zooplankton exposed to aquaculture wastewater within northern wild rice (Zizania palustris) planted mesocosms. An array of 16 mesocosms (~1400 L in volume) located at R&L Acres, Sperling, Manitoba were amended with 20 cm of soil, 30 cm of water, aquatic invertebrates from a nearby pond, fat head minnows (Pimephales promelas), and were seeded with a commercial strain of wild rice. Mesocosms (n = 10) were treated with a gradient of aquaculture wastewater, resulting in the total phosphorus and total nitrogen loads ranging from 1.15 – 17.27 g and 1.54 – 23.10 g, respectively. Six mesocosms did not receive wastewater additions to serve as references. The zooplankton communities were sampled bi-weekly using passive samplers and enumerated using classical taxonomy identification. Environmental DNA (eDNA) was also sampled from the mesocosms, and zooplankton communities will be identified using DNA metabarcoding. The results of this study will be used to identify the maximum concentration of aquaculture fertilizer that could be applied to wild rice paddies such that the freshwater ecosystem quality remains optimal.

Keywords: Ecosystem health, aquaculture effluent, zooplankton community response, environmental DNA

PLATFORM

Potential toxicity of water and pore water from a pilot-scale oilsands reclamation pond to saline-acclimated Daphnia species

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Significant amounts of tailings and oil sands process-affected water (OSPW) are generated by bitumen extraction in the Lower Athabasca Region, Alberta, Canada. These waste products are potentially toxic to aquatic organisms and require remediation after mine closure. Lake Miwasin is a pilot-scale reclamation pond consisting of consolidated fine tailings overlayed with blended OSPW and fresh water. This study assessed the potential toxicity of Lake Miwasin surface water (LMW) and pore water (LMP) using saline-acclimated Cladocera, consisting of lab strains of Daphnia magna and D. pulex, and native Daphnia species collected in brackish Lake Humboldt (HL) and Lake Miwasin (LM) itself. LMW did not display acute or chronic toxicity test for lab species and native Daphnia sp (HL). Conversely, LMP was acutely toxic to both lab species and native D. pulex (LM). In chronic tests (12-days), LMP negatively affected reproduction in D. pulex (lab), with in reductions in offspring produced. In addition to salinity being identified as a stressor in LMP, toxicity identification evaluation (TIE) phase I findings demonstrated that the additional observed toxicity for D. magna (lab) and D. pulex (LM, native) may be attributed to ammonia and metals in LMP. Further investigations are required to confirm the causality of these stressors in LMP toxicity.

Keywords: oil sands process affected water (OSPW), reclamation, pore water, sediment, toxicity, Daphnia

PLATFORM

Effectiveness of Polymer Treatment in Mitigating Oil Sands Tailings: A Comprehensive Assessment of Treated Tailing Performance in Lake Miwasin

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Waste products of bitumen extraction from oil sands include fine tailings, consisting of water, clay, fine particles, and residual bitumen. Due to their high large volume, high water content, and slow consolidation rates, these tailings have proven difficult to manage and reclaim. One approach to mitigate oil sand tailings is to use polymers to improve tailings solids content, decrease water content, and quicken the consolidation process. This study assessed the toxicity of polymer-treated tailings used as bottom substrate in Lake Miwasin, a pilot-scale end-pit lake. Water and sediment samples were collected from the littoral and limnetic zones of Lake Miwasin in 2020 and 2021. Sediment metal concentrations in both lake zones did not exceed available Canadian Council of Ministers of the Environment (CCME) Sediment Quality Guidelines for the Protection of Aquatic Life-Freshwater. Toxicity testing was conducted using larvae of Chironomus dilutus that were acclimated to reconstituted saline water (RSW) that was formulated to simulate Lake Miwasin bottom water. The larvae were exposed in 14-day static-renewal bulk sediment toxicity tests to littoral and limnetic sediments in combination with either RSW or Lake Miwasin bottom water. Results showed toxicity for both the limnetic (5% survival) and littoral (15% survival) sediments. Sediment pore water testing using static-renewal 10-d exposures of C. dilutus larvae showed minimal toxicity for both the limnetic (40% survival) and littoral (50% survival) pore waters. Whole-sediment Toxicity Identification Evaluation (TIE) was performed to identify the class of toxicant(s) most likely responsible for the observed toxicity

Keywords: Oil sands tailings, sediment toxicity, porewater, toxicity identification and evaluation

PLATFORM

Low adsorption affinity of Athabasca oil sands naphthenic acid fraction compounds to a peat-mineral mixture

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Much of the toxicity in oil sands process-affected water in Athabasca oil sands (AOS) tailings has been attributed to naphthenic acids (NAs) and associated naphthenic acid fraction compounds (NAFCs). Previous work has characterized the environmental behaviour and fate of these compounds, particularly in the context of constructed treatment wetlands. There is evidence that wetlands can attenuate NAFCs in natural and engineered contexts, but relative contributions of chemical, biotic, and physical adsorption with sequestration require deconvolution. In this work, the objective was to evaluate the extent to which prospective wetland sub-grade material may adsorb NAFCs using a peat-mineral mix (PMM) sourced from the Athabasca Oil Sands Region (AOSR). The PMM and NAFCs were first mixed and then equilibrated across a range of NAFC concentrations with moderate ionic strength and hardness that approximate wetland water chemistry. Under these experimental conditions, low sorption of NAFCs to PMM was observed. When NAFCs and PMM were mixed and equilibrated together at environmentally relevant concentrations, formula diversity increased more than could be explained by synthetically combining constituent spectra in-silico. This PMM was rich in cellulose-derived TOC, with low levels of thermally recalcitrant carbon (e.g., lignin, black carbon). The apparent enhancement of the concentration and diversity of components in PMM/NAFCs mixtures may relate to aqueous solubility of some PMM-derived organic materials, and/or matrix effects in the electrospray ionization source, where further investigation is warranted.

Keywords: Oil sands; mass spectrometry; aquatic contaminants; naphthenic acids

PLATFORM

The Long-term Chemical Fate of Crude Oil Released in the Arctic during the Baffin Island Oil Spill (BIOS) Project

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The risks of crude oil spills occurring within the Arctic heighten as ongoing impacts of climate change give rise to increasing amounts of ship traffic. The Baffin Island Oil Spill (BIOS) project was designed in 1979 to further the collective understanding regarding the short and long-term fate and behaviour of petroleum within the Arctic marine environment. A series of experimentally controlled oil releases occurred in Cape Hatt, Baffin Island, NU between 1980-1982 and were left subject to natural weathering processes. The BIOS site was revisited many times; most recently in 2019 to assess the project's original objectives. Collected samples were analyzed for Polycyclic Aromatic Hydrocarbons (PAHs), n-alkanes, branched alkanes, alkylcycloalkanes, hopanes and steranes, and alkylbenzenes using Gas Chromatography Mass Spectrometry. These hydrocarbon groups were detected in concentrations ranging from 0.0486–14.0, 1.15-1170, 0.224–51.7, 0.0643–16.9, 0.213–11.7, and 0.0171–8.60 mg/kg, respectively. Fourteen of the 16 US EPA priority PAHs were detected in concentrations exceeding the CCME marine sediment quality guideline limits, individually ranging from 7.00–640 ug/kg. The Toxic equivalency quotients from these PAHs ranged from 1.40–270 and 1.70–350 ug/kg within the surface and subsurface sediments, respectively. Comparisons with available data from the 2001 BIOS revisitation indicate losses in dimethylphenanthrene and chrysene from 240000–1000 and 8500–640 ug/kg, respectively, suggesting extensive PAH weathering over the past 18 years.

Keywords: the Arctic, Crude Oil, Weathering, Baffin Island Oil Spill (BIOS)

PLATFORM

Cattle manure application methods on hormone concentrations and activities in surface runoff and soil

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Manure application can promote transport of manure-borne hormones and growth promoters to ecosystems. Identifying agricultural practices that contribute less hormones to ecosystems will minimize environmental impacts. This study compared whether different manure application practices contributed (dis-)similar hormone concentrations and activities to water and soil. The three field treatments were 1) commercial fertilizer, 2) conventional manure application (no setbacks from watershed basin centers), and 3) precision manure application (no manure applied in basin centers). Snowmelt, rainfall runoff, and soil were collected from nine watershed basins within the three treatments. Manure materials were also collected. Chemical analysis revealed no measurable analytes in water, soil, or windrow manure. Fecal material direct from cattle contained 17α- and 17β-trenbolone, while pen manure contained 17α-trenbolone. Fecal and pen materials contained androgenic/glucocorticoid activity, and (anti-)estrogenicity, while windrow manure was mostly estrogenic and lacked androgenic/glucocorticoid activity. Snowmelt and rainfall runoff collected pre- and post-manure application was estrogenic. Estrogenicity increased in runoff after conventional, but not after precision, application. No activities were detected in soil. Results suggest precision application may protect water from hormone export if runoff occurs after recent manure-amendment, or before hormones dissipate in manured soils. This field trial supports precision application to mitigate hormone export from manure-amended soils to watersheds.

Keywords: precision agriculture, watersheds, manure-amendment, pharmaceuticals

PLATFORM

Determining Target Organ Toxicity in Sprague Dawley Rats Following Oral Exposure to Complex Groundwater Mixture: Assessment of Dose-Response Relationships of Histopathological, Biochemical, and Neurobehavioral Alterations.

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Groundwater collected from an industrial site containing a complex mixture of contaminants was characterized for toxicity via an oral exposure study with Sprague Dawley rats. Males and females were tested (n=5 /exposure) with drinking water (control group), 10% reference water (taken from edge of property) compared to 0.01, 0.1, 1 and 10% groundwater collected nearest known soil contamination (high impact) for 60 days. Plasma albumin was significantly reduced in the 10% high impact and reference groups compared to control. Plasma alpha 2 macroglobulin, an inflammatory biomarker was significantly reduced in all groups compared to the control. The histological score of hepatic inflammation was elevated in the 1% and 10% high impact groups compared to control. Histopathology revealed acute tubular necrosis in both sexes while the female rats showed a unique pattern of vacuolar degeneration along the cortico-medullary junction. Plasma symmetric dimethylarginine (SDMA), a biomarker of glomerulotoxicity, was significantly elevated in the 1% and 10% high impact groups compared to control. A novel object recognition test after 60 days showed a significant reduction in the discriminatory index at 1- and 24-hr intervals within the 10% high impact group compared to control, indicating memory impairment. Overall, the groundwater collected from both the high impact and reference wells at the study site induced hepatotoxicity, nephrotoxicity, and neurotoxicity. This pattern of effects is consistent with 2,4-dichlorophenoxy acetic acid and medium-high molecular weight hydrocarbons (F2 and F3) driving toxicity of groundwater throughout the site.

Keywords: Complex groundwater mixture, Sprague Dawley rats, Toxicity

POSTER

Not-so-simple detections of neonicotinoids and diamides in prairie streams

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In 16 lower order streams across south-central Saskatchewan, we monitored the concentrations of a suite of neonicotinoids and diamides between 2017 and 2019. Approximately 45% of all samples (176 out of 392) had at least one insecticide detected, with thiamethoxam detected most frequently. Most detection mixtures contained at least one of thiamethoxam, clothianidin, or imidacloprid (98% of detects). About 16% of samples between 2018 and 2019 had diamide detections reflecting their increasing usage in Canada. Thiamethoxam, clothianidin, imidacloprid, cyantraniliprole, and chlorantraniliprole, the five compounds with >5% detection frequency (≥20 total detections), had average concentrations ranging from 15 – 43 ng/L and maximum concentration from 76 – 370 ng/L. While thiamethoxam and clothianidin concentrations were similar between rain events and snowmelt, their average daily loads were greatest during snowmelt ($p<0.05$); suggesting overwintering and spring freshet as a significant source of neonicotinoids. Generally, sub-watersheds dominated by canola and cereals and agriculturally intensive sites had higher neonicotinoid concentrations, yet crop cover and sites only explained a small proportion of the variance. The variation of these insecticides in streams reflects crops/crop rotation practices, precipitation, prairie hydrology, agricultural practices, and environmental conditions, and highlights the need for improved monitoring programs in similar systems across Canada.

Keywords: Pesticides, monitoring, agriculture, exposure

POSTER

Comparing Effects of Chlorpyrifos on Epithelial Barrier Properties in Two Mammalian Intestinal Cell Culture Models

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Mammalian exposure to environmental chemicals occurs daily through ingested pollutants, with the intestine being the primary exposure site. Intestinal epithelial cells from porcine (IPEC-J2) or human (Caco-2) origin are widely used to study the effect of contaminants on intestinal barrier function. However, limited knowledge and comparison of their cell line-specific toxicity responses and regulations exist. In this study, we compared biological responses in both models to the effects of chlorpyrifos (CPF), an organophosphate pesticide commonly measured in water and food. Both Caco-2 and IPEC-J2 cells exhibited concentration-dependent viability reduction after 24-hour CPF treatment with no difference in calculated IC50 between the cell lines. CPF disrupted intestinal barrier function in differentiated IPEC-J2 cells as measured by decreased TEER and increased paracellular permeability to dextran; however, this response was not observed in differentiated Caco-2 cells. We also assessed the effect CPF exposure on the expression of tight-junction (TJ)-related proteins associated with barrier function. Although there was a decrease in barrier function in IPEC-J2, there was no change in expression of TJ proteins. In Caco-2 cells, CPF exposure increased the expression of TJ proteins, suggesting a protective response against CPF-induced damage. Our results show that for CPF specifically, there are differences in cell-specific responses in these two cell culture models. This research highlights the importance of considering cell type-specific sensitivity when assessing intestinal toxicity of environmental chemicals.

Keywords: Intestinal epithelial cell, IPEC-J2, Caco-2, tight junction proteins

POSTER

Exposure, Repellency, and Learned Aversion to Neonicotinoid Treated Seeds in Granivorous Birds

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Neonicotinoid seed treatments are the most widely used insecticides that show significant toxicity to vertebrate wildlife, particularly granivorous birds. This study aims to evaluate 1) the exposure of neonicotinoid seed treatments on granivorous songbirds and 2) assess whether birds avoid or acquire learned aversion to treated seeds following exposure. Field experiments were conducted in spring 2022 on five pre-seeding crop fields to simulate clothianidin-treated seed spills and document visiting wildlife activities using camera traps, RFID PIT tags and nanotags. Three experimental phases were implemented for each site: capture and release (2 days), treatment phase 1 (1 week), and treatment phase 2 (1 week). During treatment phase 1, 2 stations per field site were baited with clothianidin-treated wheat seeds and 2 had untreated seeds. During treatment phase 2, the assigned treatment was reversed for all sites. A total of 7 mammalian species and 17 avian species were recorded by cameras visiting the simulated seed spills, but the RFID and nanotags were not effective. Average consumption durations were higher at untreated stations than treated stations and the number of species occurrences declined after first exposure. Further statistical analysis will be conducted to quantify the consumption duration and number of seeds consumed at each station by species and treatment. This study will be repeated in spring 2023 to increase the sample size and number of sites and test the effect of different seed colourants on avian detection and consumption. The findings of this study will inform the regulation and use of neonicotinoid insecticides in North America.

Keywords: ecotoxicology, pesticides, neonicotinoids, songbird

POSTER

Assessment of 6PPD-quinone levels in urban runoff from different Canadian cities.

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N-(1,3-Dimethylbutyl)-N′-phenyl-p-phenylenediamine-quinone (6PPD-quinone), a transformation product of the rubber tire antioxidant 6PPD found in road runoff, has been found to be acutely toxic to some species of fish. While previous studies have shown the great importance of stormwater as a source of 6PPD-quinone, less is known about environmental and land-use drivers, and spatio-temporal dynamics across a larger study area. Therefore, this study aims to assess the levels of 6PPD-quinone in stormwater from three Canadian cities: Calgary, Edmonton, and Lethbridge. Stormwater samples were collected from these cities throughout May to October 2022 and stored frozen. Samples were extracted using solid phase extraction (SPE) and 6PPD-quinone quantified using Liquid Chromatography-High-Resolution Mass Spectrometry (LC-HRMS). Preliminary analyses show a range from 4.03 ng/L to 1.23 µg/L of 6PPD-quinone in stormwater samples from the City of Calgary. Since the median lethal concentrations (LC50s) of 6PPD-quinone in brook trout and rainbow trout, two species found in the receiving water bodies, are 0.59 and 1.00 µg/L, respectively, the presence of 6PPD quinone in stormwater might affect aquatic ecosystem health adversely. This research will provide information that will help improve the ecological and environmental risk assessment of this important contaminant of emerging concern.

Keywords: 6PPD-quinone, Stormwater, urban runoff

POSTER

Investigating Environmental Sources of the Toxic Tire-Derived Chemical 6PPD-Q

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In 2021, mass mortalities of coho salmon were linked to 6-PPDQ, a transformation product formed from an antioxidant in rubber tires. Since then, the focus of scientific efforts concerning contamination pathways has been on paved roads. However, about half of the road network in Canada consists of dirt roads. Additionally, recycled tire products are becoming common with end uses including landscaping and sports fields. We seek to understand (1) the potential for unpaved road networks to act as a source for 6PPD-Q and (2) the potential for recycled tire products to leach 6PPD-Q under environmental conditions. To investigate this, we collected dirt from the surface of unpaved roads around Saskatoon. Chemical extractions and 72-hour leachate studies will be performed on the collected road surface to detect and quantify potential 6PPD-Q concentrations in leachate. We also performed a leaching study to detect the potential for recycled tire products to leach 6PPD-Q under environmental conditions. To this end, we are collecting rainwater and meltwater that has infiltrated through a layer of rubber crumb or rubber mulch, then quantifying the 6PPD-Q concentrations in the leachate. During these studies, we expect to quantify environmentally relevant concentrations of 6PPD-Q in the collected leachate and road dirt. Preliminary results from the recycled tire product study suggests that the concentrations found in the leachate exceed the median lethal concentration for various salmonids. The results of these novel studies will fill a critical gap in our knowledge and understanding of environmental sources of 6PPD-Q.

Keywords: 6PPD-Q, unpaved roads, recycled tire material, leachate

POSTER

The Impact of 6PPD-Quinone on Cardiorespiratory Physiology of Juvenile Salmonids

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6PPD-quinone is a transformation product of the widely used tire antioxidant, 6PPD. Commonly found in road-way runoff, this compound has been reported to cause acute lethality in a variety of salmonid species. However, additional studies have shown other salmonid species to be insensitive, even at significantly higher concentrations. Sensitive species show distinctive symptoms including gasping, spiraling, increased ventilation, and loss of equilibrium, suggesting a possible impact on cardiorespiratory physiology. Here, we investigated the acute cardiorespiratory effects of 6PPD-quinone to two salmonids of differing sensitivity: a sensitive species, rainbow trout, and a tolerant species, Arctic char. Fish were exposed to 1 or 10 ug/L 6PPD-quinone in respirometry chambers for 48h to assess changes in oxygen consumption. Following exposure, cardiac ultrasound, electrocardiography, and blood gas analyses were used to analyze changes to the cardiovascular system. Data show that 6PPD-quinone exposure does not significantly impact oxygen consumption rates in either species. In rainbow trout, a significant increase in passive ventricular filling and cardiac output and a decrease in end systolic volume and PR interval length were observed, providing evidence of sympathetic stimulation. Symptoms observed following rainbow trout exposure might partly be driven by a significant increase in methemoglobin, resulting in a reduced ability to oxygenate tissues. This is the first study to analyze the toxicity of 6PPD-quinone to the cardiorespiratory system of fishes at environmentally relevant concentrations and provides information invaluable to understanding the mechanism of toxicity.

Keywords: 6PPD-quinone, rubber tire antioxidant, respirometry, cardiac ultrasound

POSTER

Evaluating the transcriptomic points of departure in early-life stage rainbow trout exposed to 6PPD-quinone

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The rubber tire derivative N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone) found in roadway runoff has recently been implicated as a driver of urban runoff mortality syndrome in coho salmon. Further studies have indicated a highly variable species sensitivity distribution. While classical exposures are integral to determining initial species sensitivity, transcriptomic analysis can provide a broader picture of potential gene expression and biological pathway disruption, as well as providing transcriptomic points of departure (tPODs). An early-life stage toxicity test was conducted on rainbow trout (*Oncorhynchus mykiss*) alevins, beginning at hatch. Treatment groups consisted of a water control, solvent control (0.01% dimethyl sulfoxide), and time-weighted average 6PPD-quinone concentrations of 2.35, 1.30, 0.44, 0.20, 0.10, and 0.06 µg/L. Whole-body alevins were sampled after 96 hours of exposure and flash-frozen for transcriptomic analysis. Analysis revealed over 1200 genes disrupted at a log2fold change of 2.0. Several biological pathways exhibited significant changes, including osteoclast differentiation, apoptosis and necroptosis, and pathways involved in inflammation and immune response.

Keywords: 6PPD-quinone, salmonids, transcriptomics, Urban Runoff Mortality Syndrome, tire wear leachate

POSTER

Evaluating the acute toxicity of emerging antibacterial compounds to the luminescent bacterium *Vibrio fischeri* using the Microtox test system

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Quaternary ammonium compounds (QACs) are emerging antimicrobials present in various products such as disinfectants and personal care products. With the surge of the SARS-CoV-2 virus, increased use of sanitizers with QACs as the main active ingredient led to their rise in wastewater treatment plant effluents and in surface waters. Currently, there is a lack of toxicological data concerning the effects of emerging antimicrobials such as QACs and their comparison to other classes of antimicrobials. In this research, the acute toxicity of selected QACs and other antimicrobials, methylisothiazolinone (MIT), chloroxylenol (PCMX) and triclosan (TCS), was assessed using the Microtox© test system with the bacteria Vibrio fischeri. The QACs evaluated in this study were alkyl dimethyl benzyl ammonium chloride (ADBAC), dimethyl dioctadecyl ammonium chloride (DODMAC), didecyl dimethyl ammonium chloride (DDAC), decyl trimethyl ammonium bromide (C10TAB), tetradecyl trimethyl ammonium bromide (C14TAB), and hexadecyl trimethyl ammonium chloride (CTAC). The luminescence activity of V. fischeri was measured at 5- (T5) and 15-minute (T15) time points. For the QACs, IC50 values (inhibitory concentration for 50% luminescence elimination) were in the range of 1.46 to 11.77 mg/L with DODMAC exhibiting the highest toxicity. However, the other antimicrobial compounds tested were marginally more toxic than QACs with TCS exposure eliciting the greatest inhibition on the bioluminescence activity with IC50 values of 0.99-1.04 mg/L. This study provides data support for the toxicological evaluation of antimicrobial compounds including emerging antimicrobials such as QACs.

Keywords: Antimicrobials, Wastewater effluent, Bacteria, Acute Toxicity

POSTER

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

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Antimicrobials are active chemical ingredients formulated to kill and/or inhibit various pathogens including bacteria and fungi. The amplified reliance on antimicrobial products and increased release through anthropogenic activities has made aquatic life increasingly vulnerable to exposure. The main objective of this research was to evaluate and compare the acute (24-hr) cytotoxicity of six antimicrobials to rainbow trout gill (RTgill-W1) epithelial cells under increasing concentrations. The neutral red uptake assay (indicating lysosomal integrity) following OECD 249 guidelines was used to determine effective concentrations causing 50% loss in cell viability (EC50). Preliminary data showed that most assayed antimicrobials were cytotoxic at concentrations above 5 ug/mL to RTgill-W1 cells except for chloroxylenol (PCMX), which showed no observable toxicity at any tested concentration. Quaternary ammonium compounds (QACs) were the most cytotoxic to RTgill-W1 cells compared to other groups of antimicrobials, raising concern that the alternatives replacing legacy triclosan may have a greater impact on fish health. In general, antimicrobial concentrations causing cytotoxicity in the RTgill-W1 cell line are higher than concentrations measured in the aquatic environment. However, these results can be used to inform future in vitro and in vivo studies to evaluate sub-lethal responses and mechanisms of action of QACs and other antimicrobials.

Keywords: Antimicrobials, Wastewater effluent, RTgill-W1, Cell viability

POSTER

Transcriptional responses and developmental effects of antimicrobial compounds on early life stages of rainbow trout (*Oncorhynchus mykiss*)

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Antimicrobial compounds are chemical substances that prevent or reduce the growth and spread of microbial organisms. They form a major part of disinfectants, soaps, and hand sanitizers and their use has increased with the rise of COVID-19. They can be released through wastewater effluent and enter aquatic systems with potential adverse effects on aquatic biota, including fish. In this study, we evaluated the transcriptional and developmental effects of three antimicrobials, triclosan (TCS), chloroxylenol (PCMX) and methylisothiazolinone (MIT), on early life stage (ELS) rainbow trout (Oncorhynchus mykiss). Newly hatched fish were exposed to graded nominal concentrations (water/solvent control, 0.38 – 400 µg/L) of each antimicrobial under static renewal conditions with 70% daily renewal of exposure water. Embryos were sub-sampled at 4 days post hatch (dph) to assess early transcriptional responses using a novel standardized reduced transcriptome qPCR array (EcoToxChip) specific to rainbow trout. The remaining fish were exposed through to 28 dph. Mortality, developmental abnormalities, and time to swim-up were evaluated throughout exposure while fish length, weight, and organ histopathology were assessed at exposure termination (28 dph). Initial histological observations indicate pathological effects of exposure on gills such as lamella fusion, shortened secondary lamella and vacuolization of primary lamella. No pathological effects on liver and small intestines were observed. Analysis of EcoToxChip data is in progress but is anticipated to reveal potential molecular toxicity pathways that could support risk assessment of emerging antimicrobial compounds.

Keywords: Keywords: Antimicrobials, Rainbow trout, EcoToxChip, Histopathology

POSTER

Impacts of Two Antimicrobials on Early-Life Stage Rainbow Trout and Lake Trout Gut Microbiome

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Antimicrobials are contaminants of emerging concern as they are continuously discharged via wastewater effluent in urban environments and their pseudo-persistence may negatively affect fish in the receiving ecosystem. In particular, antimicrobials may alter fish gut microbiomes which serve central roles in the overall condition of hosts and could have important implications for the health of fishes. This study assessed the impacts of two antimicrobials on early-life stage rainbow trout (RBT) and lake trout (LT) gut microbiome. Embryos were exposed from hatch to 28 days post-hatch to six graded concentrations of triclosan (TCS) and parachlorometaxylenol (PCMX). Excised whole gut was collected and 16S rDNA amplicon sequencing of gut microbiome was applied to investigate responses to antimicrobials. Gut microbiome of RBT was dominated by phyla Proteobacteria and Bacteroidota and genera Aeromonas and Pseudomonas, whereas LT was dominated by phyla Proteobacteria and Firmicutes and genera Aeromonas and Ahrensia. Significant changes in Shannon diversity were observed in RBT TCS-exposed fish, with RBT PCMX-exposed fish indicating a marginal response. Marginal difference was detected for LT TCS-exposed fish only. Seven genera correlated with nominal concentrations of TCS for RBT while no genera were significantly correlated for LT. Overall, TCS had the largest impact on exposed fish gut microbiome and RBT gut microbiome had the greatest response relative to LT to antimicrobials. Results of this study provide insights into the potential effects of emerging antimicrobials on alevin rainbow trout gut microbiome with implications on physiological health status of the host.

Keywords: Antimicrobials, Gut microbiome, Dysbiosis, Fish health

POSTER

Comparison of whole RNA-seq transcriptomics and targeted gene expression array following short-term exposure to fluoxetine in adult fathead minnows (Pimephales promelas)

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Traditional ecotoxicity testing programs are impeded as they predominantly rely on slow and expensive animal tests measuring adverse outcomes. Therefore, there is an increasing demand for short-term mechanistic assays that employ molecular endpoints to predict adverse outcomes of regulatory relevance. Such assays include the newly developed EcoToxChip, a qPCR array analyzing 384 targeted genes of interest, paired with the EcoToxXplorer, a web-based differential gene expression analysis tool. This study aims to correlate the transcriptomic (RNA-seq) response of fathead minnows when exposed to fluoxetine to the gene expression results obtained by the EcoToxChip. Therefore, the goal of this study was to determine the application of genetic expression tools in adult fathead minnows using fluoxetine (FLX) as a model compound. Fish were exposed to three FLX concentrations (measured: 2.42, 10.7, and 56.7 µg/L) and a negative control for 96 hours. In the livers of the male fish, RNA-seq analysis revealed 14 and 32 differentially expressed genes at medium, and high FLX treatment groups, respectively. Enrichment analysis of genes from the highest FLX group revealed dysregulation of gene terms involved in fatty acid-related pathways. Analysis of targeted gene expression via the EcoToxChip found a total of 16 dysregulated genes that are associated with immune and endocrine processes. This research demonstrates how gene expression profiling can provide insight into molecular toxicity mechanisms in fathead minnows and the potential to screen environmental contaminants of concern. This study is part of the EcoToxChip project (www.ecotoxchip.ca).

Keywords: NAMs, fluoxetine, transcriptomics, fathead minnow

POSTER

Development of molecular indicators for municipal effluent exposure responses in longnose dace (*Rhinichthys cataractae*) caged in artificial streams

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In this study we determined transcriptomic responses in longnose dace (*Rhinichthys cataractae*; LND) exposed to 5% municipal wastewater effluent (MWWE) over time to uncover molecular signals to be used as indicators of tertiary treated effluents. The Advancing Canadian Water Assets facility imbedded in the Pine Creek municipal wastewater plant (Calgary, AB) has 12 x 340 m raceways receiving treated MWWE or water from the Bow River. LND collected from a reference site in the Bow River (REF) were caged in raceways containing either 5% Pine Creek MWWE (PC) or Bow River water (BR; control) over 28 d. Liver transcriptomes were analyzed in male and female LND sampled on day 7, 14 and 28 from BR and PC, and compared to REF fish on day 0. Differently expressed unigenes (DEUs) in BR and PC female vs male, increased over time and compared to REF LND. Moreover, DEUs in female and male LND within the same treatment showed an increase over time compared to REF LND females and males. Gene Set Enrichment Analysis of LND at BR vs PC over time revealed effects on genes involved in growth, metabolism of carbohydrates and lipids, and immune system on day 7; however, by day 28, 80-90% of the enriched genes were associated with inflammation and pathogen challenging in both sexes. In this study, BR was used as control, but the water system consists of trace amounts of ESOCs from the two plants discharging upstream of Pine Creek, which was confirmed with chemical analyses. This highlights the challenges when identifying gene bioindicators of effluent exposure. Additional efforts will confirm a subset of gene bioindicators of MWWE exposure that can be used in field-based ecotoxicogenomic studies.

Keywords: liver transcriptomes, fish, municipal effluent, caging experiments

POSTER

The development of non-lethal methods for the identification of endocrine disruption in fishes

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The release of endocrine disrupting chemicals (EDCs) into aquatic environments pose a threat to fishes. As these compounds often target steroidogenic pathways, circulating steroid hormone concentrations provide useful information for characterizing the effects of these compounds. Most commonly, this is accomplished via blood sampling; however, this is problematic as fish species often used to study EDCs, such as the fathead minnow, do not provide sufficient sample volume for analyses. Ethical concerns have also been raised surrounding the sacrifice of large numbers of animals both in lab and in field-based biomonitoring programs. As such, the objective of my research is to develop lab- and field-based techniques for non-lethal quantification of steroid hormones in fishes. Specifically this will include (1) the development of a liquid chromatography tandem mass spectrometry (LCMS/MS) method for the quantification of steroid hormones excreted into holding tank water, (2) the use of similar methods for hormone quantification in two additional non-lethal media: mucus and scales, (3) the application of the above methods in-lab for the screening of EDCs, and (4) the application of the above methods in field-based biomonitoring efforts to assess the effects of EDCs on wild fish populations. Although not entirely animal-free, these methods will allow for the identification of EDC targets in whole animal models without requiring animal sacrifice and are thus in line with goals to reduce animal use in research. In addition, the use of such methods in biomonitoring programs will facilitate the conservation of essential wild fish populations.

Keywords: endocrine disruption, fish, non-lethal, steroid hormones, biomonitoring

POSTER

High throughput prediction of hepatic clearance using isolated perfused fish livers in diverse chemical mixtures

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Chemical risk assessment often focuses on screening substances for criteria of persistence, bioaccumulation potential, and toxicity (PBT). Of these criteria, bioaccumulation potential presents unique challenges in effective risk assessment due to the difficulty associated with testing compounds that undergo biotransformation. This difficulty is compounded by the diversity of chemical classes of interest, such as pharmaceuticals, ionizable organic compounds (IOCs), or complex mixtures within the environment. Presently, bioaccumulation assessment relies upon costly whole animal in vivo exposures, or new approach methodologies (NAMs) such as in vitro assays coupled with in vivo-in vitro extrapolation (IVIVE). These approaches have been used to reduce both animal use and uncertainty within assessment frameworks such as REACH, however further refinement is needed to establish the high-throughput predictive tools that are desired by modern chemical legislation. One such refinement involves the use of the isolated perfused rainbow trout liver model at an intermediate level of biological organization. This model can deliver a physiologically relevant measure of hepatic clearance and thus help build an estimate of bioaccumulation potential. In the present study, the hepatic clearance of a diverse mixture of chemicals found within the US EPA's Non-Targeted Analysis Collaborative Trial (ENTACT) trial, as well as nine psychotropic drugs were measured in both isolated perfused trout livers and isolated primary hepatocytes (RT-HEP). Samples were analyzed using liquid-chromatography-high-resolution-mass-spectrometry (LC-HRMS) to semi-quantitatively measure individual chemicals within mixture and calculate hepatic extraction fraction. Our results show that the combination of the isolated perfused liver model with mixtures is a powerful predictive tool for investigating bioaccumulation potential across a vast range of chemicals. Coupled with IVIVE modeling, this approach can achieve high-throughput screening of contaminants with minimized animal use and greater certainty than previous studies.

Keywords: IVIVE, biotransformation, pharmaceuticals, bioaccumulation

POSTER

Tank Farm: Aquatic Mesocosms as Research Tools

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In 2016, InnoTech Alberta, a subsidiary of Alberta Innovates, designed and constructed an aquatic mesocosm facility at its Vegreville site. Since the first study was commissioned in 2017, the facility has been used to investigate the effects of oil sands process affected water and tailings on model aquatic ecosystems. These studies, conducted in partnership with the COSIA Demonstration Pit Lake Joint Industry Project (DPL JIP), were intended to inform reclamation decisions in the Athabasca Oil Sands Region (AOSR) and are expected to be completed by late 2023.

This presentation will provide an overview of the facility's design and construction, emphasizing those aspects which are uniquely valuable to researchers. Informed by lessons learned over the last 6 years, elements of project execution especially important to mesocosm-based experimentats will be discussed.

Keywords: Ecotoxicology, Mesocosm, Facility, Aquatic

POSTER

Advancing the Potential of Mesocosms for Environmental Research: Insights from a Study in Alberta

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Mesocosms are simplified and replicated physical models of 'real world' ecosystems that incorporate many of the same structural and functional components. Mesocosms offer a unique balance between the control of bench-scale experimentation and the realism of field studies. An aquatic mesocosm facility comprising thirty 15 m3 tanks was used to assess the effects of soil and vegetation on aquatic ecosystems. An inaugural experiment was initiated and continued through the winter of 2017/18, utilizing the unique capability of the facility to over-winter. We will present essential methods to evaluate the physicochemical (e.g., pH and conductivity) and biological/ecological response variables (e.g., phytoplankton and macrophytes) that differed due to the presence of soil and vegetation in the mesocosms.

The results indicated that a large amount of soil (2 m3) caused significant changes in water chemistry, and water quality measures (e.g., pH) exhibited relatively large drift over time in mesocosms without soil. Therefore, a smaller volume of soil should be used for future experiments to limit surface and bottom water chemistry variations and avoid artefactual changes in water chemistry parameters. Changes in conductivity in surface and bottom water layers were linked to the brine rejection effect. An integrated depth sampling method should be used in the future to represent the entire water column better and minimize the brine rejection effect during overwintered experiments. Our study enhances the understanding of mesocosm characteristics and informs future experiment design and implementation for better environmental research outcomes.

Keywords: Aquatic mesocosms, Water chemistry, Brine rejection

POSTER

Assessing the effect of a minimally invasive oil spill remediation method on shoreline biofilm communities

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When transporting crude oil there is a probability of a spill that could damage aquatic ecosystems. While crude oil spill and remediation response effects are widely researched for marine ecosystems, there is a knowledge gap on the effects of remediation response efforts on freshwater environments. This study seeks to understand the effect of enhanced monitored natural recovery (eMNR), a non-invasive remediation method, on biofilm communities after an oil spill in freshwater shoreline environments. We used in lake shoreline enclosures and simulated a spill and the eMNR remediation method while comparing to reference enclosures. Chlorophyll a, ash free dry weight, species abundance, and species richness were measured in treated enclosures and then compared to reference enclosures. All oiled+eMNR enclosure had no statistically significant change in chlorophyll a, ash-free dry weight, species richness and abundance compared to the reference enclosures. These results shows that the eMNR remediation could be viable for use in oil spill remediation and cleanup as minimal environmental impact was observed.

Keywords: Remediation, Biofilm, Oil spill, Mesocosm, Freshwater environment

POSTER

Planning for a study into the fate and effects of diluted bitumen in model freshwater salmon-bearing river ecosystems.

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The National Academies of Science (US), the Royal Society of Canada, and others have highlighted significant knowledge gaps regarding the fate and behaviour of crude oil in freshwater environments following spill events. They specifically identified the need for ecosystem-level studies to examine the fate and effects of persistent heavy oils in rivers, and especially fish and their food resources. A multi-year research program into addressing these knowledge gaps for diluted bitumen (aka dilbit) as they pertain to driving impacts on early life stages of Chinook salmon is currently being developed at the Quesnel River Research Centre at the University of Northern British Columbia in the Cariboo Region of the BC Interior. Experiments will include investigation of the effects of suspended sediment, water velocity, turbulence, and volume of oil released, as well as the interaction and impacts of sinking oil with native sediments and associated biota. The authors are soliciting input from the research community regarding, but not limited to, the most significant knowledge gaps to address, experimental design options and modelling approaches, community engagement, and effects endpoint identification as they relate to oil entering river ecosystems. Please feel free to stop by our poster and share your thoughts and ideas!

Keywords: dilbit, Chinook salmon, spill response

POSTER

Chronic radium-226 toxicity and bioaccumulation to the aquatic invertebrate, Daphnia magna

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Uranium mining and milling constitute a vital industry in Canada. However, ore processing generates multiple by-products that contain radionuclides such as radium-226, which is mobile, long-lived, and bioaccumulative under typical environmental conditions. Canada does not currently have a water quality guideline for the protection of aquatic life for radium-226. The aim of this research project is to generate data on radium-226 toxicity to invertebrates, including Daphnia magna. Two 21-day chronic toxicity tests with D. magna were conducted using slight modifications to an OECD guideline with survival and reproduction as the endpoints. The experiments used three activity concentrations and a control treatment, each with 10 replicates with one animal per replicate. The first experiment had treatments of 50, 16, and 5 Bq/L of Ra-226, and the second, 36, 24, and 16 Bq/L of Ra-226. Results from the first experiment showed that 50 Bq/L induced 100% mortality within 48 hours of exposure, while the two other activity concentrations had no significant effects on survival or reproduction. Results from the second experiment showed no statistically significant effects for any of the activity concentrations on survival or reproduction, suggesting a toxicity threshold between 50 and 36 Bq/L of Ra-226. A separate study on Ra-226 bioaccumulation in D. magna was conducted for 21 days using a treatment of 24 Bq/L with three replicates of 80 animals each, and a comparable control treatment. Results revealed a bioaccumulation factor of 10,329 for Ra-226 in D. magna. These results show that Ra-226 can have significant effects on the survival of D. magna at high activity concentrations.

Keywords: Radium, Invertebrates, Bioaccumulation

POSTER

Characterization of periphyton from intermountain waterways in southern British Columbia using eDNA metabarcoding and selenium uptake kinetics

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Selenium (Se) is a naturally occurring element essential to organismal health but can cause toxic effects when its concentrations rise above tolerable biological thresholds due to the release of excess Se through anthropogenic activities. The critical step of Se accumulation in aquatic food webs is its uptake via microbial and algal communities, in particular periphyton, which serve as the initial point of energy fixation, as well as the uptake point of nutrients and chemicals into food webs. Selenium is then transferred via macroinvertebrates to higher trophic level consumers such as fish that are particularly sensitive to dietary exposure of this element. To better understand the dynamics that drive Se uptake at the base of a food web in natural environments, it is essential to study how the initial uptake kinetics of Se are influenced by variations in the periphyton communities. In this context and to gain insight into the relationship between Se uptake kinetics and periphyton community composition, natural periphyton communities were collected from lotic systems with natural Se concentrations in British Columbia. eDNA metabarcoding was then used to quantify the community composition followed by exposure of the periphyton to Se-spiked media to quantify Se uptake kinetics as a function of community composition. Future studies will utilize the same methodology to quantify the composition of periphyton communities from Se-enriched lotic systems and study the potential influences that differences in community composition have on Se uptake kinetics.

Keywords: periphyton, selenium, eDNA, kinetics

POSTER

Effects of macroinvertebrate and biofilm sampling strategies on tissue-based site-specific water quality benchmarks for selenium

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The United State Environmental Protection Agency (USEPA) has proposed a method to derive site-specific water quality benchmarks for total selenium (Se) based on Se bioaccumulation potential from particulate matter via invertebrates to fish. Here, we examined the potential effects of benthic macroinvertebrates (BMI) and biofilm sampling methods (Hester-Dendy artificial substrates vs sediment grab samples) and seasons (summer vs winter) on aqueous Se benchmarks derived for a boreal lake (McClean Lake) receiving continuous low-level Se (< 1 µg/L) input. In summer 2019, biofilm and BMI were sampled at 7 sampling stations on McClean Lake using artificial substrates (n=4) and sediment grab samples (n=3). In winter 2021, samples were collected at 4 stations on McClean Lake (n=3) through ice holes using a sediment grab sampler. Significant differences in tissue-based site-specific aqueous Se benchmarks were observed using data from the two sampling methods (Hester-Dendys: 0.7 µg/L, summer grab samples: 2.5 µg/L) and seasons (winter grab samples 12 µg/L). Differences between sampling methods were attributed to the higher Se bioconcentration in biofilms that grew on the Hester-Dendys relative to biofilm collected from the surface of the grab samples, whereas seasonal differences were mostly attributed to the low trophic transfer of Se to BMI in winter compared to summer.

Keywords: Selenium, water quality benchmark, benthic macroinvertebrates, biofilms

POSTER

Investigating the uptake of selenite and selenate in natural periphyton communities collected from two different lotic systems

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Selenium (Se) is a naturally occurring essential element but also a pollutant of concern in North America and elsewhere. Anthropogenic activities represent significant sources of Se in aquatic environments where it is usually found in inorganic forms (e.g., selenite, Se(IV) and selenate, Se(VI)) in the water phase. These forms of Se are biotransformed into organic Se species at the base of the food web (e.g., by periphyton). This is a critical step for Se accumulation in aquatic ecosystems to levels that can be toxic for higher and more sensitive trophic level consumers. However, little is known about how variations in water quality characteristics affect the uptake and accumulation of Se by periphyton communities in lotic systems. To study uptake characteristics at varying water quality conditions, natural periphyton was collected from two lotic systems in British Columbia, Canada, and exposed to environmentally relevant aqueous concentrations of Se (0.625 µg Se(IV)/L or 2-240 µg Se(VI)/L). Radiolabeled Se as 75Se(IV) at 0.625 µg/L, or 75Se(VI) at 2-4 µg/L, was used to measure the uptake by periphyton. Se(IV) uptake was investigated as a function of pH as well as phosphate or nitrate concentrations, while Se(VI) uptake was investigated at different sulfate concentrations. Results indicate Se(IV) uptake rates increase with higher nitrate concentrations and decrease with higher phosphate concentrations or pH. Higher sulfate concentrations decreased uptake rates of Se(VI). These data will improve our understanding of site-specific uptake kinetics of Se in periphyton and can be used to support predictions of Se accumulation under different water quality conditions.

Key words: selenium, selenite, selenate, periphyton

POSTER

Aquatic Toxicology Monitoring Program for the Faro Mine Remediation Project

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The Faro Mine is an abandoned lead-zinc mine in the Yukon that operated between 1969 and 1998 and is currently under care and maintenance. Mining activities in the area left behind 70 million tonnes of tailings and 320 million tonnes of waste rock that have the potential to leach metals and acids into the surrounding environment. The mine is located on the traditional territory of the Kaska Nations and is upstream from the Selkirk First Nation. The Government of Canada's Crown-Indigenous Relations and Northern Affairs Canada Department (CIRNAC) is responsible for the Faro Mine Remediation Project and has implemented a risk mitigation and adaptive management strategy for surface waters impacted by on-site contaminated water and runoff from waste rock piles. WSP (formerly Golder Associates) was contracted by CIRNAC to monitor surface water quality surrounding the mine site, including their potential acute and chronic toxicity to aquatic receptors, such as rainbow trout (Oncorhynchus mykiss), Daphnia magna, fathead minnow (Pimephales promelas), Ceriodaphnia dubia, Lemna minor, and Raphidocelis subcapitata. The periodic monitoring of surface waters surrounding the Faro Mine is crucial for evaluating the remediation strategies adopted by the Project and ensuring that the abandoned mine maintains acceptable water quality standards throughout the care and maintenance period.

Keywords: Mining, Monitoring, Metals, Remediation

POSTER

Rethinking the Minamata tragedy

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Minamata disease, which occurred during the 1950s and 1960s in Minamata, Japan has been cited as one of the most infamous cases of human mercury poisoning in history. Here, mercury-contaminated effluent was released by a local industrial factory into Minamata Bay, from which local human and wildlife populations consumed fish and seafood products. This resulted in thousands of cases of organometallic mercury exposure, with symptoms ranging from mild to severe with many deaths including those from congenital exposures.

The etiology of this disease was aided by experiments performed in the late 1950s on a small number of felines which were directly exposed to contaminated effluent from the factory. Only two animals were autopsied and preserved after euthanization, one being “Cat 717”. X-ray absorption spectroscopy (XAS), as well as high-energy resolution fluorescence detection-XAS (HERFD-XAS) were utilized to investigate the forms of mercury and selenium remaining within the brain tissue of Cat 717. Further to that, considerations of the chemical processes occurring within the factory resulting in the presumed production of methylmercury were investigated using density functional theory (DFT). The results of this study demonstrated that Cat 717 was exposed to organometallic mercury directly, given the large amount of organomercury present within its preserved brain tissue. As well, DFT calculations surprisingly suggested that an alternative organomercury species, α-mercuri-acetaldehyde, not methylmercury, was the most likely by-product of the chemistry occurring within the factory.

Keywords: Organometallic mercury, X-ray absorption spectroscopy, Minamata Disease, density functional theory