Platform presentations

Platform Session 1: **Contaminants of Emerging Concern**

MONDAY 13.30-13.50: **Lanthanide and pharmaceutical single and joint toxicity effects on charophyte alga, and *Ceriodaphnia dubia* over three generations**

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Nowadays, much attention is being paid to the residues of pharmaceuticals (Pharm) in the aquatic environment and their negative effects on biota. Another group of concern, lanthanides (Ln), are increasingly used in medicine and new technologies. The effects of both groups of substances on aquatic biota have been studied using conventional test organisms with conventional endpoints. However, information on these two "emerging" contaminants at concentrations close to waste and surface waters is still scarce, especially on the effects of mixtures. We conducted long-term multi-generational toxicity studies on *Ceriodaphnia dubia*, in which three subsequent generations (F1-F3) were exposed to the drugs chlorpromazine (CPZ) and diclofenac (DCF), and to lanthanide chlorides, gadolinium ($GdCl_3$) and europium ($EuCl_3$). The observation period for each generation of daphnia was up to 40 days. Environmentally relevant concentrations (0.001, 0.01 and 0.1 mg/L CPZ; 0.1, 1 and 10 mg/L DCF; 0.4, 4 and 40 µg/L Gd and Eu) were selected for treatment. Analysis of survival and two population parameters, growth and reproductive success, showed that the first generation of daphnia was the least sensitive to the selected substances. Survival in the F2 and F3 generations was reduced by both drug treatments compared to the control, while lower mortality was observed at the lowest tested concentrations of Gd and Eu in the F1 and F2 generations respectively. No inhibition of growth or reproduction was observed for the drugs in the F1 generation, whereas both lanthanides showed significant inhibitory effects on population growth. Reductions in population parameters were most often observed at the highest concentrations of each substance in generations F2 and F3. 3-generation data on daphnia will be presented for the Ln-mixture, the Pharm-mixture and their 4-component mixture. The discussion will also include data on charophyte *Nitellopsis obtusa* and crustacean *Thamnocephalus platyurus* with some of the substances.

MONDAY 13.50-14.10: **Direct and indirect effects of antimicrobials on the physiology of detritivores**

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Studies indicate that both leaf-decomposing microorganisms (i.e., bacteria and fungi) and macroinvertebrate detritivores (i.e., shredders) can be affected by antimicrobials via direct and indirect pathways (i.e., via altered microorganism-mediated food quality). However, relatively little is known about these effects on shredders. Therefore, we performed a number of studies the antibiotic ciprofloxacin, the fungicide azoxystrobin or their mixture, which aimed at unravelling the importance of waterborne and diet-related effects on the model shredder *Gammarus fossarum*. During the 24-d long term assays, sublethal effects (the shredders' energy processing and physiological fitness) were assessed when either subjected to a control treatment, to waterborne exposure, a treatment where the animals received leaves that were microbially colonized in the presence of antimicrobials, or a combination of the latter two effect pathways. While ciprofloxacin increased food quality for detritivores reflected in an elevated feeding rate and growth, azoxystrobin caused a reduction in both parameters. The mixture of the antibiotic and fungicide largely followed model predictions using independent action as reference. Ultimately, our data indicate that indirect effects of antibiotics on shredders via the diet-related effect pathway could be more relevant than waterborne exposure. The presentation will also discuss insights generated by ongoing analyses of the gut microbiome. Finally, since shredders play a key role in the leaf litter breakdown of heterotrophic stream ecosystems, diet-related effects might result in implications for the energy dynamics of these systems.

MONDAY 14.10-14.30: **Evaluating the toxicity of legacy and emerging antimicrobial compounds to early life stages of rainbow trout (*Oncorhynchus mykiss*)**

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Products with antimicrobial properties can be released with wastewater effluent and enter into freshwater systems, where they pose a risk to the health of aquatic organisms. While the effects of legacy antimicrobial compounds, such as triclosan (TCS), is well-studied, less is known about the toxicity of emerging alternative antimicrobials. This study aimed to evaluate and compare the effects of TCS and two commonly used and high-volume antimicrobials, chloroxylenol (PCMX) and methylisothiazolinone (MIT) on early life stage rainbow trout (RBT; *Oncorhynchus mykiss*). Embryos of RBT were exposed for 28 days post-hatch (dph) to TCS, PCMX, and MIT separately at nominal concentrations of 0.38 – 400 µg/L. A solvent control of 0.01% DMSO was included for PCMX and TCS while MIT did not require a solvent. Throughout the 28-day exposure, mortality along with sublethal developmental responses (e.g. presence of edema and spinal curvature, time to swim-up) were recorded and water sampled to confirm exposure concentrations. At study termination, individuals were weighed and length recorded. Samples were taken for histopathological assessment and gut microbiome profiling. Preliminary analyses show a reduction in survivability and increased incidence of developmental abnormalities in TCS and PCMX at the higher exposure concentrations as compared to MIT. Analyses of other endpoints is ongoing and will be presented. It is anticipated that this research will provide information on biological effects of antimicrobials on an ecologically relevant fish species and provide data to inform risk assessment of these compounds in Canadian freshwater systems.

MONDAY 14.30-14.50: **Emerging safeners induced toxicities in *Daphnia magna***

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Herbicide safeners have been found in water bodies, including surface water and have been implicated in causing some adverse effects in non-target species of the aquatic environment. This class of chemicals' environmental occurrence, mode of action and effect on non-target species are poorly understood; it is, therefore, important to understand their toxicities to non-target organisms such as *Daphnia magna*. Cyprosulfamide (CYP) and mefenpyr-diethyl (MEF) are emerging safeners which are becoming popular because of their ability to protect many types of crops from damage from herbicides. *D. magna* was exposed to varying concentrations of safeners and herbicides, singly and as a mixture with their co herbicides, following OECD guidelines with little modifications. Concentrations used include 0. 3,6,9,12 and 15 mg/L for MEF; 0, 5, 20, 40 and 90 mg/L for CYP for acute and chronic exposures. Results indicate that CYP did not affect the survival, growth, and heart rate of *D. magna* across all concentrations at 96 hours. However, lower concentrations of MEF increased the mortality rate of *D. magna*. CYP and MEF influence mortality for chronic exposures, while MEF leads to growth retardation and reproduction decline during chronic exposure. Thus, MEF is more toxic to *D. magna* than CYP. The high toxicity of MEF compared to CYP might be related to the high octanol partition coefficient related to cyprosulfamide. This study shows that safeners and herbicides can cause perturbations that may impact freshwater invertebrates and, therefore, should be regulated and considered potentially harmful for regulatory purposes.

MONDAY 14.50-15.10: **Ingestion of plastic by Short-tailed Shearwaters has not decreased in 8 years**

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Short-tailed shearwaters are a migratory species known to have high rates of plastic ingestion, with a 2010 study at Phillip Island, Australia, finding 100% of the fledglings assessed contained plastic in their gastrointestinal tracts. This research sought to monitor plastic ingestion by comparing beachcast fledglings at the same colony after 8 years. No significant differences in overall plastic loads were found, with 98% of individuals containing plastic (n = 52), equating to a mean of 6 particles (107.3 mg) of ingested plastic per bird. Significant changes in the types of plastic being ingested were found, with a higher proportion of user and other plastics compared to industrial pellets, which has also been observed in northern hemisphere studies. Plastics were predominantly user plastic, light in colour, fragmented in shape, and buoyant. The mean diameter of ingested particles was 4.59 mm, with particles over 10 mm being uncommon. Despite the high incidence of plastic ingestion, the majority of birds appeared to have good visceral fat stores, with 92.3% of individuals in the upper half of the condition score scale. Using Fourier-transform infrared spectroscopy, the majority of plastic was identified as polyethylene followed by polypropylene which is largely reflective of world demand, although some plastic types were found in lower proportions than expected.

Platform Session 2: **Human and Regulatory Toxicology**

MONDAY 15.40-16.00: **Assessing human exposure to hydrocarbon mixtures**

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No abstract available.

MONDAY 16.00-13.20: **Environmental compliance for licensees with cross-jurisdictional regulatory oversight**

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Environmental compliance monitoring informs the site environmental risk assessment, confirms effectiveness of mitigation measures, and ensures continued protection of the environment. Regulatory compliance verification is used to confirm that licensees are conforming with applicable laws, regulations and licence conditions, which can consist of review of environmental monitoring reports, inspections and evaluations, but there's often more than one responsible authority. Multiple regulatory bodies (e.g., Canadian Nuclear Safety Commission (CNSC), Department of Fisheries and Oceans (DFO), Environment and Climate Change Canada (ECCC), Saskatchewan Ministry of Environment (SMOE)) may have a mandate to ensure a given licensee conforms to applicable acts (e.g., Nuclear Safety and Control Act, Fisheries Act, Species at Risk Act), regulations (e.g. Canadian Environmental Protection Act 1999 Regulations), and standards (e.g., Canadian Standards Association). The CNSC collaborates and coordinates environmental compliance verification activities with other regulatory bodies for nuclear power plants and uranium mines and mills through working groups and/or implementation of Memorandums of Understanding (MOUs) to ensure effective communication and consistency with licensee enforcement activities. The presentation will provide an overview of CNSC perspective on and experience with collaborative MOUs and/or working groups (e.g., environmental risk assessments, Fisheries Act, Species at Risk Act).

MONDAY 16.20-16.40: **Evaluation of exposure to fine particulate matter and associated health risks to human populations in Eastern India**

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Particulate matter in outdoor air pollution have been classified as carcinogenic to humans by the International Agency for Research on Cancer. With increasing levels of ambient particulate matter in most Indian cities, the risk of developing cancer and other adverse health effects in non-smoking population is also increasing. Thus, the aim of this project is to evaluate the health risks from exposure to fine and ultrafine particulate matter in the Eastern Indian populations. Three different study sites in the state of West Bengal, India were selected for this study: (1) Bolpur- the non-urban reference site, (2) Durgapur – an industrial site, and (3) Kolkata – the major metropolis in Eastern India. Concentrations of various components of air pollutants including particulate matters in the ambient air were determined at each site in different seasons. To assess the human health impacts of exposure to particulate matter at each site, a spirometry test was conducted on non-smoking individuals of both sexes in the age group of 20-50 years. In addition, blood samples were also collected from these individuals for the analysis of a wide array of biomarker responses including hematology, basic blood biochemistry, metabolic and antioxidative enzymes (e.g., superoxide dismutase and myeloperoxidase), oxidation products of lipids and proteins (malonaldehyde and protein carbonyls), and cytokines (tumour necrosis factor-alpha and interleukin-6). Blood samples were also analyzed for various metals and polyaromatic hydrocarbons to understand their exposure levels and associated risks. Finally, blood transcriptomic analysis was carried out to gain insights into the key metabolic pathways that likely mediate adverse health effects due to chronic exposure to fine particulate matter. This presentation will provide an overview of this international collaborative project and discuss some of the initial findings and their implications.

Platform Sessions 3 and 4: **Toxicology of Tire-Wear Chemicals and Run-Off Waters**

TUESDAY 8.20-8.40: **Visualizing basin-scale urban runoff threats to Pacific salmon in the context of ongoing physical habitat restoration (fish passage barrier removal)**

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Salmon conservation in urbanizing watersheds poses unique challenges related to habitat availability, water quantity, and water chemistry. Urban stormwater is composed of thousands of chemicals and is often directly discharged into freshwater spawning and rearing habitats for highly migratory Pacific salmon and steelhead. Untreated runoff has been directly implicated in a basin-scale mortality syndrome affecting coho salmon, and structural equation modeling previously revealed a lethal risk gradient is close association with road density and traffic intensity, among other variables, in urban and rapidly urbanizing watersheds. The recent discovery of the tire-derived chemical 6PPD-quinone as the acutely toxic causative factor in the mortality syndrome for vulnerable salmonids (particularly coho and steelhead) raises important questions about water quality conditions upstream of dams, culverts, and other fish passage barriers lowland watersheds throughout western Washington, Oregon, and California. In Puget Sound alone, for example, this includes potentially thousands of local restoration projects across several large river basins. Accordingly, degraded water quality (in the form of 6PPD-q and related compounds in roadway runoff) has the potential to undermine the conservation goals of those currently working (at the federal, tribal, state, and local levels) to restore physical habitat conditions for salmon. This presentation will explore how salmon metapopulation dynamics and straying will likely influence the unplanned creation of ecological traps at landscape scales. We will also preview emerging geospatial visualization tools can be used to estimate current and future stormwater risks salmonids, to more effectively prioritize habitat investments against a backdrop of rapid human population growth and planned retrofits and expansions to the regional transportation grid.

TUESDAY 8.40-9.00: **Metabolomics provides insights into potential sublethal effects of 6PPD-quinone on the fathead minnow (*Pimephales promelas*)**

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N-(1,3-dimethylbutyl)-N'-phenyl-*p*-phenylenediamine-quinone (6PPD-quinone), an oxidation product of the tire-rubber antioxidant, 6PPD, is an emerging contaminant of concern. 6PPD-quinone is acutely lethal to coho salmon, brook trout, and rainbow trout, but does not cause acute lethality of other fishes, including Arctic charr and white sturgeon. The current study used H1-NMR metabolomics to investigate effects of 6PPD-quinone on the metabolome of fathead minnows, a species that we show is insensitive to acute lethality, to gain insight into potential sublethal effects of this contaminant. Sexually mature minnows were exposed for 96 h to 6PPD-quinone at nominal concentrations of 0, 0.2, 2, or 20 ug/L, and gills and livers were harvested for analysis. Pathway topology analysis identified potential disturbances in 13 biochemical pathways in the gills and 16 biochemical pathways in the livers. In gills, compared to the control, abundances of 17 metabolites were different in low and medium exposures, and abundances of 16 metabolites were different in the high exposure. In livers, abundances of 20 metabolites in the low exposure, 19 metabolites in the medium exposure, and 30 metabolites in the high exposure were different from the control. Tissue specific changes in abundances of metabolites related to one-carbon metabolism, the methionine cycle, and DNA methylation were identified. Notable metabolites include S-adenosylhomocysteine (gills: low = -6.0%, medium = -21.0%, high = -24.5%; livers: low = +32.0%, medium = +26.7%, high = +33.7%), choline (gills: low = -2.7%, medium = -17.8%, high = -23.7%; livers: low = +33.0%, medium = +28.5% high = +37.2%), methionine (gills: low = +8.5%, medium = +10.4%, high = +1.2%; livers: low = +29.0%, medium = +24.1%, high = +29.9%), and homocysteine (livers: low = +35.6%, medium = +35.4%, high: +39.5%). Reasons for tissue specific responses to 6PPD-quinone and functional impacts of these responses are being explored.

TUESDAY 9.00-9.20: **A brief history of the urban runoff mortality syndrome in Pacific salmon and steelhead**

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Many wild salmon and steelhead stocks in the western United States have been at historically low abundances for decades, and numerous distinct population segments are now listed for federal protection under the US Endangered Species Act. Many historical drivers of salmon declines are well known, including overharvest, major fish passage barriers (i.e., dams), loss of floodplain and estuarine habitat, and the proliferation of production hatcheries. The list also includes conventional indicators of poor water quality, including high temperatures, low dissolved oxygen, excessive nutrient and sediment loading, and community shifts towards pollution-tolerant taxa. However, much less is known about the limiting role of anthropogenic contaminants, particularly non-point source inputs of potentially toxic chemicals to freshwater and estuarine salmon habitats. This presentation will review two decades of research by NOAA (together with an extensive network of partners) on a novel and striking mortality syndrome, now widely documented in urban watersheds heavily influenced by runoff from roadways, parking lots, and other impervious surfaces. Field investigations into the phenomenon began in the early 2000s, spurring a series of forensic and modelling studies that eventually culminated in the discovery of a novel toxicant (6PPD-quinpone) in motor vehicle tires. This chemical is ubiquitous in urban stormwater runoff and poses a particularly severe conservation threat to coho salmon (*Oncorhynchus kisutch*). Moreover, initial studies have recently shown that steelhead (ocean-migrating *O. mykiss*) and Chinook (*O. tshawytscha*) are also vulnerable to the acutely lethal impacts of untreated stormwater. Many western US watersheds that currently support ESA-listed salmonids are increasingly under the ecosystem-scale forcing pressures of human population growth, urban/exurban development, and climate change. The urban runoff mortality syndrome has thus become an important case example how these disparate stressors are converging on iconic Pacific salmon, with far-reaching implications for recreational and commercial fishing communities, the cultural heritage of indigenous communities, the transportation sector, urban growth planning, the green economy, municipal stormwater management, and many related societal challenges

TUESDAY 9.20-9.40: **Characterizing urban stormwater-related risks to threatened and endangered salmonids in the western United States**

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Throughout the United States, urban runoff and other non-point sources of pollution have become the foremost water quality threat to aquatic habitats, protected resources, aquaculture, and fisheries. Human population growth continues to drive development and land conversion in coastal watersheds, leading to greater imperviousness and increasing toxic stormwater runoff. The Puget Sound region, for example, is expected to add 1.6 million people by 2030. Urban runoff is an extraordinarily complex chemical mixture, consisting of thousands of distinct compounds, the vast majority of which have not been identified or characterized in terms of adverse environmental effects. Nevertheless, two decades of targeted NOAA science has shown that untreated stormwater is highly toxic to aquatic species, including Pacific salmon and marine forage fish. This presentation will draw from past research on metals (e.g., copper), petroleum hydrocarbons, pesticides, and tire-derived 6PPD-quinone to review core and recurring hypotheses for assessing risk to salmonids managed by NOAA under the Endangered Species Act. Focal topics will include environmental baseline, mixture toxicity, interactions between chemical and non-chemical (e.g., climate) stressors, indirect effects via food webs, and adverse health outcomes that are sublethal or delayed in time, and yet have population-scale consequences through one or more parallel adverse outcome pathways. The connections between these related lines of research, particularly as they influence the adaptive management of ESA-listed species through green infrastructure and similar pollution reduction strategies, will also be discussed.

TUESDAY 9.40-10.00: **Environmental Levels and Acute Toxicity of the Tire Rubber-Derived Chemical 6PPD-quinone in the Canadian Context**

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N-(1,3-Dimethylbutyl)-*N*′-phenyl-*p*-phenylenediamine-quinone (6PPD-quinone) is formed in the environment as a transformation product of the rubber tire antidegradant 6PPD, and has recently been identified as the causative agent responsible for the occurrence of urban runoff mortality syndrome in coho salmon, with a median lethal concentration (LC_{50}) of <0.1 µg/L. Levels of both 6PPD and 6PPD-quinone in stormwater and snowmelt runoff, as well as receiving water bodies, are closely linked to the occurrence of tire wear particles, which despite their elastomeric properties, show similar fate and behavior to microplastic particles. Here, we provide an update on our most recent findings related to this compound in the Canadian context, both with regards to environmental levels, as well as toxicological impacts on fish species of commercial, cultural, and ecological importance.
We were one of the first groups to report the detection of 6PPD-quinone in stormwater and snowmelt runoff from a Canadian city, namely Saskatoon, in samples from 2019–2020. 6PPD-Quinone was detected in 57% (12/21) of stormwater samples with a mean concentration of approximately 600 ng L^{-1} (2019) and greater than 80% (28/31) of snowmelt samples with mean concentrations of 80–370 ng L^{-1} (2019 and 2020). Concentrations of 6PPD-quinone exceeded the acute LC_{50} for coho salmon in most of the stormwater samples. We also investigated the acute toxicity of 6PPD-quinone to rainbow trout, brook trout, Arctic char, and white sturgeon. LC_{50}s in brook trout (24 h) and rainbow trout (72 h) were 0.59 and 1.00 µg/L, respectively. Both species showed characteristic symptoms (increased ventilation, gasping, spiraling, and loss of equilibrium) shortly before death. No mortalities were observed after exposure of either Arctic char or sturgeon for 96 h at measured concentrations as high as 14.2 µg/L. This is the first study to demonstrate the acute toxicity of 6PPD-quinone to other fishes of commercial, cultural, and ecological importance beyond coho salmon, and at environmentally relevant concentrations.
Our research provides urgently needed information for environmental risk assessments of this contaminant of emerging concern and has the potential to form an integral part of potential future regulations regarding the use of 6PPD in car tires.

TUESDAY 10.30-10.50: **Ecotoxicological Assessment of Highway Stormwater Runoff and Particulate Matter using an Extended Fish Embryo Toxicity Assay Battery with Support of *in-vitro* and Chemical Data**

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Awareness and knowledge about road runoff toxicity and tire and road wear particles (TRWPs) have increased in environmental sciences. Yet, the fate and ecotoxicological effects thereof outside of communal wastewater treatment systems are insufficiently understood paired with a lack of data about how TRWPs interact with other road runoff pollutants. Consequently, aquatic environmental risk assessment of TRWP polluted runoff faces the following challenges: (1) Due to structural and chemical variability of TRWP, no standardized sample preparation protocols for (bio-)chemical assessment are available; (2) The lack of environmentally relevant effect data complicates a priori decisions on endpoints of interest to investigate. Therefore, comprehensively investigating the ecotoxicity of TRWP demands a scientific bottom-up approach generating a broad knowledge base covering both chemical and biological effect information for different environmental model scenarios. The project “RoadTox” aims for a quantitative ecotoxicological risk assessment of stormwater runoff sampled from highly frequented urban, country, and highway roads. For this purpose, a comprehensive multi-endpoint bioassay battery paired with hydrological, physical, and chemical characterization of the received runoff and its particulate matter will be performed. Addressed toxicological endpoints include dioxin-like activity, endocrine disruption, mutagenicity *in vitro*, daphnia and algae toxicity, and sublethal, transcriptional and behavioural effects in *Danio rerio* embryos. RoadTox does not solely focus on individual TRWPs alone but on the overall ecotoxicological impact of the road wastewater sample. Based on the quantitative results of the input pathways and the ecotoxicological risk assessment, interdisciplinary recommendations for mitigation measures of tire abrasion inputs will be developed. Here we present the current project state with a special focus on results obtained from an extended fish embryo toxicity assay battery on *D. rerio* embryos combining several sublethal endpoints. The project is funded by the Ministry for Environment, Agriculture, Conservation and Consumer Protection of the State of North Rhine-Westphalia (MULNV), Germany.

TUESDAY 10.50-11.10: **Toxicity of 6PPD-quinone to early life stage rainbow trout**

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N-(1,3-Dimethylbutyl)-N'-phenyl-*p*-phenylenediamine-quinone (6PPD-quinone) has recently been implicated in urban runoff mortality syndrome in coho salmon, leaching from tire wear particles into roadway runoff. Sensitivity to this toxicant is variable across fish species, but previous studies place the LC50 for rainbow trout at 1.00 µg/L. To assess early-life stage sensitivity, rainbow trout larvae were exposed for 28 days to six different concentrations of 6PPD-quinone, beginning at hatch. Larvae were maintained under semi-static conditions, with a 70% water renewal every 24 hours. Significant mortality occurred at 4, 2, and 1 µg/L starting approximately five days post exposure. A subsequent 96-hour acute study with six-week old fry exhibited mortality at 4 µg/L within four hours of exposure, and 100% mortality within 24 hours. Subsequent analysis of samples will focus on histological and transcriptome changes between concentrations and age of exposure.

Platform Session 5: **Ecotoxicology in Freshwater and Marine Systems**

TUESDAY 13.30-13.50: **A flexible pick-list approach for reducing pesticide loadings in freshwater habitats**

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Monitoring studies regularly detect current use pesticides in freshwater habitats. Broadcast pesticide applications can reach aquatic habitats through several transport pathways including aerial drift or runoff. The level of risk posed by these pesticide loadings varies depending upon the pesticide, application method and species. When the pesticide concentrations are estimated to pose a risk of harming species, particularly threatened or endangered species, steps to mitigate these effects by reducing loading are needed. A point system has been derived to arrive at sufficient risk reduction measures protective of aquatic species. The approach achieves reductions in pesticide loading needed to avoid harming species, while allowing maximum flexibility for the grower/applicator. It also rewards landowners who are already implementing reduction measures such as Best Management Practices (BMPs) that reduce loading and improve habitat. The mitigation points needed to reduce exposure is based on different application scenarios and dependent upon chemical, toxicity and application rate. A list of risk reduction measures have been identified from current practices in pesticide application and runoff management. Each risk reduction measure on the list has a point value based on its effectiveness at reducing loading from drift and runoff/drainage. In this approach, applicators look up the point value required based on certain application parameters. Then applicators choose which risk reduction measures to implement as long as the required number of points are achieved for each exposure pathway (drift and runoff/drainage). This approach has been developed with input from regulators at local, state and national levels, agrochemisty stakeholders, pesticide applicators, agricultural groups and farmers. It provides applicators flexibility to select the most feasible options, or combinations of options, for their location, land, and crops.

TUESDAY 13.50-14.10: **Transformation and Attenuation of Bitumen-Derived Naphthenic Acid Fraction Compounds in Athabasca Oil Sands Wetlands**

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During oil sands mining, surface materials and lean oil sands are displaced to overburden stockpiles to facilitate access to bitumen-rich oil sands deposits. Where landscapes are affected by legacies of historical oil sands mine development and activity, there is concern about residual bitumen-attributable toxicity. While bitumen-derived contaminants are complex and diverse, naphthenic acid fraction compounds have been consistently implicated as problematic aquatic bitumen-derived contaminants. In one reclaimed area built on piled mining overburden, named Gateway Hill, an opportunistic wetland formed adjacent to the north-south reclamation project boundary. Surface water samples were collected along Gateway Wetland's north-to-south flow pathway, which were then extracted and analyzed for NAFC concentrations and characteristics using high-resolution Orbitrap mass spectrometry. Based on concentration and assigned-formula data, attenuation of NAFCs was observed sequentially along Gateway Wetland's flow path. Concentrations of total NAFCs decreased, detectable formulae were increasingly oxygen-rich, as well as lower molecular weight. Changes in the molecular characteristics of NAFCs coincided with decreases in selected distress markers during *in-vivo* toxicity evaluations on Fathead minnow (*Pimephales promelas*), as shown by decreasing magnitudes of embryonic heart rate suppression and decreased time-to-hatch. These trends in molecular characteristics and toxicity are consistent with previous work examining the effects of engineered treatment wetlands under controlled conditions. This work provides evidence that mature wetland ecosystems on mining-affected landscapes may be capable of offering important ecosystem services in-line with those observed in engineered systems.

TUESDAY 14.10-14.30: **Estimating Selenium Body Burdens in Freshwater Fish: Do Sampling Methods and Seasons Matter?**

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Selenium (Se) is an essential micronutrient with a narrow essentiality-toxicity window known to bioaccumulate in aquatic food webs. Selenium concentrations above essential levels have been associated with impaired reproduction, development, and lethality, particularly in oviparous species. Despite extensive research, representative modelling of Se transfer to higher trophic level organisms still poses a major challenge to Se risk assessment. This study aimed to predict Se body burdens in two fish species (northern pike and white sucker) inhabiting boreal lakes (Vulture and McClean) downstream from the McClean Lake uranium milling operation. In addition, this study investigated the effects of invertebrate sampling methods (artificial substrates vs grab samples) and seasons (summer vs winter) on the predicted Se concentrations in fish tissue (muscle and ovaries). During the summer of 2019, periphyton and benthic macroinvertebrates (BMI) were sampled using both artificial substrates (n=4) and sediment grab samples (n=3) at 10 sampling stations (two in Vulture Lake and eight in McClean Lake). In winter 2021, periphyton and BMI were sampled through ice holes (n=3) using grab samples (n=4) in McClean Lake. Invertebrates were sorted into the lowest taxonomic level, and trophic transfer functions were calculated for each taxon based on dietary Se concentrations. The predicted Se body burdens in fish were compared to measured Se concentrations in fish collected from these lakes and site-specific Se benchmarks (muscle 13 µg/g d.w; egg-ovaries 19 µg/g d.w.). Selenium body burdens calculated using BMI data from the artificial substrates were overall higher for both fish species than those calculated using BMI collected from sediment grab samples. Moreover, at higher effluent exposure sites, Se concentrations in fish predicted using BMI from grab samples, but not artificial substrates, were comparable to measured Se concentrations in that species. Measured and predicted Se concentrations in fish tissue were above site-specific benchmarks only in Vulture Lake. Finally, the reduced number of winter sites sampled prevented a complete assessment of potential seasonal effects on Se bioaccumulation, but no seasonal effect was identified in this study. Further investigation is necessary to determine if the modelling of Se in fish tissue based on data from the benthic food chain is also influenced by sampling season.

TUESDAY 14.30-14.50: ***Parhyale hawaiensis* as a new model organism in marine ecotoxicology**

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Marine and estuarine areas are extremely vulnerable areas to pollution and their protections is imperative to allow the functioning of this ecosystem. In tropical regions it is common to use temperate model organisms to assess the adverse effect of chemicals and environmental quality. We started to use the circumtropical marine amphipod *Parhyale hawaiensis* in ecotoxicology because a considerable amount of knowledge has been published in the genetics, evolution/development fields. *P. hawaiensis* was collected from a pristine area in Sao Paulo state shore in 2010 and since them has been successfully cultured in our laboratory in standardized conditions. Protocols for acute (96-h) and chronic tests (42 days), both using miniaturized assays have been developed and applied to reference substances and environmental samples. Protocols for the analysis of different endpoints based on genotoxicity, gene expression and regenerative responses were developed and applied to test multiple toxicants. Methods for internal doses determination of metals and organics in hemolymph were developed. To allow the evaluation of immunotoxicity, advances in the characterization of the hemolymph cells were made. We believe that *P. hawaiensis* is a promising model in marine ecotoxicology, but the application of this tropical organism as a global model still require research.

TUESDAY 14.50-15.10: **Food web structure and mercury biomagnification in floodplain lakes of the Juruá River, Amazonas, Brazil**

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Mercury (Hg) poses health risks for fish-eating consumers. Insufficient work on food web Hg has occurred in the tropics and fish consumption is high in regions such as the Amazonian Juruá River. We studied Hg concentrations in fishes from floodplain lakes to determine rates of trophic magnification, assess if concentrations are high enough to impact humans eating fish, and examine seasonal differences. The average trophic magnification factor (increase per trophic level) was 6.6, well above the global average for freshwaters. This led to high concentrations (up to 17.6 mg/kg dry weight) in predatory pirarucu and piranha. 69% of samples had Hg concentrations above the recommended human consumption guidelines. Average concentrations were 42% higher in the dry season than the wet season, but differences varied by species. These observations point to Hg exposure for human populations here and in other tropical rainforest regions, even in the absence of local point sources.

Platform Session 6: **Endocrine Toxicity and Endocrine Disruption**

TUESDAY 15.40-16.00: **Neuroendocrine and behavioural effects of 17α-ethinylestradiol in developing sea bass larvae (*Dicentrarchus labrax*)**

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Endocrine disrupting compounds (EDCs) are a major public health and environmental problem. The regulation of these compounds is imperative and several European regulations already aim to ban them. Identifying and demonstrating the action of these compounds is an imperative and new tests and mechanisms must be deployed by the scientific community. The work presented aims to highlight the effects of a known EDC, ethinylestradiol, on the larval stages of European sea bass and to analyse its mechanisms of action on the HPG axis. Exposures were therefore carried out at different periods of larval development and the expression of genes was measured in the brain. These genes include the brain aromatase cyp19a1b, two kiss isoforms, three gnrh and er receptors. Behavioural measures were associated with the short and medium term, i.e. at 8- and 28-days post-exposure. The results show that EE2 exposure leads to modulation of the neuroendocrine regulation and behavioural disorders in the sea bass.

TUESDAY 16.00-16.20: **The use of fish scale hormone concentrations in the assessment of long-term stress and reproductive status in rainbow trout**

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As human populations continue to expand, the frequency and duration of stressors applied to fishes in both wild and captive settings is on the rise. Fishes exposed to stressful stimuli initiate a stress response mediated by the hypothalamic-pituitary-interrenal (HPI) axis that results in the release of cortisol (F) into the bloodstream. This can be advantageous in situations of acute stress, however when chronically stimulated the stress response can result in adverse effects. In addition to F, the stress response is mediated by numerous steroid hormones. This can include DHEA, an androgen and precursor steroid capable of influencing the conversion of F to the inactive metabolite cortisone (E); as well as gonadal steroids that dictate more downstream effects on reproductive status. Thus, by monitoring changes in select steroid hormones, the magnitude of long-term stress experienced by an organism as well as subsequent reproductive alterations can be assessed. Traditionally, this is done via blood sampling; however, the fish scale, now shown to incorporate F over lengthy periods of time likely provides a better media for the assessment of long-term stress. In an attempt to expand upon this approach we quantified F, E and DHEA in control and stressed trout. Increases in F, E and DHEA observed in stressed trout scales were not reflected in serum samples, adding evidence to the practicality of scale hormone concentration in long-term stress assessment. Further, we successfully quantified progesterone, testosterone and 11-ketotestosterone in rainbow trout scales to allow for the additional assessment of reproductive status. Although these methods are still under development, our results provide promising evidence of the practicality of fish scale hormone concentrations in the assessment of long-term stress in teleost fish.

TUESDAY 16.20-16.40: **Toward a better implementation of regulations on endocrine disrupting compounds: the RedPol project**

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The RedPol project is a French-English partnership funded by the European Union program Interreg. The strategy is based on the development of knowledge and methodologies in order to better implement European regulations on endocrine disruptors and thus, in the long term, to reduce environmental contamination at source. It thus aims to develop new biological tests to analyse new effects and endpoints on a range of aquatic species, including vertebrates and invertebrates, and to describe the associated (neuro)-endocrine mechanisms of action. It also aims to make methodological proposals to improve substance assessments and the determination of environmental quality standards (EQS).

Platform Session 7: **Mechanistic Toxicology**

THURSDAY 8.20-8.40: **Exploring the actions of reactive nitrogen compounds on rainbow trout through fish epithelial cell line models and strategic integration of open-source computational tools**

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The increase of anthropogenically produced reactive nitrogen compounds has raised concerns about its pollution and potential impacts on fish health in natural waters and aquacultures. Epithelial cell lines are gaining use in environmental toxicology and fish physiology research, with RTgill-W1 and RTgutGC, from respectively the gill and gut of rainbow trout, being convenient epithelial lining in vitro models for quickly and inexpensively screening environmental contaminants. This in vitro approach was used to study the effects of individual reactive nitrogen compounds (ammonia, nitrite, and nitrate) on rainbow trout cells through the evaluation of various in vitro endpoints, such as assessing cell viability, monolayer integrity, and epithelial wound healing. These endpoints characterize the *in vitro* toxicological concentration profile of ammonium chloride, sodium nitrite, and sodium nitrate on rainbow trout gill and gut cells, as well as identified unique actions of each reactive nitrogen species on these in vitro cell cultures. Additionally, open-source software platforms were explored, customized, and then implemented to facilitate the automation of rainbow trout invitroomics data processing, which has emerged as a more consistent and efficient protocol compared to conventional analysis procedures. These software additions and strategic back-end software modifications were implemented and consolidated into an open, accessible package with aims to increase the speed of data collection processes as well as improve the quality, reproducibility, and interoperability of the research. These developed protocols and results provide a framework for optimizing the *in vitro* evaluation of reactive nitrogen compounds, and other environmental contaminants, on fundamental cellular activities essential for maintaining a healthy epithelium, such as cell adherence, migration and proliferation, and for identifying the cellular mechanisms of toxicity.

THURSDAY 8.40-9.00: **The role of efflux transporters in cytotoxicity and intracellular concentration of chlorpyrifos and chlorpyrifos oxon in human cell lines**

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Chlorpyrifos (CPF) is a widely used organophosphorus pesticide and long-term exposure has been linked with adverse effects in different organ systems. The variability in toxicological outcomes and organ-specific toxicity of chemicals is due in part to differences in cellular concentration, which is determined by cell-specific transporter and/or metabolism processes. While hazard assessment of chemicals often recognizes available metabolic activity within the target system, the role of transporters and transporter-mediated interactions is only starting to be recognized as a determinant in cell-specific toxicity responses. The aim of this study was to examine how specific efflux transporters (MDR1 and BCRP) influence the cytotoxicity of CPF and its active metabolite, CPF-oxon (CPFO) in human cells. To test this, we determined the cytotoxicity of CPF/CPFO in two commonly used human cell lines, HepG2 (liver) and HK-2 (kidney). We then assessed the expression and inducibility of these efflux transporters and their transcriptional regulators with sub-cytotoxicity exposure to CPF/CPFO. Finally, we examined the functional role of MDR1 and BCRP in CPF/CPFO cytotoxicity using co-exposure of CPF/CPFO with transporter inhibitors verapamil and KO143. Cytotoxicity to CPF/CPFO differed between cell lines where HK-2 had higher IC50 to both chemicals compared to HepG2 cell and is likely attributed to overall lower basal expression and inducibility of drug transporters and nuclear receptors in HK cells. Co-exposure of CPF/CPFO with inhibitors significantly enhanced pesticide cytotoxicity in HepG2 cells, but not in HK-2 cells. Determination of CPF and CPFO concentration in cells and media is underway to identify whether transporter activity directly influences cellular concentration in HepG2 cells. Based on our results thus far, it appears that toxicity of CPF in an organ-specific cell line is affected by specific transporter activity. This raises the question of how transporters may influence mixture toxicity via interaction of CPF with other pesticides and environmental chemicals.

THURSDAY 9.00-9.20: **Validation of methods for *in vitro-in vivo* extrapolation using hepatic clearance measurements in isolated perfused fish livers**

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Bioconcentration factor (BCF) is one of the most common endpoints in chemical risk assessment, informing bioaccumulation status. While BCF as determined from *in-vivo* whole fish exposures is still considered the gold standard to inform this criterion, there is growing concern in academia, governments, and industries about the suitability and reproducibility of this test, especially for chemicals that are biotransformed. Alternative approaches using *in-vitro* biotransformation assays based on hepatocytes or liver sub-cellular fractions in combination with *in-vitro in-vivo* extrapolation (IVIVE) models have been developed as potential replacements. However, extrapolation to BCF is complicated by confounding factors, e.g., extrahepatic biotransformation and quality issues with experimental BCFs. Therefore, there is a need for an *ex-situ* model at an intermediate level of biological organization. A recently developed method is that of the rainbow trout isolated perfused liver, seeking to reduce uncertainty in IVIVE of clearance rates of chemicals. The present study seeks to obtain hepatic clearance data of five environmental contaminants of interest within the isolated perfused trout liver and cross-validate with prior standardized *in-vitro* methods. Livers of sexually immature rainbow trout were cannulated *via* the hepatic portal vein and perfused for up to six hours with a physiological buffer spiked at varying concentrations of pyrene, phenanthrene, 4-*n*-nonylphenol, deltamethrin, and methoxychlor. Afferent and efferent samples were taken in 15-minute intervals across the perfusion period. Samples were analyzed using high-performance-liquid-chromatography with fluorescence detection (HPLC-FLD) and gas-chromatography (GC) to calculate hepatic extraction fraction and clearance. Results demonstrate that this experimental method can be used to validate IVIVE models, as illustrated by the excellent fit of predicted versus measured hepatic clearance values. This study has the potential to settle an important debate in this field and enables scientists to focus on other factors to allow for confident predictions of bioconcentration in fish.

THURSDAY 9.20-9.40: **Epigenetic mechanisms underlying Benzo[*a*]pyrene-induced transgenerational bone toxicity in Japanese medaka (*Oryzias latipes*)**

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Due to bone loss in adults, fragility fractures are projected to exceed $25.3 billion in U.S. medical costs by 2025. Reports in the literature and our preliminary data indicate contributions of parental polycyclic aromatic hydrocarbons exposure to reduced bone health and increased fracture risk in the offspring. Our lab has demonstrated that parental exposure to benzo[*a*]pyrene (BaP) at environmentally relevant doses impairs bone formation in the offspring of exposed Japanese medaka (*Oryzias latipes*), a widely utilized and tractable ecotoxicology fish model. It is hypothesized that epigenetic mechanisms are responsible for bone phenotype inheritance. In a multi-biological level approach, vertebra compression (development) and reduced bone thickness (adult male) at the tissue level were likely associated with reduced osteoblast differentiation and activity, which was revealed at the cellular level through temporal and spatial assessment of bone cells in transgenic medaka strains. Analysis of the bone tissue transcriptome revealed the deregulation of (i) bone metabolism canonical pathways and (ii) BaP-responsive signaling pathways indicating the disruption of the osteoblast-osteoclast interplay during bone metabolism and associated miRNAs on the molecular level. The modified histone- and DNA methylation pattern were identified in bone tissue (H3K4me2; H3K27me2) and bone genes (Sparc) during development and in adult organisms indicating an ancestrally BaP-induced modification of the epigenetic profile in the offspring. The sperm methylome analysis indicated a reduced contribution of paternal DNA methylation to the inherited bone phenotype. The presented data will shed light on the genetic and epigenetic pathways and provide a scientific basis to reassess the impact of environmental BaP on public and environmental health, foreshadowing strategies for early detection of ancestral exposure.

THURSDAY 9.40-10.00: **Novel approaches to assessing interspecies variation in sensitivity to polycyclic aromatic hydrocarbons**

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Polycyclic aromatic hydrocarbons (PAHs) are a diverse group of naturally occurring chemicals that are ubiquitous in the environment. In certain environmental matrices alkyl PAHs are more abundant than the parent compound. Some PAHs can exert toxicity to early life-stages of fishes by activation of the aryl hydrocarbon receptor (AhR). Although there is evidence that alkylation of PAHs could increase potency of AhR activation, little is known in this regard. Additionally, most research on fishes has focused on a small number of species, which likely does not capture interspecies variation in sensitivity to AhR activation. However, assessing toxicity across phylogenetically diverse fishes is not practical due to limitations in time, cost, and challenges associated with certain species. As a step towards characterizing how alkylation affects potency of PAHs, zebrafish (*Danio rerio*) embryos were exposed to benz[*a*]anthracene (BAA) and three alkyl homologues via microinjection to assess lethality. Alkylation increased or did not affect the potency of each alkyl homologue in a position-dependent manner. The most potent alkyl PAH, 8-methylbenz[*a*]anthracene, was 5.6-fold more potent than BAA, 7,12-dimethylbenz[*a*]anthracene was 1.4-fold more potent, and 4-methylbenz[*a*]anthracene was equally potent. Measurements of activation of the zebrafish AhR2 using a standardized *in vitro* AhR transactivation assay yielded relative potencies similar to those observed *in vivo*. To investigate interspecies variation in sensitivity to AhR activation by alkyl PAHs, transactivation assays were conducted for 5 phylogenetically diverse species of fishes. The EC50 range exceeded 300-fold across all tested species-chemical combinations, with Brook trout (*Salvelinus fontinalis*) being the most sensitive, and Northern Pike (*Esox lucius*) being the least sensitive. Due to the abundance of PAHs in the environment and the vast number of species to study, development of predictive tools to assess these toxicities more pragmatically could be essential in enabling objective ecological risk assessments for this class of chemicals.

Platform Session 8: **NAMs in Chemical Screening and Risk Assessment**

THURSDAY 10.30-10.50: **Transcriptomic points-of-departure (tPODs) for chemical hazard assessment in three phylogenetically distant fishes: Case studies for 17α-ethinylestradiol and fluoxetine**

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There is urgent need for developing new approach methods (NAMs) to make chemical hazard assessment more efficient and reduce the use of live animals in toxicity testing. This study aimed to derive transcriptomic points-of-departure (tPODs) from short-term embryo-larval/alevin exposure studies with three phylogenetically distant ray-finned fishes to two contaminants of concern. tPODs were then compared to apical benchmark concentrations described in the literature. Embryos of white sturgeon (WS), rainbow trout (RBT), and fathead minnow (FHM) were exposed to graded concentrations of 17α-ethinylestradiol (EE2) and fluoxetine (FLX) until four days post-hatch and subjected to mRNA sequencing. Transcriptomic benchmark concentration (BMC) analyses yielded tPOD estimates that closely approximated apical PODs found in the literature. tPOD estimates for the median of the 20 most sensitive gene BMCs (omicBMC20) were the most protective estimates across species and across chemicals, while annotation-dependent tPOD estimate derived from pathway enrichment analyses (pathBMC) appeared to be less sensitive. tPODs from RBT were the most protective for both EE2 and FLX, while FHM was the least sensitive species, but all estimates were within the range of reported apical PODs. This study highlighted that, despite some remaining uncertainties, tPODs derived from short-term embryo-larval/alevin exposures were protective of apical PODs and therefore show significant promise as a NAM to support chemical hazard assessment and regulatory decision making.

THURSDAY 10.50-11.10: **Estimating Transcriptomic Points-of-Departure (tPODs) in embryonic rainbow trout exposed to benzo[*a*]pyrene**

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New approach methods (NAMs) are urgently needed to address the high cost, low throughput, and significant ethical concerns associated with the use of live animals in the current toxicity testing framework. A transcriptomic points-of-departure (tPOD) approach from short-term early-life stage fish studies offers a promising approach to address such issues. However, developing NAMs requires careful calibration through multiple lines of evidence. For instance, there has only been little information on the comparison of POD derived from transcriptome-wide data and apical benchmark dose, as well as the line of evidence linking the transcriptomic data to apical outcomes. Thus, this study aimed to estimate and compare tPODs from short-term rainbow trout (RBT; *Oncorhynchus mykiss*) embryo assay to benchmark doses derived from apical outcomes in chronic exposures (aPOD), and to provide weight-of-evidence in the use of tPODs in estimating aPODs. Embryos of RBT were exposed for 4 to 28 days post-hatch (dph) to benzo[a]pyrene (B[*a*]P) at measured concentrations of 0.079, 0.35, 1.5, 7.4, and 28.6 µg/L and solvent control of 0.01% DMSO. Benchmark dose analysis of toxicogenomic data (RNASeq) at 4 dph yielded tPODs of 0.02, 0.15, 1.8, and 0.07 µg/L B[*a*]P for the median of the 20 most sensitive active genes, 10^{th} percentile of all active genes, mode of the first peak of gene-level benchmark doses, and pathway-level tPOD, respectively. After 28 days, morphometric analysis showed significant growth inhibition at >7.4 µg/L B[*a*]P, with notable decreasing trend in body weight. Molecular pathways, biochemical responses, histological alterations, and physiological responses supported the canonical B[*a*]P toxicity pathway model. This study showed that molecular perturbations at 4 dph lead to biological responses at more advanced life stages, providing a strong support for the derivation and use of tPODs to estimate benchmark doses that are associated with adverse outcomes. This study is part of the EcoToxChip project (www.ecotoxchip.ca).

THURSDAY 11.10-11.30: **Maternal transfer and toxicity pathways of hexabromocyclododecane (HBCD) in the fathead minnow (*Pimephales promelas*)**

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Hexabromocyclododecane (HBCD) is a persistent organic pollutant (POP) that undergoes maternal transfer and hinders development and growth of early-life stages of fish. However, there is limited understanding of the maternal transfer kinetics and subsequent molecular mechanisms that drive the embryotoxicity of HBCD. The purpose of this study was to (1) characterize the accumulation of dietary HBCD (11.5, 36.4, 106 mg/kg, ww) in adult fathead minnows (FHM) and the subsequent maternal transfer kinetics to eggs, and (2) link transcriptomics responses to apical and physiological effects in larvae exposed through maternal transfer at seven- and 14-days post-fertilization (dpf), respectively. Maternal transfer kinetics displayed similar egg-to-muscle ratios (EMR) in the low and medium treatment groups (1.65 and 1.27, respectively). However, the high treatment group deviated from other treatments with an EMR of 4.2, potentially due to reaching diffusion and/or lipid saturation limits. A positive correlation was observed between egg HBCD concentration and time of exposure. Larvae sampled at 7dpf revealed dysregulation of pathways involved in membrane integrity (inhibition of calcium channel) and metabolic processes (downregulation of amino acid, glucose, and lipid biosynthesis), while the larvae reared for 14 days exhibited a significant decrease in survival at the highest treatment condition. These results indicate that maternal transfer of HBCD is of concern in fish, which may act through indirect mechanisms involving the inhibition of membrane transport leading to disruption in metabolic processes, collectively resulting in energy depletion and subsequently mortality. This study is part of the EcoToxChip project (www.ecotoxchip.ca).

THURSDAY 11.30-11.50: **Assessment of the effects of exposure to environmental chemicals on oxidative stress in *Caenorhabditis elegans***

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Oxidative stress is a consequence of an imbalance between the production of reactive oxygen species (ROS) and the three-phase detoxification system. The damage caused by oxidative stress is linked to various human diseases such as atherosclerosis, diabetes, as well as neurodegenerative syndromes and aging. ROS are produced by the metabolism of toxic or reactive compounds which is part of the three-phase detoxification system. Phase 1 enzymes converts a xenobiotic into metabolites that are then solubilized by phase 2 enzymes prior to excretion by phase 3 transporters. To study the environmental factors that influence oxidative stress, this project uses a transgenic strain of *Caenorhabditis elegans* engineered with green fluorescent proteins (GFP) tagged to the glutathione S-transferase 4 (gst-4p::GFP) gene encoding a phase 2 enzyme as a biomarker for oxidative stress. This strain was exposed to the US EPA ToxCast library containing ~5,000 unique chemicals at a final concentration of 100 μM at the adult stage for 24 hours in 96 well microplates at 20°C. The plates were screened automatically in a Cytation-5 microplate reader to detect changes in GFP expression. The results of the screen identified 59 chemicals that induced oxidative stress, indicated by an increase in gst-4p::GFP signal. The exposure was repeated for the 59 chemicals at concentrations ranging from 0 to 200 μM, and the top 10 chemicals demonstrating a qualitative GFP signal dose-response relationship were selected for further gene expression analysis. Quantitative PCR was used to measure the changes in mRNA expression corresponding to various enzymes and proteins involved in the three-phase detoxification system to confirm the induction of oxidative stress. Overall, this study identified new oxidative stress generating chemicals in our environment that are likely to exhibit cellular toxicity.

Poster presentations

Poster 1: **Possible causal factors for declines in intertidal invertebrate populations around Fukushima Daiichi Nuclear Power Plant After the 2011 Great East Japan Earthquake, tsunami, and nuclear disaster**

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We discuss possible causal factors for declines in intertidal invertebrate populations around Fukushima Daiichi Nuclear Power Plant (FDNPP) after the 2011 Great East Japan Earthquake and subsequent tsunami and nuclear disaster on the basis of existing knowledge about the effects of radionuclides and ionizing radiation on aquatic organisms. We found a gap between effects observed in the laboratory and those observed in natural aquatic environments, and discuss possible reasons why. Considering the complexity of the environment, we conclude that it is critical to evaluate the effects of ionizing radiation combined with other biotic and abiotic environmental factors, together with the life-history traits of the species examined, for realistic assessment of population-level effects. Finally, we present possible causal factors for a strange or abnormal phenomena observed in intertidal biota near FDNPP, namely declines in population densities and number of species of invertebrates, delayed recovery from these declines, and continuous sexual maturation in the rock shell population.

Poster 2: **Trophic transfer of metals from seaweed to shellfish in aquaculture systems**

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Kathryn Van Alstyne, Shannon Point Marine Science Center, Western Washington University

As the global human population continues to rise, there will be an increased demand for food. Some of this demand could be met through seafood aquaculture. One way to sustainably optimize the production of aquaculture systems is through integrated multitrophic aquaculture, the co-culturing of organisms, such as seaweeds and shellfish, that occupy different levels of the food web. In a seaweed-shellfish system, seaweed provides detritus for filter-feeding bivalves and bivalves provide nutrients for seaweeds. In this type of system, a potential risk to human health is that metals accumulated by seaweeds could be transferred via filter-feeding to the bivalves, which are then consumed by humans. The purpose of our study is to determine if co-culturing bivalves with seaweed causes increased metals concentrations in bivalves and whether these increases create health concern to human consumers. Specifically, we are: 1) measuring concentrations of metals in co-cultured sugar kelp (*Saccharina latissima*) and oysters (*Crassostrea gigas*), as well as in the surrounding seawater; 2) determining maximum consumption levels of sugar kelp and oysters that would be protective of human health; and 3) conducting mesocosm experiments to confirm if elevated metal concentrations in kelp can be transferred to oysters in measurable amounts. The results from the co-cultured field work will be presented along with preliminary data from the maximum consumption levels and mesocosm work.

Poster 3: **The life-cycle toxicity of binary metal mixtures on *Daphnia magna***

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Tham Hoang, Auburn University

We characterized the life-cycle toxicity of binary metal mixtures on *D. magna* using modified ASTM protocols. A titration design was used to assess the effects of Zn/Ni/Cd alone and Cd/Zn, Cd/Ni mixtures on neonate *D. magna* for 21 days. Reproduction, growth, survival, and bioaccumulation were measured as the endpoints. Cadmium was highly toxic to *D. magna* in both mixture studies. In a mixture with 40, 80, and 120 µg/L Zn, Cd toxicity was reduced in all endpoints. A similar reduction was observed in a mixture with 20, 40 and 80 µg/L Ni, but only on survival and growth. These results indicated less-than-additive toxicity. Concentrations > 120 µg/L Zn and > 80 µg/L Ni appeared to contribute to toxicity, and thus, exceeded the necessary concentration needed to induce protective effects. Metal bioaccumulation in the tissues of surviving Daphnids does infer a competitive binding mechanism (i.e., supports the Biotic Ligand Model), but embryonic effects do not support it.

Poster 4: **Radium Effects on Aquatic Organisms**

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In the nuclear fuel cycle, mining, milling, and refining uranium result in by-products (wastewater, tailings, and rock stockpiles) that are potential ecosystem hazards. Radium (^{226}Ra), a decay product of uranium, is of particular importance because of its long half-life (~1600 years), hazards associated with its immediate daughter, Radon, high geochemical mobility, and bioaccumulative potential. Although there are strict regulations for ^{226}Ra in mining effluents, no Canadian federal guideline exists for the protection of aquatic wildlife. Therefore, the overarching objective of this project is to generate essential radium ecotoxicity data for 13 key aquatic species that will subsequently be disseminated to Canadian environmental regulators and nuclear industry members. To generate this information, a series of standardized toxicological assays with fishes (*Oncorhynchus mykiss* and *Pimephales promelas*), invertebrates (*Daphnia magna* and *Chironomus dilutus*), fingernail clams (*Psidium* sp.), pond snails (*Lymnea stagnalis*), algae (*Chlamydomonas reinhardtii*, *Chlorella fusca*, and *Raphidocelis subcapitata*) and aquatic macrophyte (*Lemna minor*) will be performed, for use in the generation of a species sensitivity distribution that will be used in the establishment of a water quality guideline for ^{226}Ra in Canadian aquatic ecosystems. Preliminary data will be presented.

Poster 5: **Intergenerational effects of dietary arsenic on the reproduction and development in zebrafish (*Danio rerio*)**

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The toxicity of dietary arsenic (As) in fish is currently not very well-understood. The major objectives of the present study were: (i) to investigate the effects of chronic dietary As exposure on the reproduction in adult zebrafish (F0 generation) and early development of offspring (F1 generation), and (ii) to examine whether these effects persist in F1 generation when raised without any exposure to As. F0 zebrafish were exposed to different concentrations of dietary As (control, 30, 60 and 100 µg/g dry weight; as arsenite) for 90 days. Following exposure, As-exposed females were mated with control males (maternal exposure (ME)) and vice versa (paternal exposure (PE)). In ME groups, a concentration-dependent reduction in fecundity, hatching success, and larval survival was observed relative to the control. In contrast, fish fecundity was not affected in any PE groups, however a significant decrease in hatching success and larval survival occurred with exposure to 60 and 100 µg/g dietary As relative to the control. Dietary As exposure also induced a concentration-dependent increase in abnormal larval phenotypes in both the ME and PE groups, which mainly included cardiac edema, and spine and tail deformities. The surviving larval F1 fish from all ME and PE groups with no apparent morphological deformities were raised in clean water until they become 6-months old, and then breeding trials were conducted using males and females from the same exposure groups. In F1 generation, a significant decrease in fecundity and hatching success in conjunction with a significant increase in larval deformities were observed in both ME and PE groups relative to control. Gene expression analysis in F0 and F1 fish indicated As-mediated disruption of hypothalamus-pituitary-gonadal (HPG) axis in both male and female fish. Overall, our study provides new insights into the intergenerational effects of dietary As exposure on fish reproduction and development.

Poster 6: **Characterization of growth and the GH-IGF1 pathway in juvenile mummichog (*Fundulus heteroclitus*) exposed to ammonium chloride**

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Fish growth is influenced by internal and external factors, including contaminants such as ammonia, which enter aquatic systems through various sources including agricultural run-off. However, little is known about the mechanism by which ammonia disrupts fish growth. One important pathway that may be disrupted is the growth-hormone (GH)-insulin-like growth factor 1 (IGF1) pathway. The main aim of this study is to investigate the effect and potential mechanism of ammonium chloride (NH4Cl) on the growth of juvenile mummichog (*Fundulus heteroclitus*), a common estuarine fish model for ecotoxicology studies. A 33-day flow-through exposure of 6-month-old juvenile mummichog to 0, 125, and 200 mg/L NH4Cl resulted in significant growth differences in terms of final weights (p=0.013). However, changes in body weight over time (p=0.505), and final lengths (p=0.135) were not significantly different among treatments. Final weights between 0 and 125 mg/L NH4Cl were significant (p=0.04), however, final weights between 0 and 200 mg/L NH4Cl were not (p=0.06). Brain, liver, muscle, intestine, and gonad tissue samples will be analyzed by quantitative PCR to determine treatment-induced effects on the mRNA expression of hormones and receptors of the GH-IGF1 pathway, including growth hormone (gh), growth hormone receptor 1 (ghr1), growth hormone receptor 2 (ghr2), insulin-like growth factor 1 (igf1), insulin-like growth factor receptor 1a (igf1ra), and insulin-like growth factor receptor 1b (igf1rb). Juvenile mummichog will next be exposed to either concentrations of 0, 250, or 312 mg/L NH4Cl for a shorter period to determine if a standardized protocol of fewer exposure days at higher concentrations can be developed to inhibit growth consistently and to decrease experiment duration, thus saving time and resources making testing more efficient. The goals of these studies are to provide a standardized protocol and mechanistic information to help assess how various classes of contaminants, beginning with ammonia, affect fish growth.

Poster 7: **Use of Autonomous Sensors to Monitor Spatio-temporal Changes in Water Chemistry in an Oil Sands Demonstration Pit Lake, Alberta**

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Wireless sensor network (WSN) technology has become widely used for remote data collection and when high data frequency is desired. In the current study, we used a WSN system to monitor physical and chemical parameters (turbidity, temperature, dissolved oxygen, electrical conductivity, total dissolved solids, salinity, and ammonium ion at Suncor's Lake Miwasin (formerly Demonstration Pit Lake) in the Athabasca Oil Sands region, Alberta, Canada. Lake Miwasin is a pilot-scale pit lake containing fluid tailings (FT) treated using the permanent aquatic storage structure process capped with a blend of oil sands process-affected water and fresh surface water from the surrounding landscape. The aim of this study was to demonstrate the application of WSN to monitor water quality parameters and characterize the spatial and temporal changes in Lake Miwasin. Additionally, the project aims to identify periods of tailings resuspension at the sediment-water interface and expressed water from treated FT, as well as the possible release of ammonium from the consolidated tailings (e.g., due to the degradation of polyacrylamide compounds, or from other sources). A final goal of the study is to use the sensor data to predict future trends in key water quality parameters in Lake Miwasin. Different sensor probes were deployed at four different depths in the lake [one shallow (~0.3 m), one medium (~2.5 m), and two deep (~3.5 m and 4.0 m) depths] and hourly data were obtained for a period of 29 days and 119 days during the year 2020 and 2021, respectively. Simultaneously, manual water sampling and field measurements were carried out at each depth to validate the sensor water quality data. Also, laboratory analysis was performed for dissolved ions and metals. Results from 2020 and 2021 indicate the presence of a stratified water column profile, with one layer spanning approximately from 0 to 3.5 m and another from 3.5 to 4 m depth. There was no indication of spatial differences in parameter values within these two depth profiles in the lake. The temporal variability plots show that resuspension, represented by turbidity, occurred at the sediment-water interface, as did frequent drops and spikes in concentrations of DO and NH_4^+, respectively. Increased EC near the sediment-water interface during water column stratification suggests the expression of pore water, which has elevated salt content relative to the overlying water. A decreasing trend in EC towards the end of the monitoring season suggests water input from the surrounding catchment and possibly also a decrease in porewater expression as the tailings continue to consolidate.

Poster 8: **Effects of water from Suncore's Demonstration Pit Lake on saline-tolerant zooplankton**

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The Suncor Demonstration Pit Lake was established as a pilot-scale oil sands pit lake and later renamed Lake Miwasin. It consists of treated fluid tailings capped with a blend of oil sands process-affected water and fresh surface water. To monitor the conditions of the lake, manual water samplings were carried out in 2020 and 2021. The physicochemical analysis showed that the lake undergoes vertical stratification during the summer, where the bottom layer presents higher levels of salts and ammonia compared to the surface layer, including the presence of trace metals and organic compounds. The electrical conductivity values in the bottom layer varied from 3380 µS/cm (July 2020) to 1760 µS/cm (October 2021). This study aimed to assess the potential toxicity of the lake water to saline-acclimated standard test organisms and a comparable Cladocera species representative of The Great Plains lakes. Saline acclimated *Daphnia* species were used to test lake water sampled in 2021. Both standard test organisms, *D. magna* and *D. pulex*, were exposed in 48-hour acute and 21-day chronic static-renewal toxicity tests. Additionally, a native daphnid collected from Humboldt Lake was cultured under laboratory conditions and then exposed in a 12-day chronic toxicity test. Results indicated that none of these species showed significant adverse effects while exposed to Lake Miwasin water and saline control test solutions in acute and chronic tests based on endpoints of mortality and reproduction. Another follow-up test to evaluate salinity tolerance (NaCl) indicated that *D. magna* is more salt tolerant than *D. pulex*. Also, salinity acclimation did not reveal significant differences in the toxicity test results of acclimated and unacclimated *D. magna*; however, it did show significant differences in the toxicity to unacclimated *D. pulex*. Testing of sediment porewater is in progress to assess its potential toxicity.

Poster 9: **Zooplankton and Associated Microbiome Response to Simulated Oil Spill and Remediation Efforts**

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Oil spills can disturb aquatic ecosystems while best practices for the restoration of impacted ecosystems are continually improving. Nutrient enrichment and floating wetlands can stimulate microbial degradation of petroleum constituents assisting in the recovery of impacted aquatic ecosystems. Zooplankton provide essential functions to aquatic ecosystems and can serve as useful indicators of ecological health while zooplankton-associated microbiome and their responses to remediation practices are largely unknown. We applied DNA and RNA COI and 16s rDNA metabarcoding to profile the zooplankton and associated microbiome response to simulated oil spills and select remediation practices. The objectives of the project were to assess the response of zooplankton and associated microbiome using alpha and beta diversity metrics and compare DNA and RNA metabarcoding to measure change in the respective communities to ecosystem dynamics. Model oil spills of conventional heavy crude were applied to isolated shorelines in a pristine boreal lake and following primary recovery efforts two remediation practices, enhanced monitored natural recovery and engineering floating wetland, were employed. Five sampling time points were taken over the summer of 2021, including one pre-exposure sampling time point in June and every following month. The dominant zooplankton genera included Bosmina, while the dominant prokaryote family was Comamonadaceae overall. Differences in RNA and DNA alpha and beta diversity profiles existed between zooplankton and associated microbiomes, while RNA and DNA exhibited similar results for alpha diversity response to treatments for prokaryotes, but not for zooplankton. Overall, engineered floating wetlands had the largest negative effect on alpha diversity for the zooplankton-associated microbiome. Future steps include additional analyses using both RNA and DNA community profiles, comparison of zooplankton amplicon sequencing with morphological data, incorporation of environmental chemistry data, and a recovery time point (summer 2022).

Poster 10: **Effects of Erythromycin on Juvenile Rainbow Trout Microbiome and Fitness**

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Antibiotics are commonly discharged continuously via wastewater effluents including erythromycin, a broad-spectrum macrolide antibiotic that is one of the most commonly used antibiotics in both human and veterinary medicine. Discharged antibiotics and their pseudo-persistence could potentially result in negative effects on ecosystem health, including the condition and health of fishes. Gut microbiomes of fish serve significant roles in the overall condition of hosts, and dysbiosis could have important implications for health of host fishes. A 7-d dietary exposure of erythromycin followed by a 7-d depuration period was conducted to understand the acute effects of an erythromycin exposure on juvenile rainbow trout gut microbiome and host health using three levels of exposure (0.1, 10, and 1000 µg/g) and four collection time points (4-d, 7-d, 11-d, and 14-d). The study investigated responses of juvenile rainbow trout using 16S rDNA amplicon sequencing of gut microbiome and mRNA-seq of gut tissue. Bile samples were analyzed for changes in chemical concentrations to understand uptake and depuration of the antibiotic. Minor differences were seen in change in weight and Fulton's condition factor for juvenile rainbow trout throughout the experiment. The highest treatment had detectable levels of parent compound with levels dropping quickly at 11-d followed by no detection at 14-d. Erythromycin was hypothesized to decrease abundances of microbes and shift gut microbiome community composition of exposed fish, relative to that of the unexposed control individuals, while mRNA-seq was anticipated to provide changes in gene expression leading to altered pathways interpreted using gene enrichment analyses. Future steps include the measurement of short-chain fatty acids in collected plasma samples using target metabolomics to understand functional changes to the gut microbiome. Results of this study provide insights into potential effects of changes in microbiome communities and impacts on the intestinal tissue due to antibiotic exposure, leading to altered fitness.

Poster 11: **Comparison of whole 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, new approach methodologies (NAMs) increasingly involve short-term mechanistic assays that employ molecular endpoints to predict adverse outcomes of regulatory relevance. Such assays include the EcoToxChip, a qPCR array analyzing 384 targeted genes of interest, paired with the EcoToxXplorer, a web-based tool used to assess differential gene expression. This study aims to correlate the transcriptomic (RNAseq) response of fathead minnows when exposed to fluoxetine to the targeted gene expression results obtained by the EcoToxChip. Therefore, the overall goal of this study was to elucidate the application of NAMs 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^{-1}) and a control. After 96 hours, molecular toxicity signatures were characterized using whole transcriptomics analyses in livers of a sub-set of fish. In the livers, transcriptomic analyses of male FHM revealed 14 and 32 differentially expressed genes at 10.7, and 56.7 µg L^{-1} treatment groups, respectively. Enrichment analysis of genes from the highest FLX group revealed dysregulation of gene ontology terms involved in fatty acid-related pathways, a potentially upstream response to lipid-type vacuolation of hepatocytes, observed via histopathology. Analyses of gene expression measured via the EcoToxChip qPCR array are in progress. This study 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).

Poster 12: **A look beyond the priority: A Comprehensive Investigation of the Toxicity of Retene**

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Knowledge of the toxic potential of polycyclic aromatic hydrocarbons (PAHs) has increased over time. Although, there are other non-priority PAHs in the environment, whose toxic potential is underestimated. The United States Environmental Protection Agency (US-EPA) includes 16 priority PAHs in risk assessment or routine environmental analyses. Retene (1-methyl-7-isopropylphenanthrene; RET), a non-priority PAH, is one of the most widely produced PAHs following forest fires. At present, the toxic endpoints of RET remain unknown, especially in human health. Using human lung cells (A549), until the present time, the results revealed that RET is able to significantly decrease cell viability, increase oxidative stress, mitochondrial membrane potential, and mitochondrial contents, leading to an increased reactive oxygen species (ROS) production. Besides, RET led to a significant increase in chromosomal mutations such as micronuclei (MN), nucleoplasmic bridges (NPBs), and nuclear buds (NBUDs) frequency, as well as cell death, mainly due to necrosis. Moreover, using a toxicity pathway model, as well as through the in-silico analysis of differentially expressed genes (DEGs), interaction networks, and transcriptional profiles in A549 cells, RET induced variations in several genes related to metabolism, transcriptional and translational control, oxidative stress, cell cycle, DNA replication, and repair. Genes involved in these processes may explain the toxic phenotypes triggered by exposure to this non-priority PAH. In addition, using zebrafish (*Danio rerio*) as an experimental model, RET affected DNA generating micronuclei and provided new evidence suggesting behavioral alterations due to changes in redox status and the mRNA expression of the neurotransmitter systems. These results reinforce the risk posed by non-priority PAHs especially those whose toxic potentials remain underestimated, highlighting the importance of including RET in risk assessments and routine environmental analysis in the future.

Poster 13: **Prolonged vascular contractile response induced by the R and S-epimers of the ergot alkaloid ergocristine, and attenuation by a non-competitive antagonist**

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Ergot alkaloids exist in two molecular configurations, the R and S-epimer. Sustained vascular contractile response from R-epimer exposure has been studied previously, unlike the S-epimers which were thought to be biologically inactive. Additionally, antagonists have been utilized to attenuate vascular contraction associated with only the R-epimers of ergot alkaloids. This study utilized an arterial tissue bath to examine and compare the prolonged vascular contractile response attributed to ergocristine (R) and ergocristinine (S) using dissected bovine metatarsal arteries. The contractile blocking effect of a non-competitive alpha-adrenergic antagonist, phenoxybenzamine (POB), was also investigated in precontracted arteries. Arteries (n=6/epimer) were exposed to a single dose of ergocristine or ergocristinine (1×10^{-6} M in buffer). Each of the epimer doses were followed by a POB (1×10^{-3} M) or methanol (control) treatment at 90 min and the response was observed for a further 90 min. Both epimers produced a prolonged contractile response over the total 180 min incubation period in the control groups. The R-epimer caused a greater prolonged contractile response from 60-180 min post epimer exposure, compared to the S-epimer ($P < 0.05$), in the control groups. Phenoxybenzamine caused a decrease in the contractile response induced by ergocristine and ergocristinine from 105 – 180 min, compared to the control groups ($P < 0.05$). Overall, these results demonstrate the presence of a sustained vascular contractile response attributed to the R and S-epimer of an ergot alkaloid with differences in contractile response between the epimers, suggesting differences in receptor binding mechanisms. This study demonstrated that a non-competitive antagonist could attenuate the sustained vasocontraction effects *ex vivo*. Additional investigation into S-epimers of ergot alkaloids is needed. This research may help contribute to the understanding of the ergot epimer-vascular receptor binding mechanisms, which may support the investigation of different approaches of minimizing ergot toxicity in livestock.

Poster 14: **Combined effect of diazepam and polystyrene microplastics on the social behavior of medaka (*Oryzias latipes*)**

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The combined effect of microplastics and pharmaceuticals on aquatic organisms is an issue of concern. In this laboratory study, we evaluated the combined effect of polystyrene microplastics (2-μm diameter) and diazepam on the social behavior of medaka (*Oryzias latipes*) by using the shoaling behavior test with five treatment groups: solvent control, polystyrene microplastics exposure (0.04 mg/L), low-concentration diazepam exposure (0.03 mg/L), high-concentration diazepam exposure (0.3 mg/L), and polystyrene microplastics and low-concentration diazepam co-exposure. After 7 days of exposure, the shoal-leaving behavior of the high-concentration diazepam exposure group (8.9 ± 8.3 counts/medaka) and the co-exposure group (6.8 ± 6.7 counts/medaka) was significantly greater than that in the solvent control group (1.8 ± 2.6 counts/medaka). Even after 5 days of recovery, medaka in the co-exposure group left the shoal more often (7.3 ± 5.0 counts/medaka) than those in the solvent control group (2.6 ± 2.6 counts/medaka), whereas the shoal-leaving behavior in other exposure groups, except for the high-concentration diazepam exposure group, was restored. Our findings show that the combined effects of diazepam and polystyrene microplastics suppressed medaka social behavior, suggesting that the presence of microplastics can enhance the adverse effects of pollutants on the social behavior of aquatic organisms.

Poster 15: **Occurrence of microplastics in Swiss ground- and surface water**

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There is growing concern about microplastics (MP) and nanoplastics (NP) environmental contamination worldwide. MP and NP are found in any environmental compartment, the latter occurring even in the tissues of living organisms. While very little is known about the potential toxicity of plastics to the fauna and human beings, it is a consensus that plastics do not belong in the environment. Therefore, we should work on the assessment of pathways and the fate of plastics in and between environmental compartments to reduce unintended contamination. In particular, comprehensive MP monitoring studies in groundwater are still rare. In view of this, groundwater has historically been perceived as a secure source of freshwater for drinking water supply given the capability of porous aquifers to filter anthropogenic contaminants. However, some recent research shows the presence of MP in non-fractured, porous aquifers. Those results suggest that MP and NP have the potential to reach groundwater systems, thus putting drinking water supplies at risk. To investigate this, we have started a research project to assess the presence of MP in a Swiss river that feeds alluvial aquifers which are used for drinking water production. We will show preliminary results on the occurrence of MP in the surface water, the interface between the river and the aquifer, along the flow path from the river to the drinking water well and in the pumped groundwater.

Poster 16: **Legacy and Emerging Contaminants in Seaweeds of Interest for Washington State Aquaculture Development**

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Seaweeds are cultivated and harvested around the world for many uses including food, pharmaceuticals, cosmetics, and fuel. The seaweed aquaculture industry has been on the rise globally, and interest has been expressed in the United States in furthering the development of the industry. Because seaweeds can absorb contaminants into their tissues, an understanding of the risks to consumers is important for informing those consumers and maintaining public support for the industry. The goal of this project is to measure concentrations of legacy (PCBs, arsenic, cadmium, lead, and mercury) and emerging (per-and polyfluoroalkyl substances (PFAS)) contaminants in seaweeds that are currently or likely to be grown and harvested in Washington's (US) marine waters for consumption. The project is structured around a better understanding of the types of contaminants present in seaweeds of the Salish Sea and how they vary among species and over time. Water samples and blades of sugar kelp, *Saccharina latissima*, were collected from Blue Dot Sea Farm (US) during the growing season and post-harvest for analyses of metal content and PFAS. Additionally, five species of seaweed that are either wild-harvested or of interest to aquaculture were collected. These species include *Alaria marginata* (winged kelp), *Gracilaria* sp. (red spaghetti), *Nereocystis luetkeana* (bull kelp), *Palmaria palmata* (dulse), and *Ulva "lactuca"* (sea lettuce). Using ICP-MS and QTOS-MS, concentrations of metals (As, Cd, Pb, Hg) and PFAS compounds in seaweed tissue and the surrounding sea water were measured, and the results will from these preliminary analyses will be presented.

Poster 17: **Screening and Prioritization of Contaminants of Emerging Concern in the Puget Sound**

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Anthropogenic chemicals known as contaminants of emerging concern (CECs), are regularly released into the aquatic environment due to human activities. CECs include substances used in daily human life, such as antibiotics, personal hygiene products, and pharmaceuticals, as well as chemicals used in manufacturing, firefighting, pesticides, and thousands of other consumer products. These substances are identified as CECs because the associated environmental hazards are not well understood, and they are not commonly monitored or regulated. Considering the number and diverse range of CECs, it is important to screen and prioritize chemicals and chemical mixtures with the greatest associated risk. This work applies risk characterization to screen and prioritize CECs in the Puget Sound (United States) based on potential biological impact. Regional monitoring data for CECs was compiled across several environmental matrices such as surface water, wastewater, and mussel and fish tissue. Two novel methods to characterize risk were applied to overcome the gap in traditional toxicological data for CECs. The NORMAN ecotoxicology database was used as a source for predicted no exposure concentrations (PNEC), to compare environmental concentrations to the PNEC. Similarly, the ToxCast database was used to compare environmental concentrations to biological effects concentrations identified *in vitro*. *In vitro* response information predicts potential biological effects which do not directly translate to apical endpoints, but can be used to identify events that may lead to an adverse health outcome. This information is further applied to chemical mixtures by evaluating the effects of chemicals with common gene targets. As a result of this study, CECs are categorized into three groups, high priority chemicals with likely biological effects, watch list chemicals with a potential for biological effects, and those chemicals with low potential for biological effects. CEC screening and prioritization can then be used to direct management and regulatory efforts to priority compounds.

Poster 18: **Soil bioretention treatment of urban stormwater prevents cardiac impacts in developing Pacific herring (*Clupea pallasii*)**

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Urban stormwater runoff poses a significant water quality threat to aquatic habitats. Human population growth drives development and land conversion in coastal watersheds, leading to greater imperviousness and increasing volumes of stormwater runoff. Urban runoff is a complex chemical mixture, consisting of thousands of distinct compounds, the majority of which have not been identified or characterized in terms of adverse environmental effects. Manmade outfalls discharge stormwater directly to the nearshore marine environments which provide spawning habitat for forage fish species, including Pacific herring (*Clupea pallasi*). Our objective was to determine if a conventional green infrastructure method, soil bioretention, was protective for nearshore-spawning forage fish. We compared developmental effects in herring embryos exposed to stormwater with embryos exposed to stormwater pre-treated by soil bioretention. Results indicate that hearts of herring embryos exposed to stormwater runoff exhibited developmental abnormalities. In addition, stormwater exposures caused reductions in larval length and greater egg yolk area, consistent with a failure to mobilize embryonic energy stores (yolk). The observed effects are consistent with the known cardiotoxicity of polycyclic aromatic hydrocarbons (PAHs) to fish embryos and could result in delayed adverse outcomes such as reduced cardiorespiratory fitness and subsequent mortality. Pretreating the stormwater by passing it through a soil bioretention column with a 60:40 sand to compost ratio prevented the cardiac developmental abnormalities. Incorporating green stormwater infrastructure methods in development and redevelopment land use projects would reduce the incidence of cardiac injury to developing Pacific herring embryos.

Poster 19: **Tire-wear-particle leachate toxicity to *Americamysis bahia*: analysis of sublethal and molecular effects**

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Tire-wear particles (TWPs) are considered among the largest contributors of microplastics to the environment. They are subject to break-down due to environmental weathering, which allows for potentially toxic chemicals to be released from and sorbed onto the particles. In this study, leachate generated from “weathered” and “un-weathered” TWPs were used for sublethal toxicity tests with *Americamysis bahia*. Organisms were exposed for 2, 4, and 6 days and the endpoints were changes in respiration rate; and molecular responses (i.e., changes in transcript expression or enrichment at day 4 only) were also assessed. A threshold for stimulated respiration rate was detected for weathered leachate on day 2 only and was between 0.33 and 0.67 g/L. The threshold was on days 4 and 6 for the un-weathered leachate and was between 0.54 and 1.08 g/L. There were dysregulated transcript sequences, or contig sequences, in all tested concentrations for weathered (0.67, 1.34, and 2.68 g/L) and un-weathered (0.27, 0.54, and 1.08 g/L) TWP leachates; the contigs have sequences orthologous to specific gene descriptions in arthropods and were considered significantly dysregulated at an FDR ≤ 0.05 and $|\log_2 FC| \geq 1$. The highest levels of contig expression were observed at 2.68 g/L for weathered and 1.08 g/L for un-weathered leachates, and upregulated contigs showed enrichment compared to the de novo reference transcriptome, with potential functionality inferred via the Gene Ontology database. There were 80 dysregulated contigs across all tested weathered leachate concentrations and 139 dysregulated contigs across all tested un-weathered concentrations, with 23 unique to the weathered group, 82 unique to the un-weathered group, and 57 occurring in both groups. The tie between levels of biological organization was explored using these respiration and molecular effects, and previously observed mortality of *A. bahia* in response to the TWP leachate exposure.

Poster 20: **Effects of the tire rubber antioxidant 6PPD-quinone on cardiorespiratory physiology of juvenile salmonids**

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N-(1,3-Dimethylbutyl)-N'-phenyl-*p*-phenylenediamine-quinone (6PPD-quinone) is a transformation product of the most widely used rubber tire antioxidant, 6PPD. Commonly found in road-way runoff, this compound has been reported to cause acute lethality at roughly 1 µg/L in a variety of salmonid species including coho salmon, rainbow trout, and brook trout. However, additional studies have shown other salmonid species such as arctic char and bull trout to be insensitive, even at significantly greater concentrations (20 µg/L). Sensitive species show distinctive symptoms including gasping, spiraling, increased ventilation, and loss of equilibrium, suggesting a possible impact on cardiorespiratory physiology. This study aims to further investigate mechanisms of 6PPD-quinone toxicity to salmonids of differing sensitivity, specifically underlying cardiovascular and metabolic responses of acute exposure to 6PPD-quinone in juvenile rainbow trout and Arctic char. Fish will be exposed to 1 µg/L or 10 µg/L 6PPD-quinone in respirometry chambers for 48 h to assess temporal changes in oxygen consumption compared to unexposed controls. Following exposure, ultrahigh resolution B-mode and Doppler ultrasound will be used to characterize cardiac function by analyzing changes in ejection velocity, stroke volume, ventricular and atrial contractile rates, and cardiac output. Furthermore, electrocardiography will be used to evaluate changes in the heart's electrical activity, and blood gas analyses will be completed to determine a variety of parameters such as glucose and methemoglobin. Data will be analyzed to investigate potential linkages between 6PPD-quinone exposure and integrated cardiorespiratory responses in native salmonid species.

Poster 21: **Hepatic Biotransformation of 6PPD-Quinone in Rainbow Trout, an Acutely Sensitive Species**

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N-(1,3-Dimethylbutyl)-N'-Phenyl-*P*-Phenylenediamine-Quinone (6PPD-Q) is an abiotic transformation product of 6PPD, a tire anti-degradant. 6PPD-Q is deposited onto roads and then dispensed into waterways through stormwater and snowmelt. 6PPD-Q is acutely toxic (24 hours) to coho salmon, brook trout and rainbow trout but tolerated by many other fishes including white sturgeon and Artic char. However, little is known about its mechanisms of toxicity and whether the culprit is the parent chemical or a metabolite. Therefore, it is important to determine if 6PPD-Q is biotransformed to potentially explain its highly species-specific toxicity. In this study, an isolated liver perfusion assay was used to quantify the biotransformation of 6PPD-Q and determine associated toxicokinetic parameters in rainbow trout. This assay measured the hepatic extraction fraction (E) used to calculate clearance (Cl) of 6PPD-Q. An active transport inhibitor, Cyclosporin A (CsA), was introduced midway throughout the assay to assess active transport from plasma into hepatocytes. Furthermore, to determine the specific metabolites formed *in vivo*, bile samples from rainbow trout acutely exposed (0.70), no difference in E or Cl values with CsA administration, and discovery of an oxy-glucuronide metabolite with a hydroxy-fragment in rainbow trout bile. Further research will focus on tolerant species bile analysis in addition to *in vitro* hepatocyte and gill biotransformation assays.

Poster 22: **RobustNature Excellence Cluster Initiative - Robustness and resilience of Nature-Society Systems in the evolving Anthropocene**

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Our planet faces existential challenges such as the decline of biodiversity, climate change and chemical pollution. Although comprehensive research investigates these individual aspects, the interactions and interdependencies of these three areas on a global scale are particularly identified as major challenges for international research in the coming years. The higher-level system functionalities are highly dynamic processes, and their constant changes as well as their robustness and resilience are neither well understood nor quantified. Here we present the RobustNature Excellence Cluster Initiative, which aims to improve the understanding of robustness and resilience of Nature-Society Systems in the context of chemical pollution, biodiversity loss and climate change by means of knowledge-based transformation research. Multiscale approaches from molecules to ecosystems in the three research areas Water, Organismic Interactions and Systemic Risks are used to identify markers for multiple stressors and their specific effects. Furthermore, economic and societal impacts are investigated and strategies to manage and avoid the risks originating from imbalanced Nature-Society Systems are developed. We believe that such a holistic approach needs to combine interdisciplinary expertise from biology, geological, environmental, and social sciences, law, economics, and medicine to cover ecological, economical, and social perspectives. Thus, the RobustNature consortium, a large collection of various departments from Goethe University Frankfurt am Main (Germany) as core institution as well as international partners from academia and industry invites researchers for collaboration, expert dialogues, and scientific exchange. RobustNature is an initiative to establish a cluster of excellence with a long-term perspective, which we believe is crucial to tackle the challenges of our modern society. To successfully implement the research questions and to strengthen interdisciplinary collaborations, 11 joint SynergyFund projects are already established within RobustNature. These projects are characterized by the funding of scientists in early career stages, the participation of many working groups and (inter)national external partners.