

Proceedings of the 47th Annual Canadian Ecotoxicity Workshop: October 3 - 6, 2021, Halifax, Nova Scotia

Editors

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PROCEEDINGS OF THE 47TH ANNUAL CANADIAN ECOTOXICITY WORKSHOP:
OCTOBER 3 – 6, 2021, HALIFAX, NOVA SCOTIA

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Abstract

Rickwood, C., Mroz, R., Tupper-Ring, L., van der Jagt, A., Marteinson, S., and Miller, J. (Editors). 2024. Proceedings of the 47th Annual Canadian Ecotoxicity Workshop: October 3-6, 2021, Halifax, Nova Scotia. Can. Tech. Rep. Fish. Aquat. Sci. 3586: xviii + 101 p.

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

The 47th annual CEW was held at the Halifax Convention Centre in 2021, after a one year delay due to COVID-19. This workshop was the first hybrid CEW event and included 74 platform presentations (both virtual and in-person) and 38 virtual poster presentations. Total participation was 212 persons (85 in-person and 127 virtual attendees).

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

Résumé

Rickwood, C., Mroz, R., Tupper-Ring, L., van der Jagt, A., Marteinson, S., and Miller, J. (Editors). 2024. Proceedings of the 47th Annual Canadian Ecotoxicity Workshop: October 3-6, 2021, Halifax, Nova Scotia. Can. Tech. Rep. Fish. Aquat. Sci. 3586: xviii + 101 p.

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

Le 47^e CEW annuel s'est tenu au Centre des congrès de Halifax en 2021, après un retard d'un an dû à la pandémie de COVID-19. Cet atelier était le premier événement hybride et comprenait 74 présentations de plateforme (virtuelles et en personne) et 38 présentations d'affiches virtuelles. Le nombre total de participants était de 212 (85 participants en personne et 127 participants virtuels).

Cet atelier faisait partie d'une série continue d'ateliers annuels sur l'écotoxicologie au Canada, couvrant des sujets allant de la toxicologie aquatique de base aux applications de la surveillance des effets environnementaux (y compris la surveillance communautaire avec les Premières nations), l'établissement de règlements et de lignes directrices, l'élaboration de critères de qualité des sédiments et de l'eau et les évaluations de la toxicité. Ces ateliers mettent l'accent sur un échange informel d'idées et de connaissances sur ces sujets entre les personnes intéressées de l'industrie, des gouvernements et des universités. En outre, l'atelier permet de mettre l'accent chaque année sur les principes, les problèmes actuels et les approches de l'écotoxicologie. Ces ateliers sont administrés par un conseil d'administration et organisés chaque année par des comités d'organisation locaux. Les comptes rendus sont publiés avec le soutien de Pêches et Océans Canada.

Editors' comments

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

The editors would like to thank Remy Tadonleke for his assistance in preparing these proceedings.

Remarques des éditeurs

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

Les rédacteurs voudraient remercier Remy Tadonleke pour son aide dans la préparation de ces actes de conférence.

47th Canadian Ecotoxicity Workshop Organizing Committee / Comité organisateur du 47^e atelier canadien sur l'écotoxicité

Workshop Co-Chairs / Co-présidents de l'atelier:

Rita Mroz	Environment and Climate Change Canada
Laura Tupper-Ring	Dillon Consulting Ltd.
Abby van der Jagt	AGAT Laboratories

Workshop Organizing Committee / Comité organisateur de l'atelier:

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Troy Small	GHD Limited
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Dr. Richard C. Playle Awards for Outstanding Theses in Ecotoxicology

Impacts of chronic municipal wastewater exposure using *Mya truncata* as a biomarker of environmental change in Iqaluit, Nunavut

Christina Schaefer¹, David Deslauriers², Ken Jeffries¹

¹University of Manitoba, ²Fisheries and Oceans Canada

Municipal wastewater is a large source of pollution to Canadian waters, yet its effects on Arctic marine ecosystems remains relatively unknown. We characterized the impacts of municipal wastewater from a growing northern community, Iqaluit, Nunavut, on the Arctic truncate soft-shell clam, *Mya truncata*. Clams were sampled from six locations that varied in proximity to the wastewater treatment plant, and shell biogeochemical analysis revealed that clams nearest the wastewater treatment plant had slower growth rates, lower carbon and oxygen stable isotope ratios, and elevated concentrations of copper and lead. A parallel analysis on mRNA expression profiles characterized *M. truncata*'s physiological response to wastewater effluent. Clams nearest the wastewater treatment plant had significantly lower mRNA expression of genes associated with metabolism, antioxidants, molecular chaperones, and phase I and II detoxification, but had heightened mRNA expression in genes coding for enzymes that bind and remove contaminants. These results demonstrated a biological response to Iqaluit's wastewater effluent and highlight *M. truncata*'s potential to act as a biomonitor of municipal wastewater along Arctic coastlines in Canada.

Investigating the clearance of a chemical mixture from isolated perfused livers of rainbow trout

Zoey Bourgeois¹, Markus Brinkmann¹

¹University of Saskatchewan

The environmental risk assessment of chemicals relies on estimations of bioaccumulation, such as the bioconcentration factor (BCF). BCF is considered the gold standard metric for gauging this criterion, though scientists and regulators have been looking for alternatives due to the high cost and animal use. One such alternative might be the recently developed *in vitro* biotransformation assays. However, extrapolation of *in vitro* clearance rates to BCF has been complicated due to various factors, including the potential for extrahepatic biotransformation. Previous research has indicated that by isolating the liver, the main detoxification organ, a more reliable measure of xenobiotic biotransformation can be obtained for the creation of accurate *in vitro-in vivo* extrapolation (IVIVE) models. The purpose of this study was to obtain hepatic clearance rates of a

pharmaceutical mixture in isolated perfused fish livers, to generate urgently needed data to validate IVIVE models. Livers of juvenile rainbow trout were cannulated through the hepatic portal vein and perfused with a physiological buffer that was spiked with a mixture of nine antipsychotic, antidepressant, and anticonvulsant drugs at $5 \mu\text{g}\cdot\text{L}^{-1}$. Afferent and efferent samples were taken in 15-minute intervals for 5 hours. The perfusate was analysed using liquid chromatography-mass spectrometry (LC-MS), to determine hepatic clearance of the individual compounds over time. The findings of this experiment confirm existing studies that convey the importance of moving away from the BCF metric, and instead, shift the focus toward the isolated liver metric, allowing for more accurate extrapolation modelling. Future research using the perfused liver model will permit more reliable estimations of the impact of biotransformation on the bioaccumulation of chemicals in aquatic organisms, and thereby help advance environmental risk assessment.

CEW Outstanding Contribution Award Acceptance Speeches

CEW origins & career advice for all time

Acceptance speech for the CEW Outstanding Service Award – Halifax 2021

Gordon R. Craig¹

¹*G.R. Craig & Associates, Nanoose Bay, B.C.*

Thank you to CEW Board and my nominators for this honour, and it is great to be back in Halifax.

I first want to acknowledge the founders of CEW, John Loch¹ and John Davis² (Davis, 1998), both of the Fisheries and Marine Service, in its day, who invited toxicity lab managers from industry, academia, and government to the Freshwater Institute in August of 1974 to describe our individual capabilities and experience in toxicity testing (DFO, 1974). I had just started with the Ontario Ministry of Environment Toxicity Unit when I attended. Collectively the labs across the country reported using 23 fish species, 11 invertebrates, and 4 algae species in fresh and marine waters using acute lethal, sublethal, and *in situ* exposures. The protocols used were largely from ASTM and APHA (Standard Methods). John Sprague had published his trilogy on test methods and data interpretation in Water Research (Sprague, 1969, 1970, 1971) as well as his ABCs (Sprague, 1973). The meeting report was titled “Aquatic Toxicity Coordination Workshop” (DFO, 1974).

The next spring, I called John Davis to see if there were any offers for another meeting. There being none, I decided to host the next meeting at the Ontario Ministry of Environment facility in Toronto. We had over 90 attendees from across the country and a few from the US.

Discussion focused on QA/QC in testing, and the presentations were published under the presumptuous title “Second Annual Aquatic Toxicity Workshop” (Craig, 1975). Ed Pessah and Peter Wells, from the Environmental Protection Service of Environment Canada in Halifax, attended the Toronto meeting and committed to holding the following meeting for the first time in Halifax (Parker et al., 1977). This third annual meeting, in my mind, really cemented the tradition. That meeting chaired by Ed and Peter proved that the “workshop” met the needs of aquatic toxicologists across the country.

Later in 1979 the corporate name of the meeting was federally registered as “The Canadian National Aquatic Toxicity Workshop” as it is today. ATW, renamed CEW in 2014, has been held for 47 consecutive years with only two misses, the second being 2020 when the meeting was deferred due to the COVID-19 pandemic.

1 John S. Loch, retired as Regional Director of Science, DFO Maritime Region about 2005.

2 Dr. John C Davis, retired as Assistant Deputy Minister, Department of Fisheries and Oceans in 2008.

I am going to reiterate some of the advice from Karen Kidd and Peter Wells because it is so important.

NETWORKING

In my view the great success of the CEW meetings has been the opportunity to network with peers in your field mostly in Canada but also with visitors from other countries. We have our own national issues, regulations, and solutions, and CEW can devote program time to discuss them in a way that no other meeting can.

Networking doesn't just happen, it has to be pursued and cultivated. When you catch someone doing something well – tell them. Compliment authors whose papers you enjoy. Write to them with questions and request advice. Call them on the phone. Attend CEW meetings in person. Develop a personal relationship with contacts who will help you address the complexities of your work.

SURROUND YOURSELF WITH TALENT

CEW has always been the conference to meet talented colleagues from across the country and to include them in future projects. The presentation and poster sessions reflect the cleverness of you all.

My friend Mike McKernan, one of the founders of TetrES Consultants long since acquired by a larger environmental firm, cultivated a loyalty from his clients by inviting experts in academia, government, and consulting from across Canada and the United States to work on his projects. His clients knew that Mike would always bring the most experienced to work on their projects. He filled every room with talent.

Establish joint projects as Karen Kidd and Peter Wells³ so graphically illustrated in their work and as so many of you are already doing. Another product of networking.

PUT ECOLOGY IN ECOTOXICITY

In the history of ATW compiled in 2013 (Craig, 2014), several pages are devoted to including ecology in our characterization of toxicology. Early initiatives included Great Lakes ecosystem water quality objectives developed by an IJC committee (Ryder and Edwards, 1985) that I sat on in the 1980s. Elements of that appear in the fisheries and benthic components of environmental effects monitoring (EEM) programs². The Bay of Fundy Ecosystem Partnership (<http://www.bofep.org/wpbofep/>) that has engaged Peter Wells since 1997 is another example. We heard a wonderful presentation by Jan Ciborowski who described using biological indices to determine the success of oil sands remediation in constructed wetlands (Ciborowski et al., 2013, 2021).

We need ecosystem targets that characterize a healthy, balanced, and sustainable system. In past years we did not have the computing power to develop such a capability but I think we do now. When we have targets, we know whether the system is trending into or out of

³ Karen and Peter both received CEW Outstanding Contribution Awards and gave acceptance speeches outlining their work.

balance and by how much. We need to know quantitatively whether remediation on a broad scale is effective. I know there are those among you who will figure it out. In the conclusions of your studies, try to identify the role your discoveries play in respective ecosystems.

MISINFORMATION - YOUR GREATEST CHALLENGE

When I was your age – and I have been all of them – communication was simple. Landline phones, postage stamps and in-person conversation were all there were. Everyone listened to radio and television news and read newspapers. Sources of information were limited and considered reliable. Journalistic standards prevailed.

Not today. According to Pew Research (PEW, 2021), 40% of Americans get their news from social media. For Canadians, Ipsos says it is about 30% to 40% (Bricker, 2021). Thirty three percent of North Americans do not trust newspapers, radio, or syndicated television news (Brenan, 2021).

A significant proportion of the population get their news from Facebook, Instagram, or Twitter, or from their friends, who get their news from Facebook, Instagram, or Twitter. Communications are fast, short, and frequent. Anyone can say anything and, if repeated frequently and shared extensively, the message develops credibility among its listeners.

The 30–40% social media subscribers face contradictions from accredited academics and medical professionals; even the musings of pop star entertainers with millions of followers and no scientific training can generate credibility (Fichera, 2021).

Our present world is flooded with misinformation, confusion, and falsehoods which are accepted as fact. There are even “alternative facts” (Bradner, 2017). Many, just don’t know who or what to believe.

Our present-day example is the controversy over COVID vaccinations. The unvaccinated, 10–20% of the public, confuse possibilities with probabilities⁴. They ignore the historic value of vaccines. They don’t trust the science of testable hypotheses and reproducibility as a result of misinformation.

The same is true of climate change, a larger and longer lasting threat.

Your greatest challenge will be to cut through the fog of misinformation that pervades your world as in no past generation. Nor is it going away. You will need help. I suggest you look to the social sciences and the arts to help make the complex simple and believable.

I notice students have been leading the way in their presentations by using art forms and animation with humour in their platform and poster presentations. We saw a talking bullfrog tadpole in yesterday’s Tik Tox presentation. Karen Kidd spoke of lessons from kindergarten and Joanne Parrot put lipstick on feminized male white suckers in past years.

⁴ October 2021 – 88% Canadians 12 years and older received one dose and 84%, two doses.

These are images that capture attention and are easy for the public to understand.

Consider working with behavioural scientists, economists, journalists, and media producers to strengthen your message and to overcome the noise. Social media has great benefits but separation of fact from fiction is not one of them. Good luck – you will need some of that too.

INSPIRATION AND LEADERSHIP

This summer I participated in two symposia, one in Ontario and the other in British Columbia, that were hosted by Environment and Climate Change Canada to discuss the formation of a Canada Water Agency (ECCC, 2021). The participants were either representatives of NGOs, established institutions, academia, industry, First Nations, community groups, and individuals. Government staff organized and facilitated the symposia. I was impressed with the range of ideas and proposed solutions, and approaches to identified problems dealing with water quality and quantity. Creation of such an agency is a huge undertaking and will require leadership within all the sectors. I hope it is successful. I was truly inspired by the range of conversations and ideas.

I am also inspired after every CEW meeting. I marvel at the quality of work among environmental scientists, managers and students who share their talent in presentations and posters – we all learn so much and it leaves me wanting more. With that knowledge and talent you also have a responsibility to lead.

In fact, today, YOU, all of you, are the leaders your country is relying on.

As a result, I return home from CEW meetings each year knowing our future is in good hands.

So my message in accepting this award is that you:

- Actively Network
- Surround Yourself With Talent
- Fight The Fog Of Misinformation
- Inspire Others And Lead

It is an honour to be among you. There is no greater gratification, for me, than to be recognized by CEW members. Thank you.

Acknowledgments

This was a unique meeting for CEW over its long history in that the country was emerging from a global pandemic and for the first time CEW hosted a virtual, together with an in-person meeting. Coordinating live with pre-recorded presentations along with internet-enabled question periods was a logistical challenge that the coordinating committee carried off flawlessly. Congratulations to the 2021 organizing committee and the respective chairs.

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Reflections from a career in marine environmental science and management ⁵

Acceptance speech for the CEW Outstanding Contribution Award – Halifax 2021

Peter G. Wells¹

¹*International Ocean Institute – Canada, and the Marine Affairs Program, Faculty of Science, Dalhousie University, Halifax, NS.*

I am very honoured to receive the 2021 CEW Outstanding Contribution Award (OCA). Above all, I am really pleased to share the honour with two other OCA recipients this year, Dr. Karen Kidd and Ms. Jennifer Miller, both outstanding mid-career environmental scientists, as well as with previous recipients. Such honours are like “fuel in the tank”; they help to propel each of us forward into further environmental research, teaching, and public outreach.

The workshop organizers asked each of us to provide “three pearly words of wisdom” and perhaps some take-away messages based on our careers to date. My career of 50 years in marine ecotoxicology and environmental management (e.g., Cote and Wells, 1991, Wells, 2016 a, b, Wells and Doe, 2014, Wells *et al.*, 1998) has spanned most of the period of environmental awareness in our society⁶. During this time, the scientific fields of aquatic toxicology, ecotoxicology, environmental chemistry, and ecological risk assessment have greatly evolved and become well established, as shown by this year’s Canadian Ecotoxicity Workshop (CEW), the 47th in the series since 1974, as well as by the North America SETAC conferences to which many Canadian researchers contribute. In Canada, the science and applications of ecotoxicology have contributed to federal and provincial environmental policy and legislation, leading to much pollution prevention and control, and a much cleaner environment (e.g., especially note the many contributions of IGETG⁷ to this effort). This is surely the workshops’ greatest contribution and one that continues today, due greatly to the collective efforts of the community of scientists and managers who participate in these annual meetings and work tirelessly throughout the year on environmental problems.

What are my take-away messages (or pearls of wisdom, if any)? The first is that as ecotoxicologists, we need more understanding of the linkages between losses of biodiversity that are occurring in many aquatic and terrestrial ecosystems and the role of persistent toxic substances in these phenomena. We live in a highly industrialized, chemical-based world where only a fraction of the 80 to 100 000+ industrial chemicals in use are fully evaluated for their hazards to human and ecological health, and potential

⁵ This contribution was prepared for the Proceedings of the 47th Canadian Ecotoxicity Workshop (CEW), Halifax, NS. It is based on my brief acceptance talk on October 4th, 2021.

⁶ This period formally started around the globe at the United Nations Conference on the Human Environment, June 1972, Stockholm, Sweden (see Ward and Dubos 1972, and various websites). Note that the 50th anniversary of this pivotal event is in 2022! Of course, the environmental movement started at least a decade earlier, stimulated in large part in North America by Rachel Carson’s classic work, *Silent Spring* (Carson 1962).

⁷ IGETG –Inter-Governmental Ecotoxicological Testing Group (see Taylor *et al.* 2013).

ecological risks, despite much effort from government and industry⁸. How much of the current loss of species around the world is due to exposure to persistent, bio-accumulative and toxic chemicals, such as the endocrine disrupting chemicals (EDCs)? That concern includes exposure to newer chemical concerns, such as micro-plastics, PFAS chemicals, and other chemicals of emerging concern (the so-called CECs), as well as to chemicals that have been with us a long time and still are controversial (e.g., glyphosates). Such exposures may be contributing to habitat degradation and species loss, due to lethal and/or sublethal effects, and require further research with the most modern ecotoxicological methods, such as those discussed at these workshops.

Secondly, we need to speed up the process between the science and information about environmental problems, with possible solutions, and meaningful action at the policy, decision-making, and management stages (MacDonald *et al.*, 2016, Wells, 2021, amongst others). The poster child, of course, is climate change and its global impacts, much in the news this year due to the COP 26 Climate Conference in Glasgow, November 2021. Climate change caused by our recently industrialized society is not a new concern! I first heard of it in a marine science course at McGill University in 1965–66, and here we are, more than 50 years later, in 2021, with the 6th recently released IPCC report (IPCC, 2021) stating the facts once again and the urgency. Despite some progress, the politicians and their governments are still debating how to collectively and meaningfully mitigate and adapt to the problem. Perhaps progress will have been made at this year's COP 26 climate change meeting. However, we need to understand the multiple causes of such lag times or delays, and to try to speed up meaningful actions. Time is very short for addressing the biodiversity and the climate change problems! This challenge should be of concern to every ecotoxicologist.

The third message – collectively, we need to contribute continuously to a more scientifically literate public, so that they can convey key messages to policy and decision-makers, and to senior environmental managers at all levels of government. Despite the increasing complexity of our science, to assist in this effort, we should strive to communicate our research results effectively and urgently to the public and to all levels of decision makers. Hence, each of these ecotoxicology workshops should consider producing a policy brief, post-workshop, as well as widely distributing summaries of its Proceedings.

Finally, for the younger researchers, a few personal guiding points and philosophies may be useful in your careers and life path:

- Always thank your university mentors for their support and stay in touch with them after graduation.
- Appreciate the early contributors to this field (Sprague, 1996; Craig, 2014) as all of us truly “stand on the shoulders of giants”.
- Be sure to have a long-term vision of what you want to accomplish and stick

⁸ For example, chemical substances carried by ships are evaluated by the GESAMP Hazard Evaluation Working Group, sponsored by the International Maritime Organization (GESAMP 2002, see www.gesamp.org for updates). I was a member and chair of this group from 1988-2000 and was amazed at the huge amounts and wide variety of chemicals carried around the world, with limited knowledge about the risks involved were there to be a spill or on-board accident.

with it, despite obstacles that may (or certainly will) occur.

- Be prepared to defend our science and its institutions in politically turbulent times – always march for science (Wells, 2013).
- Think outside the box. Embrace the concept and occurrence of serendipity (McCay-Peet and Wells, 2017).
- Accept occasional failure as it provides its own lessons. We can learn as much from failure as from success (note the attitude of Elon Musk of Space X).
- Have multiple sources of inspiration “to keep the fuel topped up in your personal tank” while you work. For example, one of my constant sources has been the environmental literature, especially the works of Rachel Carson (Carson, 1962) and more recently, the books and films of the UK’s amazing David Attenborough (Attenborough, 2020).
- Above all, strive to have a life-work balance. But remember, one can still think and plan while walking in the park with family and friends.

Again, thank you very much for this distinguished award. I wish everyone who attended the workshop this year, in person or on-line, all the very best in their studies and research in ecotoxicology. See you next year at the CEW in Winnipeg!

Acknowledgments

I greatly thank members of the CEW organizing committee who supported the award nominations. This paper is dedicated to the memories of Drs. Peter Chapman and David Schindler, both giants in the field of environmental science and ecotoxicology in Canada and whose many seminal contributions will be long remembered.

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Environmental Effects Monitoring

Formalizing the reclamation assessment approach (RAA) to evaluate wetland condition in reclaimed oil sands watersheds (PL)

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Northeastern Alberta is dominated by a mosaic of wetlands and peatlands, reflecting hydrology, topography, and successional processes operating since the last glaciation. Mining companies have created reclaimed watersheds harbouring seemingly productive and biodiverse wetlands. However, methods of assessing the effectiveness or ‘functionality’ of wetland reclamation are lacking especially during early succession. The RAA is a standardized approach to quantify biological condition of an ‘at risk’ wetland of a particular age and its sustaining environmental elements. The predominant aquatic environmental assessment paradigm (reference condition approach – RCA) compares a test site’s attributes to those of a population of sites in the reference condition, whose environmental limits are determined empirically by sampling least-disturbed or ‘best-available’ locations. The RAA differs in stipulating that test sites must be evaluated relative to natural variation observed among reference sites of equivalent age as the test sites. Grant (2006; *Restoration Ecology* 14(1):28-37) addressed this constraint by proposing an environmental restoration state-and-transition (S&T) model. The RAA pairs Grant’s S&T approach with the reference-degraded continuum approach, using piecewise quantile regression to define boundaries (thresholds) and range in variation of biological community responses to environmental stress – gradients of water quality, landscape setting/topography, and permanence. Stress thresholds demarcate 3 biological wetland conditions (states; reference, degraded, intermediate) calibrated to each of several wetland age classes. A test site is at risk of degradation if its biological condition falls outside of the age-specific reference condition.

Monitoring salinity of Manitoba’s surface waters (PL)

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The concentrations of salts (e.g., NaCl, CaCl₂, KCl) in freshwater systems have been increasing globally (from both natural and anthropogenic sources), making the ecological impacts of salinization a pressing concern. Chloride ions are highly mobile, and retention times are typically longer in lakes and wetlands; therefore, relatively low inputs may result in elevated concentrations over time. Ultimately, there maybe impacts on the structure and function of food webs. We are interested in characterizing the current state of salinity and salinization in the Lake Winnipeg watershed, as evaluated through the lens of a

community-based monitoring program. Volunteers gathered samples over two field seasons (2020 and 2021), from 175 sites across Manitoba with approximately 20 samples per site collected per year. The exposure concentrations will be compared with freshwater salinity toxicity data to evaluate the risk for sensitive species. This information will be used in conjunction with geographic information systems (GIS) software to determine the possible sources and drivers of salinity in the Lake Winnipeg watershed. During the 2020 field season, multiple sites exceeded freshwater limits and can be classified as brackish water ($>1500 \mu\text{S}\cdot\text{cm}^{-1}$). Sites that experienced the greatest salinity levels were located in the Red River Valley and near the city of Winnipeg, suggesting that anthropogenic factors may be driving salinity in these regions. Lake Winnipeg is among the largest freshwater lakes in the world, and with such an expansive drainage basin, there are numerous potential pathways for dissolved salts to enter Manitoban surface waters. Therefore, high quality exposure data are needed to inform risk assessors when making regulatory decisions regarding water protection in the province.

Freshwater bivalves as an alternative to fish populations in Canadian environmental effects monitoring (EEM) (PL)

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The Canadian Environmental Effects Monitoring (EEM) program assesses whether mine effluents are associated with environmental effects to fish and fish habitats. One requirement of EEM programs is assessment of survival (age), energy use (growth and gonad size), and energy storage (condition and liver size) of at least two sentinel fish species. Fish populations are not always amenable to assessment, in which case alternatives include mesocosm (artificial stream) and caged bivalve studies. Environment and Climate Change Canada (ECCC) approved Orano Canada's proposal of an assessment of wild bivalve populations (Pisidiidae) as an alternative for the company's McClean Lake Operations Phase 6 EEM. In the Orano EEM program carried out in fall 2019, two species of fingernail clam (*Sphaerium nitidum*, *Pisidium casertanum*) were collected from both reference and exposure areas. Clam length, total weight, and ash-free dry weight were determined. Clams were also dissected to determine the number of embryos and shelled larvae they contained. Length data were used to determine age classes and to support estimates of size at age. The collection of wild bivalves took only 1 day per sampling area, while the collection of length, weight and reproductive data was similar to the effort for fish dissections. The clam sampling program produced the required EEM data and eliminated impacts on local populations of slimy sculpin and burbot, with an overall cost that was similar to that for a wild fish population survey.

Designing monitoring programs from a fish's perspective (PL)

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Prior to commencement of a development project, many countries require an assessment that evaluates potential impacts to the environment, society, and culture. This environmental impact assessment (EIA) considers both positive and negative aspects of the project to weigh the impacts against the financial gains. To assist in decision making for development, the ecosystem services (ES) concept was developed to provide a description of the benefits that the environment has for human well-being. In Canada, a dominant aspect of the environmental protection process requires meeting the needs of the *Fisheries Act*, which requires that fish, fish habitat, and the use of fish are protected. The challenge, as in many countries, is that the regulatory processes and evaluation procedures for environmental risk assessment, environmental assessment, cumulative effects assessment, and post-operational impact assessments are disjointed and poorly aligned, which creates ineffective monitoring programs filled with a discord of different approaches and metrics. It is possible that re-orienting the environmental assessment process along the lines of critical needs and benefits from the perspectives of the *Fisheries Act* could serve to better align risk, development, and monitoring assessment processes. We will present the critical ecosystem functions from a fish's perspective and suggest how a fish-oriented ES approach could be incorporated into the design of monitoring programs to better align pre-development, development, and post-operational monitoring programs.

Determination of elemental composition in soft biological tissue using laser ablation inductively coupled plasma mass spectrometry: Method validation (PL)

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Determination of elemental concentrations in biological tissue is fundamental to environmental effects monitoring (EEM). Analytical methods typically used to quantify concentrations in such studies have minimum sample volumes that necessitate lethal or impactful collection of tissues. Laser ablation inductively coupled mass spectrometry (LA-ICP-MS) has small sample-volume requirements and allows environmental practitioners to employ low-impact sampling. Environmental applications of LA-ICP-MS are limited by the lack of validated methods, partly due to the need for dry samples and the scarcity of matrix-matched certified reference materials (mCRMs). We validate an LA-ICP-MS method to determine concentrations of 30 elements in soft biological tissue. Small tissue samples were dehydrated, powdered, compressed into pellets, and analysed using LA-ICP-MS alongside three mCRMs. The method yielded concentration determinations for mCRM elements that were typically accurate to within 30% of theoretical concentrations, and

precise (relative standard deviation <20%). These results were repeatable: accuracy rarely deviated from theoretical values by >20%, and precision rarely exceeded 33%. Determinations for biological samples were replicable irrespective of tissue (fish ovary or muscle). There was good linearity between analyte signal strength and theoretical concentration (median $R^2 \geq 0.981$ for all elements) across ranges typically encountered in environmental studies. Concentrations could not be consistently obtained (i.e., determined concentrations were typically below detection limits) for boron, vanadium, molybdenum, and cadmium in muscles, and arsenic in both ovaries and muscles; nevertheless, detection limits were sufficiently low for EEM contexts. The method presented promotes the use of low-impact sample-collection methods while enabling high-quality determinations of elemental concentrations in biological tissues.

OMICS

Identifying metabolic indicators of cyanobacteria by comparing temporal changes of algal community composition in two Lake Ontario areas of concern (PL)

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Freshwater algal blooms transition into harmful algal blooms (HABs) when cyanobacteria produce toxins that impact ecosystem health. In addition to toxins, areas of hypoxia form during HAB decomposition that can impair or kill aquatic life, and predicting when toxins will be produced is resource-intensive and not often practical in smaller aquatic systems. The point at which an algal bloom transitions into a HAB is not fully understood, but we suggest that algal metabolites could be used as indicators to understand changes in the algal community over time and could give insight into what triggers a HAB. Similarly, temporal changes in algal protein abundance might help to identify a transition point. We think that combining molecular and community-level information could better predict the transition to a toxic HAB than models based on nutrients alone. This study consisted of ten weeks of sampling in 2020 from August to October in Hamilton Harbour and the Bay of Quinte, two Lake Ontario areas of concern. Algal species composition was obtained via microscopy, and water quality parameters were assessed. Metabolomics and proteomics were performed using liquid chromatography tandem mass spectroscopy (LC-MS/MS) to identify changes in metabolites and proteins over time. Our preliminary data from these analyses will be presented within the context of community-level interactions among freshwater algae. Ultimately, we expect the results of this study will expand our ability to predict when algal blooms will transition to produce toxins and will strengthen the knowledge between key water quality variables and the progression of HABs.

Assessing the toxicity of 17 α -ethinylestradiol in rainbow trout using a 4-day transcriptomics benchmark dose (BMD) embryo assay (PL)

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Societal demands and practices result in a steady increase in the production of chemical compounds and their release into aquatic environments is causing unintended adverse

effects in non-target organisms. However, current regulatory frameworks for assessing the potential toxicological hazard this ever-increasing number of chemicals poses are hampered because they are costly, time-consuming, and of significant ethical concern due to their reliance on live animal tests. Thus, there is an urgent demand for more efficient and ethical approaches in ecological risk assessment. Using 17 α -ethinylestradiol (EE2) as a model compound, this study established an embryo benchmark-dose (BMD) assay for rainbow trout (RBT; *Oncorhynchus mykiss*) to derive transcriptomic points-of-departure (tPODs) as an alternative to live-animal tests. Embryos were exposed to graded concentrations of EE2 (measured: 0, 1.13, 1.57, 6.22, 16.3, 55.1, and 169 ng·L⁻¹) from hatch to 4 and up to 60 days post-hatch (dph) to assess molecular and apical responses, respectively. Whole proteome analyses of alevins did not show clear estrogenic effects, while transcriptomics revealed responses that were in agreement with apical effects, including excessive accumulation of intravascular and hepatic proteinaceous fluid and significant increases in mortality at 55.1 and 169 ng·L⁻¹ EE2 at later time points. Transcriptomic BMD analysis estimated the median of the 20th lowest geneBMD to be 0.18 ng·L⁻¹; the most sensitive tPOD. Other tPOD estimates (0.78, 3.64, and 1.63 ng·L⁻¹ for the tenth percentile geneBMD, first peak geneBMD distribution, and median geneBMD of the most sensitive overrepresented pathway, respectively) were within the same order of magnitude as empirically derived apical PODs for EE2 in the literature. The 4-day alternative RBT embryonic assay was effective in deriving tPODs that are protective of chronic effects of EE2. This study is part of the EcoToxChip project (www.ecotoxchip.ca).

Linking transcriptomic points-of-departure (tPOD) to apical chronic responses in embryo-larval fathead minnows exposed to fluoxetine (PL)

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Current methods for chemical hazard assessment face significant challenges, given the rapidly growing number of compounds of emerging concern (CEC) requiring assessment. This is because current testing strategies rely on live animal testing, which are time-consuming, expensive, and ethically questionable. These concerns serve as an impetus to develop new approach methodologies (NAMs) to advance chemical hazard assessment that do not rely on live animal tests. This study explored the application of molecular benchmark doses (BMDs) derived from a short-term embryo-larval fathead minnow (FHM) assay to develop transcriptomic points-of-departure (tPODs) to assess the chronic effects of fluoxetine (FLX), a highly prescribed and potent selective serotonin reuptake inhibitor ubiquitously found in surface waters. Fertilized FHM eggs were exposed to graded concentrations of FLX (measured: water, 0.19, 0.74, 3.38, 10.2, and 47.5 μ g·L⁻¹) for 7 and 32

days. Whole body tissues were subjected to omics (transcriptomics and proteomics) and locomotor analyses (7 days), and to histological and biometric measurements (32 days). Overrepresentation analyses of both transcriptomics and proteomics data revealed significant perturbations in gene sets associated with serotonergic and axonal functions. Transcriptomics point-of-departure (tPOD) analyses estimated $\text{omicBMD}_{20} = 0.56$, $\text{omicBMD}_{10\text{th}} = 5.0$, $\text{omicBMD}_{\text{mode}} = 7.51$, and $\text{pathBMD} = 5.66 \mu\text{g}\cdot\text{L}^{-1}$ FLX. These tPODs are protective of apical locomotor and reduced weight effects (LOEC of $10.2 \mu\text{g}\cdot\text{L}^{-1}$) and are well within the range of chronic apical BMDs of FLX reported in the literature. Furthermore, the distribution of geneBMDs revealed a bimodal pattern, revealing disruption of sensitive neurotoxic pathways at low concentrations and metabolic pathway perturbations at higher concentrations. This testing methodology is one of the first studies using a short-term embryo assay at a life stage not considered to be a live animal under current legislations, to derive tPODs, which were protective of apical responses of FLX. This study is part of the EcoToxChip project (www.ecotoxchip.ca).

Transcriptomics of larval yellow perch (*Perca flavescens*) from seven sites differentially exposed to agricultural run-off in Lake Saint-Pierre (PL)

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Over the twentieth century, urbanization, industrialization, and agricultural activities have contributed to the deterioration of environmental quality in the St. Lawrence River. Lake Saint-Pierre, a fluvial lake in the St. Lawrence River, has been identified as having poor water quality due to agricultural activities in its drainage basin and flood plain. The population of yellow perch (*Perca flavescens*) has suffered declines over past decades, leading to fishing moratoriums. The objective of this study was to use RNA sequencing to investigate the gene expression profile of perch larvae collected from seven sites differentially affected by agricultural run-off in Lake Saint-Pierre at two different time-points (younger vs older perch). Messenger RNA was extracted from a pool of five larvae and one larva, respectively, for the younger and older fish from each site, sequenced using Illumina NovaSeq™ 6000, and transcripts were quantified using Seq2Fun (<https://www.ecoomicsanalyst.ca/>). Our analysis resulted in a total of 4048 mapped genes. Multivariate analysis revealed a clear difference in the gene expression pattern of younger and older larval perch. Within a sampling period, there was no consistent pattern in the gene expression of perch sampled in pre-determined disturbed vs. clean sites. Further linear modeling approaches are being explored to identify the biological (sex and growth rate) and water quality (nutrients, pesticides, and turbidity) variables that best predict the observed transcriptomic profiles across sites. The results of this project will provide insights into the factors that contribute to molecular-level changes of potential biological significance in the threatened yellow perch population of Lake Saint-Pierre.

Risk Assessment and Management

Method for determining available winter water use capacity for small-scale projects in the Northwest Territories (PL)

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In the Northwest Territories (NWT), winter activities such as ice road construction and exploratory drilling require the use of water from ice-covered water bodies. Excessive water withdrawal threatens fish habitat through depletion of oxygen-rich waters, loss of open water habitat volume, loss of littoral habitat, and exposure of fish eggs to freezing conditions. In order to ensure proposed water uses will not adversely affect existing users or the environment, the regulating Land and Water Boards (LWBs) of the Mackenzie Valley require water licence applicants to provide information regarding proposed water uses including identification and location of proposed water sources, timing and proposed volume of water to be used from each water source, and comparison of the proposed water use volume to the available water use capacity for each proposed source. However, volume information is not readily available for most waterbodies in the NWT, and the bathymetric survey methods described by the 2010 Fisheries and Oceans Canada (DFO) Protocol for *Winter Water Withdrawal from Ice-covered Waterbodies in the Northwest Territories and Nunavut*, which is recognized by the LWBs as best practice, require expertise and effort that may not align with the early stages or scope of smaller projects. This presentation will describe the *Method for Determining Available Water Use Capacity for Small-Scale Projects* that the LWBs and the Government of the Northwest Territories Department of Environment and Natural Resources (GNWT-ENR) have developed to be used in the event that bathymetric data is not available or is unreasonable to obtain.

An amended *in vitro-in vivo* extrapolation model to consider first pass clearance effects on chemical bioaccumulation in fish (PL)

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In vitro-in vivo extrapolation (IVIVE) approaches haven proven useful for evaluating the impact of biotransformation on chemical bioaccumulation in fish. Most IVIVE efforts to date have focused on predicting chemical bioconcentration in fish (aqueous only exposure), with less attention paid to the effect of biotransformation on predicted chemical accumulation via the diet. Following dietary exposure, biotransformation of a chemical in the gut lumen, intestinal epithelia, and liver can greatly reduce its accumulation. However,

current IVIVE models do not consider these first pass clearance effects on dietary uptake and generally only predict biotransformation impacts on whole body elimination. Failure to model these processes correctly could result in underestimation of the true impact of biotransformation on chemical accumulation. Here, we present an amended IVIVE (a-IVIVE) model that considers first pass clearance on chemical bioaccumulation in fish. The a-IVIVE model accounts for biotransformation impacts on dietary uptake using terms estimated from typical *in vitro* biotransformation assays. We then used the a-IVIVE model to simulate how biotransformation in the liver and intestine (alone or combined) may impact chemical accumulation occurring following water-only, diet-only, and combined exposures. For intermediate to high extraction efficiency chemicals, the a-IVIVE model predicted a greater influence of biotransformation on chemical accumulation following dietary exposure than conventional IVIVE methods, which may therefore be inadequate under such circumstances.

Using kelp pigment analysis as a line of evidence in aquatic ecological risk assessment (PL)

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Methods for assessing the potential for risks to aquatic macrophytes, including kelp, are limited. While standard methods such as comparing the surrounding media (water and sediment) to guidelines, comparing tissue concentrations to toxicity reference values, and toxicity testing are useful, other lines of evidence are desirable. Previous studies have presented correlations between environmental stressors and pigment content in kelp. The work presented herein assessed the potential impact on marine vegetation health due to exposure to contaminants in sediment by assessing quantities of the main components of the pigment complex. This specific study found that increasing concentrations of contaminants in sediment and kelp tissue did not have a statistically significant effect on total pigment concentrations, nor impacts on concentrations of individual kelp pigments. General considerations for the use of kelp pigment analysis in other risk assessment scenarios are also considered in this paper.

Incorporating risk-based closure into overall site management (PL)

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Risk assessment has been recognized as a practical and cost-effective approach to closure at contaminated sites in Canada, the United States, and internationally for decades. However, as focus has shifted towards sustainability in overall site management, risk assessment is also becoming increasingly important in earlier phases of contaminated sites

investigation and management. Incorporating a risk-based approach during planning and implementation of site management programs such as monitoring, environmental site investigations and assessments, remediation, permitting, decommissioning, and closure can have a multitude of advantages. This presentation will be discussing the opportunities for risk-based approaches into various project and program phases, showing it can optimize effort and spend, reduce liability, protect human health and the environment, and maximize sustainability throughout the contaminated sites management process. This will be illustrated by relevant case studies where the risk-based approach has been effectively applied.

Environmental DNA – Applications for Biomonitoring and Bioassessment

How low can you go? Establishing LOD and LOQ for samples with low copy number eDNA (PL)

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Environmental DNA (eDNA) – genetic material released from organisms into their environment – allows us to “see the unseen” species in environmental surveys and assessments in an unprecedented manner. Quantitative real-time polymerase reaction (qPCR) is a popular, highly sensitive means to detect a given organism's DNA in an environmental sample such as water, sediment, or soil. Currently, there is a drive to use qPCR data to infer species biomass or abundance by quantifying the copy number or concentration of a given target gene fragment in a sample, which is often very dilute. Cycle thresholds (C_t/C_q) on multiple technical replicates have been used to quantify eDNA amounts. However, quantification of DNA copy number has been challenging when DNA is not detected in all technical replicates. Herein, we provide a statistically robust binomial-Poisson model to create a standard curve that relates the number of qPCR-detected technical replicates to the copy number to be applied in the case of low copy number samples. Limits of detection (LOD) and quantification (LOQ) and their confidence intervals are derived using a well-accepted statistical approach thus providing a more broadly applicable and robust method for reporting eDNA abundance in the low copy number range. To date, we have applied this approach to 30+ eDNA assays from multiple labs. In this presentation, we provide a practical example on how to derive LOD and LOQ with confidence intervals and estimate copy numbers with standard errors using a standardized format and synthetic DNA to characterize an eDNA assay.

iTrackDNA: Non-destructive precision genomics for environmental impact tracking in a global climate change era (PL)

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The analysis of environmental DNA (eDNA) – genetic material shed from organisms into their environment – promises to provide rapid, non-destructive, accurate, and cost-effective biodiversity information. However, inconsistent practices and poor quality eDNA detection tools threaten end-user (regulators, industry, Indigenous Peoples, NGOs) uptake because of unacceptably high false negatives and false positives that can compromise

effective management decision-making. iTrackDNA is a new multi-year, large-scale applied research project that will address these concerns with researchers and end-users across Canada and sectors. It will build end-user capacity through innovative, accessible, socially-responsible, genomics-based analytical eDNA tools for effective decision-making by: 1) supporting the creation of a targeted eDNA detection national standard; 2) building eDNA kits to detect 100 priority invertebrates, fishes, amphibians, birds, reptiles, and mammals in Canadian coastal and inland ecosystems; 3) applying 10 eRNA kits for determining animal biosurveillance, biosanitation, and bioremediation effectiveness; 4) generating decision support software for modeling regional biodiversity changes integrating Indigenous Ecological Knowledge; 5) developing an eDNA training, certification, and inter-lab validation framework for consultants, researchers, regulators, and managers; and 6) producing a guidance document on eDNA-based methods integration into management, policy, and regulations. iTrackDNA has an unprecedented, broad stakeholder commitment to make Canada a global frontrunner on eDNA standards adoption, policy development, and transformative testing, and to confidently enable eDNA applications in coastal and inland ecological surveys and biosurveillance for mining, forestry, energy, and infrastructure projects.

Benchmarking *de novo* eDNA metabarcoding assays targeting amphibians of the Grand River watershed with various species detection methods (PL)

Nathanael Harper¹, Micheal Lynch¹, Andrew Doxey¹, Mark Servos¹, John Giesy², Paul Craig¹, Barbara Katzenback¹

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Environmental DNA (eDNA) is shed by organisms into the environment in forms such as skin, urine, scales, and feces and detection of eDNA via environmental sampling is a promising method for non-invasive species detection. However, eDNA methods must be optimized to detect local species, while minimizing detection of non-target organisms present in the same location. When multiple species are simultaneously barcoded from a single eDNA sample, it is termed eDNA metabarcoding. Here, we discuss the development of *de novo* eDNA metabarcoding assays designed to detect common amphibian species in the Grand River watershed. Multiple metabarcoding assays were designed to flank a genetic region distinctive to each of the target amphibian species, while minimizing specificity to non-target species that could occupy the same habitat. These metabarcoding assays were applied to eDNA extracted from vernal pools in the Grand River watershed. Performances of *de novo* metabarcoding assays were benchmarked against other species detection methods, including a published eDNA metabarcoding assay, species-specific quantitative polymerase chain reaction (qPCR) assays, and conventional survey methods (visual and audio observations). Species-specific eDNA qPCR assays and conventional surveys often demonstrated consistent trends in species detections. Overall, multiple eDNA metabarcoding assays generated low quality sequence reads and resulted in inconsistent

species detections, despite promising *in silico* results, demonstrating the need to rigorously validate novel eDNA metabarcoding assays under local conditions before use.

Monitoring marine pollution effects through new targeted environmental DNA (eDNA) testing in the Pacific Northwest (PL)

Neha Acharya-Patel¹, Lauren Bergman¹, Karen Cram², Shirley Lyons³, Rene Warren⁴, Lauren Coombe⁴, Chris Lowe³, Inanc Birol⁴, Tara Macdonald², Caren Helbing¹

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Globally, coastal waters experience degradation from pollution associated with multiple discharges including storm water, industrial and agricultural runoff, and nutrient enrichment from municipal wastewater. The responses of benthic communities to nutrient enrichment, and often associated low-oxygen conditions, has some striking parallels worldwide. For example, certain indicator taxa, particularly certain polychaete species, are well known to proliferate in response to high nutrient input and corresponding anoxic conditions while others, such as some echinoderms and amphipods, are sensitive to these conditions. Traditional assessment of macroinfaunal benthos involves the detailed analysis of each individual specimen within a sample by taxonomic experts. Damage to some organisms incurred during sampling at substantial depths often makes taxonomic identification very difficult or impossible. Our research aims to develop powerful and sensitive environmental DNA (eDNA) assays to detect these indicator species in an efficient and reliable way to assess organic enrichment. eDNA is extra-organismal DNA that can be isolated from environmental samples such as a scoop of water or sediment. Using whole genome sequencing we have generated mitogenome sequences for multiple benthic indicator species routinely used for monitoring programs in Pacific Northwest marine environments. We developed a pipeline for identifying unique DNA sequences conducive for robust quantitative polymerase chain reaction (qPCR) assay development. Using these new assays, we are evaluating multiple field collection and sample processing protocols to enable faster and accurate benthic biota identification and quantitation. These methods are conducive to standardization and widescale adoption of eDNA sampling into marine environmental effects monitoring in the Pacific Northwest.

Canadian Standards Association national eDNA standards program (PL)

Ken Clogg-Wright¹

¹Canadian Standards Association

eDNA use has spurred interest within industry, government, and academia because of its potential implications for moving beyond conventional species monitoring and management. eDNA technology is under continuous development, and has become

particularly beneficial in the identification of species difficult to identify through traditional survey techniques. Due to the ongoing development of the eDNA field, a variety of survey methodologies and field sampling methods have been employed. This variety can lead to variability and decreased reliability in results used to inform ecological management decisions. Lack of guidance and standards supporting the development of eDNA studies can lead to challenges in developing project appropriate survey methodology and study design, comparing results to similar studies, and interpreting results completed by other practitioners. This has led to a perceived lower acceptance by industry and regulatory agencies, as it is not sufficiently standardized or proven to regulators to become an accepted alternative to traditional survey techniques. The Canadian Standards Association (CSA Group) has developed a national eDNA standard development program to generate standards with the support of experts from across Canada. Standardization will ensure eDNA is applied in a rigorous and scientifically-defensible manner using CSA Group's formal accredited national standard development process. Standard development activities to date include: a research paper synthesising the current understanding of eDNA methods to highlight the needs of practitioners and promote continued innovation and applicability of eDNA methods; Environmental DNA Reporting Requirements and Terminology Standard; qPCR Assay Development Standard; and flagging future standards and partnerships for further standard development.

eDNA: Navigating regulatory hurdles and considerations (PL)

Jay Cashubec¹

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Before employing eDNA as a means of species capture, the inevitable questions arise: "What do the regulatory agencies think of eDNA?" and "What is their level of acceptance?" Although there may not be a universal answer to these questions due to the varying levels of eDNA understanding and comfort level with the agencies' policy frameworks, Precision Biomonitoring Inc.'s experience will help eDNA users to understand how to identify and navigate regulatory hurdles, which questions to ask, and how to account for some of the emerging standards in eDNA. Additionally, a brief commentary will be provided noting various federal agencies in Canada and the US, and their roles in approving different types of tests, covering topics from: human health and reportable diseases, veterinary pathogen and diagnostics, environmental samples, and more. Our presentation is a discussion piece that highlights the need to understand each project's intent and to implement sufficient quality control measures, and shares examples and references to consider when planning eDNA survey designs.

State of the Science for Aquatic Assessment and Monitoring for the Mining Sector

Challenges associated with risk assessment and risk management of arsenic and mercury in ecosystems affected by historic gold mining activities in Nova Scotia (PL)

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Nova Scotia had an active gold mining sector that commenced in 1860 and resulted in 64 historic mining districts across the province, operating until the mid-1940s. It has been estimated that three million tonnes of tailings were discharged into adjacent landscapes and watercourses during operations, resulting in uncontained tailings areas within the various districts. The contaminants of concern are arsenic (due to geology) and mercury (due to use of mercury amalgamation during processing). The Province of Nova Scotia implemented some risk management at two of the former mining districts over a decade ago (Montague and Goldenville), but is now moving forward with formal studies at these two sites which will include remediation of historic tailings areas. Detailed ecological risk assessments (ERAs) are underway, which together with geochemical and geotechnical considerations and human health risk assessment outcomes, will inform remedial decision-making. Our talk will focus on some of the challenges related to risk assessment and risk management at the Montague site, including consideration of elevated background levels of arsenic, tailings geochemistry, as well as natural reclamation processes that have taken place over the past 100 years. We will highlight why it was necessary to develop a multiple-lines-of-evidence-based approach for the ERA, which draws upon results from historical research programs, as well as recent sampling investigations, and incorporates the Sediment Quality Triad, bioaccessibility testing of metals, arsenic speciation, development of site-specific guidelines, food-chain modelling, and biological surveys, to inform decision making on remedial paths forward.

Evaluating toxicity of metals in mixtures – Use of the Windermere Humic Aqueous Model for Investigation of Cause (PL)

Malcolm Stephenson¹, Mary Murdoch¹, Annick St-Amand¹, Barry Wicks¹, Paul Mazzocco¹

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Trace metal concentrations in the aquatic environment frequently exceed environmental quality guidelines, and always occur as mixtures, with variable concentrations of toxicity-modifying substances such as hydrogen ions (pH), dissolved

organic carbon, and hardness cations (calcium and magnesium). Predicting the likelihood of adverse effects of metals in mixtures to aquatic species and communities is therefore challenging. The prevailing metal toxicity model used in North America (the biotic ligand model), addresses the acute toxicity of single metals only (e.g., zinc), or more rarely binary mixtures of metals (e.g., zinc and copper), to a limited number of taxa (e.g., *Ceriodaphnia dubia* or rainbow trout). There is presently a “gap” in the regulatory understanding and acceptance of models that can help us predict the chronic toxicity of metal mixtures to aquatic life more broadly, under real-world conditions. In this presentation, we demonstrate the value of the Windermere Humic Aqueous Model (WHAM) and an associated toxicity model (Ftox) to predict the potential for chronic and acute toxicity of metal mixtures to aquatic life. We present applications of the WHAM-Ftox model in Investigation of Cause (IOC) studies conducted as part of the Environmental Effects Monitoring (EEM) program for metal-mine effluents. The model can correctly predict toxicity under field and laboratory conditions, can assign primary causation to metals within effluents, and has helped to explain seemingly paradoxical results in laboratory toxicity tests.

The effects of winter cold on acute and chronic cadmium bioaccumulation and toxicity in the banded killifish (*Fundulus diaphanus*) (PL)

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¹University of New Brunswick

Trace metals like cadmium (Cd) are important contaminants to freshwater fish in temperate regions. Fish also experience large seasonal temperature fluctuations that can potentially affect their exposure and sensitivity to metals, due the profound influence of temperature on biological and chemical processes. However, temperature effects are often overlooked in ecotoxicology studies, which are mainly conducted at ambient lab temperatures (i.e., 15–20°C). This knowledge gap is even more important at the low temperature extremes experienced during winter. Our study investigates the effects of a winter cold temperature on acute and chronic Cd bioaccumulation and toxicity in a freshwater fish: the banded killifish (*Fundulus diaphanus*). Killifish were gradually acclimated to either cold winter (4°C) or warm spring (14°C) water temperatures, then exposed to either no Cd, low Cd (0.5 µg·L⁻¹) or high Cd (5 µg·L⁻¹) sublethal concentrations. After 2, 5, and 28 days of Cd exposure, we measured Cd bioaccumulation within various tissues (gills, liver, gut, kidneys, and muscle) and are currently measuring markers of oxidative stress in gills and livers. Cadmium was the most concentrated in the gills; accumulation increased over time and was typically lower in cold-acclimated fish, likely due to slower Cd uptake rates. However, this lower Cd bioaccumulation may not translate into a proportionally lower toxic response in cold-acclimated fish, because of the additional stress they may experience from winter temperatures. Overall, our study aims at

deepening our understanding of the influence of seasonal temperature on metal toxicity in aquatic organisms to improve environmental risk assessments for metals.

Unexpected modifications of rare metal internalisation kinetics (gallium, lanthanum, platinum) in the presence of natural organic matter (NOM) in a freshwater microalga (PL)

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In ecotoxicology, there is a growing interest for several rare and/or technologically critical metals including gallium, lanthanum, and platinum. Their relatively recent and increasing use in anthropic activities (e.g., catalysts, new technologies, and anti-cancer treatments) has led to an increase in their environmental mobility. As they reach aquatic systems, these metals can interact with organic ligands and especially natural organic matter (NOM). The consequent formation of organic complexes would be expected to reduce metal uptake by living cells. Indeed, according to the biotic ligand model (BLM), metal uptake can be expressed mainly as a function of free metal ion concentration when certain key assumptions are verified. This model is very robust and has been validated in many conditions in laboratory experiments, as well as in some field investigations. However, results from this work on rare metals internalization kinetics in *Chlamydomonas reinhardtii* appear to be in conflict with the BLM. For a constant total metal concentration, internalization is sometimes significantly enhanced instead of reduced in the presence of NOM. Uptake modification seems to depend on NOM composition and varies greatly from one source to the other, as demonstrated with Suwannee River standards and NOMs from Ontario, Canada. Moreover, a significantly enhanced uptake in the presence of complex organic ligands raises the issue of the impact of these metals on microalgae in realistic environmental conditions (with ubiquitous NOM). It demonstrates the importance of investigating the role on NOM in metal interactions with aquatic organisms, primary producers being of great ecological importance.

Selenium in fish: A review of data and development of a trophic transfer model in Saskatchewan (PL)

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A number of criteria and benchmarks are available from various jurisdictions that provide selenium concentrations in fish tissue associated with toxic effects, including the Federal Environmental Quality Guideline (FEQG) for selenium that was finalized in June 2021. Available data on selenium in fish from northern Saskatchewan, where there are

several uranium producers, were compiled. The regional dataset was evaluated, including looking at differences in uptake by type of fish. A trophic transfer model for selenium in fish was developed to estimate selenium fish concentrations for use in a predictive model. This allows for a comparison of predicted fish concentrations with the tissue criteria under different conditions that reflect future changes and selenium cycling in the environment. A brief history will be provided on the evolution of the selenium fish transfer model to incorporate elements of selenium speciation and cycling in the environment.

Finalization of research and preliminary selenium soil quality guideline derivation (PL)

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Selenium (Se) soil quality guidelines were reassessed based on the most recent toxicity data. Following interpretation of the results from 2015-2017 toxicity studies, the ameliorating effect of sulphate (SO₄) was further investigated with the higher Se concentration range in coarse and fine soils. The new toxicity data was included in the overall dataset, consisting of six plant species: alfalfa (*Medicago sativa*), barley (*Hordeum vulgare*), carrot (*Daucus carota*), cucumber (*Cucumis sativus*), northern wheatgrass (*Elymus lanceolatus*), red fescue (*Festuca rubra*), and two invertebrate species, earthworms (*Eisenia andrei*) and springtails (*Folsomia candida*). These species were tested in coarse and fine soils under various Se and SO₄ combinations, ranging from <0.3 to 31.2 mg·kg⁻¹ for Se and from 28 to 1500 mg·kg⁻¹ for SO₄. Benchmark Dose Software (BMDS) was used to build the dose-response curve and estimate representative toxicological endpoints for plants and invertebrates. Endpoints were plotted to derive a species sensitivity distribution (SSD) as a function of soil texture and SO₄ concentration. The 25th percentile from each SSD was used for ecological guidelines, where Se toxicity depends on SO₄ concentration in soil. The reassessed soil guidelines would improve environmental performance through decreasing greenhouse gas emission (by decreased remediation volumes). In addition, they would improve conservation efforts and reduce instances where remediation would generally be required because of guideline exceedances, although Se may not pose unacceptable risk to the environment or human health. This research was made possible through funding provided by the Petroleum Technology Alliance Canada (PTAC).

Mine sediment ponds affect selenium speciation and bioaccumulation (PL)

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Selenium occurs in natural surface waters as a variety of inorganic and organic chemical species. The oxyanions, selenate and selenite, typically predominate.

Organoselenide species, although hypothesized to be more bioavailable than the oxyanions, have rarely been identified or quantified in natural waters and little is known about their fate or bioaccumulative potential. We analyzed spatial patterns of bioaccumulation in relation to aqueous selenium speciation at more than 100 sites in southeast British Columbia, Canada. We used sites with no detectable organoselenium ($<0.01 \mu\text{g}\cdot\text{L}^{-1}$) to derive bioaccumulation models for selenate and selenite, then applied those models to the remaining sites to infer the bioavailability of the detectable organoselenides. Our analysis indicated that the methylated species dimethylselenoxide and methylseleninic acid are substantially more bioavailable than selenate or selenite. These organoselenides were associated primarily with mine sediment ponds, presumably as degradation products of selenium metabolism by algae and/or bacteria. Organoselenides exported from the ponds appear to be responsible for enhanced bioaccumulation in biota in downstream reaches. Our findings indicate that managing biological productivity in mine sediment ponds could help manage selenium risk in the receiving environment.

Development of methodology for early life stage testing with reidside shiners and evaluation of their sensitivity to maternally-derived selenium (PL)

James Elphick¹, Bonnie Lo¹, Jordana van Geest², Marc Giorgini³, Maddy Stokes³, Shari Weech³, Adrian de Bruyn², Cait Good⁴, Mariah Arnold⁴

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The reidside shiner (*Richardsonius balteatus*) is a small-bodied cyprinid found in freshwater systems of northwestern North America. Elevated concentrations of selenium have been measured in the ovaries of some fish of this species in the Koocanusa Reservoir, downstream of metallurgical coal mining operations in southeastern British Columbia, which has led to concern regarding the potential for adverse effects occurring on this species. Adverse effects associated with selenium occur primarily in developing early life stages associated with the offspring of exposed adults. Reidside shiners have not been spawned and reared before in the laboratory, therefore methods for capture, spawning, fertilization, and laboratory rearing were developed and used to assess the effects of maternally-derived selenium on development of offspring. The results of this study demonstrated that this species could be successfully spawned in the field and reared in the laboratory with a high rate of survival. There was no adverse response on survival or incidence of deformities related to selenium exposure in the developing fish at selenium concentrations of up to $28 \text{ mg}\cdot\text{kg}^{-1}$ dry weight in the egg. Concentrations of selenium in the developed eggs were approximately 50% lower than in the residual ovary, on average, demonstrating that egg selenium levels declined during final maturation of the egg.

Boat Harbour / Community-Based Monitoring with First Nations

Boat Harbour fish population assessment (PL)

Zach Hoover¹, Ezilrani Panneerselvam¹, Adekolapo Adesida¹, Andrew Carrier¹, Lucy Francis², Jasmine Hoover¹, Minh Ngoc Pham¹, Amanda Nicholson¹, Xu Zhang¹, Ken Oakes¹

¹Cape Breton University, ²Pictou Landing First Nation

Boat Harbour, surrounding wetlands and tributary watercourses, and the downstream estuary were surveyed between September 23 and October 10, 2019 to identify, enumerate, and characterize the fish community. Active and passive approaches resulted in the capture of 522 fish: 16 in Boat Harbour, 104 in the wetlands and watercourses, and 402 in the estuary. In total, five species were captured, with only three species found in Boat Harbour. Captured fish were measured (to determine their overall condition) and released alive, with a subset retained for liver somatic index (LSI), gonadosomatic index (GSI), and tissue burden (metals and organics) analyses. Overall, fish were in good condition, with similar LSI and GSI values among most locations/groups—although small sample sizes for the subset of fish lethally sampled must be acknowledged as a constraint for some endpoints. Of the nine metals measured, most did not show significant differences among locations or when compared to reference fish. Organic analyses measured 17 dioxins and furans, and 9 polycyclic aromatic hydrocarbons (PAHs). Nine dioxins and furans were found in fish tissues, but only one furan was found above the lower quantification limit. Five PAHs were found in fish tissues, with many well above the reference fish and the *post-hoc* control tomcod (*Microgadus tomcod*) concentrations. Though no statistical tests were possible due to low sample size, our organic analyses data suggest organic tissue burdens may play a role in the impoverished fish community in Boat Harbour and associated habitats.

Assessing impacts of effluent on nutrient cycling and food-web length in a coastal marine ecosystem using compound-specific isotope analysis (PL)

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Industrial pulp and paper mills discharge nutrient-rich effluent wastewater into receiving aquatic environments which can negatively impact aquatic biota. Naturally occurring stable isotopes of nitrogen ($\delta^{15}\text{N}$) are effective tools for establishing exposure of aquatic biota to effluent by measuring incorporation of nutrients into organism tissues. Boat Harbour, Nova Scotia, known as one of the most contaminated sites in Canada, was formerly a wastewater treatment facility for pulp mill effluent with subsequent discharge

from the facility into a coastal marine environment. Effluent release into Boat Harbour ceased in 2020 and remediation of the ecosystem is projected to begin in 2021. To assess ecosystem impacts and establish baselines to evaluate effectiveness of remediation activities on the aquatic system, an ecosystem-wide approach to determining spatial impacts of industrial effluent on the aquatic food web of the coastal marine environment was required. This research aimed to investigate the sources of bioavailable nitrogen along a pollution gradient to characterize impacts of historical effluent on coastal food webs, and to determine if exposure to pulp mill effluent alters the length of coastal food webs. Compound-specific nitrogen isotope analysis of amino acids was used as a methodological tool to trace energy flow and to calculate the trophic position of organisms inhabiting the coastal ecosystem, such as macroalgae, mussels, lobsters, and native fish. This study assisted in quantitatively characterizing the biogeochemistry and trophic ecology of an ecosystem impacted by pulp mill effluent prior to remediation and assessed the impacts of effluent on coastal food-web structure.

Toxicological bioassay – sublethal waterborne cadmium effects on juvenile American lobster (*Homarus americanus*) behaviour (PL)

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¹ *St. Francis Xavier University*

The health of the American lobster species is vital due to its high commercial value in Atlantic Canada. Lobsters are widespread and some will undoubtedly encounter contaminants from industrial wastewaters. Conducting lobster toxicology studies is necessary to understand the possible contaminant effects on lobsters. Behaviour integrates internal systems, thus any contaminant effects on internal systems may cause behaviour changes. We developed behavioural bioassays to determine if sublethal levels of waterborne Cadmium (Cd) would significantly affect three juvenile lobster behaviours: the escape response (tailflip), foraging, and shelter-use. Juvenile lobsters (carapace length 15–35 mm) were exposed to waterborne Cd (1 mg·L⁻¹) over 15 weeks, and two bioassays (lobster tailflip, and a combined foraging and shelter-use bioassay) were conducted weekly. The Cd dose lobsters received was lethal over time, but was preceded by behavioural changes. There were Cd effects on lobster tailflip and shelter-use behaviour, but not on foraging. In the tailflip bioassay, there was no effect on distance travelled by tailflip, but Cd caused significantly greater tailflip durations at slightly reduced velocities compared to control animals. In the shelter-use bioassay, Cd-exposed lobsters had a general decrease in shelter-use. Lobsters were found to travel the same distances outside the shelter, but Cd caused a significant decrease in lobster velocity which likely led to the significant increase in duration outside the shelter and decreased the opportunities for lobster to enter and leave the shelter. These behaviour changes that preceded mortality could be used as an early warning indicator of Cd exposure in juvenile lobsters.

Agriculture Inputs: Nutrients and Pesticides

Exposure to agricultural retention pond water disrupts metamorphic timing and gene expression in American toad tadpoles (PL)

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Retention ponds are a management strategy implemented in the agroecosystem to reduce the ecological impacts of runoff entering surface waters. However, these constructed wetlands also provide habitat for wildlife (e.g., serving as breeding grounds for amphibians) despite the concentrated burden of agrochemical contaminants. The main objective of this research project is to study the effects of water from an agricultural retention pond on native larval amphibians. At a field site adjacent to treated corn cultivation, water quality was assessed for three summers, and chemical analyses revealed a complex and dynamic mixture of agrochemicals, including herbicides (e.g., glyphosate, S-metolachlor), insecticides (e.g., neonicotinoids), and a fungicide (azoxystrobin). Chronic exposures to this retention pond water were performed on the American toad (*Anaxyrus americanus*) from the free-feeding stage throughout metamorphosis. Endpoints related to survival, growth, and morphology were assessed throughout the experiment. At larval stages, treated toads were significantly smaller in morphometric indices compared to control animals, and that rate of metamorphosis was altered by exposure. However, this effect was not observed at the 2-weeks post-metamorphosis timepoint, suggesting that the morphological impacts of agrochemical exposure are transient in nature. Exposed organisms exhibited significant alterations of hepatic gene expression related to the hypothalamus-pituitary-thyroid axis. Ongoing analyses aim to assess these genes of interest in tadpole tail tissue and to evaluate the implications of this perturbation of hormone signalling. This work provides ecologically-relevant information of the potential endocrine disrupting effects of agricultural activities on a North American amphibian species.

Development and use of epithelial cell lines from the olfactory mucosa, gill, and gut to study the actions of nitrite in rainbow trout (PL)

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High nitrite levels are an increasingly important toxicological problem for fish in aquaculture and in natural waters. Nitrite has been reported to be taken up by the gill and gut, to accumulate in olfactory tissue, and to damage epithelia. We have described cell lines from the rainbow trout gill (Rtgill-W1) and intestine (RtgutGC), and now are developing them from the olfactory mucosa, including one that is designated Rtolf. Rtolf has been passaged multiple times and successfully cryopreserved. These cell lines are being used to establish the basic parameters for exposing fish epithelial cells to nitrite *in vitro*, to describe the responses of epithelial cells to nitrite, and to study the mechanisms behind these responses. In 500 mM nitrite, cells died within 2 days, whereas with 50 mM cytotoxicity developed after 7 days. At concentrations of 10 mM and less, epithelial monolayers persisted for at least 2 weeks. When epithelial monolayers were wounded through “scratch” assays, 0.1 mM nitrite appeared to stimulate wound healing, whereas higher concentrations were inhibitory. These results provide a framework for studying the effect of nitrite on fundamental cellular activities that are essential for maintaining a healthy epithelium, such as cell adherence, migration and proliferation, and for identifying the cellular mechanisms of toxicity.

Influence of different catchment features on stream water quality in an intensive agricultural watershed (PL)

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Streams are nested, hierarchical structures wherein the larger-scale characteristics constrain the smaller components, determining instream ecology. A watershed-scale study is necessary to effectively analyze and compare the complex interactions that influence stream water quality. The objective of this study was to assess the influence of different landscape and instream characteristics on water quality across a gradient of agricultural intensity in the Grand River watershed in southern Ontario. Twenty-one sites across the central watershed were sampled in October 2020 and June 2021. A micro-basin polygon was generated for each site wherein land drainage at each site was mapped and analyzed for spatial heterogeneity (i.e., land use and riparian buffer extent) using ArcGIS®. At each site, benthic macroinvertebrate assemblages were sampled, water chemistry samples were collected and measured directly, and habitat quality was assessed. Shannon diversity index values for the October 2020 invertebrate data ranged from 1.5 to 2.7, species richness ranged from 18 to 33, and the Ephemeroptera, Plecoptera, Trichoptera (EPT) index ranged

from 10 to 50. Non-metric multidimensional scaling reflected the patterns indicated by the aforementioned metrics and lower pollution tolerance values were associated with micro-basins that had a lower gradient of agricultural land use and greater riparian buffer presence, suggesting that land use and buffer extent play a key role in determining stream health. An enhanced understanding of the characteristics that most significantly influence water quality will help watershed managers provide accurate recommendations for upstream catchments to preserve stream health.

Aquatic Toxicology – Metals

Are there downstream impacts of forest harvesting on food webs and their bioaccumulation of mercury? (PL)

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The effects of forest harvesting on headwaters are quite well understood, yet our understanding of whether impacts accumulate or dissipate downstream is limited. As part of a larger study on a suite of abiotic and biotic indicators, we investigated whether food-web structure (using carbon (C), nitrogen (N), and hydrogen (H) isotopes) and methylmercury (MeHg) or total mercury (Hg) levels in biota changed from up- to downstream sites within three river basins in New Brunswick that had different intensities of forest management. Water, food sources (leaves, biofilm), macroinvertebrates, and fish were collected at six sites representing stream orders 1–5 within each river basin. In the reference basin, the use of autochthonous C by macroinvertebrates increased from upstream to downstream, but this trend was not observed in the forestry-impacted basins. Similarly, total Hg in fish increased from upstream to downstream in the reference basin but showed no spatial trends in the basins with more forestry. Within each site, log MeHg (invertebrates) or total Hg (fish) levels were significantly predicted by their N isotope values, and lower slopes of these relationships were observed in the river basin with moderate forest management (mean of 0.27 vs 0.32 and 0.34). The latter appears to be due to higher MeHg in the lower trophic levels that did not translate into greater fish Hg in this basin. Overall, results suggest cumulative or dissipative effects of forestry on food webs and Hg levels in biota across a spatial gradient and some effects on the trophic transfer of this contaminant through food webs.

Use of otolith microchemistry in a study of heavy metal contamination in fish from a natural tundra wastewater wetland: A case study in Baker Lake, NU, Canada (PL)

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Wastewater treatment in the Canadian Arctic occurs primarily via passive tundra wetlands, in which wastewater is naturally filtered through a series of lakes and streams. Although favoured due to minimal maintenance and infrastructure, performance is constrained by extreme climates and increasing human activity. Ineffective treatment of wastewater can cause environmental degradation and influence local water use. We aim to understand contaminant accumulation in fish downstream of the wastewater treatment

lagoon in Baker Lake, NU. Most commonly, fish contaminant concentrations are measured in muscle tissue – providing only a snapshot of recent exposure. Variability in concentrations over a lifetime cannot be elucidated using muscle, or any other organ that is metabolically active, but can be elucidated using metabolically inert tissues that continuously accrete, such as otoliths. Time-resolved analysis is useful in environments that lack baseline data or where challenges preclude regular monitoring, such as in the Arctic. We will use the community of Baker Lake, NU, as a case study to 1) investigate metabolically inert fish otoliths as a method of determining lifetime exposure to contaminants, and to 2) determine metal accumulation in fish muscle from wastewater-affected (n=3) and reference (n=2) lakes. Abiotic and biotic samples were collected in 2019 and analyzed for metals in an accredited laboratory. Otolith age interpretation will be completed prior to metal determination by laser ablation inductively coupled plasma mass spectrometry. Results will provide valuable information on the current state of fish health in the Baker Lake system and help advance otolith microchemistry as an accessory monitoring technique in wastewater and Arctic environments.

Methylmercury bioaccumulation in coastal invertebrates in the Minas Basin, Bay of Fundy (PL)

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Due to their natural geochemistry, estuarine ecosystems are vulnerable to bioaccumulation of methylmercury (MeHg), a potent neurotoxin that readily bioaccumulates in organisms. Intertidal invertebrates are abundant in estuaries and are critical prey sources for migratory birds and marine fish. Determining the uptake of MeHg by intertidal invertebrates at the base of the food web is crucial in determining MeHg exposure in higher trophic level organisms like fish and birds. This research will quantify MeHg levels in sediment, porewater, and invertebrates, and relate bioaccumulation of MeHg to changes in concentrations of sulfate and dissolved organic matter in five coastal estuarine locations in the Minas Basin, Bay of Fundy (Kingsport, Hantsport, Windsor Salt Marsh, Evangeline Beach, and Blomidon). The formation of MeHg by sulfate-reducing bacteria during the reduction of sulfate to sulfide suggests that systems with sulfate loading may have increased MeHg concentrations in sediments. However, binding of Hg with sulfide has also been seen to reduce bioavailability of these complexes to methylating bacteria. Dissolved organic matter (DOM) may reduce the uptake of MeHg by invertebrates due to its size, however DOM may also increase MeHg production by acting as an energy source for methylating bacteria. I hypothesize that bioaccumulation of MeHg in invertebrates (with a focus on *Corophium volutator*, *Ilyanassa obsoleta*, and Polychaeta) will be greater in sediments and porewaters with increased sulfate and DOM. To assess the effects of sediment geochemistry on MeHg bioavailability to invertebrates, organism MeHg concentrations will be compared to DOM concentration, sulfur speciation, and Hg

speciation in porewater and sediment. This research will provide quantitative data on MeHg bioavailability in the Minas Basin, which can be used to protect both ecosystem and human health. By identifying areas that are at greater risk for increased MeHg production, this research will help protect the health of ecosystems critical to migratory birds, coastal fisheries, and many industries in Atlantic Canada.

Oil Toxicity – Impact and Assessment

CROSERF: Making a case for modernization (PL)

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Oil toxicity testing is complicated by the nature of the exposure media, comprising of a mixture of soluble and insoluble oil components with varying oil/water partitioning rates, which result in a complex mixture of dissolved components, microdroplets, and bulk oil. The Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF) published a standard protocol two decades ago to support science-based decision making on the use of dispersants. CROSERF provided detailed guidance for media preparation, experimental conduct, and chemical analyses to improve comparability and reproducibility of oil toxicity studies. While the protocol was widely adopted, more diverse data needs, advances in technology, and adaptation of the methods for use with unconventional and heavier oils have resulted in several scientific review papers suggesting revisions. Additionally, many published studies have cited CROSERF but made modifications without demonstrating or discussing the consequences to media chemistry or resulting toxicity. The CROSERF modernization project was initiated through the Multi-Partner Research Initiative (MPRI) of Canada's Oceans Protection Plan to revise the CROSERF protocols by convening working groups of international oil spill experts from academia, industry, government, and private organizations to provide the best-in-science recommendations. As the first step towards modernizing CROSERF, the various scientific reviews have been collated to ascertain what areas of the existing protocol need refinement, and why. This paper will introduce the MPRI initiative to modernize and improve the CROSERF protocol for future scientific research. Each aspect of testing will be discussed by comparing and contrasting the recommendations to the CROSERF protocol.

Latent effects of a brief exposure to physically and chemically dispersed crude oil on the survival of snow crab (*Chionoecetes opilio*) larvae (PL)

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Canada is the world's largest producer of snow crab (*Chionoecetes opilio*), accounting for nearly two-thirds of the global supply. This lucrative industry overlaps with areas of oil and gas exploration and transport in Atlantic Canada. Snow crab have planktonic larvae that undergo successive moults before settling on the benthos. The larval stages are particularly vulnerable to contaminant exposure. To understand the potential risks of an oil

spill to snow crab, toxicity tests were performed utilizing the planktonic life stage. Adult snow crab (n=30), had clutch status assessed to determine when the embryos were nearing the final developmental stage. As a clutch neared the final stage of development, the crab was isolated to retain hatched larvae. Bioassays were conducted on larvae that were less than 24 hours old. Larvae were allocated to test vessels containing dilutions of water accommodated fractions (WAF) of an offshore crude oil or a chemically enhanced-WAF (CEWAF; generated with Corexit™ 9500A). Following a 24-hour exposure, larvae were transferred to clean, seawater filled vessels. Larvae were assessed for immobilization and mortality daily for 7 days post exposure. There was a concentration-response relationship that only became apparent at 3 days post exposure. The LC₅₀s indicated that exposure to concentrations between 1 and 3 mg TPH·L⁻¹ are sufficient to cause latent mortality effects that were not observed within the first 48 hours post exposure. The results presented here provide valuable information for a less commonly studied species of great economic importance and highlight the need for generating latent effects data.

Assessing the effect of physically and chemically dispersed crude oil on the occurrence of blue-sac disease symptoms in early life stages of Atlantic cod (*Gadus morhua*) (PL)

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The petroleum industry is a major component of the Canadian economy, but with it exists the risk of spills that can impact marine ecosystems. The offshore oil industry in Atlantic Canada necessitates a greater understanding of the potential impacts of oil exposure and application of spill response measures on commercially important cold-water marine species. Our study 1) investigated sublethal effects of water-accommodated fractions (WAF) and chemically enhanced WAFs (CEWAFs) of crude oil by assessing the occurrence of blue-sac disease symptoms in early life stages of Atlantic cod (*Gadus morhua*); and 2) compared sublethal responses between cod families. Cod from five separate crosses were exposed pre-hatch (~95-degree days) to gradient dilutions of WAF and CEWAF (prepared with Corexit™ 9500A) for 24 hours. Post-exposure, live embryos were transferred into filtered seawater and monitored to hatch, at which point all live fish had sublethal endpoints (i.e., swimming ability, degree of spinal curvature, presence of yolk-sac edema) assessed using a blue-sac disease rating system. We found a concentration-dependent increase in the incidence of blue-sac disease symptoms in exposed cod at a population level (i.e., impaired swimming abilities, curved spines, yolk-sac edemas), and this response did not differ between physically vs. chemically dispersed oil exposures. Furthermore, we observed that sublethal responses varied between family crosses, with some families better able to cope with exposure. Our results suggest that sublethal effects of exposure to physically and chemically dispersed crude oil will not be

uniformly observed across cod populations and will manifest differently depending on genetic background.

Toxicity assessment of chemical dispersants to commercially valuable marine species (PL)

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A series of studies were conducted at environmentally relevant exposure concentrations expected during oil spill response operations to understand the relative hazard of commercially available chemical dispersants to economically important, cold-water species found in the North Atlantic waters. Early life stages of American lobster (*Homarus americanus*), Atlantic cod (*Gadus morhua*), lumpfish (*Cyclopterus lumpus*) and green sea-urchin (*Strongylocentrotus droebachiensis*) were exposed to four chemical dispersants, Corexit™ 9500A (COR), Finasol® OSR 52 (FIN), Slickgone EW (SLKEW), and Slickgone NS (SLKNS). The stock solutions of chemical dispersants were prepared with the ratio of 1 g of chemical dispersant to 1 L of 0.22 µm filtered seawater. A log-dilution concentration range of each dispersant stock solution was prepared and used in the toxicity testing along with a 0.22 µm filtered seawater control. Bioassays were conducted in temperature-controlled environmental chambers with species-specific photoperiods. Water quality parameters were measured in pre- and post-exposure solutions. The 50% effect concentrations are compared between dispersants and across species and are contrasted to results from standard test species and expected environmental concentrations following application. The results of this study provide information for decision making under net environmental benefit analysis (NEBA) when considering the potential use and selection of specific dispersants for oil spill response.

The development of an artificial sediment to assess the inherent toxicity of dispersants on sediment species (PL)

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In order to conduct sediment toxicity tests of chemicals or products and be able to compare results over many years, it is desirable to use an artificial substrate for reproducibility. Currently in the literature, there is a lack of information on the preparation of artificial sediments for marine toxicity testing. Environment and Climate Change Canada's Atlantic Laboratory for Environment Testing (ALET) has developed a novel artificial sediment recipe, based on the Organization for Economic Co-operation and Development (OECD) guideline 225 for the freshwater oligochaete *Lumbriculus variegatus*. Our recipe consisted of sand, clay, and peat, and was hydrated with filtered seawater. In

order to meet the test validity criteria for four marine species, we were required to reduce both the amount of peat and clay compared to those used in the OECD recipe. In addition, we removed the calcium carbonate and food that was required for the original test species. The artificial sediment has been tested on marine sediment species *Vibrio fischeri*, *Polydora cornuta*, *Lytechinus pictus*, and *Eohaustorius estuarius*. With the development of the artificial sediment recipe, we have begun to collaborate with Environment and Climate Change Canada's Emergencies Science and Technology Section (ESTS) to assess the inherent toxicity of oil spill treating agents being evaluated for regulatory use in Canada. The development of the recipe has allowed sediment toxicity testing to be performed over several years while ensuring uniformity between each batch of artificial sediment.

Implications of salinity in influencing the macroinvertebrate community in natural wetlands of the Alberta oil sands region (PL)

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Before oil sands mining began in northern Alberta, wetlands covered nearly 65% of Alberta's northern boreal landscape, about 90% of which were peatlands. Full-scale demonstration wetlands recently created in reclaimed post-mining watersheds are productive and support diverse biota. However, water in these wetlands tends to be sodic due to the presence of salts in the soils used in their construction and residual sodium from the bitumen extraction process. In order to understand how salinity influences macroinvertebrate community composition in natural systems, we sampled macroinvertebrates from 52 pools in a naturally saline fen complex. Triplicate D-frame sweep net samples were collected from each pool (conductivity range: 3757–20 170 $\mu\text{S}\cdot\text{cm}^{-1}$), preserved, and processed in the lab. Greatest richness (24 families) was observed at 9500 $\mu\text{S}\cdot\text{cm}^{-1}$. Richness was lower in more saline pools and was lowest in the most saline pools. Odonata and Gastropoda characterized the least saline pools, whereas the most saline pools were dominated by Diptera species (especially Culicidae) and Corixidae. Threshold Indicator Taxa ANalysis (TITAN) identified 21 indicator taxa, 12 of which decreased in relative abundance along the conductivity gradient and 9 of which increased. Community composition changed at an estimated threshold between 9000 $\mu\text{S}\cdot\text{cm}^{-1}$ and 11 000 $\mu\text{S}\cdot\text{cm}^{-1}$, markedly higher than values observed to date in constructed wetlands.

A battery of *in vitro* bioassays to predict the impact of oil sands processed water (OSPW) on receiving aquatic environments (PL)

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Today, oil sands process-affected water (OSPW) is stored in tailing ponds, but efforts are in motion to discharge treated OSPW into regional water bodies. OSPW consists of compounds including metals, naphthenic acids, and aromatic hydrocarbons, which can cause acute and sub-chronic toxicity to organisms including invertebrates and fish. Hence, prior to its discharge, treated OSPW must be analyzed for its potential to cause ecotoxicological effects in the receiving aquatic environment. A battery of *in vitro* bioassays is an environmental monitoring tool that is gaining traction in water quality assessment due to its potential to detect cell toxicity pathways related to chemical pollution. The bioassays will be used to analyze the organic fractions of the aqueous phases of treated and untreated OSPW, municipal wastewater, and OSPW seepage. An extensive literature review has been conducted for the compilation of the battery of *in vitro* bioassays. The chosen toxicity pathways are (1) non-specific toxicity: cytotoxicity; (2) specific toxicity: immunotoxicity, activation of xenobiotic metabolism endpoints (peroxisome proliferator-activated receptor-gamma and aryl hydrocarbon receptor), and activation of endocrine estrogen receptor; and (3) reactive modes of action: genotoxicity, mutagenicity, and oxidative stress. Our results are yet to be determined; however, the analysis of preliminary results from method development has commenced.

Transcriptomics analysis workflow for non-model species: Case study of American lobster (*Homarus americanus*) larvae after exposure to conventional heavy crude oil and polycyclic aromatic hydrocarbon exposures (PL)

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American lobster (*Homarus americanus*) is the most valuable Canadian fishery, producing \$1.3B in 2016. Due to their planktonic stage, lobster larvae are vulnerable to oil spills. While it is well known that water accommodated fractions (WAF) of oil induce developmental toxicity through cardiac defects in fish larvae, it is not clear whether similar impacts would be observed in lobster larvae. This study aims to improve our knowledge of the molecular response of American lobster larvae following an acute exposure to conventional heavy crude oil. Stage I lobster larvae were exposed for 24 hours to four concentrations of WAF (10%, 19%, 37%, and 72%), a positive control (1-methylnaphthalene, at 0.3 mg·L⁻¹), or a negative control (filtered seawater). Due to the lack of effects reported for survival, molting, and respiration after WAF exposures, only the

highest concentration of WAF (72% WAF) was considered for transcriptomics analysis. RNA extraction of whole larvae was performed using Trizol, and the transcriptomes were sequenced using Novaseq Illumina. A preliminary analysis was realized using EcoOmicsAnalyst (www.ecoomicsanalyst.ca) which supports the ultra-fast alignment tool for non-model species Seq2fun (www.seq2fun.ca). This analysis revealed a higher effect of 1-methylnaphthalene with 81 differential expressed genes (DEGs) than the 72% WAF (4 DEGs). This analysis will be repeated using aligners such as Kallisto and Salmon (www.ecoomicsanalyst.ca) and Hisat2 (<https://galaxy.ecotoxxplorer.ca>), and the results will be compared. This work will contribute knowledge to understand potential impacts of oil spills on American lobster populations and to support the use of EcoOmicsAnalyst for transcriptomics analysis in non-model species.

Ocean Plastics and Microplastics

Do plastics pose a risk to Atlantic leatherback turtles (*Dermochelys coriacea*) in coastal Canadian Northwest Atlantic waters? (PL)

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Atlantic leatherback turtles (*Dermochelys coriacea*) are faced with multiple threats while migrating through the Northwest Atlantic. This turtle disperses widely from southern nesting beaches to waters off Cape Breton Island, Nova Scotia (NS) and Newfoundland (NL). The increased plastic abundance in the ocean raises concerns about risks associated with encountering such debris and the consequent effects on their health and survival. Using 2010–2019 data from the Great Canadian Shoreline Cleanup (GSCS), an ecological risk assessment for this turtle was initiated, assuming that shoreline plastic debris largely comes in from the sea, being there for unknown times. A total of 129 225 plastic items were collected along 653 km of NS shorelines; 22 049 plastic items from 395 km of Prince Edward Island (PEI) shorelines; and 71 097 plastic items from 224 km of NL shorelines. Cigarettes/cigarette filters were the most abundant, with 37 787 items collected for NS, 19 886 for NL, and 1910 for PEI. Fishing rope was the second most abundant item, with 14 456 items collected for NS, 4369 for NL, and 3057 for PEI. Tiny plastics or styrofoam pieces were the third most abundant item, with 10 010 for NS, 4978 for NL, and 2360 for PEI. Plastic bags were the fourth most abundant item, with a total of 11 418 items from all locations. Leatherbacks may be at risk of plastic ingestion (causing lethal and sublethal effects) and entanglement. Evidence is needed on actual exposure at sea to the most common items. The ecotoxicology and risk assessment of plastics and sea turtles worldwide remain a priority for research.

Comparing levels of microplastics in caged and wild-caught organisms near a municipal wastewater treatment plant outfall (PL)

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Although microplastics are present in municipal wastewater treatment plant (MWTP) effluents, it is unclear whether these contaminants are ingested by biota living downstream of these outfalls. We examined whether microplastic levels in resident fish and caged biota were elevated near the Kitchener MWTP outfall, one of 30 that discharge to the Grand River watershed in southern Ontario. Rainbow darter (*Etheostoma caeruleum*) were

collected using a backpack electrofisher from one upstream reference site and two impacted sites downstream of the Kitchener MWTP in the fall of 2019. Amphipods (*Hyalella azteca*), fluted-shell mussels (*Lasmigona costata*), and rainbow trout (*Oncorhynchus mykiss*) were caged at the same sites for 14- to 28-day exposures. All tissues were digested in 20% potassium hydroxide prior to visual identification of microparticles. Elevated particle counts were found in rainbow trout digestive tracts at the outfall site, compared to the upstream reference ($p < 0.001$) and downstream farfield ($p = 0.005$) sites. *Hyalella* (whole body) caged upstream of the outfall had elevated particle counts compared to those caged at the downstream nearfield site ($p = 0.013$). Mussels (digestive glands) had higher particle concentrations at the reference and outfall sites, compared to the downstream farfield site ($p = 0.022$; $p = 0.047$, respectively). In contrast, particle counts in rainbow darter digestive tracts were lower at the outfall than the farfield site ($p = 0.002$). Across all samples, fibers were the most common particle morphology. Overall, results indicate that rainbow trout may be effective biomonitors for microplastics in fresh waters and can inform future studies looking at their impacts on riverine biota.

The “Trojan Horse” effect of nanoplastics: Potentiation of polycyclic aromatic hydrocarbon uptake in rainbow trout and the mitigating effects of natural organic matter (PL)

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Massive annual production of plastics worldwide, coupled with prolonged environmental degradation and poor recycling policies has resulted in serious concerns regarding the ecological impact of these materials. Moreover, weathering of macro-sized plastics results in increasing microplastics and subsequent nanoplastics in the environment. Nanoplastics have an exponentially higher specific surface area with decreasing size. Hydrophobic organic pollutants in the environment are known to sorb onto the hydrophobic surfaces of plastics. Therefore, plastics can function as a vector for the uptake of contaminants to aquatic organisms (termed the “Trojan Horse” effect). Natural organic matter (NOM) is ubiquitous in all aquatic environments and can interact with organic pollutants and nanoplastics. However, research on the effects of NOM on the “Trojan Horse” effects of nanoplastics for hydrophobic organic pollutants is still minimal. This study investigated the potentiation of phenanthrene uptake in rainbow trout fingerlings by nanoplastics and demonstrates that 20 nm polystyrene nanoplastics (PS-NPs) significantly increased phenanthrene uptake, and induced higher EROD activity in both the gill and liver when compared to either larger 500 nm PS-NPs with sorbed phenanthrene or with the phenanthrene-exposed alone group. The presence of NOM reduced both phenanthrene uptake and EROD activity, especially in the presence of 20 nm PS-NPs, demonstrating the potential mitigating effects of NOM. Finally, we demonstrate

that 20 nm plastic particles can transverse the epithelia of fish gills and are found in the liver 24 hours after initial exposure.

Aquatic Toxicology – Water and Wastewaters

Risk-based approach to hardness-modified joint action sulfate and chloride toxicity in an industrial process water discharge (PL)

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Treatment of groundwater for removal of salts by several processes leaves residual wastewater that is elevated in sulphate and chloride. At our client's industrial site, it mixes with site runoff in a reservoir, prior to discharge to the Conestogo River. Expansion of plant capacity required an amended Environmental Compliance Approval with discharge limits for the two major ions. Detailed understanding of in-plant waste streams allowed collection of a series of composite and ion-specific samples for acute toxicity testing and chemical characterization. This provided hardness-modified lethal thresholds for the joint action of chloride and sulphate exposures which were used to recommend effluent limits. Assimilation analysis of the receiver showed no impacts from past discharges or predicted impacts from discharges at the limits of 640 mg·L⁻¹ for chloride and 5140 mg·L⁻¹ for sulphate. Monitoring protocols were developed for episodic discharges, for discharges after drought periods with no runoff to dilute the process water, and to separate the role of uncontrolled road salt runoff from that of controllable inputs of process water in determining discharge characteristics.

Exploring the target lipid model to derive PAH guidelines (PL)

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Polycyclic aromatic hydrocarbons (PAHs) are a diverse group of organic compounds comprising of hundreds of individual compounds that are toxic to aquatic life at environmentally relevant concentrations. PAHs are found in petroleum products and are released into the environment primarily from spills, fuel combustion, and waste incineration. In environmental media, PAHs are found as complex mixtures yet water quality guidelines have primarily been developed only for a small set of parent PAHs. This is largely due to the lack of data that is required by most jurisdictions to develop guidelines. Estimating toxicity of PAHs based only on parent PAHs largely underestimates the effects to aquatic life. In addition, some PAHs are phototoxic and current guideline values are almost exclusively for low-light conditions. Given the large number of PAHs without guidelines and the paucity of data available for these PAHs, the use of a modelled approach in guideline development is warranted. The target lipid model (TLM) is a linear model that relates toxicity to the octanol-water partition coefficient (K_{ow}) of non-polar narcotic

substances, which was developed over 20 years ago. However, guidelines proposed in the literature, derived from the TLM, have so far not been widely adopted by jurisdictions. In this talk, we present the results of 1) testing the TLM to determine how well it predicts alkylated isomers; 2) coupling the narcotic/low-light TLM with the species sensitivity distribution; and 3) toxicity testing to extend the phototoxic target lipid model to predict chronic toxicity using newly generated acute to chronic ratios.

Seasonal characterization and ecotoxicity of wastewaters: A case study in Istanbul (PL)

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Wastewaters emerging from urban anthropogenic activities may cause a threat to the environment, and their proper treatment is essential. Physicochemical characterizations complemented by ecotoxicological tests are critical in the evaluation of wastewaters. This study aims at conducting ecotoxicity assessment of the wastewaters of Istanbul's four largest wastewater treatment plants (WWTP). For this purpose, during one year, four seasonal influent and effluent samples were taken after screening and grit removal of each WWTP and at the final discharge point, respectively. The samples were characterized by physicochemical parameters, heavy metal concentrations, and ecotoxicological acute tests. Test kits for the 48-hour EC₅₀ mobility inhibition test of *Daphnia magna*, the 72-hour EC₅₀ algae growth test of *Pseudokirchneriella subcapitata*, and the IC₅₀ bioluminescence inhibition test of *Vibrio fischeri* were used for the toxicity assessment. The test results showed that the pollution load of the wastewaters was significantly correlated with the level of toxicity in the studied test battery. WWTP influents were found to be more toxic than the WWTP effluents, showing the efficiency of the treatment to significantly suppress the acute toxicity effect to DAPHTOXKIT and BioTox. ALGALTOXKIT was found to be the most sensitive screening test. The study also resulted in proposing a test battery that includes a crustacean, an alga, and a bacterium for the future monitoring of wastewaters with respect to their toxic effect.

Development of a chloride water quality guideline based on hardness and consideration for cation toxicity (PL)

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Research on chloride (Cl) toxicity towards freshwater aquatic species, as a function of water hardness, was completed to support updating a long-term water quality guideline (WQG). Sensitive species of algae, amphibians, fishes, mussels, and aquatic insects were tested. The data were used in combination with recently published studies (up to 2021) to

derive WQGs in a manner parallel to CCME (2011). US EPA and CCME guidance for implementing hardness adjustments were incorporated. Cl guidelines ranged from 42 to 229 mg·L⁻¹ for a hardness range of 5 to 350 mg·L⁻¹. The upper Cl limit (229 mg·L⁻¹) can be applied to water with hardness levels ≥ 350 mg·L⁻¹. As per CCME (2011), the updated WQGs used data on calcium and sodium chloride where the anion drives toxicity. Potassium and magnesium chloride data where toxicity is cation driven, were excluded. For sensitive, endangered species and species of concern where toxicity limits occur at concentrations below the WQGs, the toxicity limits are applied as the WQG, based on an analysis of guideline protectiveness. Furthermore, a comparison between laboratory and field studies revealed an approximate 2-fold greater sensitivity in laboratory waters, implying an inherent safety factor for field waters. Recommendations are provided for water quality measurement locations and techniques related to guideline implementation. This research was made possible through funding provided by the Petroleum Technology Alliance Canada (PTAC).

Sediment and Soil Toxicity

Characterizing the desorption kinetics of antipsychotics in submerged sediments using diffusive gradients in thin films (PL)

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The mobility and lability of organic contaminants in sediments can have important toxicological implications in aquatic systems; yet, many of these dynamic processes remain poorly understood. In the current study, diffusive gradients in thin-films (DGT) were deployed in sandy sediments spiked with 9 antipsychotics (amitriptyline, bupropion, carbamazepine, citalopram, clozapine, duloxetine, fluoxetine, lamotrigine, and venlafaxine). Samplers were deployed for a range of times (2 hours to 30 days) to determine the flux of these compounds to DGT devices and the exchange rates between the porewater and sediment solid phase. The results showed a continuous removal of antipsychotics to a Septra-ZT binding gel, and induced a mobile flux from DGT device to the adjacent sediment solution. This flux is associated with the diffusion supply of antipsychotics from the solid phase, and the desorption kinetics between compounds in the aqueous porewater and those adsorbed to the sediment phase. A dynamic model, DGT-induced fluxes in soils and sediments, was used to derive rate constants of resupply of antipsychotics from solid phase to aqueous phase (response time, T_c), and distribution coefficients for labile antipsychotics. The largest labile pool was found for citalopram, lamotrigine, and fluoxetine. These compounds were resupplied rapidly by sediments with $T_c < 1$ min. T_c values for the other antipsychotics were longer (> 30 minutes), especially for amitriptyline and duloxetine (> 2 hours), which exhibited slow desorption rates. This study demonstrates the utility of DGT devices for characterizing the uptake of trace organic pollutants by sediment biota, which is controlled by desorption processes.

An overview of the Newfoundland and Labrador Small Craft Harbour sediment investigation program (PL)

Jody Berry¹

¹*Wood*

Small Craft Harbours (SCH) is a nationwide program administered by Fisheries and Oceans Canada (DFO). The program operates and maintains a national system of harbours to provide commercial fish harvesters and other harbour users with safe and accessible facilities. There are over 339 SCHs in the Newfoundland and Labrador (NL) region, many of which service remote communities along the coast. Sources of contamination at SCHs over

the past several decades are associated with a variety of activities, including illegal dumping of debris, discharge of effluent from seafood processing facilities and sewage outfalls, and petroleum hydrocarbon spills, among others. Sediment investigations for DFO SCH in NL have been ongoing since 2001. In 2016, Wood Environment & Infrastructure Solutions, in collaboration with DFO and Public Services and Procurement Canada (PSPC), developed a scope of work (SOW) for Marine Sediment Sampling Programs (MSSPs) to be completed at SCHs in NL. The MSSP SOW was developed for sites that were previously identified as Class 1 using the federal scoring tool. The MSSP SOW included an evaluation of four lines of evidence for site-specific human health and ecological risk assessments (HHERA) completed for the SCH waterlots: community interviews, sediment chemistry, fish/shellfish tissue chemistry, and benthic taxonomy. In 2020, Wood reviewed 137 environmental reports prepared by numerous consultants for Public Services and Procurement Canada (PSPC) at 85 sites (93 waterlot properties and 3 Public Harbours) and reference areas in NL from 2006 to 2019. Data was compiled to understand the common environmental issues at the waterlot properties (i.e., contaminants of potential concern (COPC), sources of contamination, pathways and receptors of concern) identified by the consultants. In addition, Wood developed secondary screening criteria for marine sediment and tissue chemistry utilizing recent data from a subset of the sediment and tissue chemistry databases. This presentation will discuss the key findings of Wood's review including COPC, sources of contamination, and pathways and receptors of concern, as well as the relevant findings of the associated HHERAs. Finally, the presentation will discuss how the various lines of evidence were used to develop screening criteria, which could be applied in future site investigations to determine if further collection of data and additional assessment is required.

Lucy Islands Conservatory – A focus on metals in soil for the protection of the local rhinoceros auklet population and other terrestrial receptors of concern (PL)

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The potential for contamination to adversely impact potential human and ecological receptors (including the rhinoceros auklet population) was evaluated in a preliminary human health and ecological risk assessment (HHERA), to address residual lead contamination in soil. Both direct (via soil contact) and indirect (food consumption) exposures of humans to contaminants were assessed. The ecological evaluation was preliminary; risks were characterized primarily by comparisons of mean soil concentrations to federal guidelines. The human evaluation did not identify unacceptable risks. Uncertainty was identified in the ecological evaluation in that relatively few soil samples were collected from an exposure unit occupied by auklets. Due to the concerns regarding auklet habitat destruction, there was limited characterization of contamination.

Additionally, there was uncertainty in the characterization of risks to the auklet population, during burrowing, because of unknown ingestion rates. Along with Environment and Climate Change Canada (ECCC) wildlife scientists and First Nations, blood-tissue samples (with co-located soil samples) were collected from the local auklet population to provide direct measurements of lead (and zinc) body burden at worst-case site areas. A correlation was noted between lead concentrations in soil and auklet blood-tissue lead concentrations. No correlation was found between zinc concentrations in soil vs. auklet blood-tissue. Risks were characterized based on a comparison of blood-tissue lead concentrations to toxicity reference values protective of clinical endpoints. There were no predicted risks for the rhinoceros auklet population from exposure to lead. Therefore, the remediation of lead (and zinc) in soil was not warranted.

Aquatic Toxicology – Organisms

A toxicity testing protocol for an Arctic diatom and sensitivity comparison with a standard temperate species (PL)

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There is a recognized gap in scientific knowledge regarding toxicity of contaminants in general to Arctic species, and the lack of these data to inform environmental risk assessments in the region contributes to substantial uncertainty. To help address this concern, we optimized culturing conditions and designed toxicity tests for an Arctic diatom, *Nitzschia frigida*, in order to compare sensitivities with the standard, temperate diatom species *Skeletonema costatum*. We tested six different preparations of media (Harrison's and two varieties of Guillard's f/2, each prepared with either natural or artificial seawater), four temperatures (2, 6, 10, and 14°C), and three light intensities (60, 90, and 120 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) to determine which combination resulted in the most growth within a 14-day period. We found that optimal conditions were using Harrison's medium with natural seawater at 2°C under a continuous photoperiod of 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. We then exposed *N. frigida* to copper sulphate, zinc sulphate, and methylnaphthalene, with concurrent exposures of *S. costatum* to the same compounds. To account for differences in exposure duration (96 hours and 14 days for *S. costatum* and *N. frigida*, respectively), EC₅₀ values were calculated from four time points of exposure for each species and used to calculate incipient EC₅₀ values. This research highlights the need to gather high quality, reliable toxicity data using Arctic species to inform environmental risk assessment in northern regions.

But first, let me take a *shell-fie*: Assessment of the effects of metals on embryo development in freshwater snails using macrophotography (PL)

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Despite being the most diverse class of the molluscan phylum, very little is known about gastropods, especially those that live in fresh water. Embryonic development in freshwater gastropods has yet to be characterized thoroughly and as a result, developmental milestones are not common as ecotoxicological endpoints. Throughout embryonic development, numerous complex changes occur on timescales ranging from seconds to days, and improvements in technology allow us to accurately measure these changes on increasingly finer scales. As these developmental changes reveal important underlying evolutionary and ecological processes, it is especially important now to improve our

understanding of them in light of the unprecedented local and global change that many populations are experiencing. Using novel macrophotography and image processing techniques, we identified and measured growth patterns and the timing of developmental milestones in the freshwater snail *Planorbella pilsbryi*. Copper, cadmium, and nickel were used as reference toxicants to assess the sensitivity of these novel endpoints and our macrophotography assay. We found that copper was considerably more toxic to our test species than cadmium and nickel but was relatively less toxic to the embryos than to adults and juveniles of this species. Our previous multi-generational work has shown that embryonic milestones may reveal the influence of past parental contaminant exposure, provide insight into the complex biological responses to environmental stress, and predict the health of juveniles later in life. Therefore, this technique may be a useful tool for protecting the vulnerable embryonic lifestage of this species and many other critical taxa.

Interspecies variation in sensitivity to activation of the aryl hydrocarbon receptor by polycyclic aromatic hydrocarbons in zebrafish, Japanese medaka, and fathead minnow (PL)

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Polycyclic aromatic hydrocarbons (PAHs) are a diverse group of chemicals characterized by the presence of 2 or more fused aromatic rings. Although PAHs are naturally occurring, extraction processes and human activity increase loading of PAHs into aquatic systems. Fishes can suffer a variety of toxicities due to activation of the aryl hydrocarbon receptor (AhR) by PAHs. To date, most research on fishes has focused on zebrafish (*Danio rerio*), but the sensitivity of zebrafish may not be representative of other fishes due to interspecies variation in sensitivity to AhR activation by PAHs. To address this question, a luciferase reporter gene assay was performed using AhR expression constructs from 3 phylogenetically diverse model species: zebrafish, Japanese medaka (*Oryzias latipes*), and fathead minnow (*Pimephales promelas*). The assay tested AhR activation of each species by benz(α)anthracene, 4-methylbenz(α)anthracene, 8-methylbenz(α)anthracene, or 7,12-dimethylbenz(α)anthracene. Zebrafish were the least sensitive of the investigated species to activation of the AhR by every tested chemical, but it is not yet known whether this translates to *in vivo* toxicity. To validate the AhR activation data, ongoing research will expose embryos of each species to each PAH using microinjection. The median lethal dose (LD₅₀) of each PAH at the transition to exogenous feeding will be compared across species and then used to expand understanding of interspecies variation in sensitivity to activation of the AhR by PAHs by determining the relationship between *in vitro* and *in vivo* data. This relationship will be used for informing the selection of native species of concern for subsequent studies.

The effects of temperature on nickel bioaccumulation and toxicity to the freshwater snail, *Lymnaea stagnalis* (PL)

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Lymnaea stagnalis has been reported as the most sensitive freshwater species (tested to date) to chronic nickel (Ni) exposure, with juvenile growth inhibition occurring at low $\mu\text{g}\cdot\text{L}^{-1}$ Ni concentrations. This species of snail is found in temperate regions where water temperature fluctuates widely (from near freezing to above 30°C). As temperature is known to affect biochemical reactions, it is likely to modulate Ni bioaccumulation and toxicity to *L. stagnalis*. Yet, the environmental risk of Ni remains assessed based on toxicity tests conducted at lab ambient temperature, i.e., around 20°C. Thus, current Ni water quality guidelines may not be valid in the full range of water temperatures encountered in this snail's habitats. Our research will investigate temperature effects on chronic Ni bioaccumulation and toxicity to *L. stagnalis*. We will compare Ni-induced growth inhibition and oxidative stress from snails exposed at three temperatures around their optimum growth temperature (18, 23, and 28°C). We will also investigate how temperature affects nickel uptake and elimination rates in the snails, using a radiotracing approach with Ni-63. We are anticipating our results to show an increase in Ni bioaccumulation and snail sensitivity to Ni with increasing water temperature. By providing new insight into the effect of temperature on nickel toxicity, this research will contribute much needed data on how metal environmental risk may fluctuate under various natural conditions.

Non-lethal blood sampling from rainbow trout in the laboratory and *in situ* (PL)

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There is global acknowledgment that humane methods in animal research are a priority, but few environmental effects monitoring programs use non-lethal methods with fish. While sampling blood from fish is widely conducted in research and aquaculture, it remains unexplored as to whether blood-sampling method causes significant mortalities. Thus, the goal of the present study was to determine the impacts of sampling small volumes of blood in larger-bodied fish on their survival and healing. In our approach, we housed 80 rainbow trout (*Oncorhynchus mykiss*) in our flow-through aquatic facility. We then anaesthetized fish using MS-222 and sampled $1\ \mu\text{l}\cdot\text{g}^{-1}$ body weight of blood via puncture of the caudal vasculature. We tested three different post-blood sampling treatments on the puncture wound: 1) application of liquid bandage; 2) a swab of betadine; and 3) a swab of fish mucous, and compared survival outcomes to a 4th group where no post-treatment was performed (negative control). Overall, we observed 90% survival

among all treatments, with the most effective approach being the negative control (100% survival). Based on these results, we repeated the blood sampling with no-post treatment using 20 rainbow trout (not previously tested) *in situ* using cages at a nearby creek and monitored survival for 2 weeks post sampling. The survival rate was 95% with full healing of the puncture site in all test organisms. In this presentation, we will present the detailed results of these combined studies and describe what we have determined to be the safest non-lethal blood sampling protocol.

Toxicity monitoring at Snap Lake, Northwest Territories: A historical overview (2012 – 2020) (PL)

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De Beers Canada Inc. (De Beers) owns the Snap Lake Mine (Mine), a diamond mine located approximately 220 km northeast of Yellowknife, Northwest Territories. Water used by the Mine is regulated through the issuance of a Type A Water Licence by the Mackenzie Valley Land and Water Board and the Government of the Northwest Territories. The Aquatic Effects Monitoring Program (AEMP) is a condition of De Beers' Water Licence designed to monitor Snap Lake for Mine-related effects on biota, including benthic invertebrates, fish, and plankton, and abiotic factors such as water and sediment quality. Here, we present a summary of findings of the toxicity component of the AEMP that involved aquatic toxicity testing conducted on surface water samples collected from the edge of the mixing zone, near the effluent diffusers in Snap Lake, from 2012 to 2020. Toxicity tests were performed by exposing algae (*Pseudokirchneriella subcapitata*), water fleas (*Ceriodaphnia dubia*), and fish (*Oncorhynchus mykiss* and *Pimephales promelas*) to water from Snap Lake. Toxicity to laboratory test organisms has occurred infrequently between 2012 and 2020 but has not increased in frequency or severity over time, and most lake water samples were not acutely toxic to aquatic life (e.g., 100% of samples were non-toxic to *Pseudokirchneriella subcapitata* [based on LC₅₀] and 80% of samples were non-toxic to *Pimephales promelas* [based on LC₅₀]). Laboratory toxicity testing results reported by the AEMP during Mine construction and operations indicated that there was no potential for consistent adverse effects to aquatic biota in Snap Lake.

Perfluororalkylated Substances (PFAS)

Assessing cattle health from exposure to emerging contaminants (PL)

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To assess the health of cattle from exposure to per- and polyfluoroalkyl substances (PFAS) in water, a multiple line of evidence approach was conducted. A sampling plan was designed and implemented, with stakeholder input, such that serum and milk were collected from five age groups (calves, young and older heifers, dry and milking cows) as well as collection of water source samples. In addition, a health assessment for the cattle was completed using the receptor/exposure/toxicity risk assessment framework. PFAS were detected in one water sample, one milk sample, and eight serum samples. PFAS concentrations in the water samples were similar to historical concentrations, while concentrations in the milk and serum samples were low and close to the laboratory detection limit. No trends or linkages were found between the analytical results and animal age, weight, or mother-calf relationship. Results of the supporting health assessment, based on PFAS concentrations in the source water, site-specific animal characteristics, and comparison to available toxicity metrics for select PFAS, suggest that potential risks from exposure to PFAS in water were acceptable. Based on these multiple lines of evidence, it was concluded that cattle health was not affected by PFAS in water and that the cattle could safely continue to consume the water.

Ecotoxicology in a Changing Climate

Climate change as a stress multiplier governing algal blooms in oligotrophic lakes (PL)

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The classic management approach to cyanobacterial blooms in fresh water is based on reducing surplus phosphorus loading from human activities to maintain total phosphorus concentrations below thresholds that increase the probability of blooms. This paradigm has been challenged recently by the increasing number of cyanobacterial blooms in lakes with low phosphorus levels and lakes with no human phosphorus sources. Although the increasing risk of blooms can be driven by more phosphorus loading and greater algal growth, more suitable cyanobacterial habitat and decreased algal grazing by zooplankton may also increase the risk of blooms. Warmer waters and increasing thermal stability of lakes in response to climate change favour cyanobacterial habitat and reduce grazing by heat sensitive zooplankton. Internal loading of phosphorus is increased as greater stability increases hypolimnetic anoxia in a warmer climate. More severe storms mobilize more phosphorus from the watershed, where tree health has been reduced through acid-rain-induced calcium loss. Zooplankton populations are reduced in lakes where calcium has declined; while increased road salt application in a warmer winter climate increases chloride toxicity to zooplankton to reduce filter feeding. Multiple stressors of temperature, chloride, low calcium, and invasive zooplankton predators, therefore, interact to reduce zooplankton grazing of algae, while climate change increases phosphorus loading and favours cyanobacteria habitat. Examples of all of these mechanisms will be presented using case studies from south-central Ontario lakes.

Understanding the risk of climate change on water quality in Canadian watersheds – Development of a nation-wide database for machine learning application (PL)

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Climate change is expected to affect surface water quality in Canadian watersheds. However, there is insufficient scientific knowledge to predict how these changes are likely to be manifested. The assessment of long-term trends in historical data over time and space (e.g., watershed and regional variability) may offer insights into the potential impacts of future climate change on water quality. The compilation of four to five decades of national, provincial, and territorial water quality monitoring data across Canada was initiated in

2018. Through a bootstrap approach, the ongoing study will perform multivariate analyses of the compiled data (17 465 sample locations) to attempt to answer the following questions: (1) Are there specific metals or water quality parameters that show consistent responses to climate change that could be used as ‘indicators’ for future projections? (2) Are there specific areas in Canada that are more sensitive to changes in climate (i.e., hot spots)? (3) Does underlying geology influence the extent and magnitude of climate-driven changes in water quality? During the process of collecting and compiling data for this project, the lack of inter-governmental coordination on methods related to sample collection, analysis, and data compilation have highlighted a significant barrier to establishing a national water quality database that can be used for predictive analysis. This presentation aims to provide an overview of the development of this national database with a specific emphasis on highlighting our observations and lessons learned to provide recommendations for the standardization of water quality monitoring and reporting across Canada.

Effects of oscillating temperature (short cycle: 24 hours) on metal accumulation in *Daphnia magna* in fresh water (PL)

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The accumulation of pollutants by ectothermic organisms in aquatic ecosystems is greatly influenced by environmental temperature. Although it is well known that some species experience daily temperature changes, such as migrating zooplankton, it is still unclear whether oscillating temperature can affect pollutant accumulation in these organisms, especially for trace metal pollutants, such as mercury (Hg). Here, we experimentally tested the impact of oscillating temperature (7–23°C) on the accumulation of inorganic Hg and methylmercury (MeHg) in *Daphnia magna*, compared to two constant temperatures (upper limit: 23°C and average: 15°C of the selected oscillating range). Trophic transfers were investigated by using isotopically labeled *Chlorella* with stable Hg isotopes. Uptake via water was studied by directly spiking Hg solutions into the incubators. Our results demonstrated that oscillating temperature greatly affected inorganic Hg and MeHg accumulations. For inorganic Hg, the concentrations in *Daphnia* were considerably decreased by both trophic transfers and uptake via water. For MeHg, the accumulation was reduced by trophic transfers but was enhanced by uptake via water. Our study contributes to the current knowledge on the accumulation of pollutants in aquatic organisms and how it relates to temperature. We suggest precautions be taken for such studies where aquatic ectotherm organisms are subjected to daily temperature changes. Under a global warming scenario, both the amplitude of oscillating temperature and the average temperature may rise, causing not only an overall increased accumulation of metal pollutants in aquatic ecosystems, but also an enhanced accumulation of specific pollutants such as MeHg, like what was reported here.

Pharmaceuticals and Personal Care Products (PPCPs)

Laxative and oil dispersant component dioctyl sodium sulfosuccinate is a disruptor of thyroid hormone signaling in American bullfrog tadpoles (PL)

Lorissa Corrie¹, Meaghan Kempe¹, Oxana Blajkevitch², Dayue Shang², Caren Helbing¹

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Thyroid hormones (THs), thyroxine (T₄), and triiodothyronine (T₃) regulate growth, development, and metabolism in vertebrates. Metamorphosis from a tadpole to a terrestrial juvenile frog is a TH-dependent process and is vulnerable to disruption by environmental chemicals. Dioctyl sodium sulfosuccinate (DOSS) is a constituent of numerous pharmaceuticals and food products, Corexit™ 9527 and 9500A, and is known to disrupt adipocyte differentiation signaling by targeting nuclear receptor-mediated gene expression through peroxisome proliferator-activated receptor-gamma (PPARγ), a receptor related to TH receptors. We sought to determine if DOSS disrupted TH signaling in metamorphosing American bullfrog (*Rana [Lithobates] catesbeiana*) tadpoles. Premetamorphic *R. catesbeiana* tadpoles were injected with 2 pmol·g⁻¹ body weight T₃ or 10 pmol·g⁻¹ body weight T₄ to induce precocious metamorphosis, then exposed for 48 hours to 0.5, 5, and 50 mg·L⁻¹ DOSS. Gene expression of three classical TH-responsive targets (*thra*, *thrb*, and *thibz*) was measured in liver and tail fin tissue through reverse transcription quantitative polymerase chain reaction (RT-qPCR). Exposure conditions that elicited a significant gene expression response were evaluated using RNA-seq. DOSS disrupted gene expression in liver and tail fin tissue at all concentrations tested, but the patterns of expression varied in tissue, gene transcript, and TH treatment status. To our knowledge, the current study is the first demonstration that DOSS can alter TH signaling. Further exploration into DOSS disruption of TH signaling is warranted as exposure may affect other TH-dependent processes, such as salmon smoltification, flatfish metamorphosis, bird molting, and perinatal human development.

Impacts of wastewater effluent stressors: Using reactive oxygen enzymes as a marker of neurodegeneration in darters (*Etheostoma* sp.) found in the Grand River (PL)

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The Grand River (GR) watershed extends throughout southern Ontario and accommodates 30 wastewater treatment plants (WWTP) with varying degrees of filtration. Many of them are currently unable to effectively eliminate all pharmaceutical by-products from their final effluent, leading to measurable concentrations (ng·L⁻¹ – µg·L⁻¹) in the

surface waters, leaving aquatic species chronically exposed to these pharmaceuticals. Chronic exposures to pharmaceuticals have been reported to impact oxidative stress, measurable through reactive oxygen species (ROS) production and the antioxidant defense response, which helps reduce the toxicity of ROS. This research focuses on the effects of WWTP effluent on four *Etheostoma* (darter) species endemic to the GR. Darters are excellent species to study due to their abundance, high site fidelity, and varying tolerances to anthropogenic activity. This study determined whether any neurodegenerative effects in the brain of darters, found downstream from the effluent-release point, persist compared to an upstream site from the Waterloo WWTP (Ontario, Canada). Evidence for such neurodegeneration can be indicated by increased ROS enzymatic activity, damage, or changes in specific transcript markers in the downstream darter species. An initial assessment was conducted by using transcriptional and enzyme analysis of antioxidant enzymes and an enzyme involved in serotonin synthesis. Significantly-increased expression of transcript markers was found in all downstream species compared to upstream species analyzed ($p < 0.05$). Similarly, downstream species displayed significantly increased antioxidative enzyme activity in the brains ($p < 0.05$). Continued investigation on the impacts of pharmaceutical exposures in non-target organisms is crucial to further the knowledge of WWTP effluent impacts.

Wildlife Toxicology

Invertebrate and vertebrate species sensitivity distribution for insecticides derived from insecticidal toxins of *Bacillus thuringiensis* (PL)

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Information on how insecticides containing toxic proteins from *Bacillus thuringiensis* (Bt) affect non-target organisms is largely unknown. Effects on Canadian species have also been undetermined despite the wide application of Bt insecticides. Acute toxicity tests (96-hour LC₅₀ estimates) of Bt *israelensis* (VectoBac 200G®, potency of 200 ITU·L⁻¹) on *Lithobates pipiens* and *Pseudacris maculata* tadpoles were conducted. The median lethal concentrations of these species were 75 500 and 70 560 ITU·L⁻¹, respectively. These LC₅₀ values were compared to others using species sensitivity distribution (SSD) curves constructed from median lethal concentrations of Bt insecticides (international toxic units for spp. *israelensis*, *kurstaki*, and *sphaericus*) obtained from published peer-reviewed literature. LC₅₀ estimated for anurans is approximately 400 times higher than those for certain target species such as the mosquito, *Aedes aegypti*. It was found that some non-target Dipterans and Lepidopterans may be at risk. Canadian anurans were found to be more tolerant than their South American counterparts. There are large gaps in data regarding the susceptibility of fish, amphibians, and mammals. The use of SSDs is important to assess which organisms are most at risk, and how to mitigate possible ecological effects in habitats in which these agents are applied. Funding: City of Ottawa, University of Ottawa Research Chair in Neuroendocrinology (VLT).

Identifying the mechanism of action of polycyclic aromatic compound exposure in multiple bird species exposed *in ovo* to diluted bitumen (PL)

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Most of the studies on polycyclic aromatic compound (PAC) exposure and toxicity in Canadian animal species have focused on invertebrates and fish. Embryotoxicity is a well-known effect from PAC exposure, including diluted bitumen (dilbit), an oil type transported by pipelines. However, the mechanisms of action of PAC mixtures are not fully elucidated in birds. The research objectives of this study were to assess the sublethal effects of dilbit exposure by egg injection during embryonic development, and to identify gene expression biomarkers in different bird species including domestic chicken, double-crested cormorant,

and northern gannet. Fresh non-incubated eggs were collected and injected with 1:10 to 1:10 000 dilutions of either Clearwater or Cold Lake Blend dilbit produced in Canada. Vehicle and non-injected controls were included. Total PACs measured in the injection dilutions (2–300 ng tPAC·g⁻¹ egg) covered a range of concentrations measured in wild bird eggs. Embryos were sampled at mid-incubation when the liver first formed. Expression of 10 genes involved in detoxification and oxidative stress were assessed in liver and chorioallantoic membrane. Sensitivity to PACs varied among species; mRNA levels of cytochrome P450 1a (*cyp1a*), a known biomarker of PAC exposure, was increased in both the liver and chorioallantoic membrane of dilbit-exposed chickens and cormorants (>13 ng PAH16), but *cyp1b1* decreased in dilbit-exposed gannets. Overall, the comparison of transcriptional profiles across multiple species exposed to the same oil mixtures can identify the mechanism of action for PAC exposure and develop biomarkers for environmentally realistic exposure scenarios such as an oil spill.

Water additives used in forest and municipal firefighting can cause adverse effects in terrestrial biota (PL)

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Firefighting water additives are used in firefighting to increase the extinguishing efficacy of water. As a result, their use has increased markedly over the past few decades in both wildland and municipal firefighting. Past firefighting additives that contained fluorinated constituents were used extensively, however, due to their potential to accumulate in the tissue of biota and their persistence in the environment, they are no longer permitted in Canada. New additive formulations have been developed and promoted as “eco-friendly” alternatives for fire suppression. However, there is a dearth of data on the toxicity of these new additives, particularly in relation to terrestrial biota. This study assessed the toxic effects of nine different types of firefighting water additives on four terrestrial species. The study assessed effects on survival and reproduction of the springtail species *Folsomia candida* and effects on germination and emergence of three plant species (*Picea glauca*, *Agropyron cristatum*, and *Raphanus sativus*). The *F. candida* tests revealed relative sensitivity to all products with the exception of TetraKO™ and Bio FOR N. *Picea glauca* and *A. cristatum* germination tests showed relatively high sensitivity at the lowest administered concentrations for all tested products, whereas *R. sativus* showed relatively low sensitivity to all tested products, with the exception of LC95A. Emergence tests with *R. sativus* also showed relatively high sensitivity to LC95A. The results of this study indicate that certain firefighting water additives can pose a hazard to terrestrial organisms.

Variation in habitat use and its consequences for mercury exposure in bats (*M. lucifugus* and *E. fuscus*) in the Cornwall region of eastern Ontario (PL)

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The Cornwall area is a reported mercury hotspot due to past industrial point source contamination in sediment in some areas of the St. Lawrence River. A previous nationwide survey identified elevated levels of (methyl)mercury in fur and internal organs of local bat species, including the little (*Myotis lucifugus*) and big (*Eptesicus fuscus*) brown bat. However, the question of how methylmercury biomagnification in these species occurs in the Cornwall area remains to be fully elucidated. To investigate this, spatial gradients in local bat species' fur mercury load were identified, and the hypothesis that dietary habits (aquatic vs. terrestrial insects) and foraging ecology contribute to mercury concentrations was tested using stable isotope and tracking analyses. Additionally, tissue level changes in reported molecular targets of methylmercury (global DNA methylation and mitochondrial DNA abundance) were analyzed to establish whether measured mercury concentrations in bats affect biological markers of mercury exposure. We identified significant differences in bat fur mercury concentrations, with higher concentrations in big compared to little brown bats, which furthermore decreased with distance from identified hotspots in the St. Lawrence. While significantly higher concentrations of total mercury were observed in aquatic compared to terrestrial insects, stable isotope analysis in insects and bats suggest local differences in food-web structure, limiting our ability to fully address the role of dietary contributions. No significant changes in DNA-level molecular markers were observed, suggesting that comparatively high (methyl)mercury loads in these local bat populations do not translate into molecular level changes linked to methylmercury toxicity and metabolism.

Reproductive Development

Developmental outcomes and gene expression profiling reveals early toxicological mechanisms of lead effects in an early-life stage amphibian, *Xenopus laevis* (PL)

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Reported environmental concentrations of lead can elicit a wide variety of adverse effects in amphibians, including mortality, developmental abnormalities, endocrine disruption, and altered behaviour. Toxicogenomic responses to lead exposure could help identify specific molecular mechanisms that drive the adverse effects of lead, and thus be used to predict apical outcomes of ecological and regulatory relevance for amphibians. The objectives of this research were to determine the effects of early-life stage exposure to lead on *Xenopus laevis* (XL) tadpoles, and characterize the early toxicological mechanisms of lead toxicity in amphibians. Embryos were exposed at 48 hours post-fertilization to 70, 210, or 630 $\mu\text{g}\cdot\text{L}^{-1}$ lead nitrate ($\text{Pb}(\text{NO}_3)_2$) in quintuplicates, and exposures were continued for 3 weeks. Individuals were evaluated for mortality, growth, development, and the incidence of developmental abnormalities. While there was no significant mortality in any of the lead-exposed groups, tadpoles in the high lead group (630 $\mu\text{g}\cdot\text{L}^{-1}$) had reduced total body length and increased incidence (46.3%) of developmental abnormalities. A subset of individuals (five individuals pooled per replicate) from each group was sampled at 96 hours and whole-body transcriptome profiles were assessed using high throughput RNA sequencing (RNASeq). Raw files were analyzed using alignment-based workflows in the EcoToxXplorer-hosted Galaxy server to obtain the raw counts. Differential gene expression analysis was performed using the XL-specific gene expression modules in www.ecotoxplorer.ca, and revealed 328 significantly dysregulated genes including those related to DNA repair, cell cycle, apoptosis, immune function, and metabolism. Overall, the results of the present study will provide insight into the developmental effects of lead exposure and underlying mechanisms of lead toxicity on early-life stage tadpoles. This study is part of the EcoToxChip project (www.ecotoxchip.ca).

Inhibition of oocyte maturation by organophosphate insecticides in zebrafish (*Danio rerio*) after *in vitro* and *in vivo* exposure (PL)

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Oogenesis is the process by which a primary oocyte develops into a fertilizable egg. During successful oocyte maturation, $17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one (MIH) activates the membrane progesterin receptor (mPR), which thereby induces maturation by germinal vesicle breakdown. *In vitro*, anthropogens like pesticides and phthalates can dysregulate the mPR to inhibit MIH-induced oocyte maturation. Using zebrafish as a model organism, the objective of this research was to establish whether assays of *in vitro* oocyte maturation are predictive of reproductive performance. Malathion, an organophosphate insecticide known to inhibit MIH-induced oocyte maturation *in vitro*, was used as a model chemical. It was established that the magnitude of MIH-stimulated oocyte maturation inhibition after *in vitro* exposure was highly similar to the magnitude of inhibition of *ex vivo* MIH-stimulated maturation of oocytes extracted from fish exposed to malathion *in vivo*. This identical trend was observed in response to the structurally related organophosphate, dimethoate. To determine whether the *in vitro* assay is predictive of reproductive performance, female zebrafish were exposed for 21 days to 0, 0.5, 5, or 50 $\mu\text{g}\cdot\text{L}^{-1}$ of malathion to evaluate daily fecundity and fertility. After exposure, oocytes were excised and induced to mature *ex vivo*. A significant decrease in *ex vivo* MIH-stimulated oocyte maturation was observed after a 21-day exposure in all fish exposed to malathion in comparison to controls. However, exposure did not impact fecundity or fertilization success. This study increases understanding of oogenesis as a target of chemical stressors, but the link between impairment of oocyte maturation and reproductive performance requires additional research.

Effects of exposure to conventional heavy crude oil on wild fathead minnow larvae from IISD-Experimental Lakes Area, northwestern Ontario (PL)

Vince Palace¹

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Global reserves of conventional heavy crude oil (CHV) are greatest in Venezuela and Canada, where pipelines are used to transport product between field and refinery, as well as to export for consumption. Pipelines are statistically the safest method for transporting oil, but spills can occur and present a potential threat to freshwater resources. Currently, remediation of freshwater oil spills includes invasive methods that can impact sensitive, biologically-productive shoreline habitats. These habitats are home to many vulnerable species including the eggs and early life stages of many freshwater fish. The Freshwater Oil Spill Remediation Project (FOReSt) at IISD-Experimental Lakes Area is assessing the effectiveness of two alternative secondary remedial methods to be used in conjunction with shoreline washing and sorbent collection: engineered floating wetlands (EFWs) and nutrient enhanced monitored natural recovery (eMNR). These methods were applied to large (>25 000 L) in-lake enclosures encompassing 5 m of wetland shoreline treated with ~1300 g of oil. Three unoiled enclosures served as references. Wild fathead minnow (*Pimephales promelas*) eggs were collected from a nearby pristine lake and exposed to

water from the enclosures for 7 days post-fertilization. Survivorship and developmental endpoints were monitored during exposure and malformations were assessed at the end of the 7 days. A subset of larvae was monitored for behavioural responses to light-dark stimuli, and another subset underwent respirometry trials to determine oxygen consumption. Information from the FOrEst project will provide spill responders and environmental managers with tools to assess the efficacy of less invasive oil spill clean up methods.

The effects of environmentally relevant and supra-environmental concentrations of metformin on embryonic and larval zebrafish (*Danio rerio*) (PL)

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Metformin is the most common first-line oral therapeutic agent used in the treatment of type-2 diabetes. Because of its widespread use, metformin has been increasingly detected in wastewater effluent. It is partially bio-transformed into guanyurea is subsequently released into aquatic environments. Since the literature concerning the effect of metformin and guanyl urea on early life stages of fish is scant, the aim of this research was to understand the potential influence of metformin and guanyurea on survival, and cardiometabolic and behavioural responses, in developing zebrafish embryos, from fertilization (3 hours post-fertilization (hpf)) to first feed (5 days post-fertilization (dpf)). To this end, early-stage embryos (3 hpf) were exposed to environmentally relevant (0.4, 4, and 40 $\mu\text{g}\cdot\text{L}^{-1}$) and supra-environmental (400 and 4000 $\mu\text{g}\cdot\text{L}^{-1}$) concentrations of the two chemicals, from the 4-cell stage to 120 hpf. Metformin caused an increased mortality of 20% in the highest treatment group (4000 $\mu\text{g}\cdot\text{L}^{-1}$) compared to controls. Metformin did not cause alterations in percent hatch at 48 hours, or heart rate measurements over the examined developmental stages. In addition, metformin did not cause alterations in general swimming, light-dark movement, startle response, or thigmotaxis, irrespective of exposure concentration. Exposure to guanyurea over the same developmental stages did cause significant differences in mortality or percent hatch at 48 hours. We are currently exploring the effects of the two chemicals on morphology and metabolic rates in developing zebrafish. To date, our data demonstrate that neither metformin nor guanyurea adversely impact embryonic development of zebrafish at these concentrations.

Effects of methylmercury on songbird brain during spring reproductive onset (PL)

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In spring, increasing photoperiod stimulates the hypothalamus-pituitary-gonad (HPG) axis in seasonally-breeding birds, inducing their transition from a winter physiology toward reproductive physiology. In songbird brains, spring reproductive onset is associated with an increased production of gonadotropin-releasing hormone (GnRH) that leads to HPG axis stimulation, gonad growth, and increased sex steroid hormones, which increase size and number of neurons in song control areas of the brain. Methylmercury is a neuro-toxicant known to affect sexual maturation, reproductive behaviour, and output in birds but the mechanisms behind such effect are not well understood. We hypothesized that methylmercury exposure during winter could impact songbird reproductive onset in spring via actions on GnRH and neurogenesis. We fed song sparrows (*Melospiza melodia*) a diet with an environmentally relevant level of methylmercury (0.22 ppm MeHgCl wet weight) or an uncontaminated diet during 3 months, and then photostimulated and kept them for post-exposure observation during 21 days. Preliminary results show no treatment effects on brain GnRH or testes size, suggesting that spring reproductive onset might not be affected by methylmercury in birds. We will present results of newly-produced-cells count in song control area that will indicate if MeHg may affect neurogenesis via a pathway other than the HPG axis. We will also present data of neuronal axons myelination that will indicate if methylmercury may affect other neurological aspects at this level of exposure. This study will deepen our understanding of methylmercury effects in songbird brain and spring reproductive onset.

Poster Hall

Characterization and evaluation of potential contaminants of concern in small craft harbours sediments in Nova Scotia (PO)

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Small craft harbours (SCHs) in Nova Scotia are vital for the fishing industry and have high socioeconomic and cultural importance for surrounding communities. Contamination in marine sediments can have significant impacts on biota and humans, including fishing activities and local economy. This study characterized the distribution of over 500 petroleum hydrocarbons (PHCs) and polychlorinated biphenyls (PCBs) samples in 31 SCHs sediments in Nova Scotia between 2000 and 2017. Data was acquired from marine sediment sampling programs conducted by Fisheries and Oceans Canada, and federal and regional sediment quality guidelines were used to determine exceedances. While single contaminants can be below sediment quality guidelines, interaction of multiple contaminants could exacerbate ecological risk. An ecological risk evaluation for PHCs, PCBs, and other contaminants, characterized in prior studies, was also conducted. Results show exceedances for heavy PHCs, expected given their longer permanence in sediments and lower volatility. However, only 7% of the samples exceeded 500 ppm, where benthic impairment is observed, showing low risk. PCBs do not pose high risk to biota since only 6 samples exceeded the higher effect level threshold and 25% of them exceeded the lower effect level one. Most SCHs showed low or no ecological risk, with only two SCHs presenting moderate risk. While urgent action is not needed, monitoring is recommended for these SCHs to confirm that pollution is not increasing, and to potentially identify and control contamination sources. Integrated results inform harbour authorities about historical and current state of SCHs, so future risk-management options can be developed and prioritized.

Characterizing blood plasma and organ tissue proteins in rainbow trout (*Oncorhynchus mykiss*) using a non-targeted proteomics approach (PO)

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The word “proteome” refers to the entire set of proteins expressed by a cell, tissue, or organism. The proteome is not static. Instead, protein expression patterns fluctuate and adapt to internal or external cues to meet the needs of an organism. The dynamicity of an organism’s proteome can therefore reveal significant information about an organism’s health, such as early detection of disease and environmental exposure. Proteome databases

contain limited information regarding organisms outside of medicinal biology. The Uniprot human and mouse proteomes are extensively reviewed and ~50% of both proteomes include tissue specificity, while >99% of the rainbow trout proteome is unreviewed and lacks tissue specificity. The current study aimed to discover tissue-specific information on the rainbow trout proteome using a systems-biology approach. Blood plasma and tissue proteins (brain, liver, gill, heart, and kidney) will be identified and characterized, and the potential origin of blood plasma proteins will be assessed by cross comparing plasma to tissue proteomes. Sex differences will also be considered. Blood and tissue from male and female rainbow trout were collected, plasma and tissue proteins were analyzed using liquid chromatography tandem mass spectrometry with data dependent acquisition, and peptide sequences were identified and matched to the Uniprot rainbow trout reference proteome. Preliminary data suggest distinct sub-proteomes amongst plasma and all five tissues, though sex differences were minimal. Expanding our knowledge on proteins in blood plasma and tissue specificity could help drive future research in biomarker discovery, progress current proteomic databases, and improve current methods of environmental health monitoring.

Comparative analysis of transcriptomic points-of-departure (tPODs) and apical responses in embryo-larval fathead minnows exposed to fluoxetine (PO)

Alper James Alcaraz¹, Shaina Baraniuk¹, Kamil Mikulášek², Bradley Park¹, Taylor Lane^{1,3}, Connor Burbridge¹, Jessica Ewald⁴, David Potěšil², Jianguo Xia⁴, Zbyněk Zdráhal², David Schneider¹, Doug Crump⁵, Niladri Basu⁴, Natacha Hogan¹, Markus Brinkmann¹, Markus Hecker¹

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Current approaches in chemical hazard assessment face significant challenges because they rely on live animal testing, which is time consuming, expensive, and ethically questionable. These concerns serve as an impetus to develop new approach methodologies (NAMs) that do not rely on live animal tests. This study explored a molecular benchmark dose (BMD) approach using a 7-day embryo-larval fathead minnow (FHM) assay to derive transcriptomic points-of-departure (tPODs) to predict apical BMDs of fluoxetine (FLX), a highly prescribed and potent selective serotonin reuptake inhibitor frequently detected in surface waters. Fertilized FHM embryos were exposed to graded concentrations of FLX (confirmed at <LOD, 0.19, 0.74, 3.38, 10.2, and 47.5 $\mu\text{g}\cdot\text{L}^{-1}$) for 32 days. Subsets of fish were subjected to omics and locomotor analyses at 7 days post-fertilization (dpf), and to histological and biometric measurements at 32 dpf. Enrichment analyses of transcriptomics and proteomics data revealed significant perturbations in gene sets associated with serotonergic and axonal functions. BMD analysis resulted in tPOD values of 0.56 $\mu\text{g}\cdot\text{L}^{-1}$ (median of the 20 most sensitive gene-level BMDs), 5.0 $\mu\text{g}\cdot\text{L}^{-1}$ (tenth percentile of all gene-level BMDs), 7.51 $\mu\text{g}\cdot\text{L}^{-1}$ (mode of the first peak of all gene-level BMDs), and 5.66 $\mu\text{g}\cdot\text{L}^{-1}$ (pathway-level BMD). These tPODs were protective of locomotor and reduced

body weight effects (LOEC of $10.2 \mu\text{g}\cdot\text{L}^{-1}$) observed in this study, and were reflective of chronic apical BMDs of FLX reported in the literature. The distribution of gene-level BMDs followed a bimodal pattern, revealing disruptions of neurotoxic and metabolic pathways at low and high concentrations, respectively. This is one of the first studies to derive protective tPODs for FLX using a short-term embryo assay at a life stage not considered to be a live animal under current legislations. This study is part of the EcoToxChip project (www.ecotoxchip.ca).

Effects of winter cold on acute copper bioaccumulation and toxicity in the banded killifish (*Fundulus diaphanus*) (PO)

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Disruption of Na^+ regulation has been reported in freshwater fish acutely exposed to copper (Cu) concentrations above naturally occurring levels. In addition, long term acclimation of freshwater fish to near-freezing temperatures has been shown to result in a decrease of serum sodium (Na^+) and chloride (Cl^-), suggesting impaired ionic regulation. Yet, despite the wide temperature fluctuations fish living in temperate waters experience annually, there is little known regarding the amplitude of acute Cu toxicity in winter cold. Our research will investigate cold temperature effects on acute Cu toxicity in banded killifish (*Fundulus diaphanus*). After acclimation to two temperatures (4 and 14°C), we will compare unidirectional movement of Na^+ in fish exposed to sublethal concentrations of Cu (5 and $25 \mu\text{g}\cdot\text{L}^{-1}$) using a radioisotope Na^{22} to determine influx, outflux, and net flux. Net flux of ammonia (NH_3), Cl^- , and other cations will also be investigated. Copper bioaccumulation in fish gills will also be measured. We anticipate the loss of plasma Na^+ as a result of acute Cu exposure to be exacerbated in fish acclimated to 4°C . This research project will contribute to our understanding of how cold temperatures may exacerbate the effects of acute Cu toxicity and the potential risks for fish species living in the Wolastoq | Saint John River.

Evaluation of historical mayfly (*Neocloeon triangulifer*) toxicity test results for drivers of test variability and control performance (PO)

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The EPT (Ephemeroptera, Plecoptera, and Trichoptera) taxa of insects are widely used in field biomonitoring programs because they are recognized as sensitive indicators of environmental change. Use in field programs have increased demand for laboratory-based tests of representative species; however, no standard methods for these taxa currently

exist in Canada. Progress with culturing methods and testing protocols have been made with the mayfly *Neocloeon triangulifer* (a member of Ephemeroptera), though some challenges remain. Here, we provide an overview of the unique characteristics and considerations of mayfly tests, and examine the sensitivity and variability of endpoints within and among tests. A historical dataset of 14-day sodium chloride tests used to establish mayfly culture sensitivity (reference toxicant tests) was used to compare effect concentrations for survival and growth as well as to retroactively calculate percent mean significant differences (PMSDs; an indication of test sensitivity) for each test within our database. Furthermore, all 14-day mayfly tests conducted to date were used to determine an internal expected range of control performance (survival and growth) results. These comparisons begin to elucidate differences between sensitivity of endpoints and repeatability of results, and to establish a range of PMSD values that can reasonably be anticipated with current methods. Finally, we discuss experimental design, test acceptability criteria, and endpoint selection considerations. These analyses contribute to our overall understanding of the variability within and among tests using the current methods and identify opportunities for improvement.

Mapping contaminated sediments from historic gold mines using satellite imagery (PO)

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In the mid-19th to early 20th centuries, millions of tonnes of ore were extracted from over 60 gold mining districts in Nova Scotia. This ore rock invariably contained high levels of arsenic-bearing minerals like arsenopyrite. The ore was crushed into a fine, sandy material to facilitate gold extraction. In many cases, this extraction was performed by a mercury amalgamation process which imbued sediment with high levels of this toxic element. The fine waste material, called tailings, was typically discarded into streams, wetlands, or depressions near the mining operations. These tailings fields remain a century later and may continue to leach arsenic and mercury into stream systems and their immediate environment. In this study, we employ remote sensing techniques to indicate areas that may contain tailings. Images from the Sentinel-2 satellite are classified by a random forest algorithm within Google Earth Engine to automatically indicate land cover areas that resemble known tailings, based on various input variables. The goal of this project is to help identify the presence and extent of contaminated sediments near historic mine sites across the province that are a priority for additional research and potential remediation.

Optimizing fish skin mucus sampling and protein extraction methods for proteomic analyses (PO)

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The skin mucus of fish is their primary barrier against contaminants such as industrially released metals present in the aquatic environment. The expression of proteins on the skin mucus are modulated in response to dynamic environmental conditions and can be used as tools to examine fish health. Currently, there is a growing need for sampling procedures that are minimally invasive and non-lethal. To study the changes in the proteome of rainbow trout, three different minimally invasive methods for sampling mucus (absorption with a wipe, wiping with a wipe, and scraping of the skin), and two different protein preparation methods (ultrafiltration and Bligh-Dyer liquid-liquid extraction), will be compared to ascertain the best method for protein quantification. Based on the findings, the best method will be selected to conduct non-targeted proteomic analyses on the skin mucus samples of rainbow trout exposed to environmentally relevant concentrations of waterborne nickel. By employing liquid chromatography tandem mass spectrometry (LC-MS/MS) with mucus from male and female fish, sex-specific protein and metabolite biomarkers of nickel stress occurring temporally in trout skin mucus will be investigated. The goal of this study is to advance the use of mucus sampling as a minimally invasive tool to deliver holistic information about fish health, rendering it applicable in future biomonitoring and risk assessment programs.

Pharmaceuticals and personal care products and their sublethal and lethal effects in aquatic organisms (PO)

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Pharmaceuticals and personal care products (PPCPs) include over-the-counter and prescription drugs, veterinary drugs, fragrances, and cosmetics. PPCPs have been detected in aquatic environments at low concentrations and are emerging as contaminants of concern. PPCPs are primarily released into aquatic environments via untreated sewage, wastewater treatment plants, and landfill leachate, and can affect aquatic life through persistence, bioaccumulation, and toxicity. To understand PPCP toxicity on aquatic organisms, a literature review was conducted that identified aquatic organisms known to be affected by PPCPs; concentrations of PPCPs reported as producing sublethal and lethal effects in aquatic organisms; and research gaps on PPCP aquatic toxicity. Twelve PPCPs were selected from three seminal studies for review, including bisphenol A, carbamazepine, erythromycin, fluoxetine, linear alkylbenzene sulfonate, metoprolol, naproxen, nonylphenol, ofloxacin, sertraline, sulfamethoxazole, and triclosan. Many aquatic

species were affected by PPCPs at sublethal and lethal exposures, including sublethal effects at environmentally relevant concentrations. Because lethal effects were seldom observed at environmentally relevant concentrations, many studies considered PPCPs non-toxic. Few studies have compared the effects of PPCPs on the same organisms for identical exposure parameters (time and concentration), resulting in wide variation in reported toxicity levels with limited consensus in the academic literature. Species sensitivity distributions for some PPCPs show that aquatic species are affected lethally at environmentally relevant concentrations. More studies on indirect and long-term ecological effects along with testing chronic toxicity of PPCPs at environmentally relevant concentrations are recommended. This research provided an in-depth understanding of the toxicity of PPCPs in aquatic organisms, even when present at very low concentrations, such as those resulting in sublethal effects.

Refining adverse outcome pathways (AOPs) using Japanese medaka (*Oryzias latipes*) embryos exposed to 2,3,7,8-tetrachlorodibenzodioxin (PO)

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Adverse outcome pathways (AOPs) are a framework that categorizes the impact of chemicals from the molecular to the ecosystem level. This research aims to refine two existing AOPs that are initiated when chemicals bind to the aryl hydrocarbon receptor (AhR), resulting in altered cardiovascular development. Japanese medaka (*Oryzias latipes*) hatch in nine days, and their cardiovascular system can be easily observed using a stereomicroscope due to their transparent nature. Embryos mature to morula stage only 4 hours post-fertilization (hpf). At 2 days post-fertilization (dpf), blood circulation begins distinctly with the pumping of the blood island; and at 7 dpf, cardiac development is complete marked by the development of the pericardial sac around the heart. Medaka embryos were exposed to 0.001, 0.01, 0.1, 1, and 10 ppb of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) for 1 hour at 4 hpf. Embryos were collected and observed at 2 dpf, 7 dpf, and hatch. Cardiac impairment was detected by heart rate analysis using open-source HeartBeat software on 7 dpf videos, and hatch malformation analysis was conducted by scoring the severity of pericardial edema. Our preliminary studies show that medaka embryos exposed for 1 hour to TCDD at 4 hpf display altered cardiovascular development seen as reduced blood flow, pericardial edema, and impaired angiogenesis. Targeted qPCR on AhR, COX-2, and HIF-1 α suggests activation of one of the two prospective AOPs. Refining these AOPs will benefit society by improving our ability to respond to chemical contaminants of concern more effectively.

Tracing microplastic-derived carbon in a freshwater food web (PO)

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Microplastics, a diverse suite of carbon-based contaminants, are of concern due to their ubiquity and persistence in the environment. Slowly over time, carbon (C)-containing compounds are released during microplastic degradation, some of which are biolabile and can be utilized by microbes. The ultimate fate of microplastics in the environment is difficult to study because we cannot differentiate between microplastic-derived carbon and natural carbon using traditional analytical techniques. The objective of our research is to determine whether microplastics in a boreal lake ecosystem will leach carbon over time that will subsequently be incorporated into the aquatic food web. We hypothesize that 1) microplastics in a lake will leach C over time, and 2) dissolved organic and inorganic carbon (DOC and DIC) derived from microplastics will be bioavailable and incorporated into the food web through utilization by microbes. To study the fate of microplastic-derived carbon, we set up two ~1100-L limnocorrals (*in situ* enclosures) in the littoral zone of Lake 378 at the International Institute for Sustainable Development-Experimental Lakes Area in northwestern Ontario, Canada. In one limnocorral, 3268 particles per L (or 0.05 mg·L⁻¹) of 99% ¹³C-labelled polystyrene was added in May 2021, while the second limnocorral will serve as a control. $\delta^{13}\text{C}$ -DIC and $\delta^{13}\text{C}$ -DOC in filtered water will be measured monthly to determine leaching of C from the plastic. Compound-specific isotope analysis of amino acids will be used to measure enrichment of ¹³C in zooplankton and benthic invertebrates collected monthly from each limnocorral. This project will give insight into the potential for microplastic degradation in freshwater lake ecosystems and the fate of microplastic-derived carbon in aquatic environments.

Untargeted plasma proteomics offers many insights on toxicity of PFAA mixtures, legacy and short chain compounds with PPAR β/δ , and developmental neurotoxicity as avenues of interest (PO)

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Perfluoroalkyl acids (PFAAs) are persistent environmental pollutants often being the final degradation products of many forms of per- and polyfluoroalkyl substances (PFAS). PFAAs typically make up the majority of PFAS in surface waters, and are present as extensive mixtures of structurally similar congeners. A scarcity of toxicological information still exists for short chain PFAAs, and the effects of PFAA mixtures are not established. In order to address these knowledge gaps, we performed a 3-week, aqueous exposure of

rainbow trout to 3 different concentrations of a PFAA mixture (50, 100, and 500 ng·L⁻¹) and conducted untargeted proteomics using blood plasma. Another set of exposures to individual PFAAs (25 nM PFOS, 25 nM PFOA, 25 nM PFBS, 25 nM PFBA) were conducted at higher concentrations for reference. The mixture exposures caused a relatively similar change in the number of significantly altered proteins and biological pathways (FDR<0.05) when compared to legacy contaminants PFOA and PFOS, while being 20- to 200-fold lower in terms of molarity. Biological pathway analysis revealed peroxisome proliferator-activated receptor β/δ (PPAR β/δ) to be elevated in many of the treatments along with several proteins involved in lipid metabolism. Another process, which was found to be affected across all treatments, was nervous system development with neuronal fasciculation being a common sub pathway. Overall, the current study emphasizes the need for toxicological testing of PFAA mixtures, potential neurotoxicity of PFAAs and PPAR β/δ activation as another target of PFAAs in lesser-studied vertebrates.

Using biofilms as a tool to assess the occurrence and impact of pesticides in Ontario's streams (PO)

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Monitoring the presence and concentrations of pesticides in Ontario's aquatic systems is necessary to understand the impacts on the aquatic environment. Ontario's streams are susceptible to pesticide pollutants which are transported outside of the intended area of application from surrounding agricultural fields. Biofilms are a collective of microorganisms that grow on hard surfaces in aquatic ecosystems and have been shown to bioconcentrate pesticides in water. Biofilms are highly responsive to chemical and biological changes in the environment, and therefore have the potential to act as a cost-effective, integrated sampling tool to monitor pesticide exposures in aquatic ecosystems. The objective of this study is to determine whether biofilms can be used to provide an accurate representation of pesticide exposure in Ontario's aquatic systems. Ten sites across southern Ontario were sampled between May-September 2021. At each site, water, sediment, and biofilm, colonizing both artificial and natural substrate, were collected and analyzed for the presence of ~500 pesticides. This data will be used to determine the distribution of pesticides in the three matrices (water, sediment, and biofilm) and will provide an assessment of how well each matrix characterizes the streams' exposure to pesticides. It is hypothesized that the partitioning of pesticides within water, sediment, and biofilm will be related to the physicochemical properties of the detected pesticide. The development of a novel and cost-effective sampling tool would benefit Ontario's current pesticide monitoring program and may greatly improve the reporting on pesticide pollutants and water quality in southern Ontario's aquatic environments.

Acute toxicity of polycyclic aromatic compounds to commercially spawned lumpfish (*Cyclopterus lumpus*) larvae: Application of a novel test species (PO)

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Lumpfish, *Cyclopterus lumpus*, are teleosts native to the North Atlantic and can be found as far north as Greenland and as far south as Chesapeake Bay. Due to their effectiveness at preying on sea lice, lumpfish are a species of emerging commercial importance in the aquaculture industry. The overlap of lumpfish spawning habitat with the transportation of petroleum products by sea increases the risk of exposure to this life stage, and the relative sensitivity of lumpfish larvae to exposure is poorly understood. In this study, lumpfish larvae were exposed to polycyclic aromatic compounds (PACs), thought to be the compounds primarily responsible for the aquatic toxicity of crude oil spills. Exposures were conducted for 48 hours using a passive dosing system at 10 and 4°C to mimic spatiotemporal differences in temperature observed in lumpfish habitat, and mortality was assessed at various time points during exposure. Relative species sensitivity was calculated using the critical target lipid body burden (CTLBB), described in the target lipid model (TLM). The CTLBB for lumpfish larvae was $7.7 \pm 16.6 \mu\text{mol}\cdot\text{g}^{-1}\text{octanol}$, which ranks lumpfish as one of the most sensitive species included in the TLM. The time course of effects observed during the 48-hour exposure period differed between the PACs, and exposure temperature altered the toxicity of the PACs tested. This study suggests lumpfish larvae are sensitive, and given their unique life history traits, they represent an ideal model organism that could be used in place of more difficult to culture species for risk assessment in Atlantic Canada.

An approach for the ecological risk assessment of surfactants under Canada's Chemicals Management Plan (PO)

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A surfactant is defined as an organic compound with at least one hydrophilic group and one long-chain hydrophobic group. Surfactants congregate at interfaces and disrupt cohesive forces, which results in a reduction of surface tension. Due to their surface activity, surfactants are widely used in household applications including detergents and personal care products, as well as in industrial processes such as emulsification, dispersion, and foaming and defoaming. However, the surface activity of these compounds, coupled with differences in behaviour associated with their charge status, makes them difficult to evaluate in the context of ecological risk assessment. Environment and Climate Change Canada (ECCC) has developed guidance for the risk assessment of surfactants under Canada's Chemicals Management Plan (CMP). This guidance considers the charge status of

a surfactant (anionic, cationic, or non-ionic) as well as the length of the hydrophobic carbon chain to develop recommendations on how to evaluate the fate, behaviour, ecotoxicity, and environmental exposure potential of a given substance. This presentation will provide a practical overview of ECCC's approach to evaluating surfactants, including how to interpret toxicity studies and how to adjust model parameters to account for surface activity. This approach will be illustrated using case studies from CMP substance screening assessments such as the aliphatic amines and poly(alkoxylates/ethers) groups.

Application of bioaccumulation data in ecological assessments under Canada's Chemicals Management Plan (PO)

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Bioaccumulation data have been used in the risk assessment of chemicals as part of the Chemicals Management Plan (CMP) under the *Canadian Environmental Protection Act, 1999* (CEPA) for over two decades. The science of bioaccumulation and its application in risk assessments has evolved during this time to be more versatile and comprehensive. The range of data for bioaccumulation consideration has been expanded from aquatic to terrestrial species, and with a broader application, from simply evaluating bioaccumulation potential of a substance to informing ecotoxicity and exposure. This poster describes how bioaccumulation data are applied in risk assessments moving forward. One case study will illustrate the application of bioaccumulation data in risk assessment in the context of version 2 of the ecological risk classification of organic substances (ERC2), a tool developed by Environment and Climate Change Canada (ECCC) for the purpose of prioritizing approximately 12 000 chemicals on the Domestic Substances List (DSL) that did not meet the categorization criteria used to prioritize substances for assessment in 2006 under the CMP. The other case study will highlight how bioaccumulation data, empirical or modelled with consideration of biotransformation potential, can be applied in an assessment, for example, to refine tissue residue estimation to allow for characterizing ecotoxicity potential of a substance, which can contribute to the derivation of a predicted no effect concentration. These applications use bioaccumulation data as part of the overall weight-of-evidence analysis, which enhances science-based decision-making on chemicals management.

Characterization and interpolation of PCB and PBDE sediment levels in and around resident killer whale critical habitat along the coast of BC, Canada (PO)

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British Columbia's (BC) northern and southern resident killer whale (*Orcinus orca*) populations (NRKW and SRKW, respectively) are listed as threatened and endangered in Canada, respectively, and persistent bioaccumulating contaminants, such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), pose a key threat to their survival and recovery. PCB and PBDE concentrations in subtidal surface sediments collected from 97 sites along the BC coast were analyzed to assess resident killer whale habitat quality. Total PCB and PBDE concentrations, and congener and homolog profiles in sediments, varied among sites. For PCBs, co-eluted congeners, PCB-129 + 138 + 160 + 163, were found to be the highest, followed by PCB-153 + 168, PCB-110 + 115, PCB-147 + 149, PCB-90 + 101 + 113, and PCB-118. For PBDEs, individual congeners were ranked as follows: BDE-209 > BDE-207 > BDE-206 > BDE-208 > BDE-47 > BDE-99. Congener profiles, as characterized by principal component analyses (PCA), showed more dispersion among sites for PCBs than PBDEs (PCBs: PC1 (38%) and PC2 (12%); PBDEs: PC1 (27%) and PC2 (16%)), and were correlated with octanol-water partition coefficient (LogKow; $p < 0.003$) or concentration weighted average of octanol-water partition coefficient (CWALogKow; $p < 0.001$) to PC1. Total PCB and PBDE concentrations exceeded British Columbia (BC) working sediment quality guidelines (PCBs: 3.7 pg·g⁻¹ dry weight; PBDEs: 1000 pg·g⁻¹ dry weight), developed to be protective of killer whales, at all sites and at approximately 34% of the sites by 4 (PCBs) and 2 (PBDEs) orders of magnitude, respectively. The probability of exceeding these guidelines for most of the interpolated areas over NRKW and SRKW critical habitats was >0.5, suggesting that both NRKW and SRKW populations are at risk from current levels of both PCBs and PBDEs along the BC coast. These results provide the most comprehensive assessment of PCBs and PBDEs in British Columbia waters and provide guidance that can be used for source control that will improve the quality of resident killer whale habitat.

Characterizing arsenic and mercury levels in fish tissue in the Mushkegowuk Region, northern Ontario (PO)

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Natural and anthropogenic processes can impact mercury and arsenic (As) levels in the environment and biota. Mercury bioaccumulates in fish and may present health risks for wild fish consumers. Additionally, while organic arsenic compounds are known to be less

harmful to health, inorganic arsenic is a carcinogen and is highly toxic. Through the FIShNET project, we are characterizing the links between environmental change, water quality, fish health, food safety, and food security in Mushkegowuk Region. FIShNET includes two components: 1) human health research, and 2) environmental monitoring. Due to the COVID-19 pandemic, the first component has been postponed. For the second component we will be analyzing fish samples collected in 2017. We are characterizing mercury and arsenic contamination in fish tissue samples of cisco, lake whitefish, and northern pike. Initial analyses show elevated levels of total arsenic in some species, and there are existing consumption advisories based on total mercury and total arsenic levels data, generated by the Province of Ontario. Due to a lack of information on arsenic speciation, the health risks posed by the reported arsenic levels are currently highly uncertain. The main outcomes of this work include i) quantification of inorganic arsenic using As-speciation data; and ii) communication of exposure assessment knowledge on mercury and arsenic contamination to the Mushkegowuk Region. Findings will support public health strategies that promote traditional food reliance in ways that limit contaminant exposure and will inform measures to improve food security in the context of global climate change.

Chronic effects of benzophenone (BP) sunscreens on fathead minnows (PO)

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Benzophenones (BPs) are ultraviolet filters found in sunscreens and personal care products. Concentrations up to 0.7 µg·L⁻¹ have been detected in municipal wastewater effluents (MWWEs). This study investigated the chronic effects of three BPs in early-life stages of fathead minnow. Eggs and larvae were exposed to concentrations of BP-4 at nominal concentrations of 0, 0.1, 1, 10, 100, 1000, and 10 000 µg·L⁻¹ in a daily static-renewal set-up. There were 12 replicates of control beakers and 4 replicates of each BP-4 concentration, each beaker containing 20 fathead minnow embryos/larvae. The two lowest benzophenone exposure concentrations approximate river and MWE concentrations. Mean measured concentrations of BP-4 were on average 115% of nominal exposure concentrations, and in controls were always non-detectable. Over the 21-day exposure (5 days embryo, and 16 days post-hatch (dph)), BP-4 did not affect survival or growth of the larval fish. Hatch success, time to hatch, deformities in hatched fry, and survival until 9 and 16 dph were similar across all treatments (0.340 < ANOVA p<0.981). Growth (wet weight, length, and condition factor) assessed at 9 and 16 dph was not affected by BP-4 up to 10 000 µg·L⁻¹ (0.735 < ANOVA p<0.996). Preliminary data for BP-1 and BP-3 (oxybenzone) suggest these benzophenones do affect survival, growth, and deformities but only at high exposure concentrations. These exposures show that the three tested benzophenones, at

environmentally-relevant concentrations (up to 1 $\mu\text{g}\cdot\text{L}^{-1}$), do not cause any observable negative effects in this commonly-used lab fish species.

Chronic exposure of antidepressant venlafaxine in boreal lake mesocosms (PO)

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Venlafaxine is a commonly prescribed antidepressant drug which enters freshwater ecosystems via wastewater effluent and has been detected in surface waters at $>2.0 \mu\text{g}\cdot\text{L}^{-1}$. Despite its pseudo-persistence and expected chronic exposure, studies on its environmental fate and effects on freshwater ecosystems are lacking. A 10-week exposure using in-lake mesocosms was conducted at the IISD-Experimental Lakes Area to assess venlafaxine in an ecological context (individual- to community-level). Mesocosms (n=10, 2-m diameter, 1.5-m deep) were deployed in an experimental lake and spiked with venlafaxine at concentrations in a regression design ranging from 0 $\mu\text{g}\cdot\text{L}^{-1}$ (control; in triplicate) to 100 $\mu\text{g}\cdot\text{L}^{-1}$ (n=1 per treatment). Each enclosure was stocked with 5 finescale dace (*Chrosomus neogaeus*) prior to spiking. Exposure levels were maintained at the target concentration by re-spiking weekly over the study period, as the half-life of venlafaxine was estimated at approximately 5 days. Biomass and community composition of biofilm, phytoplankton, zooplankton, and benthic and emergent invertebrates were monitored, as well as length and weight of adult finescale dace. In-lab, 7-day embryo-larval exposures were conducted using wild-collected fathead minnow (*Pimephales promelas*) eggs exposed to water from the in-lake mesocosms, assessing percent hatch, mortality, and developmental malformations. Behavioural trials with exposed *Hyaella azteca* and fathead minnow larvae were also conducted. By understanding the environmental fate, direct and indirect ecological effects, and bioaccumulation potential, we will be able to estimate the risk of venlafaxine to the environment as it is currently being discharged in wastewater treatment plant effluent.

Chronic perfluorooctanesulfonic acid (PFOS) exposure impacts anxiety-like behaviour but not boldness in zebrafish (*Danio rerio*) (PO)

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Perfluorooctanesulfonic acid (PFOS) is a widespread organic pollutant that is highly prevalent in humans, wildlife, and the environment due to its stable chemical properties that prevents degradation. While the toxicity of PFOS has been well studied in zebrafish

(*Danio rerio*), its impact on behaviour is largely unknown. To address this, zebrafish were exposed to either 0, 0.1, or 1.0 mg·L⁻¹ for 40 days. After 20 and 40 days of chronic PFOS exposure, anxiety-like behaviour and boldness were assessed using both the novel approach test and novel tank dive test, respectively. In the novel tank dive test, zebrafish exposed to a concentration of 1.0 mg·L⁻¹ PFOS for 40 days spent significantly more time in the upper zone and significantly less time in the bottom zone of the tank, indicating an anxiolytic effect. Moreover, after 20- and 40-day PFOS exposure, we observed no significant impact on zebrafish boldness at any concentration. Exposure to 1.0 mg·L⁻¹ PFOS significantly decreased locomotion in the novel tank dive test after 20 days, whereas after 40 days we observed no significant impact to locomotion at any concentration, indicating short-term effects of 1.0 mg·L⁻¹ PFOS exposure on zebrafish locomotion. In conclusion, these results provide support for an anxiolytic effect of long-term PFOS exposure on zebrafish behaviour.

Comprehensive assessments of oolichan (*Thaleichthys pacificus*) population health and range using molecular tools in impacted aquatic spawning habitat (PO)

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Protection of threatened aquatic species relies on timely, reliable, and detailed monitoring data, which can be highly challenging for species whose life histories are not well described. Oolichan (eulachon: *Thaleichthys pacificus*), an anadromous smelt with a discontinuous spawning range from California to Alaska, has experienced steeply declining population numbers over the past few decades, and the cause of this has not yet been clearly defined. In this study, we assess oolichan populations on the Central Coast in Haisla territory near Kitimat, British Columbia, which has seen significant recent development including a new oil processing plant and increased marine traffic, using a newly developed and highly sensitive environmental DNA (eDNA) assay for the purpose of detection and abundance correlation of oolichan. In addition, we perform transcriptomic analysis of larvae and non-lethal fin clips using RNA-seq and quantitative real-time polymerase chain reaction methods to address the need for more effective population health assessment methods. Caudal fin clips have successfully been used to identify and characterize exposure to water soluble components of marine oil spills. By comparing specimens from spawning habitats with different levels of ecological impact, combined with rigorous abundance estimates of eDNA sampling methods, we aim to describe species population numbers, range, and health to a degree of detail that is impossible without this new generation of cutting edge, non-invasive molecular tools.

Does exposure to microplastics have immunotoxic effects on North American wood frog (*Rana sylvatica*) tadpoles and metamorphs? (PO)

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Microplastics (<5 mm) are an emerging, ubiquitous contaminant of concern, and little is known about their impact on the immune system and health of amphibians. Our study aims to evaluate whether exposure to microplastics leads to immunotoxic effects on North American wood frog (*Rana sylvatica*) tadpoles and metamorphs. *R. sylvatica* embryos were collected and continuously exposed throughout development in quintuplet to 0, 50 000, or 500 000 particles·L⁻¹ of microplastics in an outdoor mesocosm experiment at Queen's University Biological Station. Microplastics were prepared by mixing equal parts of polypropylene, polystyrene, and polyethylene terephthalate microplastics (40–1400 µm). Tissues were sampled from wood frog larvae weekly during Gosner stage (GS) 26–42 (whole tadpole) and once from metamorphs upon reaching GS 42–45 (skin, gut, liver, and spleen) for use in downstream immune gene expression analysis. Blood was collected from metamorphs (GS 42–45) and used to prepare Wright-Giemsa-stained blood smears for determination of differential leukocyte counts. Together, tissue gene expression analysis and differential leukocyte counts will permit overall assessment of changes to tadpole and metamorph immune function. Skin swabs of tadpoles (between GS 36–41) and metamorphs (GS 45) were collected for use in DNA extraction and 16S rRNA metabarcoding to evaluate potential changes in commensal skin bacterial communities important for immune defence against environmental pathogens. Our findings will provide important new knowledge on the effects of microplastics on developing amphibians, and provide insight on whether microplastics may be contributing to amphibian susceptibility to disease and population declines. [Funded by ECCC-IKPP grant]

Effects of microplastics on viability of the larval (glochidium) stage of freshwater mussel, *Lampsilis fasciola* (PO)

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Plastic debris polluting our waterways has been a concern for decades. Recently, increased attention has been placed on microplastics (MPs) contamination of aquatic ecosystems. These small plastic particles (<5 mm) have been observed in marine and freshwater ecosystems globally. Although effects of MPs ingestion on important marine invertebrate species have been demonstrated, little is known on the effect of microplastics on freshwater species. To date, freshwater studies have focused on the presence and/or

concentration of MPs in surface waters. To assess their risk, there is a need to compare environmental concentrations of MPs to concentrations that cause adverse effects. Freshwater mussels are a group of filter-feeding organisms that have experienced a decline due to habitat destruction and poor water quality, and they are under-represented in MPs research. In this study, standard methods were used to conduct acute (48-hour) toxicity tests with glochidia (larvae) of *Lampsilis fasciola*, a species of special concern. Tests were performed with pristine MP spheres of varying polymer types and sizes. Polystyrene (6 and 90 µm), polyethylene (10–32 µm, 75–90 µm, 850–1000 µm), and cellulose acetate (1000 µm) spheres, as well as polyethylene fibers (102 µm), were used in treatment concentrations spanning 50–300 000 MP·L⁻¹. Glochidia viability (i.e., the ability to close valves) was used as a surrogate for survival. *Lampsilis fasciola* glochidia were insensitive to each type of MP tested with effect concentrations being >300 000 MP·L⁻¹ or >1000 MP·L⁻¹, depending on treatment concentrations used. This data will help inform the risk assessment of MPs to freshwater biota.

Establishing and verifying the reliability of red macroalgae (*Ceramium tenuicorne*) cultures for the purpose of developing a new Canadian regulatory test (PO)

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As required by regulations pursuant to the Fisheries Act, prescribed test methods using marine algae species must be used for sublethal toxicity testing for effluent discharged into a marine or estuarine environment. Previously, the macroalgae *Champia parvula* had been used, but this test species is no longer being offered by North American labs due to concerns regarding data quality and the lack of available new plant material. Environment and Climate Change Canada (ECCC) has mandated AquaTox Testing & Consulting Inc. (Puslinch, Ontario) to evaluate the standardized ISO 10710:2010 method using the red macroalgae *Ceramium tenuicorne*. The genus is native to Canadian coastal waters, and this species was chosen due to its relative ease to culture in the laboratory. Whereas testing with *C. parvula* was based on a reproduction endpoint, the method using *C. tenuicorne* is a 7-day growth inhibition test. To further investigate the viability of using the ISO 10710:2010 standard in Canada, cultures of *C. tenuicorne* must be established and the reliability of the organism must be verified as a regulatory test species. To achieve this, a starter culture was obtained from the Norwegian Culture Collection of Algae (Oslo, Norway) and has been established in-house. Reference toxicant testing is currently ongoing to verify the reliability of the *C. tenuicorne* cultures. Along with a procedural report, these results will assist in determining if *C. tenuicorne* can be used as a viable test species in Canadian commercial testing laboratories.

How many sister wives is too many? Optimizing sex ratios of *Hyalella azteca* to reduce variability in brood size (PO)

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Hyalella azteca is a freshwater benthic crustacean used in ecotoxicology because it is ubiquitous in North American freshwater systems and is sensitive to changes in water quality. Standard toxicological test methods for this species incorporate lethal and sublethal (growth and reproduction) endpoints, though lethal endpoints are often favoured in the context of environmental monitoring. However, sublethal endpoints are important to consider as they are ecologically relevant and are often more sensitive than lethality. It is difficult to achieve robust data for reproduction because there is a naturally high biological variability associated with reproductive yield, and because growth and reproduction are related in *H. azteca*. Furthermore, males competing for mates adds to the variability in brood sizes that females produce. The purpose of this study was to characterize the reproductive capacity of *H. azteca* by determining the role of sex ratios in reproductive yield. It was hypothesized that a lower male to female ratio will reduce intraspecific male aggression, improve reproductive success, and lower biological variability in brood size. Experiments were initiated with adults that were placed in different male to female ratios in the absence of toxicants. Reproduction was monitored weekly for 7 weeks to determine which sex ratio had the least variable reproductive output over time. Results suggested treatments with low male to female ratios had lower variability in brood size. The results will provide insight into optimizing the reproductive capacity of *H. azteca*, which will increase the ability of reproductive toxicity tests to capture effects of toxicants on amphipod reproduction.

Investigating contaminant-related health effects in killer whales in British Columbia using omics (PO)

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Killer whales (*Orcinus orca*) are an iconic species in coastal British Columbia, Canada, with three distinct populations inhabiting the area: the northern resident, southern resident, and west coast Bigg's populations. Low food availability, contaminant exposure, and noise are the major threats to these populations with the southern residents being the most vulnerable. We measured PCB and PBDE levels in blubber biopsies collected from individuals in the southern resident, northern resident, and Bigg's populations (n=10 to 12). Additionally, we are investigating trends in metabolomic profiles and gene expression

as they relate to contaminant concentration, population, and biological factors. Using RNA sequencing, we are assessing gene expression throughout the transcriptome and identifying key genes responsive to contaminant exposure. Building upon decades of research by our team, these findings will provide a clearer understanding of health effects associated with contaminants in killer whales that can be used to inform risk-based prioritization of conservation efforts.

Investigating the reproductive and developmental toxicity of naphthenic acids derived from oil sands wastewater in wood frogs (PO)

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During the process of bitumen separation, large volumes of oil sands process-affected water (OSPW) are produced in the oil sands region of Alberta and stored in man-made tailings ponds. OSPW may enter aquatic ecosystems through accidental and intentional releases, and seepage through groundwater reserves. Naphthenic acids (NAs) have been deemed a major contributor to OSPW toxicity, but little is known about their ability to interfere with reproduction in amphibians. The aim of our study is to assess the potential for NA exposure to affect reproduction and offspring development in a common wetland breeder, the wood frog (*Lithobates sylvaticus*). Adult wood frogs (n=30) were collected at the Queen's University Biological Station near Elgin, Ontario. After 24 hours of exposure to 0, 5, or 10 mg·L⁻¹ NA extract, male and female frogs were combined in outdoor lake water mesocosms with the respective NA concentrations. Mating behaviour, including time to amplexus and oviposition, was recorded. Egg masses produced during mating events were monitored in parental mesocosms for fertilization success and embryonic abnormalities. A random subsample of hatchlings (50 per mesocosm, decreased to 15 after 5 weeks) was then exposed to parental NA concentrations until completion of metamorphosis (9–12 weeks). Larval survival, behaviour, time to metamorphosis, and frequency of developmental abnormalities were examined. In addition, thyroid hormone enzyme and receptor expression were measured. The results of this study will be useful in informing evidence-based policy decisions related to the management of OSPW and the protection of aquatic ecosystems in the oil sands region of Alberta.

Recovery of duckweed after acute and chronic 2,4-D and atrazine exposure (PO)

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Duckweed (*Lemna gibba* and *Lemna minor*) were exposed for 7, 14, 21, and 28 days to herbicides (2,4-D or atrazine) and transferred to clean media for 7 days to investigate their

potential to recover in the laboratory. 2,4-D and atrazine exposure concentrations were in the range of 12–731 $\mu\text{g}\cdot\text{L}^{-1}$, and 5–187 $\mu\text{g}\cdot\text{L}^{-1}$, respectively. The EC_{50} s and NOECs for relative growth rate (RGR) following recovery phase were $\geq 558 \mu\text{g}\cdot\text{L}^{-1}$ and $\geq 117 \mu\text{g}\cdot\text{L}^{-1}$ for 2,4-D, and $\geq 271 \mu\text{g}\cdot\text{L}^{-1}$ and $\geq 89 \mu\text{g}\cdot\text{L}^{-1}$ for atrazine. The values were greater than Canadian monitoring values for both herbicides, and this was interpreted as current exposures being insufficient to have a meaningful ecological impact. We also exposed *L. gibba* and *L. minor* to each herbicide (2,4-D exposures: 2, 4, and 8 $\mu\text{g}\cdot\text{L}^{-1}$ and atrazine exposures: 3, 5, 11, and 22 $\mu\text{g}\cdot\text{L}^{-1}$) for up to 60 days continuously and found out that majority of endpoints were not significantly lowered from the control after 2,4-D or atrazine exposure. Overall, the toxicity values from 2,4-D and atrazine for all tests were greater than the regulation guidelines from the Canadian Council of Ministers of the Environment Water Quality Guidelines for the Protection of Aquatic Life from 1999 (i.e., 4 $\mu\text{g}\cdot\text{L}^{-1}$, and 1.8 $\mu\text{g}\cdot\text{L}^{-1}$ for 2,4-D and atrazine, respectively). We also found no effects at the United States Environmental Protection Agency 60-day guideline values of 10 $\mu\text{g}\cdot\text{L}^{-1}$. We conclude that current regulatory levels are likely protective of macrophytes.

Reproductive and developmental effects of chronic dietary arsenic exposure in adult Zebrafish (*Danio rerio*) (PO)

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Diet is an important source of arsenic (As) exposure and accumulation for fish living in As-contaminated environments. However, the reproductive impacts of environmentally relevant levels of dietary As exposure in fish are currently not well understood. Thus, the present study was designed to gather new insights into the reproductive and developmental effects of chronic dietary exposure to As in adult zebrafish. Fish were exposed to different concentrations of dietary As (control, 30, 60, and 100 $\mu\text{g}\cdot\text{g}^{-1}$ dry weight; as sodium arsenite) for 90 days. To understand sex specific impacts, As-exposed females were mated with control males (maternal exposure (ME)), and vice versa (paternal exposure (PE)). In ME groups, fish exposed to 60 and 100 $\mu\text{g}\cdot\text{g}^{-1}$ dietary As showed a significant reduction in fecundity (50% and 80%, respectively), hatching success (at 48, 72, and 96 hours post-fertilization (hpf)), and larval survival rate (at 120 hpf) when compared to the control fish. In contrast, in PE groups, there was no effect on fish fecundity, however similar reductions in hatching success (40% and 70%) and larval survival rate were observed in fish exposed to 60 $\mu\text{g}\cdot\text{g}^{-1}$ and 100 $\mu\text{g}\cdot\text{g}^{-1}$ dietary As, respectively. In both ME and PE groups, elevated As exposure led to a significant increase in abnormal larval phenotypes at 5 days post-fertilization, which were characterized by cardiac edema, inflamed swim bladder, dorsal curvature, and spine and tail deformities. Overall, the present study demonstrates that reproductive and developmental effects of As in adult zebrafish are mediated by chronic exposure to dietary As in males or females.

The importance of water and diet as sources of microplastics to American bullfrog (*Lithobates catesbeiana*) tadpoles (PO)

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Microplastics are an emerging pollutant of concern that amphibians may be exposed to through multiple pathways. Our study compared the relative importance of water and diet as sources of microplastics to bullfrog (*Lithobates catesbeianus*) tadpoles. We compared these two exposure routes in a triplicate 2 X 2 factorial design (control, contaminated water, contaminated food, and both contaminated water and food) in an outdoor 12-mesocosm experiment at the Queen's University Biological Station. To assess water exposure, white polyethylene fragments were added to the water column of the mesocosms (each containing 90 L of lake water) at a concentration of 100 particles·L⁻¹. To assess food exposure, green polyethylene fragments were added to a mixture of agar, spinach, and commercial tadpole food at a concentration of 3.4 µg·g⁻¹. Tadpoles (Gosner stage 25; n=60) were captured from a natural lake at the field station and exposed to treatments for 10 days in August 2020. Treatment conditions were verified by counting microplastics in samples of mesocosm water and food. Tadpoles were collected every 48 hours for microplastic quantification in the gastrointestinal tract and the rest of the body. Microplastics were isolated from tissues using a 20% potassium hydroxide solution. These data allowed us to examine microplastic body burden and uptake over time, as well as calculate bioconcentration and bioaccumulation factors for waterborne and dietary microplastics. Our study provides insights into where – and how quickly – tadpoles acquire microplastics in their environment, which is important for modelling the risk of microplastic pollution to amphibian populations.

Using epiphytic lichens as biomonitors of atmospheric mercury and arsenic associated with dust at a historical gold mine tailings site in Nova Scotia (PO)

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Between the 1860s and 1940s, over 360 gold mines in 64 formal gold districts in Nova Scotia generated approximately three million tonnes of mercury (Hg) and arsenic (As) contaminated waste (tailings) that were released directly into terrestrial and aquatic environments near mining communities. Many of these mines were abandoned by the 1940s and unremediated, leaving a legacy of environmental contamination. Today, with sparse vegetation cover and under a changing climate, there is the potential for intensified dust emissions from these tailings deposits, leading to the remobilization and distribution

of these historical contaminants to surrounding environments. Monitoring the extent of this wind-borne dust and air contamination is important for informing remediation practices and to better assess risks to public and ecosystem health. Lichens are widely used in biomonitoring spatial patterns of dust and air quality at contaminated sites due to their ability to accumulate airborne contaminants almost entirely from atmospheric deposition. In the summer of 2019, we sampled epiphytic lichens (*Platismatia* and *Usnea* spp.) over a series of gridded transects, over both tailing and reference areas surrounding the Montague gold mine tailings site near Dartmouth, Nova Scotia. Lichens were analysed for Hg and As, with the concentrations mapped to determine spatial distribution patterns. Seasonal dust and elemental levels were also determined using passive air samplers and rain collectors along a stream-based transect through the main sampling grid. We will report the geographical distribution of Hg and As in lichen and their proximity to historical gold mining sites.

Weaving knowledge systems in ecotoxicology: A synthetic review (PO)

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Post-secondary institutions and academics have been called upon to advance their understanding of reconciliation and to mainstream reconciliation in all aspects of the scientific endeavor; one way to do so in the natural sciences is to weave Indigenous and western knowledge systems. Although many studies across a wide range of disciplines have successfully woven knowledge systems, only a limited number have done so in ecotoxicology. Here, we synthesize and evaluate 17 studies that have brought together Indigenous and western knowledge systems to study environmental contaminants and wildlife health in Canada. We found that studies spanned across much of Canada's jurisdictional boundaries (but excluded British Columbia, Ontario, Nova Scotia, New Brunswick, and Prince Edward Island). The commonly studied contaminants were metals (n=6), mercury (n=3), and polycyclic aromatic hydrocarbons (PAHs) (n=3); and the commonly studied species (n=2) were lake trout, lake whitefish, arctic char, caribou, muskoxen, and common eider. Most studies wove knowledge systems at the analysis (n=11) and dissemination (n=10) stages, and credit was most often given to Indigenous community members at the data collection phase (n=14). We also explore three case studies that exemplify the advantages of weaving knowledge systems in ecotoxicology. By highlighting how previous studies have woven knowledge systems across all stages of the research process, our review demonstrates how ecotoxicologists can successfully conduct collaborative and interdisciplinary research in the future that responds meaningfully to the calls for reconciliation in Canada.

Profile of parent and alkylated polycyclic aromatic compound residue in avian tissue following *in vivo* and *in ovo* exposure to diluted bitumen (PO)

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Canadian petroleum production and exports are predominantly unconventional crude products from the oil sands region. Pipeline capacity expansions will increase the volume transported of one such unconventional crude product called diluted bitumen (dilbit). In the event of dilbit spillage, avian petroleum exposure estimates available in the literature are scarce but indicate ingestion of up to several millilitres oil per kilogram body weight (bw) per day could occur in highly exposed adult birds, and at least tenths of milligrams oil per gram in the eggs of breeding birds should they transfer oil to their eggs. In this context, we present tissue residue data from captive dosing studies with 2–12 ml dilbit·kg⁻¹ bw per day in adult zebra finch (*Taeniopygia guttata*) and 0.1–0.4 mg dilbit·g⁻¹ egg with double-crested cormorant (*Phalacrocorax auritus*) embryos. Parent and alkylated polycyclic aromatic compound (PAC) profiles in those matrices show qualitatively elevated PAC concentrations in the most highly exposed dose groups (approximately 3–300 ng·g⁻¹ liver wet weight and 1.00–3.00 ng·g⁻¹ carcass wet weight in finch and cormorant, respectively). High tissue PAC concentrations coincided with high dilbit doses at which significant mortality was observed in zebra finches; few effects in dilbit-exposed cormorant embryos were observed. PAC profiles suggest efficient biotransformation and elimination of most of the analyzed PACs in dilbit under all but the most severe exposure scenarios. This study is the first tissue PAC residue data available for avian exposure to a bulk oil sands bitumen product.

Accumulation and effects of cobalt on river biofilms (PO)

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Cobalt (Co) is an essential metal involved as a co-factor in several important biomolecules. Nevertheless, high concentrations can also be detrimental for biota, and the increase of its environmental concentrations in specific water bodies may become an issue. The aim of the present study is thus to improve our knowledge on the short- and long-term effects of Co on microorganisms living in biofilms. To that end, two approaches were conducted. In the first set of experiments, biofilms were exposed to increasing Co concentrations (between 1.10⁻⁷ M and 1.10⁻⁵ M) using outdoor mesocosms (15 L) filled with

the Gave de Pau River water. Biofilms were collected along with water at several sampling times (1, 3, and 7 days). In the second set of experiments, biofilms were exposed to 1.10^{-7} M, 5.10^{-7} M, and 10^{-6} M Co in outdoor open-stream mesocosms filled with the Gave de Pau for 28 days. Biofilms and water were collected over time (1 hour, 1, 3, 7, 14, 21, and 28 days). Water samples were analysed for the concentrations of cations (including Co), anions, and dissolved organic carbon. Biofilms were examined for their total and intracellular Co concentrations. The effect of Co on biofilms is assessed by measuring modification of the biofilm chlorophyll-a content and its community composition using targeted gene sequencing, whereas untargeted metabolomic analyses are also performed. The obtained results will help us to better understand Co impacts on aquatic biota and to identify potential new biomarkers of Co exposure and effects.

Evaluation of the use of gastropod shells as bioindicators of metal contamination in rivers using mass spectrometry imaging techniques (PO)

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Gastropod shells grow throughout their life by integrating some of the chemical elements present in their environment, including metals. Although rare, studies are available in which relationships have been established between the total concentration of metals in the shell and that present in the ambient environment, and as a function of exposure time. The goal of this work is to evaluate the possible use of shells as a tool for biomonitoring metal concentrations in fresh water. Two mass spectrometry imaging techniques are currently optimized to explore the variability of metals in shells at different spatial scales: Time-of-flight secondary ion mass spectrometry (TOF-SIMS - nanoscale), and laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS - micrometer scale). For this purpose, *Radix balthica* were collected at different sites influenced by metal input. The chosen sites are: 1) a channel in which treated waters of the sewage treatment plant of Pau are discharged, 2) a stream draining a small catchment that can be fed by industrial water discharges, and 3) the outdoor mesocosms of TotalEnergies supplied by water from the Gave de Pau. At each site, water was sampled to be analyzed for concentrations of metals, cations, anions, and dissolved organic carbon. Metal concentrations were also analyzed in the *R. balthica* whole shells and their soft bodies using acidic digestions. Finally, microchemical analyses of metals in the shells are performed with TOF-SIMS and LA-ICP-MS. Those measurements will allow us to check for the enrichment of the shell with metals according to metals concentrations in fresh water.

Do firefighting water additives used in forest and municipal firefighting pose a risk to aquatic biota? (PO)

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The use of firefighting water additives has greatly increased over past decades in both wildland and municipal fires in order to increase extinguishing efficacy. Fluorinated firefighting additives were used extensively in the past, however, due to their bioaccumulative potential and persistence in the environment they are no longer permitted in Canada for forest and municipal applications. With greater concern of the environmental fate of firefighting water additives, new formulations have been developed that are meant to be “eco-friendly” alternatives for fire suppression. There is currently very little data on the toxicity of these new additives towards aquatic biota. This study assessed the toxicity of nine different types of firefighting water additive on aquatic species. This included acute lethality testing of the aquatic species *Daphnia magna*, *Hyalella azteca*, and a 28-day reproductive test using *Tubifex tubifex*. The *D. magna* portion of the study revealed considerable risk for all tested products with the exception of Eco-Gel™ and TetraKO®. *Hyalella azteca* showed relative sensitivity to all products tested with exception of Eco-Gel™. Finally, *Tubifex* showed relatively low sensitivity to all products tested. The results of this study highlight the potential hazard that firefighting water additives could pose to aquatic organisms.

Impact of environmental factors on the bioavailability of ionizable organic chemicals: Laboratory studies and field validation (PO)

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Emerging contaminants such as pharmaceutical drugs have been detected in waters across the globe. Most pharmaceuticals are found at trace concentrations, but the continuous use and potential accumulation of some of these compounds can potentially lead to effects in aquatic organisms. Many pharmaceuticals are ionizable organic chemicals (IOCs), which makes their environmental and toxicological behaviour particularly hard to predict. This proposed research will test the hypothesis that uptake and effects of IOCs in aquatic organisms are influenced by the interaction between environmental, physicochemical, and biological factors. Relevant analytes, their concentration levels, as well as realistic ranges of physicochemical parameters, will be identified based on data and observations from previous research. Informed by this research, a model fish species, fathead minnow (*Pimephales promelas*), will be exposed to selected IOCs at environmentally relevant concentrations in combination with variations in two relevant environmental factors, pH and hardness. Chemical uptake, as well as a suite of biological

effects, will be investigated. In a subsequent field study, water and fish will be sampled, and physicochemical parameters recorded simultaneously. Water samples and fathead minnows will be obtained from Wascana Creek, a wastewater treatment plant effluent-dominated stream, as well as the South Saskatchewan River to allow for direct comparisons between lab results and field observations. This research will provide a better understanding of the effects that pharmaceuticals may cause in aquatic organisms under varying environmental conditions, and thereby aid in protecting fish species in the future.

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