

# FEDERAL PLASTICS SCIENCE KNOWLEDGE MOBILIZATION WORKSHOP

## L'ATELIER DE MOBILISATION DES CONNAISSANCES SUR LA SCIENCE DES PLASTIQUES

*Presentation Abstracts / Résumés de présentation*

**DAY 1 - Wednesday, November 30, 2022/ JOUR 1 - Le  
mercredi 30 novembre 2022**

### *Plastic pollution in biota in the Arctic*

**Dr. Jennifer Provencher, Environment and Climate Change Canada**

The Northern Contaminants Program (NCP) has supported several projects on plastic pollution which have been lead or co-lead by Environment and Climate Change Canada's Ecotoxicology and Wildlife Health Division. These projects have addressed areas of research and monitoring directed by the Canadian Plastics Science Agenda (CaPSA) with a focus on Arctic species and protected areas. These projects have explored ingested plastic pollution in several NCP core monitoring species including Arctic char, ringed seals, polar bears, thick-billed murres and northern fulmars, as well as other species that are important in the region (e.g. walrus). Plastic pollution has also been assessed in biotic and abiotic compartments within protected areas to explore how migratory birds may act as a vector for microplastics around bird colonies. Lastly, we have explored how plastic additives may be found in Arctic biota, including marine mammals, terrestrial mammals, fish and seabirds. This additives research has included retrospective studies using archived samples (seabird eggs), comparison across trophic levels (seabirds and mammals), and regional comparisons (seabirds and mammals).

### *Microplastics and Plastic Associated Contaminants in the Snow Deposition in the Canadian Arctic*

**Ingrid Granados-Galvan, L'Université du Québec à Rimouski**

Plastic pollution is a "cocktail" of contaminants that includes plastic pieces, plastic additives, and chemicals adsorbed on plastics. Microplastics (1 µm - 5 mm) and plastic-associated compounds in atmospheric deposition (e.g., snow) are pathways for airborne plastic-related contaminants to enter ecosystems. However, such knowledge is insufficient for the Canadian

Arctic. The objective of this study was to investigate the distribution and characteristics of microplastics and two groups of plastic-associated contaminants: organophosphate esters (OPEs) and per- and polyfluoroalkyl substances (PFASs) in Canadian Arctic snow. Between February 2020 and May 2021, samples were collected from Little Fox Lake (Yukon; n=6), Yellowknife (Northwest Territories; n=5), Cornwallis Island (Nunavut; n=9), and Alert (Nunavut; n=15). Filtration (>20µm), microscopic visualization and Fourier transform infrared imaging techniques were employed for microplastics analysis. OPEs and PFASs were extracted by solid phase extraction and analyzed by ultra-high performance liquid chromatography-tandem mass spectrometry. Preliminary results on microplastics found an average abundance of  $27.5 \pm 29.4$  n/L (mean  $\pm$  S.D.). Snow samples from Cornwallis Island ( $51.4 \pm 30.4$  n/L) had higher abundance and variation of microplastics, followed by Yellowknife ( $14.0 \pm 5.1$  n/L), and Alert (n=9,  $9.2 \pm 11.4$  n/L), suggesting higher abundance at the mid and low latitudinal sites in the region, possibly related to local sources. Prevalent shapes were fibers (64%) and fragments (28%), with median lengths of  $820 \pm 790$  µm (mean  $\pm$  S.D.). Diverse polymers were found, with polyester (43.6%) and polypropylene (21.8%) being the dominant polymers. Measurements and characterization of microplastics in the remaining samples are currently underway. Some OPEs and PFASs were frequently (>75%) detected in the snow samples. After all microplastic data has been obtained, correlation studies between microplastics and OPEs/PFASs will be performed. This study's findings will help to better understand the atmospheric deposition of microplastics and plastic-associated contaminants in the Canadian Arctic.

***pELastic Part I: Measuring the fate and effects of microplastics via littoral and pelagic in-lake mesocosm experiments at IISD-ELA***

**Dr. Chelsea Rochman, University of Toronto**

This presentation will introduce the pELastic Project at the International Institute for Sustainable Development's Experimental Lakes Area (IISD-ELA), aimed at testing hypotheses about the fate and effects of microplastics in a whole lake ecosystem. This presentation will specifically focus on how we are testing hypotheses about fate and transport. Since 2019 our team has been working at IISD-ELA, leading a series of projects that began with in-lake limnocorrals and will culminate in a full lake experiment. This talk will review the various experiments we have done and are planning, discuss our experimental designs, as well as share some preliminary results regarding fate and transport of microplastics in the physical environment and in organisms in a freshwater food web. The pELastic project aims to inform policies relevant to monitoring, management, and ecological risk as well as fill research gaps related to fate, transport, effects and mitigation.

***Fate and transport of microplastics on shorelines***

**Dr. Chunjiang An, Concordia University**

Shorelines provide the habitat for an array of plants, birds, and benthic species. There is an increasing concern regarding the fate and transport of microplastics in the shoreline environment. Our research team has studied the microplastic release from disposable masks

into the shoreline environment and the transport of microplastics in shoreline substrates over tidal cycles. (i) As many improperly disposed masks enter the ocean, the risk of plastics to the marine ecological system is further aggravated, especially in the shoreline environment. This is the first study to explore the changing characteristics and environmental behaviors of disposable masks when exposed to the shoreline environment. It was found a single weathered mask can release more than 1.5 million microplastics to the aquatic environment. The physical abrasion caused by sand further exacerbated the release of microplastic particles from masks. The study results indicate that shorelines are not only the main receptor of discarded masks from oceans and lands, but also act as a major area for the further transformation of masks to plastic particles. The appropriate strategy needs to be developed to better assess such emerging risk and protect the coastal ecosystem. (ii) Tidal zones providing habitat are particularly vulnerable to microplastics pollution. However, inconsistent results were identified in previous field studies concerning the impacts of key environmental characteristics on microplastics concentration in sediment. This is the first study to comprehensively explore the effects of tidal cycles on microplastics transport in porous media combining various environmental and microplastics properties. Our results highlight the significance of microplastic size, density, aging status, substrate size, porewater chemistry, and hydrodynamic forces in determining the transport patterns of microplastics under the influence of tidal cycles, which provides novel insights into sediment cleanup and field sampling in tidal zones.

### ***Quantifying and characterizing microplastics in water and zooplankton from eight lakes in British Columbia, Canada***

**Natasha Klasios, University of British Columbia**

Despite lakes having vast ecological and cultural value, data on microplastic contamination in lakes in British Columbia (BC) is scarce. We provide the first documentation of microplastics in BC lakes by quantifying and characterizing microplastics in water and zooplankton from eight lakes. By sampling both matrices, we provide insight into the fraction of microplastics entering the food web. Lakes were selected to span a gradient of urbanization to investigate if proximity to urbanization (population density per sq km) affected types or quantities observed. We collected zooplankton via 10m vertical tows and subsurface water via 12L grab samples. Samples were processed using published methodologies and all suspected microplastics underwent Raman spectroscopy. Microplastics were found in all water samples and some zooplankton samples. The mean concentration of microplastics across lakes was 0.607 (+/- 0.153 sd) and the range was 0.167-1.33 microplastics per L. From our zooplankton tows, we separated out two key zooplankton groups, Daphnia and Copepod species, and quantified microplastics in each group separately. We found Daphnia had a higher number of microplastics per individual (mean = 0.02 (+/-0.019 sd)) compared to Copepods (mean = 0.01(+/-0.013 sd)), however this difference was statistically insignificant. For all samples, we found fibers to be the most dominant morphology and polyester to be the dominant polymer observed. We found that the quantities of microplastics observed increased with increased urbanization, however the relationship was not statistically significant. Our results align with literature in terms of

concentrations and types of microplastics observed in freshwater and illustrate zooplankton retention of microplastics in nature appears low. Given our study results are reflective of a snapshot of contamination in eight lakes, further sampling is needed to better characterize microplastics in freshwater environments and food webs in BC.

### ***Microplastic composition and concentration in Canadian biosolids and agricultural biosolid applications***

**Dr. Jesse Vermaire, Carleton University**

Microplastics are a contaminant of emerging concern in ecosystems, however, the sources, sinks, and spatial distribution of microplastics remains poorly understood. Biosolids represent a potential source of microplastics to terrestrial environments, however little information exists on the concentration and composition of microplastics in biosolids in Canada or what happens to the microplastics in biosolids once they are applied to agricultural fields. We analyzed biosolids samples for microplastics from twenty-two wastewater treatment plants located across the country and two commercial fertilizer producers. The number of microplastics varied between 228 to 1353 particles per gram dry weight (mean:  $687 \pm 284$ ) with the majority (84%) of all particles being microfibrils. We also applied 25 tonnes of biosolids to an experimental  $\sim 1$  ha field in Winchester Ontario to examine the transport and fate of biosolid associated microplastics in agricultural fields. We found that the biosolid application increased microplastic concentration in the soil nearly 400% from a pre-application concentration of 0.54 plastic particles per g to 2.05 plastic particles per g of soil immediately following biosolid application. These elevated microplastic concentrations remained post application but had declined with 1.35 plastic particles per g of soil on day 28 post application and 0.99 plastic particles per g of soil on day 63 suggesting movement of microplastics either deeper into the soil or loss to the environment. The conditions were very dry during this experiment and no microplastic export was observed in the tile drainage system. Our results further show that the biosolid application did not increase the prevalence in microplastic observed in earthworms ( $n=173$ ). Further research is required to track multi-year changes in microplastic concentration at this experimental site and soil cores will be analyzed to determine if microplastics have moved deeper into the soil in this field. Importantly, our study shows that the presence of hundreds of microplastics in Canadian biosolids suggests that this is a significant source of microplastic pollution to agricultural lands in Canada, however, the microplastics are not simply staying in the surface soils of agricultural fields.

### ***Source-specific identification, characterization and control of microplastics across a remote, rural and urban gradient***

**Dr. Miriam Diamond, University of Toronto**

Canadian and international concern is mounting regarding risks associated with microplastic (MP) ubiquity, accumulation, and potential for ingestion. In response, federal and municipal strategies have been proposed to reduce plastic waste, support a circular economy, and mitigate MP pollution in the environment. A key roadblock to efficient policy implementation

remains the knowledge gap in MP sources, transport, and fate, limiting our capacity to track and remove those already in the environment. A lack of consistent analytical methods has restricted data comparability across studies. These gaps constrain our ability to use models, which help to identify source contributions and sustainable control measures, and which are needed to support policy and management initiatives. This program addresses these gaps by using novel approaches to generate the knowledge, tools and data required to establish effective control strategies. We will develop new tools for high resolution sample analysis. We will harmonise novel and existing analytical procedures across instruments, institutions, and across air, soil, and water, to establish an MP surveillance network. This will build knowledge of sources, pathways, and distribution of MPs across Ontario. We will use these tools and data to identify MP sources in remote areas (e.g., Canadian Arctic) and to calibrate watershed models. These will be used to quantify control measure effectiveness, based on experimental assessments of available technologies. Models, data, and operating procedures will be made available through a new online MP database, ensuring that this program acts as a resource to support existing and future research programs e.g., the Arctic Monitoring and Assessment Program. By working directly with municipal and federal stakeholders, and engaging with diverse groups of stakeholders, knowledge developed through this program will be directly applied to support effective MP mitigation policies which can be used across Canada.

#### ***Lagrangian tracking of Virtual Fibers in the Strait of Georgia***

**Jose Valenti, University of British Columbia**

Polyester microfibers' ubiquitous distribution in the oceans raises many questions about these negatively buoyant particles dynamics. We use SalishSeaCast, a high-resolution ocean model that resolves submesoscale dynamics in the Salish Sea; and Ocean Parcels, a Lagrangian model able to incorporate additional behaviors to build a specialized model aiming to recreate Polyester microfibers dynamics in the Salish Sea.

#### ***Degradation of consumer plastics into microplastics and nanoplastics***

**Dr. Nariman Yousefi, Toronto Metropolitan University**

Consumer plastics such as plastic shopping bags, water bottles and take-food trays can be sources of secondary microplastics and nanoplastics (MNPs) when they undergo complex environmental degradation. Under the influence of UV radiation, abrasion, atmospheric oxidation and freeze-thaw cycles, bulk plastics degrade into smaller particles with significantly increased transportation in the aquatic environment. In this study, we studied the degradation of three classes of consumer plastics, namely, plastic shopping bags (low density polyethylene), water bottles (polyethylene terephthalate) and take-out food trays (polystyrene) under simulated environmental condition. The first MNPs emerged after at least 4 weeks of simulated environmental degradation. We characterized the transformation of the surface chemistry of plastics using Fourier transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS). Our results show that environmental degradation has a significant effect on alteration of the surface chemistry of polymers by inducing a plethora of oxygen containing

groups. The morphology of the MNPs and post-degradation bulk plastics were also studied by scanning electron microscopy. Using computer-assisted image analysis, we quantified the number of particles released from the plastics after 12 weeks of degradation. We observed that the type of polymers has a marked effect on the morphology and the number of particles that are released to water.

### ***Micro- and nanoplastics as vectors for organic contaminants***

**Dr. Jeffrey Farner, University of Alberta**

This work looked to identify drivers of sorption of organic contaminants to micro- and nanoplastics and determine biological impacts of contaminant co-exposure. For 20 nm nanoplastics, co-exposure with Phenanthrene, a PAH was observed to potentiate uptake of PAHs in Zebrafish, although this did not occur with 500 nm nanoplastics. Furthermore, sorption of organic compounds onto nanoplastics were observed to depend on physico-chemical parameters such as hydrophobicity and electrostatic interactions. Ongoing work in developing weathering protocols will enhance realism in exposure scenarios.

### ***What drives microplastic toxicity?: Distinguishing the effects of plastic microparticles from their additives***

**Dr. Ludovic Hermabessiere, University of Toronto**

Microplastics are ubiquitous, including in remote locations. Once in the environment, these synthetic microscopic particles are ingested by organisms which can lead to adverse effects such as food dilution and oxidative stress. Still, what is driving the toxicity of microplastics is not well understood. Indeed, microplastics are a diverse suite of contaminants composed of several shapes, polymers, and plastic additives. Plastic additives are generally not considered in toxicity studies despite their present in plastic products (which can be up to 40% of the mass of a product – e.g., PVC). Here, we exposed fathead minnow (*Pimephales promelas*) for 60 days to four treatments: polyethylene microplastic with additive, polyethylene without plastic additive, plastic additive leachate, and a control (no plastic, no additive). The main objectives of this experiment were to understand what is driving the toxicity: the plastic itself (particles), the additives, or both in combination. We took samples at day 12, 30 and 60 days to measure bioaccumulation, gene expression, and condition indices (weight, length, condition index). We predicted that we would observe changes in gene expression that vary according to the presence of additives. We observed no significant differences between treatments at day 12, 30 and 60 for fish weigh, length or condition index ( $p > 0.05$ ). There were also no differences in survival ( $p > 0.05$ ). Bioaccumulation and gene expression analyses are underway.

This work is relevant to the session about assessing the effects of microplastics with other anthropogenic stressors as microplastics are multiple stressors, including both a physical and a chemical stressor. In addition, understanding what can drive the toxicity of microplastic (physical vs chemical) will help us understand the mechanisms of toxicity which is important for informing risk.

## DAY 2 - Thursday, December 1, 2022/ JOUR 2 - Le jeudi 1 décembre 2022

### *Can Biofilm Present on Microplastics Impact Drinking Water Consumers?*

**Dr. Robert Andrews and Dr. Husein Almuhtaram, University of Toronto**

A potential human health impact associated with microplastics (MPs) in drinking water is the presence of microorganisms (including potential pathogens) that may attach and colonize as part of biofilm. This study examined microplastic-related biofilm in drinking water sources to understand their role as vectors for the transport of potential pathogens. The primary objectives the study was to: i) determine the abundance and composition of microplastics biofilm, and ii) identify major factors impacting biofilm growth when considering a range of virgin and weathered plastic types. In this study high-density polyethylene (HDPE), low-density polyethylene (LDPE), polypropylene (PP), polyvinyl chloride (PVC), and polyethylene terephthalate (PET) microplastics (3.5 - 4.0 mm diameter, both virgin and weathered) were exposed for periods of up to 21 weeks by placement in four surface waters which serve as influent to drinking water treatment facilities. Biofilm growth was quantified by measuring adenosine triphosphate (ATP), a commonly used indicator when considering environmental samples. ATP levels increased with respect to specific types of plastics: PVC > LDPE > PP ≈ HDPE ≈ PET. Source water quality was also observed to impact the magnitude of biofilm growth. Waters with relatively high dissolved organic carbon (5 mg/L vs. 1 mg/L) typically resulted in higher ATP levels. For certain cases, elevated ATP was observed to occur on weathered microplastics, when compared to virgin counterparts. This study also analyzed microbial communities, assessed biofilm morphology, and applied gene sequencing to identify biofilm composition as well as potential pathogens that may impact drinking water consumers.

### *Long-term impacts of common microplastic polymers and plastic additives on the behaviour and proteome of juvenile Lake Trout (Salvelinus namaycush)*

**Haley Macleod, Lakehead University**

The long-term toxicity of microplastic polymers and their chemical additives are poorly understood, particularly regarding their impacts on freshwater fish species. UV-stabilizers and antioxidants are common plastic additives with the ability to bioaccumulate and act as endocrine disrupting compounds, but their effects remain understudied. Here, we exposed newly hatched Lake Trout (*Salvelinus namaycush*) to microplastics comprised of equal parts of polypropylene, polystyrene and polyethylene terephthalate polymers impregnated with common UV-stabilizers and antioxidants that ranged in size from 7 µm - 500 µm. Microplastic concentrations (0, 2, 35, 315, 2669, 22465 n/L) were selected to reflect environmentally relevant freshwater concentrations with the highest concentration mirroring those projected to occur in freshwater ecosystems if global plastic production and mishandling continues. Additional controls were implemented at the highest concentration including 1) microplastics

alone with no additives and 2) additives only with no microplastics. Lake Trout were exposed to microplastics and additives during yolk-sac absorption and for an additional 6-weeks post initial feed for a total of 12 weeks post-hatch. Whole individuals were collected for proteomic analyses at 6- and 12-week time points, with individuals assessed for behavioural and metabolic alterations at 12-weeks. Behavioural analyses found microplastics and their chemical additives led to higher proportions of individuals occupying the middle and upper portions of the water column in tanks. Preliminary proteomic analyses identifying significant ( $p < 0.001$ ) changes in cellular function, metabolism, and behaviour in individuals exposed for 12-weeks at the highest concentrations with both microplastics alone and with chemical additives. These initial results indicate that microplastics and their chemical additives can affect juvenile Lake Trout during important developmental life stages at future projected freshwater concentrations and highlights the potential vulnerability of an important Canadian freshwater fish species to microplastics and their chemical additives.

### ***The pELastic Project, Part II: Evidence for the ecological effects of microplastic pollution through ecosystem-based experimentation***

**Dr. Diane Orihel, Queen's University**

A wide variety of scientific approaches can be employed to generate evidence (or the lack thereof) for the ecological effects of contaminants. However, the most compelling evidence for establishing cause-and-effect comes from experimentation. Moreover, the greater the scale and realism of the experimentation, the more definitive and powerful the evidence, and arguably, the more useful for informing the development of sound environmental regulations. Despite rapid advances in elucidating the implications of microplastic exposure on aquatic organisms, effects of these contaminants in freshwaters at the community- and ecosystem-scale remain poorly understood. As such, our team embarked on an ambitious, decade-long study across multiple ecological scales to determine: (i) the physical, chemical, and biological fate of microplastics in lakes and their watersheds; (ii) how microplastics impact aquatic ecosystems across all levels of biological organization; (iii) how ecosystem processes and functions, such as nutrient cycling and photosynthesis, are affected by MPs; and (iv) the recovery of an ecosystem exposed to microplastics, including how they break-down and/or transform over time. Our study, named the pELastic Project, involves mesocosm, limnocorral, and whole-lake experiments situated at the IISD-Experimental Lakes Area, Queen's University Biological Station, and the Rochester Institute of Technology. Since our study began in 2019, we completed two mesocosm studies and four limnocorral studies using color-coded microplastics and conducted baseline monitoring in two study lakes in preparation for a whole-lake manipulation. In this presentation, we will first share our preliminary results from the pELastic Project on the effects of microplastics from our mesocosm and limnocorral experiments. Then, we will chart our path forward over the next 5 years as we begin our whole lake microplastic experiment, describing the comprehensive suite of effects endpoints (from the molecular level to the ecosystem level) that we are monitoring in a natural lake ecosystem and its food web (from bacteria and phytoplankton to fish and amphibians).

### ***Characterizing the exposure and effect of microplastics to freshwater and terrestrial biota***

**Dr. Ryan Prosser, University of Guelph**

Our research group has been investigating the potential effects of different types and sizes of microplastics (i.e., 10-100 µm polystyrene spheres, polyethylene sphere, polyester microfibrils) on freshwater invertebrates, soil invertebrates, plants, and mycorrhizal fungi. We have also been investigating the magnitude of microplastics in wastewater influent, effluent and biosolids from several wastewater treatment plants (WWTP) in southern Ontario, along with measuring microplastics in different tissues of freshwater mussels (Unionidae, Sphaeriidae) upstream and downstream of WWTP. We have also been characterizing the microplastics in biosolids-amended agricultural soil across several agricultural fields in Ontario and in earthworms sampled from these fields. We have conducted acute and chronic toxicity tests (2 to 56 days) with six freshwater and soil invertebrate species (i.e., *Hexagenia* spp., *Planorbella pilsbryi*, *Lampsilis siliquoidea*, *Lampsilis fasciola*, *Eisenia andrei*, *Eisenia fetida*, and *Tubifex tubifex*). We have also performed experiments to investigate the effects of microplastics on the growth of Sorghum-sudangrass (*Sorghum × drummondii*) and this plant's relationship with arbuscular mycorrhizal fungi (AMF). The greatest exposure in water was 150,000,000 microparticles (MP)/L of water and the greatest exposure in soil was 3.0% (w/w). We have not observed adverse effects on survival or reproduction within any toxicity tests with invertebrates. We have also not observed any effects on plant growth or colonization of plant roots by AMF. The concentration of microplastics ( $\geq 20$  µm) in wastewater influent was significantly greater than in effluent, ranging 7.5-34.2 microplastics/L in influent and 0.5-12 MP/L in effluent. The microplastics observed in fingernail clams ranged from 0-6.71 MP/clam. The microplastics in Unionidae hemolymph ranged from 0-16.7 MP/mL and in digestive gland from 0.4-57.8 MP/gram. Microplastics in liquid biosolids ranged from 1,219-12,035 and ranged from 673-5,806 MP/gram. Preliminary data would indicate that environmentally relevant exposures is below exposures that would cause adverse effects in the species tested

### ***Investigating microplastics as an interference agent in root-soil-microbial interactions***

**Dr. Cameron Proctor, University of Windsor**

Agricultural crops utilizing biosolids as a nutrient source are exposed to microplastics that may be drawn against the root surface causing eco-toxic effects. Crop species with higher prevalence of symbiotic partnerships with soil microbiota maybe more susceptible, but little is known about the microplastic properties (i.e., size, quantity, type, surface morphology) that cause stress. Through a dose-response study, three crops (wheat, soybeans, alfalfa) were grown in rhizoboxes amended with biosolid microplastic mimics (PET and PPE) at two concentrations (2,000-15,000 particles/kg soil) as well as biosolids. Under standard growing conditions, biophysical differences in the fine root system were investigated per treatment. At the end of the growing phase, root exudation, microbial carbon utilization using the MicroRESP system, and the concentrations of microplastics in the rhizosphere and root interior were measured.

The time-lapse imagery of the root growth pattern showed minor differences between treatments. Microbial respiration indicated unique carbon utilization patterns by plastic type and concentration, with higher concentrations eliciting a weaker microbial response.

### ***Effects of sub-chronic exposure of mice to nanoplastics on male reproductive tissues***

**Dr. Daniel Cyr, Institut national de la recherche scientifique**

The emergence of plastics as global pollutants has received considerable attention owing to their widespread presence. Nano- and microplastics (NP/MP) are ubiquitous; thus, human exposure is inevitable. The objective of this study is to assess the effects of a sub-chronic exposure to nanoplastics on the male reproductive system using a mouse model. C57BL/6 mice were treated by gavage with 0, 0.15, or 1.5 mg/day of polystyrene NPs (500 nm) for 60 days. Histopathological effects on the testis and epididymis were assessed following treatment. Stages of spermatogenesis of the testis were determined and gene expression data from the caput epididymidis was done using RNAseq. Histological sections of testis and epididymis of mice were stained with hematoxylin and eosin and revealed that NPs caused abnormalities in the seminiferous tubules of the testes, such as germ cell sloughing, increased luminal diameter, and reduced germ cell number. The distribution of stages of spermatogenesis in the testis did not differ significantly between groups. In the epididymis, greater numbers of vacuoles of varying sizes were observed throughout the epithelium of the caput, corpus, and cauda. Disintegration of the epididymal epithelium was also evident, and there were more apoptotic cells and macrophages in the NP-treated mice. Furthermore, the morphology of principal and basal cell nuclei was irregular, and the smooth muscle layer was thicker in treated versus control animals. RNA-Seq data showed that 518 genes were differentially expressed ( $p < 0.05$ ) in the caput epididymidis of mice exposed to the high dose versus control. Correspondent with the histopathology, RNA-Seq analyses revealed alterations in genes associated with various KEGG pathways, including immunological response, apoptosis, and oxidative stress response, following exposure to NPs. These data indicate that chronic exposure to polystyrene nanoparticles resulted in toxicity to the testis and epididymis of mice.

### ***Microcosm study of the effects of polyester microfibre on indigenous marine amphipods: the implication for the Strait of Georgia ecosystem***

**Dr. Oladimeji Iwalaye, University of British Columbia and Oceanwise**

Microplastics (MPs) remain contaminants of great concern because of their abundance, ubiquitous in all habitats of the world and the threats posed to organisms inhabiting these habitats. Despite amphipod's vital role in the aquatic ecosystem, and polyester (PET) being the most abundant microfibre (Mf) found in indigenous zooplankton, little is known about the effect of PET Mf on marine amphipods. We investigated the effects of microplastic (blue – PET,  $14.17 \pm 4.05 \mu\text{m}$  Mf) on marine amphipods at different concentrations (0, 10, 100, 1,000, 10,000 and 50,000 microfiber L<sup>-1</sup>) and exposure time (24, 48 and 72 h) using microcosm experiments. The effects of Mf on survival, Mf ingestion and retention, grazing, fecal pellet–volume, sinking velocity and density in relation to increasing Mf concentration and exposure

time were examined. Our study demonstrated that concentration had a greater influence on the parameters investigated than the exposure time. Exposure of amphipods to Mf did not affect their survival however, it significantly affected their feeding with a reduction in grazing rate as the concentration increased. The average ingested Mf and ingestion rate increased with concentration and exposure duration. Also, Mf encapsulation in fecal pellets (FP) significantly increased their sinking velocity and density. Exposure of amphipods to PET will increase their biological pump efficiency and carbon sequestration. Increased Mf ingestion that possibly led to reduced grazing rate is of great concern because whatever affects feeding in organisms can affect other activities such as growth and development, metabolism, reproduction, and behaviour with time and could impact the ecosystem's health. Hence, there is an urgent need for a drastic reduction of the release of fibre from laundry and the design of equipment that will efficiently trap fibres and other MPs types from laundry effluents in the wastewater treatment plants before their release into the environment.

***Polyethylene microplastic beads are pulverized in the digestive system of a cricket, but with little impact on insect growth rates***

**Dr. Heath MacMillan, Carleton University**

Interactions between terrestrial insects and microplastics (MPs) are poorly documented and understood, despite an estimated 4900 megatons of plastics being directed to terrestrial systems and insects being the most diverse group of animals on the planet. Generalist insects like crickets, for example, are also abundant in agricultural settings where wastewater sludge containing plastics is used as fertilizer. Plastics ingested by insects may be toxic and given that many insects eat a diet containing hard plant materials or other insects, their gut anatomy and physiology may make them particularly effective at transforming ingested plastics. We used a ground-dwelling generalist insect (a cricket; *Grylloides sigillatus*) and fluorescentlylabelled plastics to test whether individuals would ingest and transform MPs in their food, and what effects ingestion may have on growth and development in a laboratory setting. Polyethylene tetrphalate fibers slowed cricket growth and resource allocation to eggs at high doses, but there was little effect of ingested polyethylene beads on cricket growth, even when 10% (w/w) of the diet was plastic. By developing a novel pipeline for tracking plastic fates in animal digestive systems, we were also able to describe the effects of the cricket gut on the plastics themselves. The cricket digestive system was remarkably good at breaking down microplastics, even down to the level of nanoplastics. Taken together, our findings reinforce that plastic form is important to understanding its impacts on animal fitness, and that at least some terrestrial insects have the ability to dramatically transform microplastics. Whether this ability is realized in the wild, however, remains unclear.

***Occurrence and Ecotoxicological Effects of Micro/Nanoplastics in Saskatchewan Watersheds***

**Yao Yao, University of Regina**

Micro/nanoplastics (MP/NPs) are global pollutants found in even the most remote environments. They can travel long distances and are persistent in the environment. MP/NPs

can act as vectors for a variety of contaminants, making them a risk to ecosystems. MP/NPs pollution has been reported in the aquatic environment across Canada. Several studies have been carried out to look at MP/NPs occurrence and relevant effects in Canadian freshwater bodies. However, there are limited data on MP/NPs in Canadian prairies. Therefore, investigations on the occurrence and ecotoxicological effects of MP/NPs in Saskatchewan watersheds is desired. In this study, the occurrence, characteristics, and distribution of MPs contamination in Saskatchewan watersheds were revealed through sampling, characterization, and Synchrotron-based analytical technology. The ecotoxicological effects of NPs were comprehensively examined through factorial analysis, where the interactive effects among NPs, co-contaminants, and environmental factors on algae were revealed within the context of Saskatchewan watersheds. The findings can help enrich the understandings of the current state of MP/NPs pollution, and support related environmental policymaking in Canada, especially in the prairie regions.

### ***Plastic degradation at sea and in the laboratory***

**Dr. Emilien Pelletier, L'Université du Québec à Rimouski**

When studying weathering and fragmentation of plastics in marine environments, we observed an abyss between laboratory good practices and what is happening at sea. The three main factors contributing to the behavior and weathering of plastics at sea are photodegradation, mechanical erosion and biodegradation. Biofouling is a supra factor interfering with the three others. The chemist is facing two major constraints 1) the time available for an experience, 2) the complexity of natural factors to be considered to stay as close as possible to the real marine environment. To address these experimental constraints, we developed low-cost equipment and a simplified protocol that allows simulating in the laboratory the main natural factors affecting plastic degradation within a time window of a few months. Using this protocol, the degradation kinetics of multiple plastic samples in seawater or freshwater can be determined simultaneously with or without UV A&B exposure. Biofouling and biodegradation rates can be studied with samples already photodegraded. The degradation process is slow in contradiction with some recent papers and can be carefully monitored using photonic and electronic microscopy and ATR- $\mu$ FTR. Long-term exposures (6 to 12 months) showed discoloration (loss of organic pigments), surface photooxidation and micro-fragmentation. Multiple examples are shown including the degradation of too famous Covid procedural masks which have been produced by billions and distributed around the World. And now polluting waterways and coastal waters. Weathered microplastics have been further used to study trace metals adsorption/desorption processes in the marine environment and the transfer to aquatic organisms such as mussels. The authors conclude that a carefully designed experimental protocol can provide very useful and reliable new data on the slow degradation mechanism of plastics at sea.

### ***Novel Approaches to Detect and Image the Smallest Plastics***

**Dr. Nathalie Tufenkji, McGill University**

Weathering of a single microplastic particle can yield up to billions of nanoplastics and nanoplastic pollution is expected to be ubiquitous in the environment. Nanoplastics are potentially more hazardous than microplastics because they can cross biological membranes; yet there is little data on the occurrence, fate and impacts of nanoplastics. A key challenge in understanding the environmental burden of nanoplastics is the detection of such small, carbon-based particles in complex natural matrices such as soils or whole organisms. Our group has been working on the development of novel plastic labeling and imaging techniques for detection of nanoplastics and microplastics in complex samples. The first approach relies on stimulated emission depletion microscopy (STED) to detect labeled nanoplastics in whole organisms or other complex samples. The second approach does not require pre-labeled plastic particles. Rather, internalized unlabeled microplastics are stained with a fluorescent dye but only after uptake into the organism. A tissue clearing technique is then used to remove tissue-bound fluorescent dye while also rendering the structurally intact organism transparent. The fluorescent dye remains bound to the internalized plastics that can now be visualized in the cleared tissue. This process yields a sample with fluorescently labeled plastic that can be rapidly imaged with light sheet microscopy. This presentation will describe the new imaging approaches and show examples of nanoplastic and microplastic detection in whole organisms. Our results show the versatility of these advanced imaging techniques for detection of the smallest plastics in complex environmental samples.