

Integration and data sharing to examine the fate and transport of microplastics in the Wolastoq/Saint John River watershed

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This document serves as the final report for the Canada Water Agency Atlantic Ecosystems Initiative project: Integration and data sharing to examine the fate and transport of microplastics in the Saint John River watershed

0. Summary

Microplastics have been receiving more attention from researchers in recent years, including in Atlantic Canada, presenting a need to integrate, analyze, and publicly share this important data. In this project we developed a collaborative network of research expertise and diverse partners throughout the Maritimes to address knowledge gaps around plastic pollution and its impact on freshwater and coastal ecosystems. We brought together data on microparticles that were visually identified as potential microplastics in animal, sediment and water samples collected by multiple partner organizations throughout the Wolastoq/ Saint John River watershed. Microparticles from a subset of the archived water and bivalve samples were sent for spectroscopic analysis (micro-FTIR) to confirm whether they were plastics and identify polymer composition. We compared patterns of microparticle concentration among rivers and Saint John Harbour. For bivalve, sediment, and water samples, we found differences in concentrations of visually identified microparticles among waterbodies. Saint John Harbour sites had higher concentrations of visually identified microparticles in bivalve and water samples than freshwater sites and the lowest concentrations in sediment samples. This analysis indicated that microparticles visually identified from water samples were more likely to be accurately identified as plastics (69%) than those in bivalve (16%) samples. However, some microparticles that were not identified as plastics were composed of semisynthetic and modified natural materials, which can behave similarly to microplastics in terms of transport, fate and impacts. PET was the most commonly occurring plastic compound identified in both water and bivalve samples. Our collaborative project demonstrates the value of collaboration and data sharing across partner organizations to examine patterns in microparticles and microplastics at the watershed scale. We have shared our methods, results, and recommendations with community organizations through workshops and are making our full dataset publicly available. We are sharing our results with the general public through a web page that includes an interactive GIS map that

provides a visual summary of microparticle concentrations within the watershed. This project has enhanced knowledge of microplastic pollution in the Wolastoq/Saint John Harbour watershed and developed a strong collaborative network of microplastics researchers.

1. Introduction

Anthropogenic microparticles are a globally recognized contaminant that has now been found in all environmental and biotic matrices (reviewed by Hale et al. 2020). However, the details on how these microparticles directly impact localized species and habitat is still a question requiring further study. In the province of New Brunswick (NB), these questions could include the direct impacts on keystone species, critical habitats and the potential effects on economically significant fisheries. Furthermore, we need information on how the role of abundance, shape, size, and chemical composition of microparticle pollution may alter these impacts. In Atlantic Canada, the collection of baseline data has only recently begun for microparticle pollution in both freshwater and marine environments. The completion of this project marks the successful development of a collaborative network of diverse Maritime research partners whose concerted efforts have worked towards recognizing and addressing existing knowledge gaps.

Microparticles can be visually identified using naked eye and/or microscopes but the source material, whether anthropogenic or natural, cannot easily be distinguished (Song et al. 2015). Natural materials can resemble plastic materials and vice versa due to chemical treatments, pigmentation, and weathering processes (Song et al. 2015, Cai et al. 2019). Hence it is necessary to confirm material composition using spectroscopic methods, such as Fournier Transform Infrared Spectroscopy (FTIR) to provide specific polymer identification, which can be used to determine the origin and predict the behaviour of microparticles in the environment (Song et al. 2015, Qiu et al.

2016, Cai et al. 2019, Brandt et al. 2021). FTIR uses optical spectroscopic techniques to determine the composition of individual visually identified microparticles (Harrison et al. 2012, Tagg et al. 2015, Comnea-Stancu et al. 2017, Mai et al. 2018). Recent studies have demonstrated that visually identified microparticles are often mischaracterized as being composed of artificial materials and are instead cellulosic material (Comnea-Stancu et al. 2017, Mintenig et al. 2017, Cai et al. 2019, Lenaker et al. 2021, Baraza and Hasenmueller 2023, Costa et al. 2023). Indeed, a recent study performed in the southern Gulf of St. Lawrence (Hunt and Beardy 2022) showed that only 23% of visually identified microparticles were correctly identified as plastic confirming the need for FTIR spectroscopy despite the high cost in both time and financial resources.

In consultation with plastic pollution researchers, the Canadian federal government has identified key research priorities to identify potential areas of concern and contribute to spatial and trend analysis. These priorities include the development of inventories and reference materials and building coordination and networks; the establishment, population and promotion of an open access database or network of tools with standardized/harmonized reporting; the development of a GIS resource for the visualization of microparticle/microplastic presence in environmental matrices such as sensitive or ecologically significant species and their habitats; the establishment of technical networks/working groups to facilitate collaboration and understanding of current availability for methods and baseline with a focus on leveraging or compiling existing information and resources.

This project has brought together, in partnership, Atlantic Canadian academic institutions, nongovernmental organizations, and a First Nations community to build on past research in the region to gain a deeper understanding of the spatial variation of microparticle pollution in aquatic ecosystems within the Wolastoq/Saint John River watershed (Saint John River, Tobique River, Oromocto River, Nashwaak River, Kennebecasis River, Saint John Harbour). This project has

resulted in the streamlining of regional methodologies, the discussion of best practices among partner (and other) organizations, and has initiated and facilitated knowledge transfer and data sharing initiatives.

By leveraging previously collected microparticle data and through the compilation and mapping of existing datasets, completion of this project has allowed for comparisons within and between watershed components (freshwater tributaries to harbour) and between environmental matrices (water and sediment) and biotic samples (freshwater and marine bivalve, zooplankton, ichthyoplankton, crustacean) and lastly between three species of bivalves within Saint John Harbour. This project has also allowed for further analysis of archived microparticle samples (from previous partner projects) by micro-Fourier Transform Infrared spectroscopy (FTIR) to make the distinction between anthropogenic microparticles of natural origin and microplastics. This spectroscopic microparticle analysis identified specific polymer types, which is a vital step in determining potential pollution sources and predicting environmental fate and ecosystem impacts. While focused on the Wolastoq/Saint John River watershed, this research is relevant to freshwater and coastal ecosystems across Atlantic Canada. The completion of this project has increased Atlantic Canadian research capacity and facilitated research, monitoring and reporting, and will continue to facilitate information exchange to support continued microparticle/microplastic research in an effort to advance Canada's Zero Plastic Waste agenda.

1.1 Research Region

The Wolastoq/Saint John River is the second-longest river in northeastern North America, originating in northwestern Maine (USA) and flowing for 673 km to reach the Bay of Fundy at the City of Saint John, NB. The watershed of the Wolastoq/Saint John River has an area of > 55,000 km², of which 51% is located within New Brunswick (Labaj et al., 2021). Land use in the watershed is

largely rural, with only 2.0% of the land area settled, while 84.8% is forested and 4.4% used for agriculture. The population of the watershed is 500,000 (Labaj et al., 2021), and there are numerous small communities located along the river (most with populations of <10,000), with the largest being Fredericton (population 58,000). Sampling was conducted along a 274 km stretch in the middle reaches of the Wolastoq/Saint John River from Grand Falls to Gagetown. Within the Wolastoq/Saint John River watershed, sampling also took place at four major tributaries: the Kennebecasis, Nashwaak, Tobique, and Oromocto rivers, as well as within Saint John Harbour. Each of the tributaries are largely rural, with forested areas comprising between 78.6% (Kennebecasis) to 94.6% (Tobique) of the watershed areas. Among the tributaries, the Kennebecasis watershed is the smallest (1400 km²), contains the most agricultural and settled land (8.9% and 4.1%, respectively), and has the largest population, with the towns of Sussex and Hampton, and the Village of Norton together consisting of 10,000 residents (Fig. 1). In contrast, the Nashwaak (watershed area 1700 km²), Oromocto (watershed area 2500 km²), and Tobique (watershed area 4300 km²) rivers each have 1% agricultural area and less than 2% settled area in their watersheds, and their largest communities have less than 1000 residents (Fig. 1) (Labaj et al., 2021).

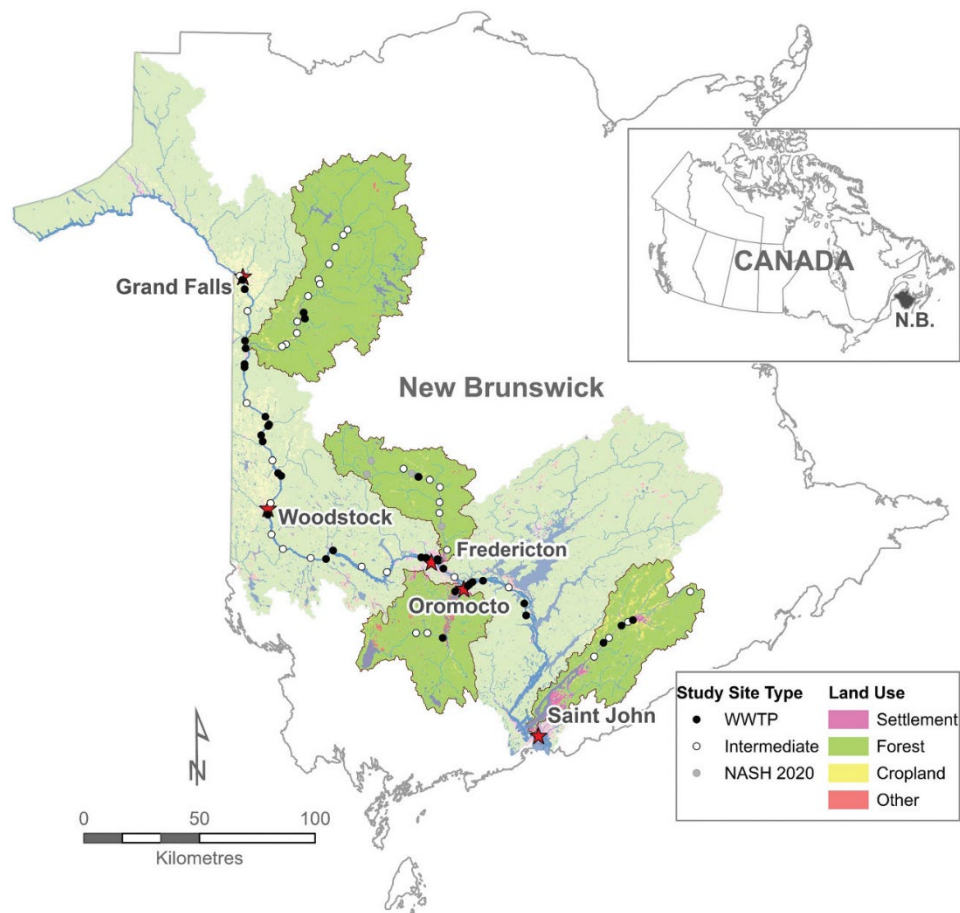


Figure 1. Map of New Brunswick, Canada, highlighting water bodies studied within the Wolastoq/Saint John River watershed, including Saint John Harbour. NASH 2020 (i.e., Nashwaak 2020) sites were sampled in triplicate in 2020 for quality control assessment, wastewater treatment plant sites (WWTP) were sampled up- and downstream from WWTP effluent pipes, and Intermediate sites were located at regular intervals along the rivers. Red stars highlight the locations of major settlements along the rivers. Map projection: NAD83 NB Double Stereographic (main map); NAD83 Lambert Conformal Conic (inset). Map data: land use: Agriculture and Agri-Food Canada (2015); Saint John River: Government of New Brunswick (2018); New Brunswick boundary: Government of New Brunswick (2005); watershed boundary: Government of New Brunswick (2018); Canadian boundary: Statistics Canada (2016) (Labaj et al., 2021).

2. Methods

Microparticle data for animal, sediment, and water samples were sourced from prior research projects completed by the five partner organizations. The University of New Brunswick, Coastal Action, and Mount Allison University researchers contributed microparticle data from bivalves and sediment along with archived microparticle samples for FTIR analysis. Mount Allison University also contributed water sample data (and archived samples for FTIR), along with ACAP and Coastal Action. The Huntsman Marine Science Centre provided microparticle data from multiple zooplankton and ichthyoplankton species and the Passamaquoddy Recognition Group Inc. contributed data collected from American lobster.

The data was combined into a series of tables in a nonrelational database. Using noSQL, five tables were created including site, animal, sediment, water, and FTIR. The sample data was separated into different tables to simplify the combination of the differing datasets. Columns (variables) were combined across partner organizations as much as possible. Columns that were unique to an organization were kept within the table, but the partner organization name was affixed to the front of the column name. The data were combined and then uploaded to Amazon Web Services to enable easy sharing among the partner organizations.

2.1 Study Sites

Sampling was carried out at 152 sites on the Wolastoq/Saint John River and four major tributary rivers were sampled (Figure 2) by Mount Allison University between 2018 and 2022 (see Table 1 for a distribution of the sites per tributary). Sites along the rivers were selected to capture a gradient of human impact, including areas with known point sources of microparticles such as wastewater treatment plants (WWTP), areas with potential diffuse microparticle input (e.g., near human

development), and intermediate sites located at regular intervals along the rivers (Figure 2). To assess whether WWTP was contributing to surface water, sediment, and mussel microparticle loads, sampling was conducted both upstream and downstream from the WWTP discharge points. On the tributary rivers (Kennebecasis, Nashwaak, Tobique, and Oromocto), sampling was conducted 20 m upstream and 20 m downstream of the WWTP discharge points. On the Saint John River, where discharge points were often located in deep water at the center of the river, sampling upstream and downstream from WWTP was conducted at the nearest possible access to the river. Saint John Harbour is the discharge point for the watershed. Biota, water, and sediment samples were taken from 40 sites in Saint John Harbour in 2018 to 2022. Partner organizations (Huntsman Marine Science Centre, Passamaquoddy Recognition Group, Mount Allison University, Coastal Action, University of New Brunswick) used separate methodologies for field work, including contamination mitigation strategies and sample storage, as the samples were originally collected for other projects prior to collaboration on this study.

2.2 Field Collection of Archived Samples

2.2.1 Mount Allison University

Freshwater Bivalve Collection

Freshwater mussels, *Margaritifera margaritifera*, *Elliptio complanate*, *Lampsilis radiata*, were sampled ~ 20 m upstream and ~20 m downstream from WWTP discharge points, as well as from additional locations along the rivers where non-point source (i.e., diffuse) inputs of microparticles may occur. When possible, five mussels of similar sizes were collected within a 5 m radius to ensure all were exposed to comparable environmental conditions. Sampling targeted the largest mussels that could be found at each site. Efforts were made to collect mussels sourced downstream of WWTPs from within the suspected effluent plume whenever possible. All collected

mussels were directly placed into individual sterile Whirl-Pak® bags to prevent cross contamination. Mussels were dispatched and preserved by freezing at $-20\text{ }^{\circ}\text{C}$. For more detailed methodology on bivalve collection and processing see Doucet et al. (2021).

River Water Sample Collection

Surface water samples were collected at each site using a 0.7 m x 0.4 m manta net with 100 μm mesh, near the center of the river channel, as wading conditions and water depth allowed. At sites located downstream from WWTP, surface water samples were collected from within the plume of the effluent pipe whenever possible. Prior to sample collection, a 1-minute average water flow rate was determined using a Höntzsch FlowTherm NT flow meter. When flow rate was sufficient for sampling with the manta net ($\geq 0.1\text{ m}\cdot\text{s}^{-1}$), the average flow rate was used to estimate sampling time (based on the dimensions of the manta net opening), with a target of between 10 000–20 000 L of surface water sampled at each site. Measured flow rates varied between 0.32 – 1.55 $\text{m}\cdot\text{s}^{-1}$ (mean: 0.64 $\text{m}\cdot\text{s}^{-1}$) on the Kennebecasis River, 0.18–1.27 $\text{m}\cdot\text{s}^{-1}$ (mean: 0.65 $\text{m}\cdot\text{s}^{-1}$) on the Nashwaak River, 0.29–1.01 $\text{m}\cdot\text{s}^{-1}$ (mean: 0.64 $\text{m}\cdot\text{s}^{-1}$) on the Tobique River, <0.1 –0.63 $\text{m}\cdot\text{s}^{-1}$ (mean: 0.49 $\text{m}\cdot\text{s}^{-1}$) on the Oromocto River, and <0.1 –0.52 $\text{m}\cdot\text{s}^{-1}$ (mean: 0.31 $\text{m}\cdot\text{s}^{-1}$) on the Wolastoq/Saint John River. When a site had sufficient water depth, the manta net was held $\sim 5\text{ cm}$ under the water's surface. In shallow-water sites where the manta net could not be fully submerged, it was held half submerged in the water, and the sampling time was doubled. When the flow rate at a site was not sufficient for deployment of the manta net ($<0.1\text{ m}\cdot\text{s}^{-1}$), an electric pump, elevated $\sim 30\text{ cm}$ off the riverbed by a cinder block, was used to pump water through the manta net. The pump was run for a period of 50 minutes, passing $\sim 4800\text{ L}$ of water through the manta net. Following the collection of the surface water sample, the material remaining in the manta net was rinsed through a 5 mm brass sieve into a 90 μm brass sieve, where it was further rinsed with river water. Material retained in the 90 μm sieve

was rinsed into a sterile Whirl-Pak bag. For more detailed methodology on water sample collection and processing see Labaj et al. (2021).

Sediment Sample Collection

Sediment samples were collected by hand from an undisturbed area at each site, generally within ~5 m of the water collection site, in water of <0.75 m depth. Due to the wide variation in river bottom substrate (e.g., silt, sand, gravel, cobble, etc.), the composition of the collected sediments varied among sites; however, whenever possible, sediment samples were collected from depositional areas. Sediments were placed into Whirl-Pak bags. For more detailed methodology on sediment sample collection and processing see Labaj et al. (2021).

Contamination Mitigation

During field work, all members of the field team wore natural fibre clothing, minimizing the risk of possible contamination of samples with microparticles from clothing. Prior to the collection of each sample, all equipment was thoroughly rinsed in river water to remove any materials remaining from previous sites. When sampling at multiple locations near WWTP, downstream sites were always sampled before upstream sites to avoid contamination caused by upstream disturbance.

2.2.2 Passamaquoddy Recognition Group Inc. (PRG)

Lobster Sample Collection

In 2019-2023, 98 lobsters (*Homarus americanus*) were sampled in the Saint John Harbour area. Standard commercial traps were baited and set. When traps were hauled after a determined soak time, individuals were banded, numbered, sexed, carapace length measured (mm), placed in

individual bags, and sealed (lobsters are to be rinsed prior to dissection so this step is not necessary in the field). Once on land, samples were transferred to a freezer.

Contamination Mitigation

Due to the ubiquitous nature of microparticles, contamination reduction was considered when conducting fieldwork. Field staff took extra precautions by wearing natural fibre, non-shedding clothes. Samples of potentially shedding plastic materials used during sampling were taken for FTIR analysis to aid in the control of field-based contamination. All sampling and storage equipment were triple rinsed with filtered deionized water prior to use. All specimens were triple rinsed prior to storage.

Contamination control samples were taken of materials used during field work which could pose a contamination risk. This included bait bags, dive bags, traps, and clothing fibres. Control samples were prepared by placing a clean filter paper in a petri slide and labelling the slide with the date, sample number, and description of the material sampled. These samples were then sent for FTIR analysis.

2.2.3 University of New Brunswick

Marine Bivalve Sample Collection

Bivalves were collected from intertidal sites in Saint John Harbour. Prior to sampling activities, all sampling equipment and storage containers were triple rinsed in filtered water. Adult soft-shelled clams (*Mya arenaria*) (> 4 cm shell length) were collected using a metal, paint-free shovel at a depth of up to 30 cm. Adult blue mussels (*Mytilus edulis*) (>5 cm shell length) were collected by hand. Each bivalve was rinsed with filtered water (47 mm polyvinylidene fluoride (PVDF) membrane with a

pore size of 0.22 μm) and placed in a glass container with a metal lid. Samples were stored in a cooler for transport back to the laboratory and were frozen (-20°C) until processed.

Contamination Mitigation

Prior to all activities, surfaces, materials and equipment were triple rinsed with filtered PVDF membrane with a pore size of 0.22 μm water. Non-shedding, natural fibre clothing was worn by field technicians and hair was tied back.

To confirm that incidental contamination from environmental microparticles were not included in the analysis, environmental controls were used throughout the fieldwork process. Environmental blanks were exposed to the field (open collection jars) environment during sample collection.

Contamination of environmental blanks resulted in the removal of microparticles of similar appearance from the final data set.

2.2.4 Huntsman Marine Science Centre

Zooplankton and Ichthyoplankton Collection

Samples were collected at six sites in Saint John Harbour using a 1 m diameter conical plankton net with a mesh size of 0.243 mm and a cod end size of 0.333 mm. A temperature-depth sensor was attached to the net along with a mechanical flow meter attached at the mouth of the net to measure water volume. Each tow was performed for 10 minutes with shallow stations towed near the surface; intermediate stations towed for five minutes at mid depth and five minutes near the surface; deepest stations towed obliquely in equal steps. For each tow, stop and start positions were recorded using GPS.

Once each tow was complete, the cod end sampler was removed and rinsed with filtered water.

Material retained in cod end was carefully transferred into glass or plastic bottles which have been

previously rinsed 3 times with ultrapure Mili-Q or filtered seawater. Live samples were immediately anaesthetized and preserved to prevent animals voiding their digestive tracts. Samples were stored at room temperature until further processing.

Contamination Mitigation

Prior to all activities, materials and equipment were triple rinsed with filtered sea water (47 mm nitrocellulose membrane with a pore size of 0.22 μm). Tyvek suits (or equivalent) with hoods were worn (over natural fibre clothing) by field technicians. All clothing and possible contaminating objects on deck were photographed for reference during analysis. Where possible, potential sources of contamination were removed from the deck (e.g., rope coils).

To confirm that incidental contamination was not included in the analysis, environmental and procedural controls were used throughout the fieldwork process. Contamination of environmental or procedural controls resulted in the removal of microparticles of similar appearance from the final data set.

2.2.5 ACAP Saint John

Water Sample Collection

An outboard LADI trawl (Civic Laboratory for Environmental Action Research 2025) equipped with a cod end (mesh size 333 μm) was used in the sampling of surface water in both the inner and outer regions of Saint John Harbour and the Kennebecasis River. A General Oceanics 2030R Flowmeter was attached to the mouth of the trawl for the duration of each tow. The reading on the flowmeter was recorded at the beginning and end of each tow to calculate the distance, average speed travelled, and the volume of surface water sampled.

The trawl was deployed for a total of 120 minutes in both inner and outer regions while the vessel traveled back and forth along the trawl trajectory. After each tow, the cod end was removed, and debris was transferred to a 355 μm sieve where larger organic materials were rinsed with freshwater and removed from the sample. The remaining debris was washed into a clean mason jar. Samples were kept frozen until processing.

Sediment Collection

Sediment samples were taken from intertidal sites in Saint John Harbour at low tide from randomly selected transects situated at the most recent high tide mark, mid beach, and back beach locations. Transects were set using premeasured, brightly coloured ropes set with pegs to mark a 1 m x 1 m area of sediment. The top 5 cm of sediment was removed using a scoop and placed in a 5-gallon bucket. Water was added to the bucket, above the sediment fill line, and the sediment was stirred. Excess water was poured through a set of nested sieves (5 mm, 1 mm, .335 mm). Large organic material was inspected, rinsed into the sieves then discarded. The remaining contents of the sieves were placed in sample bags. This process was completed 3 times per quadrat. The samples were frozen until processing.

Contamination Mitigation

Field staff avoided wearing synthetic fibre clothing or clothes which could shed easily. Clothes were covered with a fluorescent yellow jacket. Sampling and storage equipment were rinsed with distilled water prior to use. The trawl and cod end were rinsed with freshwater between tows and prior to the collection of each sample.

2.3 Lab Processing

2.3.1 Mount Allison University

Laboratory Contamination Mitigation

During laboratory processing, laundered cotton lab coats, stored within the laboratory, were worn when handling samples to reduce contamination from the clothing of lab personnel. Glass was used instead of plastic whenever possible. All glassware and other apparatus were triple rinsed with deionized (DI) water before use and between samples to reduce interior contamination. All nearby work surfaces were cleaned with 70% ethanol prior to use. Samples were covered with aluminum foil and exposed only during dissection, sieving, and visual inspection. Each activity was conducted as efficiently as possible to minimize interior exposure. Dissection was typically conducted in < 5 min, and sieving in < 2 min, while visual inspection could vary (~ 1–3 h) depending on the amount of material in the sample. One blank sample was run for every batch of five mussel samples processed (i.e., one blank per sample site) to account for interior contamination.

Blank samples were prepared in clean beakers with DI water. Each blank was sieved, rinsed, and inspected for microparticles in the same manner and for the same duration as the corresponding mussel samples and thus accounted for all stages of processing, from tissue digestion to visual analysis. Prior to data analysis, microparticle counts were corrected by categorically subtracting the microparticle counts, categorized by morphology and color, within each blank from the counts of each associated sample.

Bivalve Sample Processing

Upon removal from the freezer, mussels were defrosted for ~ 30 min, and the length (longest axis of shell), width (perpendicular to length axis), and total mass of each mussel were recorded. Once

thawed, the soft tissue of each mussel was scraped into a clean beaker to be weighed. Rather than isolating the digestive organs, all soft tissue was retained, as microparticles adherence to nondigestive tissues within the body cavity accounts for a proportion of mussel microparticle content. Any material remaining within the Whirl-Pak® bag was also rinsed into the beaker as it was assumed to have been expelled by the mussel during transport from the field. Soft tissue was submerged in a 10% potassium hydroxide (KOH) solution of approximately triple the volume of the tissue and digested for 24 h on a 60 °C hot plate. Following digestion, each beaker was partially submerged in an ultrasonic cleaner for ~3 min to expediate the disintegration of undigested tissue. Samples were then filtered through a 90-µm sieve and rinsed thoroughly with DI water before being returned to their respective beakers. As a single digestion treatment with KOH was not sufficient to adequately digest all tissue, each sample received a second digestion treatment with fresh 10% KOH, followed by the same ultrasonic bath and sieving process received in the initial treatment.

Microparticles found within the samples were identified and collected. Each sample was inspected twice under a Zeiss Stemi 508 stereomicroscope at between × 12.5 and × 50 magnification to minimize the number of particles that went undetected. Once recognized, microparticles were removed from samples using forceps, classified as fiber, fiber bundle, fragment, sphere (or bead), pellet, film, and foam, and mounted onto glass slides using clear double-sided tape for long-term storage and future micro-FTIR characterization.

Water Sample Processing

Surface water samples were poured from their Whirl-Pak bags into a clean 90 µm brass sieve, and the interior of the bag was further rinsed with deionized water. Material retained in the sieve was rinsed with DI water and transferred into a clean 600 mL beaker. Approximately 75 mL of 30% hydrogen peroxide (H₂O₂) was then added to the beaker to digest the organic matter in the sample.

The sample was then placed on a 75 °C hot plate for ~8 hours to continue breaking down organic material (modified from Masura et al. 2015). After ~8 hours in the H₂O₂ solution, the sample was again rinsed on the 90 µm sieve and backwashed into a clean beaker to await visual examination.

Sediment Sample Processing

Sediment samples were weighed into a clean 600 mL beaker, with ~20 g of wet sediment used from each site. Approximately 100 mL of 5% sodium hexametaphosphate was added to each sample as a deflocculant, and the samples were heated on a 60 °C hot plate for 2–4 hours. The samples were then immersed in an ultrasonic bath for ~15 seconds, poured onto a 90 µm sieve and rinsed with DI water, and backwashed into the 600 mL beaker. Approximately 75 mL of 30% H₂O₂ was added to the beaker to digest organic material, which was allowed to react for 8–12 hours before being placed on a hotplate at 75 °C for ~ 8 hours. The sample was again immersed in the ultrasonic bath, rinsed on the 90 µm sieve, and transferred into the 600 mL beaker. To facilitate the visual examination process (complicated in the sediment samples by the presence of small pebbles, sand, etc.), a density separation was performed. Approximately 200 mL of 30% sodium chloride (NaCl) solution (density: 1.15 g·mL⁻¹) was added to the samples (Masura et al. 2015), and they were immersed in a sonic bath for 15 seconds. After sitting undisturbed for ~24 hours, the liquid portion of the samples (containing floating material) was decanted into the 90 µm sieve, rinsed with deionized water, and backwashed into a clean 400 mL beaker. The dense (sinking) fraction of the samples was retained in the 600 mL beakers. Both the floating and dense fractions of each sample were examined for microparticles.

Visual Microparticle Examination and Enumeration

Aliquots of samples were poured into a clean glass petri dish and examined under a Zeiss Stemi 508 dissecting microscope at between 6.3 and 50x magnification and visually scanned in transects. Microparticles were recognized and distinguished from non-anthropogenic materials based on colour, shape, and texture. When a microparticle was found, it was removed with forceps and mounted on a clean glass slide with double-sided tape to be archived. All microparticles removed were counted and classified by colour and morphology (fibre, fragment, film, sphere, pellet etc.).

2.3.2 Passamaquoddy Recognition Group Inc. (PRG)

Laboratory Contamination Mitigation

Due to the ubiquitous nature of microparticles, plastic contamination reduction was considered when conducting microparticle work. Work was completed in a designated 'clean room.' Laboratory staff took extra precautions by wearing designated natural fibre, non-shedding clothes treated with a unique purple dye. Cotton lab coats were treated with the same dye and worn at all times. Samples of potentially shedding plastic materials used during processing were taken for FTIR analysis to aid in the control of laboratory-based contamination. All laboratory equipment was triple rinsed with filtered water (DI, filtered at 0.8 µm pore size) before use.

Dissections should take place in a clean and enclosed space such as a fume hood, laminar flow hood or biosafety cabinet, when possible, to minimize potential contamination. If such spaces are not available, a clean dedicated clean room/ space is suitable and will be noted in the lab book.

Environmental control samples were taken of the research environment. Control samples were prepared by placing a clean filter paper in a petri slide and labelling the slide with the date. Particles found on control filters not matching the purple dye were sent for FTIR analysis.

Lobster Preparation and Processing

The lobsters were triple rinsed prior to dissection to isolate the digestive tract. Starting at the anus, vertical cuts were made until the gonads (on either side of the anus/intestine) were reached. Tissue surrounding intestine was teased away to expose and detach the digestive tract. Tissue was kept intact around the area of the anus to avoid ripping the intestine during removal. The anus may be clamped with haemostat forceps to avoid losing any material from the inside of the intestine. However, the weight of the forceps on the end of the anus may lead to ripping the intestine from the stomach. Once removed, the anus and intestine were placed back in the cavity while the remainder of the digestive tract is removed.

Shallow vertical cuts were made upwards from the gonads to the head, following the digestive tract (should be visible) teasing away remaining tissue attachments. The lobster was then turned over (dorsal view) and a horizontal cut was made along the rostrum to remove the sharp point. A shallow vertical cut was carefully made up the rostrum towards the base of the head (the stomach lies within the head and is easily punctured). The thin layer of tissue on top and around the stomach and any remaining connective tissue was gently teased away and the stomach was carefully removed from the cavity, along with digestive tract.

A final horizontal cut was made on the anus end to completely detach the digestive tract from the body. The entire digestive tract was rinsed, placed in a clean glass jar, weighed (grams) and frozen. Using scissors, the tail was severed from the body by cutting along the lower portion of the carapace. Tail tissue was removed from shell, placed in a clean glass jar and weighed (grams) and frozen.

Lobster tissues were thawed for 20-40 minutes. Sample contents were broken apart using forceps and scissors to increase surface area contact with digestion solution. Using a fume hood,

approximately 150 ml of 10% KOH was added to the jar which was loosely covered and incubated for 24 hours at 55°C. The results of the digestions were passed through a series of sieves and then vacuum filtered onto nylon filters with a pore size of 0.8 µm. Each separate filter was placed in a clean petrislide, covered and labeled with site and sample information. Both the contents of the sieves, and each separate filter were examined under a dissecting microscope (40x magnification), and microparticles were counted and classified as fibre, fragment, film, sphere, etc.

2.3.3 University of New Brunswick

Laboratory processing of sediment, animal and water samples collected by the Huntsman Marine Science Centre, ACAP Saint John, and UNB was performed by a research technician at UNB using the following protocols.

Laboratory Contamination Mitigation

To reduce the risk of contamination, all lab activities were performed in a biosafety cabinet apart from reagent preparation (which required a fume hood) and visual analysis which was completed without removing the lid of the petrislide. Prior to all activities, surfaces, materials and equipment were triple rinsed with filtered water. Natural fibre clothing (including lab coat) was worn and hair was tied back. After preparation and prior to usage, all reagents were filtered using a 47 mm PVDF membrane with a pore size of 0.22 µm.

To confirm that incidental contamination from environmental microparticles were not included in the analysis, environmental controls were used throughout the fieldwork process. Environmental blanks were exposed to the laboratory (open petri plate with filter) environment during processing activities and procedural blanks accompanied each batch of samples to confirm the absence of contamination in reagents and filtration systems. Contamination of environmental and procedural

blanks resulted in the removal of potential microplastics of similar appearance from the final data set.

Bivalve Sample Processing

Bivalves were removed from the freezer and allowed to thaw for 30 min while remaining sealed in the glass container. For each bivalve specimen, the soft tissue (including muscle, foot, gill tissue and internal organs) was separated from the shell using forceps and weighed (g). The tissues were placed back in the glass container and a 10% KOH was added. The amount of KOH used for each digestion was dependent on the size of the specimen with 10 ml of solution used for each gram of tissue extracted. The container was covered with a metal lid and incubated at 55 °C for 24 hours.

The results of the bivalve digestions were vacuum filtered onto 47 mm PVDF membranes with a pore size of 0.22 µm. Due to the nature of the samples, some required more than one filter. Each separate membrane was placed in a clean petrislide, covered and labeled with site and sample information. The membranes were examined under a dissecting microscope (40x magnification), and microparticles were counted and classified as fibre, fragment, film, or sphere.

Sediment Sample Processing

Sediment samples were disaggregated and dried for 24 hours in a drying oven at 55 °C. A 50 g homogenized subsample was removed and placed in a clean glass container with a metal lid. The remaining dry sediment was stored in a glass container with a metal lid at room temperature (18-22 °C).

If organic matter was present, samples were pretreated by the addition of a 10% KOH solution added at a 1:3 volume (sample to solution) ratio to digest biological material. The sample was covered and incubated at 55 °C for 24 hours prior to filtration.

Microparticles were separated from the sediment by addition of high-density salt solutions. First, 150 ml of a concentrated sodium chloride solution (NaCl) was added to the sample to float off the less dense microparticles. The sediment was mixed vigorously for 5 minutes and allowed to settle for up to 5 hours. The resulting supernatant was decanted into a clean glass container and covered with a metal lid. The same sample was then resuspended with an additional 150 ml of NaCl solution by the repetition of the stirring, settling and decanting steps. This supernatant from this 2nd extraction was pooled with the first suspension. Zinc chloride solution (ZnCl_2), which has a significantly higher density than NaCl, (1.7 g/cm^3 vs. 1.2 g/cm^3) was used for the 3rd and final extraction.

The results of the sediment extractions were vacuum filtered onto 47 mm PVDF membranes filter with a pore size of $0.22 \mu\text{m}$. Due to the nature of the samples, some required more than one filter. Each separate filter was placed in a clean petrislide, covered and labeled with site and sample information. The filters were examined under a dissecting microscope (40x magnification), and microparticles were counted and classified as fibre, fragment, film or sphere.

2.3.4 Huntsman Marine Science Centre

Zooplankton/Ichthyoplankton Processing

Using filtered water ($0.22 \mu\text{m}$ glass fibre filter), plankton samples were removed from collection vials and placed in a glass Petri dish where they were rinsed with filtered water using a glass pipette to remove preservation liquid and any accompanying debris. Using a dissecting microscope, the plankton samples were inspected for microparticles and other debris that may be adhering to the carapace and appendages. Debris and microparticles were carefully removed using dissection needles and forceps. Once free from outside debris, the carapace was pulled apart using dissection needles and digested in a 10% KOH solution for 24 hours at 55°C . After the incubation

period, the samples were vacuum filtered onto a 47 mm PVDF membrane filter with a pore size of 0.22 μm , placed in a petrislide, partially covered, and allowed to dry in a laminar flow cabinet. Using a dissecting microscope, the filter was examined for microparticles which were counted, measured, and classified as fibre, fragment, film or sphere.

Seawater Sample Processing

Water samples were kept frozen (-20°C) until analysis, then thawed in the biosafety cabinet at room temperature. Each sample was inspected and transferred to a clean glass container. Samples were homogenized by gently stirring until sample contents were uniform in appearance. When required, sample contents were gently pulled apart to disperse large aggregations of organic material using fine needles. If organic content was low, samples were moved directly to the filtration step (below). If organic matter was present, water samples were pretreated by the addition of a 10% KOH solution added at a 1:3 volume (sample to solution) ratio to digest biological material. The sample was covered and incubated at 55°C for 24 hours prior to filtration.

Samples were filtered using a series of filters inserted into a vacuum filter holder which was attached to a receiver flask unit. This unit is then attached to a vacuum pump using rubber tubing. Digested samples with excessive cloudiness or visible debris (organic or otherwise) left after the digestion process were coarsely filtered ($5\ \mu\text{m} - 1\ \mu\text{m}$ pore-size) through a pre-inspected glass-fibre filter prior to filtration with PVDF membrane with a 0.22 μm pore size. After each filtration, the filter was removed and placed on a labeled petri slide, the slide was partially covered and allowed to dry in a biosafety cabinet. Once dry, the petri slide was covered and stored in a sealed container at room temperature until inspection. The samples were inspected under a dissecting microscope (40x magnification), and microparticles were identified, counted, and classified as fibre, fragment film or sphere.

2.4 Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) is an optical spectroscopy technique that allows for the determination of the chemical bonding present in a material. It is sensitive to components that are present in concentrations greater than approximately 3–5% of the total. FTIR spectroscopy relies on the sample absorbing certain wavelengths of Infrared (IR) radiation. This is measured by irradiating a sample with a polychromatic IR beam, measuring the intensity of light absorbed which is then converted to a spectrum. This spectrum is then compared to a library of known chemical spectra to identify the microparticle in question (pers. comm. Surface Science Western, 2025).

Microparticle samples were submitted to Surface Science Western either affixed to double-sided tape and placed on glass slides, or on petrislide-bound PVDF filters. Microparticles of interest were transferred to a diamond compression cell and analyzed in transmission mode under the Hyperion 2000 microscope while attached to a Bruker Tensor II spectrometer. The spectra were corrected for atmospheric water and carbon dioxide contributions and were baseline corrected. A reference of the tape adhesive was also analyzed (pers. comm. Surface Science Western, 2025).

When the spectrum was consistent with a cellulosic or cellulose material, the spectrum was examined to determine if there was a peak, a shoulder or no peak at $\sim 1105 \text{ cm}^{-1}$. If the sample showed a peak, it was considered to be of natural origin; if the sample had a shoulder or no peak, it was considered to be of a semi-synthetic nature (Cai et al., 2019, pers. comm. Surface Science Western, 2025).

For this AEI project, 1617 microparticles extracted from water (1054), sediment (39), and bivalve (563, all but 56 freshwater) tissue samples were successfully analyzed by FTIR spectroscopy which was performed by Surface Science Western at the University of Western Ontario. FTIR results determined whether particles were composed of plastic (specific polymer ID included) or of

natural/semisynthetic material. These results were added to the existing FTIR data set which included 480 samples from zooplankton, ichthyoplankton and surface water from the Huntsman Marine Science Centre, and 994 samples extracted from lobster by the Passamaquoddy Recognition Group.

2.5 Statistical Analysis

All data analyses were completed using R v.4.4.2 (R Core Team 2022). Concentrations were calculated as microparticles per g of tissue in bivalves, microparticles per g of sediment, and microparticles per mL of water. Animals other than bivalves (i.e. lobster and plankton) were not included in statistical analyses because concentrations could not be calculated. The three sample metrics: bivalves, sediment, and water, were modeled separately. Two models were completed using the bivalve data: all bivalves (species pooled), and only bivalves from the Saint John Harbour sites (species included as a factor in the analysis). A comparison was also made between freshwater and marine water samples (collected by Coastal Action), since the same methodology was used in sample collection allowing for a direct comparison between waterbody types. All models were run using concentrations for individual samples, not site averages.

Non-parametric analyses were used due to the extreme violations of normality assumptions and our inability to find adequate transformations or distributions for modeling. Kruskal-Wallis rank sum tests (“kruskal.test” function, “stats” package, R Core Team 2022) were completed to look at the spatial variation and species variation in the concentration of visually identified microparticles, as well as the microparticle morphology (fibres, fragments, films, and spheres). Pairwise post doc comparisons were completed using Conover’s all-pairs tests (“kwAllPairsConoverTest” function, “PMCMRplus” package, Pohlert 2023).

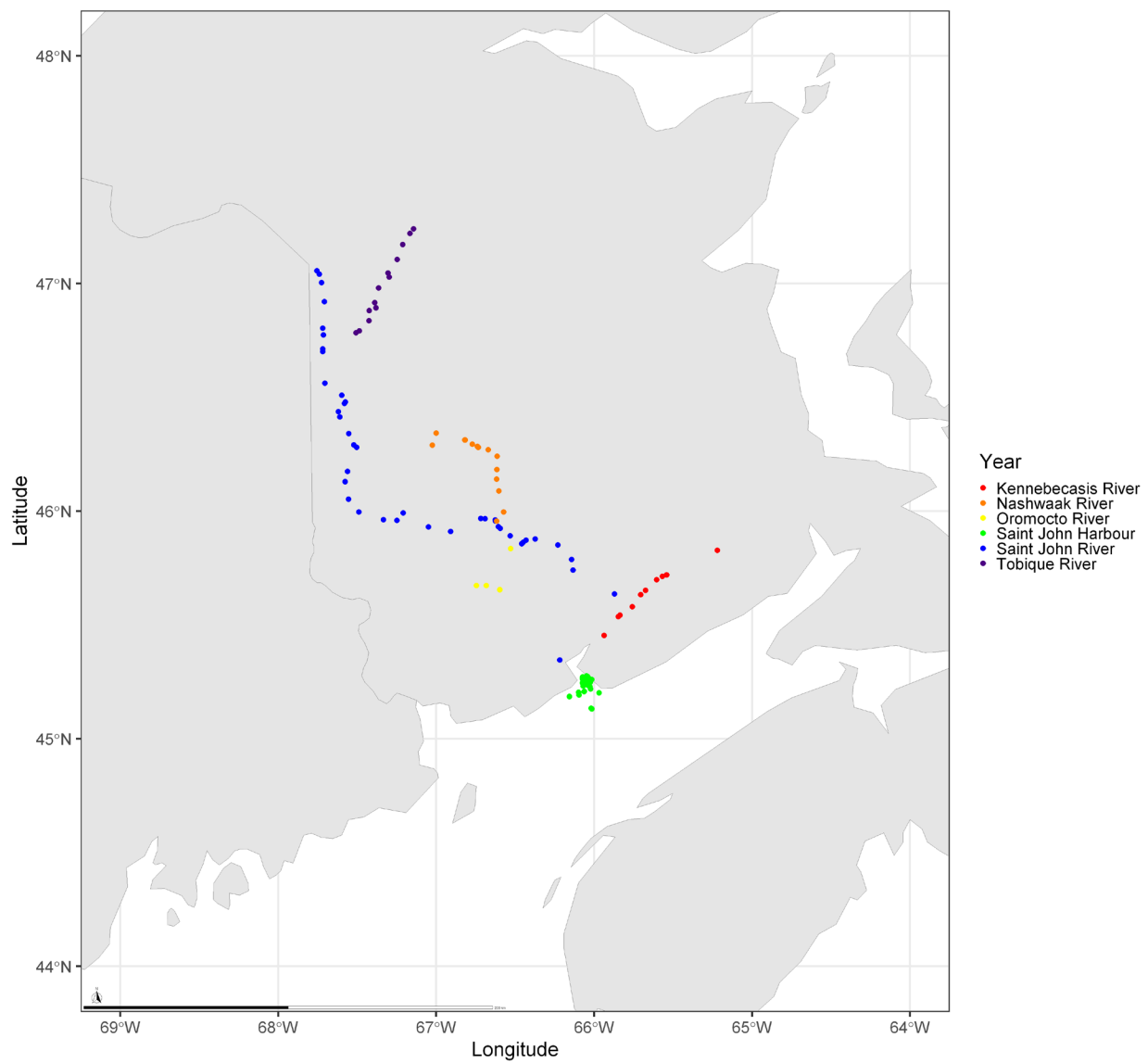


Figure 2. Map of sampling locations by all partner organizations in the Wolastoq/Saint John River watershed in New Brunswick, Canada

Table 1. Distribution of sites at each water body within the Wolastoq/Saint John River watershed sampled from 2018 to 2022.

Waterbody	Number of Sites
Saint John River	46
Tobique River	20
Nashwaak River	20
Oromocto River	9
Kennebecasis River	17
Saint John Harbour	40

3. Results

3.1 Visually Identified Microparticles

Within the Saint John River watershed, visually identified microparticles were observed in all six tributaries in all three-sample matrices, animals (bivalves), sediment, and water (Figures 3, 4, 5). Visually identified microparticles were found in bivalves in three species of bivalves in Saint John Harbour (Figure 6). The visually identified microparticles were primarily fibres, followed by fragments, with film and sphere microparticles being the least frequently found classification (Figures 3-6).

The patterns observed in the concentration and morphology of microparticles differed between the bivalve, water and sediment samples. Both the bivalve and water samples had higher concentrations of microparticles at the Saint John Harbour sites compared to the other waterbodies, but the difference between the Saint John Harbour and freshwater sites was larger for water samples than for bivalves (Figures 3, 5). In contrast, the concentration of microparticles in sediment samples was lower in Saint John Harbour samples compared to the other waterbodies (Figure 4). It should be noted that the number of sediment and water samples was lower than the number of bivalve samples collected from each waterbody.

For the bivalve samples, there were significant differences among the waterbodies for total, fibre, and fragment microparticles while there were not significant differences for film or sphere microparticles (Table 2). Saint John Harbour had a significantly higher total concentration of microparticles per g of tissue compared to the Kennebecasis River, Saint John River, and Tobique River (Supplemental Material Table S1). Additionally, the Tobique River, Saint John River, and Nashwaak River had significantly higher total concentration of microparticles compared to the Kennebecasis River (Supplemental Material Table S1). The Nashwaak River also had a significantly

higher total concentration of microparticles compared to the Tobique River (Supplemental Material Table S1). The same differences were observed with fibres, except for the previously observed differences between the Oromocto River and Saint John and Kennebecasis Rivers (Table 2, Supplemental Material Table S1). There was also a significant difference among waterbodies for fragment microparticles within bivalve samples, driven by the higher concentration of fragment microparticles in Saint John Harbour compared to the Kennebecasis, Oromocto, Saint John, and Tobique Rivers (Supplemental Material Table S1). There were no significant differences in the concentration of film or sphere microparticles among the bivalve samples (Table 2).

For the Saint John Harbour bivalves, there were statistical differences in the concentration of total and fibre microparticles among the three species collected (Table 2). Soft shell clams had significantly fewer total and fibre microparticles compared to blue mussels and fewer fibre microparticles compared to *Macoma* clams (Supplemental Material Table S1).

The sediment samples also exhibited significant differences in all visually identified microparticle morphologies, except film, among the six waterbodies (Table 2). The differences for the sediment samples were not the same as the bivalve samples. The Saint John Harbour sediment samples had a significantly lower concentration of total and fibre microparticles compared to the Kennebecasis River, Nashwaak River, Saint John River, and Tobique River (Supplemental Material Table S1). Additionally, the Nashwaak River had significantly higher concentration of total microparticles compared to the Tobique River, and higher concentration of fibres compared to the Saint John and Tobique Rivers (Supplemental Material Table S1). The Tobique River had a lower concentration of fragment microparticles compared to the Kennebecasis River and Saint John Harbour (Supplemental Material Table S1). The Oromocto River had a significantly higher concentration of sphere microparticles compared to Saint John Harbour and the Kennebecasis, Nashwaak, Saint John, and Tobique Rivers (Supplemental Material Table S1). The Saint John River also had

significantly more sphere microparticles compared to Saint John Harbour (Supplemental Material Table S1).

Lastly, there were significant differences in the concentration of microparticles for all microparticle morphologies in the water samples (Table 2). Saint John Harbour had a significantly higher concentration of total and fragment microparticles than the Kennebecasis, Nashwaak, Oromocto, and Tobique Rivers; a significantly higher concentration of fibre microparticles compared to the Nashwaak, Oromocto, Tobique, and Saint John Rivers; a higher concentration of film microparticles compared to the Nashwaak River; and a higher concentration of sphere microparticles compared to the Saint John River (Supplemental Material Table S1). The Tobique River had a significantly lower concentration of total microparticles compared to the Kennebecasis, Oromocto, and Saint John Rivers; a significantly lower concentration of fibre microparticles compared to the Kennebecasis and Saint John Rivers; a significantly lower concentration of fragment and sphere microparticles compared to the Oromocto and Saint John Rivers; and a lower concentration of film microparticles compared to the Saint John River (Supplemental Material Table S1). Nashwaak River had a lower concentration of total, fibre, fragment, film, and sphere microparticles compared to the Saint John River, and a lower concentration of fragment and film microparticles compared to the Oromocto River (Supplemental Material Table S1). There were significant differences in the concentration of total, fibre, and fragment microparticles from freshwater and marine water samples collected by ACAP ($p = 0.025, 0.025, 0.025$, respectively), with marine samples having greater concentrations. There was not a significant difference in the concentration of film microparticles ($p = 0.831$) and there were not enough sphere microparticles to run comparisons with.

3.2 FTIR

Of the 1,054 visually identified microparticles found within water samples sent for FTIR analysis for this AEI project, 729 (69.2%) of those were chemically identified as being plastic compounds, while the remaining 325 (30.8%) were identified as naturally occurring compounds (Figure 7). In contrast, of the 563 visually identified microparticles sent for FTIR that were found within bivalve samples, 92 (16.3%) were chemically identified as plastic compounds while the remaining 471 (83.7%) were microparticles composed of naturally derived compounds (Figure 7). PET was the most commonly occurring plastic compound identified in both water and bivalve samples while cellulose was the most common naturally derived compound identified for non-plastic microparticles (Figure 7). For water samples, fragment and film microparticles were much more likely to be microplastics but fibres were slightly more likely to be microplastics than naturally occurring compounds (Figure 8). Fibre and film microparticles were much more likely to be naturally occurring compounds while fragment microparticles were closer to 50% to be either plastic or natural compounds in bivalve samples (Figure 8). Approximately 90% of the bivalve samples sent for FTIR were from freshwater bivalves with the remaining 10% being marine samples. All analyses of water samples were completed on freshwater samples.

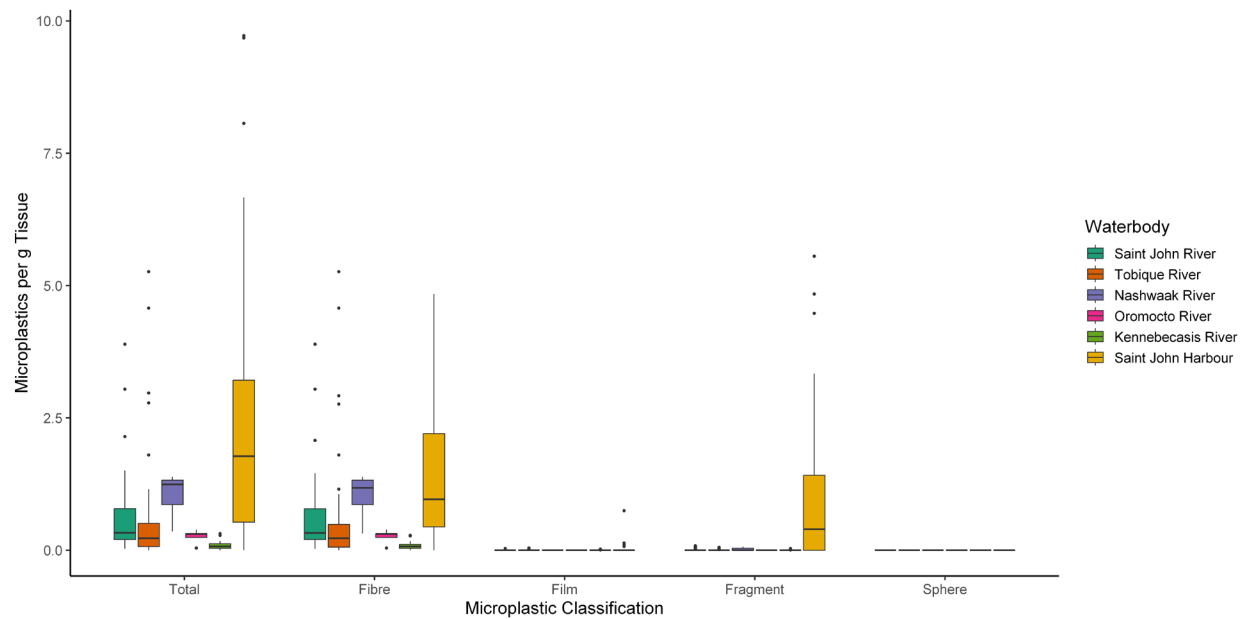


Figure 3. Boxplot of the concentration of visualized identified microparticles, classified as fibre, film, fragment, and sphere, in bivalve samples in the Saint John River watershed in New Brunswick, Canada (n = 452 samples).

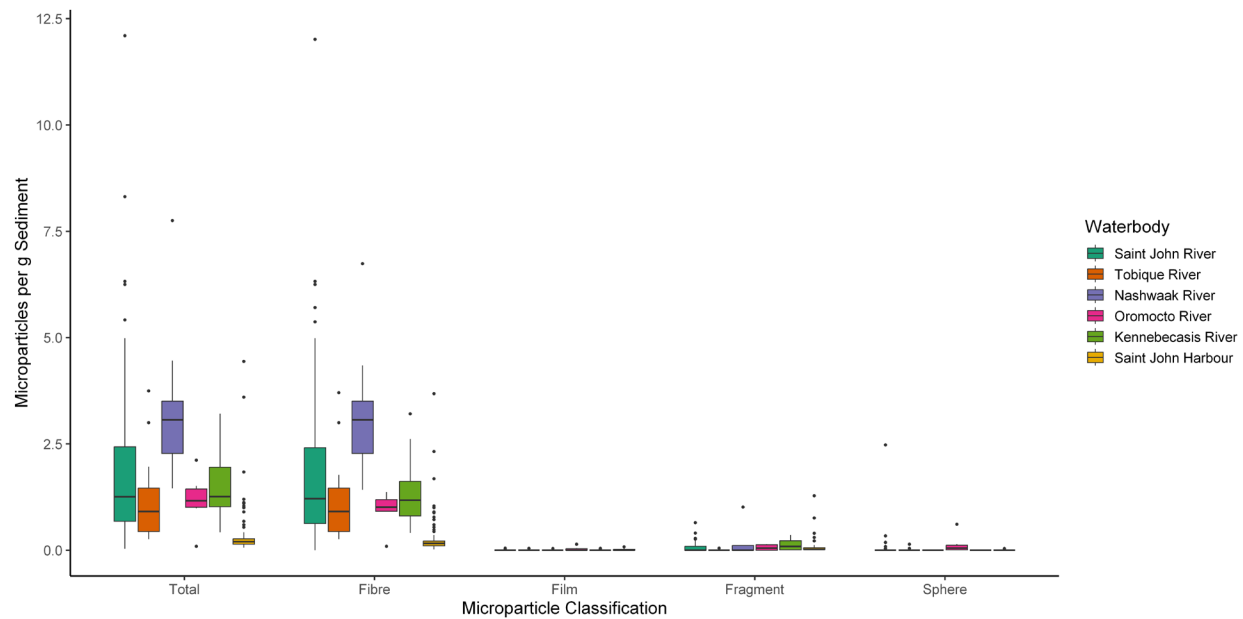


Figure 4. Boxplot of the concentration of visualized identified microparticles, classified as fibre, film, fragment, and sphere, in sediment samples in the Saint John River watershed in New Brunswick, Canada (n = 184 samples).

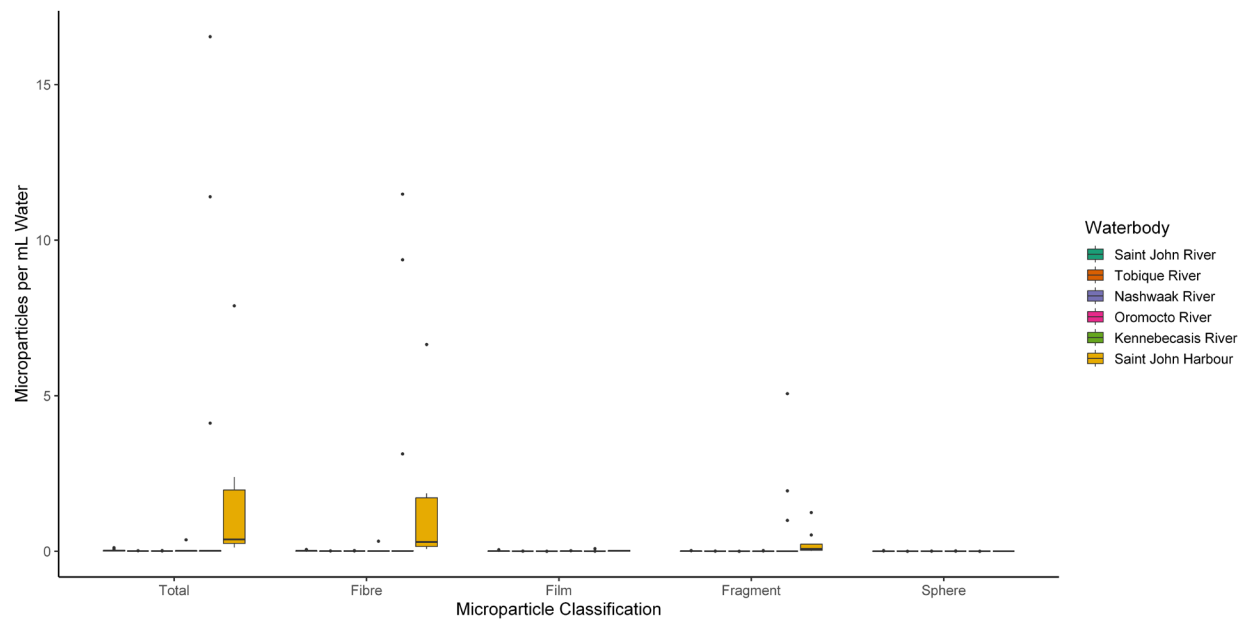


Figure 5. Boxplot of the concentration of visualized identified microparticles, classified as fibre, film, fragment, and sphere, in water samples in the Saint John River watershed in New Brunswick, Canada (n = 128 samples).

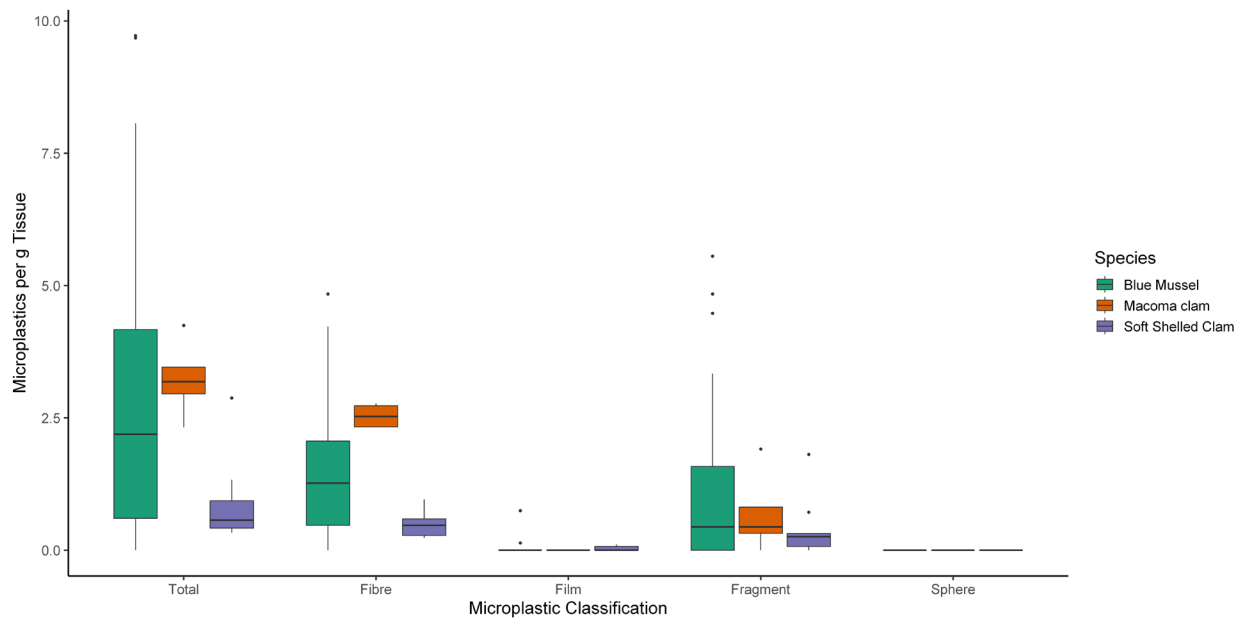


Figure 6. Boxplot of the concentration of visualized identified microparticles, classified as fibre, film, fragment, and sphere, in bivalve samples in the Saint John Harbour in New Brunswick, Canada (n = 43 samples).

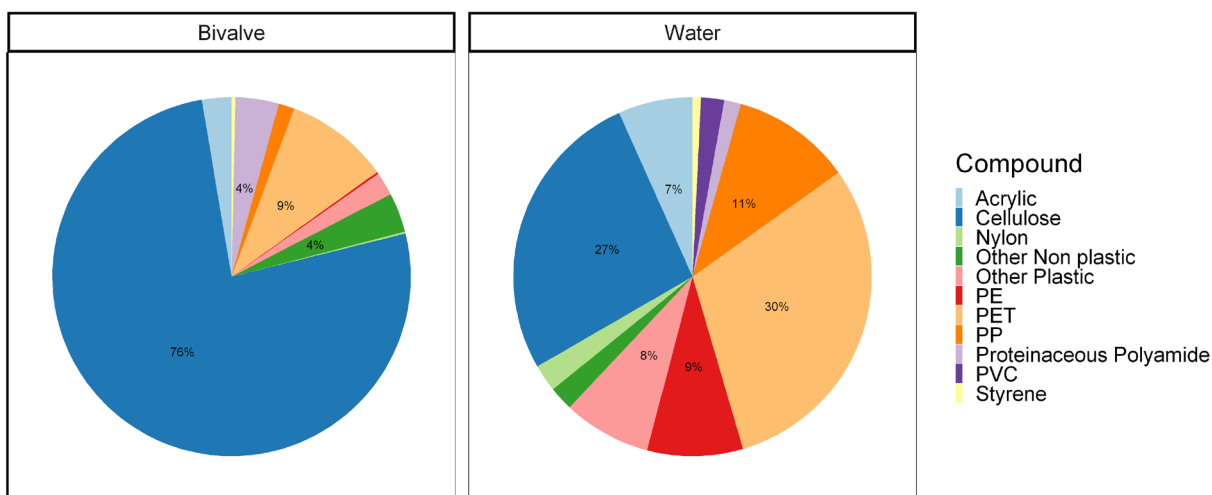


Figure 7. Pie chart of chemical compound categories for visually identified microparticles in bivalve and water samples collected in New Brunswick, Canada (n = 563 bivalve samples, 1054 water samples). Approximately 90% of the bivalve samples sent for FTIR were from freshwater bivalves with the remaining 10% being marine samples. All analyses of water samples were completed on freshwater samples.

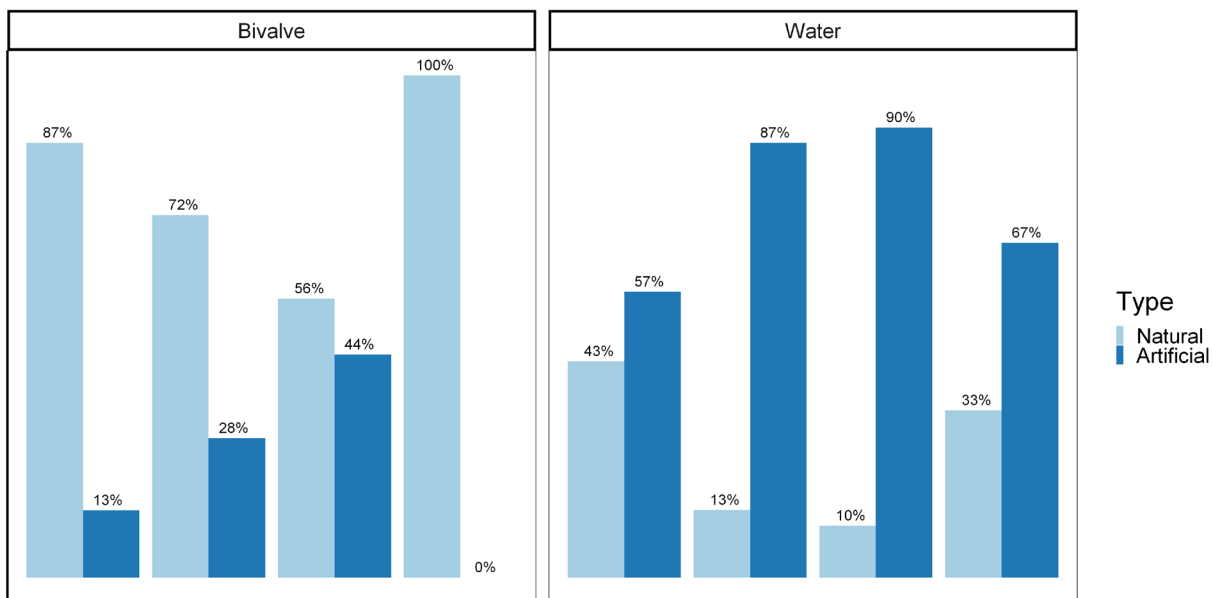


Figure 8. Grouped bar chart of chemical compound categories for visually identified microparticles in bivalve and water samples collected in New Brunswick, Canada (n = 563 bivalve samples, 1054 water samples). Approximately 90% of the bivalve samples sent for FTIR were from freshwater bivalves with the remaining 10% being marine samples. All analyses of water samples were completed on freshwater samples.

Table 2. Results of Kruskal-Wallis tests showing the spatial or species variation in visually identified microparticles, classified as fibre, film, fragment, and sphere, at sites in the Saint John River watershed in New Brunswick, Canada. Chi-sq refers to Kruskal-Wallis Chi-squared values. Bold faced text indicates significance below the chosen critical 0.05 alpha level.

Sample Matrix	Fixed factor	Visual MP Type	Chi-sq	df	p-value
All Bivalves	Waterbody	Total	95.64	5	<2.2E⁻¹⁶
		Fibre	84.329	5	<2.2E⁻¹⁶
		Fragment	71.997	5	3.933E-14
		Film	5.641	5	0.343
		Sphere	NA	NA	NA
SJH Bivalves	Species	Total	7.013	2	0.03
		Fibre	10.45	2	0.005
		Fragment	0.888	2	0.641
		Film	4.543	2	0.103
		Sphere	NA	NA	NA
Sediment	Waterbody	Total	87.283	5	<2.2E⁻¹⁶
		Fibre	82.079	5	3.081E⁻¹⁶
		Fragment	21.782	5	0.001
		Film	6.594	5	0.253
		Sphere	32.552	5	4.618E⁻⁶
Water	Waterbody	Total	63.307	5	2.515E⁻¹²
		Fibre	39.955	5	1.525E⁻⁷
		Fragment	66.411	5	5.71E⁻¹³
		Film	47.289	5	4.96E⁻⁹
		Sphere	59.244	5	1.742E⁻¹¹

4. Discussion

In this project we combined microparticle data previously collected within the Wolastoq/Saint John River watershed, which includes the Saint John River, Tobique River, Oromocto River, Nashwaak River, Kennebecasis River, and Saint John Harbour. We used this data to compare both within and between waterbodies as well as between environmental and biota matrices. We found that bivalve and water samples from Saint John Harbour had higher levels of visually identified microparticles compared to the other waterbodies, whereas sediment samples in Saint John Harbour had the lowest levels of visually identified microparticles compared to other waterbodies. This project has allowed for FTIR analysis of archived microparticle samples (from previous partner projects) to make the distinction between anthropogenic microparticles of natural origin and microplastics, which is a vital step in determining potential pollution sources and predicting environmental fate and monitoring ecosystem impacts. FTIR results found that visually identified microparticles in water samples were more likely to be identified as microplastics, while bivalve samples were more likely to be naturally derived compounds. This collaborative research effort has helped with facilitating the standardization and comparability of microparticle research efforts among Atlantic Canadian research groups.

Bivalves were the only animals included in our statistical analyses due to a lack of weights available for some taxa studied by partner organizations, specifically the plankton and American lobster, which was needed to calculate standardized concentrations of microparticles. While bivalves are susceptible to microparticles given their filtration feeding and role as primary consumers in their environment, it would have been beneficial to incorporate all taxa sampled to explore relationships between taxa. Bivalves are one of the most studied taxa in microparticle research (see review by Prokić et al. 2021), emphasizing the need for additional taxa to be studied. Another review by Bom & Sá (2021) found that the most studied species were mussels, followed by oyster, clams, with

cockles and scallops being the least studied. Marine species are more commonly studied than freshwater species (Bom & Sá 2021, Prokić et al. 2021) with no studies having compared marine and freshwater species. The inclusion of both freshwater and marine species in a single study with comparable concentration units has enabled the comparison of the microparticle loading of species from two different ecosystems. We found marine bivalves in Saint John Harbour had higher concentrations of microparticles than the freshwater mussels, which was consistent with the review by Bom & Sá (2021). Additionally, by comparing bivalve species in the Saint John Harbour samples, we found that *Macoma balthica* had higher concentrations of microparticles compared to the other two bivalve species studied but *Mytilus edulis* had a wide range of microparticles concentrations, which is consistent with other studies (review by Bom & Sá 2021).

By combining data from multiple prior projects, we were able to compare microparticle concentrations across a variety of environmental and biota matrices, which has not been done outside of literature reviews (i.e. Bom & Sá 2021, Prokić et al. 2021). The combination of data from multiple partners enabled us to examine spatial variation in microparticles across both marine and freshwater environments, another comparison that has rarely been studied. Saint John Harbour samples tended to have higher visually identified microparticle concentrations in water and bivalve samples but not sediment samples. In fact, Saint John Harbour samples had the lowest visually identified microparticle concentrations in sediment among all the waterbodies in our study area. Our comparisons between freshwater waterbodies and Saint John Harbour should be taken cautiously given the difference in methodologies between Mount Allison University, which collected almost all freshwater samples, and other partner organizations. However, our comparison of freshwater and marine water samples collected by ACAP also found significant differences in microparticle concentrations between the two types of waterbodies. It should be noted that the sample size for this comparison was extremely small. Microparticles in sediment samples are often

more abundant in densely populated areas, such as harbours and ports (Van Cauwenberghe et al. 2015). The Saint John Harbour sediment samples were not consistent with previous studies, such as Browne et al. (2011) where there was a positive relationship between human population densities and microplastic concentrations. In other water systems, such as the Saint Lawrence River, microparticle concentrations in water and bivalve samples were highest downstream in the river system closer to the Gulf of Saint Lawrence (Rowenczyk et al. 2022). This is consistent with the bivalve and water samples from Saint John Harbour in our study as the Saint John River watershed ends at Saint John Harbour, where microparticle concentrations were higher than the freshwater sites. Within the freshwater sites in the watershed, concentrations of microparticles were not significantly greater downstream of wastewater treatment sites (Labaj et al. 2021, Doucet et al. 2021). In another freshwater study (Robson et al. 2025), sites located downstream of wastewater treatment plants had lower concentrations of microparticles in water and some bivalve tissue types, contrary to what would be expected. There is a clear need for additional research into the distributional patterns of microparticles in relation to human development and other anthropogenic disturbances.

Fourier-transform infrared spectroscopy (FTIR) is a technique used to identify the composition of visually identified microparticles using their vibrational spectrums (Renner et al. 2018, Xu et al. 2019). This is becoming an increasingly important component of microparticle research as visually identified microparticles are being chemically identified as naturally occurring microparticles and not microplastics as originally believed (Comnea-Stancu et al. 2017, Mintenig et al. 2017, Cai et al. 2019, Lenaker et al. 2021, Hunt and Beardy 2022, Baraza and Hasenmueller 2023, Costa et al. 2023). By examining archived visually identified microparticle samples from the partners in our collaboration, we were able to contribute to the consensus that visually identifying microparticles should be supplemented with FTIR to determine the amount of artificial versus natural original

microparticles. Consistent with other studies, we found between 20% and 70% of the visually identified microparticles were composed of artificial compounds (Cai et al. 2019). We found visually identified microparticles were more likely to be accurately identified as microplastics for water samples than for bivalve samples. The difference in accuracy in identifying microplastics from bivalve and water samples demonstrates the need to conduct FTIR analysis on as many visually identified microparticles as possible for a given project, as there was a large difference in accuracy between water and bivalve samples. Bivalve samples would especially benefit from FTIR analysis given the higher incidence of misidentification of microparticles as plastic, especially with fibres. This also demonstrates the potential for different chemical and physical processes to cause naturally derived microparticles to be misidentified as artificial microparticles in different sample matrices.

Project communication, data sharing, and outreach has been recognized as a research priority within ECCO under the Plastic Pollution Science Framework. To promote outreach and education, data compiled and integrated throughout the course of the project will be made available shortly to the public, stakeholders, and research communities through a leading, online, open access data-sharing platform (CDOOS). In addition, we have developed an open-access, interactive map, posted on the Coastal Action (partner organization) website <https://www.coastalaction.org/atlanticecosysteminitiative.html> that provides the user with detailed and engaging visualization of project results.

The project partners have contributed to the development and compilation of public outreach resources directed towards diverse audiences, also available on the project web page. This includes research resources (i.e. field and lab procedures, best practices), educational materials for all ages including presentations, infographics, handouts, and classroom activities as well as resources for businesses (plastic reduction strategies). These resources will be made publicly

available and will provide opportunities for comparability in future research. Workshops were completed where interested groups were invited to learn and share information about project results as well as other relevant research occurring in the region. Interested community and research entities, including those within the project partner networks, have been offered the opportunity for engagement throughout the project process and will be directed to the materials shared through the Coast Action web page to ensure that the public and non-academic stakeholders have access to these data, educational resources and up-to-date best practice methodologies and quality assurance/control protocols.

This project brought together microparticle data from biota, sediment, and water samples collected by multiple partner organizations throughout the Wolastoq /Saint John River watershed to examine the spatial variation in microparticle distribution among different sample matrices and in both marine and freshwater ecosystems. We found that Saint John Harbour samples had higher concentrations of visually identified microparticles compared to the other waterbodies in bivalve and water samples and the lowest concentrations in sediment samples. A portion of visually identified microparticles were assessed using FTIR spectroscopy analysis to identify the chemical composition of the microparticles and found that microparticles in water samples were more likely to be accurately identified as plastic polymers. In contrast, microparticles in bivalve samples were more likely to be naturally derived. Our collaborative initiative demonstrates the importance of combining data across partner organizations as we were able to examine spatial variation in microparticles concentrations that would not have been feasible without partnerships. As part of this project, we conducted outreach to interested organizations and the public, through the sharing of our methods, results and data through workshops, an interactive GIS map, and web page <https://www.coastalaction.org/atlanticecosysteminitiative.html> to provide information on the extent of microparticle pollution in the Wolastoq/Saint John Harbour watershed.

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Supplemental Materials

Table S1. Tukey's test results for generalized linear models showing the spatial or species variation in visually identified microparticles, classified as fibre, film, fragment, and sphere, at sites in the Saint John River watershed in New Brunswick, Canada. Bold faced text indicates significance below the chosen critical 0.05 alpha level. NR = Nashwaak River, KR = Kennebecasis River, SJH = Saint John Harbour, SJR = Saint John River, TR = Tobique River.

Sample Matrix	Factor	Visual MP Type	Comparison	p-value
All bivalves	Waterbody	Total	NR-KR	2.5E-06
			OR-KR	0.281
			SJH-KR	<2E-16
			SJR-KR	5.0E-11
			TR-KR	4.5E-06
			OR-NR	0.139
			SJH-NR	0.999
			SJR-NR	0.209
			TR-NR	0.015
			SJH-OR	0.007
			SJR-OR	0.89
			TR-OR	1
			SJR-SJH	2.7E-07
			TR-SJH	5.9E-14
TR-SJR	0.099			
All bivalves	Waterbody	Fibre	NR-KR	5.1E-06
			OR-KR	0.22
			SJH-KR	<2E-16
			SJR-KR	5.6E-11
			TR-KR	4.7E-06
			OR-NR	0.216
			SJH-NR	0.999
			SJR-NR	0.274
			TR-NR	0.024
			SJH-OR	0.061
			SJR-OR	0.937
			TR-OR	1
			SJR-SJH	0.0002
			TR-SJH	6.9E-10
TR-SJR	0.104			
All bivalves	Waterbody	Fragment	NR-KR	0.671
			OR-KR	0.985
			SJH-KR	2.1E-12
			SJR-KR	0.814
			TR-KR	0.997
			OR-NR	0.586
			SJH-NR	0.254

			SJR-NR	0.926
			TR-NR	0.518
			SJH-OR	0.0003
			SJR-OR	0.822
			TR-OR	0.997
			SJR-SJH	4.1E-11
			TR-SJH	<2E-16
			TR-SJR	0.368
SJH Bivalves	Species	Total	Macoma clam – Blue mussel	0.487
			Soft shelled clam – Blue mussel	0.059
			Soft shelled clam – Macoma clam	0.041
SJH Bivalves	Species	Fibre	Macoma clam – Blue mussel	0.133
			Soft shelled clam – Blue mussel	0.027
			Soft shelled clam – Macoma clam	0.004
Sediment	Waterbody	Total	NR-KR	0.247
			OR-KR	0.892
			SJH-KR	2.0E-11
			SJR-KR	0.999
			TR-KR	0.805
			OR-NR	0.09
			SJH-NR	7.8E-13
			SJR-NR	0.068
			TR-NR	0.022
			SJH-OR	0.006
			SJR-OR	0.939
			TR-OR	1
			SJR-SJH	7.0E-14
			TR-SJH	1.5E-06
			TR-SJR	0.863
Sediment	Waterbody	Fibre	NR-KR	0.224
			OR-KR	0.862
			SJH-KR	2.4E-10
			SJR-KR	0.993
			TR-KR	0.956
			OR-NR	0.069
			SJH-NR	3.4E-12
			SJR-NR	0.040
			TR-NR	0.05
			SJH-OR	0.020
			SJR-OR	0.950

			TR-OR	0.996
			SJR-SJH	7.6E-14
			TR-SJH	9.8E-07
			TR-SJR	0.997
Sediment	Waterbody	Fragment	NR-KR	0.444
			OR-KR	0.862
			SJH-KR	0.386
			SJR-KR	0.055
			TR-KR	0.0001
			OR-NR	0.999
			SJH-NR	0.983
			SJR-NR	1
			TR-NR	0.313
			SJH-OR	1
			SJR-OR	0.993
			TR-OR	0.228
			SJR-SJH	0.666
			TR-SJH	0.002
			TR-SJR	0.054
Sediment	Waterbody	Sphere	NR-KR	1
			OR-KR	2.0E-05
			SJH-KR	0.997
			SJR-KR	0.08
			TR-KR	0.782
			OR-NR	0.0002
			SJH-NR	0.999
			SJR-NR	0.318
			TR-NR	0.888
			SJH-OR	6.2E-06
			SJR-OR	0.003
			TR-OR	0.002
			SJR-SJH	0.011
			TR-SJH	0.85
			TR-SJR	0.927
Water	Waterbody	Total	NR-KR	0.001
			OR-KR	0.971
			SJH-KR	0.005
			SJR-KR	0.191
			TR-KR	7.3E-05
			OR-NR	0.123
			SJH-NR	1.0E-07
			SJR-NR	1.9E-10
			TR-NR	0.638
			SJH-OR	0.002
			SJR-OR	0.467
			TR-OR	0.018

			SJR-SJH	0.078
			TR-SJH	8.2E-09
			TR-SJR	1.2E-10
Water	Waterbody	Fibre	NR-KR	0.01
			OR-KR	0.599
			SJH-KR	0.13
			SJR-KR	0.747
			TR-KR	0.0005
			OR-NR	0.921
			SJH-NR	0.0002
			SJR-NR	0.038
			TR-NR	0.596
			SJH-OR	0.004
			SJR-OR	0.977
			TR-OR	0.361
			SJR-SJH	0.001
			TR-SJH	1.2E-05
			TR-SJR	0.001
Water	Waterbody	Fragment	NR-KR	0.006
			OR-KR	0.999
			SJH-KR	0.0002
			SJR-KR	0.127
			TR-KR	0.019
			OR-NR	0.014
			SJH-NR	6.0E-09
			SJR-NR	7.0E-14
			TR-NR	1
			SJH-OR	0.004
			SJR-OR	0.451
			TR-OR	0.029
			SJR-SJH	0.03
			TR-SJH	3.5E-08
			TR-SJR	1.7E-10
Water	Waterbody	Film	NR-KR	0.274
			OR-KR	0.375
			SJH-KR	0.561
			SJR-KR	0.004
			TR-KR	1
			OR-NR	0.002
			SJH-NR	0.007
			SJR-NR	1.4E-13
			TR-NR	0.1
			SJH-OR	0.954
			SJR-OR	0.958
			TR-OR	0.416
			SJR-SJH	0.859

			TR-SJH	0.656
			TR-SJR	0.002
Water	Waterbody	Sphere	NR-KR	0.0009
			OR-KR	0.0002
			SJH-KR	0.999
			SJR-KR	8.6E-09
			TR-KR	0.568
			OR-NR	0.477
			SJH-NR	0.005
			SJR-NR	1.6E-05
			TR-NR	0.108
			SJH-OR	0.0005
			SJR-OR	0.846
			TR-OR	0.012
			SJR-SJH	8.6E-06
			TR-SJH	0.571
			TR-SJR	6.7E-09