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**Microlitter in the water, sediments, and mussels of the Saint John River (Wolastoq) watershed,
Atlantic Canada**

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0.0 Abstract

Microlitter is a widespread contaminant with implications for aquatic health; however, knowledge of its distribution in freshwaters is limited. We examined microlitter in surface water, sediment, and mussels within the Saint John River and four major tributaries. Microlitter was present at all 89 sites, with concentrations in water equivalent to other Canadian watersheds. Microlitter concentrations likely reflected differences in land use and development. Comparisons between rivers generally revealed distinct water microlitter particle compositions, suggesting that input sources were specific to each river, while microlitter in sediments and mussels was largely homogenous among rivers. There was little similarity in concentration and composition of microlitter between the three matrices sampled. Microlitter in sediments and mussels may not reliably track concentration and composition in aquatic environments. Microlitter concentration was not significantly greater downstream of wastewater treatment plants. Airborne microlitter transportation was evident, as remote sites in two tributary rivers demonstrated the highest concentrations of microlitter. Our data highlights the prevalence of microlitter in rural rivers, underscoring the ubiquity of this emerging contaminant and its integration into food webs.

1.0 Introduction

Microlitter has gained global attention as a nearly ubiquitous environmental contaminant; however, at present, the fate, transport, and extent of microlitter in freshwaters—especially within rural regions—remains poorly understood (Hoellein and Rochman, 2021), despite these regions comprising large portions of the Canadian landscape. Microlitter encompasses the suite of particles in the environment < 5 mm in size (Talvitie et al., 2017), which includes polymers and natural materials that have undergone human processing. Plastic microlitter, often referred to as microplastic, may be created through intentional production or weathering of plastics in the environment (Alimi et al., 2018), whereas microlitter comprised of natural materials is frequently generated through wear and laundering of textiles (Stanton et al., 2019). Although the majority of microlitter research to date has focused on microplastics, natural materials (e.g., cotton, wool, silk, etc.) constitute a large proportion of microlitter released to the environment, with up to 94% of textile-based microfibers comprised of natural materials (Miller et al., 2017; Stanton et al., 2019).

Due to its small size, microlitter is easily transported, and enters ecosystems through a variety of inputs, including runoff from urban (Dris et al., 2018, Grbić et al., 2020) and rural (Crossman et al., 2020; Nizzetto et al., 2015) areas, as well as by atmospheric deposition (Brahney et al., 2020; Dris et al., 2016). However, wastewater treatment plants (WWTP) have frequently been identified as key point sources of microlitter into aquatic systems (Mason et al., 2016; Murphy et al., 2016; Talvitie et al., 2017; Vassilenko et al., 2019), as large quantities of microfibers are released into municipal wastewater through laundering of textiles (Belzagui et al., 2019; Galvão et al., 2020; Vassilenko et al., 2019). Although WWTP

often have microlitter removal rates of > 98% (Murphy et al., 2016), millions of particles are released in the effluent of WWTP daily (Mason et al., 2016; Vassilenko et al., 2019).

Microplastics are persistent in the environment, as they do not easily biodegrade, and instead are weathered and broken down into progressively smaller fragments (Geyer et al., 2017). Conversely, microlitter comprised of natural materials breaks down over time, however it is frequently treated with chemicals that slow degradation (Li et al., 2010; Xu et al., 2018). In aquatic environments, microlitter is often mistaken for food and may be taken up by organisms during feeding (Au et al., 2015; Rebelein et al., 2021; Watts et al., 2015; Woods et al., 2018), where it can create digestive obstructions and a false sense of satiation, resulting in reduced growth, activity, and energy levels (Cole et al., 2013; Wright et al., 2013). In addition, microlitter comprised of plastic may include toxic polymers, and often contains toxic additives such as plasticizers and stabilizers (Rochman et al., 2013; Rochman et al., 2019), whereas microlitter comprised of natural fibers may also contain toxic preservatives, resins, dyes, and flame retardants (Correia et al., 1994; Remy et al., 2015; Schreder and La Guardia, 2014; Xu et al., 2018). Furthermore, microlitter can sorb contaminants such as PCBs and POPs from the environment, where they may then bioaccumulate in food webs (Germanov et al., 2018; Ladewig et al., 2015; Rios-Mendoza and Jones, 2015; Rios-Mendoza et al., 2007).

To date, there remains a knowledge gap regarding microlitter in Canadian freshwaters (Anderson et al., 2016; Provencher et al., 2019). Freshwater systems are vulnerable to the impacts of microlitter, and serve as important conduits for microlitter to move from the landscape to marine ecosystems (Hoellein and Rochman, 2021; Lebreton et al., 2017; Miller et al., 2017). Studies of Canadian freshwaters have frequently focused on urban regions where widespread alterations to the landscape (e.g., land clearance, construction of roads / impervious surfaces, etc.) have occurred (e.g., Castañeda et al., 2014; Corcoran et al., 2015; Crew et al., 2020; Vermaire et al., 2017). Moreover, there remains uncertainty about microlitter distribution in freshwaters of remote and rural regions, which comprise most of the Canadian landscape. Here, we apply a large-scale watershed monitoring design to understand baseline measures of microlitter distribution within the Saint John River (Wolastoq; Maliseet: *Beautiful River*), New Brunswick, one of northeastern North America's largest and least populated watersheds, as well as four of its major tributaries. We examined microlitter concentration in surface water, riverine sediment, and freshwater mussels (primarily the freshwater pearl mussel - *Margaritifera margaritifera* L.) to answer the following questions: 1) What is the distribution of microlitter in the Saint John River and its tributaries, and how does it relate to point sources (i.e., WWTP) and diffuse input sources? We anticipated higher microlitter concentrations in the Saint John River compared to the tributaries, due to greater population and land disturbance. Furthermore, we expected to observe greater microlitter concentrations downstream from wastewater treatment plant outflows, and from upstream to downstream due to accumulation in the rivers. 2) Is there a relationship between microlitter concentration and composition in water, sediment, and mussels in these rivers? We hypothesized that similarities in microlitter composition and concentration between water, sediments, and mussel tissue exist at each site. Our watershed-scale study will provide the first data of its kind from Atlantic Canada and will contribute important baseline knowledge of microlitter in rural freshwater environments.

2.0 Methods

2.1 Site Selection and Study Design

Sample collection for the study took place between June to September 2019 and from July to August 2020 for the triplicate (quality control) field sampling. Whenever possible, sample collection took place at least two to three days following rain, to avoid high flows. The Saint John River and four major tributary rivers were sampled (Figure 1). Sites along the rivers were selected to capture a gradient of human impact, including areas with known point sources of microlitter (i.e., WWTP), areas with potential diffuse microlitter input (e.g., near human development), and intermediate sites located at regular intervals along the rivers (Figure 1). To assess whether WWTP were contributing to surface water, sediment, and mussel microlitter particle loads, sampling was conducted both upstream and downstream from the WWTP discharge points. On the tributary rivers (Kennebecasis, Nashwaak, Oromocto, and Tobique), 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.

2.2 Description of Study Area

The 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, New Brunswick. The watershed of the Saint John River has an area of > 55,000 km², of which 51% is located within New Brunswick (Canadian Rivers Institute, 2011). 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 (Canadian Rivers Institute, 2011), 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 river from Grand Falls to Gagetown.

Within the Saint John River watershed, sampling also took place at four major tributaries: the Kennebecasis, Nashwaak, Tobique, and Oromocto rivers. Each of the tributaries are largely rural, with forested area comprising between 78.6% (Kennebecasis) to 94.6% (Tobique) of the watershed areas (Figure 2). Among the tributaries, the Kennebecasis watershed is the smallest (~1,400 km²), contains the most agricultural and settled land (8.9% and 4.1%, respectively), and also has the largest population, with the towns of Sussex and Hampton, and the Village of Norton together consisting of ~10,000 residents (Figure 2). In contrast, the Nashwaak (watershed area ~1,700 km²), Oromocto (watershed area ~2,500 km²), and Tobique (watershed area ~4,300 km²) rivers each have ~1% agricultural area and less than 2% settled area in their watersheds, and their largest communities have less than ~1,000 residents (Figure 2).

2.3 Field Methods

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 visually estimated plume of the effluent pipe whenever possible. Prior to sample collection, a 1-minute average water flow rate was determined with a Höntzsch FlowTherm NT flow meter. When flow rate was sufficient for sampling with the manta net (≥ 0.1 m/s), 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 m/s

(avg. 0.64 m/s) on the Kennebecasis River, 0.18 – 1.27 m/s (avg. 0.65 m/s) on the Nashwaak River, 0.29 – 1.01 m/s (avg. 0.64 m/s) on the Tobique River, < 0.1 – 0.63 m/s (avg. 0.49 m/s) on the Oromocto River, and < 0.1 – 0.52 m/s (avg. 0.31 m/s) on the Saint John River. When the site had sufficient water depth, the manta net was held ~5 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 m/s), an electric pump, elevated ~30 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 ~4,800 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- μ m brass sieve, where it was further rinsed with river water. Material retained in the 90- μ m sieve was rinsed into a sterile Whirl-Pak® bag.

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 between sites; however, whenever possible, sediment samples were collected from depositional areas. Sediments were placed into Whirl-Pak® bags.

Mussel collection methods are described in detail in Doucet et al. (2021). Briefly, when present at a site, mussels were collected near the water and sediment sampling locations (Fisheries and Oceans Canada Licence # 354349). Five mussels of the same species (primarily *M. margaritifera*) and of similar size were collected from within an ~5-m radius at each site. The largest mussels were preferentially targeted at each site to ensure sufficient tissue for digestion. Mussels were placed in individual Whirl-Pak® bags and dispatched by freezing at -20°C, preserving the integrity of their tissues without breaking down microlitter particles (Courtene-Jones et al., 2017). Mussels were stored in a cooler with ice for transportation to the laboratory.

2.4 Quality Control – Field

During field work, all members of the field team wore natural fiber clothing, minimizing the risk of possible contamination of samples with plastic microfibers 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.

Triplicate surface water samples were collected in July and August 2020 to assess the variation in microlitter between repeat samples at ten sites on the Nashwaak River. These samples were collected from the same location, and an identical amount of water was sampled for each. As these sites were collected in 2020, they do not form a part of the main analyses and are used for quality assessment purposes.

2.5 Laboratory Methods

Surface water samples were poured from their Whirl-Pak® bags into a clean 90- μ m brass sieve, and the interior of the bag was further rinsed with deionized water. Material retained in the sieve was rinsed with deionized 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 sieved through the 90-µm mesh and backwashed into a clean beaker to await visual examination.

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 to ~60 °C for 2–4 hours on a hot plate. The samples were then immersed in an ultrasonic bath for 15 seconds, poured onto a 90-µm sieve and rinsed with deionized 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 (modified from Masura et al., 2015). The sample was again immersed in the ultrasonic bath, sieved and rinsed on the 90-µm mesh, 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% NaCl solution (density: 1.15 g/mL) was added to the samples (Masura et al., 2015), and they were immersed into 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 were also retained in the 600-mL beakers. Both the floating and dense fractions were examined, with the values combined for each sample.

Laboratory processing of mussel tissue is described in detail by Doucet et al. (2021). Briefly, mussels were defrosted, opened, and their tissue was weighed. Tissue was treated with 10% KOH solution on a 60°C hotplate for 24 hours (Dehaut et al., 2016). Following the initial digestion, the beaker was submerged in an ultrasonic bath for ~3 minutes to break up the remaining material. The solution was then rinsed with deionized water on a 90-µm sieve, and the material retained on the sieve was returned to the beaker. The digestion, ultrasonic bath, and sieving were repeated a second time for each sample to ensure that all tissue was digested.

2.6 Visual 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 6.3–50x magnification. The aliquot in the petri dish was scanned multiple times: first, the bottom layer (sinking material) of the aliquot was scanned in transects, followed by the surface layer (floating material). The aliquot was then stirred with forceps, and this procedure was repeated a second time. After the second scan, the aliquot was poured into a clean beaker and retained. This was repeated until the entire sample was scanned. The material retained in the clean beaker was then poured back into the petri dish in aliquots and scanned a third and final time, with bottom and surface passes taking place, before it was discarded. Microlitter particles were recognized and distinguished from natural materials based on colour, shape, and texture (e.g., Lusher et al., 2020; Masura et al., 2015; Rochman et al., 2019). For example, particles with blue colouration were deemed to be anthropogenic due to the lack of blue pigment in nature (Newsome et al., 2014). Particles that broke or frayed during manipulation with the forceps, or those that had perfectly straight fibers, were deemed to be of natural origin (Doucet et al., 2021; Norén, 2008). When a microlitter particle was found, it was removed with forceps and mounted on a clean glass slide with double-sided tape to be archived. All microlitter particles removed were enumerated by colour and type (e.g., fiber, film, etc.)

following Rochman et al. (2019). Microlitter particles were generally within the size range of 90–5,000 μm .

2.7 Quality Control – Laboratory

All laboratory analyses used clean glass and metal implements. Prior to laboratory processing, all laboratory implements were pre-cleaned with Sparkleen® and rinsed three times with deionized water. After cleaning, glassware was covered with aluminum foil. Personnel wore white cotton lab coats to minimize the risk of sample contamination by fibers shed from clothing (Dris et al., 2016). To account for potential contamination of samples in the processing and visual examination steps, laboratory blanks were run with each batch of samples processed (Rochman et al., 2019). Blanks consisted of 600 mL beakers filled with ~150 mL of deionized water. Blanks were subject to the same processing steps (excluding the treatments with sodium hexametaphosphate, H_2O_2 , and KOH) for the same duration of time as the samples. Visual examination of all samples was timed, and the corresponding blank was exposed for the same average time as the associated samples during visual examination. Exposure time varied based on the matrix being examined as well as the amount of microlitter found, but was generally between 1 and 3 hours. All microlitter particles found in the blank were categorically subtracted by colour and type from the co-processed samples (Rochman et al., 2019). 36 blanks were processed for mussels (5 mussels / blank), 22 blanks were processed for 87 water samples (avg. ~4 samples / blank), and 15 blanks were processed for 83 sediment samples (avg. ~5.5 samples / blank).

2.8 Data Analysis and Statistical Methods

Total counts of microlitter were standardized to sampling units for surface water (particles / m^3 filtered), sediment (particles / g wet sediment) and mussels (particles / g soft tissue). Prior to statistical analysis, one outlier site (Oromocto West WWTP upstream water) was removed, as it was > 10x higher than the average of the other sites on the river (Figure S1d), and was comprised predominantly of red (66.4%) and blue (13.2%) fibers. Nested analyses of variance (ANOVAs) were carried out on surface water, sediment, and mussel data to determine whether rivers differed in their microlitter concentrations and whether sites within the rivers differed due to point source inputs from WWTP. For these analyses, river (i.e., Kennebecasis, Nashwaak, Tobique, Oromocto, and Saint John) was the primary factor, while site type (i.e., WWTP Upstream, WWTP Downstream, and Intermediate) was the nested secondary factor. Two additional nested ANOVAs were carried out each for water, sediment, and mussels: excluding Intermediate sites (testing only WWTP Upstream and WWTP Downstream sites), and excluding fibers and fiber bundles from microlitter data (as these highly abundant forms of microlitter may have obscured more nuanced trends in WWTP input). The results and their interpretation were similar to those of the nested ANOVAs with all site types and microlitter types included, and thus are not presented. Mann-Kendall trend tests were used to determine whether trends in surface water, sediment, and mussel microlitter concentrations were present from upstream to downstream in each river.

To determine how sites differed with respect to microlitter particle type and colour (i.e., composition), ordination with principal component analysis (PCA: covariance matrix) was carried out separately for surface water, sediment, and mussel microlitter data. Prior to ordination, total (i.e., non-standardized) microlitter particle counts were converted to percentage relative abundance for each site. The data were then square-root transformed to down-weight the influence of dominant microlitter particle types and colours. Arrows representing the ten microlitter particle types with the highest

absolute axis 1 and 2 scores were plotted for each PCA. Pairwise permutational multivariate analysis of variance (PERMANOVA, based on Bray-Curtis dissimilarity index and Bonferroni correction) was used to assess whether the microlitter particle composition differed between rivers for surface water, sediment, and mussels. Pairwise PERMANOVA was also used to determine whether the microlitter particle composition differed between surface water, sediment, and mussels in each of the five rivers. PERMANOVA is a non-parametric method for testing multivariate differences of pre-defined groups (Anderson, 2017), and was run using the “vegan” (Oksanen et al., 2020) and “pairwiseAdonis” (Martinez Arbizu, 2020) packages for R (version 4.0.5; R Core Team, 2021). To determine whether relationships were present between microlitter concentrations in surface water, sediment, and mussel tissue, Pearson correlations were carried out between each of these variables in the five rivers. Significance was accepted at $p \leq 0.05$.

3.0 Results

3.1 Distribution of microlitter in rivers and among sites

Microlitter was present in surface water, sediments, and mussels from all sites on the Saint John River and the four tributaries (Figure S1). Among the 87 surface water samples, mean microlitter concentrations were highest in the Saint John River (23.3 ± 23.9 microlitter particles / m^3), followed by the Oromocto (9.7 ± 11.8 p / m^3), Kennebecasis (8.4 ± 6.5 p / m^3), Nashwaak (4.9 ± 7.1 p / m^3), and Tobique (3.2 ± 3.2 p / m^3) rivers (Figure 3a). The nested ANOVA identified a significant difference in water microlitter concentration between rivers ($p < 0.01$, $F = 5.9$, d.f. = 4), but not between site types ($p = 0.29$, $F = 1.2$, d.f. = 10). A pairwise t-test (p -value Bonferroni corrected) identified a significant difference in microlitter concentration between the Saint John and Tobique rivers ($p < 0.01$), and a marginally significant difference between the Saint John and Nashwaak rivers ($p = 0.05$). Microlitter within surface water did not display a positive trend in concentration from upstream to downstream in any river (Table S1).

Within the 83 sediment samples, mean microlitter concentrations were highest in the Nashwaak River (3.3 ± 1.9 microlitter particles / gram wet sediment), followed by the Saint John (2.2 ± 2.5 p / gram wet sediment), Kennebecasis (1.5 ± 1.0 p / g wet sediment), Tobique (1.2 ± 1.0 p / g wet sediment), and Oromocto (1.2 ± 0.7 p / g wet sediment) rivers (Figure 3b). The nested ANOVA did not identify a significant difference between rivers ($p = 0.15$, $F = 1.8$, d.f. = 4) or between site types ($p = 1.00$, $F = 0.1$, d.f. = 10). Sediment microlitter displayed a significant negative trend from upstream to downstream only in the Saint John River ($\tau = -0.35$, $p < 0.01$; Table S1).

Microlitter was found in 95% (171 out of 180) of the mussels examined (after blank correction). Within the mussels, the mean microlitter concentration was highest in the Nashwaak River (1.6 ± 1.3 microlitter particles / g soft tissue), followed by the Saint John (1.3 ± 1.7 p / g soft tissue), Tobique (1.1 ± 2.0 p / g soft tissue), Oromocto (0.5 ± 0.2 p / g soft tissue), and Kennebecasis (0.2 ± 0.2 p / g soft tissue) rivers (Figure 3c). We note, however, that mussels were only collected at one site on the Nashwaak and Oromocto rivers, and these values thus do not provide a realistic assessment of mussel microlitter concentrations within these rivers. The nested ANOVA identified a significant difference in microlitter concentration within mussels at the river level ($p < 0.01$, $F = 5.7$, d.f. = 2), but not between site types ($p = 1.0$, $F = 0.1$, d.f. = 5). A pairwise t-test (p -value Bonferroni corrected) conducted between rivers determined that there was a significant difference in mussel microlitter concentration between the Tobique and Kennebecasis rivers ($p = 0.01$) and the Saint John and Kennebecasis rivers ($p < 0.01$). No

upstream to downstream trends were present in mussel microlitter concentrations in any of the rivers (Table S1).

Each of the rivers displayed distinct compositions of surface water microlitter particle types and colours (Figure 4). The surface water microlitter particle composition (Figure 4a) differed significantly ($p_{\text{adj}} = 0.01$) between all rivers, except the Nashwaak and Tobique ($p_{\text{adj}} = 0.84$, $r^2 = 0.07$, $F = 2.1$), Tobique and Oromocto ($p_{\text{adj}} = 0.24$, $r^2 = 0.11$, $F = 2.9$), and Oromocto and Saint John ($p_{\text{adj}} = 1$, $r^2 = 0.02$, $F = 1.2$), as assessed by pairwise PERMANOVA (Table S2a). Blue fibers were the most common microlitter particles (43.8%) found in the surface water samples, followed by black films (21.1%), black fibers (9.5%), black fragments (8.9%), and black foams (4.7%). In contrast to the surface water microlitter, sediment-based microlitter particles (Figure 4b) were much more variable between sites, with no distinct river-specific trends identified by pairwise PERMANOVA (Table S2b). Black fibers were the most common sediment-based microlitter particle (31.3%), followed by blue fibers (29.9%), clear fibers (23.9%), red fibers (5.5%), and green fibers (1.7%). Mussel microlitter composition (Figure 4c) differed significantly only between the Kennebecasis and Saint John rivers ($p_{\text{adj}} < 0.01$, $r^2 = 0.23$, $F = 5.5$) as assessed by pairwise PERMANOVA (Table S2c). As mussels were found at only one site on the Nashwaak and Oromocto rivers, their microlitter particle composition could not be accurately compared in these systems. Microlitter particles present in mussels were almost exclusively fibers, with blue fibers being most common (42.3%), followed by clear (26.0%), black (13.3%), red (12.2%) and green fibers (1.8%).

3.2 Relationship of microlitter particles in surface water, sediment, and mussels

A significant positive correlation was noted between surface water and mussel microlitter concentrations in the Tobique River ($p < 0.01$, $r = 0.71$, $n = 14$). No significant correlations were noted between water, sediment, or mussel microlitter concentration in any of the other rivers. The microlitter composition differed significantly between the surface water and sediments in each of the rivers (all $p < 0.01$; Table S3), as identified by PERMANOVA. Surface water and mussel microlitter composition differed significantly ($p < 0.01$) in the Kennebecasis, Tobique, and Saint John rivers, whereas sediment and mussel microlitter composition differed significantly only in the Kennebecasis ($p = 0.02$) and Saint John ($p < 0.01$) rivers (Table S3). As mussels were only found at a single site in the Nashwaak and Oromocto rivers, the mussel microlitter particle composition was not compared to water and sediments in these systems.

3.3 Triplicate water sampling

The range between samples of surface water at each of the ten Nashwaak River sites varied between 0.8 p / m^3 and 18.2 p / m^3 (Figure 5). The average range between samples was 6.6 p / m^3 (Figure 5).

4.0 Discussion

4.1 Distribution of microlitter in the Saint John River and tributaries

Microlitter was recovered from all surface water and sediment samples and 95% of mussels, highlighting the widespread nature of this emerging contaminant, even in a largely rural Atlantic Canadian watershed with ~500,000 people. Although the difference was not significant, microlitter concentrations in water were, on average, higher in the Saint John River than in the tributary rivers,

likely reflecting the greater population and settlement, intensity of land use, and large catchment size of the Saint John River relative to the tributaries.

The concentration of microlitter in the water of these rivers was similar to previously studied Canadian rivers, despite the low population and low level of watershed development around the Saint John River and its tributaries. For example, an average of 26.2 ± 18.4 p / m³ was recovered from the North Saskatchewan River in Edmonton, Alberta (Bujaczek et al., 2021), 12–16 p / m³ in the Saint Lawrence River (Ontario / Quebec; Crew et al., 2020), 5.3 p / m³ in the Red, Assiniboine, and Nelson rivers (Manitoba; Warrack et al., 2017), 4.2 p / m³ in 29 Great Lakes tributary rivers (USA; Baldwin et al., 2016), and 1.35 p / m³ in the Ottawa River (Ontario; Vermaire et al., 2017). Nonetheless, other eastern North American rivers have reported concentrations much higher, including the Hudson River (New York, USA) with concentrations of 980 p / m³, and several urban rivers near Toronto, Ontario, that averaged $15,400 \pm 7,900$ p / m³ (Grbić et al., 2020). Similar to our findings in the Saint John River and tributaries, many of these studies recognized microfibers as the most commonly recovered microlitter particle type (e.g., Baldwin et al., 2016; Bujaczek et al., 2021; Grbić et al., 2020; Vermaire et al., 2017; Warrack et al., 2017). Importantly, each of these rivers had populations of roughly one million or more people (compared to ~500,000 for the Saint John River watershed), and many have considerably more land development in their watersheds. We note, however, that these studies may not be directly comparable, due to differences in sampling apparatus, mesh and sieve size used, and processing methods. Furthermore, many of these studies focus solely on microplastics, rather than microlitter as a whole, thus their total microlitter concentration may be substantially higher when also considering microlitter comprised of natural materials. The significant differences in water microlitter composition between the rivers suggests that the main sources of microlitter are largely distinct and specific to each watershed, rather than from a regional source.

Unexpectedly, we found that the highest water microlitter concentrations in the Tobique and Nashwaak rivers occurred at the remote and relatively undisturbed “background” sites, suggesting the importance of diffuse sources of microlitter into these systems. One important source of microlitter to these remote sites may be aerial transportation and deposition, which can move microlitter up to ~100 km (Allen et al., 2019) from its sources (possibly even further), and deposit it on the landscape through wet and dry depositional processes. Aerial transport has been identified as an important source of microlitter both in urban and sub-urban environments (Cai et al., 2017; Dris et al., 2016; Dris et al., 2017; Wright et al., 2020), as well as remote sites in western North America (Brahney et al., 2020), Europe (Allen et al., 2019), and the Arctic (Bergmann et al., 2019). We suggest that—especially in their more remote stretches—aerial transportation and deposition may play a key role in microlitter transportation to these rural systems.

Sediment microlitter concentration was variable among the rivers, and unlike water microlitter concentration, does not appear to relate to land use in the watershed. Flow dynamics are an important determinant of microlitter transfer from water to sediments (Castañeda et al., 2014; Hoellein et al., 2019; Tibbetts et al., 2018; Vaughan et al., 2017), and it is likely that the sediment microlitter concentrations in the rivers are strongly influenced by patterns of flow and the depositional environment. The flow velocity varied between study rivers as well as between sites, with a faster average flow in the tributaries compared to the Saint John. However, we noted no apparent relationship between water flow velocity and sediment microlitter concentration. Microlitter movement in river environments can also vary depending on material, shape, and degree of biofouling, with pellets

travelling further than fragments and fibers, and the buildup of biofilm generally reducing travel distance (Hoellein et al., 2019). The tendency for microfibers to fall out of the water quicker than other types of microlitter may explain their abundance within our sediment samples (comprising ~94% of the sediment microlitter found), as well as the relative uniformity of the sediment microlitter composition compared to that in the water. Transfer of microlitter between water and sediments may be responsible for the lack of an upstream to downstream trend in water microlitter concentrations observed in these rivers, as well as others in North America (e.g., Bujaczek et al., 2021; Miller et al., 2017). Given that the depositional environment in rivers can vary with morphology, weather events, and season, river sediments likely act only as temporary sinks, collecting microlitter during periods of calm, slower water flow and releasing it during periods of faster, turbulent flow conditions.

Mussel microlitter concentrations were not related to watershed population or land use, but rather reflected differences in mussel size between rivers. Previous research on mussels from the Kennebecasis and Tobique rivers, using a subset of the present dataset, revealed that smaller mussels had greater microlitter concentration compared to larger mussels, likely due to differences in metabolic demands, filtration rates, and gill surface area (Doucet et al., 2021). This relationship continued to apply to mussels from the Saint John River, which had both soft tissue mass and microlitter concentrations similar to mussels in the Tobique River, both of which differed significantly from the much larger mussels with lower microlitter concentrations in the Kennebecasis River. Mussel microlitter concentrations in the present study were comparable to those from much more impacted systems elsewhere; for example, 0.4–5 p / individual were found in the Yangtze River, China (Su et al., 2018), and 4–142 p / individual were found in the Hölje River, Sweden (Berglund et al., 2019). It is important to note, however, that as mussels are living organisms capable of selective particle uptake and rejection of unwanted particles as pseudofeces, mussel microlitter concentration likely does not reflect environmental concentration (Ward et al., 2019). Nevertheless, the widespread presence of microlitter within mussel tissues in these rivers is concerning, given their importance as a common prey item for several aquatic and terrestrial species (Martel et al., 2010).

Wastewater treatment plants have previously been identified as important point source contributors of microlitter to aquatic systems (Murphy et al., 2016; Talvitie et al., 2017; Vassilenko et al., 2019), and we expected to observe greater concentrations of microlitter at sites located downstream of WWTP – a trend that has been observed in other rivers across eastern Canada and the United States (e.g., Estahbanati and Fahrenfeld, 2016; McCormick et al., 2014; Vermaire et al., 2017). However, our results revealed no statistically significant trends in microlitter concentration related to WWTP outflows, a result that has also been noted in several other rivers across North America (e.g., Bujaczek et al., 2020; Crew et al., 2020; Hoellein et al., 2017). The majority of the WWTP examined in this study are small, servicing communities of several thousand residents or less, thus their contribution of microlitter is likely much lower than the large-scale urban WWTP that are the focus of many microlitter studies. Furthermore, differences in WWTP treatment processes and their effectiveness may have resulted in different levels of microlitter being released in effluents (e.g., Talvitie et al., 2017), while site-specific flow dynamics may have influenced the amount of microlitter captured at each location (Bujaczek et al., 2021; Crew et al., 2020).

4.2 Relationship between microlitter concentration and composition among environmental matrices

There was no relationship in microlitter concentration or composition between surface water, sediment, and mussels in most of our rivers, with the exception of the Tobique River, where surface water and mussel microlitter concentrations were correlated. Furthermore, Tobique River sediment and mussel microlitter particle composition did not differ significantly. We hypothesized that the concentration and composition of microlitter would be similar among environmental matrices at sites, as microlitter must first move through the water before being incorporated into sediments or taken up by mussels. However, microlitter in sediments and mussels is influenced by additional factors that can alter their microlitter particle concentration and composition relative to the water, complicating their use as environmental indicators in river environments.

Sedimentary microlitter is likely influenced to a great extent by flow dynamics and the presence of depositional environments (Corcoran et al., 2015; Hoellein et al., 2019; Tibbetts et al., 2018; Vaughan et al., 2017). At sites with faster water flow, there may be little transfer of microlitter from water to sediments, or if deposition occurs it may only be temporary, while a greater amount of microlitter may be incorporated into the sediments in slower environments, irrespective of ambient water concentrations. As flow conditions vary as a result of precipitation events and seasonal changes, sediment-based microlitter is likely in a constant state of flux within river environments. We suggest that sediment-based measurements of microlitter are best suited for use in lower-energy lentic environments that experience minimal flow, rather than lotic environments, where flow can vary between sites and across time.

Mussels were selected for this study, as their filter feeding constantly exposes them to materials within the water and they are largely sessile. Furthermore, as an important food source for many aquatic and terrestrial organisms (Martel et al., 2010), mussels represent a route for microlitter to enter food webs. However, microlitter within mussels is not at equilibrium with that in the ambient water, as mussels are capable of expelling unwanted materials from their body as pseudofeces (Ward et al., 2019). Additionally, mussel microlitter concentration appears to vary with organism size, as greater concentrations have been observed in smaller mussels compared to their larger counterparts (Doucet et al., 2021). Since the microlitter concentration of mussels can vary considerably from levels in the surrounding environment, their use as quantitative indicators of environmental microlitter contamination is not recommended.

5.0 Conclusions

Our baseline survey of the Saint John River and four major tributaries revealed microlitter in the water, sediment, and mussels at every site examined. Despite a low human population and forested land comprising ~75–95% of the watershed, microlitter concentrations in the water were comparable to those found in more urbanized watersheds elsewhere in eastern Canada. The presence of microlitter even at the most remote sites suggests that aerial transport and diffuse movement from the landscape are likely important contributors of microlitter to these rivers. Although WWTP are frequently highlighted as important point sources of microlitter to aquatic environments, we did not observe a significant difference in microlitter in sites downstream from WWTP compared to upstream or intermediate sites in any of the rivers. There was little overlap between microlitter particle concentration and composition in surface water, sediments, and mussels, likely as a result of the varying depositional conditions in rivers, and the ability of mussels to selectively expel materials from their bodies. Nonetheless, the presence of microlitter in each of the matrices we examined highlights its

prevalence in the environment, the ease at which it is transported, and the potential for it to enter food webs, and the results of this study are a sobering reminder that microlitter is a universal issue, even in regions with relatively little human impact.

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Figure Captions

Figure 1: Map of New Brunswick, Canada, highlighting watersheds studied. NASH 2020 sites were sampled in triplicate in 2020 for quality control assessment. 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; NB Boundary: Government of New Brunswick, 2005; Watershed Boundary: Government of New Brunswick, 2018; Canadian Boundary: Statistics Canada, 2016.

Figure 2: Breakdown of land use categories in each of the five watersheds. Size of circles indicates relative watershed area (Saint John River reduced by 10x).

Figure 3: Average number of microlitter particles observed in each river, based on site type for a) water, b) sediment, and c) mussels. Error bars indicate standard deviation.

Figure 4: Principal component analysis of all sites based on microlitter particle composition in a) water, b) sediment, and c) mussels. Arrows indicate the PCA scores of the top ten types of microlitter particles in each dataset. Dotted lines are used to indicate short arrows.

Figure 5: Results of repeat (triplicate) surface water sampling at ten sites along the Nashwaak River. Numbers in brackets indicate distance along the river (in kilometers) from the most upstream site. These sites were visited in July – August, 2020.

Map of New Brunswick, Canada, highlighting watersheds studied. NASH 2020 sites were sampled in triplicate in 2020 for quality control assessment. 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; NB Boundary: Government of New Brunswick, 2005; Watershed Boundary: Government of New Brunswick, 2018; Canadian Boundary: Statistics Canada, 2016.

193x186mm (600 x 600 DPI)

Breakdown of land use categories in each of the five watersheds. Size of circles indicates relative watershed area (Saint John River reduced by 10x).

205x138mm (600 x 600 DPI)

Average number of microlitter particles observed in each river, based on site type for a) water, b) sediment, and c) mussels. Error bars indicate standard deviation.

150x112mm (600 x 600 DPI)

Average number of microlitter particles observed in each river, based on site type for a) water, b) sediment, and c) mussels. Error bars indicate standard deviation.

148x112mm (600 x 600 DPI)

Average number of microlitter particles observed in each river, based on site type for a) water, b) sediment, and c) mussels. Error bars indicate standard deviation.

148x112mm (600 x 600 DPI)

Principal component analysis of all sites based on microlitter particle composition in a) water, b) sediment, and c) mussels. Arrows indicate the PCA scores of the top ten types of microlitter particles in each dataset. Dotted lines are used to indicate short arrows.

180x180mm (600 x 600 DPI)

Principal component analysis of all sites based on microlitter particle composition in a) water, b) sediment, and c) mussels. Arrows indicate the PCA scores of the top ten types of microlitter particles in each dataset. Dotted lines are used to indicate short arrows.

180x180mm (600 x 600 DPI)

Principal component analysis of all sites based on microlitter particle composition in a) water, b) sediment, and c) mussels. Arrows indicate the PCA scores of the top ten types of microlitter particles in each dataset. Dotted lines are used to indicate short arrows.

180x180mm (600 x 600 DPI)

Results of repeat (triplicate) surface water sampling at ten sites along the Nashwaak River. Numbers in brackets indicate distance along the river (in kilometers) from the most upstream site. These sites were visited in July – August, 2020.

149x179mm (600 x 600 DPI)