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Microplastic contamination in fish from the St. Lawrence River and Estuary: Roles of semisynthetic polymers, passive uptake, and wastewater inputs

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ABSTRACT

Semisynthetic polymers, such as rayon, are inconsistently reported in microplastic monitoring as most studies focus on synthetic polymers. However, evidence is growing for their ecological impacts. We measured microplastics, including semisynthetic polymers, in water and four fish species from the St. Lawrence River and Estuary (SLRE, Canada), a freshwater-marine corridor and good model for large river-estuary systems. Microplastic abundance was 0.44 ± 1.13 (mean ± SD) in fish gastrointestinal tract, 1.34 ± 2.12 (n/sample) in fish gills, and 2.17 ± 3.68 (n/L) in water. Rayon was the dominant microplastic in both water (41 %) and fish (40–100 %), revealing an underreported but significant contribution of semisynthetic polymers to aquatic microplastic burdens. This finding underscores the need to integrate semisynthetic polymers into future monitoring frameworks. In large piscivorous fish, gill uptake contributed more to microplastic accumulation than oral ingestion, unlike most non-piscivorous species reported in the literature, which accumulate more microplastics in the gastrointestinal tract. Comparisons of sites upstream and downstream of wastewater treatment plants (WWTPs) showed no significant difference in total microplastic abundance, but downstream waters contained more particles <100 μm and a broader diversity of polymers and colors. Given the greater environmental risks of smaller microplastics, these patterns highlight gaps in WWTP performance metrics that focus solely on total counts. Our findings provide evidence to expand monitoring frameworks to include semisynthetic polymers, incorporate non-oral exposure pathways into risk assessments, and improve WWTP metrics to inform global policy against microplastic pollution.

1. Introduction

Microplastics are of concern because of their ubiquitous distribution in the environment and the potential risks they pose to ecosystems. The broadly defined microplastics refer to most anthropogenic polymer particles, including synthetic thermoplastics (e.g., all commodity plastics), thermosets (e.g., polyurethanes), elastomers (e.g., rubber), inorganic/hybrid polymers (e.g., silicone), and semisynthetic polymers derived from plant materials such as cellulose and starch (e.g., rayon and azlon), with a diameter between 1 μm and 5 mm and originating from diverse sources and pathways (Andrady, 2017; Hartmann et al., 2019). However, due to the lack of consensus on defining the polymer composition of microplastics, many global monitoring programs and research surveys typically focus on fully synthetic polymers and exclude semisynthetic materials (e.g., rayon) (Athey and Erdle, 2022; Bakir et al., 2024; Jolly et al., 2025; Stelzer et al., 2025). This is partly due to analytical and standardization challenges, such as inconsistent polymer libraries and detection protocols (Bakir et al., 2024). Consequently, the prevalence and potential environmental risks of semisynthetic polymers in aquatic ecosystems remain poorly understood. Although not considered conventional plastic, recent *in vivo* studies have identified impacts of the semisynthetic cellulose-based fibers (e.g., rayon) on fish (e.g.,

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disruption of maximal parasite burden) and mussels (e.g., induction of gill and intestinal responses), in some cases at environmentally relevant concentrations (Jiang et al., 2024; MacAulay et al., 2023). These recent findings highlight the need to include semisynthetic polymers in quantifying the exposure of organisms to microplastics in aquatic systems (Jiang et al., 2024; MacAulay et al., 2023).

Fish play a key role in aquatic ecosystems and may uptake microplastics actively or passively through feeding or breathing mechanisms (Barboza et al., 2020; Ronda et al., 2023; Yin et al., 2022). Once ingested, the physical microplastics and/or associated contaminants (e.g., additives, metals, or dyes) can be detrimental to fish health (Barboza et al., 2020; Milne et al., 2024). Eventually, microplastics can also affect human health as contaminated water or fish consumption is sometimes a direct route of human exposure to microplastics (Coffin et al., 2022; Milne et al., 2024; Thompson et al., 2009). The accumulation of microplastics in fish is species-specific and depends on geographical area (Bhatt and Chauhan, 2023; Wootton et al., 2021). The detection frequency and abundance of microplastics in fish from aquatic systems generally follow the order of freshwater > estuarine > marine environment (Wootton et al., 2021). However, although freshwater and estuarine fish may be more contaminated than marine fish, microplastic contamination status is still unknown in many freshwater systems (Blettler et al., 2018). Monitoring pollution in riverine and estuarine ecosystems is critical, as these areas act as key pathways for microplastics entering coastal and marine environments (Lebreton et al., 2017; López et al., 2021).

As the only waterway connecting the North American Great Lakes, which contain about 20 % of the world's fresh surface water by volume, to the Atlantic Ocean, the St. Lawrence River and Estuary (SLRE) supports a number of ecosystems and is vital to more than 5 million people living in the region (Great Lakes Commission, 2025; St. Lawrence Plan, 2025). Beyond its regional significance, the SLRE serves as a globally important model system for contaminant studies. It is one of the world's largest freshwater-marine continuums, draining a densely populated and industrialized watershed, and faces human-induced pressures similar to those of other major estuarine systems, such as the Yangtze, Thames, and Mississippi. Microplastics have been found in the SLRE's water, sediment, and bivalves, indicating they are distributed throughout this system (Crew et al., 2020; Rowenczyk et al., 2022). A recent study also found various synthetic thermoplastics and thermosets in two benthic species, the channel catfish (*Ictalurus punctatus*) and the Atlantic tomcod (*Microgadus tomcod*), in the SLRE (Kabir et al., 2025). However, semisynthetic polymers were not included in that study and the status of the contamination with microplastics in fish living in the upper water column of the SLRE is unclear. In addition, although wastewater treatment plants (WWTPs) are known to be an important factor influencing contaminant exposure and accumulation in SLRE fish (Dépatie et al., 2020; Houde et al., 2014), their impacts on the microplastic contamination in water and fish are unknown.

According to a global review by Bhatt and Chauhan (Bhatt and Chauhan, 2023), the most studied fish orders for microplastics are Cypriniformes (~30 %), Characiformes (~14 %) and Siluriformes (~13 %), while Esociformes and Perciformes remain less examined. Several ecologically and economically important species in the SLRE belong to these under-studied orders, including northern pike (*Esox lucius*), walleye (*Sander vitreus*), yellow perch (*Perca flavescens*) and white perch (*Morone americana*). Northern pike are sedentary piscivorous predators distributed in shallow upper waters, sometimes close to the surface (Harvey, 2009), and commonly prey on yellow perch, making them susceptible to both direct microplastic uptake via gills and indirect uptake through prey. Walleye are also piscivorous, inhabiting shallow, turbid upper waters and feeding on insects and fish, including white perch, resulting in similar dual exposure routes. Yellow perch occupies 1–10 m depths and feed on zooplankton as larvae and shifting to invertebrates and small fish as adults, mainly in littoral zones (Brown et al., 2009), where they may encounter microplastics both suspended in the water and through diverse prey. White perch prefer shallow habitats and are opportunistic feeders on zooplankton, crustaceans, insect larvae, and small fish, with diets varying by size and location (Couture and Watzin, 2008; St-Hilaire et al., 2002). Small individuals (<50 mm) feed mainly in the upper water column, whereas larger ones are partly epibenthic (St-Hilaire et al., 2002), potentially encountering different microplastic sources.

The role of active ingestion and passive uptake of microplastics by these fish species, which influences the fate and toxicity of microplastics in the aquatic environment remains unknown. The analysis of microplastics in fish gastrointestinal tract (GIT) is a tool to record oral ingestion-related exposure. Through the analysis of gills, real-time contamination in the surrounding water column can be assessed. The tissues of these two organs are relevant bioindicators of microplastic contamination in a given ecosystem and useful for ecological risk assessment (Ferreira et al., 2018; Mizraji et al., 2017; Yin et al., 2022). Therefore, investigating the distribution of microplastics in the tissues of these less-studied fish species in the SLRE will improve the understanding of the factors affecting microplastic contamination in fish.

Despite growing recognition of microplastic pollution, many monitoring programs exclude semisynthetic polymers such as rayon, and few evaluate non-oral exposure pathways like gill uptake. WWTPs are often assessed solely on total microplastic counts, overlooking size and compositional shifts that may influence ecological risks in receiving downstream environment. These gaps limit our ability to fully characterize microplastic sources, transport, and biological exposure in large freshwater-marine systems. This study addresses these gaps using the SLRE as a globally relevant model by (1) quantifying microplastic abundance and characteristics, including semisynthetic polymers, in water and upper water column fish near Montreal and Quebec City, Canada; (2) examining GIT and gill tissues to determine the relative roles of passive and active uptake in SLRE fish; and (3) comparing water and fish samples collected upstream and downstream of the major WWTPs to access their influence on the microplastic distribution.

2. Materials and methods

2.1. Sample collection and preparation

In the riverine part of the SLRE, northern pike ($n = 41$) and yellow perch ($n = 12$) were collected upstream (northern pike $n = 20$; yellow perch $n = 6$) and downstream (northern pike $n = 21$; yellow perch $n = 6$) of Montreal's Jean-R.-Marcotte WWTP, one of the largest primary WWTP in the world (~2.5–7.5 million m^3 wastewater treated per day) (Mulligan and Sharifi-Nistanak, 2016), between June and August 2022 (Fig. 1). Fish were collected by seine fishing. Once caught, they were placed in a water tank with a solution of 400 mg L^{-1} eugenol. A spinal severance with a scalpel at the junction of the skull and the first vertebrae was then done. The total length, caudal length, and weight of the fish were recorded. All dissections were performed on a boat using purple nitrile gloves, and the GIT and gill samples were stored in pre-cleaned glass jars in coolers with ice before returning to the laboratory. GIT and gill samples were stored at −20 °C until analysis.

For the upper estuary part of the SLRE, walleye ($n = 24$) and white perch ($n = 10$) were collected upstream (walleye $n = 4$; white perch $n = 10$) and downstream (walleye $n = 20$) of Quebec City WWTP eastern station (primary treatment with ozonation; ~0.24 million m^3 wastewater treated per day) (City of Quebec, 2025), during the R/V *Lampsilis* survey in September and October 2022 (Fig. 1). Fish were caught using a trawl net, measured (total length) and weighted (total weight) in the boat's laboratory and then frozen in aluminum foil. Later, the fish were dissected under a chemical fume hood, both gills and GIT were collected and individually weighted. The tissues were preserved at −20 °C in aluminum foil until analysis. All fish handling procedures followed the Canadian Council of Animal Protection guidelines (Batt et al., 2005). Fish within each species were selected to be similar in size (Table 1) to

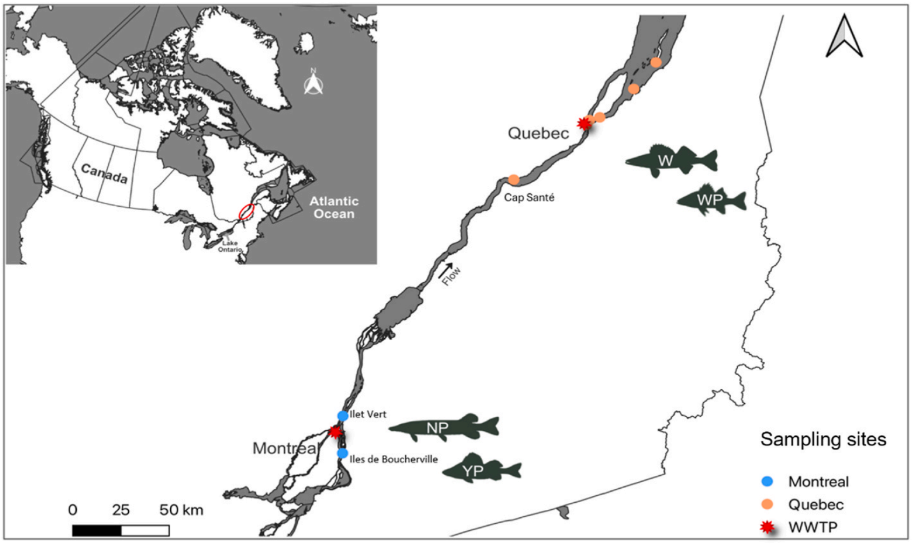

Fig. 1. Sampling sites for fish ($n = 87$ total) and surface water ($n = 18$ total) in the St. Lawrence River and Estuary. Northern pike (NP) and yellow perch (YP) were collected from upstream (Iles de Boucherville; NP $n = 20$; YP $n = 6$) (blue dots) and downstream (Ilet Vert; NP $n = 21$; YP $n = 6$) (blue dots) of Montreal's WWTP (red point). Walleye (W) were collected from upstream (Cap Santé; $n = 4$) (orange point) and downstream (orange points) of the WWTP (red point) of Quebec City ($n = 20$). White perch (WP) ($n = 10$) was only collected from upstream (Cap Santé) (orange point) of Quebec City. Map was generated using QGIS (V 3.34.12) and geographical data from Statistics Canada (v2022-09-19).

Table 1
Biometric data (wet weight based) and microplastic abundance in fish tissues collected in the St. Lawrence River and Estuary.

Species	Sample size (n)	Region	Mean weight (g) ± SD	Mean Length (cm)) ± SD	Mean GIT weight (g) ± SD	Mean Gills weight (g) ± SD	Total MPs	MPs/individual	
								Mean ± SD	Median
Northern pike	41	Montreal	1396 ± 1140	59 ± 9.4	55.7 ± 50.7	29.3 ± 22.3	76	1.85 ± 2.12	1
Yellow perch	12	Montreal	74 ± 53	NA	3.3 ± 2.11	1.4 ± 1.0	7	0.58 ± 1.08	0
Walleye	24	Quebec	443 ± 216	33 ± 4.8	19.7 ± 11.9	8.9 ± 4.7	71	2.96 ± 3.47	2
White perch	10	Quebec	249 ± 55	23 ± 2.1	5.3 ± 1.0	5.5 ± 1.5	1	0.10 ± 0.32	0

Species	Sample size (n)	Region	MPs/GIT		MPs/Gills		MPs/g GIT (ww)		MPs/g Gills (ww)	
			Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median
Northern pike	41	Montreal	0.61 ± 1.5	0	1.24 ± 1.5	1	0.02 ± 0.05	0	0.04 ± 0.04	0
Yellow perch	12	Montreal	0.33 ± 0.65	0	0.25 ± 0.62	0	0.19 ± 0.39	0	0.20 ± 0.47	0
Walleye	24	Quebec	0.33 ± 0.7	0	2.62 ± 3.09	1.5	0.03 ± 0.06	0	0.32 ± 0.37	0
White perch	10	Quebec	0.10 ± 0.32	0	ND	0	0	0	ND	ND

NA: not available; ND: not detected; MPs: microplastics.

minimize age-related bias.

Along with the fish sampling, surface water ($n = 18$) was collected from each sampling area (2.0 L per sample) (Fig. 1), except for Cap Santé (upstream of Quebec City) for logistical reasons. Water sampling was done using bulk water sampling using pre-cleaned amber glass bottles, which were stored at 4 °C until analysis. Samples included water from the surface, and just below the surface down to approximately 30 cm and therefore represent the very top layer of water.

2.2. *Chemicals and reagents*

Hydrogen peroxide (H_2O_2, 30 % w/w, stabilized reagent grade), potassium hydroxide (KOH, ACS reagent, ≥85 %, pellets), zinc chloride ($ZnCl_2$, anhydrous, reagent grade, ≥98 %) and iron (II) sulfate heptahydrate ($FeSO_4.7H_2O$, ≥99 %), were purchased at Fisher Scientific (Ottawa, Canada). Polycarbonate (PCTE, 10.0 μm pore size, filter diam. 47 mm) membrane filters were purchased at Sterlitech (Auburn, USA). Microscope Low-e slides used for μFTIR analysis of the particles were purchased at Kevley Technologies (Chesterland, USA).

2.3. *Microplastic analyses*

Fish GIT and gills (both gills analyzed together) were digested and combined with density separation based on previously published methods with some modifications (Foekema et al., 2013; Jaafar et al., 2020; Karami et al., 2017). Briefly, fish tissues were digested using 10 % KOH (1:3 organ volume to solution). Herein, the weight of the sample was used as a proxy to acquire the volume of the sample with 1 g of tissues equivalent to 1 mL of tissues. The GIT and gills were digested separately under a chemical fume hood for approximately 48 h on a 65 °C hotplate in beakers covered with aluminum foil to avoid airborne contamination. Samples were filtered on 10 μm PCTE membranes and placed in a glass Petri dish and wrapped in aluminum foil to avoid air contamination. Gill samples were further processed using a $ZnCl_2$

solution (density ≈ 1.56 g cm^{-3}, 20 °C), which allowed microplastics to float and separate from undigested fragments (Coppock et al., 2017; Kabir et al., 2025). Then, the top layer of the solution was filtered on a 10 μm PCTE filter (Kabir et al., 2025). The $ZnCl_2$ solution used in this study has a higher density than most common microplastics, including semisynthetic polymers such as rayon (~1.50–1.52 g cm^{-3}), which enables effective flotation and separation of microplastics during density separation.

Water samples were first filtered using 10 μm PCTE membrane filters, then organic matter present on the filter was digested overnight by wet peroxide oxidation (WPO) (7.5 g of $FeSO_4·7H_2O$/500 mL+ 3 mL H_2SO_4, 20 mL H_2O_2 at approximately 70 °C). This digestion was followed by an additional density separation process as described above. Final extraction was performed on a 10 μm PTCE filter membrane.

Characterization of the shape, size, and color of each particle was conducted using Leica S9i digital stereo microscope (Leica Microsystems Inc., Whitby, Canada). Briefly, suspected particles were transferred, using tweezers, from the filter membrane to a slide where a number was attributed to them for later correlation between microscope analysis and μFTIR identification. The identification of the polymer type was performed by micro-Fourier Transform Infrared Spectroscopy (μFTIR) (Spotlight 200i; PerkinElmer Scientific Canada ULC, Woodbridge, Canada). Background signals were collected using a blank measurement at the start of each analytical batch during setup, and repeated whenever the measurement mode switched between reflectance and attenuated total reflection (ATR) within the same batch. Spectra were then generated at a resolution of 8 cm^{-1} with 64 acquisitions, and the infrared wave numbers ranged from 4000 to 650 cm^{-1}. The spectral data were collected using the Quant V2.0 software, and results of all particles were subsequently compared to the spectral reference library (COMPARE).

2.4. Quality assurance and quality control (QA/QC)

To prevent laboratory contamination, nitrile gloves and a 100 % cotton lab coat were worn during the experiment. All glassware was cleaned with detergent (Contrad® 70), that will act as a surfactant making sure microplastics do not stick to the glassware, rinsed in distilled water, followed by nano-pure water (Arium® Pro Ultrapure Water Systems) (Sartorius Canada Inc., Oakville, Canada) to ensure no remaining contamination by microplastics from previous rinsing steps, and dried at 100 °C overnight before being covered by aluminum foil (previously baked at 450 °C). The samples were filtered under a chemical fume hood to limit airborne particle contamination. This method has been shown to reduce the contamination of the samples by fibers present in the laboratory by 50 % (Wesch et al., 2017).

For each sampling site, field blanks (one for GIT and one for gills) ($n = 10$ total) were collected for fish samples and one field blank ($n = 5$ total) for water samples. In the Montreal region, field blanks consisted of two glass jars left open near the dissection station for fish samples, and an amber glass jar left open during water sampling. In the Quebec City region, as whole fish were wrapped in aluminum foil, blanks consisted of an aluminum sheet left open near the fish processing station, and an amber glass jar left open during water collection. During sample processing, a procedural blank (a Petri dish, containing a PCTE membrane filter) was left under the fume hood near the samples for each batch to assess airborne contamination. This procedural blank was added per batch of samples to ensure the quality of the experiment. All blanks were handled the same as samples.

A total of 7 particles were found in procedural and field blanks for fish and water samples altogether, while no particles were detected in fume hood blanks. The 3 particles detected in procedural blanks were cellulose and not microplastics. In addition, 1 cellulose particle and 3 rayon particles were found in the field blanks collected in Quebec City for fish sampling. The microplastics (3 rayon particles) found in all blanks accounted for 1.9 % of the total microplastics recorded in this study ($n = 155$ in fish tissues), which is below the 10 % threshold recommended in the literature (Dimitrijevic et al., 2019). Several methods exist for using and interpreting blanks, such as particle deduction per category (e.g., shape, polymer, size) or using a minimum detectable amount (MDA) calculation (Lao and Wong, 2023; Shruti and Kutralam-Muniasamy, 2023). The MDA method is based on statistical analysis. Because only 3 rayon particles were found in field blanks from one site, the MDA method was not applicable in the present study. It has been reported that blank corrections based on particle color showed large variation among laboratories and sample matrices, making color an unreliable criterion for correction (Lao and Wong, 2023). Because color is a subjective characteristic and can be influenced by extract purity or other background interference, color-based correction was not used (Lao and Wong, 2023). Instead, correction by particle shape, which provided more consistent results across studies (Lao and Wong, 2023), was adopted in this work. Therefore, the 3 rayon microplastics identified in the field blanks were subtracted from the data for fish samples collected in Quebec City, based on particle shape (fibers).

During method development, the efficiency of digestion and filtration was tested. The spike-recovery tests using purple polyethylene (PE) fibers ($n = 4$), translucent polyethylene terephthalate (PET) fragments ($n = 2$), orange PE fragments ($n = 2$), brown PE fragments ($n = 2$) and translucent PE film ($n = 2$) were based on recorded environmental abundance and performed in the GIT and gills of Atlantic mackerel (*Scomber scombrus*) collected from previous studies in the laboratory in order to test in a matrix similar to the samples (Barboza et al., 2020; Lusher et al., 2013). The digestion efficiency and spike recovery were between 90 and 100 %. Additionally, due to the high abundance of rayon particles found in the samples, and their degradation potential, spike recovery of rayon fibers was tested to ensure no underestimation of recorded particles. Blue rayon fibers ($n = 2$) were spiked under the same conditions as other polymers. Recovery was 100 %, and no particle showed signs of degradation (e.g., discoloration) by the digestion process.

2.5. Data analysis

Statistical analyses were carried out in RStudio (V2023.12.1), and all significant levels were set at $\alpha = 0.05$. Both mean (± standard deviation (SD)) and median are reported to facilitate comparisons with literature. Since the data did not follow a normal distribution, the Kruskal–Wallis's test followed by a post hoc Dunn's test was used to determine whether there were significant differences in the abundance distribution in the same type of tissue among species. Comparisons between two groups were conducted by the Mann-Whitney test. The correlation between the different quantitative variables (i.e., fish length, weight, or whole tissue weight) and the abundance of each microplastic shape category was assessed using a Spearman correlation test. A Generalized Linear Mixed Model (GLMM) assuming a Poisson distribution for microplastic counts and a log-link function was used to test interaction between microplastic abundance data (y) and tissues weight for gill and GIT (categorical variable) samples, analyzed separately. All collected fish samples were analyzed together within each tissue type, with species included as a random effect to account for interspecific variation. Analyses were conducted using the *lme4* package in R. Multivariate analysis of polymer and color composition was conducted using the *ecoCopula* package in R (Popovic et al., 2022). This approach simultaneously models multiple response variables and accounts for latent correlations among them. Separate generalized linear models (GLMs) were fitted to each response variable (individual polymer types and colors) using a binomial distribution, as the abundance data were sparse and zero-inflated, and thus transformed into presence/absence data. These GLMs were then integrated through the copula framework, and ordinations were generated to visualize the results. In the ordinations, data were grouped by tissue type (gills or GIT), stream (site) location (upstream or downstream of WWTPs), and particle type (fiber, fiber bundle, film, and fragment) to show compositional patterns. Particle size was not included in the analysis because it is a continuous variable and not suitable for the binomial copula framework. Plots were generated using Prism (V10.1.1, 270) and R (*ggplot2* package and the *circlize* package).

3. Results and discussion

3.1. Microplastic abundance in SLRE fish tissues and surface water

3.1.1. Detection frequency and body burden

Of the 87 fish samples analyzed, microplastics were found in at least one tissue in 59 % of the fish. Specifically, microplastics were found in at least one tissue of 71 % of northern pike, followed by 67 % of the walleye, 25 % of yellow perch and 10 % of white perch. The proportion of occurrence was higher (69 %) in predatory fish (northern pike and walleye) and lower (18 %) in prey fish (yellow perch and white perch). While examining tissue distribution, 75 % of recorded microplastics were observed in the gills, while only 25 % were found in the GIT of all fish samples. The occurrence of microplastics was the lowest in the white perch, with only one microplastic particle found in their GIT. These detection frequencies were lower than those reported in the benthic catfish and tomcod in the SLRE (91 %), even when semisynthetic polymers were not included in the measurements for benthic fish (Kabir et al., 2025). These results suggest that fish in the upper water column of the SLRE water were less exposed to and/or accumulated less microplastics than benthic fish from the same ecosystem.

A total of 155 microplastics were detected in the collected SLRE fish. Microplastic abundance in northern pike, yellow perch, walleye, and white perch individuals (i.e., GIT and gills calculated together) were 1.85 ± 2.12 (median: 1.0), 0.58 ± 1.08 (median: 0.0), 2.96 ± 3.47 (median: 2.0), and 0.10 ± 0.32 (median: 0.0) (n/fish), respectively (Table 1). The predator fish walleye from the Quebec City area accumulated significantly more microplastics than the prey white perch (Mann-Whitney $p < 0.01$). The predatory northern pike also showed a trend of accumulating more microplastics than the yellow perch prey from the Montreal area, albeit not statistically significant (Mann-Whitney $p = 0.06$).

3.1.2. Microplastic abundance in fish GIT

The microplastics in all the analyzed GIT of SLRE fish was 0.44 ± 1.13 n/GIT (median: 0.0). The mean abundance of microplastics in the GIT of SLRE fish from the upper water column was much lower than that reported for SLRE benthic fish (1.4 ± 0.27 n/GIT in tomcod, 1.8 ± 0.24 n/GIT in catfish) (Kabir et al., 2025) (Table S1). The microplastic abundances in the GIT of all studied SLRE fish were much lower than those for fish from the upstream Great Lakes. The mean abundance of microplastics in the GIT of fish from Lake Ontario (Canada), located near Toronto and upstream of the SLRE, was 93 ± 226 (n/GIT), which was about 1–2 orders of magnitude higher than that in the SLRE fish (Milne et al., 2024) (Table S1). The higher levels of microplastics found in Lake Ontario fish are probably due to differences in source inputs, hydrodynamic conditions, and the feeding behavior and habitat use of the fish studied in both systems. The Lake Ontario fish were collected from Humber Bay near Toronto (Milne et al., 2024), a large city with a population of around 7 million (Greater Toronto Area). It receives WWTP effluents and urban runoff from heavily urbanized tributaries, including several plastic manufacturers around (Ballent et al., 2016; Corcoran et al., 2015; Milne et al., 2024; Yu et al., 2024). By comparison, Montreal and Quebec City have populations of approximately 1.7 million and 0.5 million, respectively, indicating there may be fewer microplastic inputs from human activities. Lake Ontario has an average hydraulic residence time of roughly 8 years, and even the shortest particle residence times are around 60 days (McKenna and Chalupnicki, 2011). Such prolonged retention may increase particle accumulation in the lake basin, consequently resulting in greater exposure of fish to microplastics. In contrast, the St. Lawrence River and the fluvial estuary have high water flow (0.5–1 m/s), and water travel times from the Lake Ontario outlet to Quebec City (~550 km) are estimated at just 1–2 weeks, with particle residence times of about 15 days (Hudon et al., 2017; Simons et al., 2006). These shorter retention periods, combined with stronger dilution, may decrease the availability of microplastics to aquatic organisms in the upper layer of St. Lawrence River and the fluvial estuary. Additionally, the Lake Ontario study included several benthic and littoral species, such as brown bullhead (*Ameiurus nebulosus*), white sucker (*Catostomus commersonii*), and rock bass (*Ambloplites rupestris*), which feed near sediments where microplastics tend to accumulate and ingest microplastics indirectly through benthic prey (Milne et al., 2024). In contrast, most species analyzed in this study have limited contact with sediments, which likely decreases their exposure to sediment-associated microplastics. Methodological differences are unlikely to explain the observed differences, as both studies used KOH digestion, and the Lake Ontario study used a 45 μm filter compared to the 10 μm filter in our research. This difference in the filter would likely affect our results towards higher counts, not lower.

The mean abundance of microplastics in the analyzed SLRE fish from the upper water column was also generally lower than the fish from most other freshwater and estuarine environments, such as the Minho River and Estuary (Spain) (3 species; 6 ± 7 n/GIT), the Mondego Estuary (Portugal) (3 species; 1.27 ± 0.27 n/GIT), and the River Thames/River Stour (East Anglia) estuaries (UK) (3 species; 1.47 ± 3.17- 2.46 ± 3.10 n/GIT), which also connect to the North Atlantic Ocean (Bessa et al., 2018; Guilhermino et al., 2021; Horton et al., 2024) (Table S1). A previous study examined microplastic abundance in the GIT of northern pike from a prairie creek in central Canada, finding an average of about 5 microplastics/GIT. This is also higher than the levels observed in the northern pike from SLRE (Table S1) (Campbell et al., 2017). In contrast, the microplastic abundance in the fish GIT observed in this study was comparable to the average levels reported for 26 species from the Pearl River Estuary (China) (0.37 ± 0.24 n/GIT) and more than those (69 species) found in the Paraiba (0.07 ± 0.27 n/GIT) and Mamanguape (0.12 ± 0.37 n/GIT) Estuaries (Brazil), which are connected to the South Atlantic Ocean (Table S1) (Lin et al., 2020; Vendel et al., 2017). These comparisons suggest that microplastics in the GITs of the upper-water-column fish in the SLRE are at the lower end of the global range reported for freshwater and estuarine environments, but still within the levels observed in relatively less impacted systems.

In the present study, the abundance of microplastics in the GIT of northern pike (0.61 ± 1.50 n/GIT; median: 0.0) and walleye (0.33 ± 0.70 n/GIT; median: 0.0) was not statistically different than that observed in their respective prey fish, the yellow perch (0.33 ± 0.65 n/GIT; median: 0.0) and white perch (0.10 ± 0.32 n/GIT; median: 0.0). Microplastic abundance in fish GIT showed no significant variation across sampling locations, except in downstream walleye, which exhibited higher detection frequency and levels than their upstream counterparts (Fig. S1). Moreover, the abundance of microplastics in the GIT of the four studied SLRE fish species was not correlated with GIT weight or fish size.

Microplastic accumulation in fish GIT is often attributed to dietary preferences, active ingestion, and body size (Lusher et al., 2013; Sun et al., 2019; Yin et al., 2022). However, in the SLRE we found no spatial trends and no significant differences between predator and prey fish for GIT microplastic abundance. In the present study, the overlap of habitat and some diet resources between prey and predator fish likely contributes to similar exposure pathways and comparable GIT microplastic burdens across trophic levels (Brown et al., 2009; Couture and Watzin, 2008; Harvey, 2009; St-Hilaire et al., 2002). Additionally, the rapid excretion of ingested microplastics from the fish digestive tract may also decrease the differences between predator and prey fish in terms of microplastic accumulation in the GIT (Güven et al., 2017; Jovanović, 2017). This pattern also suggests that GIT content in SLRE fish primarily reflects recent, opportunistic ingestion rather than local contamination gradients which would be accumulated over time. As a result, GIT measurements appear poorly suited for tracking spatial patterns of microplastic pollution in this system.

3.1.3. *Microplastic abundance in fish gills*

As gill contamination is more directly linked to the surrounding water and less influenced by feeding activity (Yin et al., 2022), we also examined gills as a potential alternative indicator of microplastic exposure. Microplastics in the gills of the four target SLRE fish species were 1.34 ± 2.12 n/Gills (median: 1.0) and varied significantly among species (Kruskal-Wallis $p = 0.001$). More microplastics were found in the gills of predatory fish, northern pike (1.24 ± 1.50 n/Gills; median: 1.0) and the walleye (2.62 ± 3.09 n/Gills; median: 1.5) than the prey fish (yellow perch: 0.25 ± 0.62 n/Gills, median 0.0; white perch: not detected) (Fig. 2A). For the two predatory species, no difference of microplastic abundance was found between northern pike and walleye gills (Mann–Whitney $p = 0.08$). Microplastic abundance in fish gills showed no significant variation across sampling locations; downstream walleye exhibited slightly higher levels than upstream individuals, though this difference was not statistically significant (Fig. S1). Microplastics in fish gills have been relatively understudied. Kabir et al. (2025) reported that the average microplastic abundance in the SLRE benthic catfish (predator) and tomcod (prey) was 1.9 and 0.5 n/Gills, respectively. These levels and the pattern of greater accumulation in the gills of large predators than prey are similar to the results of the present study. Considering that semisynthetic polymers were not included in the previous research, benthic fish in the SLRE may accumulate more microplastics on their gills than fish from the upper water column. This is likely because sediment generally accumulates much more microplastics than the water column, acting as a long-term sink for microplastics (Rochman et al., 2024). Sediment resuspension (e.g., caused by tide or seasonal flow variations) can remobilize microplastics to the benthic layer, exposing benthic species to higher concentrations of microplastics (Horton et al., 2024). Benthic fish also ingest sediment during foraging, and their prey are likely to contain high levels of microplastics (Horton et al., 2024). All these factors contribute to higher levels of microplastics in benthic fish compared to those in the upper water column. In contrast to the GIT comparison results, the average microplastic abundance in the gills of SLRE fish tends to be higher than that reported for fish from the Minho River and Estuary (Spain) (3 species; 0.5 ± 1.1 n/Gills) and the Pearl River Estuary (China) (26 species; 0.21 ± 0.19 n/Gills), and comparable to that reported for fish from Hangzhou Bay and the Yangtze Estuary (China) (14 species; 0.3 ± 0.5–2.6 ± 1.6 n/Gills) (Table S1) (Guilhermino et al., 2021; Lin et al., 2020; Su et al., 2019). These findings suggest that SLRE fish may experience comparatively higher gill-associated microplastic exposure, highlighting the potential importance of non-oral uptake pathways in this system.

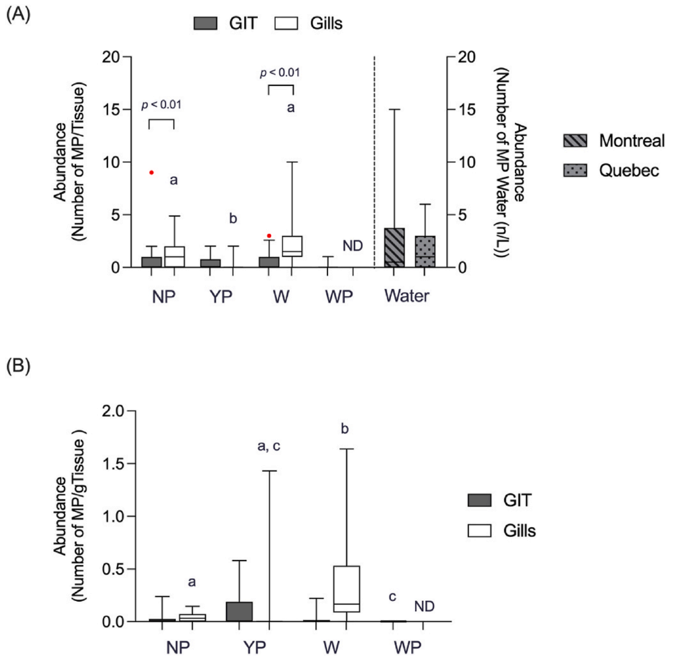

Fig. 2. Abundance of microplastics (MPs) in (A) whole fish tissues and surface water, and (B) fish GIT and gills based on tissue weight. Box plots are defined as follows: center line, median; box plot edges, 25th and 75th quartiles; whiskers, 5th and 95th quartiles of the distribution. Outliers are shown in red. Northern pike (NP) and yellow perch (YP) were from the SLRE Montreal area, while walleye (W) and white perch (WP) were from the Quebec City area. ND: not detected. Letters above bars indicate significant differences of microplastic abundance in gills among species.

A comparison of the microplastic abundance per unit of tissue weight revealed a significantly lower abundance of microplastic in the gills of the northern pike and the yellow perch from the Montreal region than in the walleye from the Quebec City area ($p < 0.01$) (Fig. 2B). A positive Spearman's rank correlation was found between the weight of gills and the abundance of microplastics detected in the gills ($\rho = 0.32$, $p < 0.01$) (all fish analyzed together), indicating larger fish gills accumulated more microplastics. The GLMM results showed a significant positive interaction between gill weight and microplastic abundance ($\beta = 0.01 \pm 0.003$, $z = 3.32$, $p < 0.001$, $R^2 = 0.67$), suggesting that larger gills tended to accumulate more microplastics. The interaction was influenced by a few high-value outliers, but the overall trend remained significant ($\beta = 0.02 \pm 0.01$, $z = 1.98$, $p = 0.05$, $R^2 = 0.63$) after their removal and is consistent with the biological expectation that greater gills provide more surface area for particle retention. Since fish gill size is proportional to fish body size, larger fish would accumulate more microplastics in their gills in the SLRE. As a result, predatory fish species would be at higher contamination risk due to passive gill uptake of microplastics than their fish prey.

3.1.4. *Tissue comparisons and relationships with microplastic abundance in water*

When comparing microplastic contamination between tissues, average abundance in the northern pike was significantly higher in gills (1.24 ± 1.50 n/Gills; median: 1.0) than in the GIT (0.61 ± 1.50 n/GIT; median: 0.0) (Mann–Whitney $p < 0.01$). A similar difference was observed for the walleye, for which gills had more microplastics (2.62 ± 3.09 n/Gills; median: 1.5) than GIT (0.33 ± 0.70 n/GIT; median: 0.0) (Mann–Whitney $p < 0.01$) (Table 1 and Fig. 2A). In prey fish, however, no significant differences were observed between tissues. This pattern implies that ambient water contamination plays a greater role in determining overall microplastic burden than trophic transfer and oral ingestion in large piscivorous fish, which differs from fish with other feeding habits (e.g., detritivorous, herbivorous, and omnivorous), the latter usually accumulating more microplastics in the GIT than in gills (Bhatt and Chauhan, 2023; Wootton et al., 2021). Our observation that gill uptake exceeds GIT accumulation in large piscivorous fish reveals a dominant contribution of respiratory exposure pathways to total microplastic burden. This finding provides a new mechanistic insight into how microplastics bioaccumulate in fish, questioning the traditional emphasis on oral ingestion and highlighting the need to integrate respiratory exposure routes in future risk assessment.

Fish gills accumulate microplastics on the rakers when water is filtered during the eating and breathing process. Therefore, the contamination pattern of microplastics in water and fish gills should be similar (Buwono et al., 2021; Yin et al., 2022). Analysis of microplastics in surface water showed no significant difference in their abundance between samples from the Montreal (2.42 ± 4.27 n/L; median: 0.5) and Quebec City (1.67 ± 2.34 n/L; median 1.0) regions (Fig. 2A), which was consistent with the similar microplastic abundance in the gills between northern pike and walleye. There were no differences in microplastic abundance in the water (Fig. S1) or fish, except for the GIT of walleye, between sampling sites upstream and downstream of the WWTPs. These results indicated that the release of urban effluents from WWTPs or the larger human population in the Montreal area may not significantly influence the total microplastic abundance in the receiving downstream environment, at least for those microplastics >10 μm in size. Another possibility is that water and fish tissue might not be effective matrices for detecting such effects. A recent study found that most microplastics sink to the bottom within a few weeks, with only 1 % remaining in the water and 0.0001 % found in fish in a large in-lake mesocosm experiment (Rochman et al., 2024). In contrast to that study, where microplastics were added once to a static lake, WWTPs continuously released microplastics into the SLRE. Despite these differences, sediment is likely an important sink for microplastics in the SLRE (Crew et al., 2020) and may better reflect the impact of municipal effluents on microplastic levels, which should be investigated further.

3.2. *Characterization of detected microplastics: shape, color and size*

3.2.1. *Shape*

Fiber was the most frequently detected microplastic shape in fish tissues (57 %–100 % of total microplastics) and surface water (61 %), followed by films (0–28 % in fish; 17 % in water) and fragments (7–14 % in fish; 15 % in water) (Fig. 3A). The distribution of shapes was similar in both GIT and gills, with fiber being the dominant shape (50 %–100 % in GIT; 66 %–77 % in gills) followed by film (2 %–25 % in the GIT; 11 %–33 % in gills). This composition closely aligns with previous studies on microplastics in benthic fish from the SLRE and in fish from the upstream of SLRE (i.e., Lake Ontario and Lake Superior of the North American Great Lakes), where fibers accounted for 66 % and 65–80 % of the microplastics found, respectively (Kabir et al., 2025; Munno et al., 2022) (Table S1).

Water downstream of the Montreal WWTP showed a higher prevalence of films and spherical microplastics, along with reduced fibers and fragments, compared to upstream (Fig. S2). This trend was reflected in yellow perch GIT, where films dominated downstream specimens, aligning with the elevated film percentage observed in water downstream of the WWTP (Fig. S2). However, gill tissues in yellow perch showed divergent patterns: downstream samples contained exclusively fibers, contrasting with upstream samples, which had only films (Fig. S2). These tissue-specific differences in microplastic morphology should be interpreted with caution due to the low microplastic abundance detected in both GIT and gills in yellow perch. In contrast, northern pike and walleye displayed no spatial variation in microplastic shape composition between upstream and downstream locations (Fig. S2).

3.2.2. *Color*

The dominant colors of the detected microplastics in fish were blue (28 %–100 %) and black (10 %–42 %) (Fig. 3B). Lower proportions of

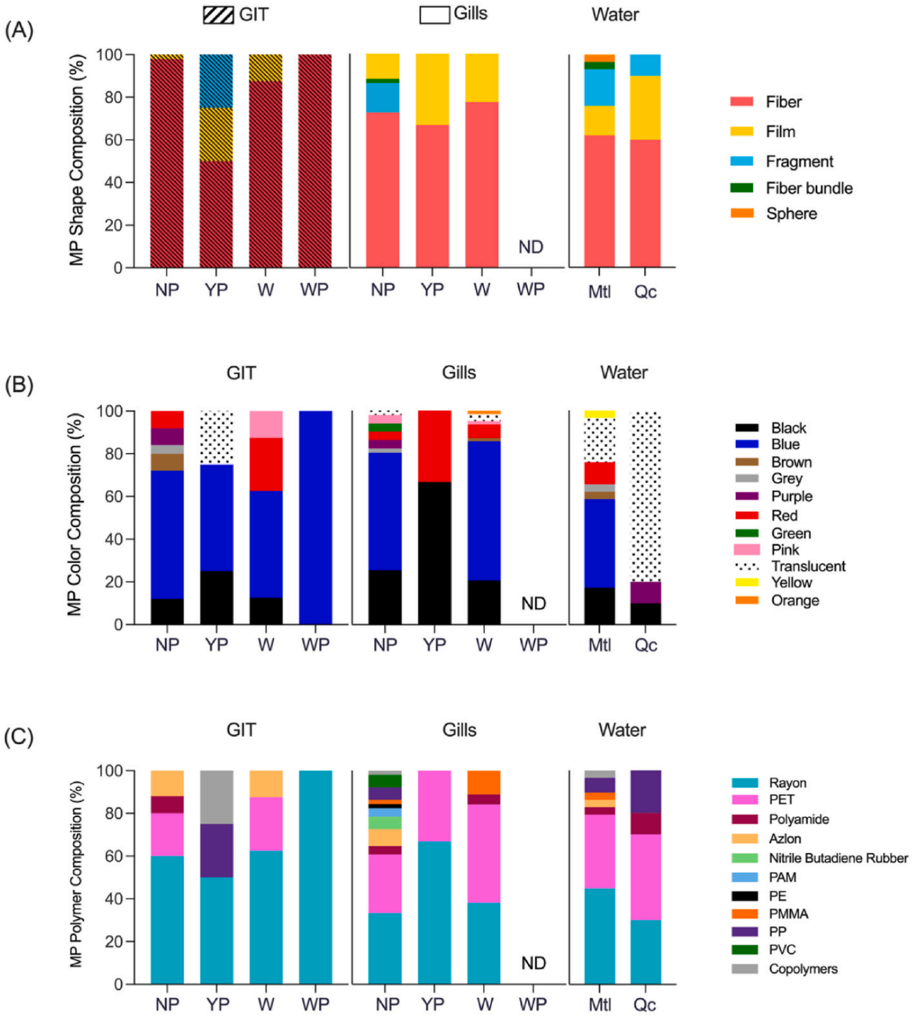

Fig. 3. Shape (A), color (B) and polymer (C) compositions of microplastics (MP) in GIT and gills of northern pike (NP), yellow perch (YP), walleye (W), and white perch (WP), as well as surface water from Montreal (Mtl) and Quebec City (Qc) areas in the SLRE. ND: not detected. PAM: polyacrylamide; PE: polyethylene; PET: polyethylene terephthalate; PMMA: poly (methyl methacrylate); PP: polypropylene; PVC: polyvinyl chloride.

translucent microplastics were found in fish tissues compared to surface water (Fig. 3B). This is generally consistent with previous studies, but translucent or clear microplastics also played a dominant role in some fish from other study areas, which differs from our results (Table S1). The lower proportion of translucent microplastics in fish in our study may partly result from analytical limitations, as translucent particles are more difficult to visually identify under the microscope, especially when attached to fish residues or filter surfaces (Kotar et al., 2022). Additionally, colored particles like blue or black microplastics are more visible in the water column and may look like natural food for fish, leading to increased ingestion (Ory et al., 2017; Ríos et al., 2022).

Blue microplastics were the most often found color in both fish GIT (50 %–100 %) and gills (54 %–65 %). Microplastics found in predators showed greater color diversity than their potential prey fish (Fig. 3B). In addition, the colors of microplastics were more diverse in the gills of the predatory fish than in their GIT (Fig. 3B). The color of microplastics observed in surface water samples were comparable to those of fish, except that the most found color was translucent (35 %) followed by blue (30 %). A chord diagram was used to understand the relation between different characteristics of the microplastics found across fish and surface water samples (Fig. 4A). The diagram revealed that when looking at color and shape, fibers had the highest color diversity, followed by film (Fig. 4A). However, both shapes of microplastics were in majority blue and black (Fig. 4A).

The model-based ordination (Fig. 5) shows similarities in microplastic color composition across samples. Each point represents a sample, and samples closer together have more similar color distributions. The labeled color names (e.g., blue, black, red) represent the color variables, and their positions show how these colors covary across the dataset. The two axes (latent variables (LV) 1 and 2) represent the main gradients of variation in color composition and do not have fixed physical units. Fig. 5A shows the ordination grouped by tissue type (gills, GIT) and sampling position relative to WWTPs (upstream, downstream). The color labels blue, black, and red were all positioned at the upper right of the ordination (LV1 ≈ 0.8–1.0; LV2 ≈ 0.8–1.2), indicating that these colors contributed the most to the overall variation in color composition. Blue showed high loadings on both LVs and was associated with many samples from both upstream and downstream sites, reflecting its widespread presence across the study area. Black and red clustered together and were closer to downstream GIT samples, suggesting an enrichment of microplastics with these colors near the WWTP downstream. Colors such as purple, grey, green, and orange have LV1 and/or LV2 values near 0–0.5, indicating weak contributions to the overall variation and limited differentiation between sites. Fig. 5B groups samples by tissue type and particle shape (fibers, fiber bundles, films, fragments). Fibers were mainly associated with blue, black, and red colors, confirming that these dyed particles dominated the color variability in fish tissues. Films and fragments were more dispersed across the ordination space and showed no strong association with any particular color category. This pattern indicates that colored fibers were the main drivers of color differences among microplastics in the SLRE fish. Colors near LV1 and/or LV2 around 0–0.5, such as purple, grey, green, and orange, contributed minimally to overall variation and showed limited differences among microplastic shapes. Pink and translucent colors were located near the positive end of the LV1 (close to 1) but showed little association with any sample cluster, suggesting that these colors contributed to model variance but were rarely observed in the samples.

SLRE surface water from the Montreal area had a higher color diversity than did that from the Quebec City area (Fig. 3B). Blue and black remained dominant across most sampled locations, except for water samples from Quebec City, where translucent microplastics predominated (Fig. S3). However, some spatial variation was evident in microplastic color compositions. Downstream of the WWTP, northern pike GIT and gills, as well as walleye gills, exhibited greater color diversity compared to their upstream counterparts (Fig. S3). In contrast, less color diversity was found in Montreal downstream water samples (Fig. S3). It is possible that other sources upstream of the Montreal WWTP (e.g., the WWTP of the City of Longueuil) masked the impacts of the Montreal WWTP regarding microplastic color diversity. Another possibility is that the limited water sampling volume (e.g., 2 L per sample), combined with single-time collection, may be insufficient to capture the full diversity of microplastic color profiles. This represents a limitation of the present study, as the limited sampling volume, time frame, and area coverage may have underestimated the variability of waterborne microplastics. Future studies should account for temporal and spatial variability and

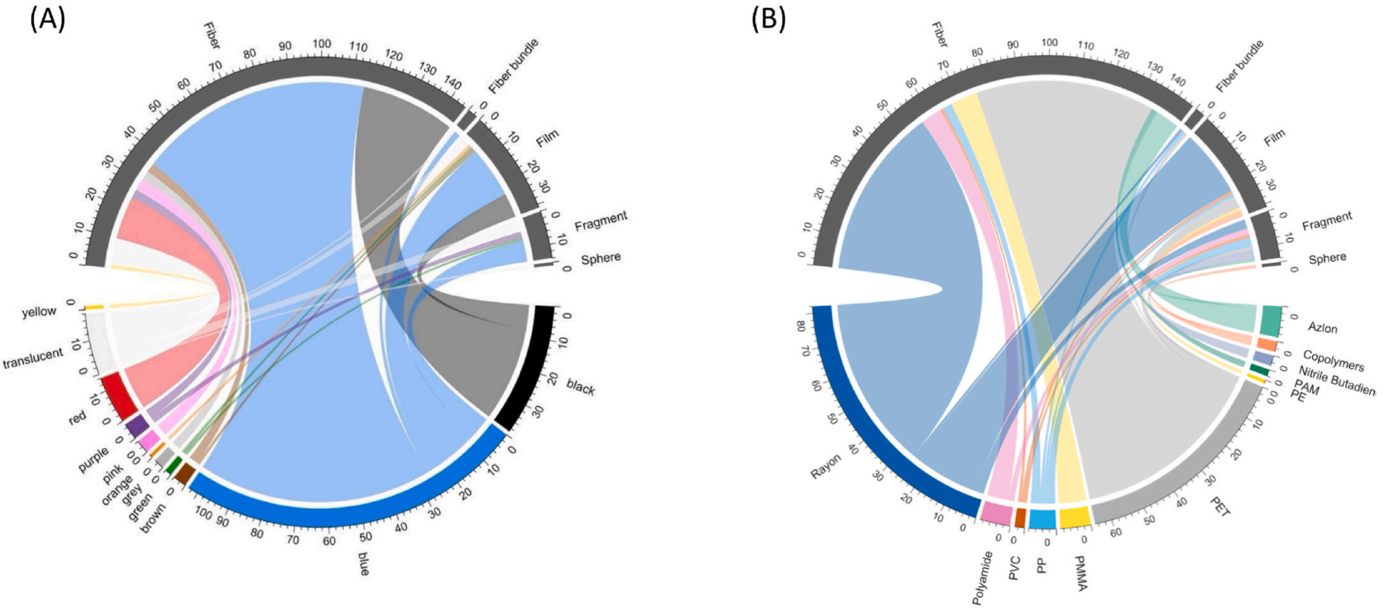

Fig. 4. Chord diagrams representing (A) the relationships between the color of microplastics (bottom of the diagram) and shape characteristics (top of the diagram), and (B) the relationship between the microplastic polymer type (bottom of the diagram) and shape (top of the diagram). The axis represents the number of particles per category. All fish tissues from both sites and surface water samples are accounted for together. PAM: polyacrylamide; PE: polyethylene; PET: polyethylene terephthalate; PMMA: poly (methyl methacrylate); PP: polypropylene; PVC: polyvinyl chloride.

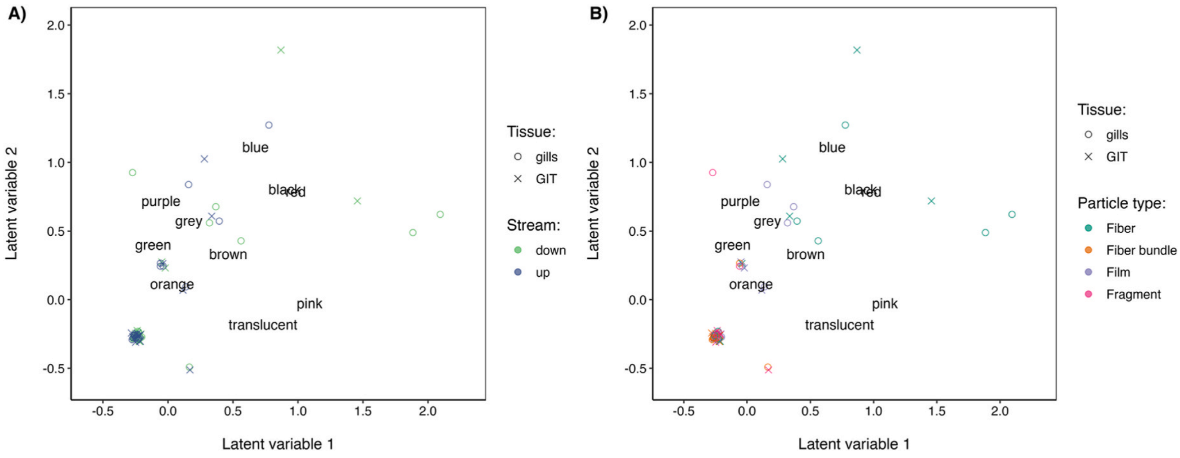

Fig. 5. Model-based ordination of microplastic color composition derived from a binomial copula model. Each point represents a sample positioned according to similarities in color composition based on residual correlations estimated by the model. The text labels (e.g., blue, black, red) represent individual color categories; their positions indicate how these colors covary and contribute to overall patterns in the dataset. (A) Ordination grouped by tissue type (gills, GIT) and sampling position relative to WWTPs (upstream, downstream). (B) Ordination grouped by tissue type (gills, GIT) and particle type (fiber, fiber bundle, film, fragment).

prioritize replicating sampling efforts with larger water volumes to better evaluate the accumulation of microplastics in fish via water.

3.2.3. Size

The average size of all microplastics found in the fish and surface water was 1381 ± 1072 μm (median: 1078 μm) and 813 ± 633 μm (median: 582 μm), respectively. The mean size of microplastics found in SLRE fish in this study was comparable to that reported for Lake Ontario fish (1.3 ± 3.5 mm) (Milne et al., 2024). When all fish species and tissues were considered together, microplastics found in fish from the Quebec City area (1699 ± 1165 μm) (median: 1378 μm) were significantly bigger than those from the Montreal area (1121 ± 917 μm) (median: 819 μm) (Mann-Whitney $p < 0.01$). This difference was driven by the significantly larger sizes of microplastics in the gills of walleye (1752 ± 1182 μm) than those in the gills of northern pike (1150 ± 967 μm) (Mann-Whitney $p = 0.01$) and yellow perch (419 ± 14 μm) (Mann-Whitney $p < 0.01$) (Fig. 6), as no differences were found for microplastic size in the GIT among different species (Kruskal-Wallis $p = 0.08$). Despite not being significant, a trend for larger microplastics was also observed in surface water from Quebec City (1031 ± 516 μm; median 1046 μm) relative to Montreal (735 ± 661 μm; median 477 μm) (Mann-Whitney $p = 0.06$) (Fig. 6). Smaller microplastics generally had higher mobility and toxicity and may pose greater risks to aquatic species in the Montreal area than those larger microplastics in the Quebec City area (Liu et al., 2024; Luo et al., 2025).

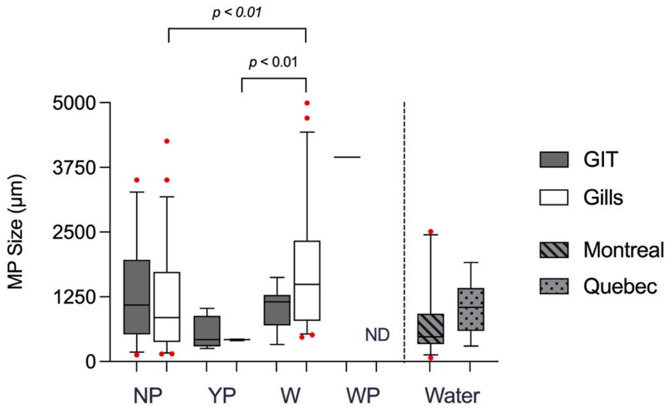

Fig. 6. Size distribution of the microplastics (MPs) found in the GIT and gills of northern pike (NP), yellow perch (YP), walleye (W), and white perch (WP), as well as in the SLRE surface water from Montreal and Quebec City areas. Box plots are defined as follows: center line, median; box plot edges, 25th and 75th quartiles; whiskers, 5th and 95th quartiles of the distribution. Outliers are shown in red. ND: not detected.

Regarding the impacts of effluent release from the WWTPs, no significant differences in size distribution were found between upstream and downstream samples (Fig. S4). It has been reported that WWTPs are effective in removing larger microplastics (e.g., >150 μm) and less effective for smaller microplastics, as larger particles are more readily trapped during sedimentation, coagulation, and filtration processes (Iyare et al., 2020; Kwon et al., 2022; Reza et al., 2024). Therefore, the influence of WWTPs on the distribution of microplastics may only focus on those with small sizes. To test this, we further compared the microplastic size composition (<100, 101–150, 151–500, 501–1500, 1501–3000, 3001–5000 μm) in water and fish between upstream and downstream sites, when samples where available. Nearly 20 % of the microplastics in the water downstream of the Montreal's WWTP were <100 μm, which was not found in the upstream water (Fig. S5). These results suggest that effluents from WWTPs in this region introduce smaller microplastics to the downstream aquatic environment and increase the abundance and composition of microplastics <100 μm. This novel observation provides rare field evidence that WWTPs are sources/vectors of 10–100 μm microplastics, a size fraction often overlooked in monitoring but critical for exposure and risk assessments. However, such differences were not detected in fish tissues (Fig. S5). Since the same density separation and filters were used for both water and fish samples, the detection of 10–100 μm microplastics in water and their absence in fish GIT and gills in this study were unlikely to be caused by the performance of density separation and filters for that size range. Therefore, the lack of 10–100 μm microplastics in the fish tissues may be partly due to the KOH digestion method, which differed from the WPO treatment of water samples. Although KOH digestion has been reported to cause surface degradation or decrease the recovery of certain microplastics under some conditions (Karami et al., 2017), there is no evidence showing the complete removal of microplastics in this size range due to KOH digestion. Our spike-recovery test showed a recovery rate of 90–100 %, indicating a high efficiency of the method. However, this test was not conducted for the size range of 10–100 μm. A more likely explanation is that biological processes, such as size-selective and tissue-specific retention or possible translocation of small microplastics, contributed to their absence in the fish GIT and gills (Luo et al., 2025; McIlwraith et al., 2021). This also aligns with McIlwraith et al. (2021), who used KOH digestion and reported the absence of <100 μm microplastics in the GIT of fish collected from Lake Simcoe, Ontario, Canada, while <100 μm microplastics were found in the fish fillet.

3.3. Chemical composition of detected microplastics

Ten types of polymers and copolymers were identified among the detected microplastics, with rayon being the predominant polymer (40–100 % in fish and 41 % in water) followed by PET (14–43 % in fish and 40 % in water) (Fig. 3C). These results suggest that semisynthetic polymers such as rayon are prevalent in aquatic environments and should be included in future microplastic monitoring programs rather than focusing exclusively on synthetic polymers. Predatory species (10 types of polymers and copolymers) were characterized by a greater polymer diversity than their potential fish prey (3 types of polymers and copolymers). Northern pike (10 types of polymers and copolymers) was the species with the highest diversity among all analyzed fish species (Fig. 3C). In surface water, seven polymers and copolymers were identified. Of the polymers identified, rayon constituted most particles such as fibers and films (Fig. 4B), revealing a potentially dominant but underreported role of semisynthetic polymer contamination in the aquatic environment. PET was only associated with the composition of fibers and a few films (Fig. 4B). Additionally, poly(methyl methacrylate) (PMMA) was only identified in fibers (Fig. 4B).

Fig. 7 illustrates the model-based ordination of polymer composition across samples. Fig. 7A shows that the polymers PET, rayon, and polyamide are located along the positive side of LV1 at around 1.0–1.5, indicating they are the main contributors to the observed variation. PET is located farthest to the right (LV1 ≈ 1.5, LV2 ≈ 0.2), suggesting PET largely drives the differences between samples. Rayon (LV1 ≈ 1.1, LV2 ≈ 0.4) also makes a large contribution, consistent with its widespread distribution throughout the system. Polyamide (LV1 ≈ 1.0, LV2 ≈ 0.0) is situated closer to downstream samples, implying its influence near WWTP discharges. Other polymers (e.g., PMMA, PE, copolymers, polyacrylamide (PAM)) cluster around LV1/LV2 at 0–0.5, indicating weaker contributions and minimal variation between sites or tissues. Fig. 7B compares tissues and particle shapes (fibers, fiber bundles, films, fragments). The polymers PET, rayon, and polyamide clustered near the fiber, confirming that fibrous microplastics dominated polymer variability in fish tissues. Films and fragments were spread across the ordination space and showed no consistent association with any specific polymer, indicating a mixed or low-abundance composition.

Analysis of microplastic polymer composition in water and fish gills from sites near the WWTP in Montreal, revealed greater diversity in downstream samples compared to those from upstream (Fig. S6). A quantitative source apportionment was not feasible due to data limitations and the scope of this study. Therefore, the discussion below focuses on qualitative evidence to identify the likely sources of microplastics in the SLRE water and fish. The dominant rayon and PET found in fish and water samples, especially fibers, are likely reflect inputs from textile manufacturing, pulp and paper effluents, and laundry discharges. In 2019, 270 textiles factories were registered in Quebec and these industries are centered around Montreal, Montérégie and Chaudière-Appalaches regions, all places close to our study areas (Ministère de l'Économie, de l'Innovation et de l'Énergie, 2022). Pulp and paper production is also a major industry in Quebec, with 38 mills operating as of 2020, many located near the SLRE (Nadon-Roger and Dufaux, 2022). Although no Quebec-specific microplastic emission data exist, a recent study of pulp and paper WWTPs in Finland found high microplastic concentrations in sludge (900-1600 particles/g dry weight in primary sludge; up to 210 particles/g dry weight in biosludge) (Yli-Rantala et al., 2024), suggesting that this sector can contribute substantial microplastic waste that may enter the SLRE. The co-occurrence of both rayon and PET in the aquatic system is frequently the results of water contaminated by sewage discharges (De Falco et al., 2020; Gies et al., 2018; Hernandez et al., 2017; McIlwraith et al., 2019). This is consistent with the findings that modified cellulose (e.g., rayon) and polyester (i.e., PET fibers) were the dominant microplastics in Canadian wastewater (Gies et al., 2018). In Montreal specifically, a citizen-science study estimated that installing washing-machine filters citywide could prevent ~12.8 tons of plastic microfibers per year from entering the wastewater network, highlighting laundry as important local source and pathway (Polytechnique Montreal, 2023). In addition, semisynthetic polymers such as rayon (i.e., modified cellulose) have relatively higher biodegradability compared to other polymers (Zambrano et al., 2019). Larger pieces of semisynthetic polymers could be broken down into particles smaller than 5 mm in the environment, increasing the occurrence of microplastics observed in aquatic systems (Lusher et al., 2013; Park et al., 2004).

In the present study, the polymer compositions in fish and water samples (i.e., dominated by rayon (density: ~1.50–1.52 g cm^{-3}) and PET (density: ~1.37–1.45 g cm^{-3})) were different from the polymer distribution observed in some other freshwater and estuarine systems, where low-density polymers (e.g., PE (density: ~0.91–0.94 g cm^{-3}) and polypropylene (PP) (density: ~0.90 g cm^{-3})) were found in surface water and fish (Oza et al., 2024; Shamskhany et al., 2021) (Table S1). For example, polymers identified in the GIT of fish from the Great Lakes, upstream of the SLRE, were predominantly PE (Munno et al., 2022), while PE was only a small proportion of the polymers found in fish from the SLRE. In contrast, like our results, previous studies found that the dominant polymer in SLRE benthic fish was also PET (rayon was not

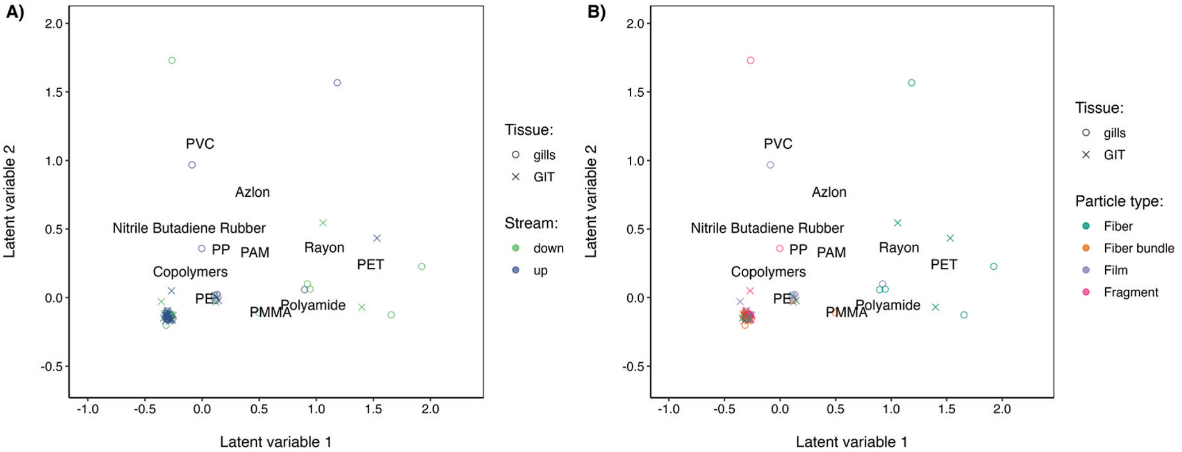

Fig. 7. Model-based ordination of microplastic polymer composition derived from a binomial copula model. Each point represents a sample positioned according to similarities in polymer composition based on residual correlations estimated by the model. The text labels (e.g., PET, rayon, polyamide) represent individual polymer types; their positions indicate how these polymers covary and contribute to overall patterns in the dataset. (A) Ordination grouped by tissue type (gills, GIT) and sampling position relative to wastewater treatment plants (upstream, downstream). (B) Ordination grouped by tissue type (gills, GIT) and particle type (fiber, fiber bundle, film, fragment).

analyzed), while rayon and PET were also found in SLRE mussels (Kabir et al., 2025; Rowenczyk et al., 2022). Our results are also consistent with observations from the Minho River and Estuary (Spain), the Mondego Estuary (Portugal) (Table S1), the South China Sea, where rayon and PET were prevalent polymers in water and/or fish GIT and gills, as well as water from the western English Channel, where rayon was the dominant microplastic (Bessa et al., 2018; Ding et al., 2019; Guilhermino et al., 2021; Steer et al., 2017). These observations might be explained by the fact that fibers are usually made of polymers with a density greater than that of water and are expected to sink in systems with low turbulence and stable water (e.g., lakes) (Lenaker et al., 2021; Rajak et al., 2022). But in highly dynamic systems like SLRE, with mean flows of ~12,200 m^3/s at Quebec City and a mean tidal of ~4 m at Quebec City (Pascal et al., 2017; Ryan et al., 2019), those fibers made of high-density polymers will remain in surface waters for a longer time due to strong mixing (Khatmullina and Chubarenko, 2021). These results also highlight the spatial variation of microplastic polymer compositions in aquatic environments and the need to improve microplastic monitoring in different regions to develop localized management policies against microplastic pollution.

3.4. Limitations, uncertainties and future work

In field studies, sample size of aquatic organisms is often limited by their catchability and by ethics guidelines. We acknowledge that the relatively small sample size for some species (e.g., white perch, $n = 10$) may limit statistical power and the ability to detect differences among species or sites. Therefore, the results should be interpreted with caution. Although statistically significant results were observed in our study, a larger sample size and a broader diversity of species, would likely have allowed us to confirm the significance of several additional observed trends. While the northern pike and yellow perch's sedentary nature (Leclerc et al., 2008; Ouellet-Cauchon et al., 2014) make them potentially effective bioindicators for assessing microplastic contamination from urban effluents, the movements of walleye remain poorly understood within the study area. This uncertainty represents an important limitation, as walleye migrating between zones may mask the spatial gradients in microplastic exposure and accumulation, potentially causing a biased estimation of the differences between upstream and downstream sites for this species. Nevertheless, given that microplastic concentrations primarily reflect short-term exposure dynamics (McIlwraith et al., 2021), unlike the bioaccumulation of many other contaminants, we argue that the comparative analysis of upstream and downstream sites is of ecological relevance for evaluating impacts of municipal effluents.

Using additional methods to analyze microplastic contamination in other tissues that are consumed, such as the muscle, could give accumulation information in these different fish species and the potential risk of microplastics for human health (Milne et al., 2024). Laboratory exposure studies have found that microplastics larger than 20 μm are unlikely to translocate from GIT to other tissues, whereas microplastics found in the muscle and liver of wild fish are generally in the range of 100–400 μm (McIlwraith et al., 2021). Analyzing microplastics in other tissues of wild fish from different systems would provide further information on both contamination mechanisms and toxicokinetics of microplastics.

As the exposure of species to microplastics during their lifespan may be affected by changing concentrations of microplastics in surface waters with time, temporal analyses would constitute a further step to this study to assess potential changes in microplastic contamination over different time scales.

Microplastic contamination often raises concerns about potential direct and indirect human exposure by their associated chemical additives. Therefore, measuring microplastic additives in the tissues of these species and associating the results with microplastic levels is integral to monitoring these habitats concerning microplastic pollution.

4. Conclusion

This study provides the first comprehensive investigation of microplastics, including semisynthetic polymers, in fish and surface water from the SLRE, a globally significant freshwater-marine continuum. Rayon, a semisynthetic material often excluded from microplastic studies, was the dominant polymer detected in both water and fish. This result underscores that omission of semisynthetic polymers from monitoring programs can underestimate environmental microplastic burdens. The higher microplastic abundance in gills compared to GITs in large upper-water-column piscivorous fish indicates that passive uptake is a major exposure pathway, which has implications for improving exposure assessment, toxicity tests, and ecological risk models. The predominance of blue and black rayon and PET fibers suggests major sources from textile industries, laundry effluents, and possibly pulp and paper operations in the SLRE region. Effluents from WWTPs did not significantly influence total microplastic abundance downstream but shifted size distributions toward particles <100 μm and increased polymer diversity. Such changes highlight that total microplastic counts alone may not adequately reflect WWTP performance or capture metrics relevant for ecological risk assessment. Given the higher potential of <100 μm microplastics for tissue translocation and toxicity, regulatory frameworks and waste treatment performance assessments should include size-specific microplastic measurements. Future research should enhance the method to reduce detection limits for particle size, enabling better identification of the smallest microplastics released from WWTPs and improving understanding of their distribution, fate, and risks. These results also highlight the need to further evaluate the adverse effects of these microplastics on the species in the SLRE at environmentally relevant concentrations and sizes. This is particularly necessary for semisynthetic polymers such as rayon, as very little information is available on the toxicity of the physical rayon fibers and associated chemicals (e.g., dyes or other associated contaminants) to aquatic species.

CRediT authorship contribution statement

Elisa Michon: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis. **A.H.M.Enamul Kabir:** Writing – review & editing, Methodology. **Magali Houde:** Writing – review & editing, Resources, Methodology, Conceptualization. **Marc Mingelbier:** Writing – review & editing, Resources. **Youssouf D. Soubaneh:** Writing – review & editing, Resources, Methodology. **Jennifer F. Provencher:** Writing – review & editing, Methodology, Funding acquisition. **Huixiang Xie:** Writing – review & editing, Resources, Funding acquisition. **Dominique Robert:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Zhe Lu:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could appear to have influenced the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2025.123170.

Data availability

Data will be made available on request.

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