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Microplastics in the benthic fish from the Canadian St. Lawrence River and Estuary: Occurrence, spatial distribution and ecological risk assessment

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ABSTRACT

Microplastic contamination in the St. Lawrence River and Estuary (SLRE), Canada, poses potential risks to aquatic species. However, limited understanding of microplastic contamination in benthic fish, potentially more vulnerable than pelagic species, impedes effective risk assessment in this crucial ecosystem. This study addressed knowledge gaps by analyzing microplastics in the gastrointestinal tracts (GIT) and gills of Channel Catfish (*Ictalurus punctatus*) and Atlantic Tomcod (*Microgadus tomcod*) in the SLRE. Forty-two fish from ten stations were examined using KOH digestion, density separation, wet-peroxidation, and spectroscopy. Results indicated an average abundance of 3.0 ± 0.4 (mean ± SE) microplastic particles per individual fish. Most detected particles were small microplastics (<809 μm) and fibers, with blue and transparent colors. Major polymers identified included polyethylene terephthalate and polyethylene. While catfish showed higher microplastic abundances per individual than tomcod, data based on GIT weight do not support microplastic biomagnification in this predator-prey relationship. Catfish from downstream of Québec City showed elevated levels of microplastics and more variations in their characteristics compared to average abundance found from a site located 50 km upstream. Urban activity may increase microplastic accumulation in downstream benthic fish and others. This highlights the need for further studies on the migratory capacities of fish species. Ecological risk assessment revealed medium to high-risks for the catfish stations close to the Québec City due to the prevalence of smaller microplastics <809 μm and highly toxic polymers (polymethyl methacrylate, polyvinylchloride, polyurethane, acrylonitrile butadiene styrene). This study provides a baseline for monitoring plastic pollution in the SLRE fish and assessing ecological risks.

1. Introduction

Microplastics, tiny plastic particles ranging from 1 to 5000 μm, constitute a pervasive pollution impacting aquatic environments worldwide, either in their original or after undergoing degradation (Auta et al., 2017; Bergmann et al., 2017). In aquatic environments, microplastics can travel long distances, accumulate in water and sediments, and be ingested by various organisms (e.g., zooplankton, bivalves, shrimp, fish, etc.) (Ribeiro et al., 2019; Gao et al., 2023). With varying sizes, chemical compositions, densities, shapes, and colors, microplastics are persistent, bio-accumulative, toxic, and transport hazardous contaminants (e.g., pathogens, organic pollutants, metals) in the environment (Osman et al., 2023; Miller et al., 2020; Rochman et al., 2019). Consequently, microplastics poses a significant and growing threat to aquatic life.

Microplastics have been found in different fish organs (e.g., liver, muscles, brain, and gonads) in various aquatic systems (Ding et al., 2018; Su et al., 2019; Solomando et al., 2022), with the GIT and gills receiving the most attention due to their importance in elucidating exposure pathways (e.g., ingestion and entanglement) and risks through passive or active ingestion (Zazouli et al., 2022; Lin et al., 2023; Roch et al., 2020). Ingested microplastics can temporarily reside in the GIT and translocate within body through intestine systems, contributing to the prolongation of tissue exposure to microplastics and associated

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contaminants in the fish body (Ma et al., 2020; McIlwraith et al., 2021). Additionally, gills can provide another pathway of exposure to microplastics (Zhang et al., 2021). Batel et al. (2018) found that plastics of 1–20 μm were consistently filtered through zebrafish (*Danio rerio*) gills, adhering superficially to fish filaments. Studies also found that microplastics could lead to various adverse physiological effects in fish, including digestive tract blockage (Wright et al., 2013); neurotoxicity, oxidative damage and energy-related changes (Barboza et al., 2018); decreased feeding, gills impairment, weakened immunity, and reduced reproduction (Ma et al., 2020; Mallik et al., 2021). Moreover, microplastics associated toxic substance (e.g., additives, adsorbed contaminants) can impact feeding behavior, growth, and mortality rate of fish (Ma et al., 2020; Jovanović, 2017). Since the toxicological risks of microplastics in fish depend on exposure and accumulation, it is essential to understand the occurrence, distribution, and fate of microplastics in fish organs such as the GIT and gills.

Benthic aquatic environments, located at the bottom of water bodies, serve as critical sinks for contaminants such as microplastics and persistent organic pollutants (Lenaker et al., 2021; Krasnobaev et al., 2020). These environments are also ecologically critical as they support diverse species, including fish. Benthic species are potentially more vulnerable to microplastics contamination than pelagic species due to their close interaction with both sediment beds and the water column (Keerthika et al., 2023; Merga et al., 2020; Bellasi et al., 2020; Bessa et al., 2018). However, there is still a lack of comprehensive understanding regarding the occurrence, distribution, and fate of microplastics in benthic fish within freshwater and estuarine environments. The environmental behavior of microplastics is influenced by factors such as particle polymer type & density, shape, size, fluid density, environmental and hydrodynamic processes such as biofouling, aggregation, current, tides, waves etc. These properties influence the migration, settling, resuspension, and dispersion of microplastics within the water column and sediment layers (Chubarenko et al., 2016, 2018; Kooi et al., 2018; Kowalski et al., 2016). For instance, high-density particles (>1.0 g cm^{-3}) tend to sink and accumulate at the bottom of aquatic systems quickly (Chubarenko et al., 2016), potentially impacting their interactions with various species. Measuring the occurrence of microplastics in benthic freshwater and estuarine species can provide insights into what types of microplastics and compositional characteristics have the largest potential impacts on benthic ecosystems and their associated biota. This knowledge can in turn inform on the risks of microplastic contamination and facilitate the development of policies to address these challenges.

The St. Lawrence River and Estuary (SLRE) in Canada, originating from the Laurentian Great Lakes, sustains diverse ecosystems crucial for millions of Canadians. The St. Lawrence Estuary is one of the largest estuaries in North America and a highly dynamic part of the SLRE, stretching from west to east for 655 km, from the St. Lawrence River (Lake Saint-Pierre) to the Gulf of St. Lawrence (Pointe des Monts) in Québec, Canada. The circulation of the estuary is driven by strong tidal currents due to a large volume of water flowing through a narrow section (Simons et al., 2010). Microplastics have recently been detected in the water and sediment in the SLRE, raising concerns about the potential exposure of benthic aquatic species to microplastics (Rowenczyk et al., 2022; Crew et al., 2020; Castañeda et al., 2014). However, little is known about microplastic contamination in benthic species in this highly dynamic and energetic part (i.e., the St. Lawrence Estuary) of the SLRE. Addressing this knowledge gap is vital for a comprehensive understanding of the microplastic contamination and risk assessment in the SLRE as well as informing policy decisions.

The channel catfish (*Ictalurus punctatus*) (hereafter catfish) is a crucial benthic predator in the SLRE, consuming a variety of prey like insects, snails, aquatic plants, and small fish (Page and Burr, 1991; Wellborn, 1988). It is globally significant for fishing and aquaculture, driving the catfish farming industry in North America. In 2022, catfish contributed 57.61 million Canadian dollars in freshwater fish imports to Canada (Fisheries and Oceans Canada, 2023) and is the most targeted catfish species in the U.S. by 8 million anglers annually, leading to rapid aquaculture expansion (Carlander, 1969; USDA, 2024). Another important benthic fish in the SLRE is the Atlantic tomcod (*Microgadus tomcod*) (hereafter tomcod), a prey species for the catfish. Native to the western Atlantic Ocean, from the Gulf of St. Lawrence to the Hudson River, tomcod is notable for its adaptability to a wide range of salinities and genetic resilience to toxic pollutants such as polychlorinated biphenyls and 2,3,7,8-tetrachlorodibenzodioxin, making it a crucial bioindicator in pollution research (Wirgin et al., 2023, 2011). Overall, both catfish and tomcod are ecologically and economically significant, important for human consumption across the Atlantic Ocean and North America. However, uncertainties about microplastic contamination in these two species persist.

Fish species ingest a wide range of microplastics with various characteristics, such as shapes, sizes, and polymer types, in aquatic environments (Parker et al., 2020), posing ecological risks. Recent studies utilize metrics like the Pollution Load Index *(PLI)* (Tomlinson et al., 1980), Polymeric Hazard Index (*PHI*) (Lithner et al., 2011), and Ecological Risk Index *(ERI)* (Hakanson, 1980) to evaluate microplastic ecological risks (Pan et al., 2021; Peng et al., 2018; Pandey et al., 2023). These models identify which plastics are highly toxic and at what abundances microplastics become low to highly hazardous (Pan et al., 2021; Peng et al., 2018). They can also link toxic plastics to specific land-use sources, helping to determine which land-use sources could impact certain ecosystem parts. This information could aid in understanding and managing microplastic pollution and risks. Furthermore, while the shape and size of microplastic particles significantly influences their risks (e.g., smaller microplastics and fibers are more prone to be ingested by aquatic life), current ecological risk assessment models often exclude microplastic particle size as a risk factor, which could lead to incomplete evaluations of ecological risks. Incorporating particle size into the risk models could advance the understanding of ecological risks, as well as inform policy decisions and management.

The objectives of this study were to (1) investigate the profiles of microplastic contamination in two benthic fish species (catfish and tomcod) from the SLRE, focusing on their occurrence, distribution, and land-use-related sources; and (2) assess the ecological risks of microplastics to the benthic environment across the study area using catfish and tomcod as indicators using modified risk indices. We hypothesized that (1) microplastic contamination profiles differ between the gills and GIT of benthic fish due to varied exposure pathways, (2) urban activities increase microplastic accumulation abundance and diversity in downstream benthic fish, and (3) microplastic size is a significant factor influencing ecological risk modeling results, with smaller microplastics posing higher ecological risks. To our knowledge, this is the first study on microplastic contamination in fish from the SLRE and on ecological risk assessment in this region.

2. Materials and methods

2.1. Sampling

Catfish (n = 26) and the forage species tomcod (n = 16) were collected from the SLRE, close to Québec City, aboard the *R/V Lampsilis* between 22 September and 4 October 2022. We selected study sites near Québec City, including upstream, downstream, and distant locations, to examine whether varying levels of land use (e.g., urban) activity affect microplastic contamination profiles. For catfish, samples were captured from Cap-Santé (CC1, $n = 10$), Québec City (CC2, $n = 1$; CC3, $n = 2$; CC4, $n = 4$), and east of Île d'Orléans, (CC5, $n = 5$; CC6, $n = 3$; CC7, $n = 1$) (Fig. 1). Tomcod samples were collected at Pointe-au-Pic (AT1, $n = 5$ and AT2, $n = 10$) and Rivière-du-Loup (AT3, $n = 1$) (Fig. 1). Fish were caught using a bottom trawl (depth: 10–50 m from the water surface), transported to the laboratory after landing, wrapped in aluminum foil and refrigerated immediately at −20 °C.

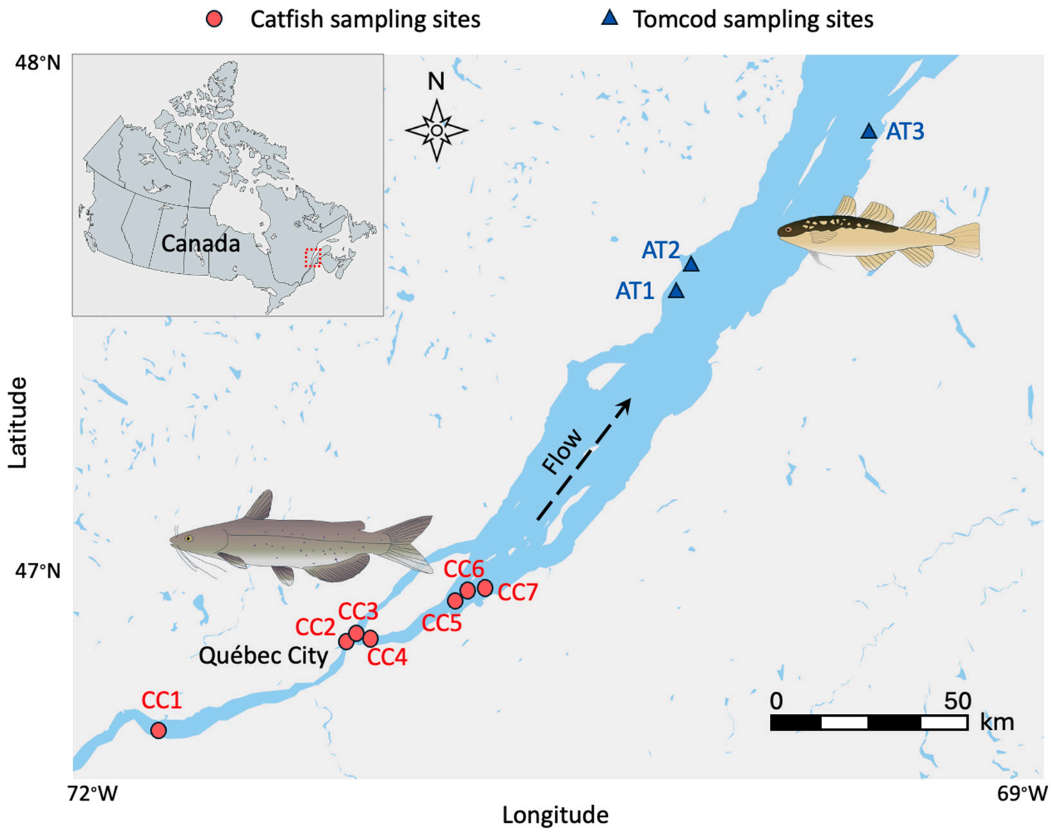

Fig. 1. Sampling stations of channel catfish and Atlantic tomcod in the St. Lawrence River and Estuary, Québec, Canada. CC and AT represent the sampling stations for the channel catfish and Atlantic tomcod, respectively.

2.2. Materials and chemicals

Zinc chloride ($ZnCl_2$, anhydrous, reagent grade, ≥98 %), potassium hydroxide (KOH, ACS reagent, ≥85 %, pellets), hydrogen peroxide (30 % *w/w*, stabilized reagent grade), sulfuric acid (H_2SO_4, ACS reagent, 95.0–98.0 %), iron (II) sulphate heptahydrate ($FeSO_4.7H_2O$, ≥99 %), polytetrafluoroethylene (PTFE, 5.0 μm pore size, Omnipore, filter diam. 47 mm) and mixed cellulose ester (MCE, 1.2 μm pore size, MF-Millipore™, filter diam. 47 mm, hydrophilic) membrane filter papers were purchased from Sigma-Aldrich Canada (Oakville, Ontario, Canada). Standard Brass Sieves (aperture size of 20 μm) with steel cloth were purchased from Fisher Scientific (Ottawa, Ontario, Canada).

2.3. Sample preparation

Each fish was thawed for 12–24 h and cleaned using nanopure water to eliminate any externally attached materials on the fish body (Lusher et al., 2016). The body length (cm) and weight (g) of the fish were recorded (Table 1). The fish was dissected on an aluminum tray using scissors, scalpels, and forceps. The GIT and gills were carefully removed, transferred to a 500 mL clean glass beaker, weighed (g) (Table 1), and covered with aluminum foil to prevent external contamination. The GITs and gills were then prepared for the extraction of microplastic candidates (Section 2.4). All gills per fish were analyzed together and the results were thus based on the total gills per fish.

2.4. Microplastics extraction

The GIT and gill samples underwent KOH digestion, wet peroxidation (WPO), and density separation to extract the microplastic candidate particles. In brief, the GITs and gills were digested in 10 % KOH at 40 °C over 72 h. After complete digestion, the liquid was filtered through a stainless-steel sieve (aperture size: 20 μm) and all the remaining solids were transferred to a clean beaker. Then, 100 mL of $ZnCl_2$ solution with a density of 1.5 g cm^{-3} (972 g $ZnCl_2$ per liter of H_2O) were poured into the beaker (Coppock et al., 2017), and the solids were allowed to settle for at least 24 h. The solids from the supernatant were collected onto a 5-μm PTFE filter. Organics in the collected solids were removed by subjecting the solids to WPO at 65 °C in a mixture of 20 mL of $FeSO_4{\cdot}7H_2O$ solution and 20 mL of 30 % H_2O_2 (Masura et al., 2015). The WPO process was repeated, if needed, in a smaller volume of the mixture of the $FeSO_4{\cdot}7H_2O$ solution (10 mL) and 30 % H_2O_2 (10 mL) until the

Table 1
Information on studied fish species and their corresponding levels of microplastics ingestion.

	Sample (n)	Length Range [Mean (cm) ± SE]	Weight Range [Mean (g) ± SE]	GIT Weight [Mean (g) ± SE]	Gills Weight [Mean (g) ± SE]	Mean Microplastics/g BW	Mean Microplastics/g GIT	Mean Microplastics/g Gills
Atlantic Tomcod	16	11–25 (20.3 ± 0.8)	19.7–115 (80.1 ± 5.2)	1.07–18 (10.3 ± 1.0)	0.6–6.5 (2.6 ± 0.3)	0.03 ± 0.01	0.21 ± 0.06	0.71 ± 0.52
Channel Catfish	26	24–59 (40.31 ± 2.4)	170–1950 (768.46 ± 93.7)	5.68–140 (56.21 ± 8.01)	30.1 ± 3.89	0.01 ± 0.001	0.07 ± 0.01	0.07 ± 0.02

organics are visually eliminated. The candidate microplastic particles in the WPO-treated liquid were collected onto a 1.2-μm MCE membrane filter. All steps of sample preparation and microplastics extraction were completed under a controlled biological and chemical fume hood with stainless-steel surface.

2.5. Microscopic observation, identification, and characterization

The extracted particles were observed using a microscope (BX 53, Olympus, Japan) equipped with a camera (DP71, Olympus, Japan) and operated with Stream Essential 2.5 version software. The isolated microplastic candidate particles were examined and/or quantified for shapes, sizes, and colors. Five size classes (26–809 μm, −1102 μm, −1395 μm, −1688 μm, and − 5000 μm) were used to conduct size-based ecological risks assessment, in accordance with Yuan et al., 2022. Subsequently, the particles underwent micro-Fourier transform infrared spectroscopy (μ-FTIR, Perkin Elmer 200i Spotlight) and Thermo DXR Raman Microscope (μ-Raman) analysis to confirm the identities of microplastics and their polymer composition through chemical imaging. Before each μ-FTIR run, the instrument was filled with liquid nitrogen and allowed to stabilize for 30 min. Background signals were acquired using a blank sample for each measurement. Spectra were generated at a resolution of 8 cm^{-1} with 32 background scans, and infrared wavenumbers ranged from 4000 to 700 cm^{-1}. After μ-FTIR analysis for each particle, spectrum data were collected using Perkin Elmer's Quant v.2.0 software. The gathered spectra were then compared to the spectral reference. μ-Raman spectra were acquired using the 532 nm laser line. Standard parameters included a green line, 25 & 50 μm aperture, 10 mm focal length, 10 mW laser power, and 10-50× microscopic objects. Laser power was adjusted to prevent sample damage or particle degradation. Exposure time and repetitions, and objects were optimized for noise-to-signal ratios. Spectra were processed with OMNIC for background removal and identified using OMNIC polymer library.

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

Nanopure water was employed to rinse fish dissecting materials (e.g., aluminum tray, scissors, and forceps) thoroughly before, during, and after use. Cotton-made laboratory coats, masks, and nitrile gloves were used. The $ZnCl_2$, KOH and $FeSO_4.7H_2O$ solutions were passed through a 5-μm PTFE membrane filter before each use. The extracted microplastic particles were stored in glass Petri dishes and/or glass vials, covered with aluminum foil to prevent external contamination.

Different control samples were prepared to examine background contamination of Microplastics. Laboratory blanks ($n = 8$) were obtained with MCE filter papers exposed to the air in the fume hood. Procedural blanks ($n = 8$) were acquired using Nanopure water (250 mL/sample) as clean samples to detect contamination during analytical procedures. Field blanks ($n = 9$) were collected by exposing aluminum sheets to open air during sample collection and handling of fish samples. To measure the field blanks, each aluminum foil was opened under the fume hood, rinsed with Nanopure water, and processed through filtration (MCE, 1.2 μm pore size, MF-Millipore™, filter diam. 47 mm, hydrophilic). All laboratory, procedural and field blank samples underwent the filtration and WPO described earlier to extract the potential microplastic particles. Only synthetic plastic particles were considered in the total microplastic particle count. Non-microplastic particles (e.g., cellulose, rayon, azlon and others) were identified and subtracted from the particle count. Spike-recovery test ($n = 3$) was performed by digesting fragments, films, and fibers of high-density polyethylene (HDPE), low-density polyethylene (LDPE), polyethylene terephthalate (PET), and polypropylene (PP) polymers with sizes between 100 and 5000 μm and colors of transparent, red, white and green, yellow with fish soft tissue in a 10 % KOH solution over 72 h to measure digestion efficiency and recovery, following Karami et al. (2017) guidelines.

2.7. Biomagnification factor (BMF)

BMF was determined by dividing the concentration of microplastics within the GIT of the predator catfish i.e., number of microplastic particles per kilogram fish body weight (n/kg) and tissue weight (n/kg) within the GIT of the prey tomcod multiplied by the trophic level difference between the predator and the prey. The BMF formula can be expressed as:

$$BMF = C_i / C_j \cdot (TL_i - TL_j) \tag{1}$$

where C_i signifies the microplastic concentration in the predator, C_j the microplastic concentration in the prey, TL_i the trophic level of the predator, and TL_j the trophic level of the prey. As the trophic level data for the SLRE is unavailable, values were sourced from https://fishbase.mnhn.fr/. A BMF value >1 indicates likely biomagnification, signifying an elevation in microplastic concentration at higher trophic levels, as exemplified by the catfish in this investigation.

2.8. Microplastic contamination ecological risk assessment

In this study, we improved ecological risk models by incorporating size-based risk factors *(SRI)* alongside microplastic abundance, *PLI, PHI,* and *ERI* (Table 2). We applied these models to assess microplastic contamination ecological risks in the benthic areas of the SLRE, using catfish and tomcod as indicator species. This approach provides broader insights into the ecological risks of microplastic contamination.

2.9. Data analysis

Statistical analyses were conducted using Microsoft Excel (Version: 16.82, 2,402,116), and PAleontological STatistics (PAST) software (Version 4.16) (Hammer et al., 2001). Descriptive analysis included calculation of maximum, minimum, median, mean, and standard error (SE) values. Because residuals did not distribute normally, a non-parametric Kruskal-Wallis *H* test was employed to identify significant differences in microplastic abundance. To explore relationships, a non-parametric Spearman correlation test was applied to assess the associations. The significance threshold *(p)* for all statistical tests was set at 0.05. The reporting unit for microplastics abundances was expressed as 'n' where 'n' signifies the number of microplastic particles per individual fish (n/fish) or GIT (n/GIT) or gill (n/gill).

3. Results

3.1. QA/QC results

QA/QC testing assessed spike-recovery performance and background contamination across the laboratory (fume hood), analytical procedures, and field sampling. The KOH digestion of fish soft tissues over 72 h achieved a digestion efficiency of 99.0 ± 0.00 % ($n = 3$), based on pre- and post-digestion tissue weight differences. Recovery tests yielded 97.4 ± 0.02 % (n = 3) particle recovery for HDPE, LDPE, PET, and PP polymers, aligning with acceptable limits (Karami et al., 2017).

A total of 18 particles were detected in fume hood, procedural, and field blanks, of which 14 (77.8 %) were non-microplastic particles (e.g., cellulose, rayon, azlon), and 4 (22.2 %) were microplastics, specifically PET (n = 3) and PE ($n = 1$). The fume hood filter paper blanks ($n = 8$; 1 blank per batch experiment) contained only one PET particle, averaging 0.13 ± 0.1 particles per 47 mm diameter filter paper, indicating minimal laboratory-derived microplastic contamination. Similarly, procedural blanks (n = 8) showed negligible procedural contamination, with one PET particle, averaging 0.13 ± 0.1 particles per 250 mL sample. Field blanks ($n = 9$) demonstrated minimal environmental contamination, with an average of 0.2 ± 0.15 microplastic particles per aluminum sheet (1 PE and 1 PET particle in total). Blank analysis results are summarized

Table 2
Risk indices, their limit values, and levels used to assess microplastic contamination risk in the benthic areas from the SLRE, Canada.

Indices and Equations	Explanations	Limit Values and level	Score	Reference (s)
Contamination Factor (CF) and Pollution Load Index (PLI)				
$CFi = C_i/C_o$ $PLI_s = \sqrt[n]{PLI_1 \times PLI_2 \times PLI_3 \cdots\cdots PLI_n}$	CF_i is the contamination factor at the individual fish i; C_i is the abundance of MP particles at the individual fish i; C_o is the minimum mean background abundance baseline concentration ($C_o = 0.7$ MPs/marine bivalve was taken from Rowenczyk et al. 2022 due to its similarity to the species and same ecosystems); PLIs (Pollution Load Index) is the pollution load index at the station s;	< 1 Low >1 Contaminated 1 – 3 Medium 3 – 6 High >6 Very High		Tomlinson et al., 1980; Hakanson, 1980
Risk Factor 1 (RF 1): Particle Size & Abundance				
$SRI_i = \sum_{j=1}^{m}\{(S_{ji}/C_i) \times R_j\}$ $SRI_i = SRI_i \times PLI_i$ $SRI_s = \sqrt[n]{SRI_1 \times SRI_2 \times SRI_3 \ldots\ldots \times SRI_n}$	S_{ji} is the number (m) of particles for each single size range (j) identified at the fish individual i; R_j is the hazard score for each size range type. SRF_i represent the size risk index at the fish individual i; SRF_s represent the size risk index at the station s	**Size (µm)** > 26 – <809 Very High >809 – <1102 High >1102 – <1395 Moderate >1395 – <1688 Low >1688 – <5000 very Low	 5 4 3 2 1	This study following Yuan et al., 2022
Risk Factor 2 (RF 2): Polymer Toxicity & Abundance Polymeric hazard Index (PHI)				
$PHI_i = \sum_{j=1}^{m}\{(P_{ji}/C_i) \times S_j\}$ $PHI_s = \sqrt[n]{PHI_1 \times PHI_2 \times PHI_3 \ldots\ldots \times PHI_n}$	P_{ji} is the number (m) of particles for each single polymer (j) identified at station i; S_j is the hazard score for each polymer type; PHIs (Polymeric Hazard Index) represent the polymeric hazard index at the station s	< 10 Very Low 10 – 100 Low 101 – 1000 Medium 1001 – 10,000 High >10,000 Very High	1 2 3 4 5	Lithner et al., 2011
Contamination Risk Index (CRI)				
$CRI_i = PHI_i \times PLI_i$ $ERI_i = \sqrt[n]{CRI_1 \times CRI_2 \times CRI_3 \ldots\ldots \times CRI_n}$ $ERI_s = \sqrt[n]{CRI_1 \times CRI_2 \times CRI_3 \ldots\ldots \times CRI_n}$	ERI_i is the MP ecological risk index at the fish individual i; ERIs (Ecological Risk Index) is the risk index at station s	<150 Very Low 150 – 300 Low 300 – 600 Medium 600 – 1200 High >1200 Very High	1 2 3 4 5	Kabir et al., 2022; Pan et al., 2021; Peng et al., 2018; Hakanson, 1980
Cumulative Contamination Risk (CCR)				
$CCR_s = \sqrt[n]{RF_1 \times RF_2 \times \ldots\ldots \times RF_n}$	CCRs (Cumulative Contamination Risk) represents the cumulative contamination risk at the station s	Very Low Low Medium High Very High	1 2 3 4 5	

in Fig. 2A.

Notably, PTFE particles were absent from all fish samples, confirming that the PTFE membranes used in the experiments did not contribute microplastics. Microplastics in all blanks represented 3.1 % of the total microplastic particles detected in fish samples, well below the recommended threshold of <10 % (Dimitrijevic et al., 2019). Corrections for microplastic contamination in controls were made by subtracting blank results from total plastic particle counts based on shape, color, and polymer. Overall, the control results indicated a negligible presence of the background contamination of microplastics, suggesting that the microplastic abundance in fish samples significantly exceeded that in controls (Kruskal-Wallis *H* Test, *p*-value = 0.0001, df = 9).

3.2. Microplastic abundance, spatial and tissue distributions, and biomagnification factor in the benthic fish from the SLRE

In total, 125 microplastic particles were extracted from the GIT and gills of the catfish and tomcod, with 91 % of fish containing at least one microplastic particle. Microplastic abundances varied from 0 to 11 n/fish (GIT and gills are considered together; mean ± SE: 3.0 ± 0.4 n/fish), with a median of 2.0 n/fish across all sites and species. The highest microplastic abundance (11 n/fish) was found in the catfish at the station CC7, while tomcod showed the lowest abundance (1.6 ± 0.8 n/fish) at the station AT2. Although no statistically significant difference was observed (Kruskal-Wallis *H* Test, *p*-value = 0.146, df = 41), catfish showed a trend of higher microplastic abundances (3.6 ± 0.6 n/fish) compared to tomcod (1.9 ± 0.3 n/fish).

The microplastic distribution results in fish revealed an average microplastic abundance of 1.4 ± 0.27 n/GIT in tomcod, which was significantly higher than in their gills (0.50 ± 0.16 n/gills) (Kruskal-Wallis *H* Test, *p*-value = 0.01 < 0.05, df = 31). On the other hand, the microplastics abundance was not significantly different between the GIT (1.8 ± 0.24 n/GIT) and gills (1.9 ± 0.47 n/gills) of catfish (Kruskal-Wallis *H* Test, *p*-value = 0.3 > 0.05, df = 51). There was no significant difference in microplastic abundance in GIT (Kruskal-Wallis *H* Test, *p*-value = 0.36 > 0.05, df = 41) or gills (Kruskal-Wallis *H* Test, *p*-value = 0.09 > 0.05, df = 31) between catfish and tomcod, implying a similar level of ingestion of microplastics by the GIT or gills between the two species.

Microplastic distribution based on body weight (BW), or tissue weight showed that tomcod had a higher abundance (0.03 ± 0.01 n/g fish BW) than catfish (0.01 ± 0.00 n/g fish BW). Also, the occurrence and uptake of microplastics by GIT and gills differed between the species. Tomcod had higher microplastic abundances in both their GIT (0.21 ± 0.06 n/g GIT) and gills (0.71 ± 0.52 n/g gills) compared to catfish (0.06 ± 0.01 n/g GIT and 0.07 ± 0.02 n/g gills), on a tissue

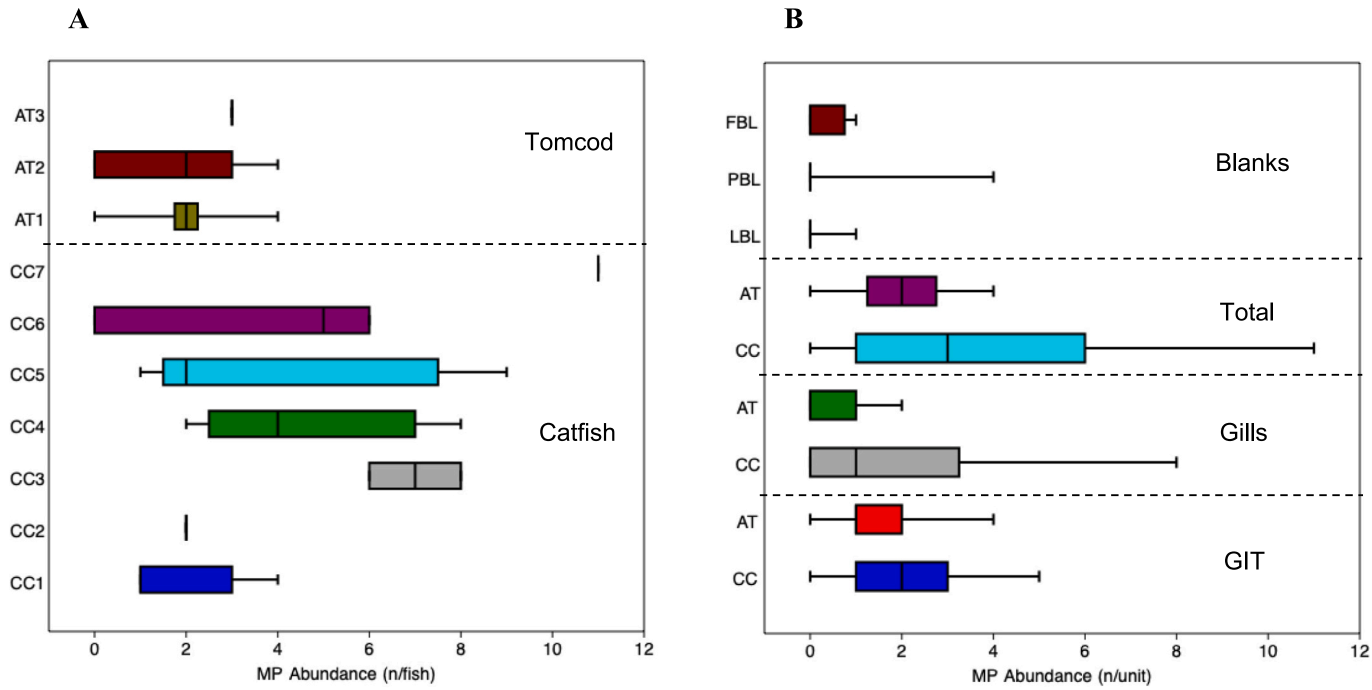

Fig. 2. Distribution of microplastics in the (A) fish from different study sites and in the (B) channel catfish (CC) and Atlantic tomcod (AT) tissues. Boxplots are defined as follows: center line, median; boxplot edges, 25th and 75th percentiles; whiskers, 5th and 95th percentiles. LBL, PBL, and FBL represent laboratory, procedural, and field blanks, respectively.

weight basis (Table 1).

Considering both species together, our analysis revealed a positive correlation between biological variables and the abundance of microplastics among analyzed fish. Specifically, we found positive correlations between microplastic abundances and fish body weight (Spearman Rho correlation, $r = 0.33$, p-value $= 0.02 < 0.05$, df $= 41$), length (Spearman Rho correlation, $r = 0.34$, p-value $= 0.03 < 0.05$, df $= 41$), and gills weight (Spearman Rho correlation, r $= 0.33$, p-value $= 0.03 < 0.05$, df $= 41$) (Fig. 3A–D), thus influencing microplastic accumulation in fish. Therefore, although not statistically significant (catfish gills vs. tomcod gills: Kruskal-Wallis H Test, p-value $= 0.09 > 0.05$, df $= 31$) in this study, catfish with larger gills might accumulate a higher number of microplastics (1.9 ± 0.47 particles/gills) compared to tomcod (0.50 ± 0.16 particles/gills) with smaller gill.

Spatially, for catfish, the samples from the downstream sites (CC2–7) of Québec City showed significantly higher microplastic abundance (4.75 ± 0.79 n/fish) than those from 50 km upstream (CC1) (1.9 ± 0.38 n/fish) (Kruskal-Wallis H Test, p-value $= 0.02 < 0.05$, df $= 25$) (Fig. 1, Fig. 2A). No significant spatial variations were found among three sampling sites for tomcod (Fig. 2A).

Predator-prey BMF analysis revealed fish BW based BMF value $= 0.5 < 1$ (GIT weight based BMF value $= 0.57 < 1$; gill weight based BMF value $= 0.38 < 1$) between the catfish and tomcod in the SLRE.

3.3. Microplastic characteristics: Shape-size-color-polymers

3.3.1. Shape

Analysis of extracted microplastic particles revealed three distinct shapes: fragments, films, and fibers. Fibers emerged as the predominant shape in both the GIT and gills of catfish and tomcod, constituting 66.4 % of the total microplastics, followed by films at 17.6 %, and fragments at 16.0 %. Spatial distribution showed that the catfish from the upstream counterpart CC1 were exclusively contaminated with fibers, whereas downstream CC2–7 fish samples showed contamination by fragments (28 %) and films (31 %) in addition to fibers (41 %). Regarding the tomcod, station AT1 indicated primary contamination with microplastic fibers, accompanied by a few films, while AT2-AT3 samples were solely contaminated with fibers. The shape-based results are summarized in Fig. 4A.

3.3.2. Size

Smaller microplastics, particularly in the range of 26–809 μm, dominated, constituting >40 % of all microplastics, followed by 809–1102 μm (20 %), 1102–1395 μm (14 %), 1395–1688 μm (11 %), and 1688–5000 μm (15 %) when considering all fish GIT and gills together. The prevalence of smaller microplastics (26–809 μm) was consistent in the GIT of both catfish (48 % of total microplastics in GIT) and tomcod (43 %), indicating similar size-based occurrence of microplastics in the GIT of these species. However, in gills, particles >809 μm dominated in the tomcod (38 %), while particles <809 μm were prevalent in the gills of catfish and consistent with the GIT (Fig. 4B). The across-sites size distribution of microplastics showed a predominance of smaller microplastics of 26–809 μm in the catfish over all sites from CC1 to CC7 (42–100 %), exhibiting the higher abundance of smaller particles from 26 to 809 μm. Remarkably, all the particles from the CC2 were smaller (26–809 μm) microplastics. In contrast, tomcod exhibited distinct distribution based on size across sites, with AT1 showing a prevalence of smaller particles <809 μm, whereas AT2 and AT3 showed most microplastics ranging from 809 to 1102 μm and 1688 to 5000 μm, respectively (Fig. 4B).

3.3.3. Color

The microplastic particles extracted from the catfish and tomcod samples exhibited a diverse range of colors, with blue (50 %) and transparent (19 %) emerging as the major colors, followed by white (8 %), red (6 %), black (6 %), violet (6 %), yellow (2 %), green (2 %), and grey (1 %) (Fig. 4C). For each fish species, at least seven distinct colors of microplastics were found, with blue being the sole color present across all samples and species. A comparative analysis of fish species showed a higher prevalence of transparent and white particles in the gills compared to the GIT (Fig. 4C).

The color-based distribution of microplastic particles revealed

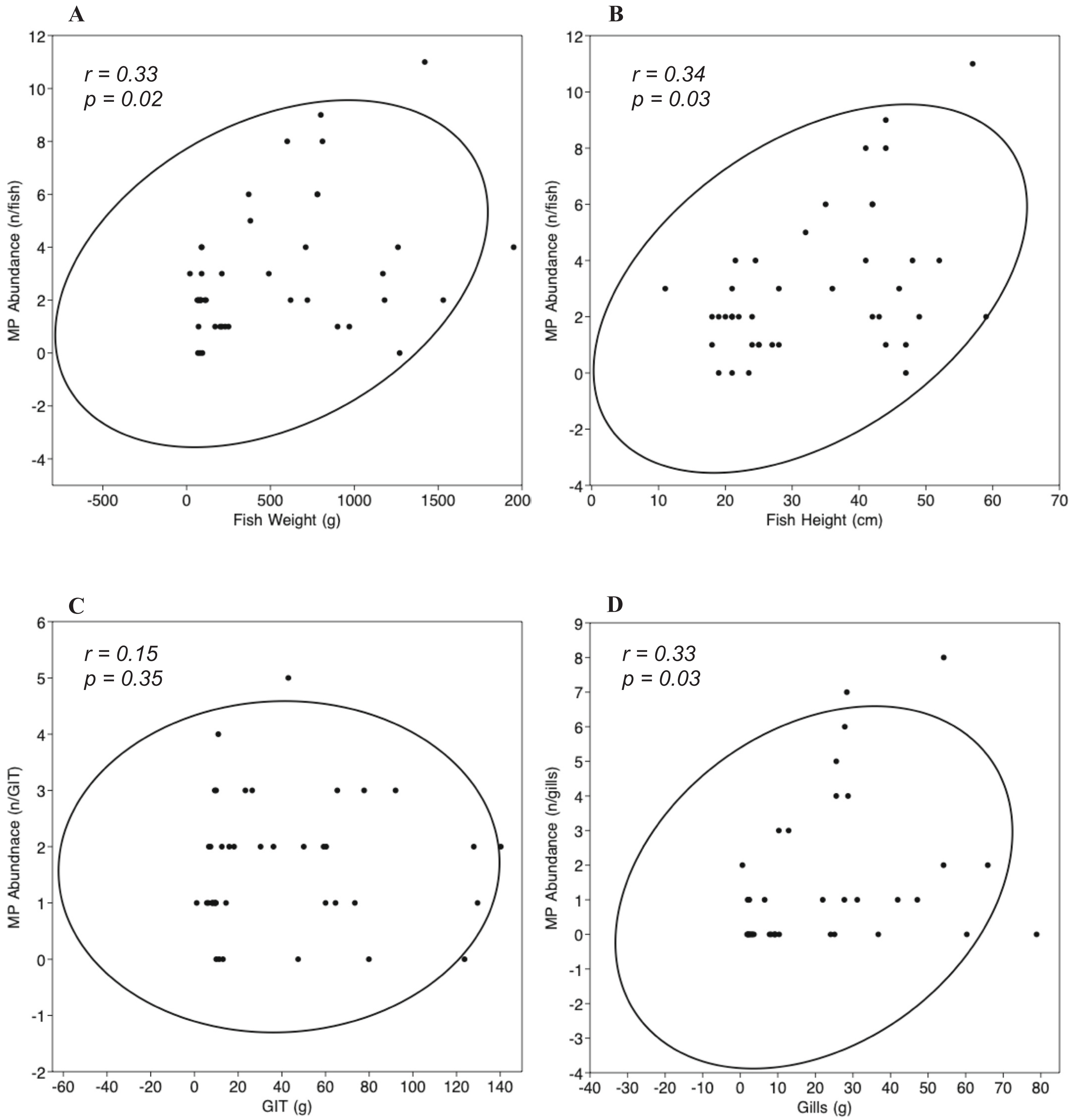

Fig. 3. Relationships between microplastic levels and fish metrics including, (A) body weight, (B) height, (C) gastrointestinal tract (GIT) weight, and (D) gills weight. The ellipses represent 95 % confidence intervals around the centroid of each data cluster. 'MP' stands for 'microplastic'.

notable disparities among the sampled stations. Predominantly blue, transparent, and white particles were observed in catfish across the various stations. A comparative analysis between the upstream counterpart CC1 and downstream stations highlighted differences, with blue particles being dominant in CC1, whereas transparent, white, and blue particles were major in CC2–4. CC5–7 exhibited contamination primarily by blue and transparent particles. In tomcod, blue and black particles were predominant.

3.3.4. Polymer

The μ-FTIR and μ-Raman analyses uncovered the presence of 11 distinct polymers in the catfish and tomcod (Fig. 4D; Fig. S1). Although PET (42 %) and PE (20 %) were the dominant polymers in the tissues of both species, polymer compositions in fish tissues were different, with more variations in catfish than in tomcod. Microplastics in catfish gills showed more polymer types than their GIT and both tissues of tomcod (Fig. 4D). For example, acrylonitrile butadiene styrene (ABS), polyurethane (PUR), ethylene-vinyl acetate (EVA), and polyamide (PA) were only found in catfish gills among all tissues analyzed. In contrast,

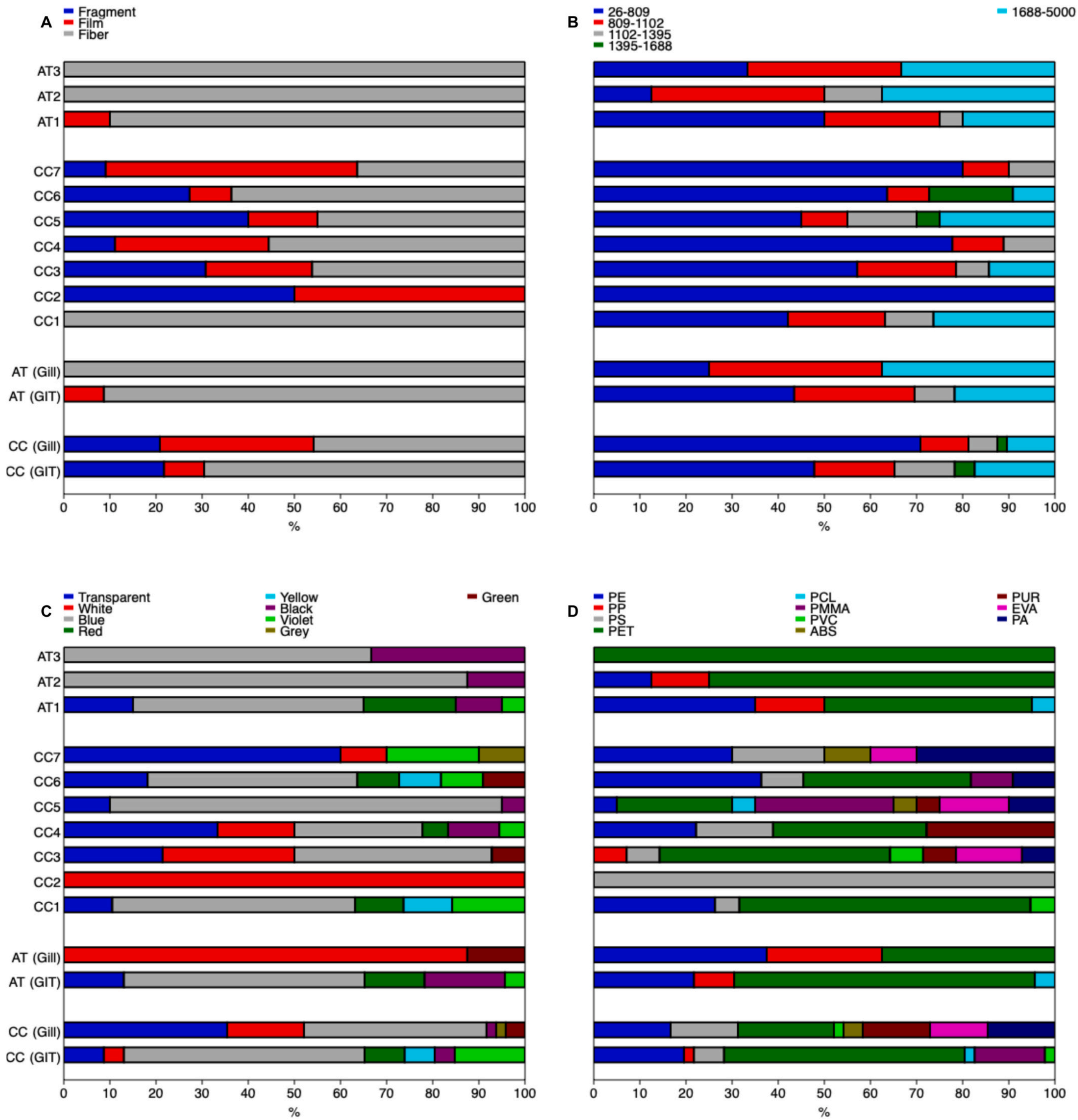

Fig. 4. Compositions of microplastic (A) shapes, (B) sizes, (C) colors, and (D) polymers along the sampling sites as well as in the channel catfish (CC) and Atlantic tomcod (AT) tissues. All the size measurements in (B) were in 'μm'.

polymethyl methacrylate (PMMA) and polycaprolactone (PCL) were only found in catfish GIT but not in their gills. In addition, polystyrene (PS), and polyvinyl chloride (PVC) were only found in catfish but not in any tissue of tomcod, while tomcod accumulated more polypropylene (PP) than catfish in both GIT and gills (Fig. 4D). However, only the PET fibers correlated with the total microplastic abundances across all the studying sites (all fish tissues considered together), and thus could be identified as a potential marker of microplastic contamination (Spearman Rho correlation, $r = 0.53$, p-value $= 0.0003$) in the benthic fish of the SLRE (Fig. 5A).

A correlational analysis between polymer density (g cm^{-3}) and microplastic particle abundance across all sites, considering all fish combined, revealed no significant relationship (Spearman's Rho: $r = 0.05$, p-value $= 0.88$) (Fig. 5B).

A comparative analysis of polymer distribution along the catfish (CC1–7) and tomcod (AT1–3) study areas within the SLRE revealed notable variations in polymer diversity. In catfish sampling sites, PET (63 %) and PE (26 %) were dominant in the samples from the upstream of Québec City (CC1). However, downstream samples exhibited differences: CC2–4 showed a prevalence of PS and PET, along with PUR, EVA, and PA. A diverse array of polymers, including PMMA, ABS, and PCL along with the PE, PET, PUR, EVA, were detected in catfish from CC5–7.

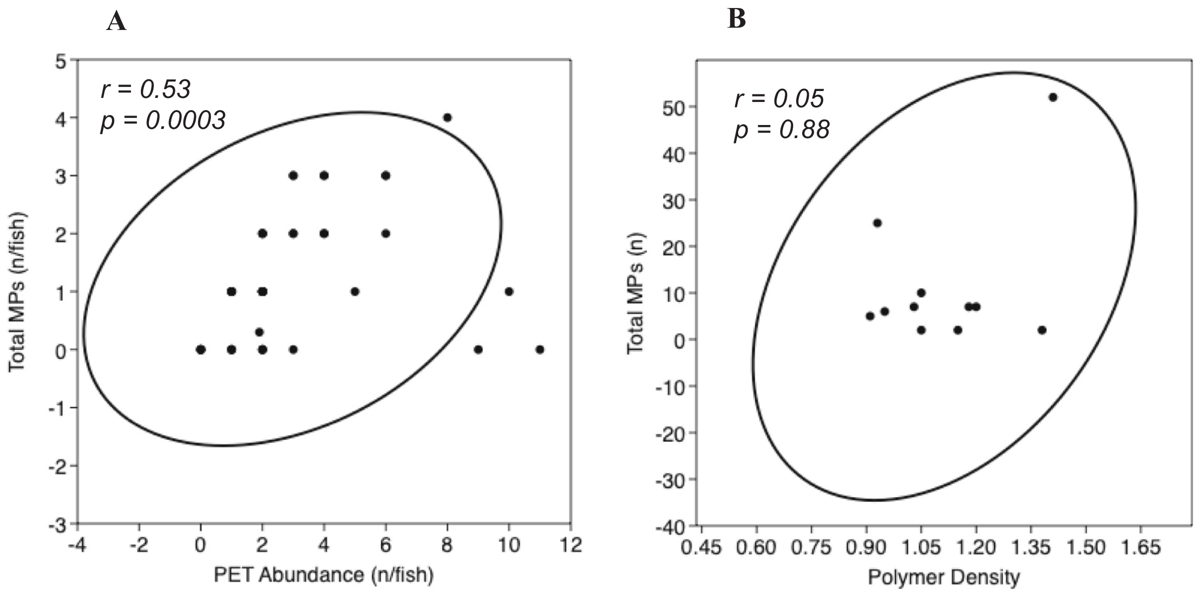

Fig. 5. Relationships among microplastic abundance, polymer abundance, and polymer density (g cm^{-3}) in the two benthic fish. (A) Microplastic and PET polymer abundance in the two fish species across all study sites. (B) Microplastic abundance across sites, considering all fish combined, plotted against the density of all detected polymers. Ellipses indicate 95 % confidence intervals around the centroid of each data cluster. 'MPs' refers to microplastics.

Overall, downstream catfish displayed a diverse array of polymer types than those from the upstream. In contrast, tomcod samples at different sites generally presented less diverse polymers, though still detectable, with PE, PET, and PP being major constituents (Fig. 4D).

3.4. Microplastic contamination ecological risk assessment

3.4.1. Risk factor 1 (RF1): microplastic size and abundance-based risk assessment

Microplastic size and abundance-based risk assessment revealed that smaller particles (<809 μm) were the major contributors to ecological risks in the SLRE benthic environment (Table 4; Fig. S3).

3.4.2. Risk factor 2 (RF2): abundance and polymeric toxicity-based risk assessment (PLI, PHI, and ERI)

The *PLI* results indicated that all study sites were contaminated with microplastics, with *PLI* values exceeding 1 per fish at certain locations. Stations CC2, CC6, and CC7 showed very high contamination levels, while CC4 and CC5 exhibited medium levels, and CC1 and CC3 displayed low levels. Tomcod stations exhibited an overall medium level of contamination. Stations near Québec City (CC2, CC4, CC6, and CC7) demonstrated higher *PLI* values compared to other stations, reflecting increased microplastic contamination in those urban land-use affected areas (Fig. 1; Table 4).

PHI analysis showed site-dependent variations in polymeric hazards, with heightened hazard levels at CC2, CC4, and CC5 due to the presence of specific polymer types. The *ERI,* which integrates microplastic abundance and polymeric hazard levels, indicated elevated contamination risks at CC2, CC4, CC5, CC6, and CC7 (Fig. 1; Table 4). Positive correlations were observed between *PHI* and *ERI* ($p = 0.00002$; $r = 0.95$; df = 41) and between PLI and ERI ($p = 0.01$; $r = 0.54$; df = 41).

3.4.3. Cumulative contamination risk (CCR) and land-use

CCR assessments which includes microplastic particle size, abundance and polymeric hazard scores, identified benthic catfish study sites as medium-high risk areas for microplastic contamination, while tomcod study areas were deemed lower risk (Table 4). Stations near Québec City (CC2, CC4, CC5, CC6, and CC7) were classified as higher risk, reflecting increased microplastic abundances and the presence of highly toxic polymers (Fig. 1; Table 4).

4. Discussion

4.1. Limitations and uncertainties

This study provides valuable insights and establishes a baseline for the occurrence, spatial distribution, and ecological risk assessment of microplastic contamination in benthic species within the SLRE. However, several limitations should be noted. Due to logistical sampling constraints and low observed benthic fish abundance at sampling stations in the SLRE, we captured a low number of individual fish during a limited period. For this reason, we decided to focus our analysis only on the two most abundant species encountered in the study area, instead of the full benthic fish assemblage. Moreover, the absence of a *PHI* score for polycaprolactone and shape factor-based limit values most likely affected the certainty of ecological risk assessment. To build on the baseline provided in the present study and reveal causal relationships between microplastic contamination and urban activity, further investigations should rely on a more comprehensive spatio-temporal sampling effort within the SLRE, including a parallel analysis of microplastic concentration in bottom water and sediment. Also, future research involving a broader range of benthic species is needed to conclusively determine microplastics' presence across trophic levels and better reflect the condition of SLRE benthic ecosystems.

4.2. Microplastic abundance, spatial and tissue distributions in SLRE benthic fish

Microplastic contamination in benthic fish highlights its pervasiveness in the SLRE and the vulnerability of benthic species. The overall abundance of microplastics varied widely between species, with results suggesting that catfish accumulate microplastics at higher rates than tomcod (Fig. 2A). This difference may stem from variations in feeding behavior, habitat, physiology, and exposure routes (Zazouli et al., 2022; Lin et al., 2023; Roch et al., 2020). In this study, the differences in microplastic accumulation between catfish and tomcod, and their GIT and gills tissues highlight species-specific exposure routes and accumulation patterns. For instance, tomcod exhibited significantly higher microplastic abundances in their GIT compared to gills (Fig. 2B), suggesting that mouth intake is the primary exposure route for this species. This observation aligns with previous studies from other regions such as

Caspian Sea (Northern Iran) (Rasta et al., 2023) and Guangdong (South China) (Pan et al., 2021). In contrast, catfish showed no significant difference in microplastic abundance between their GIT and gills, indicating that both oral ingestion and gill filtration may contribute equally to their exposure. These findings support the idea that the relative contributions of GIT and gill-based microplastic uptake can vary across species. In addition, there was no significant difference in microplastic abundance in GIT or gills between catfish and tomcod, implying a similar level of ingestion of microplastics by the GIT or uptake by gills between the two species. However, tomcod's higher microplastic abundance per unit of body or tissue weight likely stems from biodilution effects, as their smaller, lighter bodies, GITs, and gills accumulate more microplastics per unit weight due to lower overall body/tissue weight (Table 1). This aligns with McIlwraith et al. (2021), which found that smaller fish exhibit higher microplastic abundances per gram of tissue wet weight than larger and heavier fish. This indicates that body size, weight, and feeding behavior influence microplastic exposure, accumulation and distribution in fish species.

Further to that, a positive correlation between microplastic abundance and biological variables such as fish body weight & length, and GIT & gill weight, highlighted the influence of fish size and anatomical features on microplastic accumulation. The findings suggest that larger catfish, with heavier GITs and gills, tend to accumulate greater quantities of microplastics, whereas smaller tomcod accumulate fewer. This aligns with previous studies on benthic fish from the Thames River (UK) (Horton et al., 2018), Spain (Alomar et al., 2017), Lake Ontario (Canada) (Munno et al., 2022), and the Han River (Republic of Korea) (Park et al., 2022). Regarding gills, microplastic accumulation may be linked to the greater surface area and filtering efficiency of larger individuals. Larger gills provide more surface area for microplastic adherence and enhance the capacity to entangle and retain particles from flowing water, as suggested by earlier studies (Collard et al., 2017; Vasanthi et al., 2021; Gregory, 2009; Kolandhasamy et al., 2018). Although the difference in gill microplastic accumulation between catfish and tomcod was not statistically significant, the observed trend of higher microplastic quantities in catfish gills supported our hypothesis that microplastic accumulation differs between gills and GITs. Additionally, larger fish, which consume more food overall, may face higher contamination risks due to increased ingestion rates and trophic exposure, making them more likely to ingest microplastics through prey. This strengthens the connection between fish size and microplastic accumulation (Alomar et al., 2017; Munno et al., 2022; Park et al., 2022). These findings underscore the importance of considering anatomical features, such as fish size and tissue weight, when evaluating microplastic exposure and retention in fish and assessing the ecological risks of microplastic pollution. Further research is needed to clarify the mechanisms behind these interspecies differences in microplastic accumulation and to examine the long-term ecological consequences, particularly in larger benthic species, to better understand the potential risks to ecosystems and food webs.

A key finding from the spatial analysis of microplastic abundance was the significant variation between downstream and upstream sites. Catfish collected from the downstream stations near Québec City (CC2–7) showed significantly higher microplastic abundances (Fig. 1, Fig. 2A). This spatial difference likely reflects the influence of urban land-use on microplastic contamination, as downstream sites near Québec City are more affected by industrial and urban activities, could increase the influx of microplastics into the SLRE, supported by the previous study in the SLRE by Crew et al., 2020. Crew et al. (2020) found higher levels of microplastic particles from the sediment in the downstream of Québec City (site 26 in that study representing 76.3 % urban land-use which is close to CC2–4 in the present study) compared to the less urbanized upstream area (site 23 in Crew et al. (2020) representing 10.0 % urban land-use which is close to CC1 in this study) in the SLRE. The consistency between the microplastic contamination results from the SLRE sediments by Crew et al. (2020) and our catfish samples suggests that the Québec City urban land-use likely contributes to increased microplastic input into the aquatic ecosystem, affecting sediment and biota, and underscores the habitat's role in microplastic uptake by the benthic catfish in the SLRE. However, it is worth noting that catfish's migratory activity may also affect their exposure to microplastics, although the present study could not track such affecting factors. Even though they are generally considered sedentary, catfish can migrate over 150 km in the SLR (Scott and Crossman, 1973), potentially encountering microplastic other than the sampling areas during their migration. This should be validated by further studies focusing on tracking how migration of catfish in SLRE affect their exposure and accumulation of microplastics. In contrast to catfish, no significant spatial variations were found among three sampling sites for tomcod (Fig. 2A), possibly due to the consistency of land-use around the sampling areas of AT1–AT3. Future research should aim to expand on these findings by including a broader range of species, temporal data, and sediment and water quality analyses to fully assess the ecological risks posed by microplastic contamination in the SLRE and similar ecosystems.

The predator-prey BMF analysis in this study revealed no significant biomagnification of microplastics between catfish and tomcod in the SLRE. The calculated BMF values based on fish body weight ($0.5 < 1.0$), GIT weight ($0.57 < 1.0$), and gill weight ($0.38 < 1.0$) all suggested no evidence of microplastic biomagnification. This aligns with similar findings from McIlwraith et al. (2021), which showed no definitive biomagnification in seven fish species from Lake Simcoe, Ontario. Additionally, a study by Covernton et al. (2022) found that large-sized microplastics (>100 μm) did not biomagnify across different trophic levels in bivalves, crabs, echinoderms, and fish from southern Vancouver Island (British Columbia, Canada), which is consistent with our findings. This could happen as most of the microplastics detected in this study were > 100 μm. However, given that this analysis was confined to just two species, further research involving a broader range of species is necessary to conclusively determine whether biomagnification of microplastics occurs across trophic levels in the SLRE ecosystems.

4.3. *Microplastic characteristics: shape-size-color-polymers*

4.3.1. *Shape*

The analysis of microplastic shapes revealed significant variations in particle types, highlighting fibers as the predominant shape across all samples, followed by films and fragments. This finding aligns with global studies on benthic fish, which consistently report fibers as the most common microplastic shape ingested by benthic fish species (Table 3). The dominance of fibers may be attributed to their widespread presence in aquatic environments, originating from textile fibers, fishing gear, and industrial discharges (GESAMP, 2016).

Differences in microplastic shape contamination were also observed between species and tissues. Catfish showed contamination by all three shapes, fibers, films, and fragments, in both GITs and gills, whereas tomcod were primarily contaminated by fibers, with only a few films detected in the GIT and no fragments in any samples (Fig. 4A). These differences may reflect the anatomical and behavioral characteristics of the species. The larger mouths and gills of catfish, compared to tomcod, likely increase their exposure to a broader range of microplastic shapes, consistent with Siddique et al., 2024. This finding highlights the importance of considering species-specific traits when evaluating microplastic exposure risks.

Spatial variation in microplastic shapes across sampling sites highlights the influence of local environmental factors on contamination patterns. Upstream locations (e.g., CC1) showed exclusively fiber contamination in catfish, whereas downstream sites (CC2–7) exhibited contamination by all three shapes: fibers, fragments, and films. A comparison with Crew et al. (2020) revealed similar trends, where increased abundances of fibers were recorded upstream (site 23 in Crew et al. (2020), close to CC1 in the present study) and higher numbers of

Table 3
A comparison of microplastic contamination in the benthic fish with previous studies around the world.

Benthic Species	Study Area	Abundance (Mean ± SD)			Characteristics				References
		n/GIT	n/gills	n/Fish	Shape	Size	Color	Polymer	
Microgadus tomcod	SLRE, Canada	1.4 ± 0.27	0.5 ± 0.16	1.9 ± 0.3	Fiber	<809	Blue, Black	PET, PE	This study
Ictalurus punctatu	SLRE, Canada	1.8 ± 0.34	1.9 ± 0.47	3.6 ± 0.6	Fiber	<809	Blue, Black	PET, PE	This study
C. lyra	NW Iberian shelf, Spain	2.53 ± 1.88			Fiber				Filgueiras et al., 2020
M. surmuletus (Predator)	NW Iberian shelf, Spain	1.56 ± 0.53							Filgueiras et al., 2020
Leiognathus brevirostris	Thoothukudi, Tamil Nadu, India	0.58 ± 0.24							Keerthika et al., 2023
Siganus canaliculatus	Thoothukudi, Tamil Nadu, India	1.88 ± 1.27							Keerthika et al., 2023
Ammodytes personatus and *Gobiidae*	Jiaozhou Bay, China			3.68 ± 0.79	Fiber	< 1000	Black, Blue	PE, PET,	Zhang et al., 2023
Mullus barbatus	Adriatic Sea, Tyrrhenian Sea, and Sardinia, Italy	0.14 ± 0.04			Fiber	<1000	Black, Blue	PET/ Polyester	Valente et al., 2022
Misgurnus anguillicaudatus and *Johnius belangerii*	Yangtze River estuary (near Chongming Island and offshore area), China				Fiber	<1000		PET, PE	Li et al., 2022
Clarias gariepinus) and benthopelagic (Cyprinus carpio and Carassius Carassius	Lake Ziway, Ethiopia	4.4 ± 3.6			Fragment, Fiber	< 200	Transparent white, Blue	PE, PP, PET	Merga et al., 2020
Gobiidae,	Haizhou Bay, Yellow Sea, China	2.54 ± 0.89			Fiber	< 1000	Blue	PET	Zhang et al., 2022
Cleisthenes herzensteini	South Yellow Sea, China				Fiber	< 500	Transparent, Black, Blue	PP, PET, PAM, PS	Wang et al., 2019
Trigla lyra, Boops boops, Trachurus picturatus, Scyliorhinus canicular, and *Merluccius merluccius*	Portuguese coast, Portugal	0.03 ± 0.18–0.67 ± 0.58			Fiber			PP, PE, PET, Acrylic, PAM	Neves et al., 2015
Mastacembelus armatus Cirrhinus reba Glossogobius giuris	River Old Brahmaputra, Bangladesh	2.33 ± 0.57–10.31 ± 0.75			Fiber	<1000	Black, Blue, Transparent, Red	PE, PAM	Ferdous et al., 2023
Platichthys flesus	River Thames and River Stour, UK	1.98 ± 3.50						PP	Horton et al., 2024
Solea solea	Adriatic Sea, Italy	1.73 ± 0.05–1.64 ± 0.1			Fragment, Fiber	<500		PVC, PP, PE, PET, and PAM	Pellini et al., 2018

fragments and films were observed downstream (site 26 in Crew et al. (2020), near CC2–4 in this study) of Québec City. The findings in this study mirrored this trend, with catfish from CC1 solely contaminated with fibers, while those from CC2–7 were contaminated by fibers, fragments, and films. These results suggest that Québec City's urban land-use practices may contribute to the prevalence of fragments at downstream sites and influence the uptake of various microplastic shapes by benthic species in the SLRE. Similarly, for tomcod, fibers were the dominant shape at all sites, with films occasionally detected at specific locations. These spatial differences likely result from variations in land-use practices and urbanization in the SLRE, which contribute distinct shapes of microplastic pollution.

Overall, the predominance of fibers, coupled with spatial and species-specific variations in microplastic shapes, provides valuable insights into the ecological impacts of microplastic pollution. Future research should aim to identify the sources, transport mechanisms, and degradation pathways of different microplastic shapes to better evaluate their ecological risks. Understanding these dynamics is essential for developing targeted mitigation strategies to address microplastic pollution in aquatic ecosystems.

4.3.2. Size

This study highlights the predominance of smaller microplastics 26–809 μm (small microplastics defined as <1 mm, while larger microplastics range from 1 to 5 mm; Naji et al., 2019; Piehl et al., 2019) in the GITs and gills of benthic fish from the SLRE. The observed size distribution aligns with previous research, which consistently reports that microplastics smaller than 1 mm dominate in benthic species (Table 3; Ryan, 2016; Roch et al., 2021; Lin et al., 2023). The consistent detection of microplastics ranging from 26 to 809 μm in the GITs of both catfish and tomcod suggests these particles are highly ingestible by these two benthic species. This can be attributed to several factors: (1) the potential high environmental prevalence of particles between 26 and 809 μm in the SLRE, which suggests prioritizing the analysis of surrounding water and sediment in future studies; (2) this size range closely resembles natural food sources, or their prey items, such as zooplankton, phytoplankton (Ory et al., 2017), and their secondary ingestion through prey contaminated with microplastics increasing their likelihood of mixing with food and being preferentially ingested (Koongolla et al., 2020); (3) mechanical actions, such as chewing and ingestion, may break larger microplastics into smaller fragments, further amplifying their abundance (Zhang et al., 2021). Additionally, smaller microplastics tend to have longer retention times in fish GITs, further explaining their higher prevalence (Roch et al., 2021). Interestingly, the size distribution in gills varied between species. Tomcod showed a dominance of larger particles (>809 μm) in gills, which could be attributed to species-specific differences in respiratory filtration mechanisms.

The prevalence of smaller particles highlights the pervasive nature of microplastics in the SLRE, driven by processes such as breakdown, degradation, transportation, and dispersion across benthic habitats (Katija et al., 2017; Roch et al., 2021; Lin et al., 2023). However, the variability observed between the catfish and tomcod stations reflects site-specific factors, including localized microplastic sources, hydrodynamic sorting, and differences in feeding behaviors, physiology, and anatomy (Roch et al., 2021). These findings underscore the ecological significance of smaller microplastics in aquatic ecosystems. The elevated ingestion of smaller microplastic particles by benthic fish is particularly concerning due to their increased potential for bioaccumulation, biological transport, and prolonged retention in tissues (Lei et al., 2018; Katija et al., 2017; Zheng et al., 2020; Roch et al., 2021). These characteristics render smaller particles more toxic and impactful in benthic ecosystems, which not only threatens benthic fish but also poses risks to the broader food web in the SLRE. Therefore, this study highlights the need for targeted mitigation strategies to reduce the input of smaller microplastics into aquatic environments, considering their persistence and disproportionate impact on aquatic biota. Future research should prioritize understanding the mechanisms driving size-selective ingestion of microplastics across species and their physiological and ecological consequences. Additionally, investigating the sources and pathways of smaller microplastics, particularly in high-contamination areas of the SLRE, will be crucial for informing effective pollution management strategies.

4.3.3. Color

The diverse color spectrum of microplastic particles extracted from catfish and tomcod underscores the complexity of microplastic contamination in the SLRE's benthic ecosystem. Blue particles emerged as the predominant color across all samples, followed by transparent and white particles, while other colors such as red, black, violet, yellow, green, and grey were also present, albeit in smaller proportions. This pattern aligns with findings from previous studies, which have identified blue, and transparent microplastic as the major ones ingested by benthic fish (Table 3). The consistent dominance of blue-colored microplastic across all stations and species in this study may indicate a potential link to their resemblance to natural prey items or food-like stimuli (Ory et al., 2017; Roch et al., 2020; Boerger et al., 2010). For example, research has shown that foraging fish are more likely to ingest microplastic with colors resembling those of their prey or direct ingestion from prey already contaminated with microplastics, such as algae, insects, aquatic plants, phytoplankton, zooplankton, etc. (Merga et al., 2020; Roch et al., 2020). This ingestion could explain the widespread presence of blue microplastic in both catfish and tomcod (Boerger et al., 2010). Furthermore, alongside the prevalence of blue particles, the color distribution of microplastic in this study aligns with findings from other research, with red, black, and transparent being the other major colors (Bellas et al., 2016; Table 3). Additionally, transparent and white particles were prevalent in the gills compared to the GIT. The differences in the color of microplastics between the GIT and gills, for instance the higher prevalence of blue microplastics in the GIT, indicated varied exposure pathways. We thought that blue microplastics, resembling natural aquatic particles, are predominantly ingested orally, making them more abundant in the GIT, while transparent and white particles might be entangled or retained from the surrounding environment by the gills in respiratory filtration mechanism, given their higher abundances in the gills. These indicated that differences in microplastic color distribution between the GIT and gill tissues potentially highlight variations in exposure, uptake and accumulation mechanisms in benthic fish bodies.

Spatially, blue particles were predominant across all sites, differences were noted between upstream (CC1) and downstream stations (CC2–7). Upstream samples primarily contained blue particles, whereas downstream locations exhibited more diverse contamination, including transparent and white microplastic. This variation could reflect differences in microplastic sources, transport pathways, or degradation processes, as downstream areas are more likely to accumulate microplastic from multiple sources, including urban runoff and industrial discharges. For tomcod, the predominance of blue and black particles across stations further suggests species-specific interactions with microplastics, potentially influenced by their habitat preferences and feeding behaviors. The absence of sediment microplastic color data from the study region (Crew et al., 2020) limits direct comparisons but emphasizes the importance of characterizing sediment microplastics in future research to establish potential links between environmental microplastics and those ingested by fish. Overall, the color distribution of microplastics provides insights into the interactions between aquatic organisms and microplastics in their environment. The prevalence of specific colors, particularly blue, underscores the need for further studies on how visual cues influence microplastic ingestion in the benthic species form the SLRE. Understanding these dynamics can inform mitigation strategies aimed at reducing the prevalence of high-risk microplastic particles in aquatic ecosystems.

4.3.4. Polymer

This study underscores the complexity of microplastic contamination in benthic fish, highlighting the diversity of polymer types and their spatial variability across the SLRE. The identification of 11 distinct polymer types in catfish and tomcod reflects the pervasive nature of microplastic pollution and its diverse sources in the SLRE's benthic ecosystem.

The predominance of PET and PE in benthic fish, alongside other major polymers such as PP and PS, aligns with global trends (Table 3). However, the detection of less commonly reported polymers in fish like PCL, PMMA, PVC, ABS, PUR, and PA is distinct for the benthic fish in the SLRE, particularly in catfish. Notably, some polymers, such as EVA, PUR, and PA, were exclusively found in catfish gills, suggesting species-specific exposure pathways. These results are consistent with prior findings by Rowenczyk et al. (2022), which reported a diverse array of microplastic polymers, including PET, PP, PS, PE, PUR, EVA, and PVC, in SLRE surface waters and PET, PS, PE, PP and EVA in benthic bivalves. The study areas of Rowenczyk et al. (2022) were similar to the downstream of the catfish and tomcod sampling sites in this study. This suggests that benthic species may encounter microplastics primarily from the SLRE's surrounding water-sediment habitat.

Further discussion on the diversity and distinct occurrence of microplastic polymers considered two key aspects: (1) the environmental behavior of microplastic polymers and (2) the localized exposure of benthic fish to specific microplastic sources in the SLRE.

Firstly, the settling behavior of microplastic particles is influenced by their density (Chubarenko et al., 2016; Kooi et al., 2018; Kowalski et al., 2016; Morét-Ferguson et al., 2010; Cózar et al., 2014; Zettler et al., 2013). The high-density PET (1.37—1.45 g cm^{-3}) was the predominant type of microplastic found in the two benthic fish species (all fish GIT and gills considered together), and abundances significantly correlated with the total microplastic abundances across all study sites (Fig. 5A). Other high-density polymers (>1 g cm^{-3}), such as PCL (1.15 g cm^{-3}), PMMA (1.17–1.20 g cm^{-3}), PVC (1.16–1.58 g cm^{-3}), ABS (1.05 g cm^{-3}), PUR (1.2 g cm^{-3}), and PA (1.02–1.05 g cm^{-3}), were abundant (Fig. 4D), and largely contributed to the total microplastic load in the two benthic fish. When comparing the total abundance of all polymers in the fish, polymers with densities >1 g cm^{-3} accounted for 89 out of 125 total microplastic particles (71.2 %), whereas polymers with densities <1 g cm^{-3} accounted for 36 particles (28.8 %). This finding underscores the critical role of high-density polymers in the composition of microplastics ingested by benthic fish. To further explore this relationship, although the correlation analysis between polymer density and microplastic abundance did not reveal a statistically significant correlation (Fig. 5B), we thought that polymer density plays an indirect but important role in microplastic accumulation in benthic fish. Factors such as rapid sedimentation of high-density polymers to the bottom aquatic environments could amplify the exposure of bottom-dwelling fish to high-density microplastics (Chubarenko et al., 2016; Kooi et al., 2018; Kowalski et al., 2016; Morét-Ferguson et al., 2010; Cózar et al., 2014; Zettler et al., 2013), whereas low-density polymers (<1 g cm^{-3}), such as PE, PP, PS, and EVA, typically require biofouling, the attachment of organic and inorganic matter, or that of organic aggregates to alter their buoyancy and allow them to reach the sediment. Thus, the elevated presence of high-density polymers in SLRE benthic fish indicates that the benthic habitat likely plays a role in fish uptake from their surroundings, highlighting sediment and water column deposition as critical pathways for microplastic exposure in benthic species and understanding the habitat-specific contamination pathways. Future studies incorporating hydrodynamic modeling and dietary analyses could provide deeper insights into the mechanisms driving microplastic accumulation in benthic ecosystems.

Secondly, the polymers PE, PP, PET, and PS, widely referred to as 'single-use plastics,' are extensively utilized across various sectors, including commercial, industrial, household, food packaging, and agriculture. These materials are consumed on a massive scale globally, have a short useful life, and quickly enter waste streams, contributing significantly to microplastic emissions and environmental pollution (UNEP, 2018; GESAMP, 2016; Plastics Europe, 2020). Other detected polymers have more specialized origins; for example, PMMA may derive from medical applications, while PVC is associated with building materials, water distribution pipes, floor coverings, cable insulation, and packaging. Similarly, PUR is linked to the production of leather goods, sports equipment, car seats, tires, and biomedical devices, while ABS is used in automotive components, toys, household goods, and 3D printing. PA is primarily used in textiles, packaging, engineering, and agriculture, and EVA is commonly found in footwear, automobiles, toys, and packaging (Plastics Europe, 2020) (detailed descriptions of these polymers are provided in Table S1). Therefore, the findings in this study highlight the pervasive impact of single-use plastic pollution, with PE, PP, PET, and PS comprising the majority of detected microplastics in the benthic fish from the SLRE. The presence of distinct polymers such as PUR, ABS, and EVA further underscores the variety of contamination sources, from urban runoff to industrial discharges. Spatially, catfish samples from downstream areas (CC3–7) displayed greater polymer diversity compared to upstream sites (CC1), reflecting the impact of urban land-use, particularly around Québec City. The presence of polymers such as ABS, PUR, and PA in these areas suggests inputs from urban and industrial activities, including construction, automotive manufacturing, and textile production. In contrast, tomcod showed less polymer diversity across sites, with PE, PET, and PP dominating at all locations. This interspecies difference likely reflects variations in habitat preferences, feeding behaviors, or proximity to microplastic sources. Notably, the strong correlation between PET fibers and total microplastic abundance suggests that PET could serve as a reliable marker for microplastic pollution in the SLRE, providing a valuable tool for future monitoring of microplastic in the SLRE.

Overall, the study underscores the diverse land-based, both point and non-point, contributions to microplastic pollution in the SLRE, which could significantly influence the spatial distribution and fate of microplastics. Targeted management strategies are needed to mitigate microplastic contamination, focusing on urban land-use practices and waste management. Future research should prioritize exploring the pathways and mechanisms driving polymer-specific microplastic accumulation in aquatic organisms, particularly under varying environmental conditions. Enhanced understanding of these dynamics will enable the development of effective policies to reduce the ecological and environmental impacts of microplastic pollution.

4.3.5. Potential ecotoxicological threats of microplastics in benthic fish in the SLRE

Small microplastics pose significant hazards due to their increased likelihood of ingestion, translocation within tissues, and biological transport by aquatic organisms (Katija et al., 2017; Lei et al., 2018; Zheng et al., 2020). Microplastic fibers are linked to respiratory and reproductive issues (Hu et al., 2020). For example, PET fiber ingestion can trigger inflammatory responses and oxidative stress, with fibers proving more toxic than fragments by disrupting oxidative processes, immune function, and hematopoiesis in fish (Choi et al., 2022; Liang et al., 2023; Ziajahromi et al., 2017). Fibers and fragments may also cause physical damage, including intestinal blockages, internal abrasions (de Sa et al., 2018; Wright et al., 2013). Various polymers such as PE, PP, PS and PET can cause neurotoxicity, reproductive and growth impairments, and oxidative stress (de Sa et al., 2018; Avio et al., 2015; Barboza et al., 2018), while others, like PS, PMMA, PVC, ABS, PUR, and PAN, carry additional risks with carcinogenic, mutagenic, and endocrine-disrupting effects (Lithner et al., 2011; Gallo et al., 2018). Human exposure concerns arise from fish consumption, impacting fisheries-dependent communities (Rist et al., 2018). Thus, the microplastics observed in this study may pose ecotoxicological threats to fish in the SLRE, warrants for targeted research to assess ecotoxicity at these realistic exposure levels. Such studies would clarify risks to fish populations and aid fisheries management and aquatic wildlife conservation in the region.

4.4. Microplastic contamination ecological risk assessment

The ecological risk assessment of microplastic contamination in the SLRE highlights the complexity and variability of risks associated with microplastic size, abundance, and polymeric toxicity. Smaller microplastic particles (<809 μm) emerged as significant contributors to heightened ecological risks along the SLRE benthic environments and supported our hypothesis (Table 4; Fig. S3). Their elevated ingestion by benthic fish is particularly concerning, as smaller particles are more prone to pose unique ecotoxicological challenges such as bioaccumulation, biological transport, and prolonged retention in tissues like the GIT (Lei et al., 2018; Katija et al., 2017; Zheng et al., 2020; Roch et al., 2021). Therefore, this size-based ecological risk assessment highlights the critical role of size distribution in understanding microplastic contamination risks, especially in benthic habitats. Further, the microplastic contamination ecological risk assessment also highlights the significant role of polymeric composition in influencing contamination risks. Elevated *PHI* values in downstream stations near Québec City point to the presence of highly toxic polymers, such as PVC, PMMA, ABS, and PUR, which contributed to increased *PHI* and *ERI* values; thus, these highly toxic polymers, despite their relatively low abundance, emerged as key drivers of ecological risks. The correlations observed between *PLI, PHI,* and *ERI* further emphasized the interplay between microplastic abundance and polymeric hazards levels in shaping ecological risk levels (Lithner et al., 2011). Additionally, the elevated ecological risks in downstream stations near Québec City underscore the impact of urban land-use activities, which not only contribute to the release of highly toxic polymers but also increase microplastic abundance and diversity in these areas within the SLRE ecosystems. Overall, the cumulative contamination risk (CCR) assessment, integrating size, abundance, and polymeric hazard scores, identified downstream catfish study sites as medium- to high-risk areas in the SLRE for microplastic contamination (Table 4). Conversely, tomcod study sites exhibited lower risks, consistent with their reduced exposure to urban land-use impacts. Stations near Québec City (downstream CC2, CC4–7), classified as higher risk, which could attribute to higher microplastic abundances and the presence of highly toxic polymers (Fig. 1 and Table 4). This suggests that urban land-use could elevate ecological risks by increasing microplastic abundances and releasing diverse, potentially highly toxic polymers into SLRE systems, impacting benthic fish species. Effective

Table 4
Microplastic pollution load index (PLI), polymeric hazard index (PHI), ecological risk index (ERI), cumulative contamination risk (CCR), and the risk levels posed to the benthic environment in the SLRE.

Species	PLI	RF1: Abundance & Particle Size		RF2: Abundance & Polymeric Toxicity			Cumulative Contamination Risk (CCR)	
		Risk Score	Risk Level	PHI	CRI	Risk Level	Risk Score	Risk Level
Atlantic Tomcod								
AT1	3.0	11.5	5	5.5	16.5	1	2	Low
AT2	3.6	10.3	5	4.5	18.0	1	2	Low
AT3	4.3	14.3	5	4.0	17.1	1	2	Low
Channel Catfish								
CC1	2.3	8.0	5	562.0	1283.0	5	4	High
CC2	9.9	40.3	5	1750.0	17324.7	5	5	Very High
CC3	2.9	14.3	5	30.0	85.7	1	2	Low
CC4	5.1	26.7	5	3854.0	19583.1	5	5	Very High
CC5	4.2	14.4	5	1333.0	5579.0	5	5	Very High
CC6	7.8	31.3	5	106.0	825.9	4	4	High
CC7	15.8	72.3	5	680.0	10679.4	5	5	Very High
Fish Total	3.7	14.5	5	1124.1	2248.3	5	5	Very High

Very Low | Low | Medium | High | Very High

management of microplastic contamination must address both the quantity of microplastics and their toxicological profiles to mitigate potential risks to aquatic ecosystems and human health.

This study enhanced the ecological risk assessment framework by incorporating a size-based model to address size-dependent ecological risks, offering a more comprehensive approach to microplastic contamination. Overall, by linking abundance, size, and polymeric hazard levels with land-use sources, the framework identified Québec City's urban areas as significant contributors of highly toxic polymers in the SLRE. It enabled the mapping of microplastic risk zones, provided predictive insights into contamination trends under varying environmental and land-use scenarios, and supported targeted risk management strategies within the SLRE. This approach contributes to guiding efforts to mitigate microplastic pollution and safeguard aquatic ecosystems. Future refinements, such as integrating additional microplastic characteristics like shape, could further enhance the precision and effectiveness of ecological risk assessments.

5. Conclusion

This study provides a comprehensive baseline for understanding microplastic contamination in benthic fish species from the SLRE, highlighting the occurrence, spatial distribution, and associated ecological risks. The findings revealed species-specific accumulation patterns, with catfish exhibiting higher microplastic loads than tomcod, influenced by differences in feeding behaviors, anatomical features (size, weight, and length of the fish bodies and tissues), and exposure pathways. Tomcod were primarily exposed to microplastics via oral ingestion, while catfish were exposed through both GIT and gill uptake. Spatially, downstream sites near Québec City exhibited heightened contamination levels, reflecting urban land-use impacts. No evidence of microplastic biomagnification was observed between the predator-prey pair (catfish and tomcod), suggesting that consuming tomcod was not a significant factor affecting the accumulation of microplastics in catfish. Fibers dominated the detected microplastic shapes, with small-sized particles (<809 μm) and colors such as blue, red, and black being most prevalent. PET and PE were the most common polymers, alongside a diverse range of types, including PMMA, PUR, PVC, ABS, and PS, implying diverse point and non-point sources of different polymers in this area. Fragment-shaped microplastics appeared to be linked to urban activities, while the presence of high-density polymers (e.g., PVC, PMMA, PUR, ABS, PS) likely resulted from sediment deposition. Smaller particles (<809 μm) and highly toxic polymers (e.g., PVC, PUR, PMMA, ABS) as the primary contributors to elevated ecological risks. Elevated contamination and ecological risks at downstream sites near Québec City underscore the urgent need for targeted management strategies to mitigate microplastic pollution in the SLRE. Future research should address existing knowledge gaps by analyzing sediment and water in addition to biota, expanding the spatial and temporal scope of investigations, including hydrodynamic understanding of microplastic advection, and examining the long-term impacts of microplastics on fish health, food webs, and ecosystem dynamics. These efforts are vital for informing policies aimed at protecting aquatic biodiversity and sustaining ecosystem services in the SLRE and similar environments worldwide.

CRediT authorship contribution statement

A. H. M. Enamul Kabir: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Elisa Michon:** Writing – review & editing, Investigation. **Marc Mingelbier:** Writing – review & editing, Resources, Investigation. **Dominique Robert:** Writing – review & editing, Resources, Conceptualization. **Youssouf D. Soubaneh:** Writing – review & editing, Resources. **Huixiang Xie:** Writing – review & editing, Supervision, Resources, Funding acquisition. **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 have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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Data availability

Data will be made available on request.

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