Supplementary Materials

Use of a fluorescent molecular rotor probe for nanoplastics assessment in epiphytic biofilms growing on submerged vegetation of Lake Saint Pierre (St. Lawrence River)

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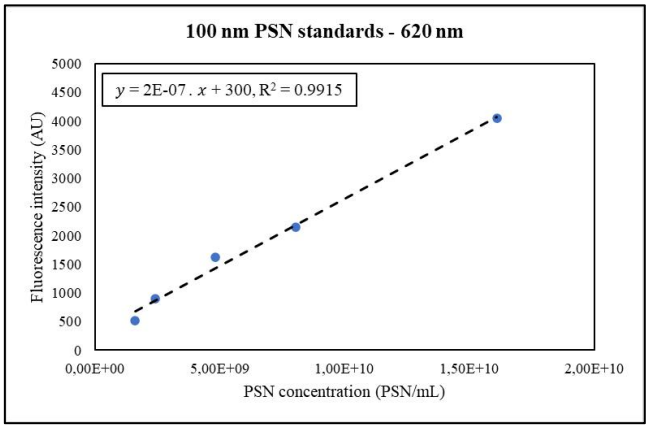
Supplementary Table 1. GPS coordinates and water physiochemistry of sampling stations

Sampling station	Latitude	Longitude	Conductivity (µs/cm)	Temperature (°C)	pH
A1	46,024188	-73, 180070	155,3	25,0	7,85
A2	46,050885	-73,179695	150,7	21,7	7,43
A3	46,055768	-73,157048	160,7	21,8	7,61
A4	46,064772	-73,112587	108,3	23,4	8,55
A5	46,078133	-73,031250	276,8	21,5	8,17
A6	46,118567	-73,039450	221,2	22,7	8,03
A7	46,089245	-72,984235	239,9	23,1	8,12
A8	46,146750	-72,992450	205,5	22,5	7,43
A9	46,123967	-72,968100	251,3	22,0	8,38
N1	46,179758	-72,972755	154,4	21,1	7,35
N2	46,261111	-72,825833	231,2	20,4	7,44
S1	46,049417	-73,125900	245,4	23,8	8,45
S2	46,133733	-72,857917	164,2	23,2	7,56
S3	46,202500	-72,724722	167,5	21,4	8,20

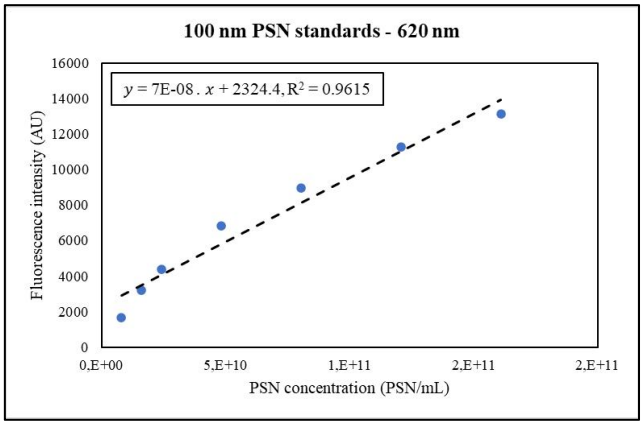
Supplementary Table 2. Autofluorescence of plastic materials assay. A 40 µL sample of standard polystyrene beads (Polyscience, Polybead® Microspheres) of different sizes was analyzed by spectrophotometry (excitation wavelength 450 nm), both with and without the DCVJ fluorescent probe. Fluorescence intensities are expressed as arbitrary units (AU)

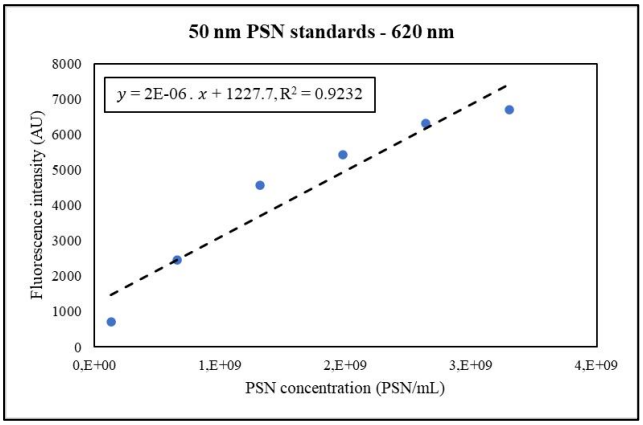
Probe	Sample type	Emission 620 nm (AU)	Final concentration (particles/mL)
In the presence of the DCVJ probe	Deionized water	1434	NA
	50 nm	4282	$6.37x10^{10}$
	100 nm	5074	$8.35x10^{10}$
In the absence of the DCVJ probe	Deionized water	1	NA
	50 nm	92	$-5.23x10^{9}$
	100 nm	18	$-7.08x10^{9}$

A



B (i) **(ii)**





Supplementary Figure 1. Standard curves obtained using suspensions of PSN of 100 nm at low (A) and high [B(i)] fluorescence intensity and PSN of 50 nm [B(ii)] in concentrations ranging from 2E+09 to 2E+11 PSN/mL. Fluorescence was determined at 450 nm excitation and 620 nm excitation. Black lines represent the linear regression and blue dots represent the PSN spiked concentrations. The curve for 100 nm at low fluorescence intensity (i.e., below 5 000) was used to calculate polystyrene-like nanoplastics concentration in biofilm samples. The curves for 100 nm at high fluorescence intensity (i.e., above 5 000) and 50 nm were used to calculate PSN concentration in QAQC samples whose raw fluorescence signal fit in these calibration curves.

Supplementary Table 3. Raw data for polystyrene-like nanoplastics (PSNP) detected in biofilm samples at all sampling stations. Results are expressed as numbers of particles per ml of digested biofilm (PSNP/mL) or numbers of particles per mg biofilm dry weight (PSNP/mg). The corresponding fluorescence intensities are expressed as arbitrary units (AU) and were determined at 450 nm excitation wavelength; the 620 nm fluorescence intensity was used for nanoplastic quantification and 512nm as an estimate for samples' viscosity.

Sampling station	Sampling point	Emission 620 nm (AU)	PSNP concentration (PSNP/mL)	PSNP concentration (PSNP/mg)
A1	A	2321	2,02E+11	1,01E+09
	B	2830	2,53E+11	1,26E+09
	C	3855,5	3,55E+11	1,78E+09
A1	A	1756	1,45E+11	7,27E+08
	B	2751	2,45E+11	1,22E+09
	C	1454	1,15E+11	5,76E+08
A3	A	4251	3,95E+11	1,97E+09
	B	3766	3,46E+11	1,73E+09
	C	3826	3,52E+11	1,76E+09
A4	A	2377	2,07E+11	1,04E+09
	B	1862	1,56E+11	7,80E+08
	C	2824	2,52E+11	1,26E+09
A5	A	833	5,32E+10	2,66E+08
	B	748	4,48E+10	2,24E+08
	C	321	2,10E+09	1,05E+07
A6	A	1994	1,69E+11	8,46E+08
	B	2232	1,93E+11	9,65E+08
	C	1806	1,50E+11	7,52E+08
A7	A	1647	1,35E+11	6,73E+08
	B	1350	1,05E+11	5,24E+08
	C	2414	2,11E+11	1,06E+09
A8	A	4513	4,21E+11	2,10E+09
	B	4781	4,48E+11	2,24E+09
	C	3719	3,42E+11	1,71E+09
A9	A	2365	2,06E+11	1,03E+09
	B	2629	2,33E+11	1,16E+09
	C	2857	2,55E+11	1,28E+09
N1	A	3497	3,19E+11	1,60E+09
	B	1822	1,52E+11	7,60E+08
	C	3017	2,71E+11	1,36E+09
N2	A	1397	1,10E+11	5,48E+08
	B	1577	1,28E+11	6,38E+08
	C	1919	1,62E+11	8,09E+08
S1	A	1297	9,96E+10	4,98E+08

	B	1667	1,37E+11	6,83E+08
	C	1457	1,16E+11	5,78E+08
	A	6253	1,12E+12	5,61E+09
S2	B	5316	8,54E+11	4,27E+09
	C	5355	8,65E+11	4,33E+09
	A	3177	2,87E+11	1,44E+09
S3	B	3603	3,30E+11	1,65E+09
	C	3833	3,53E+11	1,76E+09
Blank 1		-655	-2,83E+11	
Blank 2		-627	-2,81E+11	
Blank 3		-514	-2,70E+11	