

Fish community shifts along a strong fluvial environmental gradient revealed by eDNA metabarcoding

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Abstract

Large rivers and their estuaries are structurally complex and comprise a diversity of habitats supporting a rich biodiversity. As a result, identifying and monitoring fish communities using traditional methods in such systems may often be logistically challenging. Using the mitochondrial DNA 12S MiFish primers, we performed an eDNA metabarcoding analysis to assess the effect of spatial and environmental factors on the variation of the fish community structure along most of the St. Lawrence River/Estuary/Gulf (Québec Canada), a transect spanning 1300 km across a diversity of habitats from a fluvial non-tidal section to a marine environment. A total of 129 species were identified including freshwater and marine species. For the freshwater sectors, eDNA identified 80 species compared with the 85 species previously reported based on conventional sampling. eDNA also revealed similar species diversity and communities in the fluvial section of the St. Lawrence River. Furthermore, our study improved current knowledge about the brackish and marine sections by describing community transition between freshwater and marine fish communities in association with a drastic shift in environmental conditions observed between the end of the fluvial estuary and the beginning of the middle (brackish) estuary. Altogether, this study exemplifies how eDNA metabarcoding is a powerful tool to document fish community shifts in large temperate lotic ecosystems.

KEYWORDS

biodiversity, biomonitoring, community survey, environmental DNA, freshwater fishes, lotic system, marine fishes

1 | INTRODUCTION

Because so many urban areas were developed around them, a large proportion of the human population depends on river services and resources (Kummu et al., 2011). Rivers have provided water, food, and transportation pathways and have supported agricultural

development from the origin of civilization (Gleick, 1996). Despite covering a small area of our planet, rivers harbor a rich biodiversity playing fundamental roles in ecosystem function (Blanchet et al., 2020; Dudgeon et al., 2006; Lundberg et al., 2000; Reid et al., 2019). However, human infrastructures (e.g., channels, dams), contamination, increasing freshwater usage, over-exploitation

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of living resources, the introduction of non-indigenous species, and climate changes have caused ecosystem disturbances driving habitat degradation and the extinction of thousands of freshwater species all over the world (Brismar, 2002; Burkhead, 2012; Dudgeon et al., 2006; Jelks et al., 2008; Reid et al., 2019; Sala, 2000; Su et al., 2021).

In Canada, the St. Lawrence River did not escape this reality and numerous sources of stress have impacted its ecosystems over the last 160 years (Côté & Morin, 2007; Foubert et al., 2020; Marty et al., 2010; Paradis et al., 2020). Dragging 25% of the world's freshwater reserves, the St. Lawrence River is one of the largest rivers in the world (Centre Saint-Laurent, 1996). It drains the Laurentian Great Lakes and a vast hydrographic network composed of more than 200 tributaries that support an important number of aquatic species of great socioeconomic and environmental value (Centre Saint-Laurent, 1996). In Québec (Canada), concerns about fish species conservation status compelled authorities to create, in 1995, the Fish Monitoring Network (FMN) of the freshwater section of St. Lawrence River. A long-term netting (i.e., gillnets and seine nets) program has delivered valuable information on community structure, biodiversity, and stock status of fishes throughout several sectors of the river (Foubert et al., 2018; Mingelbier et al., 2016; Paradis et al., 2020). Due to its large scale and complexity, the fish communities of the St. Lawrence River are multidimensionally heterogeneous. Several fish assemblages have been identified both along the river's main axis (Foubert et al., 2018) but also transversally (Berger et al., 2020; Foubert et al., 2018). The interplay between various factors can explain the strong structuration of fish communities in the St. Lawrence River. The differences between water masses from tributaries (e.g., the "green waters" from the Great Lakes and the "brown waters" from the Ottawa River) that flow side by side without mixing along an important part of the fluvial section of the river, as well as the influence of tides and changes in salinity, are among the most relevant (Berger et al., 2020; Centre Saint-Laurent, 1996; Vincent et al., 1996).

Precise and swift monitoring of fish communities to understand their structure and response to natural and anthropogenic environmental changes is costly and logistically challenging in large river systems such as the St. Lawrence River, Estuary, and Gulf (Thomsen & Willerslev, 2015; Valentini et al., 2016). Traditional sampling methods (TSM) such as direct observation and capture using nets (i.e., gillnets and seines) or electrofishing are the most current practices for collecting, identifying, and counting fish species (Gunzburger, 2007). However, these techniques are invasive, perturb habitat, and communities, and in most cases are lethal (Dalu et al., 2015; Portt et al., 2006; Snyder, 2003). Moreover, the complexity of some ecosystems (e.g., depth, high-speed, and water turbidity in large rivers), and practical limitations of TSM (e.g., species selectivity and species-specific behavior) may bias appraisals of fish assemblage diversity (Allard et al., 2014; Fujii et al., 2019; Hercos et al., 2013; Hubert et al., 2012; Pope et al., 2010).

eDNA metabarcoding can efficiently overcome the challenges of TSM while providing accurate identification and, in some cases, quantification of species (Beng & Corlett, 2020; Coble et al., 2019; Deiner et al., 2017; Hänfling et al., 2016; Pont et al., 2020). This approach progressively emerges as a useful tool to reveal ecosystem-level processes and dynamics and to provide assessments of biodiversity (Bohmann et al., 2014; Deiner et al., 2021). However, hydrological conditions have profound effects on eDNA dynamics, which may affect the efficiency of eDNA metabarcoding for monitoring fish communities (Harrison et al., 2019). In lotic environments, eDNA can be rapidly transported from a few meters to many kilometers from its source, depending on the environmental characteristics of the system (Civade et al., 2016; Minshall et al., 2000; Pont et al., 2018; Wilcox et al., 2016). Newly available information highlights the effects of discharge intensity and distance on eDNA detectability, evidencing that under high current velocities eDNA rapidly fades under the effect of dilution (Thalinger et al., 2021) but see also (Wood et al., 2020). Similarly, lateral eDNA distribution heterogeneities due to hydrodynamic characteristics of river flow and seasonal variations have been described for temperate rivers (Laporte et al., 2020; Thalinger et al., 2021). Despite high downstream transport and evidence that information obtained from eDNA can unify spatially structured biodiversity (Deiner et al., 2016), a growing number of studies portray eDNA metabarcoding as a useful tool to obtain accurate descriptions of varied fish assemblages and biodiversity patterns at different geographic scales in temperate streams and large rivers (Civade et al., 2016; Li et al., 2018; Olds et al., 2016; Shaw et al., 2016). However, most studies published so far in fluvial systems focused on relatively small geographic scales where environmental variables are spatially relatively homogenous, with very few studies demonstrating the reliability of eDNA metabarcoding to describe the longitudinal pattern of fish assemblages in large heterogeneous systems like the 500 km of the Rhone River (Pont et al., 2018).

The present study aims to use eDNA metabarcoding to assess the effect of spatial and environmental factors on the composition of fish communities along a nearly 1300 km transect of the St. Lawrence River system that comprises three distinct aquatic realms (freshwater, estuary, and saltwater) from the Island of Montréal to the northwest of Newfoundland in the Gulf of St. Lawrence. We tested the ability of eDNA metabarcoding to identify and delineate fish communities associated with distinct hydro-morphological structures (i.e., corridors, lake, estuaries, and gulf) and assess the role of environmental factors shaping fish assemblages along the river/sea/scape. Then, we tested whether the pattern of differentiation and fish biodiversity follows a gradient from headwaters to the Gulf of St. Lawrence. We finally compared and discussed the eDNA metabarcoding results with those obtained by the long-term survey program conducted by the Fish Monitoring Network in the fluvial sectors of the river (Foubert et al., 2018) and evaluate the utility of eDNA metabarcoding as an approach for fish biomonitoring in large temperate lotic ecosystems.

2 | MATERIAL AND METHODS

2.1 | Study area

We compiled eDNA metabarcoding sequencing from four sampling campaigns to characterize fish communities over a distance of 1300 km comprising the fluvial, estuarine, and gulf sections of the system between the Island of Montréal and the northwest of Newfoundland (Figure 1). Following an upriver-to-downriver direction, five sections, mainly distinguished by the level of salinity and the influence of marine tides, are recognized between Cornwall (Ontario) and the Gulf of St. Lawrence (Québec): Fluvial section, Fluvial Estuary, Middle Estuary, Marine Estuary, and Gulf of Saint Laurent (Centre Saint-Laurent, 1996). First, the Fluvial section spans 240 km of freshwater flowing unidirectionally with a limited admixture of the waters from the major tributaries draining in the system (Figure 1). We considered three sectors within this river section as defined in Foubert et al., (2018): Montréal – Sorel (MS-1), the Archipelago of Lake Saint-Pierre (ALSP-2), and Lake Saint-Pierre (LSP-3) (Table 1).

The second section is the Fluvial Estuary (160 km), which is delimited downstream by the eastern end of Orléans Island near Québec City. We distinguished three sectors for this second river section: Trois-Rivières – Grondines (TG-4), Grondines – Saint-Nicholas (GSN-5) as defined in Foubert et al., (2018), and Québec – Orléans Island (QIdO-6). The TG-4 sector is microtidal, and the water

from tributaries is still largely unmixed. The GSN-5 is a mesotidal sector though, downstream from Grondines, the progressive effect of freshwater tides produces a reversal of the current and there is water mass mixing (Centre Saint-Laurent, 1996). The QIdO-6 is the last freshwater sector of the river and is macrotidal (Gauthier, 2000).

The third section is the Middle Estuary (sector MiE-7), a 150 km long river stretch ranging from the eastern end of Orléans Island to Tadoussac and Cacouna (Figure 1), whose brackish waters define the transition zone between the river and the marine waters. In this sector, the effect of tides is maximal, reaching up to 6.9 m at the eastern end of Orléans Island (DesGranges & Ducruc, 1998; Godin, 1979). The fourth section we analyzed is the Marine Estuary (sector MaE-8), which spans 230 km from Tadoussac to Pointe-des-Monts and is mesotidal (Figure 1). Finally, the Gulf of St. Lawrence (also sector GSStL-9) is a semi-closed and mostly shallow sea of about 263 km² (Figure 1; Appendix S1A for details).

2.2 | Water sampling and processing

A total of 130 sampling stations from the four sampling campaigns were combined for subsequent analyses (see Figure 1; Table 1; Appendix S2 for details). The first sampling event (September 13 and 15, 2017) was in a 5 km region about 40 km downstream of Montréal (Berger et al., 2020), and 3 sampling stations were randomly selected to represent this sector. The second sampling event

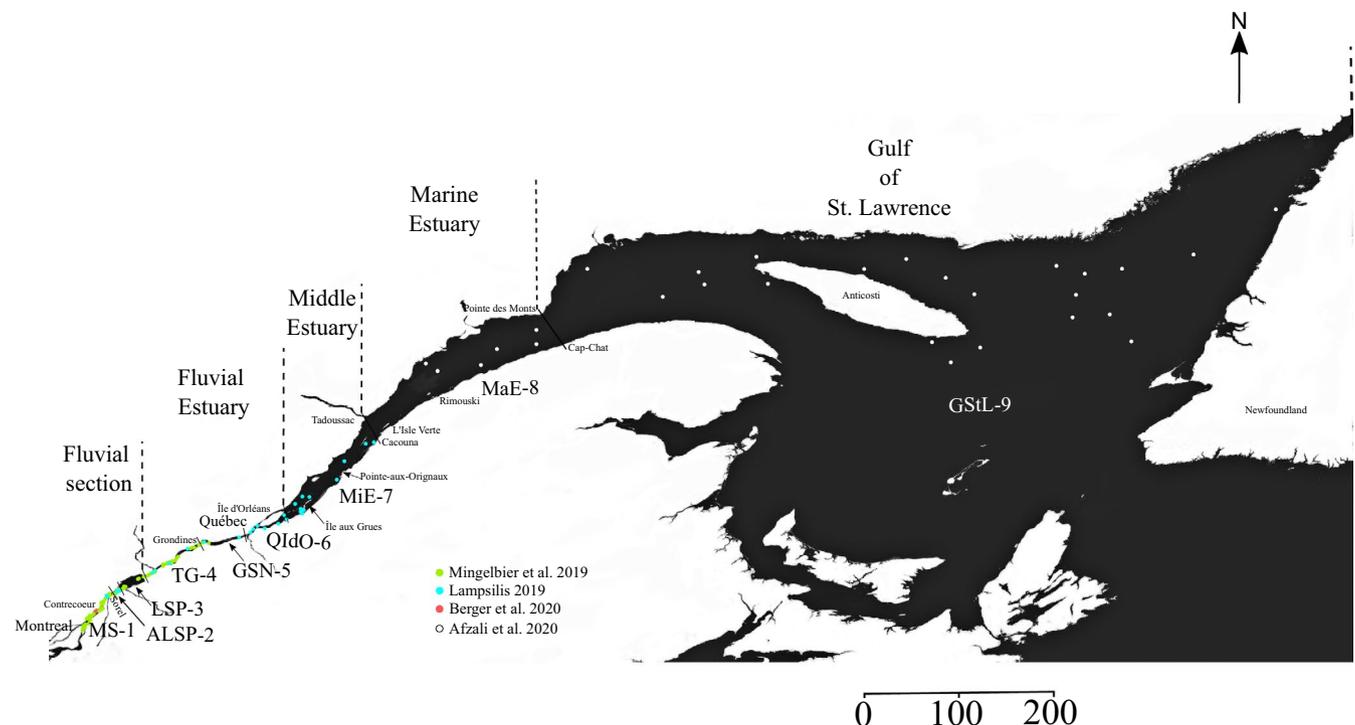


FIGURE 1 Study area of the St. Lawrence River. The different sections recognized along the river are delimited by vertical dashed bars. The sectors are delimited by the continuous bars and identified by acronyms: ALSP-2, Archipelago of Lac Saint-Pierre; GSN-5, Grondines – Saint-Nicholas; GSStL-9, Gulf of St. Lawrence; LSP-3, Lac Saint-Pierre; MaE-8, Marine Estuary; MiE-7, Middle Estuary; MS-1, Montréal-Sorel; QIdO-6, Québec – Île d'Orléans; TG-4, Trois-Rivières – Grondines

TABLE 1 Sampling sites, number of samples, and number of species identified by eDNA at each sector

	Sectors									Total
	MS ^a 1	ALSP 2	LSP 3	TG 3	GSN 4	QldO 5	MiE 6	MaE 7	GStL 9	
Length (km)	65	22.4	25.6	65	55	35.4	42.3	213.8	770	1294.5
No. stations	26	14	7	19	9	8	10	12	23	130
No. samples	43	20	14	35	13	16	20	12	23	196
No. species (richness)	73	68	59	72	59	69	73	35	39	127

Abbreviations: ALSP-2, Lake Saint-Pierre Archipelago; GSN-5, Grondines – Saint-Nicolas; GStL-9, Gulf of St. Lawrence; LSP-3, Lake Saint-Pierre; MaE-8, Marine Estuary; MiE-7, Middle Estuary; MS-1, Montréal – Sorel; QldO-6, Québec – Orléans Island; TG-4, Trois-Rivières – Grondines.

^aFirst upstream station near the Old Port of Montréal.

(July 3 to 13, 2018) consisted of 61 sampling stations covering the river stretch between Montréal and Donnacona, which is about 40 km upstream of Québec City (Mingelbier et al., 2019). The third (identified as Lampsilis 2019 expedition) consisted of 29 stations sampled between August 31 and September 11, 2019, covering the river stretch between Sorel and Cacoua. Results of eDNA metabarcoding analyses from these last two sampling campaigns are new and have not been published before. The last set was a sampling campaign covering the Marine Estuary and the Gulf of St. Lawrence (August 2 to September 2, 2017) (Afzali et al., 2021), and 35 sampling sites out of 84 were randomly selected to represent this sector. All samples of Afzali et al. (2021) were collected using a bottom trawler at a mean depth of about 150 m. Subsampling from the studies of Berger et al. (2020) and Afzali et al. (2021) was done to balance the sampling representation across the different defined sectors. Additionally, we tested both data sets (i.e., subsample and full data set) from the Marine Estuary and the Gulf of St. Lawrence for differences in estimates of diversity and they did not differ statistically (rarefaction index $t = 0.41487$, $df = 117$, p -value = 0.679; Simpson (1-D) index $t = 0.72273$, $df = 117$, p -value = 0.4713; Simpson evenness index $t = -0.51091$, $df = 117$, p -value = 0.6104) although some species were missed (see Section 4 and Figure 1). The water volume collected (i.e., 250 ml to 2 L), filtration procedure (i.e., in situ filtration or frozen water and laboratory filtration), and filter storage (i.e., direct freezing, Longmire buffer preservation, and freezing) varied among the four studies (Appendix S2 for details). As the number of eDNA reads can be affected by differences in protocols, the number of eDNA reads was transformed in relative abundances for all subsequent analyses to mitigate biases between species with high and low numbers of counts (Laporte et al., 2021, see also Data processing below). For all four sampling campaigns, field negative controls were performed as detailed in Berger et al., (2020) and Afzali et al., (2021).

2.3 | eDNA extraction, amplification, sequencing

The eDNA from the full set (i.e., published and unpublished data) of water samples was extracted following the same protocols and sterilization methods, as well as methods for field and laboratory negative controls were as described by Afzali et al. (2021) and Berger

et al. (2020) and based on Goldberg et al. (2011) and Spens et al. (2017) protocols. The MiFish primer pair (Miya et al. 2015) amplifying a hypervariable segment (approx. 170 bp) of the 12S RNA mitochondrial gene was used, and detailed protocols of amplification and high-throughput sequencing were described elsewhere (Afzali et al. 2021; Berger et al. 2020) (see Appendix S1B for further details).

2.4 | Data processing

We filtered raw sequencing reads to remove primer sequences and demultiplexed using the MiSeq Control software v2.3. We trimmed, merged, and extracted reads from 5' and 3' to keep only sequences containing the 12S MiFish primers and analyzed the sequences using the Barque v1.5.2 pipeline developed in our research group (www.github.com/enormandeau/barque). When compared with other pre-build pipelines, Barque was shown to be an efficient alternative to some of the existing and highly used pipelines. Of the pipelines tested, Barque had the best sensitivity and specificity metrics as well as run times (Mathon et al., 2021). Detailed Barque settings for sequence analysis can be found on the GitHub webpage. We used default settings from v1.5.2. Taxonomic assignment was performed by searching in our reference database (also available as a part of Barque at www.github.com/enormandeau/barque), which is composed of sequences from the MitoFish database (Iwasaki et al., 2013), the GenBank database (Benson et al., 2012), and the Barcode of Life (Ratnasingham and Hebert, 2007), supplemented with new sequences generated in our laboratory to ensure that all freshwater/diadromous fish and most marine species in the study area were represented in the database.

We used a 97% sequence similarity as a threshold for species assignment. We used genus or family level classification when closely related species showed identical sequences. We used the reads detected in the negative controls from the field and laboratory to minimize false positives. We corrected each species number of reads by subtracting the maximum number of sequence reads for this species observed in any of the negative controls. To standardize data across data sets, we translated the matrix of the number of reads (the response matrix) (Appendix S3) into a matrix of relative abundances by applying the Hellinger transformation of data ($y'_{ij} = \text{SQR}(y_{ij}/y_{i+})$),

where y_{ij} is the abundance of species j in site i , and y_{i+} is the sum of abundances in site i (Legendre & Gallagher, 2001) using the *deco-stand* function in *vegan* 2.5-7 package (Oksanen et al., 2020). The Hellinger transformation corrects for biases produced by high read values and double 0 similarities and shows better performance with eDNA metabarcoding data set (Laporte et al., 2021; Legendre & Legendre, 2012).

2.5 | Environmental variables

We considered four environmental variables that were available for the entire study area that were likely to influence the distribution of fish communities: salinity, temperature, tide, and the number of growing days. We obtained the salinity and temperature data from the Government of Canada website (<https://open.canada.ca/data/en/dataset/8a3dc9e5-f3af-4270-8c09-43fa2c25848b>) and from Afzali et al. (2021). We obtained tide information from the Fisheries and Oceans Canada website (<https://www.marinfo.gc.ca/e-nav/stl-stl/stl-glf3-eng.php?pedisable=true#>). We also included the maximum number of growing days (Lepage et al., 2012), a measure of the length of the growing season for plants and cultures for a given region which begins when the temperature remains stable over 5°C. This agrometeorological index implicitly involves climate variations (i.e., temperature and precipitations) that may have an impact on temperature requirements for fish reproduction and growth, etc., and thus affect the geographic distribution of species.

2.6 | Community analysis

2.6.1 | Longitudinal variation of fish communities detected by eDNA

We used a shading matrix (heatmap) diagram to visualize the spatial distribution of the relative number of eDNA sequence reads for each species along the total experimental transect. We log-transformed the response matrix using *deco-stand* function implemented in the *vegan* 2.5-7 R package (Oksanen et al., 2020). We arranged species from higher to lower numbers of reads and according to three main fish groups; strictly freshwater, marine/brackish, and diadromous. For the heatmap and all subsequent analyses, we arranged sampling stations according to their relative geographic position along the river. We performed a principal component analysis (PCA) to portray the main fish assemblages along the river according to the distribution of the Hellinger transformed response matrix of eDNA read counts.

To further assess the boundaries between fish communities occurring along the upstream–downstream axis of the river without a priori delimitation of sectors defined in previous studies, we used a multivariate regression tree (MRT) (Borcard et al., 2018; Moisen, 2008) (function *mvpart* from the homonymous package) constrained

by the environmental descriptors (i.e., tide, temperature, salinity, and the maximum number of growing days). We used the Hellinger transformed response matrix and the environmental variables standardized to a matrix with mean of zero and a variance of one using the *standardize* option of the *deco-stand* function implemented in the *vegan* 2.5-7 R package (Oksanen et al., 2020). We selected the tree with the smallest cross-validated relative error (CVRE) (De'ath & Fabricius, 2000). We also evaluated the tree whose CVRE was within one standard error (± 1 SE) of the tree with the smallest CVRE after 100 iterations, which is considered the tree with the highest predictive power.

To test for differences in fish assemblages among predefined hydro-morphological structures described above and assess the impact of environmental descriptors (i.e., Sector, Salinity, Temperature, Tide) on community structure, we performed a Permutational Multivariate Analysis of Variance (PERMANOVA) (Anderson, 2001), implemented in the *vegan* 2.5-7 R package, using the *adonis* function with 9999 permutations. This analysis was based on Bray-Curtis distances calculated from the Hellinger transformed response matrix. As our sampling design is unbalanced (i.e., unequal number of observations for the fixed effect factor: sector), and this can strongly bias the results of this analysis (Anderson & Walsh, 2013), we also tested the homogeneity of the groups using *betadisper* function (with 1000 permutations) in *vegan* 2.5-7, which is an implementation of the PERMDISP2 routine based on centroids (Anderson, 2006).

2.6.2 | Indicator species

We used the indicator value species analysis (IndVal) (Dufrene & Legendre, 1997) using *multipatt* (*IndVal.g* function) implemented in the package *indicspecies* (ver. 1.7.8) (De Cáceres & Legendre, 2009) to test for species association to one or more sampling site combinations. Indicator species are determined as the product of their relative abundance and their occurrence in the different sites of a sector. Values close to 1 have the strongest predictive value. We also performed a multilevel pattern analysis (function *IndVal.g*) to estimate the specificity predictive value A, which corresponds to the probability that a given site belongs to the target sector and the fidelity value B which is the probability that the species appears at the sites of a target sector. We calculated the statistical significance for each species in a multigroup comparison analysis by using a random permutation procedure (9999 permutations) (De Cáceres et al., 2010). We applied Holm correction (Holm, 1979) to correct the significance values for multiple comparisons using the function *p.adjust* in R.

2.6.3 | Community diversity

We used three different measures of fish community diversity to shed light on different aspects of biodiversity, for example by giving different importance to very abundant and very rare species. The

rarefaction index (Hurlbert, 1971; Shimadzu, 2018) is a measure of the expected diversity, which allows the comparison of species richness under unequal sampling efforts. We used the function *rarefy* in *vegan* 2.5-7, fixing the number of samples to 6286, the smallest value of sequence reads obtained for a station. The Simpson index (1-D) (Simpson, 1949) is a dominance measure that provides the probability that two individuals (i.e., eDNA reads in this case) taken at random from a community belong to the same species. It is less sensitive than the species richness index (S) and Shannon diversity index (H') to different sample sizes and species richness and is strongly weighted toward the most abundant species (Magurran, 2004). Finally, we calculated the Simpson evenness index ($E_{1/D}$), which expresses how similar in numbers each species are in a given site and is not sensitive to the species richness (Smith & Wilson, 1996). We estimated these two latter indices using the *specnumber* and *diversity* functions in the *vegan* R package. To assess the relationship between diversity and longitude (used as the proxy for upstream-downstream location), we applied a non-parametric Spearman rank correlation (R function *cor.test*).

To further examine the trends in fish community diversity along the river's environmental gradients, we translated the response matrix into an occurrence matrix (i.e., "1" and "0"), and calculated the β -diversity and its two additive partitions (i.e., species replacement or turnover and nestedness) (Baselga, 2013; Legendre, 2014). We used the Sørensen dissimilarity index (β_{sor}) as a measure of the number of shared species between sites, being $\beta_{sor} = 0$ if two sites share the same species compositions, and $\beta_{sor} = 1$ if species compositions are completely different. The replacement, β_{sim} and the nestedness, β_{sne} components were computed following Baselga (2012) using the R package *betapart* (Baselga & Orme, 2012) (See Appendix S1C). We used the Mantel test (999 permutations of spatial distances) implemented in *vegan* to assess the effect of the geographic distance on β_{sor} . We also used the R package *geosphere*, and its function *geodesic* to build a matrix of geographic distances between stations based on the shortest distance between points of an ellipsoid. Additionally, we computed a Mantel correlogram using the function *mpmcorrelogram* in *vegan* to assess the geographic distance where fish community composition tends to differentiate.

3 | RESULTS

3.1 | Species and distribution

We identified 129 species representing 37 families and 22 orders (Appendix S4). For the freshwater sectors, Cyprinids species were the most represented (20% of the total number of species detected), followed by the Percidae (11%) and Catostomidae (9%) families. All freshwater, brackish, and marine species identified by eDNA are known to occur in the St. Lawrence River. Among all freshwater sectors combined, 86 species were identified when merging eDNA data and those from TSM in Foubert et al. (2018) and MMFP (unpublished data). A total of 80 of these (94%) were common to both methods,

1 was detected by eDNA only, and 5 were detected by TSM only (Appendix S3). The five species not detected with eDNA were the American gizzard shad (*Dorosoma cepedianum*), the cutlips minnow (*Exoglossum maxillingua*), the sand shiner (*Notropis stramineus*), the blacknose shiner (*N. heterolepis*), and the American pickerel (*Esox americanus americanus*). Among them, we could not discriminate the sequences of *N. heterolepis* from that of several other cyprinid species (i.e., *Luxilus cornutus*, *N. rubellus*, *N. volucellus*, *Pimephales notatus*). The number of eDNA reads in these cases of poor taxonomic resolution was marginal and thus excluded from the analysis. Ambiguous species identification also concerned *Percina caprodes*–*P. copelandi* and *Ictalurus punctatus*–*Ameiurus nebulosus*, but most sequences for those were properly assigned to one or the other species (see more details in Berger et al., 2020). Rainbow trout (*Oncorhynchus mykiss*) was detected by eDNA (5492 reads) but not by TSM although the species is known to occur in the St. Lawrence River. It is noteworthy that we also detected the Capelin (*Mallotus villosus*) (151 668 reads), an essentially marine species, in the most downstream freshwater sector. Finally, we identified a total of 41 species from the subsample of stations ($n = 35$) from the marine sectors MaE-8 and GSTL-9. This number was lower than the 72 species recognized using the full data set that was covering a broader part of the southern part of the Gulf of St. Lawrence (Afzali et al., 2021). Water sampling for the selected stations was done at the bottom only, which might contribute to the lower number of species detected. So, while randomly selecting stations from this study to balance the sampling effort with that of other sectors led to underestimating the total number of species present in the marine sectors, the diversity estimates did not differ from those that were estimated based on the 85 samples analyzed by Afzali et al. (2021), namely because the additional species had a low abundance (see details provided in the methods section).

The first inspection of the fish eDNA relative abundances along the study system offers an illustrated summary of the distribution and abundance of the main fish groups detected by eDNA according to their salinity preferences (Figure 2). As expected from the gradient spanning from freshwater to brackish to marine environments, the freshwater species were progressively replaced by diadromous and marine estuarine species in the transition zone represented by the QldO- Middle Estuary (MiE-7) sectors, followed by an almost exclusive occurrence of marine species (except for a few diadromous species) in the Marine Estuary (MaE-8) and the Gulf of St. Lawrence (GSTL-9).

The detection and relative abundance of eDNA reads of the different species varied widely along the study system. The eDNA of several species was detected throughout the freshwater and brackish water sectors but many showed more restricted distributions (Figure 3). For example, reads of *Acipenser fulvescens*, *Catostomus catostomus*, *Ictalurus punctatus*, and *Sander vitreus* were recorded in all freshwater and brackish water sectors (i.e., MS-1 to MiE-7), followed by the reads of *Moxostoma anisurum*, and *M. macrolepidotum*, which were also among the most abundant from MS-1 to QldO-6 (but not found in MiE-7). In the freshwater/brackishwater sectors, reads of some species (e.g., *Micropterus salmoides*) were more abundant in

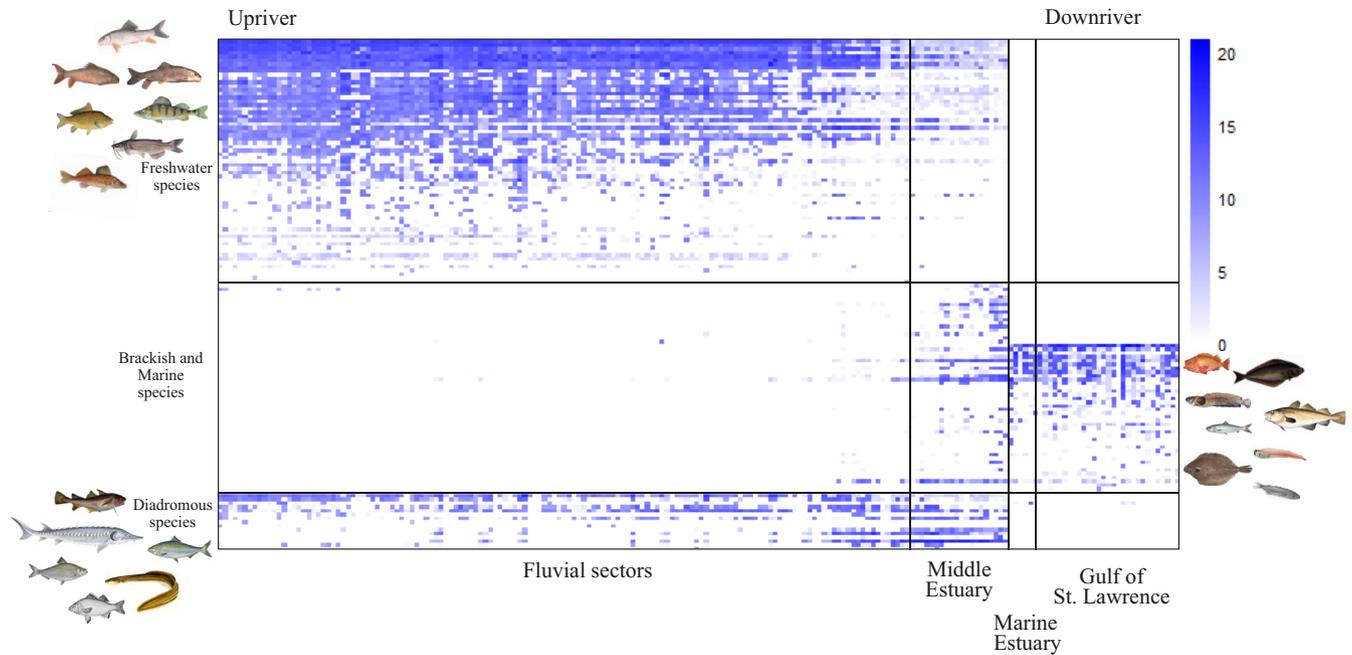


FIGURE 2 Shading matrix diagram showing the distribution of the fish eDNA sequence reads along the river. The species, in each main class group (i.e., freshwater, brackish–marine, and diadromous), were arranged according to their decreasing relative numbers of reads (i.e., from high to low). The most abundant species of each group are illustrated. The blue shading represents the spatial distribution of species along sectors with color intensity emphasizing relative abundance of eDNA sequence reads

upriver sectors (i.e., MS-1 to LSP-3) while others were more abundant downriver sectors such as QldO-6 (e.g., *Sander canadensis* and *Morone americana*) and MiE-7 (e.g., *Microgadus tomcod*, *Morone saxatilis*, and *Osmerus mordax*), as also reported by Foubert et al. (2018) from TSM data. The broad detection of eDNA from either the naturalized *Cyprinus carpio* and the invasive *Neogobius melanostomus*, a species for which eDNA reads were abundant in all stations from MS-1 to MiE-7 is noteworthy, also as reported by Foubert et al. (2018). At the scale of sectors (i.e., MS-1 to GSN-5 of the Fluvial section and Fluvial Estuary), eDNA detected on average 23.7% more species per sector compared with 7.1% detected on average for TSM, independently of the sector analyzed ($\chi^2 = 12.285$, $df = 4$, $p = 0.015$).

3.2 | Longitudinal differentiation of fish communities

The principal components ordination of the full set of stations showed that the first two components (PC1 and PC2) accounted for 58.06% of the total variation of the data matrix. We identified four main clusters, ordered along the main river axis (Figure 4). From an upstream–downstream direction, the first cluster combined sectors MS-1 to GSN-5 from the Fluvial Section and the Fluvial Estuary. The second cluster comprised only QldO-6 sampling stations located in the last portion of the Fluvial Estuary while the third cluster comprised the Middle Estuary (MiE-7) stations. Finally, the fourth cluster comprised sampling stations from the Marine Estuary (MaE-8)

and the Gulf of St. Lawrence (GSStL-9). The first component (PC1) differentiated freshwater sectors (MS-1 - GSN-5 and QldO-6) from brackish/marine waters (MiE-7 and MaE-8 - GSStL-9) fish communities while PC2 mainly distinguished intermediate sectors QldO-6 and MiE-7 from upstream and downstream sectors (Figure 4). Overall, these results illustrated that MiE-7 and QldO-6 sectors form a transition zone between fluvial and marine fish communities in the study system. Nine species showed high scores loads associated with the two main principal components: *Acipenser fulvescens*, *Catostomus commersonii*, *Cyprinus carpio*, *Moxostoma anisurum*, *M. macrolipeditum*, *Sander vitreus*, and *Neogobius melanostomus* (score: 0.89–0.34). Those were more associated with the freshwater fluvial sectors while *Microgadus tomcod* and *Sebastes* sp. (score: –0.89 to –0.34) were characteristic of more downriver saline sectors.

3.3 | Multivariate regression trees (MRTs)

The MRT with the smallest error (Figure 5) was a slightly more accurate predictor than the ± 1 SE CVRE tree (0.431 vs. 0.459, respectively), providing a finer resolution of community structure. The variation of the four environmental descriptors (tide, temperature, salinity, and the maximum number of growing days (grdays) used to constrain the MRT tree were highly correlated all along the river axis and were associated with several of the detected community splits (Figure 5; Appendix S5b). Tide, temperature, and salinity were strongly related to the community split detected among downriver sectors from QldO-6 (in the Fluvial Estuary) to GSStL-9 (Gulf), while

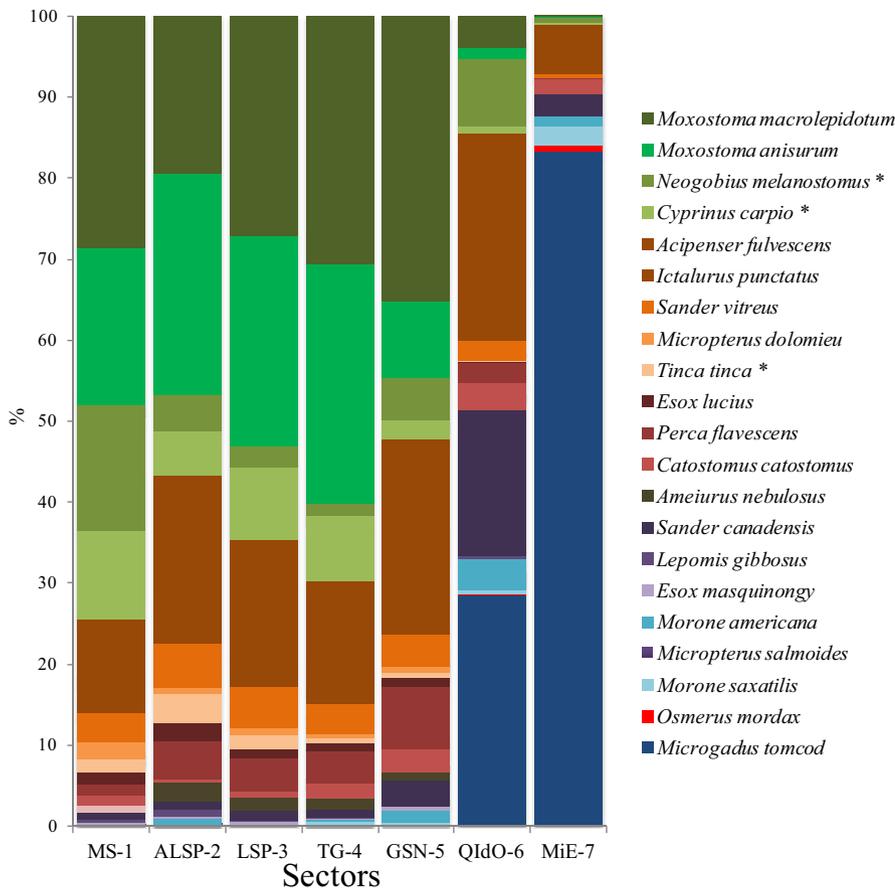


FIGURE 3 The proportion of eDNA sequence reads scaled for a selected group of species distributed all along the fluvial and brackish water sectors of the Saint Lawrence River. Asterisks point out the invasive and naturalized species. ALSP-2, Archipelago of Lake Saint-Pierre; GSN-5, Grondines – Saint-Nicholas; LSP-3, Lake Saint-Pierre; MiE-7, Middle Estuary; MS-1, Montréal-Sorel; QldO-6, Québec – Orléans Island; TG-4, Trois-Rivières – Grondines

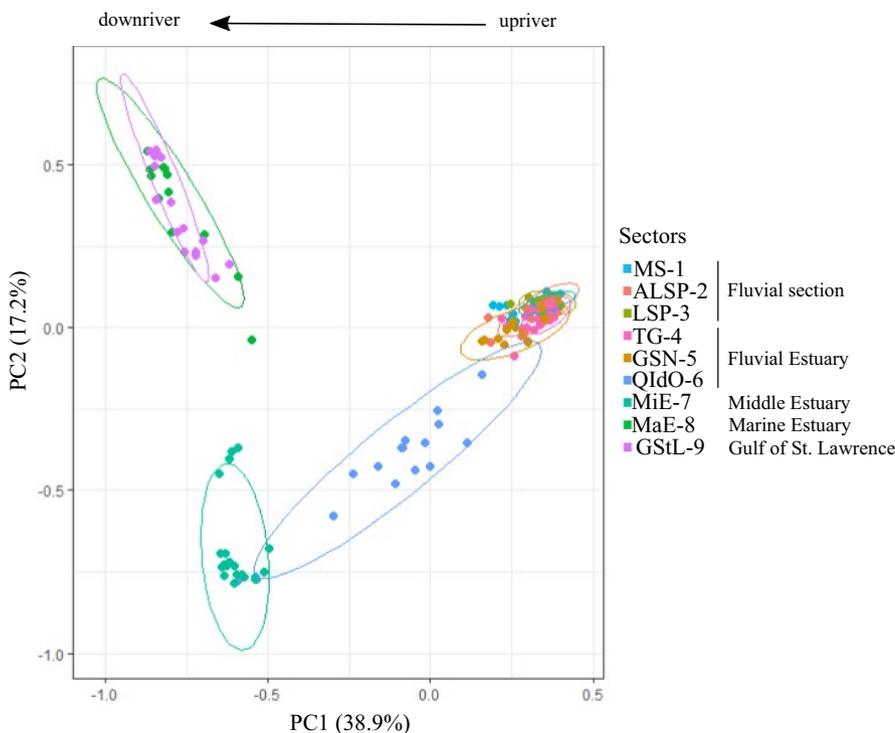


FIGURE 4 Ordination analysis of the Hellinger transformed eDNA sequence reads. The sectors and their 95% confidence ellipses are highlighted with different colors

local hydro-morphological structures (e.g., corridors, archipelago, lake) determined fish community composition in the upriver sectors (i.e., MS-1 to GSN-5) (Appendix S5a). The ten-leaf tree showed

the first division of sampling stations, explaining 36% of the variation, with the QldO-6 sector marking the limit between fluvial and brackish–marine fish communities. Here, the split was mainly

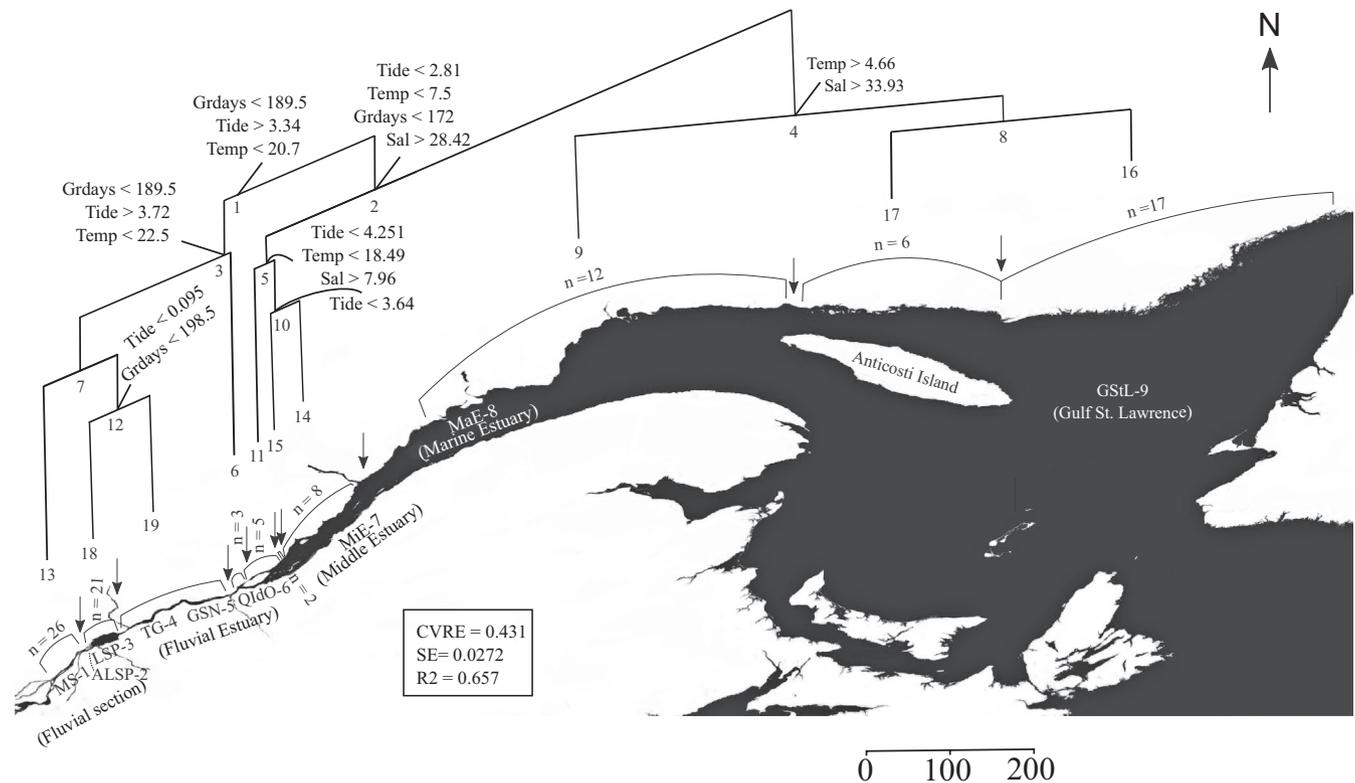


FIGURE 5 Multivariate regression tree (minimum standard error) describing the main fish community partitions inferred along the Saint Lawrence River. The arrows indicate the point of the split between communities. Nodes and leaves are identified by numbers, and the number of stations (n) is indicated for each leaf. The splits determined by the alternative predictor variables are indicated, and the values refer to the right side of each node (i.e., upstream sector to the referred split point). Tide (Tide), temperature (Temp), salinity (Sal), and the maximum number of growing days (Grdays)

driven by the effects of a lower number of growing days, but the effect of the increased tide (>3.34 m), and reduced mean temperature ($<20.7^{\circ}\text{C}$) on downstream sectors was also strong. In general, a higher number of growing days, no or very low salinity, and higher temperatures characterized the environment in the upriver sectors. The ten partitions were not completely in agreement with the predefined hydro-morphological sectors. The QldO-6 sector split into two communities, as were the MiE-7, and GStL-9 sectors. The split of MaE-8 and GStL-9 marine communities from the previous upstream community occurred at a converging point of a drop in temperature, a dramatic increase in salinity, and a lower number of growing days, and a progressive reduction in the magnitude of tides. The split between the last two communities (i.e., MaE-8 and GStL-9) was associated with a shift in mean temperature, which was relatively lower in the GStL-9 sector, and an increase in salinity. This also revealed that the Marine Estuary (MaE-8) fish community extended east up to the western extreme of the Anticosti Island instead of being constrained to the predetermined limits (i.e., Pointe-des-Monts on the north shore and Cap-Chat on the south shore of this sector) (Figure 1). More upriver, the analysis identified three splits: MS-1, ALSP-2 + LSP-3, and TG-4 + GSN-5, the last two being under the effect of tides (>0.095 m increase downriver), and a lower number of growing days (<198.5) downriver.

3.4 | Community differentiation among hydro-morphological sectors

For this and all subsequent analyses, we kept the predefined limits among sectors but extending the limit of the Marine Estuary (MaE-8) to Anticosti Island according to the result of the MRT. For simplicity, we did not consider the additional subdivisions inferred by the MRT for QldO-6, MiE-7, and GStL-9 sectors. Here, paired comparisons among contiguous sectors performed using PERMANOVA showed that all sectors were significantly characterized by distinct fish communities ($p < 0.001 - p < 0.02$). The multivariate homogeneity test of group dispersions was significant ($p < 0.001$) for the TG-4 - GSN-5 comparison only, suggesting that data dispersion was not homogeneous. The pairwise comparisons provided more details on the role of environmental descriptors potentially shaping community structure along the study system. The effects of sector, tide, and temperature were significant ($p < 0.05 - p < 0.001$) and explained the differentiation between sectors in the following pairwise comparisons: LSP-3 - TG-4, TG-4 - GSN-5, and GSN-5 - QldO-6. Similarly, all descriptors were highly significant ($p < 0.001$) when comparing QldO-6 - MiE-7, and MiE-7 - MaE-8 sectors. Overall, these results corroborated and complement the inferences obtained by the MRT analysis.

3.5 | Indicator species

A total of 52 indicator species were identified for one sector or a composite of two or more sectors (Appendix S6). Among them, we identified several of the most abundant species characterizing the groups class identified (see Figures 2 and 3). Only two marine species were strongly linked to a single sector; *Melanostigma atlanticum* in MaE-8 (Marine Estuary) and *Scomber scombrus* in GStL-9 (Gulf) although only *M. atlanticum* ($A = 0.98$, $B = 1$) was a good indicator of its associated sector. The reads of several species showed both high specificity and high fidelity with two sectors upstream of those two marine sectors. *Microgadus tomcod* (estuarine dependant) and *Acipenser oxyrinchus* (diadromous) ($A \geq 0.98$; $B = 1$) were indicator species for the QIdO-6 and MiE-7 transition sectors; *Mallotus villosus* (a marine species tolerating lower salinity conditions) ($A = 0.94$, $B = 1$) was a good indicator for the MiE-7 and MaE-8 sectors while *Glyptocephalus cynoglossus*, *Sebastes* sp., *Reinhardtius hippoglossoides*, and *Gadus morhua* (all marine species) (A range 0.99–1.00; B range 0.89–1.00) were good indicators of MaE-8 and GStL-9 sectors. In contrast, the strongest associations for the fluvial sectors were found for composite groups of five to seven sectors. Five mostly freshwater native species (*Ameiurus nebulosus*, *Esox lucius*, *Hiodon tergisus*, *Aplodinotus grunniens*, *Moxostoma macrolepidotum*), and two introduced species (*Cyprinus carpio* and *Tinca tinca*) showed high specificity (A range 0.98–0.99) and fidelity (B range 0.86–1.00) to all fluvial sectors from MS-1 to GSN-5. Seven freshwater and low-salinity-tolerant species (*Perca flavescens*, *Catostomus commersonii*, *Carpionides cyprinus*, *Lota lota*, *Percopsis omiscomaycus*, *Noturus flavus*, and *Percina* sp.) showed high specificity (A range 0.98–0.99) and fidelity (B range 0.86–1.00) to all sectors from MS-1 to QIdO-6 (freshwater sectors), while nine species (*Acipenser fulvescens*, *Anguilla rostrata*, *Catostomus catostomus*, *Ictalurus punctatus*, *Moxostoma anisurum*, *M. macrolepidotum*, *Sander vitreus*, *S. canadensis*, and the invasive species *Neogobius melanostomus*) were recorded across most of the stations of all sectors from MS-1 to MiE-7 (Freshwater to Middle Estuary sectors) (Figure 3, Appendix S6).

3.6 | Upstream–downstream variation of fish diversity

All three measures of species richness and equitability (i.e., evenness) showed lower values toward the downriver sectors as indicated by a negative and highly significant ($p < 0.001$) correlation between each index and the longitude (Figure 6). All measures dropped drastically in the brackish and marine water sectors suggesting lower diversity and lower equitability. In contrast, Simpson's evenness correlations were not different from zero when brackish and marine water sectors were excluded (Appendix S7). Among the freshwater sectors (i.e., MS-1 to QIdO-6), species diversity was higher and its distribution was relatively homogenous among stations. The fish community of ALSP-2 tended to exhibit the highest value of species richness in contrast to that of LSP-3 and GSN-5 which showed lower values of

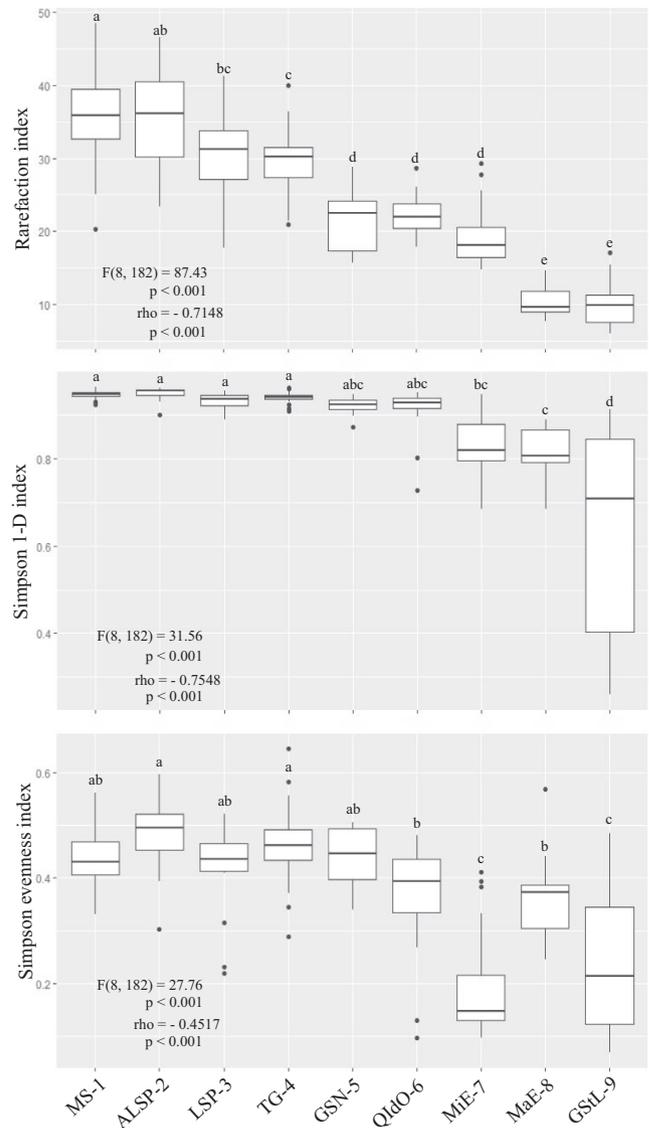


FIGURE 6 Boxplots showing the variation of the diversity indices used. (A) Rarefaction index estimated for a sample size of 80 reads; (B) Simpson diversity index; (C) Simpson evenness index. Spearman correlation coefficients (ρ) and significance values were obtained for the full set of sectors. One-way ANOVA and Tukey post hoc tests were used for multiple comparisons analysis with $\alpha = 0.05$ cutoff. Different letters indicate statistically significant differences

diversity and equitability as indicated by rarefaction and Simpson evenness indices (Figure 6). Further downstream, the Middle Estuary (MiE-7) sector showed contrasting values of diversity estimates with relatively high richness and low equitability value. This result agreed with the overwhelming relative number of reads of the species *Microgadus tomcod* compared with the high number of species detected in this sector (Figure 3, Table 1). The Gulf (GStL-9) showed the lowest diversity estimates together with pronounced differences among stations, as indicated by the tall boxplots of Simpson (1-D) index and Simpson evenness index (Figure 6). The contrast between these two indices evidenced a relatively large proportion of stations

characterized by low diversity estimates and strong dominance by few species.

The beta-diversity (proportion of diversity due to differences among sampling sites within sectors) estimates (β_{sor}) were high and the turnover (β_{sim}) component largely dominated over the nestedness all through the study system which determined the pattern variation of the beta-diversity as well (Table 2). The sectors MS-1, TG-4, MiE-7, and GStL-9 showed the highest diversity values. The Mantel test showed a positive and significant ($\rho = 0.88$, 97.5% quantile = 0.0879; $p = 0.001$) correlation between the pairwise β_{sor} dissimilarity matrix among stations and the geographic distance indicating the increment of the beta-diversity with distance. Following the same trend, the results of the Mantel correlogram (Appendix S8) showed significant and positive spatial correlation at distance classes 87.4 and 174.8 km and a significant and negative correlation at longer distances.

4 | DISCUSSION

Although in later years the use of eDNA metabarcoding to study fish communities has considerably increased, studies of large river communities are still uncommon and limited to the freshwater portion. Here, we use eDNA metabarcoding to document the fish communities along a 1300 km transect of the St. Lawrence River, including its large estuary and part of its gulf. Despite several limitations associated with combining data from different studies (see methodological considerations below), we demonstrate the ability of the eDNA metabarcoding approach based on MiFish 12S primers to efficiently identify the vast majority of species previously known to occur in the system as well as deciphering the spatial structure of fish communities along the longitudinal axis of this large temperate river ecosystem. eDNA metabarcoding identified a pattern of fish community differentiation that was strongly associated with a series of longitudinal hydro-morphological structures and to the upstream-downstream effect of a gradient of salinity, temperature, and tide amplitude variations. Our results showed that eDNA metabarcoding evidenced an upstream-downstream reduction in fish diversity indices which can be explained by a switch of fish composition in the transition zone between freshwater and marine waters and the overwhelming dominance of few species in most downriver sectors. Below, we compare and discuss the results of eDNA metabarcoding survey with those obtained by the long-term survey program conducted by FMN in the freshwater sectors of the river (Foubert et al.,

2018) and demonstrate that eDNA metabarcoding is a useful and alternative tool to document fish community shifts in large temperate lotic ecosystems such as the St. Lawrence River. We do not compare and discuss eDNA and TSM data for the marine sectors as this was fully covered in Afzali et al. (2021). However, eDNA results for the marine sectors are discussed in interpreting patterns of community shifts as seen by eDNA across the entire study area.

4.1 | Species detection

In the MS-1 to GSN-5 sectors, also used in Foubert et al. (2018), eDNA detected 80 of the 86 fish species occurring across those sectors, which represents an excellent agreement considering such a large number of species. Similar results were reported for the Rhône River (Pont et al., 2018) that is home to about half the number of species of the St. Lawrence River. Additionally, eDNA efficiently detected species (river redhorse *Moxostoma carinatum* and four salmonid species), which are only occasionally detected by the FMN (Ministère des Forêts, de la Faune et des Parcs, unpublished data). Similarly, the American eel (*A. rostrata*), a common species in the St. Lawrence River, was detected in all freshwater sectors and the Middle Estuary (MiE-7) using eDNA, in contrast with Foubert et al. (2018) results that only reported this species in Lake Saint-Louis, outside the area studied here. At the sector scale, the eDNA survey detected on average more species than the TSM survey. Several studies evaluating different sampling methods have already emphasized the ability of eDNA to provide more exhaustive appraisals of biodiversity (Civade et al., 2016; Hänfling et al., 2016; Hinlo et al., 2017; Valentini et al., 2016; Wilcox et al., 2016). This likely results from the capability of eDNA to integrate local-level information, its sensitivity to detect fish species for a wide range of abundances, and reduced selectivity compared with TSM (Cantera et al., 2019). This is also particularly true for large aquatic ecosystems where susceptibility of species to capture, topological complexity, and variable depth can reduce TSM efficacy (Pont et al., 2018). Nonetheless, when we inspected the species assemblages characterizing the freshwater sectors both eDNA and TSM surveys produced a similar list of indicator species and similar distributions of these species across sectors.

In recent years, the use of eDNA for the detection and monitoring of invasive species has rapidly expanded (Mahon & Jerde, 2016). Here, we efficiently detected two non-native species (i.e., *Neogobius melanostomus* and *Tinca tinca*) that are of major concerns for the

TABLE 2 Beta-diversity (β_{sor}) and its turnover (β_{sim}) and nestedness (β_{sne}) components estimated between stations at the different sectors

	Sectors								
	MS 1	ALSP 2	LSP 3	TG 4	GSN 5	QldO 6	MiE 7	MaE 8	GStL 9
β_{sor}	0.80	0.70	0.62	0.80	0.68	0.69	0.75	0.67	0.83
β_{sim}	0.73	0.58	0.47	0.72	0.54	0.58	0.70	0.50	0.75
β_{sne}	0.07	0.12	0.14	0.08	0.14	0.11	0.06	0.18	0.07

conservation of native fish communities in the St. Lawrence River and its tributaries since they likely represent threats to local species (Kipp & Riccardi, 2012; Masson et al., 2013; Paradis, 2018). Both were detected across freshwater and brackish water sectors; however, the number of sequence reads was much more important for the upriver fluvial sectors (i.e., MS-1 and ALSP-2), in agreement with the estimates based on TSM (Paradis, 2018; Pelletier et al., 2012).

4.2 | Longitudinal differentiation of fish communities

The potential homogenizing effect of downstream transportation of eDNA from upstream sources is one of the most important challenges in using eDNA metabarcoding to study aquatic communities in lotic ecosystems (Deiner & Altermatt, 2014; Deiner et al., 2016; Jane et al., 2015; Laporte et al., 2020). Despite the obvious potential for downstream eDNA transportation, our results show that the community structure information contained in the eDNA data was robust enough to depict pronounced shifts in fish assemblages. The large size and the hydrodynamic and structural complexity of the St. Lawrence River provide a variety of habitats that may contribute to shaping fish assemblage compositions (Berger et al., 2020; Foubert et al., 2018). Along upriver sectors, fish community differentiation was determined by the succession of corridors, archipelagos, and a large lake, almost exactly as previously found using TSM in the river transect comprised between Montréal and Saint-Nicolas (i.e., MS-1 to GSN-5) (Foubert et al., 2018). However, eDNA failed to differentiate sectors TG-4 and GSN-5 as comprising different fish communities. Known as the first mesotidal sector of the river with water reversal, the GSN-5 sector was identified by TSM data as harboring a highly differentiated fish community among the freshwater sectors (Foubert et al., 2018). While this result can be interpreted as a low sensitivity of eDNA metabarcoding compared with the TSM, perhaps due to the eDNA dispersal effect, the low number ($n = 9$), and the clustered spatial distribution (i.e., distributed upstream near the limit with TG-4 sector) of the eDNA sampling stations can partly explain this discrepancy. Our results suggest that future surveys should optimize eDNA sampling strategies to improve the assessment of the community structure in this ecosystem (Bylemans et al., 2018; Cantera et al., 2019; Grey et al., 2018).

Downstream, at the freshwater–saltwater transition, the joint effect of sharp gradients in tides height, temperature, and salinity set unique environmental conditions. Here, eDNA metabarcoding identified QldO-6 and MiE-7 sectors as the home of two completely different fish communities, characteristic of estuarine, and transition waters regions (Elliott et al., 2007). Three species were identified as indicator species of these two sectors: tomcod (*Microgadus tomcod*); Atlantic sturgeon (*Acipenser oxyrinchus*); and rainbow smelt (*Osmerus mordax*). The tomcod eDNA signature largely dominated the Middle Estuary (MiE-7) where this diadromous species is known to be abundant along with rainbow smelt. For example, MiE-7 is also known to be the main larval retention zone for these two species in the

St. Lawrence River system (Laprise & Dodson, 1990; Winkler et al., 2003). In contrast, the fish community of QldO-6, which is under a strong tidal influence, shows a balanced composition of freshwater (e.g., *I. punctatus*; *M. anisurum*; *S. canadensis*), euryhaline (e.g., *A. fulvescens*; *C. catostomus*; *C. commersoni*; *L. lota*), and diadromous (e.g., *A. rostrata*; *M. tomcod*; *Morone saxatilis*, *O. mordax*) species. This result highlights the uniqueness of this downstream transition sector of the Fluvial Estuary. Indeed, fluvial estuaries are well known to be home of fish communities comprising species from a diverse number of functional groups (Elliott et al., 2007). Finally, our results revealed at least two well-differentiated fish assemblages in the Marine Estuary (MaE-8), and Gulf (GStL-9) sectors, providing new information about the fish communities in this Northwestern Atlantic marine ecosystem.

4.3 | Upstream–downstream variation of fish diversity

In addition to deciphering community structure and largely corroborating previous observations based on TSM, our results revealed an overall pattern of upstream to downstream reduction in rarefaction and Simpson diversity indices estimated from eDNA data. This trend was comparable to that observed previously using TSM along with the fluvial sectors (i.e., MS-1 to GSN-5) (Foubert et al., 2018). Indeed, eDNA species richness estimate in the Archipelago of Lake St. Pierre (ASLP-2) sector was also higher than that of all other sectors, Lake St. Pierre (LSP-3) also shows lower diversity, followed by Grondines-Saint Nicolas (GSN-5) sector. Similar patterns of higher fish biodiversity in freshwater communities at the middle part of large rivers were described for the Ganges (Das et al., 2013) and the Mekong (Chea et al., 2017) rivers. In contrast, the estimates of equitability were similar across all fluvial sectors showing a significant decrease in downstream sectors. In these sectors, eDNA of a few species was dominant: Atlantic tomcod (*M. tomcod*) and Atlantic sturgeon (*Acipenser oxyrinchus*) in the MiE-7, as previously noted; Atlantic soft pout (*Melanostigma atlanticum*), Redfish (*Sebastes* sp.), and Greenland halibut (*Reinhardtius hippoglossoides*) in the MaE-8 sector; and *Sebastes* sp., Atlantic cod (*Gadus morhua*), *Pleuronectide* sp., and Atlantic herring (*Clupea harengus*) in the GStL-9 sector. Generally speaking, those species are also the most abundant in those sectors, as revealed by TSM surveys (e.g., Afzali et al., 2021).

When we analyzed beta-diversity (Sørensen dissimilarity), the turnover component largely dominates over evenness all along the river. As this pattern implies that species are replaced between the sites, it also indicates that eDNA transportation was not sufficient to erase signatures of community shifts (Mächler et al., 2019). Nonetheless, we found a significant spatial auto-correlation between stations located at distances below 175 km, which is roughly in the range of the maximal eDNA transportation inferred in other studies (Pont et al., 2018). At that scale, we should then expect that eDNA transportation could contribute to homogenize contiguous community compositions, thus blurring the signal of

local differentiation (Deiner et al., 2016). However, and as discussed above, this was apparently not the case in our study. Nonetheless, the information provided by eDNA metabarcoding might not be accurate enough to determine species specific distributions due to upstream–downstream eDNA transport in such a large fluvial ecosystem. However, and despite the partial blurring due to potential eDNA transport, our study highlights the ability of eDNA metabarcoding analysis to document major shifts in fish community and biodiversity gradients in large lotic ecosystems. This supports the view of large rivers as a dis-continuum of longitudinal series of alternating geomorphological structures shaping local aquatic communities (Poole 2002; Ward and Stanford 1995).

4.4 | Methodological considerations

In this study, we merged the results of four studies conducted in different years, months of the years, each with different goals, sampling strategies, and eDNA sampling effort (see Appendix S2). Regarding this, although the average number of reads per sample was similar among most studies, in one case (i.e., Mingelbier et al., 2019) the sequencing effort was lower and could have affected the detection of rare species in the most upriver sectors. It is noteworthy that despite lower sequencing effort per sample, species diversity was nevertheless the highest in those most upstream sampling locations covered by (Mingelbier et al., 2019), suggesting that it did not affect importantly our overall interpretations in community shifts and patterns of diversity. Yet, this might certainly represent a methodological handicap for the quality of the data set (Bylemans et al., 2018; Gotelli & Colwell, 2001; Laporte et al., 2020). But despite these potential caveats, eDNA revealed a pattern of fish biodiversity and community structure that concur well with the patterns described by a long-term survey program using TSM, giving confidence to our findings and suggest that even better correspondence with results from TSM could be achieved by performing a more standardized sampling protocol. Another possible limitation of our study is that a growing number of studies has suggested that the use of a single pair of universal primers targeting only one mtDNA segment may represent a limiting factor of eDNA metabarcoding, particularly when the study aimed to assess complex and diverse communities (Alberdi et al., 2018; Collins et al., 2019; Li et al., 2019; Stat et al., 2017). Here, we used Mifish (Miya et al., 2015), a fish-targeting primer pair that amplifies a segment (approx. 171 bp) of the mitochondrial 12S rRNA gene widely used in fish eDNA studies (Bylemans et al., 2018; Miya et al., 2020; Ushio et al., 2018; Zhang et al., 2020). Our results demonstrate that this universal primer pair and sequence segment successfully amplifies and distinguishes the vast majority of species present in the system with a few exceptions that could have possibly been resolved by the analysis of one or more mtDNA segments. This, however, would come as a trade-off between the gain in improved information and substantially increased cost in sequencing. Additionally, because we have only taken a subsample of all stations from Afzali et al. (2021) in the marine sectors to balance our sampling

design with the more upstream sectors, we have underrepresented the overall species diversity in the Gulf of St. Lawrence. Yet, this did not significantly impact the comparison of diversity indices because in general the results revealed that, while there are more species in the marine sectors overall (Afzali et al., 2021; Centre Saint-Laurent, 1996), the brackish and marine fish communities sectors are largely influenced, as mentioned above, by a few species of overwhelming abundance. For instance, in the study of Afzali et al. (2021), four species represented 96% of the entire biomass of fish sampled using bottom trawl, and those four species represented 78% of all eDNA sequence reads.

5 | CONCLUSIONS

Biodiversity surveys of large fluvial ecosystems are logistically complex requiring substantial amounts of resources and time (Zajicek & Wolter, 2018). This probably explains why, despite their importance, relatively few studies about fish communities of large rivers have been published (Chea et al., 2017; Das et al., 2013; Erős et al., 2017; Galat et al., 2005; Whitten & Gibson-Reinemer, 2018). The relative paucity of research on fish communities in large fluvial ecosystems is very consequential for the conservation of freshwater fish biodiversity, which has recently been coined as “the world's forgotten fish” (WWF, 2021).

Despite the aforementioned limitations, the results of our study corroborate the growing evidence demonstrating that eDNA metabarcoding is a useful complementary (and sometimes replacement) tool to study aquatic communities. Particularly, we show that eDNA can provide equivalent information in a shorter time than long-term fish surveys using TSM, as previously demonstrated for the Rhône River (Pont et al., 2018). We also demonstrate that eDNA sampling was efficient to detect and provide relative abundance profiles of most fish species living in the St. Lawrence River. This is expressly useful to survey species of particular interest for management and conservation, as is the case of the American eel and the river redhorse among others, some of them inefficiently quantified or detected using gillnets and seine nets (Foubert et al., 2018). eDNA also makes it possible to sample and analyze fish communities in some river stretches where the use of TSM is difficult or impossible, such as rapids in freshwater or rocky bottoms in marine waters, as well as sampling during harsh weather times of the year (i.e., during the harsh Canadian winter), to better understand the dynamics of fish communities in space and time (Buxton et al., 2017; Takahashi et al., 2018). This is particularly relevant considering the spatiotemporal shifts observed in freshwater fish communities as a response to climate changes (Maire et al., 2019). Integrating eDNA decay dynamics, shedding, and transportation rates coupled with hydrological and geomorphological information will enable a better knowledge of biodiversity and the improvement of the conservation plans, especially in large and highly diverse aquatic ecosystems (e.g., Carraro et al., 2020).

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CONFLICT OF INTEREST

We have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

EG-M: have made major contributions to analysis, interpretation of the data, and writing of the manuscript. ML and EN: have made major contribution to analysis of the data and writing of the manuscript. CH: have made major contributions to the acquisition of the data and writing of the manuscript. GC: have made major contribution to the acquisition of the data. YP: have made major contributions to the acquisition of the data and writing of the manuscript. MM: have made major contributions to the acquisition of the data and writing of the manuscript. LB: have made major contributions to the conception of the study, interpretation of the data, and writing of the manuscript.

DATA AVAILABILITY STATEMENT

All eDNA metabarcoding data generated are available in Appendix S3.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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