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Fine-scale environmental heterogeneity shapes fluvial fish communities as revealed by eDNA metabarcoding

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Abstract

Conservation of freshwater biodiversity requires being able to track the presence and abundance of entire fish communities. However, studying fish community composition within rivers remains a technical challenge because of high spatial and temporal physico-chemical variability, anthropic activities and connections with other river catchments, which may all contribute to important variations in local ecology and communities. Here, we used environmental DNA metabarcoding to document spatial variation in fish communities at a small geographic scale in a large river system. The study was conducted in the Contrecoeur sector (5.5 km long and approximately 1–1.5 km wide) of the St. Lawrence River (Québec, Canada), where two water masses with different physico-chemical properties, known as "brown waters" and "green waters," flow in parallel with limited admixing. Water samples were collected during two consecutive days at 53 stations located in both water masses. Using universal PCR MiFish 12S primers, Illumina MiSeq sequencing, and the Barque (www.github.com/enormandeau/barque) eDNA analysis software developed by our group, a total of 67 fish species were detected. PERMANOVA and redundancy analyses (RDA) performed on relative read abundance revealed that each water mass comprised distinct communities that depended on turbidity, depth, and to a lesser extent on the upstream versus downstream position along the study area. eDNA metabarcoding results were compared with those of traditional surveys conducted previously in the sector and up to 40 km upstream of it. As previously reported, higher species diversity was detected by eDNA and with substantially lower sampling effort. Our results represent one of the few studies documenting the potential of eDNA metabarcoding to investigate small-scale variation in 2D spatial patterns of distribution of whole fish communities associated with habitat characteristics within a lotic system.

KEYWORDS

environmental DNA, fish communities, metabarcoding, river

Chloé Suzanne Berger and Cecilia Hernandez contributed equally to this work.

1 | INTRODUCTION

Biodiversity in freshwater ecosystems has been decreasing globally for the last 50 years (McLellan, Iyengar, Jeffries, & Oerlemans, 2014), and its assessment and monitoring will help to implement appropriate conservation and fisheries management policies (Geist, 2011). Estimating biodiversity requires studying not only the presence and abundance of specific species, but also the entire fish assemblage that forms a community. Because fish interact as prey, predators, and competitors, disruptions in a community (e.g., introduction of an invasive species or reduction of a native species) are likely to have strong impacts on the whole ecosystem (Cook, 2008). Since 2005, the global will to protect freshwater biodiversity (Dudgeon et al., 2006) has led to the emergence of monitoring programs that are mainly based on visual detection, fishing, and counting (Bonar, Hubert, & Willis, 2009). They include trawl surveys (Yule, Adams, Stockwell, & Gorman, 2008) and camera-based approaches (Ebner & Morgan, 2013). While certainly useful, these methods are often either highly invasive, time-consuming (Rees, Maddison, Middleditch, Patmore, & Gough, 2014), and/or dependent on the availability of taxonomic experts (Hopkins & Freckleton, 2002). They also frequently have the disadvantage to restrict the estimates of biodiversity mainly to easily accessible areas. This may lead to missing important components of communities, for instance, deep-water fish communities (Dudgeon et al., 2006). Additionally, recently introduced invasive species and endangered native species are generally underrepresented with these methods because of their low abundance in the communities. Moreover, underestimation of rare species may be exacerbated in river systems because of unidirectional water flows as well as high spatial and temporal physico-chemical variability, ultimately generating important variations in local ecosystems (Kumar & Prabhahar, 2012; Liu, Chen, Dong, & Peng, 2012). Yet, rivers are of particular interest because of the high impact of nearby anthropic activities and the connection with other river catchments that are likely to facilitate the spread of invasive species (Leuven et al., 2009). In this context, there is a need for noninvasive methods that would allow describing fish communities, including potential rare species, at a fine-scale in highly dynamic river systems.

The emergence of environmental DNA (eDNA) metabarcoding offers new possibilities to monitor aquatic biodiversity (Deiner et al., 2017; Rees et al., 2014; Taberlet, Bonin, Zinger, & Coissac, 2018). Environmental DNA is released by organisms from their skin cells, mucus, metabolic waste, and gonads, which can be used for the detection of species by collecting environmental samples (Lodge et al., 2012). In freshwater ecosystems, eDNA sequencing has been successfully applied to detect rare species, including newly invasive species in wetlands (e.g., amphibians: Ficetola, Miaud, Pompanon, & Taberlet, 2008) and river systems (e.g., fish: Mahon et al., 2013). Endangered species have also been tracked in streams (e.g., amphibians: Pilliod, Goldberg, Arkle, & Waits, 2013) and rivers (e.g., fish: Strickland & Roberts, 2019). Recent advances in next-generation sequencing (NGS) are also offering the possibility to assess overall biodiversity by simultaneously identifying multiple species through eDNA metabarcoding. Using this approach, the DNA of multiple species is extracted and amplified at the same time using universal polymerase chain reaction (PCR) primers and species presence and in some cases abundance are inferred from the sequenced reads (Ruppert, Kline, & Rahman, 2019). Indeed, sequencing read abundance has been previously demonstrated to be a fair proxy of species abundance in freshwater ecosystems (Evans et al., 2016; Hänfling et al., 2016; Li et al., 2019) and environmental DNA metabarcoding has already been used in several studies to document fish communities in freshwater ecosystems (reviewed in Ruppert et al., 2019). Specifically, previous studies have documented the large-scale organization of fish communities along a longitudinal gradient in lotic environments (Civade et al., 2016; Nakagawa et al., 2018), as well as the seasonal dynamics of riverine fish communities (Milhau et al., 2019). Environmental DNA metabarcoding has also been successfully used in a wide range of lake types for community-level analysis (Li et al., 2019). To our knowledge, however, eDNA metabarcoding has never been applied to document variation in fish communities at small geographic scales (<1 km) and considering both longitudinal and lateral variations in large river systems, which may allow determining how fish biodiversity is influenced by local environmental conditions (e.g., turbidity, depth, or distance from and along river bank). It may also help monitoring potential invasive or endangered species in relation to the environmental conditions and the other species detected.

The objective of this study was to investigate the relative role of different environmental factors in shaping variation in fish community composition in a large river using eDNA metabarcoding at a small geographic scale. Our study was conducted in the Contrecoeur sector (5.5 km long and approximately 1–1.5 km wide) of the St. Lawrence River (Québec, Canada). The St. Lawrence River runs 3,058 km, its drainage area, which includes the Great Lakes, the world's largest system of freshwater lakes, is 1,344,200 km^2, and its average discharge in our study area is approximately 10,000 m^3/s. There, it is characterized by two water masses with very distinct physico-chemical properties and that are known to flow in parallel with limited admixing (Hudon, 2000; Laporte et al., 2020; Rondeau, 1999). Using eDNA metabarcoding, we found significant differences in fish community composition between these two water masses. Moreover, we found further variation within each water mass that was significantly associated with turbidity, depth, and to a lesser extent with the upstream–downstream position along the study area. The accuracy of the eDNA metabarcoding results was compared to traditional surveys (i.e., gillnet and seine) previously performed in the sector, as well as up to 40 km upstream of it, as part of a standardized government biomonitoring program. Together, our results represent one of the few studies documenting the potential of eDNA metabarcoding to investigate small-scale variation in spatial patterns of distribution of a whole fish community, even within a lotic system as complex as the St. Lawrence River. Metabarcoding and biomonitoring studies will benefit from our results, which illustrate how a more comprehensive knowledge of eDNA distribution at a small geographic scale—especially lateral distribution (from shore

toward center of the river) of eDNA—is crucial to precisely detect variation in fish community composition in rivers.

2 | MATERIALS AND METHODS

2.1 | Collection of water samples

Water samples were collected from between September 13th and 15th, 2017, at 53 stations in the Contrecoeur sector (about 40 km downstream of Montréal) where two water masses, the Ottawa River or "brown waters" and the Great Lakes or "green waters," flow in parallel with limited admixing (Figure 1). These two water masses differ in their physico-chemical properties, in particular turbidity and conductivity (Hudon, 2000; Laporte et al., 2020; Rondeau, 1999) (Table 1). Ottawa River waters have high suspended solids concentrations (>8 mg/L) and turbidity (4.2 NTU) with lower clarity (K = 1.3 m^{-1}) and conductivity (<160 mS/cm). On the contrary, Great Lakes waters are characterized by their extremely low suspended solids concentrations (<1 mg/L) and relatively low turbidity (1.3 NTU) with a very high clarity (K = 0.3 m^{-1}) and moderately high conductivity (>250 mS/cm; Hudon, 2000; Laporte et al., 2020; Rondeau, 1999). The sampled region was 5.5 km long and varied in width between 1.0 and 1.5 km. Twenty-seven stations were sampled in the brown waters (mean depth: 3.6 m) and 26 in the green waters (mean depth: 5.0 m; Table 1). In each water mass, water samples were collected at six transects across the river from shore to shore and separated by distances varying between 10 m and 5,000 m (Figure 1). This sampling scheme was first designed as part of an experiment to document patterns of 2D dilution of eDNA in this system from a eDNA source consisting of caged fish using species not usually present in the system (Brown trout *Salmo trutta* and Rainbow trout *Oncorhynchus mykiss*) (Laporte et al., 2020). This experiment revealed very limited lateral diffusion from the point source (caged

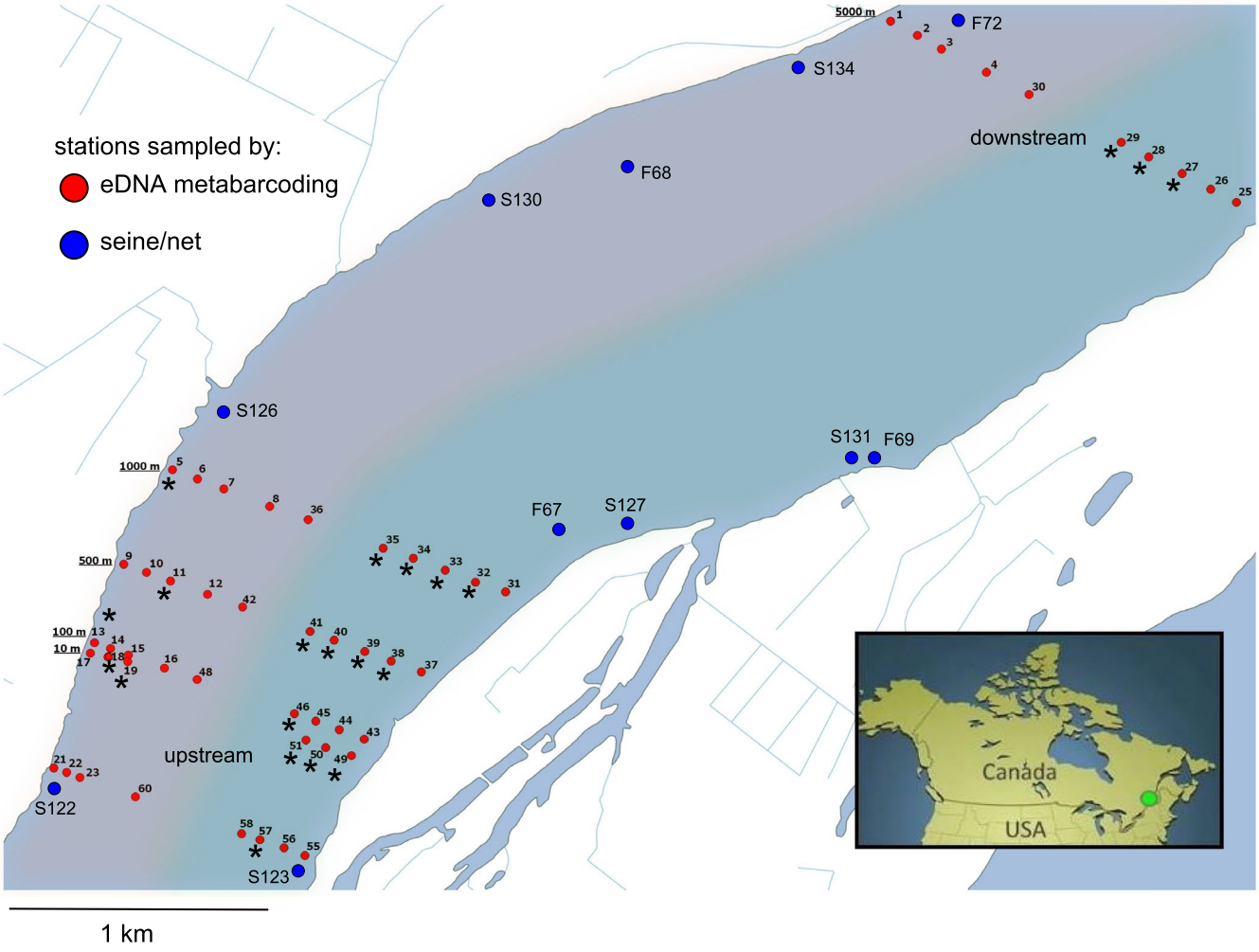

FIGURE 1 Location of the 53 environmental metabarcoding sampling stations in the St. Lawrence River. Stations are located in the Contrecoeur sector where two very distinct water masses flow in parallel: the Ottawa River or "brown waters" and the Great Lakes or "green waters." A total of 27 stations are located in the brown waters, and 26 stations are found in the green waters. In the map are also indicated the sites that were previously sampled by traditional surveys (seine (S) or gillnet (F)) in 2001 and 2015 and that were used to compare species composition with the eDNA metabarcoding approach. Stations for which marine species were detected are indicated with an asterisk (*) [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Physico-chemical characteristics of the brown and green water masses in the Contrecoeur sector of the St. Lawrence River

	Conductivity (mS/cm)	Depth (m)				Turbidity (NTU)			
Water mass	Value	Mean	Max	Min	*SD*	Mean	Max	Min	*SD*
Brown	<160	3.6	8.0	0.5	2.8	4.4	7.0	1.7	1.3
Green	>250	5.0	7.5	1.5	1.5	1.3	1.8	0.0	0.3

Note: Conductivity (mS/cm) is reported from Hudon (2000) and Rondeau (1999), while depth (m) and turbidity (NTU, Nephelometric Turbidity Unit) were measured in this study. For depth and turbidity, the mean, maximum value, minimum value, and standard deviation (*SD*) were calculated using the values measured at 27 stations in brown waters and at 26 stations in green waters, including their temporal replicates. Turbidity and depth differ significantly between the two water mass types (*U* test $p = 1.64\text{-}14$ for turbidity, *U* test $p = .008$ for depth).

fish). This prompted the present study by indicating that it may be possible to detect lateral (e.g., from shore toward center of the river) variation in fish community composition in this sector of the St. Lawrence River. At each sampling station, water samples were collected near the shore and at various distances from both shores (from 25 m near shore, up to 550 m away from shore) (Figure 1). A 250 ml integrated water column sample (from bottom to top) was taken from a boat at each of the 53 stations. Depth (m) at each station was inferred using modeling based on high summer water flows (Bouchard & Morin, 2000), and turbidity was measured simultaneously to water sampling using a turbidity meter (model "Lamotte 2020i"). Among the 53 stations, 12 stations in the brown waters and 15 stations in the green waters were sampled twice, once each day on two consecutive days, for a total of 80 samples. In addition, 14 negative field controls (blanks) used distilled water that were treated in the same way as the real samples. Water filtration using single-use syringes with 1.2 µm glass microfiber filter (Whatman, 25 mm) was performed directly in the field. Syringe heads were bleached, sterilized, and UV-treated. In total, 95 filters (80 eDNA samples and 14 field negative controls) were kept in "Longmire" buffer (Longmire, Baker, & Maltbie, 1997) and stored at −20°C until eDNA extraction.

2.2 | eDNA extraction

eDNA extraction was performed using a QIAshredder and DNeasy Blood and Tissue kit (Qiagen) according to a previously developed protocol (Goldberg, Pilliod, Arkle, & Waits, 2011; Spens et al., 2017). Extractions were performed under a UV hood with bleached and/or UV-treated instruments to eliminate any possible contamination. The extracted eDNA was stored at −20°C during 1 year until amplification. For each extraction batch, a negative extraction control was added to account for possible contamination.

2.3 | PCR amplification

We used the MiFish primers that target a hypervariable region of the 12S rRNA gene (174 bp), which allows identifying all fishes at the species levels except for seven closely related species (these species are listed in the Results section) (Miya et al., 2015): MiFish-U-F 5′-GTC GGT AAA ACT CGT GCC AGC-3′ and MiFish-U-R 5′-CAT AGT GGG GTA TCT AAT CCC AGT TTG-3′. For each sample, a unique dual indexing approach was used and a 8 bp barcode was added during PCR to the amplicon. The PCR reaction was conducted in a total volume of 25 µl including 12.5 µl of MasterMix (Qiagen), 2 µl of each primer (10 µM), 5.5 µl of diH_20, and 3 µl of eDNA sample. The PCR program was run under these conditions: 15 min at 95°C, 35 cycles of amplification (30 s at 94°C, 90 s at 65°C, 60 s at 72°C), and a final elongation step of 10 min at 72°C. For each eDNA and field negative control sample, five PCR replicates were performed and pooled after amplification, a PCR negative control was also added for each index combination to confirm the absence of laboratory contamination. No PCR positive control was added. With over 60 species in the system with a wide range of variation in abundance, designing a mock community as a control would have been nearly impossible. Also, while positive controls are particularly crucial in studies aiming to detect the presence of particular species (presence–absence), this is less of a concern in a community analysis where all samples analyzed generated thousands of sequences, as in this study. Here, the two nonnative species (Brown trout and Rainbow trout) used in the caged fish experiment described above were considered as two external positive controls for which amplification was expected to validate the success of the experiment and of the taxonomic identification. PCR products were visualized on a 1.5% agarose gel. No amplification of the PCR negative controls was observed. PCR products were purified using a paramagnetic bead-based post-PCR clean up kit (Axygen). After elution in 35 µl of water, DNA concentration of each sample was determined using the TECAN Spark 10 M 223 Reader and the Ultra High Sensitivity dsDNA Quantitation kit (AccuClear). Samples were pooled in equal quantities and repurified in order to equalize sequencing depths across sampling sites. The concentration and fragment size distribution of the library were analyzed on an Agilent 2100 Bioanalyzer.

2.4 | Sequencing

Samples were sequenced at the genomic platform of the Institut de Biologie Intégrative et des Système (IBIS), Université Laval, Québec

(http://www.ibis.ulaval.ca/). Sequencing was performed using Illumina MiSeq (Illumina) and the MiSeq Reagent Kit V3 with paired-end 300 bp reads (Illumina). More information about sequencing methods is available in Appendix S1 and on the GitHub webpage of our research group (www.github.com/enormandeau/barque).

2.5 | Data processing and cleaning

Raw sequencing reads were filtered to remove primer sequences and demultiplexed using the MiSeq Control software v2.3. Reads from 5′ and 3′ were merged, and the sequences were analyzed using the Barque v1.5.2 pipeline developed in our research group (www.github.com/enormandeau/barque). Detailed settings for sequence analysis can be found on the GitHub webpage. The settings of the Barque pipeline used for this project are also available in Appendix S1, as well as information about the primers and the barcodes used. Taxonomic assignment was performed by searching the sequences among the MitoFish database (Iwasaki et al., 2013), the GENBank database (Benson et al., 2012), and the Barcode of Life. A minimum similarity threshold of 97% between the sequences of interest and the species sequences available in the database was used to assign taxonomic identifications. Seven species that could not be unambiguously identified because of high sequence similarities with other species were further investigated. If the number of sequences detected for them was lower than for the other species, and/or if they had never been reported in Québec rivers, they were removed from subsequent analyses. In order to be conservative, species for which sequences were detected 10 times or less were also removed from the analysis as they could be caused by sequencing artifacts (Brown et al., 2015) or sample misidentification (Schnell, Bohmann, & Gilbert, 2015). Sequences attributed to nonfish species were also discarded. Finally, sequences attributed to Brown trout and Rainbow trout were also not considered in further analyses for reasons explained above.

2.6 | Data analysis

Statistical analyses were performed using the R software version 3.5.2 (R Core & Team, 2018) and the package *vegan* (Oksanen et al., 2015). We used relative read abundance after correction to account for biological or technical biases (see below) as a proxy of species abundance, as it was previously done in other studies that applied eDNA metabarcoding and MiFish primers to infer fish abundance from sequence read abundance (Evans et al., 2016; Hänfling et al., 2016; Li et al., 2019; Ushio et al., 2018). Furthermore, because turbidity is the main factor that discriminates between brown and green waters (*U* test p = 1.64-14; Table 1), we used turbidity as a proxy of the water mass type, which allowed to include in all analyses a continuous variable (i.e., turbidity level) instead of a categorical variable (i.e., water mass type). Permutational multivariate analysis of variance (PERMANOVA) was performed based on Bray–Curtis similarity to test patterns of dissimilarities among fish species composition (based on relative read abundance, RRA per species) depending on the following: (a) turbidity (NTU), (b) depth (m), and (c) downstream distance (m) (number of permutations: 999; turbidity, depth, downstream distance, and their interactions as fixed factors) (Bray & Curtis, 1957; Clarke, Gorley, Somerfield, & Warwick, 2001). To take potential amplification biases during sequencing into account, the number of reads obtained per species was transformed into a relative read abundance (RRA) obtained per species. This was done before PERMANOVA, in order to reduce the importance of species with very high number of reads (presumably the most abundant ones) compared to those with few reads (presumably rarer ones). The use of RRA, instead of the direct number of reads, has previously been suggested to be a more accurate approach to interpret count data in metabarcoding studies (Cavallo et al., 2018; Deagle et al., 2019). To obtain RRA, the number of reads of an individual species in an eDNA sample was divided by the total number of reads measured in that eDNA sample and multiplied by 100, giving the percentage, or relative read abundance (RRA), of each species in each eDNA sample (Cavallo et al., 2018; Deagle et al., 2019). We included in the model all 80 samples (i.e., including temporal replicates).

Redundancy analyses (RDAs) were performed to determine associations between stations and community composition. A first analysis was conducted with all the sampling stations including their temporal replicates, as well as turbidity, depth, and downstream distance as constraint predictors. Then, for each environmental variable separately (turbidity, depth, and downstream distance), RDAs were performed within both water masses (brown or green waters, respectively) for a total of six RDAs. This second step allowed to document the contribution of each fish species to the segregation observed between (a) stations located in less turbid waters versus those located in more turbid waters; (b) stations located in shallow waters versus those located in deep waters away from shore; and (c) stations located downstream versus those located upstream in both water masses separately. For each analysis, we used the number of eDNA reads per species (Evans et al., 2016; Hänfling et al., 2016; Li et al., 2019; Ushio et al., 2018) that was corrected using a Hellinger's transformation as the response matrix. Hellinger's transformations were produced in order to reduce the importance of species with very high number of reads compared to species with smaller number of reads, which is problematic with the Euclidean distance that is used by default in principal component and RDA analyses (Legendre & Gallagher, 2001; Legendre & Legendre, 1998). Finally, the species that contributed the most to the observed dissimilarity (most negative and positive RDA scores on axis 1 and axis 2) were plotted, with a maximum of 30 species plotted by constraint predictor.

2.7 | Comparison of eDNA metabarcoding data with traditional surveys

The metabarcoding dataset was compared to data of fish abundance (number of fish caught) available from the “Réseau de Suivi

Ichtyologique" (RSI) (https://catalogue.ogsl.ca/dataset/17b68796-fcd2-4888-8653-ecbcaadc8a91), which is part of a standardized fish biomonitoring operated by the Ministère des Forêts, de la Faune et des Parcs (MFFP) du Québec. In the Contrecoeur sector, seven stations were sampled by seine (four in brown waters and three in green waters) and four stations were sampled by gillnet (two in brown waters and two in green waters) (Morissette, 2018). These samplings occurred twice, once in 2001 and then in 2015 (Figure 1), and the number of fish caught by species was determined for each station. The total number of species detected was estimated at each station sampled either using eDNA, seine, or gillnet. We plotted the occurrence frequency (in terms of number of stations) of the total number of fish species detected.

The eDNA metabarcoding dataset collected on the studied 5.5 km sector was also compared to a dataset of fish biodiversity observed as part of the RSI survey in the Contrecoeur sector at a larger spatial scale that is a region of 50 km including a distance of 40 km upstream of the Contrecoeur sector and a distance of 4.5 km downstream of the sector (Figure S1). For this comparison, we only selected RSI data from 2015. Comparison with RSI data from 2001 was also performed, and the same results as for 2015 were obtained (data not shown). As it was previously demonstrated that eDNA can disperse up to 5,000 m downstream from its source in this sector (Laporte et al., 2020) and that previous studies have documented fish eDNA detection up to 60 km downstream from a source in freshwater ecosystems (Deiner & Altermatt, 2014; Pont et al., 2018), we wanted to verify whether species not caught by fishing gears in the 5.5 km sector, but for which eDNA was detected, were caught further upstream. Again, at each station sampled using eDNA metabarcoding, seine or gillnet, the total number of fish species detected was estimated. A Monte Carlo statistical resampling approach was used to estimate the number of species detected by each method under various levels of sampling intensity (Jackson & Harvey, 1997; Sard et al., 2019). For each sampling method, the mean (± *SD*) number of species detected was based on 30 simulations for each level of sampling effort. We then plotted the cumulative species count by sampling gear for various levels of sampling efforts in order to compare the efficiency of sampling methods.

3 | RESULTS

3.1 | Negative controls

Positive amplifications were detected in all field negative controls. However, the numbers of reads were much lower than for the actual samples. Thus, seven out of 14 field controls had a total of less than 100 sequences detected (representing about 0.00083% of the mean number of sequences (n = 119,810) detected in one real eDNA sample) and six others had a total of less than 300 sequences detected (representing 0.0025% of the mean number of sequences detected in one real eDNA sample), which is negligible. Field negative control 07 had higher levels of total sequences detected (1 479 sequences representing 0.012% of the mean number of reads per sample). Detection in this sample was mainly attributed to Copper redhorse *Moxostoma hubbsi* (1,290 sequences), which is an endangered species potentially present in the study system but that was not detected in the real samples (Table S1). The high level of detection for this negative sample was probably the result of material contamination for this specific sample, which had no impact on the real samples. All extraction negative controls and PCR negative controls showed no positive amplification indicating the absence of contamination during sample extraction and amplification. The results can therefore be taken with confidence for further analyses.

3.2 | Composition of fish communities at a small geographic scale

After sequencing on the Illumina MiSeq platform and data filtering using the Barque pipeline, a total of 9,847,660 sequences were obtained. A total of 110 fish species belonging to 44 genera were identified (Table S1):

Firstly, seven species either had 12S rRNA gene sequences that differed only by a few nucleotides with other species and therefore could not be unambiguously identified, and/or corresponded to species never reported in Québec and were very unlikely to correspond to a new occurrence based on their geographically distant range of distribution (Table S2). The number of sequences detected for those species were lower than for the alternative species known to occur in the study system and that were selected for analysis (Tables S1 and S2). Consequently, these species were not taken into account for further analyses. Second, 17 species had sequences that were detected 10 times or less, which were removed from the study (Table S1). Third, sequences attributed to either Brown trout or Rainbow trout were also discarded for the reasons detailed above (Table S1). Finally, 17 marine fish species were detected with ten sequences or more, but these are obviously unlikely to naturally occur in this freshwater system (Figure 1 and Table S1). Environmental DNA molecules for these marine species were detected at 21 stations in total and showed a nonrandom pattern of distribution with 16 out of those 21 being located in green waters. These 17 marine species were also discarded from all analyses. As a result, a total of 67 fish species were kept for analyses. In addition, 20 nonfish species including 13 mammal, five bird, and two reptile species were also removed for the subsequent analyses (Table S1).

PERMANOVA and RDA analyses with turbidity (as a proxy of water mass type), depth, and downstream distance as environmental factors were performed on the retained 67 fish species. We found that each of these environmental variables had a significant effect on fish community composition. PERMANOVA analysis detected significant differences in fish community composition depending mostly on turbidity (proxy for brown vs. green waters) (adj.R^2 = 0.29; p = .001***), then on depth (adj.R^2 = 0.07; p = .001***) and to a lesser extent on the downstream distance along the river (adj.R^2 = 0.02; p = .01*). A significant interaction between turbidity

and depth (p = .006**) and turbidity and the downstream distance (p = .001***) was also detected (Table 2). RDA analysis returned three significant axes explaining 31% of the total variation in eDNA levels (adj.R^2 = 0.31; p = .001***). To account for potential pseudoreplication issues in our dataset, we performed PERMANOVA and RDA analyses again, using the mean of the RRAs (for PERMANOVAs) or the mean of the number of reads corrected with a Hellinger's transformation (for RDAs) per species of day 1 and day 2, for each station sampled twice. We found the same results as previously, except for the effect of downstream distance on fish community composition that was no longer significant (adj.R^2 = 0.02; p = .09). We therefore decided to use the corrected number of reads per species obtained for each temporal replicate separately for further analyses (instead of the mean values of day 1 and day 2), which allowed a more accurate interpretation of the results. To account for spatial autocorrelation, we also ran PERMANOVAs by including latitude and longitude as predictor variables, and we found that the effects of the environmental factors (turbidity, depth, downstream distance) were still significant.

Stations sampled in brown waters and stations sampled in green waters formed two distinct clusters, and fish community dissimilarity between brown and green waters was mainly explained by the predominance of eDNA from Tessellated darter *Etheostoma olmstedi* (Percidae), Trout-perch *Percopsis omiscomaycus* (Percopsidae), and Fantail darter *Etheostoma flabellare* (Percidae) in brown waters, versus the predominance of eDNA molecules from Round goby *Neogobius melanostomus* (Gobiidae), Longnose sucker *Catostomus catostomus* (Catostomidae), and Lake sturgeon *Acipenser fulvescens* (Acipenseridae) in green waters (Figure 2). Only four stations from the brown waters were more similar to the green stations (stations 30-1, 42-1, 48-1, and 60-1 sampled the first day; Figure 2), and those were all located toward the middle of the river near the interface between the two water masses (Figure 1). Furthermore, seven stations sampled in the green water mass differed from the other green stations: station 25-1 and its temporal replicate 25-2, station 26-1 and its temporal replicate 26-2, station 31-1, station 37-2 sampled the second day, station 43-1 and its temporal replicate 43-2, station 49-1 and its temporal replicate 49-2, and station 55-2 sampled the second day (Figure 2 green circle). All of these stations were among those closest to shore (Figure 1). In brown waters, stations that were sampled near shore (station 1-1, station 5-1 and its temporal replicate 5-2, station 9-2 sampled the second day, station 13-1 and its temporal replicate 13-2, station 17-1 and its temporal replicate 17-2, station 21-1 and its temporal replicate 21-2) also tended to spatially segregate, but the separation with the other brown stations was weaker (Figures 1 and 2 brown circle).

3.2.1 | Parallel variation in species composition within brown versus green waters

RDAs revealed that eDNA of three species from various genera was mainly detected in less turbid sampling stations in parallel between both water masses (i.e., Mooneye *Hiodon tergisus*, Smallmouth bass *Micropterus dolomieu* and Round goby) (Table 3 panel A; Figure 3 species in blue), while eDNA of six species was found in more turbid stations in parallel between both water masses (i.e., Rock bass *Ambloplites rupestris*, Quillback *Carpiodes cyprinus*, Northern pike *Esox lucius*, Tessellated darter, Emerald shiner *Notropis atherinoides*, and Walleye *Sander vitreus* (Table 3 panel A; Figure 3 species in purple)).

The eDNA of 11 species belonging mostly to the family Cyprinidae was mainly found in stations of lower water depths in both water masses, while eight species primarily belonging to the family Catostomidae were predominantly associated with stations of greater water depths, again in both water masses. Species with eDNA predominantly associated with shallower stations in both water masses were Rock bass, Brown bullhead *Ameiurus nebulosus*, Common carp *Cyprinus carpio*, Northern pike, Tessellated darter, Eastern silvery minnow *Hybognathus regius*, Pumpkinseed *Lepomis gibbosus*, Golden shiner *Notemigonus crysoleucas*, Bridle shiner *Notropis bifrenatus*, Trout-perch, and Tench *Tinca tinca* (Table 3 panel B; Figure 4 species in blue). Species with eDNA predominantly associated with stations of greater water depths in both water masses were Lake sturgeon, American eel *Anguilla rostrata*, Longnose sucker, White sucker *Catostomus commersonii*, Channel catfish *Ictalurus punctatus*, Smallmouth bass, Shorthead redhorse *Moxostoma macrolepidotum*, and Round goby (Table 3 panel B; Figure 4 species in purple).

TABLE 2 Results of PERMANOVA based on Bray–Curtis similarity testing patterns of dissimilarities among fish species composition (based on the corrected number of reads per species) depending on turbidity (NTU), depth (m), and downstream distance along the river (m) and their interactions in the Contrecoeur sector of the St. Lawrence River

Source of variation	*df*	MS	F.Model	adj.R^2	*p*-value
Turbidity	1	2.40	39.02	0.29	.001
Depth	1	0.60	9.80	0.07	.001
Downstream distance	1	0.18	2.88	0.02	.01
Turbidity × depth	1	0.25	4.13	0.03	.006
Turbidity × downstream distance	1	0.33	5.33	0.04	.001
Downstream distance × depth	1	0.10	1.55	0.01	.15
Residuals	73	0.06		0.54	
Total	79			1.00	

Abbreviations: *df*, degrees of freedom; MS, mean square.

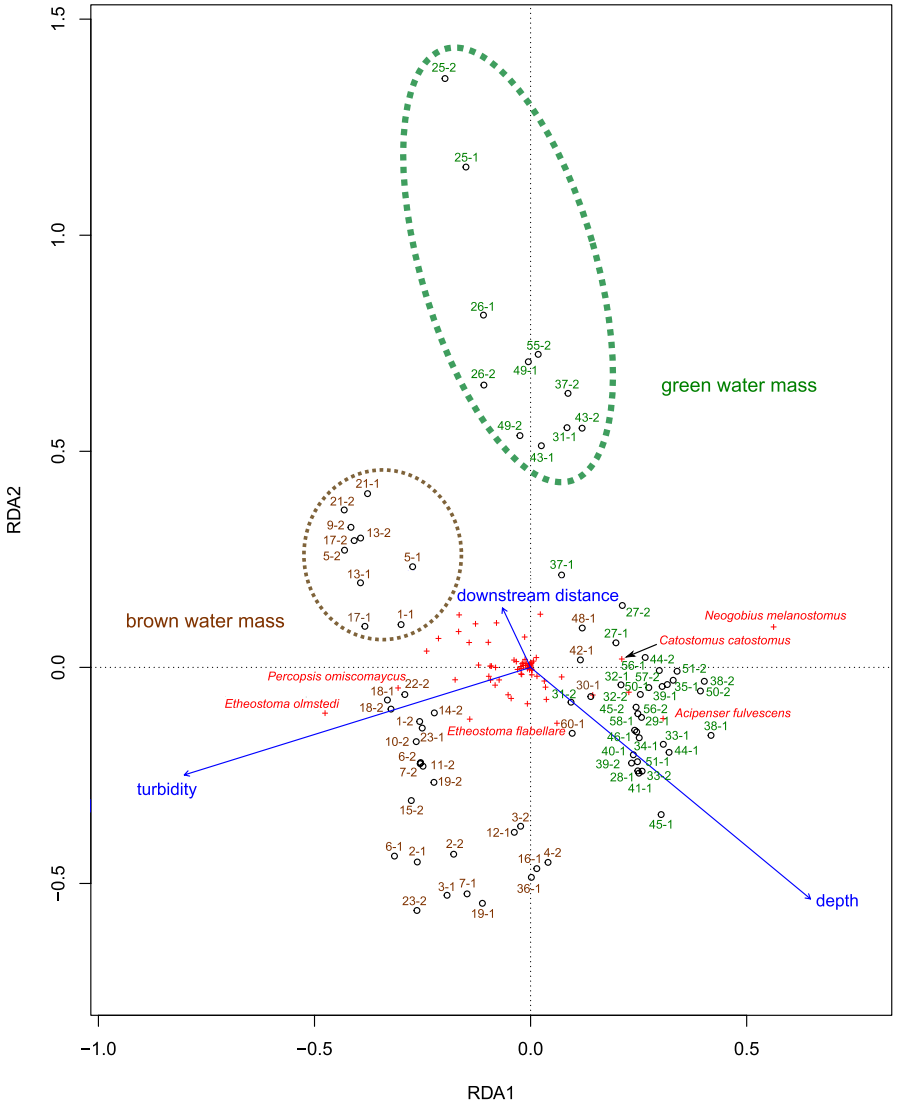

FIGURE 2 Redundancy analysis (RDA) based on the Hellinger distance matrix of the number of reads per species with turbidity (NTU), depth (m), and downstream distance (m) as constraint predictors. All sampling stations (53) and their temporal replicates were included into the analysis that was performed on 67 fish species. For each station, temporal replicates are indicated by −1 (day 1) and −2 (day 2). Brown and green colors indicate the water types where the stations were sampled. In each water mass, stations near the shore were circled (see Results section for explanations). The species for which eDNA abundance contributes the most to the observed dissimilarity are in red [Colour figure can be viewed at wileyonlinelibrary.com]

Finally, eDNA of five species belonging to the Catostomidae or Cyprinidae families was primarily detected in downstream locations in both water masses (Catostomidae: White sucker and River redhorse *Moxostoma carinatum*; Cyprinidae: Eastern silvery minnow, Golden shiner, and Bridle shiner) (Table 3 panel C; Figure 5 species in blue). Finally, eDNA of six other species belonging to various genera (i.e., American eel, Mooneye, Smallmouth bass, Round goby, Common logperch *Percina caprodes*, and Trout-perch) was primarily detected upstream in both water masses (Table 3 panel C; Figure 5 species in purple).

Temporal replicates showed that fish community composition did not differ markedly at a given sampling station (Figures 2–5; Table S1). In the PERMANOVA, we did not find any significant difference in fish community composition between day 1 and day 2 of sampling for each station sampled twice ($p > .5$). In the RDA, both temporal replicates for a given station tended to position nearby each other, albeit sometimes slightly differing (Figures 2–5). The largest difference between temporal replicates was observed for station 31 located in green waters (Figure 2). This is likely explained by the much lower number of sequences detected on the second day (station 31-2: total of 146 sequences for 30 detected species; the lowest level of sequences measured in our study) compared to the first day (station 31-1: total of 109 696 sequences for 39 detected species; Table S1).

3.3 | Comparison of environmental metabarcoding with traditional surveys

Using gillnet and seine, a total of 42 fish species were detected in the 5.5 km Contrecoeur sector in 2001 and 2015 combined (Table S3). These 42 species were all identified using eDNA metabarcoding, and none of the species that we discarded from the analysis were detected with these gears. For stations sampled by gillnet and seine in brown waters, the species that were predominantly caught were Yellow perch *Perca flavescens* (191 individuals) and Trout-perch (189 individuals). In green waters, the most predominant species were Bluntnose minnow *Pimephales notatus* and Rock bass, for which a total of 192 and 155 individuals were respectively caught with gillnet and seine combined. At each station,

TABLE 3 Summary of the fish community composition depending on A. turbidity, B. depth of sampling stations, C. downstream distance along the river and that was common to brown and green waters in the Contrecoeur sector of the St. Lawrence River

A. Species with more eDNA in less turbid stations	Species with more eDNA in more turbid stations
Mooneye *Hiodon tergisus* (Hiodontidae)	Rock bass *Ambloplites rupestris* (Centrarchidae)
Smallmouth bass *Micropterus dolomieu* (Centrarchidae)	Quillback *Carpiodes cyprinus* (Catostomidae)
Round goby *Neogobius melanostomus* (Gobiidae)	Northern pike *Esox lucius* (Esocidae)
	Tessellated darter *Etheostoma olmstedi* (Percidae)
	Emerald shiner *Notropis atherinoides* (Cyprinidae)
	Walleye *Sander vitreus* (Percidae)
B. Species with more eDNA in stations with low water depths	**Species with more eDNA in stations with greater water depths**
Rock bass *Ambloplites rupestris* (Centrarchidae)	Lake sturgeon *Acipenser fulvescens* (Acipenseridae)
Brown bullhead *Ameiurus nebulosus* (Ictaluridae)	American eel *Anguilla rostrata* (Anguillidae)
Common carp *Cyprinus carpio* (Cyprinidae)	Longnose sucker *Catostomus catostomus* (Catostomidae)
Northern pike *Esox lucius* (Esocidae)	White sucker *Catostomus commersonii* (Catostomidae)
Tessellated darter *Etheostoma olmstedi* (Percidae)	Channel catfish *Ictalurus punctatus* (Ictaluridae)
Eastern silvery minnow *Hybognathus regius* (Cyprinidae)	Smallmouth bass *Micropterus dolomieu* (Centrarchidae)
Pumpkinseed *Lepomis gibbosus* (Centrarchidae)	Shorthead redhorse *Moxostoma macrolepidotum* (Catostomidae)
Golden shiner *Notemigonus crysoleucas* (Cyprinidae)	Round goby *Neogobius melanostomus* (Gobiidae)
Bridle shiner *Notropis bifrenatus* (Cyprinidae)	
Trout-perch *Percopsis omiscomaycus* (Percopsidae)	
Tench *Tinca tinca* (Cyprinidae)	
C. Species with more eDNA in stations located downstream	**Species with more eDNA in stations located upstream**
White sucker *Catostomus commersonii* (Catostomidae)	American eel *Anguilla rostrata* (Anguillidae)
Eastern silvery minnow *Hybognathus regius* (Cyprinidae)	Mooneye *Hiodon tergisus* (Hiodontidae)
River redhorse *Moxostoma carinatum* (Catostomidae)	Smallmouth bass *Micropterus dolomieu* (Centrarchidae)
Golden shiner *Notemigonus crysoleucas* (Cyprinidae)	Round goby *Neogobius melanostomus* (Gobiidae)
Bridle shiner *Notropis bifrenatus* (Cyprinidae)	Common logperch *Percina caprodes* (Percidae)
	Trout-perch *Percopsis omiscomaycus* (Percopsidae)

Note: A. Species for which eDNA was predominantly detected in less turbid (left) or in more turbid (right) stations in both water masses. B. Species for which eDNA was predominantly detected in stations with lower (left) or greater (right) water depths in both water masses. C. Species for which eDNA was predominantly detected in stations located downstream (left) or upstream (right) in both water masses. Results are summarized from six redundancy analyses (RDA) performed on 67 fish species, with each environmental variable (turbidity, depth, downstream distance) and water mass type (brown, green) analyzed separately.

the total number of species caught by traditional surveys (mean of 8.4 species per station) was always lower than the total number of species detected by eDNA metabarcoding (mean of 40.5 species per station). Using traditional surveys, the highest number of species detected was 20 (station S122, seine) whereas the highest number of species detected by eDNA at a given station was 52 (station 9) and the lowest number of detected species was 29 (stations 38 and 45) (Figure 6).

We compared the cumulative counts of species as a function of sampling stations between the eDNA data obtained in the 5.5 km studied sector and the traditional survey data obtained in a 50 km sector comprising the 5.5 km Contrecoeur sector and up to 40 km upstream of it. Monte Carlo statistical resampling showed that eDNA metabarcoding required less sampling efforts (in terms of number of stations sampled) to reach a plateau with a higher number of detected species compared to either gillnet or seine surveys. Using eDNA metabarcoding, a plateau of 67 species detected was reached with 80 stations sampled (Figure 7 black curve). In contrast, 47 and 23 species were respectively detected with 110 and 87 stations sampled with seine (Figure 7 blue curve) or gillnet (Figure 7 red curve). A total of 54 species were caught using gillnet and seine combined at 197 stations (Figure 7 green curve). We found that 51 species were detected both using eDNA and traditional gears, while 14 species were only detected using eDNA (Figure 7, Table S4) and three species were only caught using gillnet and seine (Figure 7, Table S4). These three species (namely, Cutlips minnow *Exoglossum maxillingua*, Common shiner *Luxilus cornutus*, and Sand shiner *Notropis stramineus*) were caught outside the 5.5 km area sampled for eDNA metabarcoding, mainly upstream near Montréal (Figure S2). The sequences of these three species were present in the databases used for taxonomic assignment, confirming that the lack of eDNA detection for these species reflects fish absence (or

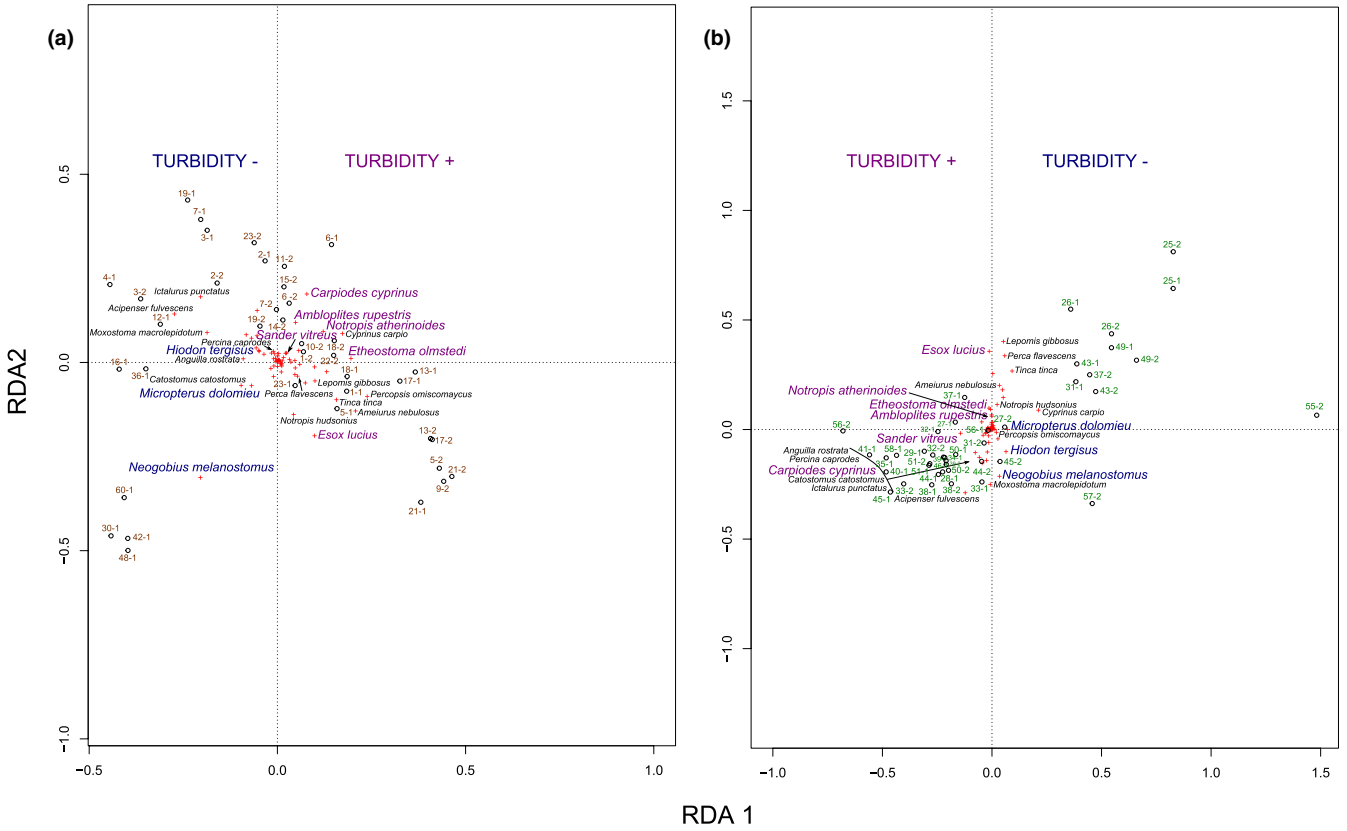

FIGURE 3 Redundancy analysis (RDA) based on the Hellinger distance matrix of the number of reads per species with turbidity (NTU) as a constraint predictor. (a) Stations sampled in brown waters (27) and their temporal replicates. (b) Stations sampled in green waters (26) and their temporal replicates. For each station, temporal replicates are indicated by −1 (day 1) and −2 (day 2). In (a) and (b), analysis was performed independently on 67 fish species. Species names in blue: fishes for which eDNA was predominantly detected in less turbid stations in both water masses. Species names in purple: fishes for which eDNA was predominantly detected in more turbid stations in both water masses. Species names in black: fishes for which eDNA was predominantly detected in less turbid stations or in more turbid stations in one of the water mass only [Colour figure can be viewed at wileyonlinelibrary.com]

at least very low abundance), rather than missing information in the databases used for taxonomic assignment.

4 | DISCUSSION

Few studies have attempted to document the role of environmental factors in structuring fish communities spatially at small geographic scales and considering both longitudinal and lateral variation within rivers using eDNA methods. Here, we used environmental DNA metabarcoding to document fish community composition (in terms of presence and relative read abundance) in the Contrecoeur sector of the St. Lawrence River (Québec, Canada).

The use of sequence read number detected by eDNA metabarcoding as a proxy to document variation in fish community composition has been subject to intense debates (Fonseca, 2018). This is primarily because biotic, abiotic, and technical parameters can affect sequence read number (Fonseca, 2018). Among biotic constraints, the relative numbers of sequences measured by eDNA metabarcoding for different species are influenced by the rate at which each species excretes DNA depending on its physiology and developmental state (Pilliod, Goldberg, Arkle, & Waits, 2014; Rees et al., 2014). Environmental abiotic factors, such as temperature and pH, are also likely to influence the concentration of eDNA measured (Barnes et al., 2014; Lacoursière-Roussel, Rosabal, & Bernatchez, 2019; Rees et al., 2014). Among technical biases happening during amplification, primer specificity can affect abundance estimates as this specificity varies with the PCR conditions (e.g., number of cycles, type of polymerase master mix) and with the GC content of the targeted sequences (Kelly, Shelton, & Gallego, 2019; Nichols et al., 2018). In other words, the sequences of certain species could be amplified more efficiently than others (Deagle et al., 2014). Keeping these constraints in mind, evidence is growing that sequence read abundance measured by eDNA metabarcoding can be used as a fair proxy to estimate fish abundance and biomass in nature (Afzali et al., 2020; Boivin-Delisle et al., 2020; Evans et al., 2016; Hänfling et al., 2016; Lacoursière-Roussel, Côté, Leclerc, & Bernatchez, 2016; Lacoursière-Roussel et al., 2019; Li et al., 2019; Takahara, Minamoto, Yamanaka, Doi, & Kawabata, 2012; Yates, Fraser, & Derry, 2019). For example, Ushio et al., (2018) showed that variation in eDNA sequence read numbers measured with the MishFish primers can be used as a rough

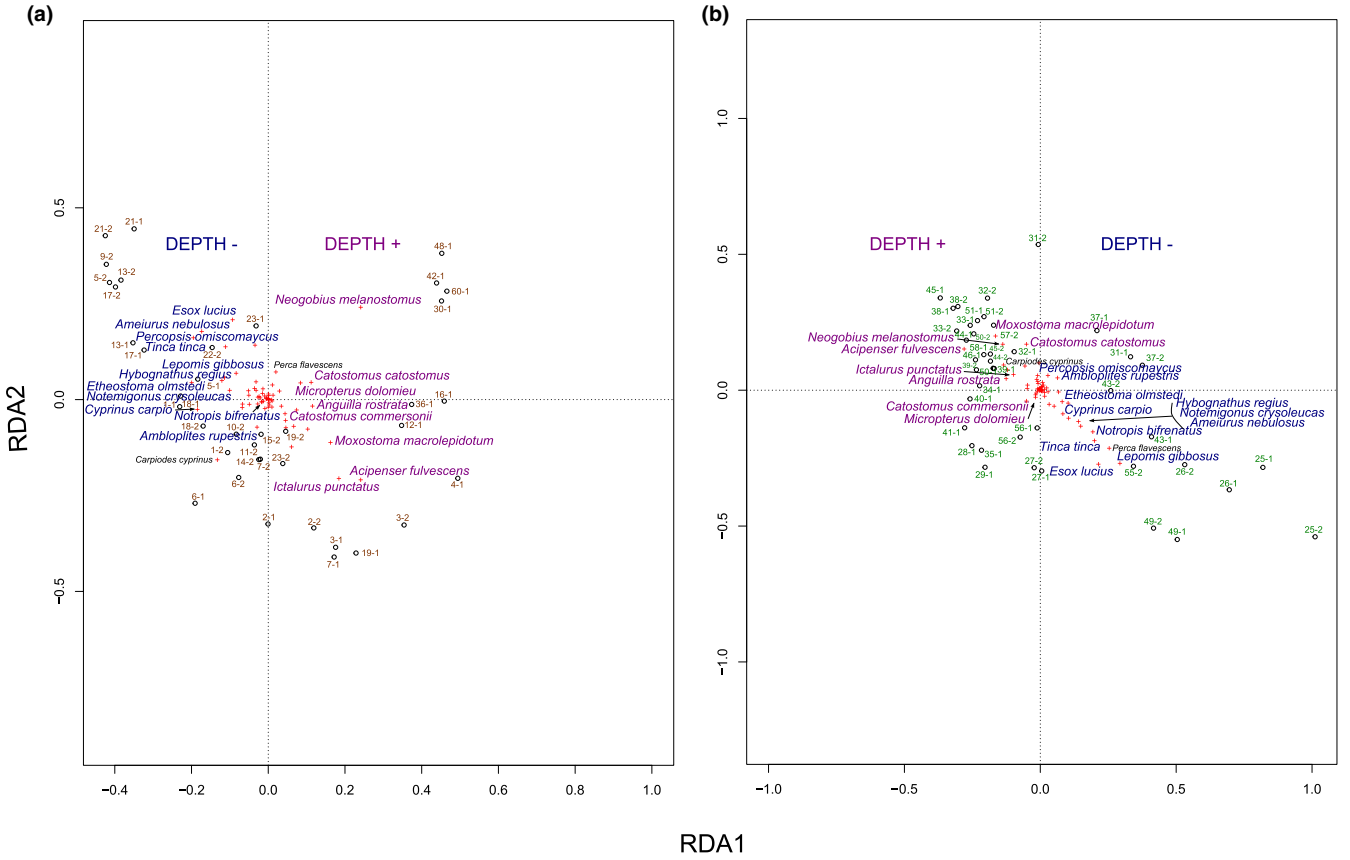

FIGURE 4 Redundancy analysis (RDA) based on the Hellinger distance matrix of the number of reads per species with depth (m) as a constraint predictor. (a) Stations sampled in brown waters (27) and their temporal replicates. (b) Stations sampled in green waters (26) and their temporal replicates. For each station, temporal replicates are indicated by −1 (day 1) and −2 (day 2). In (a) and (b), analysis was performed independently on 67 fish species. Species names in blue: fishes for which eDNA was predominantly detected in stations with low water depths in both water masses. Species names in purple: fishes for which eDNA was predominantly detected in stations with greater water depths in both water masses. Species names in black: fishes for which eDNA was predominantly detected in shallow stations or in deep stations of one of the water mass only [Colour figure can be viewed at wileyonlinelibrary.com]

indicator of species abundance, when appropriate external controls are included into the protocol and analyses. Boivin-Delisle et al. (2020) also used MishFish primers to demonstrate that fish communities' complexity (in terms of fish presence and abundance) was better described using eDNA metabarcoding compared to gillnets in a Canadian northern freshwater system and that eDNA metabarcoding performed as good as quantitative PCR in reflecting abundance of the most common species (Walleye). Knudsen et al. (2019) demonstrated that although eDNA concentrations do not directly correlate with the biomass of fish caught by trawls in marine environments, associations can be observed between concentrations of eDNA and the known abundance of species living in the area. Afzali et al. (2020) compared eDNA metabarcoding and trawling data to evaluate their efficiency to characterize demersal fish communities in the Estuary and Gulf of Saint-Lawrence, Canada. Their results indicated that the relative abundance estimated by eDNA and trawl is significantly correlated for species detected by both methods, while the relationship was also influenced by environmental variables (temperature, depth, salinity, and oxygen). In our study, eDNA levels measured in the control samples were much lower than in the actual samples, confirming that our results are not significantly affected by contamination problems. Furthermore, we found that the species with the highest numbers of sequences measured (i.e., Silver redhorse *Moxostoma anisurum* with 1,726,150 of total raw sequences (1,411.8 relative read abundances RRAs); Shorthead redhorse with 1,112,228 of total raw sequences (949.8 RRAs); and Lake sturgeon with 758,786 of total raw sequences (669.4 RRAs)) are among the most common fish species in the St. Lawrence River (Fortin, Mongeau, Desjardins, & Dumont, 1993; Hatry et al., 2014). In the same trend, species with the lowest levels of eDNA sequences (i.e., Striped bass *Morone saxatilis* with 17 of total raw sequences (0.01 RRAs); Goldfish *Carassius auratus* with 213 of total sequences (0.2 RRAs)) are respectively either less common, endangered (Douglas, Caissie, & Chaput, 2006), or early invasive (Gertzen, Familiar, & Leung, 2008) species in Québec rivers. Also, if certain species were more efficiently amplified than others because of variation in primer specificity, then these biases should manifest themselves in all sampled stations. However, this was not the case (either with raw read counts or RRAs). For example, the Shorthead redhorse, which was

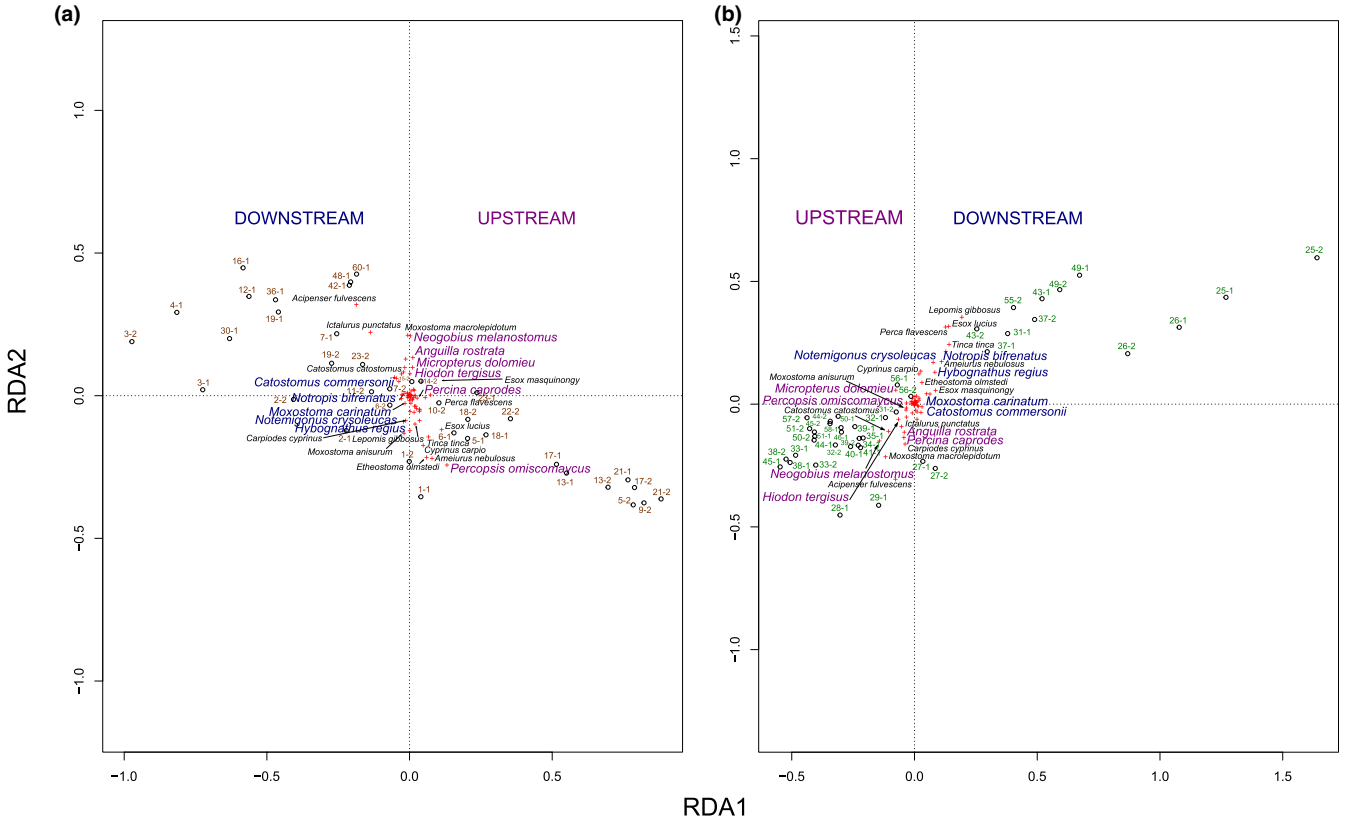

FIGURE 5 Redundancy analysis (RDA) based on the Hellinger distance matrix of the number of reads per species with downstream distance (m) as a constraint predictor. A. Stations sampled in brown waters (27) and their temporal replicates. B. Stations sampled in green waters (26) and their temporal replicates. For each station, temporal replicates are indicated by −1 (day 1) and −2 (day 2). In (a) and (b), analysis was performed independently on 67 fish species. Species names in blue: fishes for which eDNA was predominantly detected in stations located downstream in both water masses. Species names in purple: fishes for which eDNA was predominantly detected in stations located upstream in both water masses. Species names in black: fishes for which eDNA was predominantly detected in downstream stations or in upstream stations of one of the water mass only [Colour figure can be viewed at wileyonlinelibrary.com]

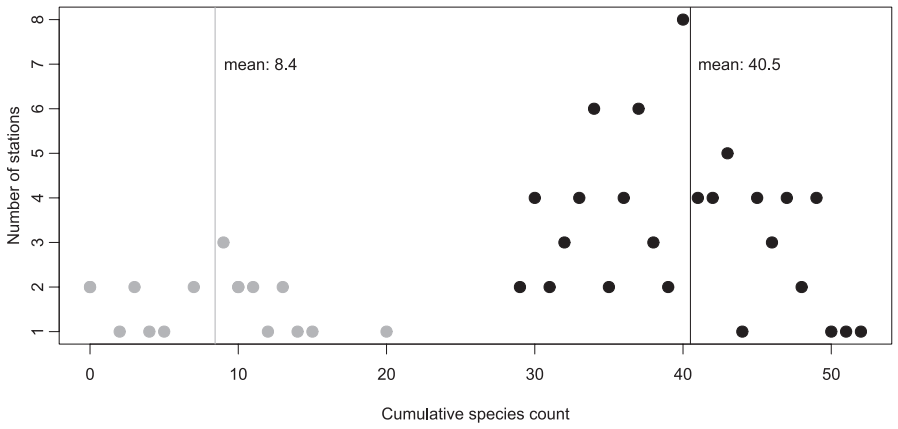

FIGURE 6 Comparison between traditional fish community survey data and eDNA metabarcoding in the Contrecoeur sector of the St. Lawrence River. The occurrence frequency in terms of number of stations is plotted against the total number of fish species detected at each station. For each survey, the vertical bar represents the mean count of species detected by station. Gray: traditional surveys (gillnet + seine sampled in 2001 and 2015). Black: eDNA metabarcoding

one of the most detected species with over one million sequence reads in total, had only 30 raw sequences (20.5 RRAs) detected at station 31 and 57 raw sequences (0.3 RRAs) detected at station 25. Quillback showed a wide range of detection levels from 1 raw sequence (0 RRA) to more than 10,000 raw sequences (15 RRAs) amplified depending on the stations. Finally, we observed a strong parallelism in the number of species detected and in the number of sequences measured when we studied the roles of the environmental factors in structuring fish communities separately in each water mass, which adds further support to the validity of our results that can be replicated in distinct environments. Moreover, we normalized the raw read counts obtained (using relative read abundance (RRA) for PERMANOVAs, and Hellinger's transformation for RDAs), which allowed to correct for potential biotic, abiotic, or technical biases that would result in higher amplification rates of some species compared to others (Cavallo et al., 2018; Deagle et al., 2019). Therefore, we are confident that despite uncertainties, as with any sampling methods, eDNA metabarcoding

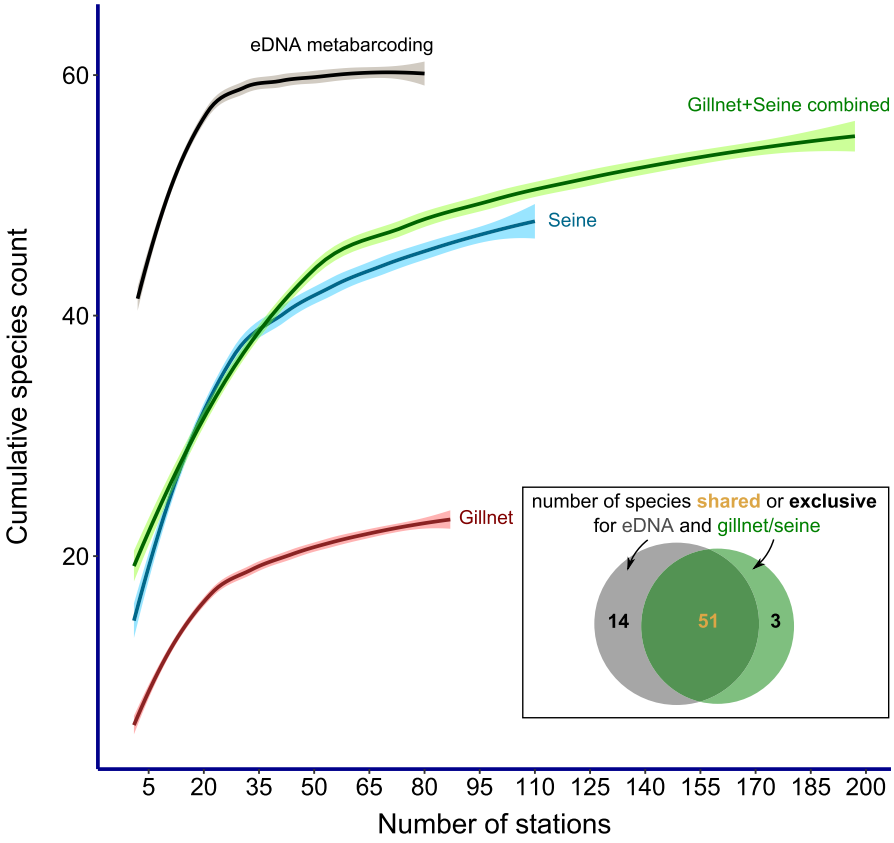

FIGURE 7 Cumulative species count as a function of sampling stations for eDNA metabarcoding and traditional (gillnet and seine) sampling methods. Simulations of sampling efforts (in terms of numbers of stations sampled) are based on eDNA metabarcoding data measured in the Contrecoeur sector, and on data collected in 2015 in a 50 km sector, up to 40 km upstream of the Contrecoeur sector for traditional surveys (gillnet and seine analyzed separately or in combination). The values represent the average from a total of 30 simulations for each method and intensity. The shaded area around each curve represents 95% confidence interval. Venn diagram shows the number of species detected both with eDNA and gillnet/seine, with eDNA only or with gillnet/seine only [Colour figure can be viewed at wileyonlinelibrary.com]

can be used to reliably infer spatial variation in community composition, at least in our study system.

4.1 | eDNA metabarcoding highlights the role of environmental factors in shaping 2D variation in fish community composition at a small geographic scale

Using eDNA metabarcoding, a total of 67 fish species were detected in the Contrecoeur sector, that is, 57% of all 118 freshwater fishes of Québec (https://mffp.gouv.qc.ca/la-faune/especes/). These fishes appeared to form distinct communities within the approximately 5 km^2 river surface area that we studied. Indeed, fish community composition was most significantly affected by turbidity (a proxy for brown vs. green waters), followed by water depth and to a lower extent by downstream distance along the river.

We demonstrated that the stations located in brown waters (i.e., more turbid waters) were dominated by the eDNA of Tessellated darter, Trout-perch, and Fantail darter. Tessellated darter is a common fish in Québec rivers that is known to live in sand or mud environments (Bernatchez & Giroux, 2012; Cole, 1967), which is in accordance with the high turbidity levels of the brown waters. In the same way, Trout-perch and Fantail darter occupy sand and mud substrates (Bernatchez & Giroux, 2012; Magnuson & Smith, 1963; Strange, 1993). In contrast, eDNA of Round goby, Longnose sucker, and Lake sturgeon predominated in stations located in green waters (i.e., less turbid waters). Round goby is an invasive species native to central Eurasia, the Black Sea and Caspian Sea whose presence in the St. Lawrence River has been reported since 1997. It has been shown to strongly impact on native fish community structures (Morissette, 2018) and other aquatic species (sculpins: Janssen & Jude, 2001; zebral mussels: Ray & Corkum, 1997). In accordance with the low turbidity levels of green waters, Round Goby preferentially inhabits rocky and clear substrates (Ray & Corkum, 2001). Similarly, Longnose sucker lives in very clear and clean water (Edwards, 1983). Lake sturgeon is also reported in clear waters where its prey is abundant (Peterson, Vecsei, & Jennings, 2007).

Depth of sampling stations was the second main environmental factor explaining differences in fish community composition in both water masses of the Contrecoeur sector. The higher importance of depth in our RDA analysis compared to the effect of downstream distance along the river to structure fish communities in both water masses is supported by previously published hydrologic bidimensional models conducted in the sector (Laporte et al., 2020; Matte, Secretan, & Morin, 2017). These models first demonstrated a low widthwise dilution (i.e., advection) in the 5.5 km sector, meaning that little (if any) eDNA from a fish measured near the shore of the river would be detected in the center. This characteristic is advantageous to study species habitat preference depending on depth, which increases with the distance from the shore. They also showed a downstream laminar flow dispersion of eDNA along the 5.5 km sector, such that the downstream distance alone cannot be used to predict presence and abundance of aquatic species (Laporte et al., 2020; Matte et al., 2017). In each water mass, variability in fish community composition was mainly driven by the stations localized

near the shore that tended to be distinct from the other stations. Since depth increases with distance from the shore, our results suggest fish vertical zonation, the structuration of species communities as a function of depth (Chappuis, Terradas, Cefalì, Mariani, & Ballesteros, 2014), in parallel between the two water masses of the Contrecoeur sector. Previous studies conducted at a regional scale reported pelagic fish vertical zonation during scuba diving (Edwards & Rosewell, 1981) or video footage (Torquato et al., 2017). To our knowledge, our study is the first to document repeated differences in freshwater fish communities between stations sampled in deep waters versus stations sampled in low water depths using eDNA metabarcoding. However, it is important to keep in mind that we sampled integrated water column samples (from bottom to top) at each station. Sampling water at multiple depths would allow an exhaustive estimation of eDNA distribution in the water column as well as of fish presence and abundance depending on depth.

Previous studies using traditional sampling methods (including electrofishing, cages and nets) and multivariate analyses also demonstrated the importance of turbidity, depth and downstream distance in shaping differences in fish communities in freshwater ecosystems. For example, a 3-year study reported that fish species composition varied with turbidity in the Laurentian Great Lakes (Janetski & Ruetz, 2015). Martin-Smith (1998) also showed that depth was one of the main factors responsible for the structuration of distinct fish assemblages in streams of Malaysia. The importance of depth in fish community structure was likewise highlighted in lagoons and rivers of the Pantanal ecosystem in Brazil (Súarez, Petrere, & Catella, 2001). Finally, a 3-year sampling study in three rivers of Portugal demonstrated changes in fish community structures with the downstream distance along the rivers (Pires, Cowx, & Coelho, 1999). At the scale of the St. Lawrence River, Foubert, Lecomte, Legendre, and Cusson (2018) conducted between 1995 and 2012 a multivariate analysis of datasets of fish biodiversity obtained from either gillnet or seine in the 550 km freshwater portion of the river. They demonstrated that fish community structure changed with the downstream distance along the river and that, depending on whether seine or gillnet was used, different fishes would be targeted in shallower shoreline habitats and deeper areas, respectively, underlining the importance of downstream distance and depth in structuring fish communities in this system. Turbidity, a factor typically discriminating brown and green water masses, was not taken into account in their analyses (Foubert et al., 2018). Compared to all these studies that successfully highlighted the roles of environmental factors in shaping overall fish communities, the eDNA metabarcoding approach has the advantage of reducing sampling efforts (in terms of number of stations sampled and of time) while also being noninvasive.

4.2 | eDNA metabarcoding reveals parallel variation in species composition between brown and green waters

Using eDNA metabarcoding, we were able to define distinct fish communities whose compositions are well in accordance both with the biology of the detected species and with the local environmental conditions in our 5 km^2 study area.

Turbidity was the main environmental factor that discriminated between green (i.e., less turbid) and brown (i.e., more turbid) waters. Furthermore, turbidity contributed to the segregation of fish communities within each water mass, and eDNA of the same species was detected in parallel between stations with similar turbidity level within each water mass. In addition to eDNA of Round Goby that was detected in green (i.e., less turbid) waters, we detected eDNA from Smallmouth bass and Mooneye at less turbid stations in both water masses, in accordance with their habitat preferences for clear environments (Carter, Shoup, Dettmers, & Wahl, 2010; Laplante-Albert, Rodríguez, & Magnan, 2010). In stations located in more turbid waters, we especially detected the eDNA of Rock bass, Northern pike, and Walleye, which are known to live in mesotrophic or eutrophic environments with a high level of suspended particles in the littoral zone (Casselman & Lewis, 1996; Gross & Nowell, 1980; Pandit, Zhao, Ciborowski, Gorman, & Knight, 2013), along with eDNA of Tessellated darter, which was previously found to be one of the most dominant species in brown (i.e., turbid) waters.

Then, water column depth at sampling stations was mainly responsible for the parallel segregation of fish communities within both water masses. In stations with low water depths of both brown and green waters, eDNA of species belonging to the family Cyprinidae predominated. In particular, we detected eDNA of Eastern silvery minnow, Golden shiner, and Bridle shiner that are typical of shallow and calm environments (Whittier, Halliwell, & Daniels, 2000). We also detected eDNA from Common carp and Tench, two invasive species that are typical of shallow waters where they can tolerate low oxygen levels and consequently colonize environments that are hostile for many other fishes (Avlijaš, Ricciardi, & Mandrak, 2018; Bajer & Sorensen, 2010). Tench raises major concerns as it has negative impacts through competition on Copper redhorse *Moxostoma hubbsi*, the only endemic fish species in Québec (Avlijaš et al., 2018; Masson et al., 2013). On the other hand, the family Catostomidae was typical of stations with greater water depths in both brown and green waters: we detected eDNA of White sucker, which is considered the most abundant species in Québec rivers, demonstrates a high capacity of adaptation to variable environments and is generally found over muddy or rocky bottoms (Bernatchez & Giroux, 2012; Stewart, 1926). eDNA molecules of Longnose sucker and Shorthead redhorse were also detected more abundantly at greater depths. Longnose sucker is known to inhabit deep and cold waters (Edwards, 1983). Shorthead redhorse is usually found in shallower areas; however, it often reaches the bottom of deeper waters to feed on aquatic insects (Hubert & O'Shea, 1992; Sule & Skelly, 1985). Two other species that were detected by eDNA metabarcoding in stations with greater water depths are also reported to be benthic feeders: Lake sturgeon and American eel (Busch, Lary, Castilione, & McDonald, 1998; Chiasson, Noakes, & Beamish, 1997; Miller & Casselman, 2014). American eel is also famous for burying itself in the mud (Busch et al., 1998; Miller & Casselman, 2014). Finally, Smallmouth bass eDNA was also

predominantly detected in stations with deeper waters. This species migrates in deep and colder areas during summer as it does not tolerate high temperatures (Brown, Runciman, Pollard, Grant, & Bradford, 2009; Scott & Crossman, 1973). Because field sampling was done at the end of summer, the abundance of eDNA molecules detected for Smallmouth bass at stations with greater water depths probably results from this migrating behavior.

The importance of the downstream location in structuring fish communities in the Contrecoeur sector was low compared to that of turbidity and depth. In fact, the effect of downstream distance was no longer significant when PERMANOVA and RDA analyses were performed using the corrected mean of the number of reads per species of day 1 and day 2 for stations that were sampled twice. Therefore, we remain cautious concerning the capacity of eDNA to inform on the importance of the downstream distance to structure fish communities in both water masses. Indeed, Laporte et al. (2020) reported a downstream laminar flow dispersion of eDNA along the 5.5 km sector. It is thus likely that the pattern of distribution of eDNA molecules (upstream vs. downstream) that we reported in both water masses along the 5.5 km sector is influenced by a source located further upstream of the Contrecoeur sector.

4.3 | Environmental DNA metabarcoding highlights the stability of fish communities in a fluctuating environment

Environmental DNA metabarcoding revealed stability in fish communities that appeared to be shaped by the same environmental factors in parallel in the two water masses. Moreover, repeated sampling over two consecutive days confirmed that local fish community composition did not change markedly between the two sampling periods. Variation in environmental properties of the sector is likely to influence eDNA dynamics in water such as its shedding (Sansom & Sassoubre, 2017), dispersion (Laporte et al., 2020; Shogren et al., 2017), degradation (Eichmiller, Best, & Sorensen, 2016), and sedimentation (Lacoursière-Roussel & Deiner, 2019; Turner, Uy, & Everhart, 2015). Yet, eDNA signals were stable enough to confirm the temporal consistency in local fish community composition and as such bring further support to the robustness of our assessment of fish community composition based on eDNA metabarcoding. Admittedly, the experiment was performed only over two consecutive days and it could be extended over a longer period to document either seasonal (e.g., Campos et al., 1997; Sigsgaard et al., 2017) or interannual variation, which was beyond the scope of this study (Hurst, McKown, & Conover, 2004).

4.4 | Environmental DNA metabarcoding complements traditional fishing surveys for monitoring fish community composition

Data obtained with eDNA metabarcoding were compared to data obtained with traditional surveys (i.e., gillnet and seine) in 2001 and 2015. Traditional surveys supported the results obtained with eDNA metabarcoding as all 42 fish species that were caught by gillnet or seine in the 5.5 km area were also detected by eDNA. Traditional surveys primarily caught fish living in shallow environments, including Trout-perch (Hall & Rudstam, 1999) in brown waters and Rock bass (Gross & Nowell, 1980) in green waters, whose eDNA molecules were also primarily detected in shallow water stations. Conversely, eDNA metabarcoding detected species that are difficult to catch with gillnets or seine because of biological and/or technical constraints (Thomsen et al., 2016). This included American eel, whose anguillid body shape, protective mucous coat, and lack of rays and spines reduce the efficiency of its physical capture by traditional fish sampling gears (Busch et al., 1998; Miller & Casselman, 2014). This also comprised River redhorse, which is a species of conservation concern in Canada that lives on rocky bottoms hardly accessible by traditional gears (Campbell, 2002). Therefore, the use of eDNA metabarcoding allowed obtaining a more complete picture of fish community composition as previously demonstrated in other freshwater systems (Baker et al., 2016; Keskin, Unal, & Atar, 2016; Sigsgaard, Carl, Møller, & Thomsen, 2015).

By comparing our eDNA metabarcoding dataset with that of fish biodiversity observed at a larger spatial scale (50 km, including 40 km upstream of the studied area), we confirmed that most of the species not caught by fishing gears in our study sector, but for which eDNA was detected, were caught further upstream. Fourteen species were detected by eDNA metabarcoding but not by traditional surveys. Conversely, three species were caught only using gillnet or seine in 2015 and were not detected by eDNA metabarcoding. These species were all caught in stations that were located outside our 5 km^2 study area, and mainly near Montréal, 40 km upstream of the studied site. Either these species have habitat preferences too far outside from our studied sector so that their eDNA was not detected or they were absent when the eDNA metabarcoding sampling was performed in 2017.

Overall, our results add to the building evidence that eDNA metabarcoding allows detecting higher species diversity with substantially lower sampling effort than traditional capture methods. Yet, these methods are still a requirement to obtain an accurate description of fish biomass and abundance (Knudsen et al., 2019). As such, our study highlights the relevance of coupling eDNA metabarcoding to traditional surveys for monitoring biodiversity.

4.5 | Detection of eDNA emanating from urban wastewaters

We identified 17 fish marine species obviously not naturally present in the freshwater section of the St. Lawrence River and that were consequently not taken into account for the analyses. None of these species were detected in the negative controls. Essentially, all of these species are exploited commercially for food consumption (Greenberg, 2011) and are common in fish markets and groceries of Montréal in Québec. Two species (Streamline chub *Erimystax*

dissimilis and Blackchin tilapia *Sarotherodon melanotheron*) are also commonly found in amateur aquaria (Sakurai, Sakamoto, & Mori, 1993). The vast majority of sampled stations that were positive for these species were localized in green waters. Interestingly, wastewaters from Montréal are discharged in green waters at a major municipal effluent about 40 km upstream from our sampling site (Gust, Fortier, Garric, Fournier, & Gagné, 2013). Therefore, these positive detections most likely reflect the contamination of wastewater effluents with DNA from fish that are consumed by humans or kept in aquaria. Maximal eDNA dispersion reported so far in freshwater ecosystems ranges between 12 and 60 km from its source (Deiner & Altermatt, 2014; Pont et al., 2018). In the St. Lawrence River, dispersion of waste waters may be exacerbated by the strong average daily flow (2.5 million cubic meters) of the effluent of Montréal (Gagnon & Saulnier, 2003). Furthermore, weak lateral dilution, preventing admixture of eDNA between the two water masses in our study area (Laporte et al., 2020), probably explains why detection of marine species was mostly restricted to stations located in green waters. We are thus confident that the detection of these marine species shows that fish eDNA can disperse and be detected at least 40 km from its source in this section of the St. Lawrence River.

5 | CONCLUSION

Our results demonstrate that it is possible to detect lateral (from shore toward center) variation in eDNA and consequently in fish community composition at a small geographic scale in large rivers. This is a key information that will help to implement biomonitoring tools that can track the presence and relative abundance of organisms in rivers and improve our understanding of the ecology and evolution of fish communities. Clearly, eDNA metabarcoding can complement traditional capture methods and help documenting with higher precision the role of physico-chemical factors in shaping local fish community composition in large fluvial systems such as the St. Lawrence River.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

G.C. and L.B. designed the study. G.C. and Y.P. performed field sampling. C.H. performed the laboratory work. E.N. performed the bioinformatic analyses. C.S.B. analyzed the results in collaboration with M.L. for eDNA and with D.W.K.T. for RSI data. C.S.B. wrote the manuscript with collaboration from all the authors.

DATA AVAILABILITY STATEMENT

All eDNA metabarcoding data generated are available in Table S1.

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SUPPORTING INFORMATION

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

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