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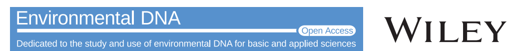

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Caged fish experiment and hydrodynamic bidimensional modeling highlight the importance to consider 2D dispersion in fluvial environmental DNA studies

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Abstract

The analysis of environmental DNA (eDNA) is a powerful tool to increase the efficiency of species detection and monitoring in aquatic ecosystems. Yet, several points remain to be clarified in order to estimate with better precision the distribution and abundance of targeted species, such as the dispersion and dilution of eDNA in large lotic systems. This study aimed to document the dispersion patterns of eDNA in the St. Lawrence River, the largest fluvial system in eastern North America. Caged Brown trout (*Salmo trutta*) were placed in two different water masses present in this part of the river, the Ottawa River, and water from the outlet of the Laurentian Great Lakes. eDNA detection of the caged fish was performed for two days following cage removal at 53 sampling stations located at 500 upstream, and 10, 100, 500, 1,000, and 5,000 m downstream from the cages. Quantitative PCR analysis using a Brown trout specific assay revealed a positive detection only at downstream stations and up to 5,000 m. To further investigate patterns of dispersion, the relative concentrations of eDNA were predicted using a bidimensional hydrodynamic model, calibrated for downstream advection and lateral mixing of particles (*i.e.*, quantification of 2D dispersion). The detection and the quantities of eDNA obtained by qPCR analyses were compared with the model predictions. Our model which predicts a low lateral mixing and a downstream flow in direct line from the eDNA source best fits the results. We discuss how such studies can improve our capacity to produce more precise estimates of species abundance and distribution in order to better interpret eDNA signals in large lotic systems.

KEYWORDS

aquatic biodiversity, conservation, eDNA ecology, Environmental DNA, management, qPCR, River, *Salmo trutta*

Laporte and Bougas are co-first authors.

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1 | INTRODUCTION

Freshwater biodiversity shows an alarming decline, and its protection is considered to be an ultimate conservation challenge (Dias et al., 2017; Dudgeon et al., 2006; Vörösmarty et al., 2010; WWF, 2018). Prompt and precise evaluation of spatial distribution and abundance of species is a first step to better understand the biology of aquatic species, leading to more efficient protection of their habitat and a better management of biodiversity. Reliable surveys of endangered or newly invading aquatic species remain challenging because of their inherent rarity. This is particularly true in river systems showing spatial and temporal variability of unidirectional water flow that can be affected by human activities (Feng, Shenliang, Ping, & Jun, 2012). Large rivers are of special interest for their high biodiversity level and their connectivity among drainages that cause the perturbation of local fauna, for instance by spreading of invasive species in new areas (Heino, Paavola, Virtanen, & Muotka, 2005; Leuven et al., 2009). The evaluation of spatial distribution and abundance in river systems is traditionally performed using electric-fishing, gill nets, trap nets, and scuba diving, but those methods are generally effort-intensive and are not without their own biases (Kubečka et al., 2009, 2012; Smith, Quist, & Hardy, 2015). The development of less effort-intensive methods is thus necessary for prompt surveys of aquatic fauna in river systems, which may ultimately help to protect global freshwater biodiversity.

The analysis of environmental DNA (eDNA) is a revolutionary approach that can alleviate some of the logistical constraints associated to more traditional survey methods. eDNA analysis allows tracing residual DNA collected in environmental samples (e.g., water, soil, and air) that are expelled from organisms via their epidermis, feces, mucus, hair, gametes, and other various sources (Levy-Booth et al., 2007; Rees, Maddison, Middleditch, Patmore, & Gough, 2014). Biological information can thus be obtained without manipulating organisms and disturbing their ecosystems. The presence of DNA of a target species (including harvested, rare, or invasive species) can be detected in water samples using PCR amplification with specific primers, that is quantitative PCR or metabarcoding methods (Balasingham, Walter, Mandrak, & Heath, 2018; Deiner et al., 2018; Erickson et al., 2016; Lacoursière-Roussel, Dubois, Normandeau, & Bernatchez, 2016). However, in order to use the full potential of eDNA analyses for biomonitoring, the distribution and abundance of targeted species need to be estimated with precision. A growing number of studies confirmed a positive relationship between eDNA concentration and biomass in aquatic environment for both experimental (Evans et al., 2016; Kelly, Port, Yamahara, & Crowder, 2014; Lacoursière-Roussel, Côté, Leclerc, & Bernatchez, 2016; Takahara, Minamoto, Yamanaka, Doi, & Kawabata, 2012) and field studies (Doi et al., 2017; Klobucar, Rodgers, & Budy, 2017; Maruyama, Sugatani, Watanabe, Yamanaka, & Imamura, 2018; Thomsen et al., 2012, 2016; Wilcox et al., 2016) but the strength of association can vary considerably depending on environmental characteristics (temperature: Lacoursière-Roussel, Rosabal, & Bernatchez, 2016; mesocosm: Chambert, Pilliod, Goldberg, Doi, & Takahara, 2018; lotic: Pilliod, Goldberg, Arkle, Waits, & Richardson, 2013; also see Lamb et al., 2019 and Yates, Fraser, & Derry, 2019 for metanalyses). Thus, eDNA dynamics in water, which include shedding, dispersion, degradation, and sedimentation mechanisms, need to be better understood, particularly in lotic environment where eDNA can be transported over long distance (Carraro, Hartikainen, Jokela, Bertuzzo, & Rinaldo, 2018; Lacoursière-Roussel & Deiner, 2019; Sansom & Sassoubre, 2017; Shogren et al., 2017).

A first step toward precisely estimating the distribution and abundance of species in river systems requires a thorough understanding of the effects of water flow on eDNA dispersion and dilution. eDNA in river systems has been detected at variable scales and up to 60 km away from its source (Deiner & Altermatt, 2014; Jane et al., 2015; Pont et al., 2018) while dispersion in lentic and marine systems is mostly observed within a few hundred meters (Eichmiller, Bajer, & Sorensen, 2014; Murakami et al., 2019; O'Donnell et al., 2017), which implies that water flow has a major effect on eDNA dispersion. Models including hydro-geomorphological features as well as degradation and/or sedimentation mechanisms have recently been used in small river systems to reconstruct upstream distribution and abundance of target species (Carraro et al., 2018; Sansom & Sassoubre, 2017). However, downstream continuous widthwise dispersion and dilution of eDNA in large river systems with large volume discharge (e.g. thousands of cubic m/s) may be more complex and is not well documented, which could complicate the location of eDNA sources as well as its correlated biomasses abundance (Shogren et al., 2017).

The main goal of this study was to compare the dispersion of eDNA from point sources with simulations obtained from a hydrodynamic model. More specifically, relative concentrations of eDNA were predicted with a bidimensional hydrodynamic model, calibrated for downstream advection and lateral dispersion of particles (*i.e.*, quantification of 2D dispersion) in one of the largest rivers in the world, the St. Lawrence River (Québec, Canada). Model predictions of eDNA relative concentration were then compared with downstream distance in their capacity to explain; (a) the detection, and (b) the amount of eDNA quantified by means of qPCR analyses. An experiment in natural conditions was set up by positioning two caged fish present in two different water masses and collecting water samples at upstream (8 locations used as control), and downstream (45 locations) for two days. eDNA detection and concentration measured downstream of caged fish were simulated by the bidimensional hydrodynamic model, but not by downstream distance solely. This result highlights the importance of considering hydraulic processes, namely flow path and lateral dispersion (*i.e.*, lateral mixing) in order to better understand eDNA dispersion in fluvial environment.

2 | MATERIALS AND METHODS

2.1 | Sampling

2.1.1 | Field sampling and water filtration

A natural transplantation experiment was conducted to estimate DNA dispersion in the St. Lawrence River in the Contrecoeur sector

(approximately 50 km downstream of Montréal, Québec, Canada) where the two water masses (Ottawa River or "brown waters" and the Great Lakes or "green waters") described in the Introduction flow in parallel, largely without admixing (Figure 1). The experiment lasted from September 11th to September 14th, 2017. Caged fish were put in place September 11th and eDNA sampling took place on September 13th and 14th. A total of 50 Brown trout (*Salmo trutta*; total biomass of 27.9 kg) were kept in one cage placed in the Ottawa River water mass and 49 *S. trutta* (total biomass of 28 kg) were kept in another cage placed in the Great Lakes water mass. 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) and moderately high conductivity (>250 mS/cm), while Ottawa River waters exhibit higher suspended solids concentrations (>8 mg/L) and turbidity (4.2 NTU) with lower clarity (K = 1.3/m) and conductivity (<160 mS/cm; Rondeau, 1999; Hudon, 2000). Note that Brown trout has not been reported in this area and eDNA detection of this species should only come from these two fish-caged sources. Negative controls upstream of the two cages have also been tested to support the absence of the species in the area (see below). The cages measured 10 m long and 2 m in diameter with 1.9 cm mesh size, which allowed zooplankton, small fish, and aquatic insects to freely enter the cages. To prevent abrasion of fish skin, the nylon mesh was knotless.

A schematic view of the experiment with visual explanations of downstream advection and lateral dispersion terms is presented in Figure 1. Stations for water collection were located at 500 m upstream, and 10, 100, 1,000, and 5,000 m downstream from the cages (Figures 1 and 2). Integrated water samples were taken near the bottom of water (1 m) from a boat using 1 L single use bottle attached to a rope. Water samples (250 ml) were filtered directly in the field after collection using a single-use syringe with a 1.2 µm glass microfiber filter (Whatman, 25 mm) as detailed in Leduc et al. (2019). Briefly, syringe heads were bleached, sterilized, and UV-treated. After filtration, filters were conserved in the Longmire buffer and frozen at −20°C until eDNA extraction using a syringe mounted with a XY filtering. Water sampling was conducted at 53 stations (10 stations on average for each distance) for a total of 112 samples including eight negative controls (−500 m) with up to two temporal/day replicates per station (see Figures 1 and 2; Appendix S1). In addition, a total of 4 negative bottle controls consisting of sterile water treated in the same way as the real samples were taken.

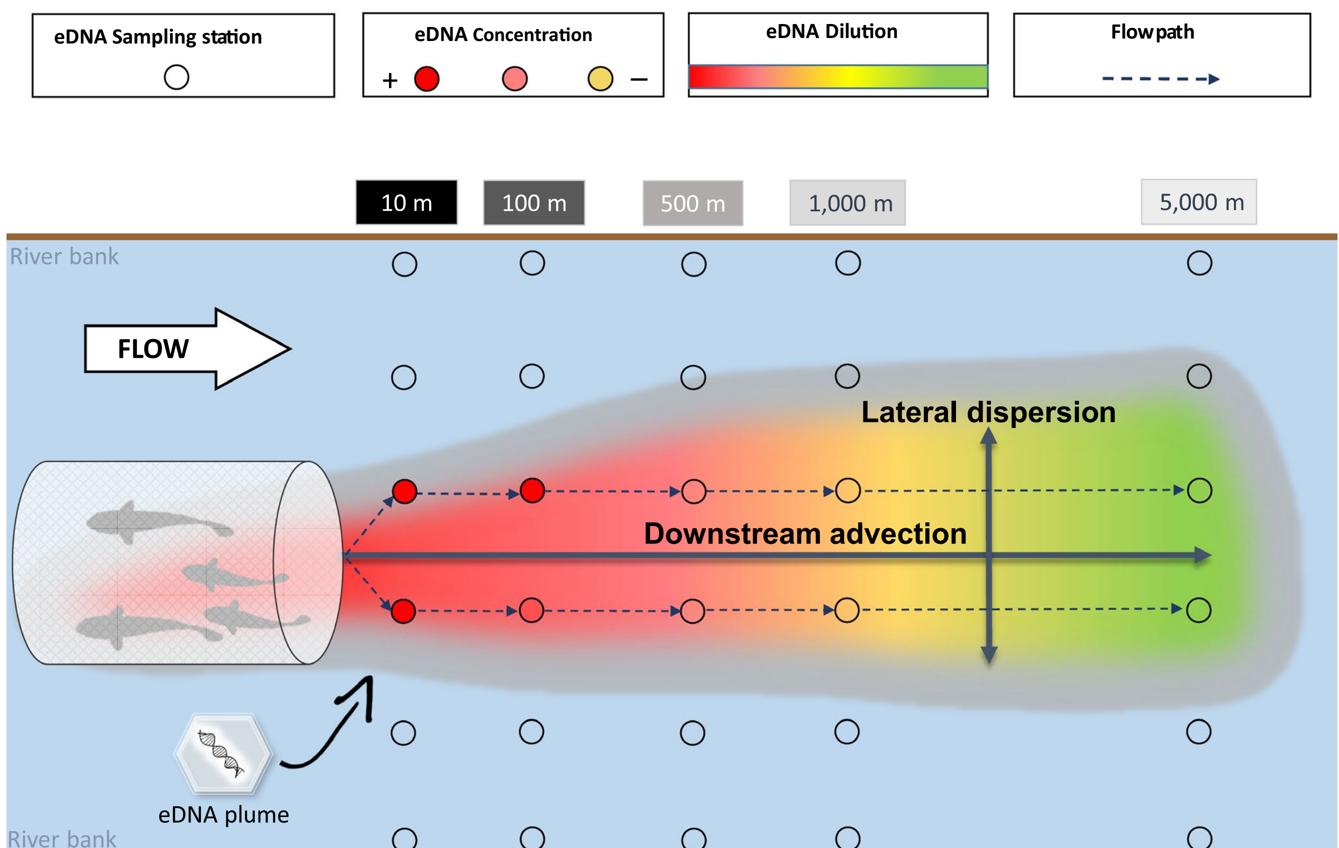

FIGURE 1 Schematic aerial view of the experimental setup and the eDNA hydrodynamic transportation model

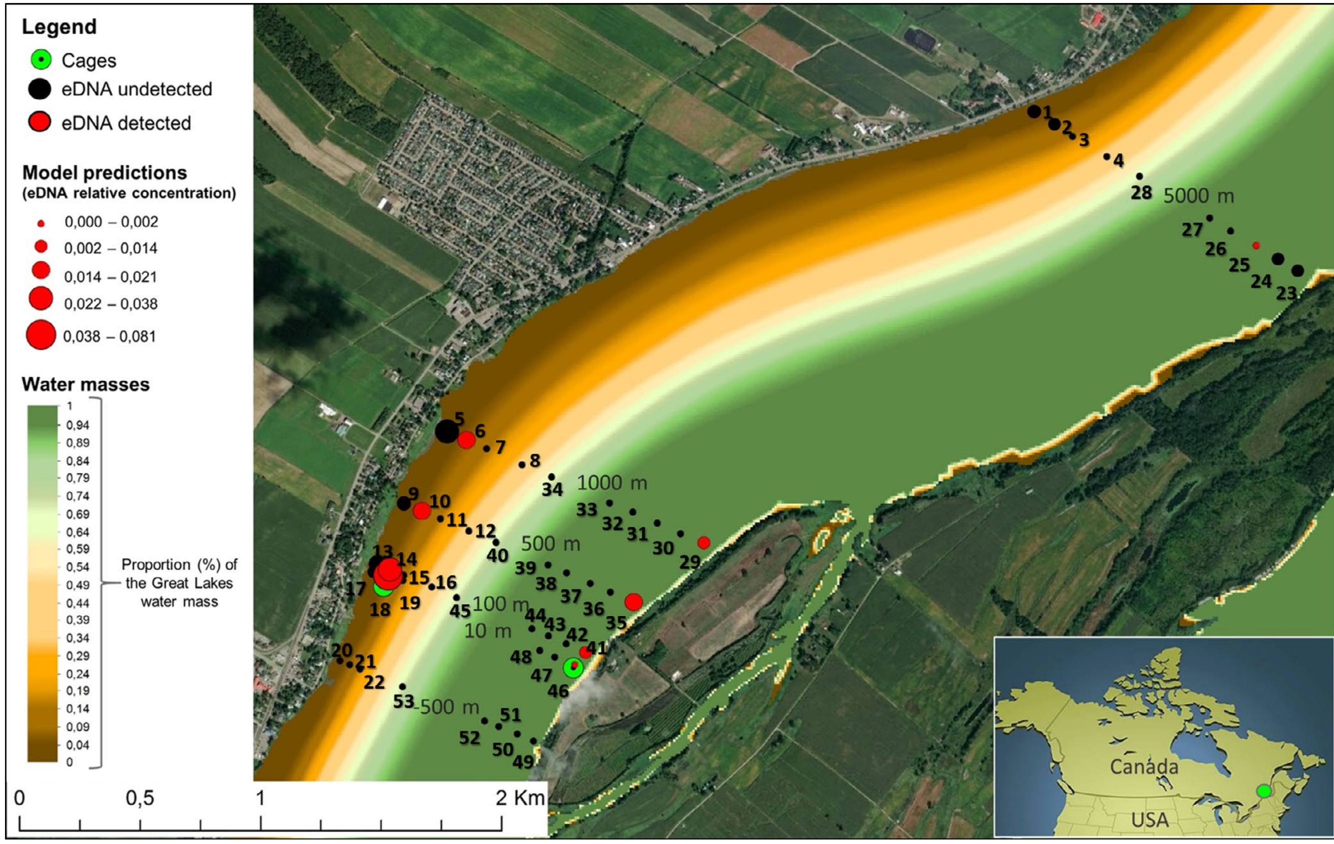

FIGURE 2 Geographic location of sampling sites. Circle size represents the relative concentration (where a value of 1 is attributed to the sources) estimated with the bidimensional hydrodynamic model and the color represents detection of eDNA with qPCR (red for positive and black for negative detection). The proportion from Great Lakes water masses (green) and Ottawa river (brown) estimated from hydrodynamic modeling are also presented. More information on model prediction is available in Figure 3

2.2 | Bidimensional hydrodynamic model

A previously developed high-resolution, bidimensional, time-dependent hydrodynamic model for the St. Lawrence River (Québec, Canada) was used with the objective of simulating eDNA dispersion and dilution (Matte, Secretan, & Morin, 2017). Briefly, topography and friction data were assembled onto mesh composed of triangular elements with a grid resolution that can go down to 1m, which allowed us to account for bidimensional local variations in topography friction and hydrodynamic properties (Matte et al., 2017). The hydrodynamic model uses the conservative H2D2 scheme (H2D2 CNN) for eDNA tracking, which allows the conservation of all properties of the tracked element. Hydrological conditions (flows and levels) during the time of sampling were inputted into the hydrodynamic model as boundary conditions and forcing since temporal environmental conditions can affect model results. The water levels along several locations of the river are measured by the Department of Fisheries and Ocean Canada (DFO) and by the Water Survey of Canada and are available at https://www.waterlevels.gc.ca/. Moreover, the discharge measurements are provided by the Quebec ministry of “Environnement and Lutte Contre les Changements Climatiques”, Water Survey of Canada and Hydro-Québec and are available at https://eau.ec.gc.ca/search/real_time_f.html. To note, semi-diurnal water-level fluctuation is less than 0.02m and there is no tidal flow reversal in this area (Morin & Bouchard, 2000). Model predictions take the form of a relative concentration (between 0 and 1) of eDNA that should pass in each position (here sampling location) from a source (here caged fish). Further details of model setup, calibration, and validation are presented in previous studies (Frenette, Arts, Morin, Gratton, & Martin, 2006; Martin, Frenette, & Morin, 2005; Matte et al., 2017; Morin et al.., 2003; Ouellet, Secretan, St-Hilaire, & Morin, 2014).

2.2.1 | eDNA extractions

To reduce potential laboratory contamination, procedures for eDNA extraction from filters, qPCR preparation, and cycling were all performed in three different rooms. Bench spaces were bleached, and UV treated prior to proceed to extraction batch. Equipment and materials were sterilized, and UV treated prior to proceed to the extraction batch. DNA was extracted using the QIAshredder and DNeasy Blood and Tissue kit (Qiagen, Inc.) method from Golberg, Pilliod, Arkle, and Waits (2011) adapted for glass microfiber filters. To isolate eDNA, 500 µl of ATL buffer and 50µl of Proteinase K (Qiagen) were added to the tubes containing the filters and the extraction control

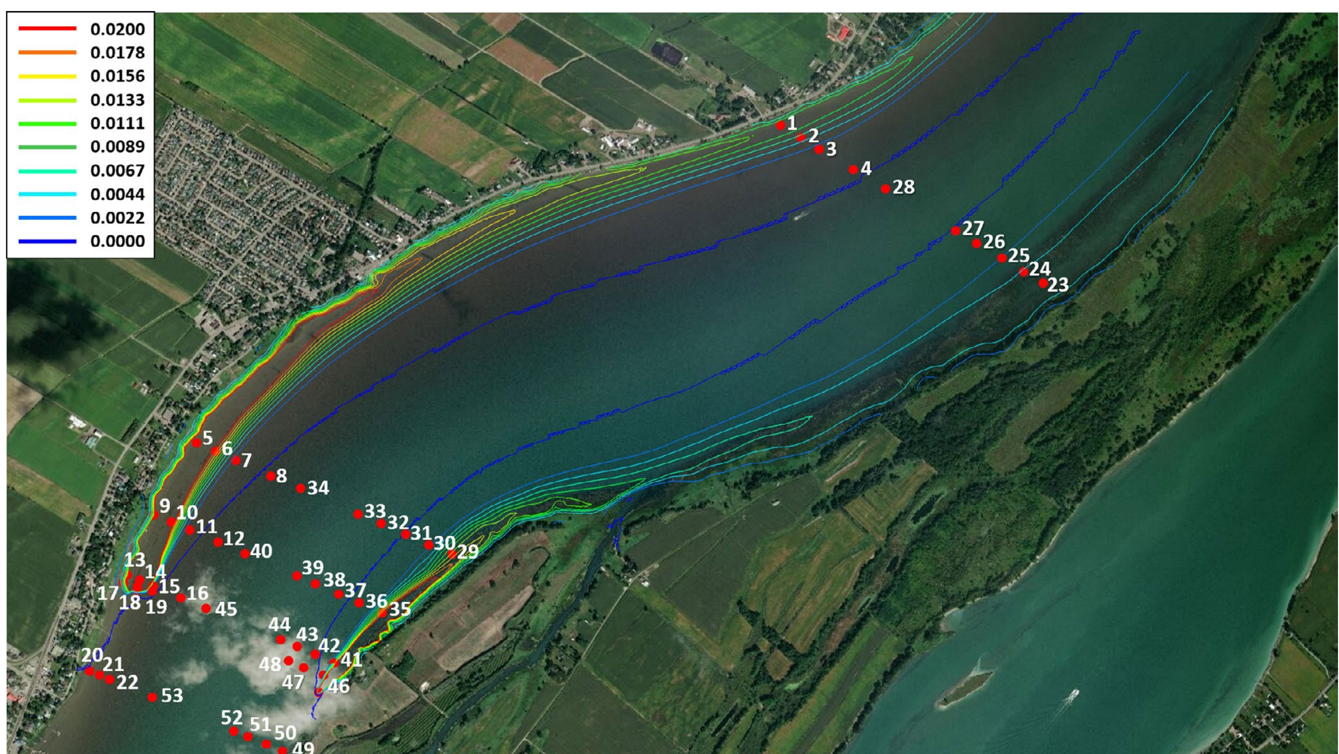

FIGURE 3 Mapping of the model predictions using isoline. Each color represents a relative level of concentration from the sources (i.e., the two fish-caged, which have a relative concentration of 1.000). The red points are the sampling sites

tubes. One extraction negative control tube was prepared for each extraction batch of 11 filters each on average. Tubes were vortexed and incubated at 56°C overnight. Filters and lysis solution were centrifuged at 19,000*g* and equally separated in four tubes. A total of 400 µl of AL buffer were added to each tube, vortexed, and incubated at 70°C for 10 min. Then 400 µl of 95% EtOH was added to each tube and the liquid was vortexed. All the liquid was transferred to a DNeasy Mini spin column (Qiagen) and centrifuged at 19,000 *g*. The spin column was washed with 500 µl of AW1 buffer and centrifuged 1 min at 19,000 *g* followed by a last washing with 500 µl of AW2 buffer and centrifugation 1 min at 19,000 *g*. Purified DNA was eluted in 80 µl of nuclease-free water heated to 37°C, incubated 10 min at room temperature, and centrifuged 1 min at 19,000 *g*. The extracted eDNA was then frozen at −20°C until amplification.

2.3 | eDNA samples analyses by qPCR method

The qPCR assay specific to *S. trutta* was designed in our laboratory and the following primer sequences were used to detect eDNA with the qPCR method: forward primer 5′ GCTTCTGACTCCTCCCTCCG 3′, reverse primer 5′ AAGTGGAGTTTGATATTGGGAGATG 3′, probe 5′ FAM-CTAGCAGGTAATCTTGCC-MGB 3′ (Hernandez et al., submitted). In brief, this assay was tested by searching possible nonspecific oligonucleotide hybridization using multiple alignments of the target species DNA sequences with sequences of related species available in online DNA databases and by predicting probe performance. They were also tested by amplifying tissue-derived DNA from related salmonid species representing five different genera (*Salmo salar*, *Salvelinus fontinalis*, *Salvelinus namaycush*, *Salvelinus alpinus*, *Coregonus artedi*, *Coregonus clupeaformis*, *Prosopium cylindraceum*, *Oncorhynchus clarkii*, *Oncorhynchus nerka*, *Oncorhynchus tshawytsha*, and *Oncorhynchus kisutch*).

To detect and quantify the collected eDNA samples, the TaqMan qPCR method was used with the addition of SPUD assay to the reaction as well as a standard curve. Brown trout eDNA detection was performed for each eDNA sample and extraction control. The SPUD assay (Sigma) is used as an internal positive control to evaluate the efficiency of reaction and to identify the presence of inhibitors in the samples. Amplification was performed in a final volume reaction of 20 µl including 1.8 µl of each primer (10 µM), 0.5 µl of probe (10 µM), 10 µl of Environmental Master Mix 2.0 (Life Technologies), 3.9 µl of SPUD, and 2 µl of DNA following these conditions: 2 min at 50°C, 10 min at 95°C, 50 cycles of 15 s at 95°C and 60 s at 60°C. Six technical replicates were processed for each sample. Six negative control wells containing all reagents but no eDNA sample and one positive control well containing all reagent and DNA extracted from Brown trout were included on each qPCR plate.

A synthetic DNA template of 500 base pairs (gBlocks, IDT) was designed from the COI sequence to be used as a standard curve for quantification. Using the gBlocks, the amplification efficiency and the limit of detection for the assay were determined by serial dilution (1,000 to 0.5 molecule/µl; 10 technical replicates) (Forootan et al., 2017). All positive amplifications detected were sequenced to eliminate the potential

PCR artefacts. Finally, all qPCR results were quantified using a standard curve of known synthetic DNA quantities. The standard curve was created using 5-point serial dilution (200,000 to 20 molecules per reaction) of the synthetic DNA template. The latter allowed us to quantify positive PCR amplification in terms of number of molecules.

2.4 | Statistical analyses

Probabilities of detection were tested via logistic regressions ("glmm" function from the "glmm" R package, Knudson, 2018; R v3.6.0 software, R core Team, www.r-project.com) with; (a) the fixed variables "predicted eDNA concentration" (obtained from the bidimensional hydrodynamic model, which quantifies downstream advection and lateral dispersion from the source), and (b) the "downstream distance" from the source (10, 50, 100, 1,000, and 5,000 m) in order to explain presence/absence of Brown trout eDNA in our qPCR analyses. Because sampling distances were not equally distributed, we applied a logarithmic transformation on the variable "downstream distance." We considered both eDNA sources as independent experiments, thus the variables "water mass" (Ottawa River or Great Lakes) and "station" were added as random effect. In order to evaluate how the probability of detection was affected when resampling, we merged day of sampling (when possible) and re-run the logistic regressions with only "water mass" as random effect. For this last analysis, the variable "relative predicted eDNA concentration" obtained by hydrodynamic modeling was also averaged among samples for each station and the detection of eDNA in a single sample of a given site was considered as a presence of eDNA for that station. Finally, we tested for the fixed effect of "predicted eDNA concentration" and "downstream distance" on the quantity of eDNA obtained from qPCR analyses. Only cases of detection were considered in the analysis in order to remove the excess of 0 in the dataset. Indeed, an excess of no detections by qPCR analyses combined with several predictions of no eDNA concentration by the model would inflate the correlation between those two variables (Legendre & Legendre, 1998). In this way, we ran a two-way mixed model analysis of variance (ANOVA) with "predicted eDNA concentration" and "downstream distance" as fixed effects, and "water masses" and "qPCR run within a sample" (which will control for the variation of the six qPCR replicated for a given sample) included as random effects ("lme" function from "nlme" R package, Pinheiro, Bates, DebRoy, & Sarkar, 2015). Datasets and R code are available in Appendices S2–S4.

3 | RESULTS

3.1 | Hydrodynamic bidimensional model predictions for eDNA detections

The bidimensional hydrodynamic model predicted that eDNA dispersion should follow a pattern of flow in direct line from the source with low lateral mixing (Figures 2 and 3). Indeed, predicted concentrations were higher 5,000 m downstream in a direct line than at sites located 10 and 100 m from the cages but positioned laterally from the cages. These predicted dispersion plumes showed similar trends in both green and brown water masses, whereby plumes dispersed in direct line from the source, suggesting that the physicochemical factors differentiating these water masses do not have a major influence on the lateral dispersion of the plumes (Figures 2 and 3). Moreover, low lateral dispersion is expected in this area of St. Lawrence River and the model predicted that eDNA originating from both cages will not cross water masses after 5,000 m of downstream dispersion (Figure 3).

3.2 | eDNA detection and associations with distances and model predictions

The Brown trout specific assay showed an amplification efficiency of 98.7%, a limit of detection of 20 mtDNA copies/rxn (with >95% amplification success), and no DNA amplification from the 12 related species (Hernandez et al. submitted). Therefore, we are confident that detection in the wild will be specific to the species. Using this qPCR assay, Brown trout eDNA was detected at all distances downstream of the cages and it was mostly detected at the stations located near of the shore in shallower waters (Figure 2). No detection was observed in the eight negative controls (*i.e.*, −500 m), confirming that the caged fish are the only sources of Brown trout eDNA in this study. More precisely, nine out of the 45 sites tested showed positive detections (Figure 2). As the model predicted, eDNA detection also appeared to follow a flow in direct line from the source (Figure 2). All controls tested were negative, standard curves had $R^2 > .99$ and an average efficiency of 91% and no qPCR inhibition was observed. Results of sequencing of all positive detections confirmed the amplification of *S. trutta* DNA.

Significant logistic regressions explained detection at both "sample" and "station" levels when using the variable "predicted eDNA concentration" (sample level: $z = 4.21$, $p < .001$; station level: $z = 2.77$, $p = .006$; Figure 4a,c). At the sample level, 50% detection probability was reached with a modeled relative particle concentration of 0.025 (Figure 4a) in comparison to 0.017 at the station level, indicating that the partial resampling of our dataset reduced the level of eDNA concentration needed for detection by 1.5 (Figure 4c). In contrast, the logistic regressions were not significant when using the "downstream distance" variable to explain detection at both "samples" and "sites" levels (Figure 4b,d). Finally, amounts of eDNA that were quantified by qPCR quantities were significantly explained by the variable "relative predicted eDNA concentration" ($F = 14.53$, $p < .001$), but not by "downstream distance" ($F = 0.25$, $p = .624$; Table 1).

4 | DISCUSSION

In this study, we used a specific qPCR assay to detect the presence and estimate the quantity of eDNA from two cages containing Brown trout in one of the largest rivers in the world. A total of 53 stations

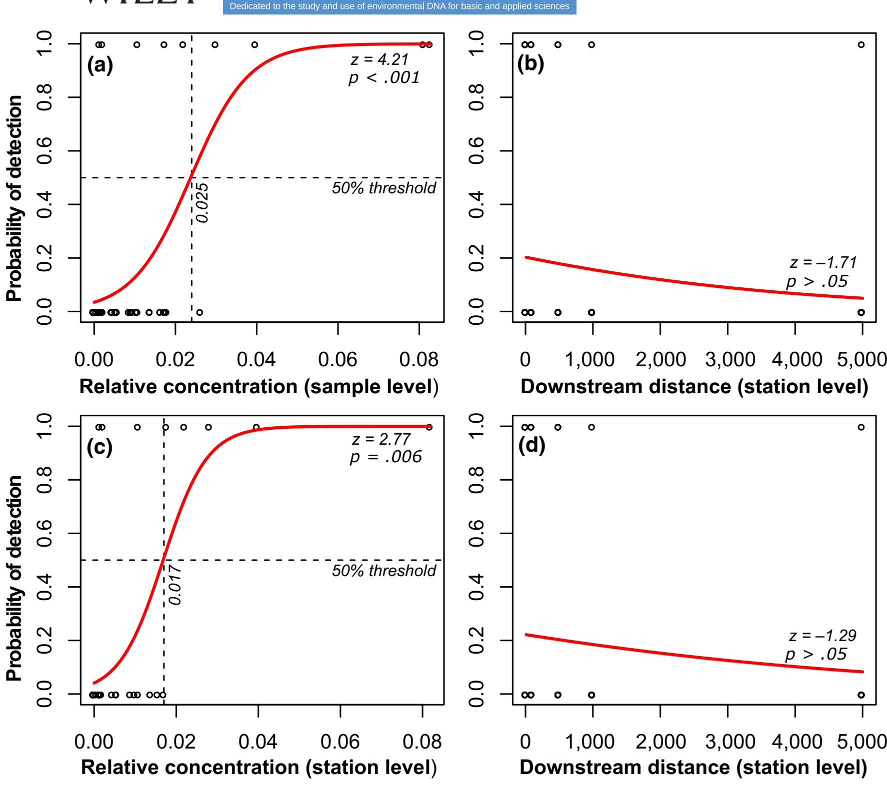

FIGURE 4 Logistic regressions estimating probabilities of eDNA detection with (a, c) bidimensional hydrodynamic model predictions and (b, d) downstream distance from eDNA sources. A and B were tested at sample level (all samples where used in the analyses), correction for random effect of "station" and "water masses," while "c" and "d" were tested at station level (with up to two temporal samples merged within a given station) with only a correction for random effect of water masses

TABLE 1 Fixed effects of bidimensional hydrodynamic model predictions and distance on quantitative estimation of eDNA from qPCR analyses tested with a two-way mixed model analysis of variance (ANOVA) with "water masses" and "qPCR run" as random effects

	DFs	*F*-value	*p*-value
Intercept	1, 28	22.02	<.001
Model predictions	1, 28	14.53	<.001
Distance	1, 28	0.25	.624

Abbreviation: DFs, numerator and denominator degrees of freedom.

(including eight upstream control) were sampled up to 2 days in two different water masses and at six distances from the caged fish (−500, 10, 100, 500, 1,000, and 5,000 m). Because of a low lateral dispersion, we found that downstream distances from the source cannot explain the probabilities to detect or estimate quantities of eDNA when large transversal sampling transects are used. On the other hand, bidimensional hydrodynamic model quantifying downstream advection and lateral dispersion showed significant relationships with both detection and quantities of eDNA. These results underline the importance of quantifying both downstream advection and lateral dispersion in large river systems in eDNA studies, contributing to the understanding of precise eDNA distribution and abundance estimation in such systems.

4.1 | Applying Brown trout assay for eDNA studies

Genetic resources (*e.g.*, microsatellites primer pairs, genome…) have been historically used as nonintrusive tools (*i.e.*, without the need to kill specimen) to study different evolutionary and ecological processes (*e.g.*, population structure, effective migration, natural selection…). This is particularly true for the eDNA analysis sequencing that goes further in this direction and for which it is not needed to capture and potentially injure specimens. Indeed, sampling aquatic resources with conventional tools (*e.g.*, gill nets) is not species specific and individuals from vulnerable species can be killed and wounded even though they are not specifically targeted during a sampling campaign. Moreover, analysis of eDNA samples can facilitate the detection of species that are difficult to monitor with conventional methods (Andruszkiewicz, Sassoubre, & Boehm, 2017; Bohmann et al., 2014; Pikitch, 2018). The Brown trout specific assay was applied to eDNA samples from sites where the target species was absent (eight upstream controls) and sites downstream from the cages. Here, we showed parallel positive detection up to 5,000 m from sources containing approximatively 28 kg of fish biomass.

We demonstrated the reliability of this assay specifically developed to detect and quantify Brown trout eDNA in the wild. Carim et al. (2016) have previously published a qPCR assay specific to *S. trutta* that produced no amplification on 17 related species found in Western North America and demonstrated positive amplification on eDNA samples. However, because the amplicon was too short to allow verification of the positive amplification by Sanger sequencing, we used the assay of Hernandez et al. (submitted) designed in our laboratory, which was also tested with 12 related species found in Eastern Canada. Besides its use in the present study, this assay will allow to track the geographic expansion of Brown trout, a non-native species introduced into several rivers in Québec since 1890 and where the species now breeds naturally.

4.2 | Weak widthwise diffusion affects eDNA dispersion

Waterflow in river is affected by bidimensional water mixing (downstream lateral dispersion), which can result in a homogeneous widthwise diffusion (Sun, Wells, Bailey, & Anderson, 2013). The longest distances reported for eDNA detection from its source in a river are 12.3 km for an invertebrate (Deiner & Altermatt, 2014; *Daphnia longispina*) and up to 60 km for a fish (Pont et al., 2018; *Coregonus lavaretus*), but the processes of eDNA lateral dispersion during downstream dispersion in large river are poorly documented. In a case of strong lateral dispersion, widthwise complete mixing of eDNA will occur fast and downstream distances alone should show high association with the detection and the quantities of eDNA. Past studies found variable results on downstream eDNA dispersion which goes from a good association between the distance downstream of source and eDNA quantities (Nukazawa, Hamasuna, & Suzuki, 2018; Tillotson et al., 2018) to no significant decreasing concentrations with distance up to 9 km (Sansom & Sassoubre, 2017; Wacker et al., 2019). Here, our results show the importance of hydrodynamic modeling to follow eDNA dispersion, particularly on large rivers and can explain, at least partly, those previous results of no significant association between distance and eDNA quantities.

Here, our eDNA detection in direct line to the eDNA source suggests that lateral dispersion is weak up to 5,000 m downstream, which is supported by the previously published hydrodynamic bidimensional model. More precisely, the model simulation shows that higher relative concentration is expected up to 5,000 m of distance in direct line from its source in comparison to sites not in direct line, no matter their distance from the source. The model also predicted that the eDNA from the two cages did not merge at the center of the river after 5,000 m of downstream dispersion (Figure 3), indicating that no eDNA from a fish present near one river bank will be found on the other river bank, at least up to 5,000 m from its source of origin. Admittedly, it is possible that eDNA mixing could occur between the two water masses after those 5,000 m if the rate of eDNA decay in both environmental conditions is lower than the rate of water masses mixing. Furthermore, in the Great Lakes water masses, sampling stations in 10–100 m downstream of caged fish showed lower eDNA concentrations than some stations located along the riverbank 500–1,000 m downstream (Figures 1 and 2). Indeed, the model predicted that most of eDNA will disperse near the riverbank and not in the closest stations at 10 and 100 m of distance that are not exactly downstream in the flow in direct line trajectory (Figure 2). Thus, eDNA samples collected at stations located 500 and 1,000 m downstream of cages were predicted to have higher concentrations since they are in direct line with the cages. This explains why eDNA predictions from this model showed a significant association with eDNA detection and quantities obtained from qPCR analyses, while distance explained neither eDNA detection nor quantities. Moreover, the proximity of the source to the riverbank could explain why eDNA concentration predictions are lower in one of the water masses at 5,000 m of distance, given that the riverbank can somehow "retain" the eDNA plume because of its much slower water velocity. Together, this supports the importance of considering bidimensional dispersion to more accurately predict upstream eDNA distribution and abundance of aquatic species in large rivers such as the St. Lawrence River. For example, hydraulic processes can influence how many samples should be included in an experimental design, as well as their position. In the case of the St. Lawrence River, the weak lateral dispersion between and within water masses makes it possible to document species habitat preference in this system, for instance for documenting differences in community composition between "green" and "brown" waters, or between littoral and "offshore" habitats using eDNA metabarcoding (Berger et al., submitted). Moreover, eDNA surveys of endangered and invasive species in large river systems should also benefit from this study. More particularly, the use of this eDNA 2D dispersion model could help predicting the localization of invasive (*e.g.*, Grass carp: *Ctenopharyngodon idella* and Round goby: *Neogobius melanostomus*; Paradis, 2018) or endemic (Copper redhorse: *Moxostoma hubbsi*, Froese & Pauly, 2019) species that are present in the St. Lawrence River, and consequently, it would help defining actions to be taken for the monitoring of these species. Admittedly, the model used in this study does not currently take in consideration decay and settling factors that could have an impact on the eDNA transport in this system. Physicochemical factors such as UV, temperature, and/or turbulence contribute to DNA degradation and affect the eDNA persistence (Barnes et al., 2014; Li et al., 2019). Decay and settling factors could thus differ between the two water masses. In addition, a recent study on modeling environmental DNA transport in marine coastal areas found a higher impact of decay and settling on the eDNA transport comparatively to the mixing effect (Andruszkiewicz et al., 2019). Therefore, integration of decay and settling rates to the present model should allow a better estimation of downstream localization of eDNA sources, as previously showed in smaller rivers (Carraro et al., 2018; Sansom & Sassoubre, 2017; Shogren et al., 2017). We are currently performing experimental work to address this issue for the St. Lawrence River system (Caza-Allard et al. in preparation).

4.3 | Effect of multiple sampling on eDNA detection probability

We took advantage that 20 stations were resampled twice in two consecutive days to see how resampling could impact the probability of eDNA detection. Temporal resampling permitted 50% of detection for samples that had 1.5 times lower eDNA concentrations compared with one-day sampling. This supports the idea that temporal resampling provides better estimates of species distribution in large rivers such as the St. Lawrence River. Indeed, eDNA sampling is a punctual event and probabilities to miss low concentrations of eDNA molecules are non-negligible, as observed both by Wilcox et al. (2016) and Baldigo, Sporn, Geaorge, and Ball (2017) in their studies on salmonids. In addition, it has been shown that vertical distribution of eDNA in the water column depends on the behavior

of eDNA, the latter being dependent on its state (e.g., intra- or extra-membranous, particulate, free/ dissolved) and physicochemical characteristics of the environment (Murakami et al., 2019). Thus, a more thorough estimate of eDNA distribution in the St. Lawrence River should be achieved by performing a vertical water sampling at multiple depths in future studies. Thus, despite associated costs, we suggest that having repeated field replicates taken at the same stations would improve the precision of estimates of abundance and distribution in rivers.

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CONFLICT OF INTEREST

None declared.

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SUPPORTING INFORMATION

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

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