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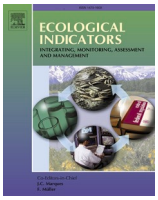

Cage transplant experiment shows weak transport effect on relative abundance of fish community composition as revealed by eDNA metabarcoding

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ABSTRACT

Protection of freshwater fish diversity is a global conservation priority in face of its alarming decline in the last decades. A crucial step to protect freshwater fish diversity is the production of prompt and precise evaluation of community composition and spatial distribution. Metabarcoding of environmental DNA (eDNA metabarcoding) generally surpasses traditional methods for documenting diversity and community composition in aquatic environments. Nevertheless, empirical evidence evaluating how eDNA transportation in water affect community composition and structure via eDNA metabarcoding data remains scarce. Using a brown trout (*Salmo trutta*) cage transplant experiment in the St. Lawrence River (Canada), a large fluvial system, we tested the detection and relative abundance of species' eDNA along 15 sampling locations. We detected brown trout eDNA in five localities up to 5,000 m from the cage, but only one sampling location situated 10 m downstream and in the direct line of the cage was affected at the community composition level. This locality showed a relative abundance of brown trout eDNA of 13.1%, while the four others showed a relative abundance under 1.0%. K-means cluster analysis confirmed the impact of brown trout eDNA on community composition by separating this locality from all others. Based on species loading of a redundancy analysis, we showed that this different k-means group was associated with the high relative abundance of brown trout. No evidence of transport effect of brown trout eDNA on fish community composition was observed in any other sampling locations. Together, our results support the view that eDNA metabarcoding can be both a conveyor belt of biodiversity information and a precise tool to study the composition and structure of fish communities in river.

1. Introduction

Protection of freshwater fish diversity is a high conservation priority considering its alarming decline of the last decades (Dias et al., 2017; Dudgeon et al., 2006; Vörösmarty et al., 2010; WWF, 2021). A crucial step in freshwater fish conservation is the prompt and precise evaluation of community composition and spatial distribution. Fish community assesments also lead to a better understanding of aquatic ecosystems and more efficient protection habitat. The analysis of environmental DNA (i.e., DNA collected in environmental samples that are expelled from organisms) has shown promising results in lentic environments, generally outperforming traditional methods to document the species composition of fish communities (Hänfling et al., 2016; Handley et al., 2019; Harper et al., 2018; Boivin-Delisle et al., 2021; Czeglédi et al., 2021). Thus, this non-invasive and less labour-intensive approach to describe fish community structure is increasingly considered in conservation and biomonitoring. Moreover, a single traditional method (i.e., electro-fishing, gill nets, seine, or scuba diving) cannot be used to sample all habitats in a large lotic environment (Casselman et al., 1990). The use of eDNA metabarcoding (i.e., eDNA methods based on multispecies DNA amplification with massive parallel amplicon sequencing) can solve the problem of describing the structure of fish communities in lotic ecosystems via a single method. To this end, the effect of eDNA transportation in lotic environments on the structure of fish communities need to be better understood (Goldberg et al., 2016).

Environmental DNA of aquatic organisms (including fishes) can be transported on several kilometers (km) in river (Deiner and Altermatt, 2014; Jane et al., 2015; Carraro et al., 2018; Pont et al., 2018; Laporte

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et al., 2020). Those upstream sources of eDNA can then affect the observed fish community composition at downstream sites. In parallel, spatial variation of fish community composition has been demonstrated in rivers with metabarcoding eDNA analysis (Pont et al., 2018; Berger et al., 2020; García-Machado et al., 2022; Laporte et al., 2021; Hallam et al., 2021; Cantera et al., in press). These contradictory observations are not necessarily mutually exclusive if eDNA dilution diminishes the impact of transport at the level of relative abundance in the community composition. Indeed, one can expect that the more distant the source of fish eDNA is from a given sampling location, the more it will result in a weak relative abundance of sequences and, hence, a decreased impact on the overall observed composition of the fish community (Laporte et al., 2021; Cantera et al., in press). Generally speaking, empirical evidence allowing a better understanding of why fish community composition and structure could be coherent despite eDNA transportation from upstream are lacking and are necessary to develop this ecological indicator of community composition in rivers.

Here, using a cage experiment containing high biomass (27.9 kg) of an exogenous species, the brown trout (*Salmo trutta*), we specifically test the effect of eDNA transport on the fish community composition using 15 samples distanced between 10 and 5000 m downstream of the cage via metabarcoding analyses. More precisely, we first test for brown trout eDNA detection and its relative eDNA abundance for each sample. We predict that detection of brown trout eDNA should be observed as far as 5 km downstream as already observed using a qPCR approach in a previous study (Laporte et al., 2020), but that relative proportion of brown trout eDNA in metabarcoding analyses will decrease from the source via dispersion and dilution. Then, using k-means clustering, we study community composition and identify species that differentiate fish composition in relative abundance using an ordination approach (redundancy analysis; RDA). Localities associated to brown trout eDNA relative abundance will be considered as impacted by eDNA transportation.

2. Material and methods

2.1. Localities, sampling, and eDNA dataset

A caging experiment was conducted in September 2017 to estimate DNA dispersion in the St. Lawrence River in the Contrecoeur sector (approximately 50 km downstream of Montréal, Québec, Canada; Table 1). Contrecoeur sector have a mean depth of 3.6 m SD ± 2.8 and a mean turbidity of 4.4 NTU SD ± 1.3 with about 60 fish species inhabiting the area (Berger et al., 2020).The complete experimental setup is described in Berger et al. (2020) and Laporte et al. (2020). The cage, measuring 10 m long by 2 m in diameter made of 1.9 cm knotless mesh, was positioned parallel to the riverbank at the bottom (approximately 2.5 m). A total of 50 brown trout (*Salmo trutta*) weighting 27.9 kg were kept in the cage. Based on a 20-years sampling campaign in this river section using gill nets and seines, brown trout are considered absent of this area (Mingelbier et al., 2016). Moreover, no detection of the species in upstream sampling sites from the cage was observed using qPCR methodology during the time of the experimentation (Laporte et al., 2020). We used the dataset from Berger et al. (2020) (that excluded brown trout DNA sequences for their analyses) with the following differences. First, we kept *S. trutta* sequences in the database. Second, to ensure that fish were not under stress caused by their manipulation, we only selected samples that were collected the 15th September (i.e., three days after the installation of the cage). The final dataset comprised 15 samples at five longitudinal distances from the source (10 m, 100 m, 500 m, 1000 m, and 5000 m) localized in three latitudinal positions distanced at approximatively 100 m (Table 1; Fig. 1; Supplementary Materials 1). Briefly, a 250 ml integrated water column sample (from bottom to top; range [0.5 – 8 m]) was taken from a boat for each sample in addition to a total of 14 negative controls. For conservative purpose, all negative controls from Berger et al. (2020) were kept here. Negative controls were distilled water samples treated with the same field and laboratory protocols as the real samples (overall, <100 sequences were detected (0.00083%), suggesting a low level of contamination). Water filtrations on 1.2 µm glass microfiber (Whatman, 25 mm) were performed on the field using single-use syringes (previously bleached, sterilized and UV treated). DNA extraction was performed using a QIAshredder and DNeasy Blood and Tissue kit (Qiagen) according to a previously developed protocol, all performed under a UV hood with bleached and UV-treated instruments (Goldberg et al., 2011; Spens et al., 2017). DNA amplification used MiFish primers, targeting a hypervariable region of the 12S rRNA gene (174 bp) (Miya et al., 2020). Sequencing was performed at the platform of the Institut de Biologie Intégrative et des Système (IBIS) at Université Laval, Québec (https://www.ibis.ulaval.ca), using Illumina MiSeq sequencer and the MiSeq Reagent Kit V3 with paired end 300 bp reads (Illumina). Finally, the 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 (https://www.github.com/enormandeau/barque; Mathon et al., 2021). Detailed settings for sequence analysis can be found on the GitHub webpage. See Berger et al. (2020) for further details on sampling methodology, DNA extraction, sequencing, and bioinformatic analyses.

Table 1
Geographic coordinates of the 15 sampling locations, distances from the source and bidimensional hydrodynamic model preditions (concentration after dispersion and dilution where simulated) for September 14, 2017.

Station	Lattitude	Longitude	Distance	Model predictions
1	45.814479	−73.369238	10	0.0087
2	45.814337	−73.368496	10	0.0811
3	45.814167	−73.367692	10	0.0001
4	45.814777	−73.369037	100	0.0139
5	45.814580	−73.368378	100	0.0398
6	45.814354	−73.367647	100	0.0019
7	45.817031	−73.367606	500	0.0109
8	45.816753	−73.366675	500	0.0221
9	45.816455	−73.365686	500	0.0007
10	45.819721	−73.365320	1000	0.0263
11	45.819403	−73.364293	1000	0.0164
12	45.819065	−73.363209	1000	0.0014
13	45.831601	−73.333972	5000	0.0096
14	45.831137	−73.332878	5000	0.0054
15	45.830690	−73.331904	5000	0.0014

2.2. Statistical analyses

First, we transformed raw numbers of sequenced eDNA in relative abundance to illustrate detection and relative proportion of brown trout eDNA along our transect. Then, we test for an association between both, raw number of sequences and average relative proportion of brown trout eDNA with the prediction of a high resolution, bidimensional, time-dependant hydrodynamic model simulating dispersion and dilution in the St. Lawrence River (see Matte et al., 2017 for a description of the model). This model has recently been used to predict patterns of eDNA detection and concentration in this study system (Laporte et al., 2020). For further investigation, a Hellinger transformation was performed on the raw eDNA metabarcoding dataset as detailed by Laporte et al. (2021). Hellinger transformation is considered the gold standard to study species abundance in ordinate analyses (Legendre and Gallagher, 2001; Legendre and Legendre, 2012). This transformation is the square root of relative abundance that results in a Hellinger distance when transformed data are projected in ordination analyses based on Euclidean distance (e.g. principal component or redundancy analysis). It corrects for the 'double 0 problem' when assessing similarities among sampling sites, which is caused by the fact that species have unimodal distributions along a gradient of environmental conditions and are

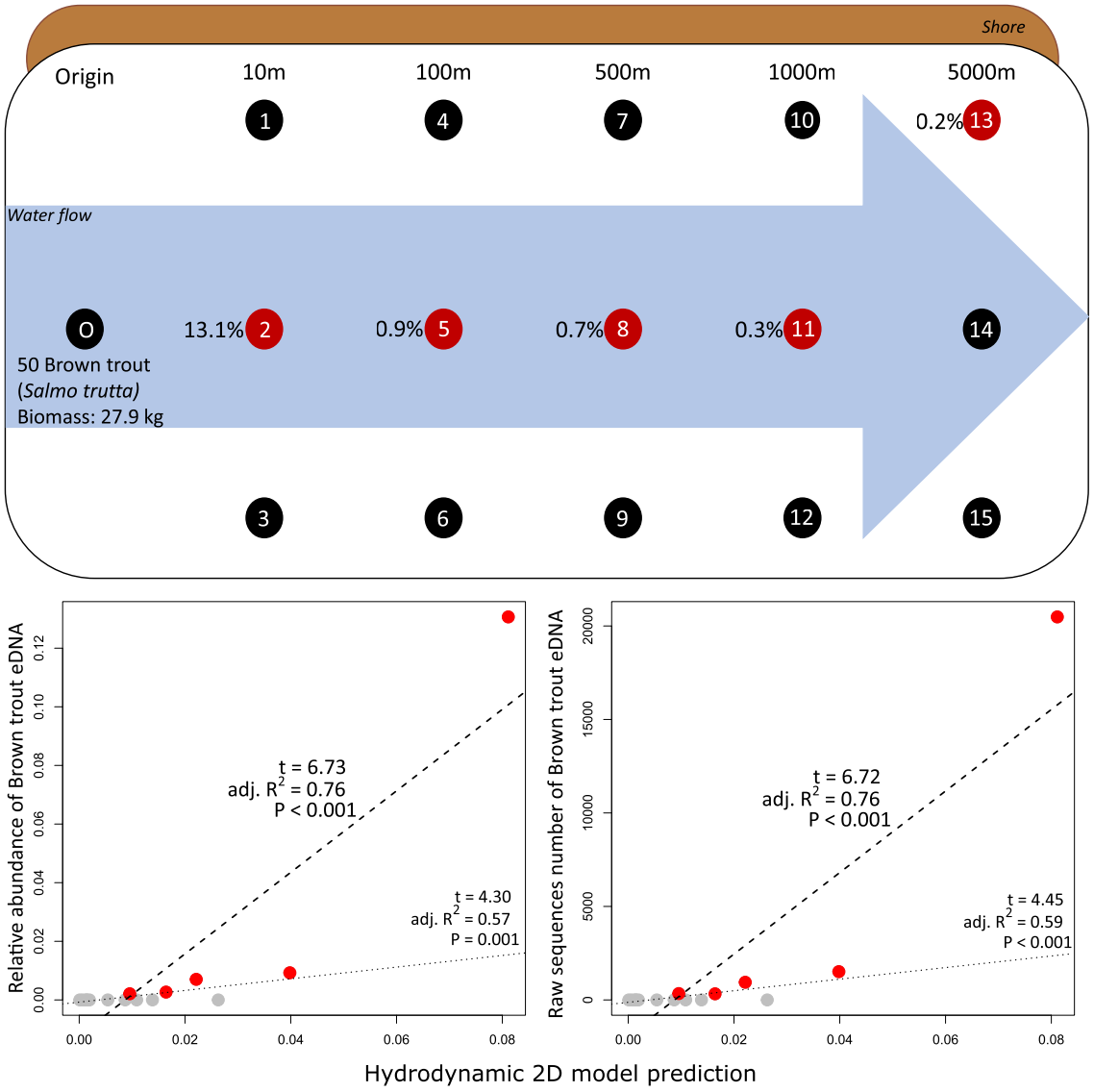

Fig. 1. Top: Schematic representation of sampling locations. Black and red points represent respectively localities without and with brown trout eDNA detection. Percentage before red point corresponds to the relative proportion of brown trout eDNA in the fish community. Bottom: Linear regressions where hydrodynamic 2D model explain relative abundance (left) and raw number sequences (right) of eDNA brown trout. Dashed lines represent full datatset while doted lines represent dataset without the sample positioned at the top-right in the graph to ensure significativity of relationships without this point.

absent from sites that differ in their environmental conditions (ter Braak and Prentice, 1988). Following the Hellinger transformation, we conducted a k-means/redundancy analyses (RDA) procedure for the identification and the description of community structure as presented by Laporte et al. (2021). The 'kmeans' function in R software v3.6 (R core Team, 2018) was used with 1,000 iterations, to perform K-means analyses (Hartigan-Wong algorithm) to identify groups of sites sharing similar species community (Legendre and Legendre, 2012). The number of groups tested was between 2 and 10 (K = 2 to 10). The optimal number of clusters used for subsequent analyses was estimated based on the "elbow method" using the within-group sum of squares (WSS; Legendre and Legendre, 2012). We then produced an RDA using the function 'rda' with package 'vegan' in R software v3.6 (Oksanen et al., 2019). Response matrix was the Hellinger transformed dataset and the explanatory was the variable 'group' (i.e., association of a site to the groups obtained from the optimal K-means analysis). More specifically, this approach aims at estimating the proportion of eDNA variation that is significantly explained by k-means clustering as well as the identification of species differentiating those groups. To identify which species significantly contribute to differentiate the groups of communities, we used a method allowing to find outliers ($P < 0.1$) in species loadings of each significant RDA-axis, as presented in Forester et al. (2018) and modified by Laporte et al. (2021) for eDNA metabarcoding. Finally, to estimate the effect of eDNA shedding from caged brown trout on fish community composition, we computed and compared Hellinger distances between dataset with and without brown trout eDNA for sample where brown trout eDNA was detected, and compare them to the Hellinger distances of samples within a k-means group and between k-means groups.

3. Results

Five of the 15 sampling sites showed detection of brown trout eDNA, where four of them were in direct line downstream from the source at 10 m, 100 m, 500 m, and 1000 m, while the last one was a detection at 5000 m near the shore (Fig. 1 - top). Relative abundance of brown trout eDNA sequences varied from 13.1% for the site immediately downstream (10 m) of the cage, to<1% for all other sites (Fig. 1 - top). Linear

regression between average relative abundance or raw number of sequences of brown trout eDNA and bidimensional hydrodynamic model predictions was highly significant (both adj. $R^2 = 0.76$, $P < 0.001$; Fig. 1 - bottom). K-means WSS elbow method suggested five clusters (K = 5) and sites with brown trout eDNA did not cluster differently between K = 5 to K = 10. Therefore we chose K = 5 as optimal and kept this structure for the subsequent analyses (Fig. 2). Briefly, in K = 2, a single site located at 5000 m downstream of the cage clustered separately from the rest, at K = 3, four of the five sites close to the shore formed a new cluster and at K = 5, the site at 10 m and in a direct line from caged fish created a new group. These groups remained intact for all remaining clusters tested.

Using clustering based on K = 5 significantly explained 75.9% of eDNA variation with redundancy analysis ($P < 0.001$; Fig. 3). The first RDA axis (51.6% of the variation) separates the 'purple' group (#2 in Fig. 2) from other sampling locations by a higher relative abundance of lake sturgeon (*Acipenser fulvescens*) eDNA, while the second axis (16.0% of the variation) separates mostly the 'brown' group (#3 in Fig. 2) from the rest by its higher relative abundance of northern pike (*Esox lucius*), brown bullhead (*Ameiurus nebulosus*) and the trout-perch (*Percopsis omiscomaycus*) eDNA (Fig. 3; Fig. 4). These two major axes show correspondence with K-means clustering observable at K = 2 and K = 3, respectively (Fig. 2). Interestingly, the 'red' group (#5 in Fig. 2) includes a single site at 10 m from the brown trout cage and is strongly associated with higher brown trout eDNA relative abundance on RDA axes 3 (10.5% of the variation) and 4 (4.6% of the variation) (Fig. 3; Fig. 4). Based on RDA axis 2, 'green' (#1 in Fig. 2) and 'blue' (#4 in Fig. 2) groups show a high relative abundance of eDNA from the shorthead redhorse (*Moxostoma macrolepidotum*) but are differentiated by the higher relative abundance of silver redhorse (*Moxostoma anisurum*) in the 'green' group on RDA axis 4. Finally, the longnose dace (*Rhinichthys cataractae*), the tench (*Tinca tinca*), and the common carp (*Cyprius carpio*) eDNA are also abundant in 'blue' group sites according to the RDA axis 4 (Fig. 3; Fig. 4).

Hellinger distance between dataset with and without brown trout eDNA for site #2 (10 m from the encaged Brown trout; 0.34) was higher than the average of Hellinger distances between two sampling locations within a k-means group (0.31; 95% CI [0.25–0.36]) but lower than the average of Hellinger distances between two sampling locations from different k-means groups (0.51; 95% CI [0.49–0.53]; Fig. 5). In comparison, the average of Hellinger distance between dataset with and without brown trout eDNA for site #5,8,11 and 13 (100 – 5000 m from the encaged brown trout; 0.07; 95% CI [0.05–0.09]) was low (Fig. 5).

4. Discussion

Our results show that 27.9 kg of caged brown trout can be detected as far as 5000 m downstream, but its relative abundance significantly influenced the detected community composition only at the nearest sampling site, located 10 m downstream of the experimental cage. At 100 m downstream its influence on community composition was insignificant, given its low relative abundance (<1.0%). This shows that exogenous eDNA was rapidly diluted following release by the experimental trout, as predicted by the bidimensional hydrodynamic model. Therefore, relative abundance of eDNA metabarcoding seems to provide a local signal of community composition with a resolution between 10 and 100 m in the St. Lawrence River. Altogether, this supports the view that eDNA metabarcoding can be a precise tool for the study of fish community in large rivers.

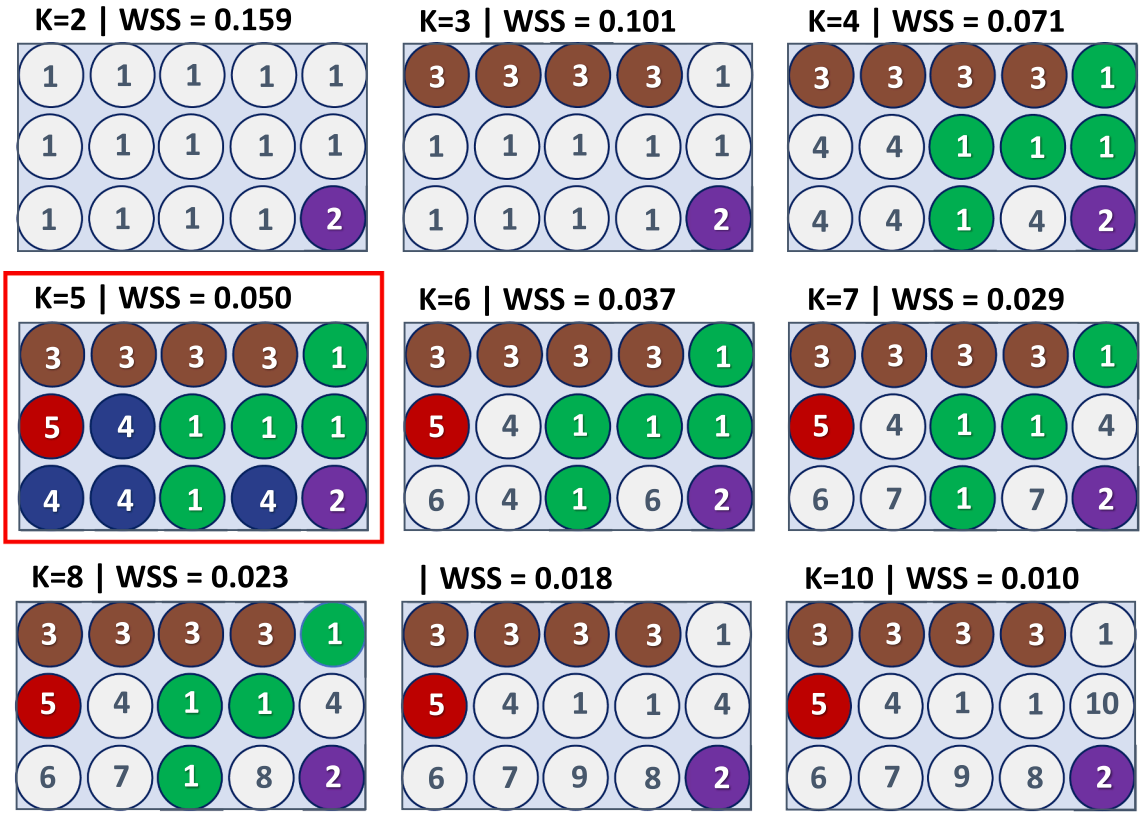

Fig. 2. Visual representation of k-means analyses results for a number of cluster (K) between two and ten. Within sum of square (WSS) have been used to select the most likely number of communities in the area (framed in red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

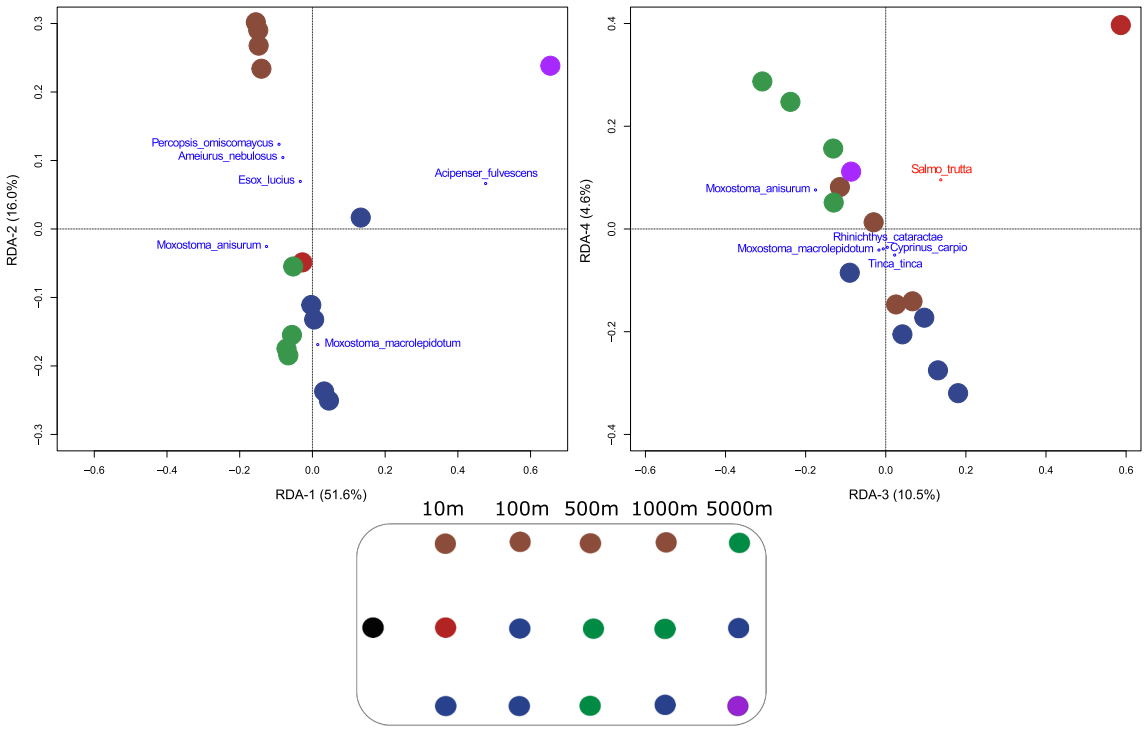

Fig. 3. Biplots of redundancy analyses representing the four RDA axes (left: axes 1 and 2, right: axes 3 and 4). Five communities (colors) were obtained via k-means analysis explained 75.9% of sequence reads variation among sampling localities using the dataset including *Salmo trutta* (brook tout) eDNA sequences. Species with significant loadings differentiating sites are indicated in blue in the exception of *S. trutta* in red. Schematic representation of the spatial repartition of communities is presented below the RDA biplots. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

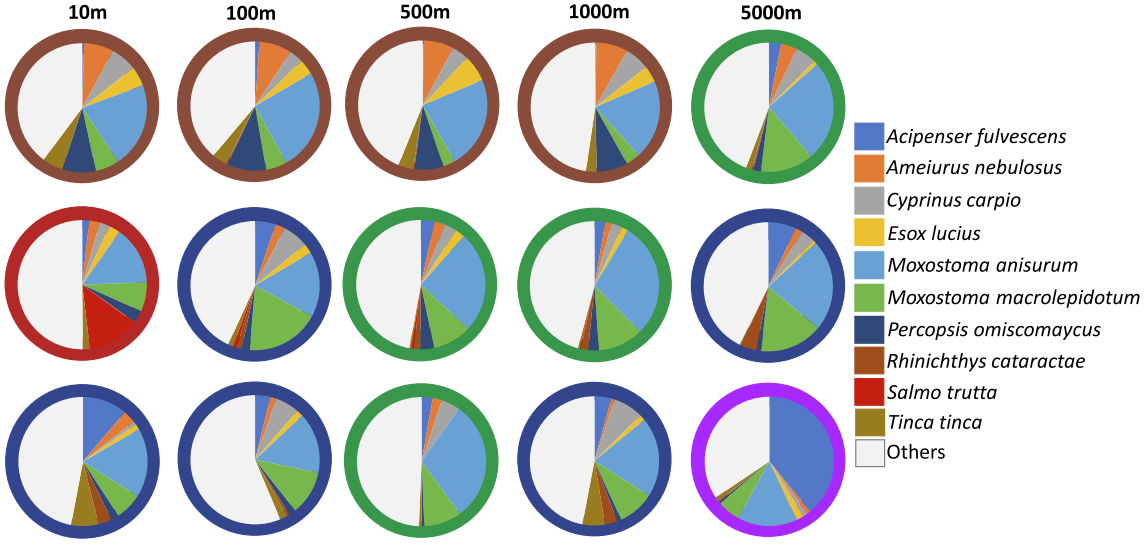

Fig. 4. Pie charts representing in situ relative abundance of the 10 species that significantly differentiated the five fish communities. Fish communities are represented by circling the pie charts with the color used in Fig. 3.

4.1. *High resolution of fish community detection in the St. Lawrence River*

Without considering DNA sequences from the caged brown trout, we detected four distinct fish communities. The 'brown' community was characterized by the higher relative abundance of northern pike (*Esox lucius*), brown bullhead (*Ameiurus nebulosus*), and the trout-perch (*Percopsis omiscomaycus*) eDNA and was located near the shore. These three species are known to prefer shallow habitats with vegetation, where trout-perch is an abundant prey species, namely for northern pike (Scott and Crossman 1976, Bernatchez and Giroux, 2012). The 'purple' community encompasses a single offshore site characterized by a high relative abundance of lake sturgeon (*Acipenser fulvescens*) eDNA, a species inhabiting deeper water (Berger et al., 2020). The shorthead redhorse (*Moxostoma macrolepidotum*) and the silver redhorse (*Moxostoma anisurum*) eDNA, which respectively characterized the 'blue' and the 'green' communities are also more abundant in deeper lotic habitat (Scott and Crossman 1976, Bernatchez and Giroux, 2012). This is in accordance with Berger et al. (2020) who found that depth was a main

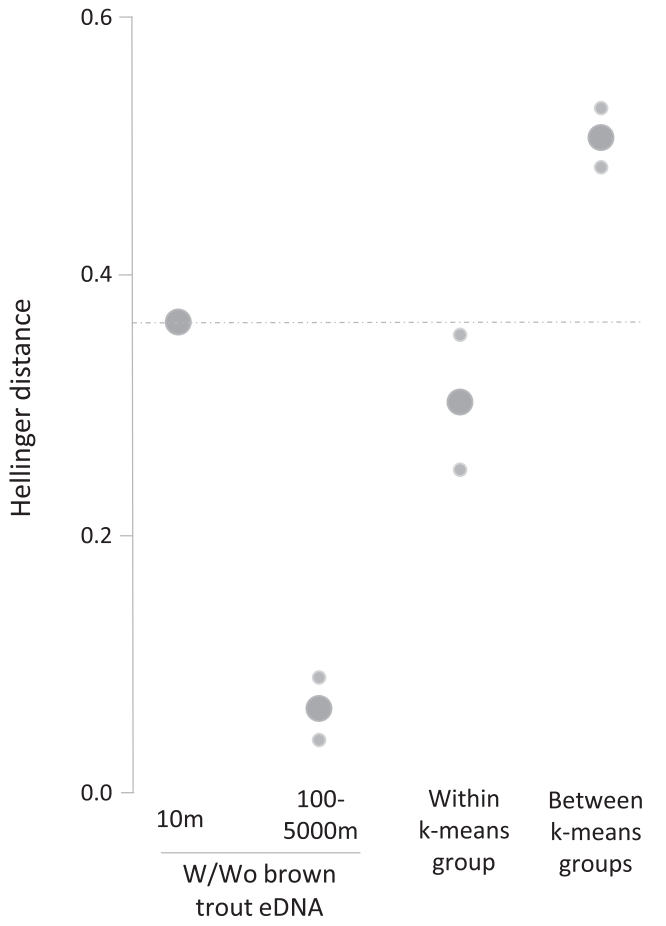

Fig. 5. Hellinger distance of i) site #2 (10 m downstream): comparison with versus without brown trout eDNA, ii) average for sites #5,8,11,13 (100 – 5000 m): comparison with versus without brown trout eDNA. iii) average among sites within a k-mean group and iv) average among sites from different k-mean groups. For iii) and iv) computations, only the dataset with brown trout eDNA was use to avoid pseudo replications. Small dots represents 95% confidence interval.

factor structuring fish communities in the area. Finally, the brown trout DNA sequences produced an artificiel community (the 'red' group) formed by a single site located at 10 m downstream of the encaged brown trout where it represented 13.1% of the sequence relative abundance. The rest of the fish community structure remained intact, with a brown trout maximum relative abundance under 1% for the four other sampling sites. Indeed, Hellinger distances between dataset with and without brown trout eDNA of those sites (#5, 8, 11 and 13) (0.07; 95% CI [0.05–0.09]) was lower than Hellinger distances between sites inside a same k-means group (0.31; 95% CI [0.25–0.36]).

In order to associate eDNA detection and/or quantification with distances from the source, others caged fish experiments showed the importance of considering several effects such as eDNA decay, retention and resuspension, as well as several hydrodynamic and hydrogeomorphic factors in lotic ecosystems (Janes et al., 2015; Wilcox et al., 2016; Shogren et al., 2017; Sansom and Sassoubre, 2017; Fremier et al., 2019; Robinson et al., 2019; Thalinger et al., 2021; Wood et al., 2021). However, the water flow of St. Lawrence River (16 800 m^3/s) is several orders of magnitude higher than the ones of those studies, showing the importance to investigate the effect of downstream advection and lateral dispersion on eDNA plume in our system. We showed that a high-resolution, bidimensional, time-dependent hydrodynamic model specifically developed for this area (Matte et al., 2017) was strongly correlated with number of brown trout eDNA copies from qPCR quantification (Laporte et al. 2020), and both relative abundance and raw sequences number of brown trout eDNA metabarcoding (present study). Therefore, we suggest that high dilution levels are largely responsible for the observations made here, and in previous eDNA metabarcoding studies that revealed fine-scale resolution of community structure in the area (Berger et al., 2020) and all along the St. Lawrence River (García-Machado et al., 2022). Of course, eDNA degradation can also explain the low proportion of upstream exogenous eDNA, but likely represent a minor factor considering the high flow rate and the distance tested in this study. This is also supported by eDNA persistence up to 16 days in a controlled experiment conducted using water of the study area (Caza-Allard et al., 2022). Finally, the bed of the St. Lawrence River mainly composed of fine substrate (clay, silt and sand) and the presence of submerged macrophytes in the area could also potentially explain the observed pattern by retaining a higher proportion of eDNA plume (Shogren et al., 2017; Matte et al., 2017; Laporte et al., 2020).

4.2. *Resolving species community structure in other river systems*

The study of fish communities through eDNA analysis has been performed in few other riverine systems. Pont et al. (2018) demonstrated the ability of eDNA metabarcoding to reveal fish assemblage structures with a spatial autocorrelation of 70 km along 500 km of the Rhône River, in France. They found variation in species richness, Shannon index, and Eveness index among sites associated with a spatial structure revealed by a principal component analysis. More recently, Laporte et al. (2021) resolved eight fish communities in a small dendritic river, the St. Charles River, Québec, Canada, which is a tributary of the St. Lawrence River at approximately 150 km downstream of the sampling area of the present study. The study revealed that pronounced change in fish communities can be observed at a sampling sites separated by approximately 5 km, with a global spatial autocorrelation of 16 km. In two large and species rich Neotropical rivers (Oyapock and Maroni rivers; Amazonian region; ≈ 53 species) Canterra et al. (*in press*) showed that eDNA metabarcoding can describe fish fauna with a spatial signal over a few hundreds meters only, which is comparable to that gathered with traditional methods. Finally, in the Thames River, UK, Hallam et al. (2021) observed only minor variations in the capacity to detect fish communities between traditional methods and eDNA metabarcoding, supporting the idea that eDNA can reliably identify fish communities in rivers. Recent studies also documented non-fish communities in rivers. Using stringent criteria to estimate macroinvertebrate eDNA in Swiss rivers, Mächler et al. (2019) found a strong relationship in local richness between eDNA and kicknet captures, with a weak downstream transport effect. Moreover, in the Conwy Catchment river drainage, Seymour et al. (2021) concluded that eDNA enables complex spatio-temporal studies of community diversity and ecosystem function, previously infeasible using traditional methods. The present study adds to the growing evidence pertaining to the usefulness of eDNA metabarcoding to study riverine community assemblages. However, a recent meta-analysis across 215 datatsets revealed that eDNA metabarcoding show congruence and outperformed traditional methods for documenting fish community composition, but not for plankton, microphytobenthos and macroinvertebrates communities (Keck et al., in press). This variation among taxonomic commununities could be explained by higher diversity and divergence in those non-fish taxonomic groups, which suggest that a better understanding of eDNA ecology including the origin, state and fate of eDNA should be incorporated in addition to transport effect is necessary for an optimal use of eDNA analysis in lotic ecosystems (Barnes and Turner, 2016).

4.3. *How can local fish communities be resolved under the hypothesis of a conveyor belt of biodiversity?*

In contrast with this capacity of eDNA metabarcoding to document species community structure in lotic environment, Deiner and Altermatt

(2014) and Pont et al. (2018) respectively reported that eDNA could be detected a long distance from its source, namely up to 12.3 km for the invertebrate *Daphnia longispina* and up to 60 km for the European whitefish (*Coregonus lavaretus*). Moreover, Laporte et al. (2021) reported that eDNA of 84% of fish species present in the 550 km^2 basin of the St. Charles River was detected at its mouth (around 60 km from the most upstream section). Similarly, Nakagawa et al. (2018) detected 86.4% of reported species within 6 km upstream of the eDNA sites in 51 rivers around Lake Biwa, Japan. These observations support the concept that eDNA in riverine systems is a conveyor belt of biodiversity, where samples from a river mouth can provide biodiversity information of the entire basin (Deiner et al., 2016). We thus suggest that eDNA metabarcoding can be both: i) a conveyor belt of biodiversity information and yet; ii) an efficient approach to document community structure in lotic environment. Thus, documenting community structure may be possible with a relatively weak transport effect on local relative abundance resulting in a low proportion of exogenous eDNA having a limited impact on the signal of fish communities among localities. Here, we show that brown trout eDNA was detected to a distance up to 5 km (the maximum tested in this study), but the relative proportion of brown trout eDNA detected at only 100 m from the shedding source was too low to influence the fish community clustering. More specifially, the relative abundance of brown trout eDNA was 13.1% for the first sampling location 10 m downstream from its source but decreased to<1% at 100 m downstream, following the prediction of dispersion and dilution of the bidimensional hydrodynamic model. Therefore, dilution seems to explain this result, but further investigation is needed to fully understand how eDNA transport can affect eDNA metabarcoding community composition for both species richness and relative abundance. Indeed, a single cage experiment cannot allow a full understanding of eDNA transport dynamics in lotic ecosystems, but in our view, this study represents a valuable step toward this goal considering the high number of individuals and biomass we used in comparison to other cage experiments (Jo et al., 2022). Nevertheless, multispecies transplant experiments are needed as next steps to better estimate the transport influence from punctual sources of extra-organismal eDNA on detected community composition. Finally, analyzing rivers with different biological (e.g., fish abundance and biomass, species richness) and physicochemical characteristics (e.g., temperature, flow, sinuosity, chemistry) is needed to fully understand the effectiveness of estimating fish community composition via eDNA metabarcoding in lotic ecosystems.

CRediT authorship contribution statement

M. Laporte: Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing. **C.S. Berger:** Formal analysis, Writing – review & editing. **E. García-Machado:** Formal analysis, Writing – review & editing. **G. Côté:** Conceptualization, Writing – review & editing. **O. Morissette:** Writing – review & editing. **L. Bernatchez:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

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Appendix A. Supplementary data

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