Using environmental DNA for biomonitoring of freshwater fish communities: Comparison with established gillnet surveys in a boreal hydroelectric impoundment

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Abstract
Accurate data characterizing species distribution and abundance are critical for conservation and management of aquatic resources. Inventory methods, such as gillnet surveys, are widely used to estimate distribution and abundance of fish. However, gillnet surveys can be costly in terms of material and human resources, may cause unwanted mortality in the fish communities being studied, and is subject to size and species selection bias. Detecting allochthonous DNA released by species in their environment (i.e., environmental DNA, hereafter eDNA) could be used as a noninvasive and less costly alternative. In this study, we directly compare eDNA metabarcoding and gillnets for monitoring freshwater fish communities in terms of species richness and relative species abundance. Metabarcoding was performed with the 12S Mifish primers. We also used species-specific quantitative PCR (qPCR) for the most abundant species, the walleye (Sander vitreus), to compare estimated relative abundance with metabarcoding and gillnet captures. Water sample collection, prior to gillnet assessment, was performed on 17 sites in the hydroelectric impoundment of the Rupert River (James Bay, Canada), comparing two water filtration methods. After controlling for amplification biases and repeatability, we show that fish communities’ complexity is better represented using eDNA metabarcoding than previously recorded gillnet data and that metabarcoding read count correlates with qPCR ($r = 0.78, p < .001$) in reflecting walleye abundance. Finally, based on partial redundancy analysis, we identified alpha chlorophyll, pH, and dissolved oxygen as environmental variable candidates that may influence differences in fish relative abundance between metabarcoding and gillnets. Altogether, our study demonstrates that the proposed eDNA metabarcoding method can be used as an efficient alternative or complementary technique adapted to the biomonitoring of the fish communities in boreal aquatic ecosystems.

KEYWORDS
environmental DNA, freshwater fish communities, metabarcoding, qPCR
1 | INTRODUCTION

A fundamental goal in conservation and wildlife management is to perform efficient biodiversity monitoring to obtain accurate knowledge of species distribution and abundance. This is especially relevant in a context of anthropogenic modifications of aquatic habitats (Chatterjee, 2017; Dias et al., 2017; Dudgeon et al., 2006; WWF, 2018). Such habitat modifications are often due to dam and powerhouse construction with reservoir impoundment and change of water flow downstream, where extensive monitoring of fish assemblages is required to track change in abundance and community composition (Dejean et al., 2011; Shelton et al., 2016; Ushio et al., 2018). Broadly used conventional methods for inventories, such as gillnets, are based on capture and morphological identification. However, gillnets may introduce biases due to gear selectivity for species and individual behavioral traits. This, in turn, may reduce the accuracy of detection and estimates of species abundance (Fuji et al., 2019; Knudsen et al., 2019; Shaw et al., 2016; Shelton et al., 2016). In addition, gillnet surveys can be costly in terms of material and human resources, and may cause unwanted mortality in the fish communities being studied (Hänfling et al., 2016; Thomsen and Willerslev, 2015; Valentini et al., 2016). It is therefore pressing to develop more accurate, reliable, noninvasive, and cheaper monitoring tools that can rapidly assess fish communities to better direct management and conservation strategies (Menning, Simmons, & Talbot, 2018; Pimm et al., 2015; Ruppert, Kline, & Rahman, 2019).

Environmental DNA sampling (eDNA) entails collecting traces of DNA shed by an organism through their epidermis, feces, mucus, hair, gametes, and other various sources in an environmental matrix, such as filtered water samples (Levy-Booth et al., 2007; Lodge et al., 2012; Rees, Maddison, Middleditch, Patmore, & Gough, 2014). eDNA is rapidly gaining interest for freshwater fish monitoring because it is cost-effective, noninvasive, and potentially more accurate for species detection than conventional methods (e.g., electric fishing, gillnets, traptnets, and scuba diving; Kelly, Port, Yamahara, & Rodgers, 2012; Thomsen & Leese, 2015; Taylor & Gemmell, 2016). Indeed, eDNA is already broadly used as a complementary tool and is likely to become a recognized method of biodiversity assessment in “a new era of biomonitoring 2.0” (Baird & Hajibabaei, 2012; Chamberl, Pilliod, Goldberg, Doi, & Takahara, 2018).

The two most common methods for eDNA detection in water samples are i) quantitative real-time polymerase chain reaction (qPCR) to detect and estimate the quantity of a target species (Shelton et al., 2016; Thomsen, Kielgast, Iversen, Möller, et al., 2012; Thomsen, Kielgast, Iversen, Wiuf, et al., 2012) and ii) metabarcoding, which consists of using “universal primers” targeting a taxonomic group combined with high-throughput sequencing to amplify DNA of a whole community to recover species composition (Deiner et al., 2017; Elbrecht & Leese, 2015; Hänfling et al., 2016; Riaz et al., 2011). The qPCR approach is highly sensitive and is being used very successfully to detect species where traditional inventories have failed (Doi et al., 2017; Kelly et al., 2014; Klobucar, Rodgers, & Budy, 2017; Lacoursière-Roussel, Rosabal, & Bernatchez, 2016; Maruyama, Sugatani, Watanabe, Yamanaka, & Imamura, 2018; Takahara, Minamoto, Yamanaka, Doi, & Kawabata, 2012; Thomsen et al., 2016; Wilcox et al., 2016). Metabarcoding is also increasingly used to document fish assemblages with more accuracy and better resolution than established, conventional methods (Evans et al., 2016; Hänfling et al., 2016; Miya et al., 2015). However, an important frontier of eDNA applications that remains to be fully addressed is whether quantitative eDNA data can reliably inform on species abundance (Chambert et al., 2018; Fukaya et al., 2018; Shelton et al., 2016). After more than a decade, no consensus has been reached on this controversial topic. For instance, a weak quantitative relationship was found in a recent meta-analysis based on 22 articles dealing with the quantification of biomass using metabarcoding (Lamb et al., 2019). The authors concluded that “additional research is required before metabarcoding can be confidently utilized for quantitative applications.” Nevertheless, there is increasing evidence that fish density or biomass is linked to eDNA concentration (Evans et al., 2016; Hänfling et al., 2016; Lacoursière-Roussel, Rosabal, et al., 2016; Handley et al., 2019). Similarly, a meta-analysis of the qPCR literature revealed a trend in field- and laboratory-controlled experiments ($R^2 = 57\%$ in nature and $81\%$ in the laboratory, $p < .05$) based on 19 articles comparing density and biomass with qPCR (Yates, Fraser, & Derry, 2019). The observed higher correlation in laboratory experiments can be explained by (a) the complexity of the interactions of eDNA with its environment and (b) the unknown true abundance of organisms in nature. Indeed, controlled mesocosm experiments have shown that the detectability of eDNA, which depends on shedding rate by individuals and degradation in the system, is modulated by local environmental factors (Barnes et al., 2014; Diaz-Ferguson et al., 2014; Goldberg, Strickler, & Pilliod, 2015; Jo, Murakami, Yamamoto, Masuda, & Minamoto, 2019; Lacoursière-Roussel, Côté, Leclerc, & Bernatchez, 2016; Lacoursière-Roussel & Deiner, 2019; Strickler, Fremier, & Goldberg, 2015). However, very few studies have considered the effect of environmental factors on the relationship between abundance and eDNA in natural freshwater ecosystems (but see Lacoursière-Roussel, Rosabal, et al., 2016).

To assess those potential biases and to propose a comprehensive methodology to use metabarcoding as a monitoring tool in natural conditions, it is important to compare and validate metabarcoding data with established methods (Fujii et al., 2019). In this study, we conducted a direct, simultaneous comparison (same sampling locations and sampling time) of metabarcoding and an experimental standardized gillnet survey was performed in a reservoir environment caused by the construction of hydropower dams. Our main goal was to investigate the quantitative potential of metabarcoding obtained from eDNA as a proxy of fish abundance or biomass. Toward this goal, we first assessed amplification biases using a mock community. Secondly, we quantified the spatial autocorrelation between two biological replicates taken at each of the 17 sampling locations to verify the repeatability of the method. Thirdly, we compared the efficiency of two different strategies of eDNA collection. Fourthly, we compared the quantitative estimates (in terms of molecular concentration) measured from qPCR with those obtained.
from metabarcoding data (number of sequence reads) to investigate consistency between these two techniques of quantification, using the walleye (Sander vitreus) as focal species. Finally, we statistically assessed the effect of several biotic and abiotic factors on the abundance of eDNA, after controlling for fish abundance quantified in gillnets to identify environmental variables that can contribute to explain observed differences in fish abundance between the two methods.

2 | MATERIALS AND METHODS

2.1 | Gillnet survey

The Rupert River (James Bay, Canada) was partially diverted in its upstream section in 2009 in the context of the Eastmain-Rupert hydroelectric project. An impoundment of approximately 350 km² was created to divert the flow into the La Grande Complex. Before the impoundment and since then, a fish community monitoring is being performed every two years to document the long-term impact of the impoundment on fish populations. In this context, an experimental gillnet survey was conducted at 17 sampling locations in August 2016 (Figure 1; Table 1). At every site, we set up an experimental gillnet (45.7 m long by 2.4 m high) composed of six panels of 25 mm, 38 mm, 51 mm, 64 mm, 76 mm, and 102 mm mesh size, coupled with a uniform 76-mm or 102-mm mesh size gillnet of the same dimension. Nets were deployed in the bottom of the river, from the shore to the offshore. Thus, such nets can capture fish of a very broad size range. Fishing effort was 24 hr per sampling site, and catch per unit effort (CPUE) and biomass per unit effort (BPUE) were calculated from the survey.

2.2 | eDNA sampling

Prior to the deployment of the nets, two identical biological replicates of water sample were taken at each of the 17 sampling locations using two different Niskin bottles (four liters per Niskin bottle and each sample). Niskin bottles were disinfected before each sample with 10% bleach (0.6% sodium hypochlorite) for 30 min and rinsed three times with distilled water. Niskin bottles were dropped at the middle point of the net location. Water samples were taken at one meter above the bottom and careful attention was given to not hit the bottom to avoid sediment suspension and release of sediment DNA. Water samples were stored at 4°C until filtration. Using a multiparameter probe, we also measured abiotic (pH, turbidity, dissolved oxygen, temperature, depth) and biotic (alpha chlorophyll) water characteristics at each station in order to evaluate the possible effect of these factors on eDNA concentration (Table 1).

2.3 | Water filtration

Water samples were filtered within 24 hr of collection in a dedicated room where no fish were handled, at the Eastmain Hydro-Quebec camp located in the same area of the sampling locations. Two liters of water for each replicate was filtered with a vacuum pump on a glass fiber 1.2-µm filter (Whatman GF/C), and 1 L of distilled water was filtered for each site as a field-negative filtration control for a total of 51 filters. The vacuum pump was disinfected before each filtration with 10% bleach and rinsed three times with distilled water. Along with the two Niskin bottle 2 L replicates, an additional 250 ml water was filtered using a piston syringe with a glass fiber 0.7-µm filter (Whatman GF/F) for each site for a total of 17 filters. Piston syringe is commonly used as an alternative to pump filtration because of its easier and simpler application in many contexts, although it comes with the possible limitations of filtering a smaller volume of
Prior to usage, the 17 disposable piston syringes were sterilized with UV light for 1 hr and individually sealed for the 250-ml filtration. All filters were individually stored in aluminum foil, sealed, and stored in −20°C until DNA extraction.

2.4 DNA extraction

DNA extractions were performed in a dedicated hood at Université Laval located in an area of the laboratory strictly used for pre-PCR eDNA manipulation. DNA extractions of the vacuum pump-replicated filters were performed following a modified salt extraction protocol of Qiagen DNeasy Blood and Tissue Kit (see Goldberg, Pilliod, Arkle, & Waits, 2011; Lacoursière-Roussel, Dubois, Normandeau, & Bernatchez, 2016 for further details). Three negative controls of distilled water were added to account for contamination during the extraction step. The DNA from the 17 GF/F syringe filters was extracted following a standard phenol–chloroform protocol. However, six of those syringe filters were contaminated with chloroform during the extraction step due to a manipulation mistake. Those six samples (EM356A, RP030B, RP042B, RP114A, RP116A, and RP220A) have been removed, and further analyses involving the syringe are based on 11 sampling sites. A negative control of distilled water was added to account for contamination during the extraction step.

<table>
<thead>
<tr>
<th>Site</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Sampling date</th>
<th>Depth (m)</th>
<th>pH</th>
<th>Temperature (°C)</th>
<th>Dissolved O₂ (mg/L)</th>
<th>Turbidity (FTU)</th>
<th>Chlorophyll α (µg/L)</th>
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<td>51.87494</td>
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<td>2016–08–17</td>
<td>3.4</td>
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<td>17.09</td>
<td>8.88</td>
<td>0.68</td>
<td>4.00</td>
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<td>EM355A</td>
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<td>−75.20277</td>
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<td>8.07</td>
<td>0.47</td>
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<td>7.5</td>
<td>7.37</td>
<td>17.17</td>
<td>8.78</td>
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<td>EM354A</td>
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<td>−75.32803</td>
<td>2016–08–19</td>
<td>7.9</td>
<td>7.02</td>
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<td>8.85</td>
<td>0.6</td>
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<td>EM360B</td>
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<td>2016–08–19</td>
<td>5.3</td>
<td>6.92</td>
<td>18.3</td>
<td>8.18</td>
<td>0.45</td>
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<td>6.35</td>
<td>7.07</td>
<td>18.2</td>
<td>8.81</td>
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<td>2016–08–22</td>
<td>19.7</td>
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<td>7.02</td>
<td>0.16</td>
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<td>2016–08–26</td>
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</table>
added to account for contamination. Figure 2 provides a schematic illustration of the different filtration and extraction methods used in this study.

2.5 | Metabarcoding

For the metabarcoding DNA amplification, we used the “Mifish” universal primers (Miya et al., 2015), which targeted a ~135-bp fragment from a variable region of the 12S gene. We first validated the specificity of the primers in silico by aligning target species sequences known to occur in the research area with the Mifish primer sequences using the Geneious (http://www.geneious.com). We also validated the primer specificity in vitro using DNA from the tissues of the five most abundant species occurring in the gillnet survey (i.e., walleye, Sander vitreus; northern pike, Esox lucius; yellow perch, Perca fluviatilis; lake whitefish, Coregonus clupeaformis; and white sucker, Catostomus commersonii). PCR was performed on a T1 Thermocycler (Biometra, Whatman) using fusion tag primers consisting of the Illumina adapters, single-indexed barcodes for each sample, and the Mifish primers following a one-step PCR amplification protocol. We used Qiagen Multiplex Taq Polymerase as suggested by Nichols et al. (2018) to diminish any GC content bias. Five amplification replicates were performed for each sample and for a PCR blank for each sampling site, accounting for contamination through PCR step. Cycling conditions were as follows: (i) 95°C for 15 min; (ii) 94°C for 30 s; (iii) 65°C for 90 s; (iv) 72°C for 60 s; (v) repeat steps (i)-(iv) for 34 additional cycles; (vi) 72°C for 10 min; and hold at 12°C. PCR products were visualized on a 2% agarose gel electrophoresis with ethidium bromide under a UV light. Negative control samples were also visualized on gel and showed no signal of amplification. The five PCR replicates were then pooled to reduce potential bias of stochastic variation along the PCR amplification step. PCR products were cleaned up with magnetic beads (Axygen) in a ratio of 1.8 to remove PCR artifacts such as primer dimers and eluted in 50 μl of sterilized RNase- and DNase-free water (diH2O). All 64 samples (including 15 field-negative controls and 4 laboratory-negative controls) were then quantified with a Qubit fluorometer, normalized, and pooled altogether in a final eluate for a single run of Illumina MiSeq sequencing performed at the genomic platform of IBIS (Institut de Biologie Intégrative et des Systèmes) at University Laval using a paired-end “MiSeq Reagent Kit V3” (Illumina; sequence length = 300 bp) and following the manufacturer’s instructions. The amplicon pool was diluted to 4 nM with molecular-grade water, denatured, and then sequenced at 10 pM following the manufacturer’s instructions inclusive of spiking the samples with 15% of PhiX.

2.6 | Quantitative PCR (qPCR)

In order to evaluate the relationship between the quantitative information obtained from metabarcoding data (number of reads per species) and that measured by qPCR (number of eDNA molecules), we selected the walleye (S. vitreus) as a focal species because it was the most abundant species of the gillnet survey. Also, there was not enough DNA volume left in the samples to perform analyses for other species after the metabarcoding analysis. We used the walleye-specific “ND2” qPCR primers (175-bp fragment) developed by Carim, Dysthe, Young, McKelvey, and Schwartz (2017). In that paper, they also tested for coamplification with other percids and validated that the primers were specific to walleye. In addition, we tested the primers and probe specificity with DNA extracted from walleye muscle tissues collected from the two most distant locations (Rupert River and Mistassini Lake separated by about 200 km) to assess the possible effect of genetic variation on the primers and probe specificity. We also included SPUD (Sigma-Genosys; Nolan, Hands, Ogunkolade, & Bustin, 2006) in each sample to allow detection for possible inhibitors in the sample. TaqMan qPCR amplifications were performed with a 7500 Fast Real-Time PCR System (Life Technologies). Six technical replicates of each sample were performed. For each individual qPCR reaction, we used 2 μl of sample DNA; 10 μl of TaqMan Environmental Master Mix 2.0 (Applied Biosystems); 1.8 μl of forward and reverse primers (10 μM); 0.5 μl of probe, mixed with SPUD reaction composed of 1.2 μl of SPUD forward and SPUD reverse primers; 0.5 μl of SPUD probe; and 1 μl of SPUD template. We also added positive control (DNA extracted from muscle tissues) and negative controls to track for contamination during the qPCR amplification. For the standard curve preparation, we used a dilution series of five points of 10-fold, with three replicates per point (ranging from 10 to 100,000 molecules) of synthetic gBlock DNA for quantification, and samples were randomized over six qPCR plates.

2.7 | Mock community preparation

In order to investigate possible amplification bias of metabarcoding due to the difference in affinity between the primers and the binding site of the different species sequences of the primer regions, we built a mock community and sequenced it on a separate MiSeq run. The mock community was composed of the five most abundant species found in the gillnet survey: walleye, northern pike, yellow perch, lake whitefish, and white sucker, and DNA concentration was split in equimolar quantity of 5 ng/μl for each species. Given the pronounced differences in total genome size (e.g., about 3 gigabases for lake whitefish and about 700 megabases for walleye) and to avoid the possibility of creating uneven concentration of mitochondrial DNA among the five species, we first amplified the 12S region targeted by the Mifish primers using DNA extracted from muscle tissues of the five species and the PCR products were cleaned up with beads (Axygen Magnetic Beads). We then quantified by fluorescence the amplicons and normalized the samples to 5 ng/μl using a NanoDrop. We pooled the amplified mitochondrial 12S DNA of each five species in equimolar concentration. Five technical replicates of this mock community were sequenced separately following the same procedure as described above for the field samples.
2.8 | Bioinformatics

Mifish primers sequences and Illumina adapters were removed, and raw sequencing reads were demultiplexed using the MiSeq Control software v2.3. Raw reads were then analyzed using Barque pipeline v1.5.1 (https://github.com/enornmandeau/barque). Briefly, the following steps were performed: (a) trimming and filtering of the raw reads (forward and reverse) using Trimmomatic (LEADING:20, TRAILING:20, SLIDINGWINDOW:20:20, MINLEN:100, CROP:200; Bolger, Lohse, & Usadel, 2014), (b) merging of the paired-end reads using Flash (-z, -m 30, -M 280; Magoc & Salzberg, 2011), (c) splitting reads by amplicon using a dedicated python script found in Barque (split_amblicons_one_file.py), and (d) chimera sequence removal and merging of the remaining unique reads was done with vsearch (https://github.com/torognes/vsearch). Finally, using the mifish 12S database (http://mitofish.aori.u-tokyo.ac.jp/), supplemented by a homemade database of fish 12S sequences (https://github.com/enornmandeau/barque), reads with 97%–100% identity match at the species level were conserved for further analyses. Reads of species detected in negative controls were considered as potential contamination, and the number of reads was subtracted from the corresponding number of reads of the species within the associated samples. Finally, we ensured that all species analyzed further were present in the two biological replicates with the goal of minimizing the possibility of false positives.

2.9 | Statistical analyses

All statistical analyses were performed using R, version 3.4.3 (R Core Team, 2016), mainly with the vegan package (Oksanen et al., 2016). The accumulation curves for the species richness comparison were done with "specaccum" function using the vegan package (Oksanen et al., 2016). We used a chi-squared ($\chi^2$) test with the function "chisq.test" to verify whether the relative abundance of reads sequenced corresponds to the initial proportion of the five species composing the mock community. For the comparison of the true biological eDNA replicates, we performed multiple Mantel’s correlation test for all the pairwise eDNA methods using the function "mantel" from the vegan package (Oksanen et al., 2016). We used nonmetric multidimensional scaling (nMDS) to visualize the fish communities depicted by the different methods. After standardization of the data on total proportion ("decostand" function from vegan package) and using the 10 shared species between both approaches (eDNA and gillnets), we compared the different communities using a Bray–Curtis distance ("vegdist" function from vegan package) while grouping by those approaches. Permutational multivariate analysis of variance (PERMANOVA) was then performed to statistically assess the strength of associations. To highlight which species were causing discrepancy between the methods, we performed a similarity percentage analysis (SIMPER). Those results were also visually represented on the MDS ordination using the "envfit" function from vegan package. We performed a regression analysis between both abundance (CPUE) and biomass (BPUE) gillnet data to assess the correlation (Pearson’s $r$) against the number of sequence reads (using the mean of the pump replicates) for the four most abundant species captured by gillnets: walleye, $n$ = 180; lake whitefish, $n$ = 70; white sucker, $n$ = 58; and northern pike, $n$ = 49. Other species were not analyzed because of their low sample size (total $n$ < 20 over 17 sampling locations) and the absence of several sites that can affect the power of statistical tests. Regression analyses were also used to compare metabarcoding with qPCR using the walleye as a focal species. We used the mean value of the six technical replicates of qPCR with the mean value of the pump replicates, followed by a log transformation of qPCR to meet the statistical assumption of normality.

Finally, the effect of environmental parameters on the eDNA reads was assessed using a partial redundancy analysis (pRDA). Briefly, the Hellinger transformation using the function "decostand" was performed on both eDNA metabarcoding and gillnet CPUE datasets as proposed by Legendre and Legendre (2012). Backward selection using the function "ordistep" in vegan package was performed to detect the best environmental variables explaining at least one of the relative abundance datasets. To test whether environmental variables ($pH$, chlorophyll $a$, and dissolved oxygen) can explain the difference in relative abundance between eDNA metabarcoding and CPUE datasets, we performed a partial redundancy analysis (pRDA), using the "rda" function in vegan package. The response matrix was eDNA metabarcoding relative abundance, which we explained with the environmental variables after controlling for the CPUE relative abundance matrix.

3 | RESULTS

3.1 | Sequences quality

A total of 11,459,443 reads were obtained from a single run on the Illumina MiSeq platform comprising all 64 samples. Following the filtration steps performed with the Barque pipeline, 8,672,220 reads were assigned to fish species with a 100% identity match, while 2,787,223 were unassigned. We achieved an accurate taxonomic resolution given that identification was possible for almost all fish sequences and that only very few taxonomic uncertainties were observed; namely, 14 reads associated with Salvelinus alpinus (Arctic char), which has never been reported in the Rupert River system, were discarded from further analyses. Because of the extremely low number of reads recovered, it is more plausible that those represented sequencing errors corresponding to the closely related S. namaycush (lake trout), which has only one nucleotide difference and has been captured in the study area. Similarly, Cottus bairdi and C. cognatus, two common sculpin species known to occur at those latitudes, cannot be discriminated by the 12S MiFish marker, which were therefore treated as a single taxon. Also, MiFish cannot discriminate four of the species within the genus Oncorhynchus. We therefore grouped associated reads as Oncorhynchus sp. However, O. mykiss (rainbow trout; introduced species throughout eastern
North America is the only Oncorhynchus that would likely be detected in our study area. Yet, because it has never been recorded in the Rupert River system and it was present at one site and in one pump replicate only (site RP042B replicate B), we considered those reads as a contamination and discarded them from further analyses, even if the number of reads was high (71,132 reads). Finally, 82 reads of White Perch (Morone americana) were detected in two different sampling locations. In Québec, this species only occurs in the St. Lawrence R. and was therefore considered as contamination also. Some mammals (moose: Alces alces; cattle: Bos Taurus; human: Homo sapiens; and pig Sus scrofa) and the northern two-lined salamander (Eurycea bislineata) were also detected. Overall, we adopted a conservative approach to remove possible contamination biases by adding a filtration blank (field-negative control) at every sampling site and by subtracting the number of reads found in the blank to both replicates of the corresponding blank. Due to a higher level of contamination in the filtration blank of site RP115A (199,274 reads), we excluded it from further analyses. Global contamination from the filtration blanks represented only 0.5% of the reads with the exclusion of this sampling site (2.7% when included). We performed all statistical analyses with and without removal of the contamination (for the other 16 sites) and obtained very similar results without any modification of our main interpretations, indicating that the overall low contamination level does not impact on the main outcomes of this study. Raw data of metabarcoding and gillnet captures are provided in the Data S1.

### 3.2 Species richness comparison

Combining all methods, a total of 20 species were detected in the study area, and only 10 of these were caught by gillnets, while both eDNA collecting methods detected all of them. However, the 250-ml syringe method missed two species detected as very low frequency by both the pump filtering and gillnet (Acipenser fulvescens: lake sturgeon; and S. namaycush: lake trout). Three species were detected only by the pump method (Notropis atherinoides: Emerald Shiner; Rhinichthys cataractae: Longnose Dace; and S. fontinalis: Brook Char). No species were exclusively detected by gillnets at the scale of the whole study area. At the sampling site level however, both eDNA sampling methods missed two species (false negatives) that were locally captured by gillnets: lake trout at site RP062B and lake sturgeon at site RP014A (Figure 3).

When considering the sampling effort, the accumulation curves (Figure 4) showed that both eDNA metabarcoding approaches reached a plateau at about 11 sampling locations (Figure 4). In contrast, gillnet did not reach a plateau, even on the predictive curve (dashed line), thus indicating that sampling effort was insufficient to detect all fish present in the community in comparison with eDNA. Curve patterns between eDNA pump replicates (VAC-1 and VAC-2) were very similar and depicted the highest fish diversity. The syringe method also reached a plateau but detected less species than the pump method, which can be explained by the smaller volume of water filtrated by syringe (syringe: 250 ml; pump 1 L) and possibly also by the lower sampling effort (11 sites instead of 17).

### 3.3 Amplification bias assessment and biological replicate comparison

There was no amplification bias observed after sequencing the mock community. Indeed, the DNA proportion of the five species of the mock community yielded the same proportion of reads after sequencing (Figure S1; \( \chi^2 = 0.005, p > .05 \)). Amplification bias is mainly driven by the number of mismatches between the primers and the target amplicons (Piñol, Mir, Gomez-Polo, & Agustí, 2018). Here, inspection of the 12S sequences of the 20 species recovered in this study with the Geneious software revealed that no species had more than one mismatch between their sequence and that of the primers that all occurred at the same cytosine position. Together, this suggests that while it cannot entirely be ruled out, amplification bias in our study system should not significantly impact on our results.
and interpretations. Also, we found a highly significant correlation in all pairwise comparisons of Niskin bottles *duplicata* (Mantel test: \( r = 0.80; \) \( p < .001; \) Figure 5) and between filtering methods (pump, two samples) versus syringe (mantel test: \( r = 0.82 \) and \( r = 0.86; \) all \( p < .001; \) Figure 5).

### 3.4 | qPCR versus Metabarcoding

No amplification was detected in the laboratory-negative controls while all positive controls were amplified, and SPUD showed constant amplification in all samples, thus confirming the absence of inhibition. For the resulting metrics of the six standard curves, the slope ranged from \(-3.384\) to \(-3.491\) (mean = \(-3.44, SD = 0.04\)) and \( R^2 \) between 0.998 and 1 (mean = 0.999, \( SD = 0.0008\)). The walleye eDNA concentration measured by qPCR is illustrated by the mean value of positive amplification in the six technical replicates, followed by a log transformation. The scatterplot showed a strong association between qPCR concentration and the number of walleye metabarcoding sequence reads (\( r = 0.78, p < .001; \) Figure S2).

### 3.5 | Relative abundance between eDNA and gillnet captures at the community level

To compare the relative abundance of different species at the community level, we used the ten species detected both by gillnets and by metabarcoding. The nMDS ordination showed a modest overlap among methods (global PERMANOVA: \( R^2 = 0.35; p < .001\), indicating that an important proportion of variance in relative abundance differed between them (Figure 6). The pairwise PERMANOVA comparison based on the Bray–Curtis dissimilarity on the standardized relative sequence read abundance per species revealed no significant difference between pump replicates (\( F = 0.53; p = .74\)), syringe and pump replicates (pump A: \( F = 0.14, p = .97\); pump B: \( F = 0.29; p = .90\)), and CPUE with BPUE (\( F = 1.38; p = .24\)), while all other comparisons between eDNA methods and gillnets were significantly different (Table 2). In addition, results showed that eDNA methods produce similar results and that eDNA metabarcoding and gillnets captured a significantly different portrait of fish communities (Figure 3). This was also confirmed by the SIMPER analysis revealing that the relative abundance of Yellow Perch and Cisco (*Coregonus artedi*) as determined by the number of metabarcoding sequence reads was significantly higher relative to gillnet data, whereas the opposite was observed for walleye (Figure 6). Indeed, more variation and complexity in community composition was depicted by both eDNA metabarcoding methods in comparison with gillnet.

### 3.6 | Correlation between eDNA sequence reads and gillnet captures at the species level

The four abundant species (walleye, lake whitefish, white sucker, and northern pike) with number of fish caught by gillnets higher than 20 were tested for an association between the number of sequence reads/species (from the pump method) and gillnet captures. Walleye and white sucker showed a stronger correlation between the number of sequence reads/species and abundance (CPUE) than with biomass (Figure 7). Lake whitefish showed similar level of correlation between eDNA and CPUE or biomass (Figure 7). No significant correlation was observed for the Northern Pike. No significant correlation was observed between the number of sequence reads/species obtained from the syringe method and either abundance (CPUE) or biomass.

### 3.7 | Environmental factors

The pRDA showed that 18% of the eDNA variation was explained by a combined effect of pH (\( p = .005\)), chlorophyll \( \alpha \) (\( p = .04\)), and dissolved oxygen (\( p = .029\)), after controlling with fish abundance (CPUE) variation observed with gillnets (global model significance: adj\( R^2 = 0.18; p < .001; \) Figure 8). Therefore, pH, chlorophyll \( \alpha \), and dissolved oxygen are potential candidates affecting the correlation...
between specific gillnet abundance and metabarcoding eDNA number of reads.

### 4 DISCUSSION

In this study, we evaluated the performance of metabarcoding to describe freshwater fish communities by comparing eDNA data (number of sequence reads/species) with that of gillnet captures performed as part of a “real-life” experimental standardized gillnet survey in a boreal hydroelectric impoundment environment. We found that eDNA metabarcoding allowed detecting many species at most of the sampling locations that were totally missed by the gillnet survey. Thus, eDNA metabarcoding sequence reads and gillnet captures showed important differences in fish community assemblage; nevertheless, significant positive associations at the individual species level were observed. Our results also point out that site variation in chlorophyll, pH, and dissolved oxygen may partly explain the discrepancies in relative abundance between these two methods. In addition, we found that metabarcoding and qPCR were equivalent in terms of depicted relationship between DNA abundance and gillnet catches, using the walleye as a focus species of comparison. Finally, we demonstrated a high reproducibility of the method when comparing replicates of eDNA and we detected no amplification bias between the Mifish primers and five of the most abundant species of the Rupert River using a mock community. By having investigated several different technical aspects of metabarcoding within the same experiment, this study establishes that eDNA metabarcoding can be a usable and efficient methodology for monitoring freshwater fish communities of the Canadian boreal ecosystem.

#### 4.1 eDNA metabarcoding detects higher species richness

Our main purpose was to evaluate the quality of usable information that we can extract from eDNA metabarcoding to be used in a context of applied field biomonitoring. To determine whether there was a gain in species detection using eDNA, we performed a direct comparison between metabarcoding and an established experimental gillnet survey for the description of fish assemblages. Our results demonstrated that metabarcoding generally detected twice as many species at every sampling site compared with gillnet catches, thus revealing a more accurate portrait of local fish biodiversity. These results are consistent with the growing literature showing that eDNA metabarcoding allows a more exhaustive characterization of fish communities (Andruszkiewicz, Sassoubre, et al., 2017; Andruszkiewicz, Starks, et al., 2017; Civade et al., 2016; Hänfling et al., 2016; Handley et al., 2019; Miya et al., 2015; Port et al., 2016; Shaw et al., 2016; Thomsen, Kielgast, Iversen, Møller, et al., 2012; Thomsen, Kielgast, Iversen,
Admittedly, the occurrence of false negatives, albeit modest, should be considered and eDNA metabarcoding results cautiously interpreted accordingly. Also, our results show that sampling effort can be considerably reduced with eDNA metabarcoding to recover the entire fish community compared with conventional gillnet surveys. Indeed, it is much more time-consuming and costly to set gillnets one day and come back to collect all captured fish at each station than simply take a water sample on a single visit (instead of two) and then performing water sample filtration. Recent literature also found similar advantages in reducing sampling effort (see Sard et al., 2019). Of course, eDNA needs laboratory and bioinformatic analyses that are not required for gillnet surveys, but constraints of those extra steps remain modest in comparison with benefits of substantially reducing the logistics, manpower, and travel costs required for conventional gillnet surveys in remote areas. The eDNA pump filtering method recovered more species than the syringe method, most likely because of the larger water volume being filtered, but also possibly because of the smaller sampling effort of the syringe method caused by the loss of six samples. Moreover, we cannot rule out the possibility that the different DNA extraction protocols used in each method can affect the number of species detected. In any case, despite the fact that the

**TABLE 2** Pairwise PERMANOVA based on the Bray–Curtis dissimilarity on the standardized relative abundance

<table>
<thead>
<tr>
<th>Paired method</th>
<th>df</th>
<th>F</th>
<th>Adjusted R²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pump A versus Pump B</td>
<td>33</td>
<td>0.55</td>
<td>0.02</td>
<td>.738</td>
</tr>
<tr>
<td>Pump A versus SYR</td>
<td>27</td>
<td>0.14</td>
<td>0.01</td>
<td>.968</td>
</tr>
<tr>
<td>Pump A versus CPUE</td>
<td>33</td>
<td>16.85</td>
<td>0.35</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Pump A versus BPUE</td>
<td>33</td>
<td>14.23</td>
<td>0.31</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Pump B versus SYR</td>
<td>27</td>
<td>0.29</td>
<td>0.01</td>
<td>.901</td>
</tr>
<tr>
<td>Pump B versus CPUE</td>
<td>33</td>
<td>18.53</td>
<td>0.37</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Pump B versus BPUE</td>
<td>33</td>
<td>16.42</td>
<td>0.34</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>SYR versus CPUE</td>
<td>27</td>
<td>13.40</td>
<td>0.34</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>SYR versus BPUE</td>
<td>27</td>
<td>10.67</td>
<td>0.29</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>CPUE versus BPUE</td>
<td>33</td>
<td>1.39</td>
<td>0.04</td>
<td>.241</td>
</tr>
</tbody>
</table>

Note: Significant tests (p < .001) are in bold.
syringe method is easier and cheaper to use in the field compared with the pump filtering approach, based on our results we suggest using the pump method in subsequent studies in order to obtain a more complete and accurate portrait of local fish communities.

4.2 | No detection of amplification bias

Many studies previously showed that the occurrence of amplification bias is imputable to a differential affinity with primer binding sites
Our results demonstrated that no difference was found between initial proportion of DNA and proportion of reads after sequencing in all the replicates, which can support the reliability of our eDNA metabarcoding dataset. As mentioned in the section PCR amplification, all species of our system had a single mismatch with the same cytosine position of the MiFish primers, so we hypothesize that the amplification probabilities were similar for all species, which was supported by our mock community results. Nevertheless, our mock community was limited to the use of the five most abundant species in equimolar proportions. It would be relevant in the future to use the same methodology but to incorporate more species and to vary the proportions of each to further support the absence of amplification bias for all the fish communities in the area. However, this represents an entire study on its own, which was beyond the scope of this study. In addition, different DNA polymerases may have different affinities for sequences with specific GC nucleotides, which can be a source of bias in relative abundance estimates by metabarcoding (Nichols et al., 2018). This should also be considered and investigated in future research to confirm the absence of amplification bias.

4.3 High reproducibility of eDNA sample replicates

Replication is a key parameter to increase confidence in eDNA metabarcoding analysis (Andruszkiewicz, Sassoubre, et al., 2017; Andruszkiewicz, Starks, et al., 2017; Dickie et al., 2018; Goldberg et al., 2016; Ruppert et al., 2019). Thus, we collected true biological replicates of 2 L water samples using two distinct Niskin bottles at every site and filtered using a pump filtration method. We also filtered a third 250 ml water sample from the first Niskin bottle using a sterile syringe. The samples taken for each sampling location were then sequenced and analyzed separately. Our results showed a high reproducibility between sample replicates (see Barnes et al., 2014). Therefore, despite additional cost, we suggest adding replicates in further eDNA studies to ensure the reliability of the results, as suggested in Ficetola et al. (2015) and Laporte et al. (2020).

4.4 Strong quantitative congruence between eDNA metabarcoding reads and qPCR

Few studies previously compared directly quantitative information that can potentially be obtained from metabarcoding with qPCR from eDNA collected in the field. This is particularly relevant given that most abundance inference with eDNA in aquatic environment comes from qPCR analyses (Yates et al., 2019), whereas little is known about the quantitative resolution offered by eDNA metabarcoding (but see Lamb et al., 2019). Our analysis revealed a strong correlation between qPCR and metabarcoding for walleye, the most abundant species in the system. In addition, the relationships between qPCR and CPUE or BPUE were similar to those observed with metabarcoding. Those results indicate that, in some circumstances at least, metabarcoding may have the same quantitative resolution than qPCR, which was also recently reported in Harper et al. (2018).

4.5 Extant association between the number of eDNA metabarcoding sequence reads and gillnet captures

When we compared the relative abundance of standardized data of the ten shared species, we observed some discrepancies between eDNA and gillnet surveys. Without knowledge of the real abundance in the system, it is challenging to determine whether one method is more accurate than the other. But indirectly, metabarcoding gives a more plausible interpretation of a predator-prey community, whereby, in contrast to gillnets, it depicted higher abundance of prey (Cisco, Yellow perch) than predators (walleye, northern pike) (York & Anderson, 1973), and also revealed the common occurrence of the Burbot (Lota lota), a predatory fish that is difficult to catch using conventional methods such as gillnets. Indeed, the Burbot is an eel-like round and very slippery fish without external spines or hard rays that can easily slip through the net mesh. However, no obvious reason can explain why Cisco and Yellow Perch would avoid gillnets, especially given the fact that mesh sizes were appropriate to catch these species. In addition, eDNA can better represent broader fish communities since it can be transported up to several km in lotic ecosystems (Deiner & Altermatt, 2014; Laporte et al., 2020; Pont et al., 2018), and over several hundred meters in lentic systems (Eichmiller, Bajer, & Sorensen, 2014). Based on these observations, we propose that eDNA metabarcoding provides a better insight of species richness than gillnets, at least in boreal freshwater ecosystems.

Despite these differences, we also found a statistically significant increase in the number of eDNA sequence reads with fish abundance for the three most abundant species out of four using the pump method, thus providing further evidence that eDNA metabarcoding can be used to obtain some quantitative information about relative abundance in some circumstances (see also Afzali et al., 2020; Evans et al., 2016; Hänfling et al., 2016; Handley et al., 2019). However, no significant correlation was found between eDNA sequence reads obtained from the 250-ml syringe samples and gillnet catches, suggesting that small volumes of water may not be suitable for quantitative analyses and that the pump method returns better quantitative estimates.

4.6 Effect of environmental factors on eDNA metabarcoding reads

“Ecology” of eDNA is a complex matter that needs to be further investigated (Barnes and Turner, 2016; Lacoursière-Roussel & Deiner, 2019). Using a partial redundancy analysis (pRDA), we found that pH,
chlorophyll α, and dissolved oxygen explained 18% of the variance in eDNA read distribution after controlling for variation in fish abundance in gillnets. These variables are good candidates in partially explaining how gillnets and eDNA metabarcoding may differ in reflecting the composition and relative abundance of fish community. Chlorophyll α is the most commonly used proxy for primary production in aquatic environments. A more productive environment can potentially affect degradation of eDNA by increasing the presence of microorganisms using eDNA as a source of energy. Dissolved oxygen modulates the physiological tolerance of aquatic organisms to their environment with a decrease in dissolved oxygen leading to hypoxia (Stewart, 2019). This can modulate the distribution of fishes in the water column and the recovery of their eDNA, as shown by Port et al. (2016) in the marine environment and by Handley et al. (2019) in lakes. Also, Seymour et al. (2018) showed that eDNA degradation increases with acidity, although Buxton, Groombridge, Zakaria, and Griffiths (2017) found a very little influence of pH on the abundance amphibian eDNA in mesocosms. Nevertheless, similar investigations of the effect of environmental parameters should be performed in future studies with the goal of better understanding the ecology of eDNA.

5 | CONCLUSION

Properly executed, metabarcoding of environmental DNA performed with the 125 Mifish primers (Miya et al., 2015) can provide insightful information on fish biodiversity and can be used at least as a semiquantitative tool for monitoring fish communities. The methodology proposed in this study can already be used as a complementary or even an alternative to established surveys, with the benefits of a more accurate species detection, noninvasive practices, and less cumbersome means of obtaining information on relative abundance. We still need more empirical studies comparing the performance of eDNA metabarcoding versus conventional capture methods toward the goal of standardizing eDNA metabarcoding for biomonitoring purposes and its use in regulatory contexts such as impact studies or governmental policies (Looza-Quintana, Abbott, Heath, Bernatchez, & Hanner, 2020). Moreover, eDNA metabarcoding should be used to create baseline data of biodiversity at a landscape scale to monitor long-term modifications in communities in the context of a rapidly changing environment.

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CONFLICT OF INTEREST

None declared.

DATA AVAILABILITY STATEMENT

All raw sequences have been deposit in NCBI.

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REFERENCES


SUPPORTING INFORMATION

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

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