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Comparing environmental metabarcoding and trawling survey of demersal fish communities in the Gulf of St. Lawrence, Canada

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[Correction added on 21 July 2020, after first online publication: The correspondence section has been updated.]

Abstract

Biodiversity assessment is an important part of conservation management that ideally can be accomplished with noninvasive methods without influencing the structure and functioning of ecosystems. Environmental DNA (eDNA) metabarcoding has provided a promising tool to enable fast and comprehensive monitoring of entire ecosystems, but widespread adoption of this technique requires performance evaluations that compare it with conventional surveys. We compared eDNA metabarcoding and trawling data to evaluate their efficiency to characterize demersal fish communities in the Estuary and Gulf of Saint-Lawrence, Canada. Seawater and bottom trawling samples were collected in parallel at 84 stations. For a subset of 30 of these stations, water was also collected at three different depths (15, 50, and 250 m) across the water column. An eDNA metabarcoding assay based on the 12S mitochondrial gene using the MiFish-U primers was applied to detect fish eDNA. We detected a total of 88 fish species with both methods combined, with 72 species being detected by eDNA, 64 species detected by trawl, and 47 species (53%) overlapped between both methods. eDNA was more efficient for quantifying species richness, mainly because it detected species known to be less vulnerable to trawling gear. Our results indicated that the relative abundance estimated by eDNA and trawl is significantly correlated for species detected by both methods, while the relationship was also influenced by environmental variables (temperature, depth, salinity, and oxygen). Integrating eDNA metabarcoding to bottom trawling surveys could provide additional information on vertical fish distribution in the water column. Environmental DNA metabarcoding thus appears to be a reliable and complementary approach to trawling surveys for documenting fish biodiversity, including for obtaining relative quantitative estimates in the marine environment.

KEYWORDS

biodiversity, biomonitoring, eDNA, fisheries, marine fish

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1 | INTRODUCTION

Assessing and monitoring biodiversity, especially for commercially important, endangered, rare, or invasive species, is an important part of conservation and management goals (Danielsen et al., 2005). Ideally, this should be fulfilled by applying standardized methods in the least noninvasive way possible (Pauli, Whiteman, Riley, & Middleton, 2010). The structure and functioning of marine ecosystems are affected by human activities (Mullon, Fréon, & Cury, 2005; Zhou et al., 2010). Overfishing resulting from industrialized fisheries is known to be the main cause of decline for major fish stocks where biomass has been reduced by 80% within 15 years of exploitation (Myers & Worm, 2003). Trawling is the most common fishing technique in deep-sea waters, but it is known to damage the seabed and benthic communities (Clark et al., 2016; Kaiser, Collie, Hall, Jennings, & Poiner, 2002; Thurstan, Brockington, & Roberts, 2010; Tillin, Hiddink, Jennings, & Kaiser, 2006; Trenkel et al., 2019; Yesson et al., 2017). However, recent shifts in shrimp trawling with higher gear selectivity have decreased certain impacts on benthic communities (Moritz et al., 2015). Spatial analysis of trawling effects in Canadian waters of the Atlantic and Pacific Oceans has demonstrated benthos disturbances due to the interaction of fishing gears with the bottom, and its impact on groundfish bycatch (Kulka & Pitcher, 2001). Canada's groundfish fisheries catch numerous other nontarget species, some of which are overfished, threatened, or endangered. Several management strategies are in place, such as annual catch limits, to regulate fishing, but they have only been moderately effective, and fishing still remains a prominent threat (DFO, 2011). For example, in 2013, *Hippoglossoides platessoides* (American plaice), *Gadus morhua* (Atlantic cod), and *Urophycis tenuis* (White hake) stocks declined because of bottom trawling bycatch impact within other groundfish species fisheries of Gulf of St. Lawrence, Canada (GSL) (Seafood watch, 2014). Bycatch of *G. morhua*, a species that is considered "endangered" in 2003 by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC), is of high concern due to overfishing especially in the Maritimes regions (DFO, 2012).

The Gulf of St. Lawrence (GSL) with a surface area of 235,689 m^2 and an average depth of 148 m is a semi-enclosed sea that connects to the Atlantic Ocean through the Cabot Strait and the Strait of Belle Isle (Figure 1). Since the 17th century, the GSL has been exploited by traditional fishing operations including netting, dredging, longline, and trawl fishing activities (Benoît, Gagné, Savenkoff, Ouellet, & Bourassa, 2012). A large amount of freshwater drainage from the Laurentian Great Lakes and St. Lawrence Basin into the GSL coupled with high biological diversity and productivity creates its unique marine environment (DFO, 2005). Fisheries and Oceans Canada (DFO) has conducted surveys for groundfish biodiversity and stock assessments annually in the estuary and the northern part of the GSL since 1990 (Archambault et al., 2014; Bourdages et al., 2016, 2018; Bourdages, Savard, Archambault, & Valois, 2007; Scallon-Chouinard, Dutil, & Hurtubise, 2007). These data are used for several stock assessments, in particular for the most abundant and commercially important species such as *G. morhua*, *Sebastes* spp. (Redfish), *Reinhardtius hippoglossoides* (Greenland halibut), and *Hippoglossus hippoglossus* (Atlantic halibut). Collecting such time-series data is essential to understand global changes in community composition and abundance.

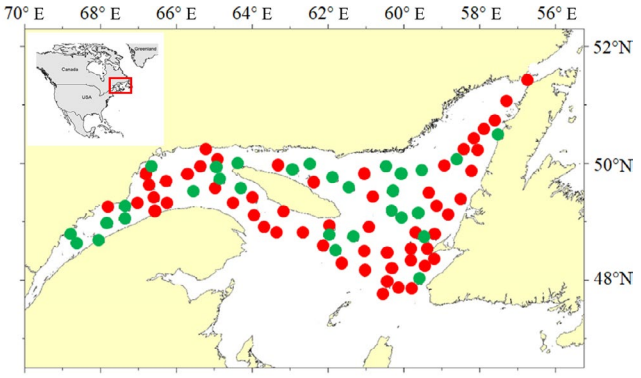

FIGURE 1 Map of the 84 sampling sites. Detailed map of the 84 sampling sites in Estuary and Gulf of St. Lawrence (EGSL). Red circles represent stations where trawling and eDNA sampling were done simultaneously only at bottom ($n = 54$). Green circles represent stations where trawling and eDNA sampling were done simultaneously at bottom and where eDNA sampling was additionally performed at different depths in the water column ($n = 30$). The insert shows the location of the sampling area within North America (red rectangle)

Monitoring species diversity has traditionally relied on sampling and counting individuals. For instance, surveys of demersal fish community in the GSL have been conducted to date using bottom trawling (Bourdages et al., 2007, 2018). Yet, this method is known to be invasive and may affect the structure and function of the ecosystems (Danielsen et al., 2005). Additionally, traditional surveys must rely on an highly specialized expertise in species identification based on morphological traits, which may lead to identification errors if such expertise is not available (Baird & Hajibabaei, 2012; Deiner et al., 2017). Moreover, parts of oceanic ecosystems may be difficult to monitor, for instance, in regions that are hard to access by conventional fishing gears (e.g., steep slopes and hard rocky areas) or deep-sea habitats that are logistically very costly and difficult to assess (Carugati, Corinaldesi, Dell'Anno, & Danovaro, 2015; Danovaro, Snelgrove, & Tyler, 2014). Such limitations highlight the critical need to develop approaches to complement conventional methods for quantitative stock assessments. Extracting organisms' genetic material directly from water samples without the need to collect organisms could be an alternative approach that is gaining more attention in biodiversity studies (Beja-Pereira, Oliveira, Alves, Schwartz, & Luikart, 2009; Maruyama, Sugatani, Watanabe, Yamanaka, & Imamura, 2018).

In recent decades, environmental DNA (eDNA) techniques have turned out to be a revolutionary additional tool for large-scale biodiversity assessments (Gibson et al., 2015). A combination of computational and molecular biology allows for comprehensive and cost-effective (Pawlowski et al., 2018) screening of entire ecosystems (e.g., Deiner & Altermatt, 2014; Ficetola, Miaud, Pompanon, &

Taberlet, 2008; Fujii et al., 2019; Hajibabaei, Shokralla, Zhou, Singer, & Baird, 2011; Lacoursière-Roussel et al., 2018; Miya et al., 2015; Thomsen et al., 2012; Valentini et al., 2016; Watts et al., 2019). High-throughput multiple species identification using genetic material (Cristescu, 2014; Porter & Hajibabaei, 2018; Shendure & Ji, 2008; Shokralla, Spall, Gibson, & Hajibabaei, 2012) found in water samples (eDNA metabarcoding) can be done by polymerase chain reaction (PCR) amplification of extracted eDNA with indexed universal primers that align to short regions of the genomes (e.g., mitochondrial 12S, 16S, and 18S ribosomal RNA gene) of target taxonomic groups followed by next-generation sequencing (NGS) (Evans et al., 2016; Hajibabaei et al., 2011; Kelly, Port, Yamahara, & Crowder, 2014; Li et al., 2019; Miya et al., 2015; Valentini et al., 2016). The design and selection of universal markers capable to detect eDNA from a taxonomically diverse set of target species are an important part of metabarcoding studies (reviewed in Freeland, 2017). Toward this goal, Miya et al. (2015) developed MiFish-U/E universal primers which amplify short variable regions of the mitochondrial DNA 12S rRNA gene (with amplicon size of ~170 bp) which provided sufficient resolution to identify fishes to species level in many coastal marine ecosystems (Andruszkiewicz et al., 2017; Ushio et al., 2017; Yamamoto et al., 2017) or freshwater habitats (Lecaudey, Schletterer, Kuzovlev, Hahn, & Weiss, 2019; Nakagawa et al., 2018; Sun et al., 2019). It should be noted that, as for about any others, this universal primer set may have a higher binding affinity toward certain taxonomical groups that might affect interpretation of final results to conclude the prevalence of the different species. Previous findings proved that different primer sets revealed different suites of taxa from a given environment (e. g., Kelly, Shelton, & Gallego, 2019). Other eDNA shortcomings such as availability of reference sequences deposited on public databases, or identical sequences of sister species, may also limit the efficiency of eDNA methods.

eDNA metabarcoding enables understanding spatial and temporal patterns of fish biodiversity (reviewed in Bálint et al., 2018; Handley et al., 2019), both qualitatively (presence/absence) and quantitatively (abundance/biomass) (Hanfling et al., 2016; Li et al., 2019). The estimation of seasonal species abundance (Bista et al., 2017; Buxton, Groombridge, Zakaria, & Griffiths, 2017), relative species richness (Pont et al., 2018), detection of invasive species (Smart, Tingley, Weeks, van Rooyen, & McCarthy, 2015), vertical distribution patterns associated with latitudinal and depth gradients (Jeunen, 2018; Zintzen, Anderson, Roberts, Harvey, & Stewart, 2017), ancient DNA (Haile et al., 2009; Pedersen et al., 2015), zoonotic disease outbreaks (Sato et al., 2019), and host–microbiome interactions (Deagle, Kirkwood, & Jarman, 2009; Johny, Saidumohamed, Sasidharan, & Bhat, 2018; Van der Reis, Laroche, Jeffs, & Lavery, 2018) that are critical objectives in ecological studies (Gaston, 2000) have all been explored by eDNA-based approaches. Comparing eDNA metabarcoding success with existing conventional monitoring methods is necessary to validate and calibrate the application of eDNA for conservation biology. Toward this end, multiple studies that have compared the efficiency of eDNA to conventional sampling methods (e.g., Erdozaina et al., 2019; Fujii et al., 2019; Knudsen et al., 2019; Port et al., 2016; Shaw et al., 2016; Thomsen et al., 2016; Valentini et al., 2016; Yamamoto et al., 2017) detected up to 50% more species by eDNA metabarcoding and also identified species missed by traditional monitoring methods (e.g., Andruszkiewicz et al., 2017). Therefore, a growing body of literature provides evidence that eDNA metabarcoding often outperforms conventional surveys in terms of getting a more complete picture of community composition (e.g., Fujii et al., 2019; Handley et al., 2019; Li et al., 2019; Olds et al., 2016; Wilcox et al., 2016). From a quantitative standpoint, Thomsen et al. (2016) compared eDNA detection performance to parallel trawling catch data which revealed a positive relationship between marine fish density and eDNA reads abundance in Greenland waters. Our study underlines the important findings presented by Thomsen et al. (2016), while it highlights the ability of eDNA metabarcoding in characterization of fish communities in different depths and also the effects of environmental variables on eDNA studies that are very important for further interpretation of metabarcoding datasets, and are only provided by few studies.

Recently, Knudsen et al. (2019) compared the results of specific qPCR assays with bottom trawling in the Baltic Sea to detect *G. morhua*, *Clupea harengus* (Atlantic herring), *Platichthys flesus* (European flounder), *Pleuronectes platessa* (European plaice), *Scomber scombrus* (Atlantic mackerel), and *Anguilla anguilla* (European eel). Despite these recent and very promising advances, uncertainties and limitations associated with eDNA metabarcoding surveys that could lead to misinterpretation of the results must be investigated further, notably in different environments (Cristescu & Hebert, 2018; Darling & Mahon, 2011; Goldberg et al., 2016; Li et al., 2019; Seymour et al., 2018; Shaw et al., 2016; Taberlet, Coissac, Pompanon, Brochmann, & Willerslev, 2012; Yoon et al., 2017). In particular, a better understanding of the influence of environmental variables on eDNA production and persistence in the environment might facilitate comprehensive application of the method for conservation and management (Laporte et al., 2020;Stewart, 2019).

Given that no previous study has yet characterized fish eDNA distribution in the western North Atlantic, including the Estuary and Gulf of St. Lawrence (EGSL), our main objective was to perform eDNA metabarcoding analysis of seawater samples and compared demersal fish communities with those detected in a parallel trawling survey. More specifically, multisurvey sampling data collected as part of DFO's annual research trawling survey in August 2017 were used (a) to compare the efficiency of eDNA metabarcoding and trawling methods applied conjointly at 84 sites for detecting and characterizing qualitatively and quantitatively groundfish communities and (b) to assess the ability of eDNA to describe vertical variation in community composition across the water column by analyzing eDNA samples at three different depths at a subset of 30 sites. We also addressed the effect of four abiotic factors (temperature, depth, salinity, and oxygen) on eDNA and trawl surveys and on the variation observed between the two methods.

2 | METHODS

2.1 | Data collection and sampling

Trawling was performed by DFO (Maurice Lamontagne Institute (IML), Mont-Joli, Quebec) at 84 stations (Figure 1) within trawlable habitats (ranged from 48.79°N to 51.43°N in latitude and from 60 m to 498 m in depth). This is part of the DFO annual research trawl survey in EGSL performed from 2 August to 2 September aboard the CCGS Teleost (Canadian Coast Guard Fisheries Research Vessel) in 2017 (Bourdages et al., 2018). A Campelen 1,800 (4-sided) shrimp trawl (McCallum & Walsh, 1996) equipped with "Scanmar™ hydroacoustic" system was used for the survey that had 44-mm meshes, rockhopper ground gear, a wingspread of 16–17 m, and headrope length of 29.5 m. The extension and the end of the trawl were equipped with a knotless nylon liner with a mesh opening of 12.7 mm (cod-end liner mesh size). The trawling speed was fixed at 3 knots. The horizontal opening between the trawl wings was about 16.94 m that is used to estimate the area swept by the trawl. The duration of each trawling was 15 min that calculated from the time the Scanmar sensor signaled that the trawl had hit bottom (Bourdages et al., 2007). An average of eight fishing tows per day was done, and catches were processed in the wet laboratory located under the main deck at the stern of the vessel. Fish morphological identification to the lowest possible taxonomic level was done by two fish taxonomists with more than 10 years of experience in fish identification. They also contributed to the identification guide (Nozères, 2018) for the species in the northern Gulf of St. Lawrence.

Water sample collection and filtration for eDNA were undertaken by DFO in parallel with the trawling survey at each of the 84 stations. The water samples were taken either before or after the fishing tow, depending on the workload in the wet laboratory (fish measurement). So the oceanographic position was either close to the position at the beginning or end of the tow. A total of 156 seawater samples were collected for eDNA metabarcoding in a sterile 2-L Niskin bottle at 84 stations in EGSL (Figure 1). Of these, 84 water samples were obtained in parallel with bottom trawling at all stations (n = 84), and 72 water samples were collected within the water column at three more depths at 30 of these 84 stations when possible (30 samples at 15 m, 30 samples at 50 m, and 12 samples at 250 m). Water samples were stored on ice before filtration while avoiding exposure to light to prevent eDNA degradation. On the same day (<8 hr), seawater samples were vacuum-filtered onto a small pore size Whatman glass microfiber filter (GF/C; 47 mm diameter; 1.2 μm pore size; Whatman, Maidstone, UK). The filter papers were wrapped in commercial aluminum foil placed in small plastic ziplock bags stored at −80°C until used for DNA extraction. As a negative control, one filtration blank consisting of 2-L deionized water (n = 45) was performed during each filtration session to monitor any cross-contamination between field samples during filtration. All working surfaces and equipment (e.g., Niskin bottles and containers) were decontaminated using 10% bleach before and after each sample collection and filtration.

2.2 | Molecular analyses

eDNA metabarcoding experiments were done in L. Bernatchez Laboratory at Université Laval where is specialized for eDNA studies and follows important decontamination routines including UV light, DNA decontaminant solution (Molecular BioProducts™ DNA AWAY™), UV hood, and isolated pre- and post-PCR rooms. eDNA from the filters was extracted using QIAshredder and DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) (Lacoursière-Roussel, Dubois, Normandeau, & Bernatchez, 2016). Extraction negative controls (consisting of the same extraction materials with no filter, added with 80 μl nuclease-free water) were run for each extraction batch (n = 32) to monitor any contamination. A total of 450 μl of ATL Buffer and 50 μl of Proteinase K (Qiagen) were added to tubes containing half of a filter and incubated at 56°C overnight. After incubation, the tubes were centrifuged at 15,800 ***g*** within a QIAshredder tube (to retain any fragment and insoluble material in the pellet which ensures a clear lysate), and the solution aliquoted into equal volumes in three different tubes.

A total of 400 μl of AL Buffer was added to each tube, vortexed, and incubated at 70°C for 10 min. After the incubation, 400 μl of ethanol was added and the mixture was transferred to a DNeasy Mini Spin Column (Qiagen) and centrifuged at 15,800 ***g***. The spin-column filter was washed using 500 μl of washing buffer (AW1) and centrifuged at 15,800 ***g***. Subsequently, the spin-column filter was washed again using 500 μl of washing buffer (AW2) and centrifuged at 15,800 ***g***. Purified DNA was then eluted in 80 μl of nuclease-free water, incubated at room temperature for 5 min, and centrifuged at 15,800 ***g***. The extracted DNA was then stored at −20°C until further use. To reduce the risk of laboratory cross-contamination, all bench spaces and laboratory tools were bleached and exposed to UV for 30 min before and after each batch of extraction.

A segment of the mitochondrial DNA 12S gene (with amplicon size of ~170 bp) was amplified using indexed universal primers (MiFish-U) designed by Miya et al. (2015). A unique 6-bp barcode was ligated to the primers during PCR amplification for each environmental sample. Five PCR replicates and a negative control were performed for each eDNA sample [(5 replicates + negative per sample) × (156 water samples + 45 field negative samples + 32 extraction negative controls) = 1,404 total amplicons]. The final reaction volume for each PCR replicate was 50 μl, including 25 μl Qiagen Multiplex Mastermix, 18 μl diH20, 2.0 μl of each primer (10 μM), and 3.0 μl of DNA. The PCR mixture was denatured at 95°C for 15 min, followed by 35 cycles (94°C for 30 s, 65°C for 90 s, and 72°C for 60 s) and a final elongation at 72°C for 10 min. Products of the five replicates of each sample were pooled together on a 96-well PCR plate (total of 234 amplicons), while PCR-negative control of each sample was transferred to separate 96-well PCR plate. All amplifications were visualized on a 1.5% agarose gel electrophoresis, and the correct length of bands was confirmed. No positive amplification of the PCR-negative controls was observed, so they were not sequenced. Field negative controls were treated exactly the same as regular samples and were also sequenced. The Axygen PCR Clean-up Kit

was used to purify pooled PCR product. DNA concentration was calculated with AccuClear Ultra High Sensitivity dsDNA Quantitation Kit using a Tecan Spark 10 M Reader for each sample. The concentration and fragment size distribution of the libraries were verified on an "Agilent 2100 Bioanalyzer" and were pooled in equal molar concentrations to maximize equal sequence depth per sample and to have equal coverage for all samples (Harris et al., 2010). Three MiSeq runs (each containing 78 samples) were performed and sequenced using on Illumina MiSeq (Illumina, San Diego, USA) at the IBIS genomic analysis platform (Université Laval, Quebec, Canada) using a paired-end "MiSeq Reagent Kit V3" (Illumina, San Diego, USA; sequence length = 300 bp) and following the manufacturer's instructions. For sequencing, the amplicon pool was diluted to 4 nM with molecular grade water, denatured, and then sequenced at 10 pM following manufacturer's instructions inclusive of spiking the samples with 15% of PhiX.

2.3 | Data analyses

Adaptor and primer sequences were removed, and raw sequencing reads were demultiplexed using the MiSeq Control software v2.3 into independent read files. Direct taxonomic assignment of each merged read with ≥97% base identity was performed using the Barque v1.5.2 pipeline (www.github.com/enormandeau/barque). The procedure followed by this pipeline is detailed in Lacoursière-Roussel et al. (2018). Briefly, reads were filtered for quality using Trimmomatic 0.36 (LEADING:20, TRAILING:20, SLIDINGWINDOW:20:20, MINLEN:100, CROP:300). Paired-end reads were then merged using flash v1.2.11 (-z -m 30 -M 280). Amplicons were then split by sample using the barcodes in the sequences. Chimera sequences were removed using vsearch v2.14.1 (vsearch –uchime_denovo, –fasta_width 0; Rognes, Flouri, Nichols, Quince, & Mahé, 2016). Reads were compared against a supplemented version of the complete BOLD database available to the public using vsearch v2.14.1 (vsearch –usearch_global, –qmask none, –dbmask none, –id 20, –maxaccepts 20, –maxrejects 20, –maxhits 20, –query_cov 0.6, –fasta_width 0). The filtered eDNA read counts were summed over all three libraries for each taxon and reads were analyzed at the level of lowest possible taxonomic assignment, which is the species level for the vast majority of taxon (94%). In some cases, however, the lowest taxonomic level was the genus or family due to the relatively low species-level resolution of the primers, namely Cyclopteridae (1 species identified at genus level), or due to sequence similarities, for example, in the case of Pleuronectidae (2 species with identical barcode identified at family level), Anarhichadidae (3 species with identical barcode identified at genus level), or Cottidae (2 species with identical barcode identified at genus level). All statistical analyses were conducted in the R programming environment version 3.5.1. (R Core Team, 2018) except for the permutational multivariate analysis of variance (PERMANOVA) that was conducted using PRIMER 6. version 6.4.7.0 & PERMANOVA+ (Clarke & Gorley, 2006).

The detection rate was first calculated for all taxa (presence/absence) identified either by eDNA metabarcoding or by trawling, and we plotted the cumulative empirical density function (CEDF) of all taxa (n = 84) using *labdsv* package (Roberts, 2015) (see Figure_2_Rcode in supplementary information). Then, for the 47 shared taxa, the detection rate was compared between eDNA and trawl methods using a linear regression model (Faraway, 2016). First, data were log-transformed and distribution was checked using the Shapiro–Wilk normality test and Q-Q plot. Then, the linear model was plotted using *lm* function and p-value was calculated for data with normal distribution using an ANOVA (see Figure_E1_Rcode in supplementary information).

Species richness by sampling method, sampling site, and depth was calculated using the *vegan* package (Oksanen et al., 2016; see Figure_H1_Rcode in supplementary information). Species composition (presence/absence) was analyzed with a PERMANOVA based on Bray–Curtis similarity with transformed data (square root) to test patterns of dissimilarities among fish species composition detected by eDNA and trawl across the sampling sites (number of permutations: 999; survey methods as fixed factor and stations as random factor). Fish composition differences and overlap within sites were visualized using circular heat maps computed with *heatmap.plus*, *RCircos*, *base*, *lattice*, and *RColorBrewer* packages (Krzywinski et al., 2009; see Figure_3_Rcode in supplementary information). Stations were ordered according to the depth from shallowest water to deepest depth (from 60 m to 498 m), to show changes in fish composition within stations by depth.

Species composition (with relative abundance data) was analyzed with redundancy analyses (RDAs) that were performed on the 47 shared taxa at 84 sites to compare the variation in trawl relative abundance and biomass with eDNA relative reads abundance, and reported with the adjusted R^2 (Adj.R^2). Hellinger's transformation was performed before the RDAs to deal with community composition, in particular to be able to account for the high number of zero characterizing this kind of data, as suggested by Legendre and Legendre (2012). These analyses were reperformed with trawl biomass instead of trawl number of catch for further comparison and visualized using heat-scale representation on bathymetric maps within the sampling sites (n = 84; see Figure_F1_Rcode in supplementary information). The relationship between eDNA relative abundance and trawl relative abundance of the most abundant taxa was visualized using bathymetric heat maps with eDNA level colored in circles within the sampling sites (n = 84). The map was computed with oceanographic data from the ETOPO1 database hosted on the National Oceanic and Atmospheric Administration (NOAA) website (www.noaa.gov) using packages *marmap*, oce, and *ocedata* (Levitus & Oort, 1977). Map data were added with the package *mapdata*, and relative eDNA counts and trawl catch relative abundance at each sampling site were represented using the *ggplot2* package (Wickhan, 2018). The strength of the correlation between relative eDNA abundance and relative trawl abundance was estimated using nonparametric Spearman's rank correlation coefficient (rho). The

data normality was checked using Q-Q plot, and data were log-transformed before checking for correlation (see Figure_5_Rcode in supplementary information).

In addition, for each site, within-site Hellinger distances between eDNA relative abundance and trawl relative abundance were calculated to assess similarity between the two methods and to test for the effect of environmental variables (depth, temperature, salinity, and oxygen) on the difference between eDNA and trawl shared species datasets. The effects of those variables were also tested on species richness obtained from eDNA and trawl sampling by applying a linear model. These outputs were plotted using *dplyr*, *patchwork*, and *ggplot2* packages (see Figure_G1_Rcode and Figure_6_Rcode in supplementary information).

For the 30 stations where vertical variation in the water column was analyzed, a linear mixed model was performed using *lme4* and *lmertest* (Kuznetsova, Brockhoff, & Christensen, 2017), with depth as factor and station as random factor to evaluate whether species richness differs between depths. Dissimilarity in fish community composition across these 30 stations at different depths was visualized by nonmetric multidimensional scaling (NMDS) analyses, based on Bray–Curtis dissimilarity matrix using the *decostand"*, *vegdist*, and *envfit* functions (based on 999 random permutations; see Figure_7_Rcode in supplementary information). The similarity percentage (SIMPER) test based on "Bray–Curtis dissimilarity index" was performed using the vegan package to estimate the contribution of each species to the observed dissimilarity between depths (see Figure_D1_Rcode in supplementary information).

3 | RESULTS

3.1 | Trawling and eDNA metabarcoding data

A total of 401,805 individuals representing 64 taxa in 35 families were caught by trawling at 84 stations, at bottom depths ranging from 60 to 498 m (Table 1). The total biomass catch was approximately 38,256 kg. A total of 18,020,351 raw paired-end reads were produced on the Illumina MiSeq platform. After data filtering using the Barque pipeline, 81% of the total reads were retained from the three libraries. From these, 90.5% of the reads matched one single target taxa, 9.5% of the reads hits multiple species but more than 9% (of the total) were successfully assigned to the finest possible taxonomic level relying visual inspection of the "Geneious v.9" software (Kearse et al., 2012) outputs and fish geographical distribution information as explained in Appendix S1 and Table A1. The remaining 0.4% of reads that still hit multiple species were removed (Table A1 for further details). Sequences not representing marine fishes were comprised of mammals and birds, 28 freshwater fish species, as well as some false-positive detections representing nonlocal fish species and positive field negative controls which were excluded from study, as explained in Appendix S1 (see also Table A2), thus retaining 79% of total reads for subsequent analyses.

3.2 | Species detection sensitivity by eDNA and trawl

A total of 87 fish species belonging to 39 families and 19 orders were identified when combining both trawl and eDNA information (Table 1). Of these, 71 taxa (82%) were detected by eDNA, 64 taxa (74%) were detected by trawl, and 47 taxa (53%) from 25 families were shared between both methods. To further compare communities consistently between the two methods, species from the same genus that were detected only by one technique and could not be identified up to the species level with the other techniques were taken at genus level in our analysis, resulting in a total of 84 taxa in subsequent analyses (Table 1).

Detection sensitivity was higher with eDNA than with trawling, since eDNA detected more species in total and the number of each species present at a given site was generally higher in the eDNA survey (Figure 2). This difference was mostly due to four rare species that were not caught by trawl but sampled with few eDNA reads, such as *Alosa sapidissima* (American shad), as well as three small species abundantly detected by eDNA but missed by trawl likely due to larger mesh size, such as *Lycenchelys verrillii* (Wolf eelpout). Other species detected by eDNA but missed by benthic trawling included three pelagic species such as *Gymnelus viridis* (Fish doctor), or four anadromous species including *Salmo salar* (Atlantic salmon) as explained in Appendix S3 (see also Table 1). On the contrary, some species were detected by trawling but missed by eDNA as explained in Appendix S2 (see also Table 1). This concerned three species for which the 12S sequences were not available in the NCBI GenBank database, for example, *Polymetme thaeocoryla* (Lightfishes), and four species which are known to amplify poorly due to a lower primer sensitivity, for example, *Amblyraja radiata* (Thorny skate), and eight species with very low biomass (individual catch ≤ 3), for example, *Chauliodus sloani* (Sloane's viperfish).

For the 47 shared taxa, there was a positive relationship between the number of stations where a given taxon was detected by eDNA and the number of stations where it was captured by trawl when identified at the lowest possible taxonomic level ($p = .0001$, adj. $R^2 = 0.54$; see Figure E1 in Appendix S5), or at the family level ($p = .0001$, $R^2 = 0.44$). However, there were some incongruent observations such as *Ammodytes* spp. (Sand lances) that were detected by eDNA in 41 stations (61,032 reads; abundance rank: 15th out of 69 taxa) despite only four individuals being caught by trawl in four stations (four individuals caught; abundance rank: 42th out of 64 taxa). We assumed they may escape trawl net because of their small size and pencil-like body shape. *Limanda ferruginea* (Yellowtail flounder) is another species detected at 55 stations by eDNA (2,845 reads; abundance rank: 40th out of 71 taxa), but only in one station by trawl (three individual catches; abundance rank: 44th out of 64 taxa). Since this species prefers sandy bottoms, it may have been missed by trawl because of the lowest trawling efficiency in sandy or muddy seabed. In contrast, *Nezumia bairdii* (Common grenadier) was frequently captured by trawl at 49 stations (1,008 individuals caught; abundance rank: 9th out of 64 taxa), while it was detected by eDNA at ten stations only (638 reads; abundance rank: 56th out of 71 taxa).

TABLE 1 Overview of quantitative species detection from parallel bottom trawling and eDNA metabarcoding obtained from 84 stations (total of 84 samples). "×" indicates detected, and "NA" indicates not detected. Lines are merged when one method does not allow identification of the taxon to the finest taxonomic level (see Page et al. 2013 for fish species authority names list)

			Detection (84 stations)		Edna	Trawl catch data	
Family	Species	Common name	eDNA	Trawl	No. of reads	No. of individual	Biomass (kg)
Agonidae	*Aspidophoroides monopterygius*	Alligator fish	×	×	7,798	90	0.04
	Leptagonus decagonus	Atlantic poacher	×	×	8,076	148	3.52
	Ulcina olrikii	Arctic alligator fish	×	NA	6	0	0.00
Ammodytidae	*Ammodytes americanus*	American sand lance	×	*Ammodytes* sp.	31,542	4	0.01
	Ammodytes dubius	Northern sand lance	×		22,092		
	Ammodytes hexapterus	Pacific sand lance	×		7,398		
Anarhichidae	*Anarhichas lupus*	Atlantic wolfish	*Anarhichas* sp.	×	98,711	87	31.72
	Anarhichas minor	Spotted wolfish		×		2	0.02
Argentinidae	*Argentina silus*	Atlantic argentine	×	×	2,608	18	1.29
Clupeidae	*Alosa sapidissima*	American shad	×	NA	3	0	0.00
	Clupea harengus	Atlantic herring	×	×	471,341	6,317	1,326.37
Cottidae	*Artediellus atlanticus*	Atlantic hookear sculpin	×	×	12,884	45	0.23
	Artediellus uncinatus	Arctic hookear sculpin	×	×	2,814	12	0.05
	Gymnocanthus tricuspis	Arctic staghorn sculpin	×	×	1,240	43	2.01
	Icelus spatula	Spatulate sculpin	×	×	9,194	1	0.00
	Myoxocephalus scorpius	Shorthorn sculpin	×	×	1,128	25	9.67
	Triglops murrayi	Moustache sculpin	*Triglops* sp.	×	53,701	969	8.83
Cryptacanthodidae	*Cryptacanthodes maculatus*	Wrymouth	×	×	56,189	10	5.61
Cyclopteridae	*Cyclopterus lumpus*	Lumpfish	×	×	68,874	31	18.49
	Eumicrotremus spinosus	Atlantic spiny lumpsucker	*Eumicrotremus* sp.	×	1715	45	0.45
Etmopteridae	*Centroscyllium fabricii*	Black dogfish	×	×	137	482	313.98
Gadidae	*Arctogadus glacialis*	Polar cod	×	NA	9	0	0.00
	Boreogadus saida	Arctic cod	×	×	2072	78	0.85
	Gadus morhua	Atlantic cod	×	×	729,910	3,241	1551.86
	Gadus ogac	Greenland cod	×	×	22	1	0.45
	Melanogrammus aeglefinus	Haddock	×	NA	25	0	0.00
	Pollachius virens	Pollock	×	×	1561	2	3.41
Gasterosteidae	*Gasterosteus aculeatus*	Threespine stickleback	×	×	2	1	0.00
Gonostomatidae	*Cyclothone microdon*	Small-Toothed bristlemouth	×	×	559	9	0.01
Hemitripteridae	*Hemitripterus americanus*	Sea raven	×	NA	1736	0	0.00
Labridae	*Tautogolabrus adspersus*	Cunner	×	NA	192	0	0.00

(Continues)

TABLE 1 (Continued)

Family	Species	Common name	Detection (84 stations)		Edna	Trawl catch data	
			eDNA	Trawl	No. of reads	No. of individual	Biomass (kg)
Liparidae	*Careproctus reinhardti*	Sea tadpole	×	×	1922	1	0.00
	Liparis gibbus	Variegated snailfish	×	×	7,957	1	0.01
	Liparis fabrici	Gelatinous snailfish	×	NA	3,009	0	0.00
	Paraliparis calidus	Lowfin snailfish	NA	×	0	1	0.01
	Paraliparis copei	Blacksnout snailfish	×	×	3,883	4	0.04
Lophiidae	*Lophius americanus*	Monkfish/ Goosefish	×	×	18,363	7	33.09
Lotidae	*Enchelyopus cimbrius*	Fourbeard rockling	×	×	296,156	621	21.40
	Gaidropsarus ensis	Threadfin rockling	×	NA	1867	0	0.00
Macrouridae	*Nezumia bairdii*	Common grenadier	×	×	638	1,008	39.67
Merlucciidae	*Merluccius bilinearis*	Silver hake	×	×	2,303	96	21.47
	Microgadus tomcod	Atlantic tomcod	×	NA	7,434	0	0.00
Myctophidae	*Notoscopelus kroyeri*	Lancet fish	NA	×	0	3	0.08
	Protomyctophum arcticum	Arctic telescope	×	NA	40,974	0	0.00
	Myctophidae sp.	Lanternfishes	NA	×	0	49	0.55
Osmeridae	*Mallotus villosus*	Capelin	×	×	156,961	63,083	658.58
Pholidae	*Pholis gunnellus*	Rock gunnel	×	NA	8	0	0.00
Phycidae	*Phycis chesteri*	Longfin hake	×	×	9,988	592	44.35
	Urophycis tenuis	White hake	×	×	72,912	328	156.80
Pleuronectidae	*Glyptocephalus cynoglossus*	Witch flounder	×	×	363,040	1598	236.25
	Hippoglossoides platessoides	American plaice	*Pleuronectidae* sp.	×	374,917	4,681	463.01
	Hippoglossus hippoglossus	Atlantic halibut	×	×	209,555	69	350.27
	Limanda ferruginea	Yellowtail flounder	×	×	2,845	3	0.12
	Reinhardtius hippoglossoides	Greenland halibut	×	×	609,277	4,836	1,437.77
Salmonidae	*Salmo salar*	Atlantic salmon	×	NA	3,994	0	0.00
	Salmo trutta	Brown trout	×		755		
	Salvelinus alpinus	Arctic char	×		9		
Scombridae	*Scomber scombrus*	Atlantic mackerel	×	×	39,172	139	1.24
	Scomberesox saurus	Atlantic saury	×	×	853	1	0.02
	Thunnus thynnus	Atlantic bluefin tuna	×	NA	7,311	0	0.00
Sebastidae	*Sebastes fasciatus*	Atlantic redfish	×	*Sebastes* spp.	6,554,008	309,842	30,875.70
	Sebastes mentella	Deepwater redfish	×		635,921		
Stichaeidae	*Eumesogrammus praecisus*	Fourline snakeblenny	×	×	6,094	19	0.62
	Leptoclinus maculatus	Daubed shanny	×	×	46,419	101	0.36
	Lumpenus lampretaeformis	Snakeblenny	×	×	41,026	124	3.92
	Stichaeus punctatus	Arctic shanny	×	NA	8	0	0.00

(Continues)

TABLE 1 (Continued)

Family	Species	Common name	Detection (84 stations)		Edna	Trawl catch data	
			eDNA	Trawl	No. of reads	No. of individual	Biomass (kg)
Zoarcidae	*Gymnelus retrodorsalis*	Aurora unernak	×	NA	2086	0	0.00
	Gymnelus viridis	Fish doctor	×	NA	7,177	0	0.00
	Lycenchelys verrillii	Wolf eelpout	×	NA	23,051	0	0.00
	Lycodes esmarkii	Esmark's eelpout	×	×	43	3	0.61
	Lycodes lavalaei	Newfoundland eelpout	×	×	27,148	19	3.34
	Lycodes polaris	Canadian eelpout	×	NA	7,393	0	0.00
	Lycodes terraenovae	Atlantic eelpout	×	×	6,499	2	0.43
	Lycodes vahlii	Vahl's eelpout	×	×	107,930	120	10.43
	Melanostigma atlanticum	Atlantic soft pout	×	×	247,346	128	0.40
Ceratiidae	*Cryptopsaras couesii*	Triplewart seadevil	NA	×	0	1	0.11
Myxinidae	*Myxine glutinosa*	Northern hagfish	NA	×	0	1,129	61.73
Nemichthyidae	*Nemichthys scolopaceus*	Atlantic snipe eel	NA	×	0	2	0.09
Paralepididae	*Arctozenus risso*	White barracudina	NA	×	0	403	7.05
Phosichthyidae	*Polymetme thaeocoryla*	Lightfishes	NA	×	0	1	0.02
Psychrolutidae	*Cottunculus microps*	Polar sculpin	NA	×	0	1	0.02
Rajidae	*Amblyraja radiata*	Thorny skate	NA	×	0	820	463.92
	Bathyraja spinicauda	Spinytail skate	NA	×	0	2	27.56
	Malacoraja senta	Smooth skate	NA	×	0	322	55.74
Sternoptychidae	*Polyipnus clarus*	Slope hatchetfish	NA	×	0	2	0.00
Stomiidae	*Chauliodus sloani*	Sloane's viperfish	NA	×	0	1	0.00
Synaphobranchidae	*Synaphobranchus kaupii*	Northern cutthroat eel	NA	×	0	2	0.03

3.3 | Community richness and composition as characterized by eDNA and trawl

Species richness per station was significantly higher ($p < .000009$, Student's paired t test) when detected by eDNA (mean = 18; standard deviation = 6) than when detected by trawl (mean = 14; standard deviation = 3.5). Shannon's diversity index also showed a higher mean value for eDNA richness per sampling site (mean = 1.0, standard deviation = 0.64) compared with trawl richness (mean = 0.8, standard deviation = 0.63; $p < .00004$, Student's paired t test).

Site-by-site community composition (presence/absence) differed significantly when characterized by eDNA or trawl methods (Figure 3), as indicated by a PERMANOVA (df = 83, P(perm) = 0.001). The difference in fish composition remained significant when estimated for only the shared species detected by both methods, while similarity increased from 15% to 17%, indicating the differences may have occurred due to differences in species identity and the efficiency of each method to detect fish species in different seabeds.

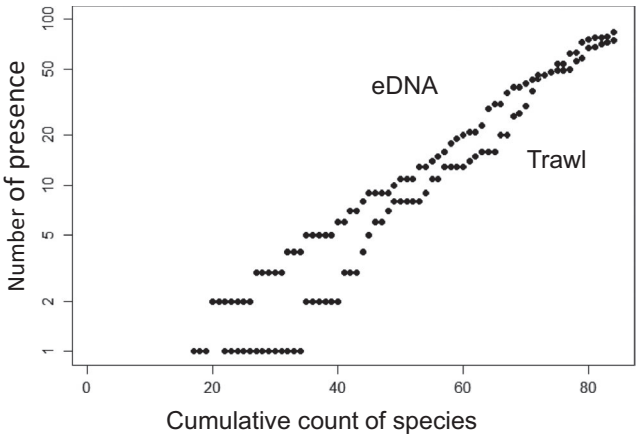

FIGURE 2 A plot of the cumulative empirical density function (CEDF) for species presences. Comparison of cumulative distribution of species occurrence for all taxa detected by eDNA and trawl within 84 stations. The x-axis is total taxa detected by both techniques (n = 84), and the y-axis is the average number of presence for each taxon. Data were square-root-transformed to de-emphasize dominant species. The y-axis is log-scaled, but retains the units in the original scale

3.4 | Community composition and relative abundance as characterized by eDNA and trawl

When comparing the relative abundance of the 47 shared taxa across 84 sampling sites, we observed a significant positive correlation between the two methods (see Figure F1 in Appendix S6). Indeed, RDA analyses showed that eDNA explained 76% of the variance in trawl individual catch (Adj. $R^2 = 0.76$; $p = .001$) and 72% of the variance in biomass data (Adj. $R^2 = 0.72$; $p = .001$), whereas 58% and 53% of eDNA variation were explained by trawl individual catch (Adj. $R^2 = 0.58$; $p = .001$) and biomass data (Adj. $R^2 = 0.53$; $p = .001$), respectively.

For the most abundant species, the two survey methods also provided a consistent overall pattern (Figure 4). *Sebastes* spp. was by far the most abundant taxon detected by both methods. The species *C. harengus* represented the third and fourth most abundant species detected by trawl and eDNA, respectively, whereas *R. hippoglossoides* was the fourth most abundant species detected by trawl and

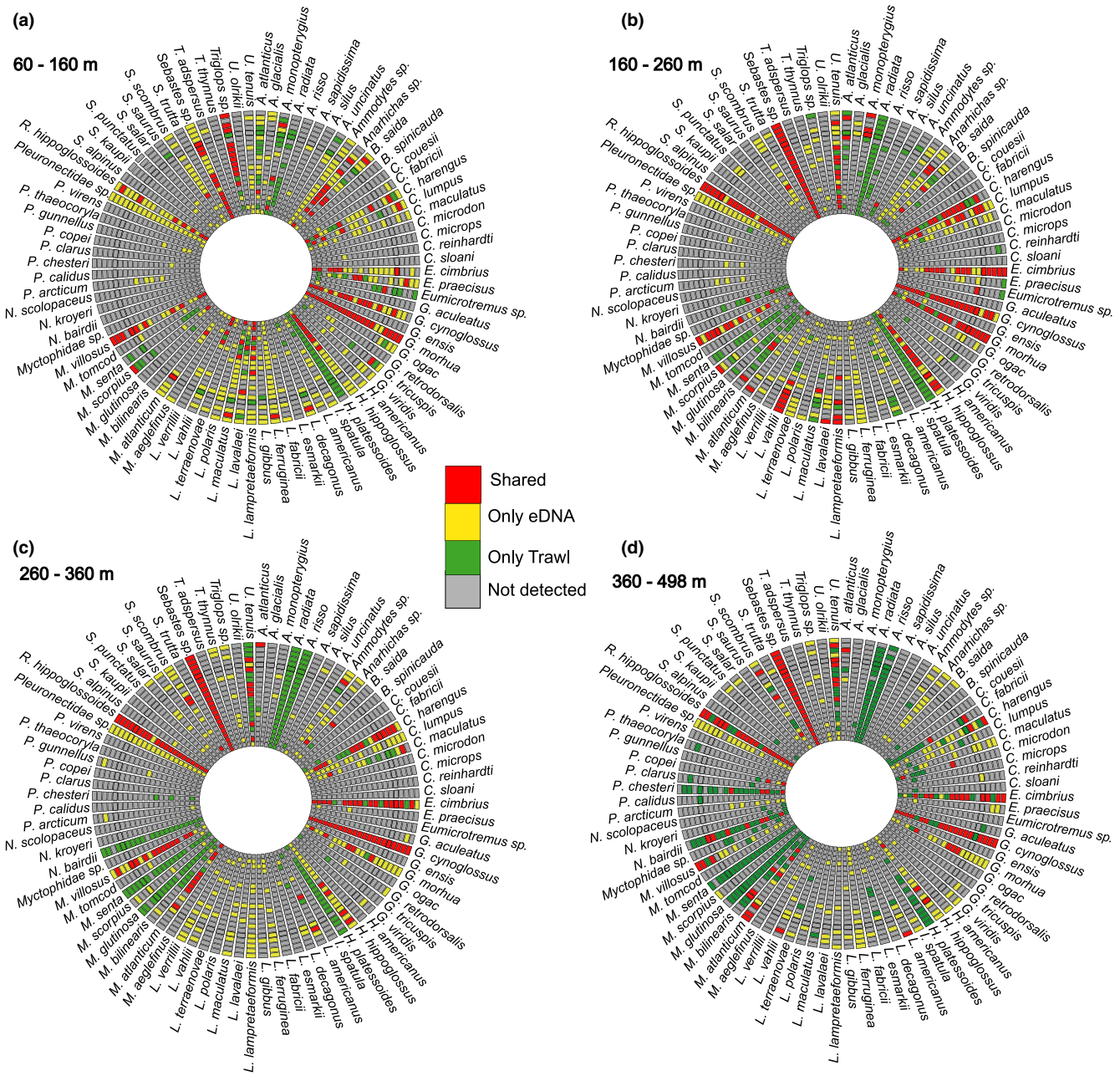

FIGURE 3 Circular heat map showing fish composition similarities (red and gray colors) and dissimilarities (yellow and green colors) of total fish taxa detected at each bottom station ($n = 84$) based on eDNA metabarcoding presence versus. trawling individual catch data. Each map (a, b, c, d) represents distribution of all taxa ($n = 84$) detected by both surveys within 21 stations (out of the 84) arranged from lower (60 m) depth to higher (498 m) depth. Color of each cell (station) indicates whether corresponding taxa is detected by both surveys (red), only eDNA (yellow), or only trawl (green) in that station. The gray cell indicates the taxa were not detected by any survey at the corresponding station

the third detected by eDNA. *Mallotus villosus* (Capelin) was the second most abundant species by trawl (10th most abundant species of eDNA), whereas *G. morhua* was the second most abundant species detected by eDNA (6th most abundant species by trawl). Other species represented only 4% of total trawl catches but 22% of the total eDNA reads. For the most abundant species, which are also commercially important fish, a highly significant Spearman's rank correlation coefficient (rho) was observed between relative eDNA reads and trawl catch relative abundance (*Sebastes* spp.: $\rho = 0.55$, $p = .0000004$; *R. hippoglossoides*: $\rho = 0.59$, $p = .0000004$, *C. harengus*: $\rho = 0.68$, $p = .00003$; *G. morhua*: $\rho = 0.59$, $p = .00002$; Figure 5).

3.5 | Effects of environmental variables on the consistency between eDNA and trawl surveys

The environment significantly affected the similarity between eDNA characterization and trawl characterization of the species community. However, given the high correlation ($r > 0.63$, $p < .05$; see Figure G1 in Appendix S7) between the four environmental variables recorded (depth, water temperature, salinity, and oxygen concentration), we cannot disentangle their respective effects.

Thus, the similarity between eDNA and trawl data for abundance of shared taxa (Figure 6a) was significantly influenced by environmental parameters (linear regression model; $p < .05$). The within-site Hellinger distance between eDNA relative abundance and trawl relative abundance decreased as a function of increased depth, water temperature, or salinity, and as a function of decreased oxygen concentration. Species richness was also more consistent between the two methods at stations of intermediate and deeper depth, that is, stations with higher temperature and salinity and lower oxygen concentration (Figure 6b).

3.6 | eDNA metabarcoding for assessing vertical variation in fish community along the water column

For the 30 stations sampled for eDNA throughout the water column at three depths (15 m, 50 m, and 250 m), a total of 2,957,751 reads identified 66 species representing 26 families (see Table B1 in Appendix S2). *Sebastes* spp. was the most abundant taxon in all three depths (44%, 49%, and 75% of total reads at 15, 50, and 250 m depths, respectively). Three other most abundant species were *M. villosus* (7%), *R. hippoglossoides* (6%), and *S. scombrus* (6%) at 15 m depth, *G. morhua* (10%), *R. hippoglossoides* (10%), and *H. hippoglossus*, 7%) at 50 m depth, and *C. harengus* (4%), *G. morhua* (4%), and *Melanostigma atlanticum* (Atlantic soft pout, 3%). Other species comprised 35%, 24%, and 11% of all reads at 15, 50, and 250 m depths, respectively. *Osmerus mordax* (Rainbow smelt, 0.03% of total reads) was an additional species relative to the previous analyses since it was not detected by either the bottom eDNA or the trawling survey but it was detected at depth 15 m in one station (94 reads).

We found no significant difference in species richness between the three different depths within the water column across stations (linear mixed model, $p = .64$; see Figure H1 in Appendix S8). By contrast, fish community composition (presence/absence) significantly differed between depths (PERMANOVA; 15 and 50 m: $p = .04$, 15 and 250 m: $p = .02$, 50 and 250 m: $p = .05$). Such differences in community composition between depths were mainly explained by the occurrence of 11 species (the blue vectors) based on Bray–Curtis dissimilarity matrix (Figure 7). Consequently, the overall dissimilarities between 15 and 50 m, 15 and 250 m, and 50 and 250 m depth samples were 69%, 59%, and 58%, respectively (SIMPER test, see Table D1 in Appendix S4), and *Sebastes* spp. was the main taxon explaining these dissimilarities.

4 | DISCUSSION

Given the current threat on marine ecosystem and aquatic resources (Jackson et al., 2001), there is an urgent need for improved marine conservation and effective conservation and management practices require reliable, noninvasive, and comprehensive survey methods. The present work demonstrated the efficiency and the accuracy of a noninvasive novel molecular tool, environmental DNA metabarcoding, in comparison with bottom trawling for conducting fish biomonitoring studies in marine ecosystem. Our results show that eDNA was able to detect a higher number of species than trawl, notably providing a promising tool for detecting rare species characterized by low abundance. On the other

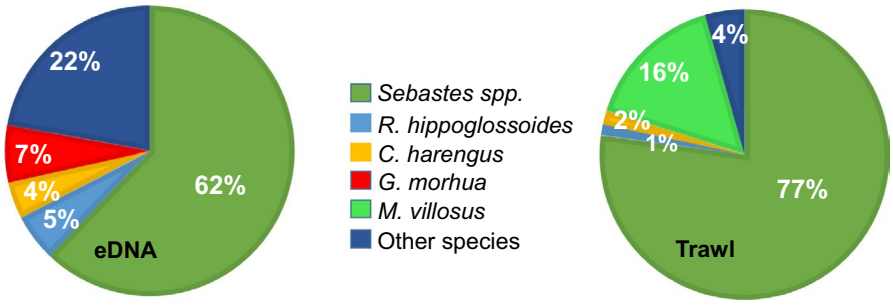

FIGURE 4 Proportion of the most abundant species detected by eDNA metabarcoding and trawling surveys. The relative proportion of trawling individual count of each taxon was compared to the relative abundance of each taxon detected by eDNA across all 84 sites. Three out of four most abundant species were similar between two methods, while the second most abundant species were different

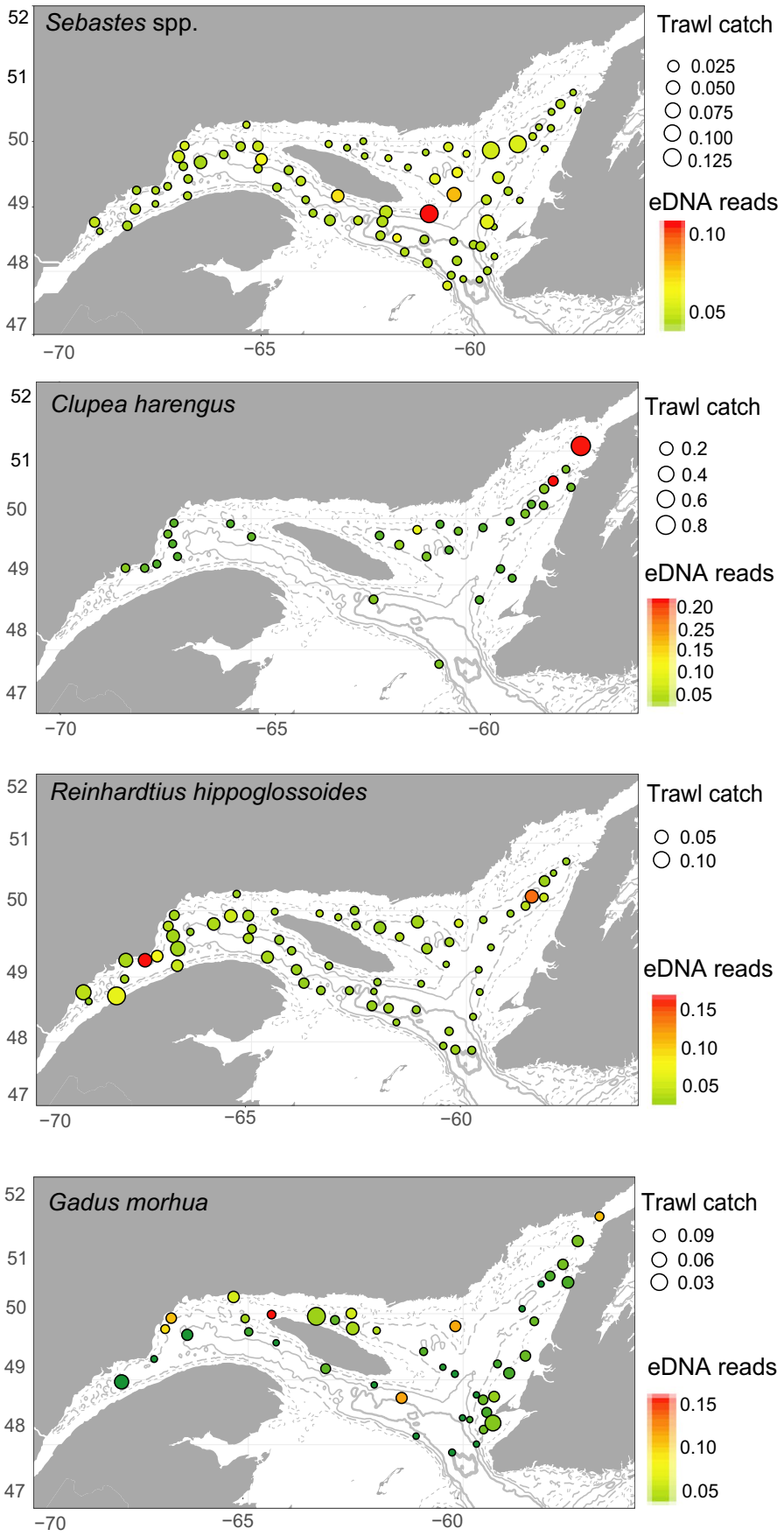

FIGURE 5 Bathymetric maps comparing the relative abundance estimated by eDNA and trawl for the four most abundant species distributed among 84 stations. The size of the circles indicates the trawl catch relative abundance, and color (green to red) indicates eDNA relative read abundance at a given site

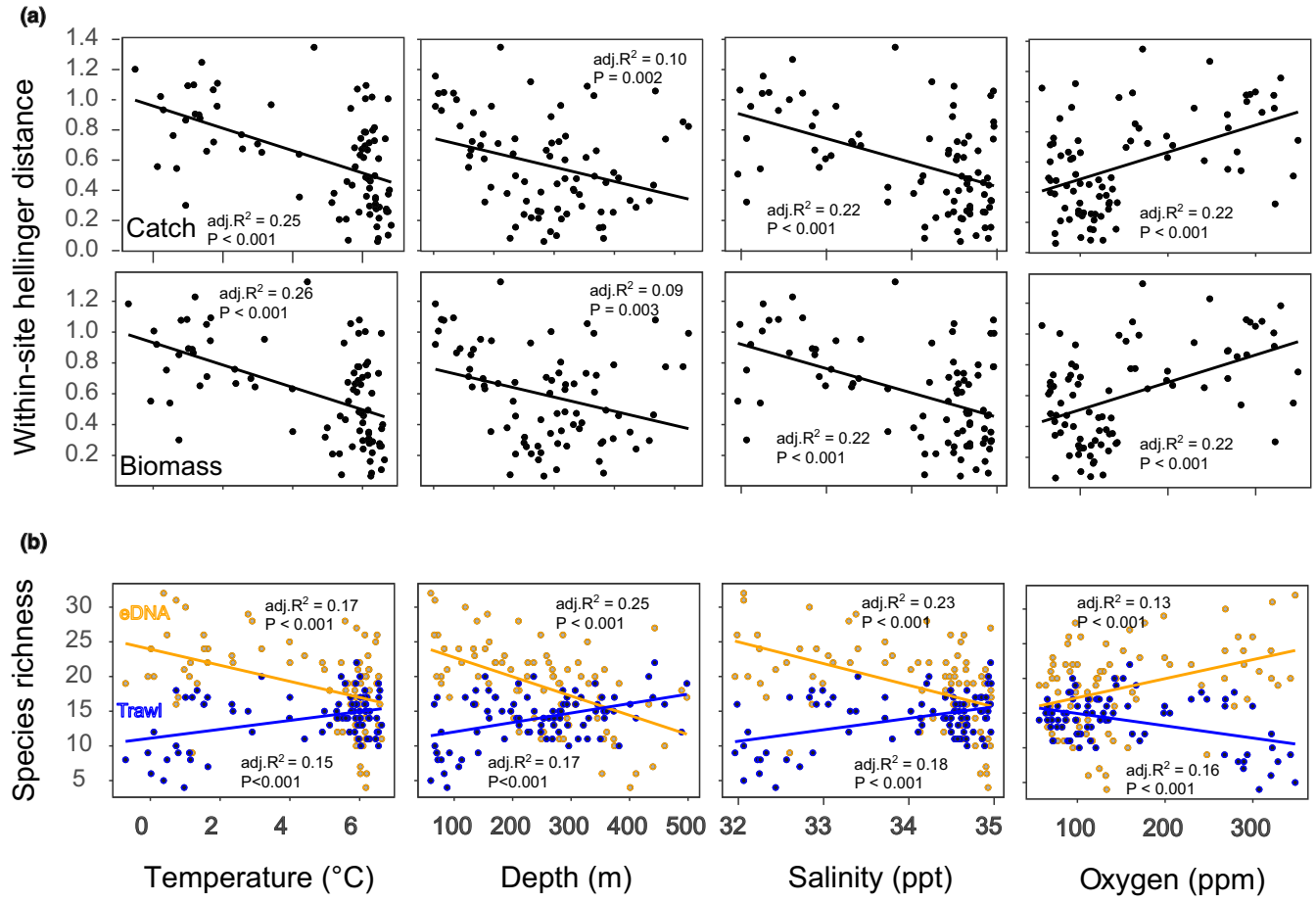

FIGURE 6 Linear regression plots depicting the effects of environmental variables on (a) the within-site Hellinger distance between eDNA metabarcoding and trawling abundance data and (b) species richness for the 84 bottom stations characterized by both trawl and eDNA methods

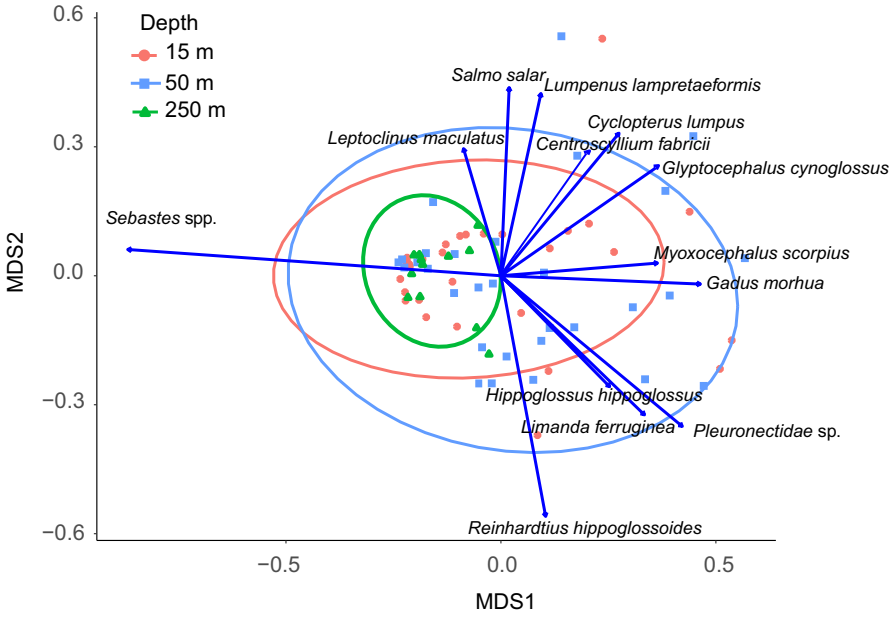

FIGURE 7 Nonmetric multidimensional scaling (nMDS) plot representing fish composition (presence/absence) dissimilarities across the water column, at different depths at the 30 stations in which only eDNA was analyzed. Points with different colors represent samples at different depths in the water column, and the blue vectors represent the species that are significantly different between the 3 depths based on Bray–Curtis dissimilarity matrix and 999 random permutations (stress value = 0.1)

hand, we also report a large overlap between the two methods at the community level and at species level (for the most abundant species) for abundance data, suggesting that eDNA metabarcoding could also be used for quantitative surveys. Moreover, the variation in species abundance in the trawling dataset was better captured by the variation in species abundance observed in the

eDNA dataset compared with the reverse, suggesting that eDNA is a promising tool for estimating community complexity in marine environment. We discuss hereafter the limitations and the advantages of an eDNA metabarcoding survey inferred from our compared analysis with trawling survey in the EGSL, as well as the other possibilities offered by the method, such as expanding the survey to area inaccessible to traditional methods, for instance, different depths within the water column.

4.1 | eDNA is a highly efficient tool to detect a wide range of species and to assess species richness

A total of 88 fish species were detected in our study by the combination of both techniques, among which eDNA recovered 82% (72 taxa) and trawl 73% (64 taxa of these 88 species). In our study, eDNA metabarcoding thus appears to efficiently complete trawling survey to monitor marine fish diversity that inhabits different sea environments or with different sizes, abundances, or behaviors. Our results are in accordance with several other studies that also identified more species with eDNA compared with other conventional methods in different ecosystems (e.g., Andruszkiewicz et al., 2017; Civade et al., 2016; Handley et al., 2019; Valentini et al., 2016). In addition, eDNA metabarcoding detected 72% of species captured by trawling, which is also congruent with previous comparative studies showing that eDNA detects between 60% and 100% of the species that were detected by other conventional methods (e.g., Fujii et al., 2019; Shaw et al., 2016; Sun et al., 2019; Thomsen et al., 2016; Valentini et al., 2016; Yamamoto et al., 2017). Here, it should be noted that eDNA lower detection of species that were observed by conventional methods could be explained by different sampling efforts. Indeed, Yamamoto et al. (2017) detected ~63% species that had been observed over 14 years of underwater visual censuses with eDNA, whereas Thomsen et al. (2016) detected 100% of species that had been caught by trawl in Greenland waters based on an equivalent numbers of sampling sites. Valentini et al. (2016) showed higher detection efficiency of eDNA metabarcoding when detecting bony fishes and amphibians compared with traditional or historical survey data. Fujii et al. (2019) compared eDNA with seven conventional methods and showed a 70% overlap between fish composition detected by eDNA compared with the other techniques. Sun et al. (2019) demonstrated that DNA metabarcoding may identify more than twice the number of taxa compared with net-based morphological identification methods. Altogether, these findings strongly suggest that eDNA at the very least offers similar but often higher performance of detection than those traditional methods. Yet, eDNA results must be interpreted cautiously and parsimoniously given: (a) the potential danger of introducing incorrect identifications from badly curated databases with incorrect assigned taxonomic identities, (b) the risk of overlooking species not being present in databases, or (c) inferring the presence of unlikely species being present because of a broad and global gene database is used as reference (e.g., inferring tropical species being present in polar seas or vice versa).

4.2 | Discrepancies between eDNA and trawl provide evidence for both limitations and advantages for assessing species composition

Despite the similarities in some aspects of the general patterns observed between both methods, we also observed discrepancies. Thus, species detected by eDNA but missed by trawl were mostly anadromous, pelagic, small, rare, or those inhabiting rocky and muddy areas (see details in Appendix S3). This highlights the limited ability of trawl to capture taxa in particular type of sea habitats, or fish with different sizes and behaviors, while eDNA could theoretically detect fish in any type of habitat, with different swimming behaviors and sizes when the metabarcoding protocols are well-established. These dissimilarities may also be explained by the ability of eDNA-based approach to detect organisms at different life stages (different sizes) compared with net-based traditional methods that only catch mature individuals with specific size ranges. Also, previous research demonstrated eDNA power to detect aquatic species, especially endangered (Plough et al., 2018), invading (Dejean et al., 2012; Mahon et al., 2013; Piaggio et al., 2014; Takahara, Minamoto, & Doi, 2013; Wilcox et al., 2018), or rare species (Jerde, Mahon, Chadderton, & Lodge, 2011; Rees, Maddison, Middleditch, Patmore, & Gough, 2014; Thomsen & Willerslev, 2015).

In contrast, Erdozain et al. (2019) identified significantly fewer genera (15.9%) and families (11.5%) than conventional method when compared to stream macroinvertebrate community based on DNA metabarcoding. In our study, although eDNA metabarcoding detected a higher number of species overall, it also failed to identify several species that were captured by trawling survey. This was likely due to a combination of some limitations related to some primer biases, incompleteness of reference database, or possible stochasticity for species with low biomass. The MiFish primer sets (U) that were used in the present work were able to identify sequence reads at the species level for 94% of the taxa, but eDNA also failed to detect species inside the Rajidae family. This is because, with the exception of the *Centroscyllium fabricii* (Black dogfish), this primer set could not decipher most of the rays and shark species (Miya et al., 2015). Using multiple universal primers (e.g., a combination of MiFish-U and MiFish-E) is a solution that contributes to increase in the species detection rates in eDNA studies (Miya et al., 2015; Shaw et al., 2016; Valentini et al., 2016). This is particularly true for taxa that shared the same sequences at the species level for a given marker. For example, in our study, two species of Pleuronectidae, that is, *H. platessoides* and the *Pseudopleuronectes americanus* (Winter flounder), were 100% identical for the 12S marker. Adding a new marker (and developing the related reference database for it) would have likely resulted in the detection of the *H. platessoides* since only this species was captured by trawl.

The false-negative detections often occur when the affinity between primer and primer-binding sites during PCR is low. Due to the high risk of primer bias or design deficiency, a thorough in silico and in vivo evaluation of universal primers is needed to test their biodiversity coverage before selecting suitable primer sets minimizing PCR biases for specific DNA metabarcoding protocol fitted to geographic regions and taxonomic groups of interest (Elbrecht & Leese, 2017).

Moreover, the incompleteness of the 12S reference database available in GenBank is another problem encountered by the eDNA method in our study. Previous eDNA metabarcoding studies also attributed misidentification to the gap in reference database (e.g., Miya et al., 2015; Thomsen et al., 2016). Therefore, the improvement of reference sequences is critical for increasing assay quality in metabarcoding eDNA studies (Hajibabaei, Singer, Hebert, & Hickey, 2007; Tytgat et al., 2019). However, the generation and submission of reference sequence will keep increasing the representation of missing taxa over time, but it is crucial to examine the quality of submitted sequences from correctly identified specimens to prevent deposition of insufficient or incorrect annotated barcode sequences in public databases (Bengtsson-Palme et al., 2016; Porter & Hajibabaei, 2018), leading to misidentifications of species. Therefore, it is important to engage taxonomic experts to insure the reliability of database before submitting a reference sequence to a public database (Collins & Cruickshank, 2013).

Lack of sampling replication may also influence species detection by eDNA (Shaw et al., 2016). Previous eDNA metabarcoding studies found that the number of detected taxa is higher and is more representative of the actual diversity when sampling multiple replicates per sampling location rather than increasing the volume of water sampled per site (Kelly et al., 2014; Shaw et al., 2016). Here, because of time and logistical constraints we collected 2 L water samples only once, and therefore, we recommend increasing of the number sample replications in the future studies (e.g., 3 × 1 L volume), when at all possible. Beentjes, Speksnijder, Schilthuizen, Hoogeveen, and van der Hoorn (2019) compared the effects of spatial and temporal eDNA sampling and concluded that the dissimilarity of temporal replicates at a one-week interval was comparable to that of spatial replicate samples. These authors showed that replication leads to better estimations of total biodiversity, where the effects of spatio-temporal sampling replicates are significantly greater than PCR replications, resulting in a substantial increase in richness. In our study, although our sampling was done within a one-month period only, the monthly variation in eDNA composition could still partly impact on the results. In fact, this is an important consideration for any eDNA study with different sampling time points.

Moreover, it has been shown that different eDNA metabarcoding experimental methods with various filter material, pore size, and extraction method affect the marine taxa eDNA yield (Deiner et al., 2018; Hinlo, Furlan, Suitor, & Gleeson, 2017; Lacoursière-Roussel, Côté, Leclerc, & Bernatchez, 2015), so multiple methods may be needed for different locations to increase the detection efficiency (Deiner et al., 2018). Bush et al. (2019) reviewed the factors of uncertainties and challenges underlying eDNA metabarcoding and traditional methods, and concluded that sources of uncertainty associated with metabarcoding can be minimized more easily than traditional approaches by applying standard and automated operating procedures. Overall, this supports that metabarcoding eDNA is a promising tool that will enhance our comprehension of marine and other aquatic ecosystems despite a need for further development of methods and databases to increase the accuracy of species detection.

4.3 | eDNA metabarcoding provides a quantitative assessment of species composition

Despite the discrepancies discussed above, we found a strong association between eDNA relative number of reads and trawl relative abundance and biomass in our 47 shared species dataset. This positive association suggests that eDNA metabarcoding holds the ability to reflect a quantitative estimate of biodiversity for marine or aquatic ecosystems. Estimating biomass and abundance provides important information for conservation of rare and endangered species and in the management of population sizes (Jerde et al., 2011; Takahara, Minamoto, Yamanaka, Doi, & Kawabata, 2012; Tréguier et al., 2014). A positive correlation between eDNA and biomass or abundance datasets has also been reported previously in different aquatic environments (Kelly et al., 2014; Lacoursière-Roussel et al., 2015; Thomsen et al., 2016; Yates, Fraser, & Derry, 2019). Furthermore, Takahara et al. (2012) demonstrated that species biomass in natural environments could be estimated by eDNA more easily and more rapidly than using traditional methods. In contrast, Knudsen et al. (2019) did not find any significant correlation between eDNA concentrations and the biomass of the targeted marine fish caught by the simultaneous trawling, suggesting that improving the molecular assays protocols may be needed in some cases. Also, Lamb et al. (2019) conducted a meta-analysis and suggested a weak quantitative relationship between the biomass and eDNA sequence reads with large degree of uncertainties.

Nevertheless, the present study revealed a significant correlation between eDNA reads and trawl catch data at both the community level and the species-specific level (for the most abundant and most commercially important species), suggesting the increased value of eDNA technique as a monitoring tool for broad application. For example, *Sebastes* spp. was the most abundant species, representing 62% of eDNA total detection and 77% of trawl capture. This corroborates a recent report of increases in the abundance and the biomass of *Sebastes* spp. (*Sebastes fasciatus* and *Sebastes mentella* which are managed by DFO as a single species of Redfish) (Bourdages et al., 2018). This indicates that eDNA tools could increasingly be used to monitor quantitatively marine population to support fisheries management programs.

Interestingly, we found more concordance between the two surveys in terms of species richness and relative abundance in warmer and deeper waters, with higher salinity and lower oxygen rates. On the one hand, this observation could possibly be explained by the lower efficiency of trawling in shallower waters (Hoffman, Bonzek,

& Latour, 2009), or to different communities including fish more or less detected by one of the two methods. On the other hand, this trend may also be linked to the effect of environmental factors on the production and degradation of eDNA, an area under active research (Jo, Murakami, Yamamoto, Masuda, & Minamoto, 2019; Lacoursière-Roussel, Rosabal, & Bernatchez, 2016; Strickler, Fremier, & Goldberg, 2015; Tsuji, Ushio, Sakurai, Minamoto, & Yamanaka, 2017). For instance, Collins et al. (2018) reported that eDNA degrades 1.6 times faster in the inshore environment than the offshore. Since eDNA detectability over time depends on its degradation rates in water (see Barnes et al., 2014), the interpretation of eDNA outputs therefore requires to fully understand the biotic and abiotic factors affecting eDNA detectability to develop eDNA protocols suited for different types of sampling environment (Ruppert, Kline, & Rahman, 2019; Stewart, 2019). Also, Lacoursière-Roussel, Rosabal, et al. (2016) showed that fish release more eDNA in warm water, such that eDNA concentration could better reflect fish abundance or biomass at higher temperature. Moreover, it can be argued that fish body surface area could potentially play a role in eDNA production. Clearly, further research is needed to clarify the mechanisms underlying the higher differentiation estimate between eDNA tool and conventional methods depending on the environmental conditions (Lacoursière-Roussel & Deiner, 2019).

4.4 | Beyond traditional surveys, the assets of eDNA metabarcoding

One major advantage of eDNA methods for marine biomonitoring is that they can be performed anywhere in a standardized way, including areas inaccessible to trawling. Here, we took advantage of the eDNA survey to explore fish communities along the water column and provided a comprehensive description of the vertical distribution of marine fish communities that is not possible with traditional techniques. This was further proved by detecting *O. mordax*, a common coastal species of EGSL, around estuary area at depth 15 m, which was missed by trawl. Similarly, Andruszkiewicz et al. (2017) documented vertical variation in marine vertebrate communities using eDNA metabarcoding throughout different depths resulting in identification of new taxa missed by traditional surveys. In contrast, when we compared species richness obtained from different depths at 30 stations with those obtained by eDNA from bottom at the same stations, we found six fish species that had been found at bottom, but were not detected across the depth gradient (see Table B1 in Appendix S2). All of these species were benthic or demersal fish species that feed and live on the seafloor or close to the bottom or deeper than 250 m, suggesting that a fine-scale sampling of eDNA may provide a biologically relevant and comprehensive characterization of fish with different depth profiles. The application of eDNA metabarcoding at finer temporal and spatial resolution compared to traditional biomonitoring has been demonstrated by a number of scientific works (e.g., Andruszkiewicz et al., 2017; DiBattista et al., 2019; Hanfling et al., 2016). We here confirmed the usefulness of eDNA metabarcoding to determine marine fish distribution in different water layers and to characterize fish communities' composition, richness, and relative abundance at different depths that might be fruitful for marine biodiversity assessments.

5 | CONCLUSION

In this study, eDNA metabarcoding applied in a complex marine ecosystem outperformed trawling survey by enabling detection of more species, including rare and endangered taxa. This technique could allow exploration of fish communities in different seabeds that were missed or were not accessible by trawl gears. eDNA metabarcoding applied in this study also characterized fish taxa across different water strata. The value of eDNA to provide a comprehensive overview of marine species distribution was further confirmed by good correlations between detection sensitivity and relative abundance of both surveys. We further demonstrated that eDNA could go beyond species presence/absence reports since it reflects the local biomass, which may have important implication for fisheries managements. Although eDNA metabarcoding techniques must still be improved (e.g., need to more complete reference databases and correct taxonomic identities associated with sequences deposited) and cannot replace traditional methods to provide information on phenotypes, and exact location of target species, we suggest that modern biomonitoring programs should integrate eDNA metabarcoding with traditional surveys to provide more reliable and clearer pictures of fish diversity for science-based policy decisions. Given its noninvasive nature and its potential to diminish costs and time, we recommend eDNA metabarcoding as a complementary tool to collect information on species richness, and distribution, and to some extent, relative abundance in the context of large-scale biomonitoring of multiple species in marine ecosystems. Meanwhile, continued research and development is required to better interpret the information provided by eDNA and its limitations (Lacoursière-Roussel & Deiner, 2019), including the improvement of reference sequence databases.

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DATA AVAILABILITY STATEMENT

All analytical R codes are available as supplementary files accompanying this manuscript. The data that support the findings of this study will be openly available through the NCBI's Sequence Read Archive PRJNA639118 (SRA, http://www.ncbi.nlm.nih.gov/sra).

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SUPPORTING INFORMATION

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

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