

Groundtruthing of pelagic forage fish detected by hydroacoustics in a whale feeding area using environmental DNA

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Abstract

Conservation of whales, considered as umbrella species in marine environments, requires to be able to understand their relationships with ecosystem components such as prey species, including pelagic fish. However, studying such relationships in nature is a technical challenge. In this study, we used two noninvasive methods in combination, namely hydroacoustics and environmental DNA (eDNA), to detect five pelagic or semipelagic fish species in the Saguenay–St. Lawrence Marine Park (Québec, Canada): the sandlance *Ammodytes* sp., the Atlantic herring *Clupea harengus*, the capelin *Mallotus villosus*, the rainbow smelt *Osmerus mordax*, and the redfish *Sebastes* sp. The Marine Park is a major summer feeding ground for a wide diversity of marine wildlife species, including the endangered St. Lawrence beluga whale population. Up to now, scarce research efforts have been dedicated to the estimation of pelagic fish abundance and diversity in this area. Hydroacoustics allowed to easily discriminate the classification of echoes from fish, and with certain limitations to distinguish swim bladder fish from fish without swim bladder. We used eDNA to groundtruth acoustics data and to improve species identification. eDNA analyses especially demonstrated that the capelin was the most predominant species, while the abundance of the redfish and the sandlance was strongly variable over the 2 years of the study. Our results also suggest that there are annual fluctuations in prey availability that marine mammals encounter in this area. Although the approach we used is not without constraints that should be addressed in future studies, we hope that this study will contribute to science-based conservation and fisheries management policies.

KEYWORDS

environmental DNA, hydroacoustics, pelagic fish, whales

C. S. Berger and B. Bougas contributed equally to this work.

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1 | INTRODUCTION

Conservation of marine biodiversity requires obtaining an accurate description of the trophic ecology between marine predators and their prey (Terborgh & Estes, 2013). Since the 70s, the global will to protect marine mammals, namely whale populations depleted by hunting, has specifically led to regulatory measures in whale catching for commercial purposes (Birnie, 1989). Although these measures favored recovery in some whale populations, others remain critically endangered (Attard, Beheregaray, & Möller, 2016; Clapham, Young, & Brownell, 1999). The increase in contemporary threats is thought to impede the recovery of depleted whale populations such as increased contamination in coastal areas, maritime traffic, overfishing of prey sought out by whale species, and the impacts of climate change. Conservation of whales, considered as umbrella species, has been a strong motivation for wider marine ecosystem conservation objectives. In this context, it has been proposed that regulatory measures must take into account the unique characteristics of each marine mammal population, including their relationships with ecosystem components such as prey species like other fish. However, tracking such relationships in nature remains a technical challenge (Birnie, 1989).

The Saguenay–St. Lawrence Marine Park (Québec, Canada) and surrounding waters are recognized as a major summer feeding ground for a wide diversity of marine wildlife species. Marine mammals converge to the head of the Laurentian Channel to feed from spring to fall, indicating that particular bathymetric features and oceanographic processes (e.g., tidal upwellings) have an influence on pelagic prey availability such as pelagic fish and euphausiids (Ménard, 1998; Simard, 2009). Schooling pelagic fish are major components of marine trophic webs that are consumed by higher trophic levels. Among the common wildlife species in the St. Lawrence Estuary (SLE), several species of whales, seals, and seabirds are pelagic fish predators. Changes in the availability of pelagic fish have been suggested as a possible explanation for the observed decline in fin whale abundance in their traditional feeding areas at the head of the Laurentian Channel observed since the early 2000s (Martins, Turgeon, Michaud, & Ménard, 2018). Since the same period, shifts in environmental regimes at the scale of the Gulf of the St. Lawrence Estuary, namely demersal and pelagic fish availability and composition, have also been proposed as a contributing factor in the decline of the St. Lawrence beluga whale population (*Delphinapterus leucas*) (Plourde et al., 2014). In this context, the need to understand the influence of pelagic prey availability in sustaining marine wildlife biodiversity in the SLE catalyzed the development of studies in the Saguenay–St. Lawrence Marine Park (Ménard, 2009).

Although significant work has been done in the SLE on zooplankton communities (Gagné et al., 2013), scarce research efforts have been dedicated to pelagic forage fish. The community of schooling pelagic forage fish is relatively low in diversity in the SLE, being essentially composed of five species: the sandlance *Ammodytes* sp., the Atlantic herring *Clupea harengus*, the capelin *Mallotus villosus*, the rainbow smelt *Osmerus mordax*, and the Atlantic mackerel *Scomber scombrus*. These five fish species are considered as prey for cetaceans, although the cold upwelled waters at the head of the Laurentian Channel are

considered inadequate habitat for both the rainbow smelt, found in surrounding warmer areas, and the Atlantic mackerel, found mainly in the lower SLE and Gulf of St. Lawrence. The redfish *Sebastes* sp. is a semipelagic species which has undergone a spectacular rebound of its population, with the presence of juvenile redfish abundance from 2011 to 2013 cohorts that demonstrate record biomass (Senay et al., 2019). Redfish is not generally considered as an important prey species for cetaceans, although juveniles are pelagic and can form schools that could be consumed by whales.

In 2009, initiated largely for conservation purposes because of the observed decline in the number of fin whales and to better understand the ecological needs of the endangered St. Lawrence beluga whales, a monitoring of the spatial distribution and abundance of potential prey of marine wildlife predators, namely euphausiids and pelagic fish, was launched by Parks Canada in the Saguenay–St. Lawrence Marine Park. This monitoring program relied on hydroacoustic technology and systematic simultaneous visual surveys of seabirds and marine mammals. The objective was to better understand how prey spatiotemporal variability influenced marine wildlife predators in terms of abundance and diversity and to locate and characterize the environmental conditions of feeding areas for marine mammals.

Fisheries acoustic methods have been widely used for the monitoring of fish and zooplankton, and to estimate their abundance and distribution (Holliday, Pieper, & Kleppel, 1989; Masse, 1996). They offer the advantages of continuously sampling at high-resolution large volumes of water and being a noninvasive method. However, one of the main challenges and limitations in fisheries acoustics is species identification (Horne, 2000). It is now well documented that acoustic backscatter strength from fish and zooplankton is frequency-dependent. Differencing the mean volume backscattering strength ($\Delta MVBS$) has been used as a multifrequency classification method to discriminate backscatter from fish to zooplankton (Madureira, Everson, & Murphy, 1993), between euphausiid species (McQuinn, Dion, & St. Pierre, 2013), and between fish species (Korneliussen & Ona, 2003). Since the swim bladder contributes to at least 90% of the sound scattered, fish species can be separated in two groups depending on the presence or absence of a swim bladder (Foote, 1980). With the exception of sandlance, a pelagic fish which is also characterized by its particular burrowing behavior (Robards, 1999), all the other species which form the pelagic fish communities at the head of the Laurentian Channel harbor an internal swim bladder. Previous studies documented that fish with swim bladder have a weakly decreasing volume backscattering coefficient (S_v) with increasing frequency, while fish without swim bladder have a S_v essentially frequency independent at 18–120 kHz and peaked at 200 kHz (Korneliussen & Ona, 2002).

However, species discrimination by multifrequency classification methods requires to be groundtruthed. This is traditionally performed by conventional capture methods such as trawling, which has the advantage of providing information on organism size but also the disadvantage of being invasive. Moreover, trawls are selective (Wileman, 1996), particularly for size (Bethke, Arrhenius, Cardinale, & Håkansson, 1999), and many fish can escape (Engås & Godø, 1989)

or avoid nets altogether (Fonseca, Sanches Fernandes, Fontainhas-Fernandes, Monteiro, & Pacheco, 2016; Kaartvedt, Staby, & Aksnes, 2012). Due to local environmental conditions characterized by strong currents and a rugged seafloor, trawling has proven to be ineffective in the area studied here. Another method to obtain additional evidence for species identification, organism size and tilt angles, is imagery. Because of strong currents and dark and turbid waters, it might remain difficult or impossible to groundtruth acoustic signals using video imagery techniques. Thus, after numerous attempts to groundtruth acoustic signals by various fishing methods and imagery, there was a need to find a method that could effectively be used in combination with hydroacoustics to groundtruth the acoustic backscatter and improve the interpretation of acoustic data.

Recent developments in environmental DNA (eDNA) (Deiner et al., 2017; Rees, Maddison, Middleditch, Patmore, & Gough, 2014; Taberlet, Bonin, Zinger, & Coissac, 2018) offer the potential to groundtruth acoustic data of pelagic fish in marine ecosystems. DNA is released by organisms in the environment from skin cells, mucus, metabolic waste, or gonads. In other words, eDNA is a signature of the organism's presence in the environment. Therefore, eDNA studies are used to detect DNA from organisms without the need to capture them by collecting samples from their environment (Lodge et al., 2012). eDNA allows to estimate fish diversity in aquatic environments (Li et al., 2018), as well as to predict the biomass of organisms (Coulter et al., 2019; Lacoursière-Roussel, Côté, Leclerc, & Bernatchez, 2016; Lacoursière-Roussel, Rosabal, & Bernatchez, 2016).

The objective of this study was to evaluate the efficiency of eDNA to identify the species composition of pelagic forage fish shoals detected by hydroacoustics in the Saguenay–St. Lawrence Marine Park. eDNA and hydroacoustics have been recently used in combination to assess the distribution and the abundance of the Japanese jack mackerel *Trachurus japonicus* (Fukaya et al., 2018; Yamamoto et al., 2016), and they have been described as an innovative approach to comprehensively detect aquatic species (Miksis-Olds & Watts, 2019). But to our knowledge, their ability to noninvasively detect several fish species simultaneously has never been tested so far. Here, we used eDNA and hydroacoustics to detect five pelagic or semipelagic fish species (the sandlance *Ammodytes* sp., the Atlantic herring *C. harengus*, the capelin *M. villosus*, the rainbow smelt *O. mordax*, and the redfish *Sebastes* sp.). Our study, which was conducted over a 2-year period in different areas of the marine park, highlights the spatiotemporal variability in food resources that marine mammals encounter in the SLE.

2 | MATERIALS AND METHODS

2.1 | Collection of acoustic data and water samples in fish shoals

A total of 20 shoals were sampled by acoustics and for environmental DNA, including 12 shoals in summer 2017 (Figure 1, orange circle;

sampled between 18 July and 13 October 2017) and eight shoals in summer 2018 (Figure 1, red triangle; sampled between 17 July and 20 September 2018). The fish shoals were detected by hydroacoustics in areas known for fish aggregation and in different areas to insure variety in species composition. Information about each shoal (date of sampling, GPS coordinates, mean temperature (°C), mean salinity (PPM), and mean depth (m) measured at each location) is reported in Table S1.

Acoustic data were collected continuously and simultaneously to the water sampling from the Park's Canada vessel *L'Alliance* using a Simrad EK60 multifrequency acoustic system operating at 38, 120, and 200 kHz. Calibrations of the system were performed every year with the standard-target method (Foote, 1990; Simmonds & MacLennan, 2005), using a 38.1 mm tungsten-carbide (6%) sphere suspended from motorized tripoint lines. The transducers were installed at 1 m below the surface on the port side of the vessel. The acoustic system configurations were adjusted to sample equivalent volumes on all transducers (Korneliusson, Diner, Ona, Berger, & Fernandes, 2008). All frequencies were transmitted simultaneously once per second with a 1.0 ms pulse length. Raw volume backscattering strength (S_v dB re 1 m^{-3}) data were collected in Simrad format using the ER60 software. Additionally, a conductivity–temperature–depth (CTD) profiler was deployed in all selected shoals to record environmental parameters (Figure 2). Only the first CTD cast within each shoal was selected since it was the only one to respect the soaking time.

For environmental DNA, water samples were collected on the same boat with a sterilized Niskin bottle (1 L) deployed along the CTD cable at the desired depth of sampling in fish shoals (Figure 2). No fish are being collected or processed on this boat or elsewhere by the members of the crew. Negative field controls (blanks) using distilled water were done for every shoal. These controls were performed on the boat in the field and treated in the same way as the real samples to estimate the possible contamination of the filtration material. Within a given shoal, water samples were taken at four locations (T1, T2, T3, and T4) at depths corresponding to the core of the shoal as detected by hydroacoustics, as well as at one location upstream of the shoal by taking into account the general direction of surface currents. The upstream water sample was taken outside of the shoal of interest (minimum distance of 323.71 m, mean distance of 1,275.05 m between the upstream location and the shoal of interest), in an area exhibiting highly similar physico-chemical properties (in terms of temperature, salinity, depth) as the core of the shoal (Table S1). Because physico-chemical properties were similar between the upstream location and the core of the shoal, we expected the eDNA concentrations not to be affected by these environmental factors. The upstream location was sampled to serve as an external reference of the shoal of interest, for which no or very low levels of environmental DNA detection of the five species were expected. For four shoals in 2017 (IDs: 2017_05, 2017_07, 2017_09 and 2017_12) and one shoal in 2018 (ID: 2018_03), no upstream sampling was done because of the large size of the shoals that prevented to

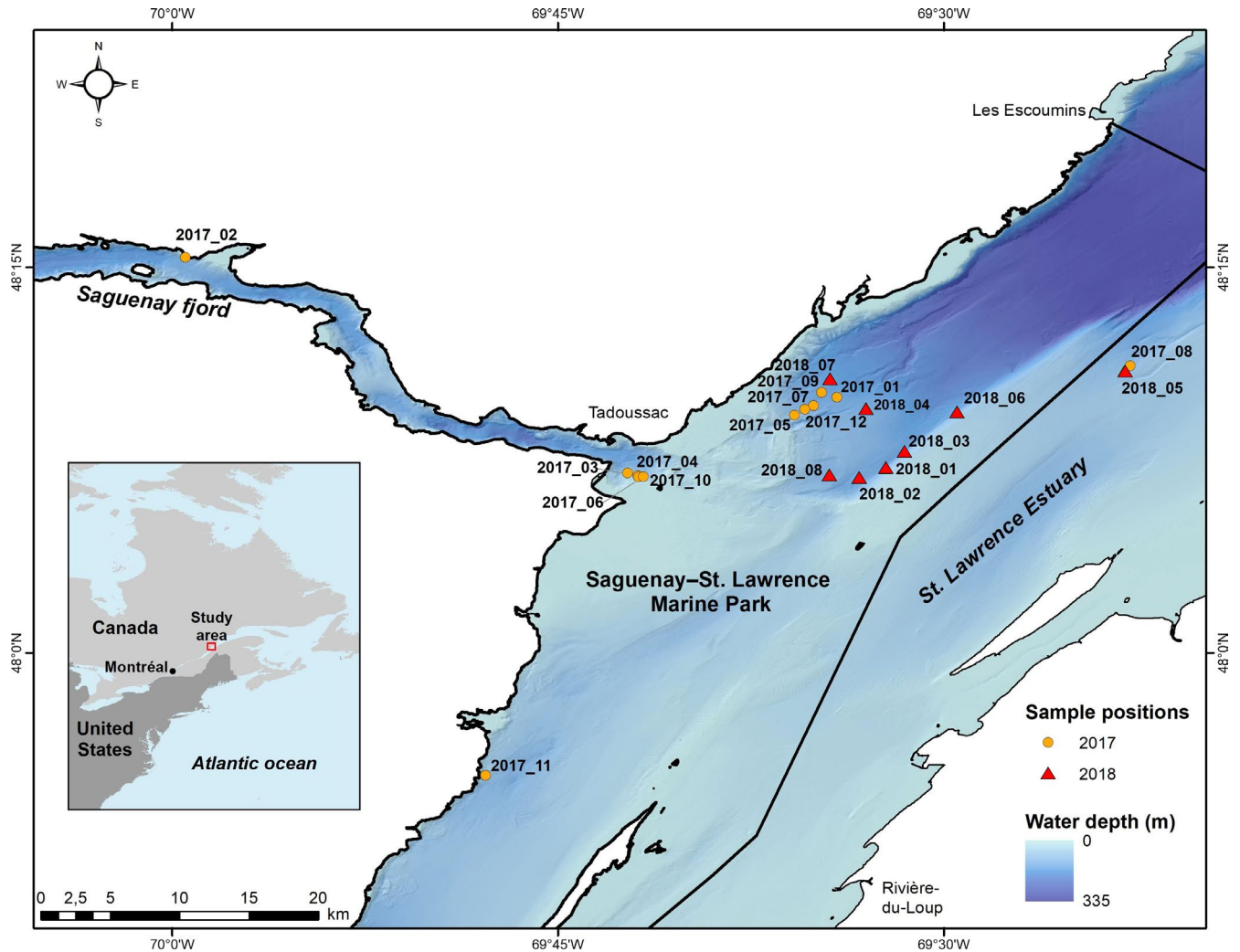


FIGURE 1 Location of the 20 shoals that were sampled by acoustics and for environmental DNA in the Saguenay–St. Lawrence Marine Park. Orange circles indicate the shoals sampled in 2017. Red triangles indicate the shoals sampled in 2018. The Saguenay–St. Lawrence Marine Park is delimited by solid black lines. Most of the shoals are located near the head of the Laurentian Channel

clearly define the end of the shoals. Water sampling and filtration using a peristaltic pump were performed within 24 hr in a Parks Canada laboratory space and according to the protocol described in Lacoursière-Roussel, Côté, et al. (2016), Lacoursière-Roussel, Rosabal, et al. (2016). Water samples were vacuum-filtered onto a small pore size Whatman glass microfiber filter (47 mm diameter; 1.2 μm pore size; Whatman). In total, 115 filters (95 eDNA samples and 20-field negative controls) were frozen and stored at -20°C until eDNA extraction.

2.2 | Acoustic data processing

Acoustic data were processed using Echoview Software version 6.1 (Myriax Software) and standard cleaning techniques (Simmonds & MacLennan, 2005). The seafloor was automatically detected, and an offset of 0.5 m was applied. Then, the bottom line was manually edited to exclude all false bottom detection. Data shallower than 5 m, which is twice the transducer near-field, were also removed from

analysis to eliminate the transmit pulse and minimize backscatter from surface bubbles (Simmonds & MacLennan, 2005). In order to minimize the effects of background noise, we estimated the signal-to-noise ratio (SNR) at each frequency using a previously developed method (De Robertis & Higginbottom, 2007) and excluded all data with $\text{SNR} < 10$ dB for either frequency. In addition, a background threshold of -80 dB was applied. Finally, the echograms were manually edited to remove logging artifacts (e.g., surface bubbles, side lobes). For each fish shoal sampled, a postcalibration was applied to the acoustic data in order to modify the sound speed according to the CTD profile. One shoal (ID: 2017_02) was completely excluded from the acoustics analysis since the most of the backscatter was included in the side lobes.

2.3 | Acoustic multifrequency response

Fish shoals sampled were delineated manually on the echograms by creating region around them. Each frequency of the processed

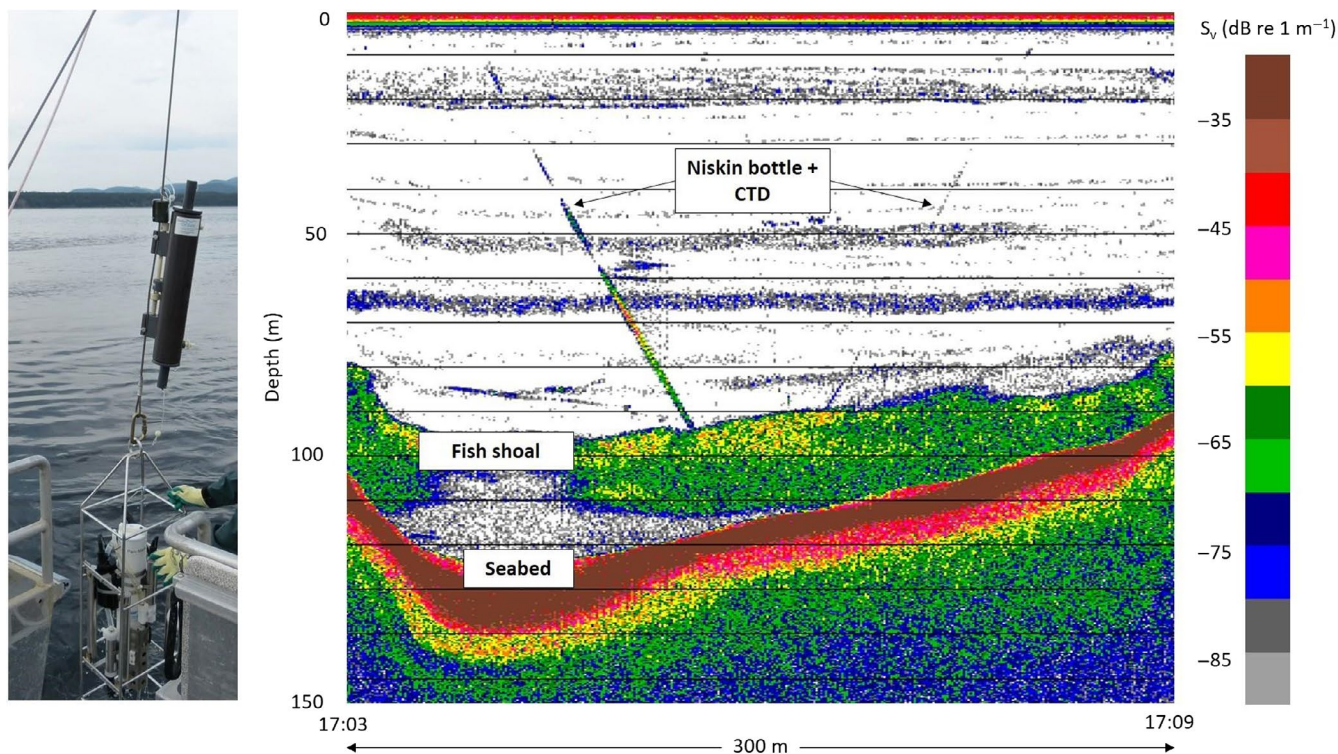


FIGURE 2 Measuring hydroacoustics and eDNA in the Saguenay–St. Lawrence Marine Park. Left: The conductivity–temperature–depth (CTD) profiler used in the study, with a sterilized Niskin bottle (1 L) deployed along the CTD cable. Right: 38 kHz echogram showing the water sampling into a fish shoal, the line to the left indicating the cable descending into the shoal and the right line while it is ascending. The color scale illustrates the variation in volume backscattering coefficient (S_v) that ultimately allows to discriminate shoals, their composition, and their density

data was then integrated by region and into 25×2 m (horizontal \times vertical) echointegration units (EIU), referenced to the sea surface. Echo classification was conducted using dB differencing method and applied on pairs of S_v values for EIU at 38, 120, and 200 kHz:

$$\Delta S_{v,F2-F1} = S_{v,F2} - S_{v,F1}$$

where $F2 = 120$ or 200 kHz and $F1 = 38$ or 120 kHz, three different combinations are possible. Then, we investigated the acoustic multifrequency response of fish shoals by plotting EIU frequency pairs of ΔS_v values in a 0.5 dB two-dimensional gridded surface where $x = \Delta S_{v,120-38}$ and $y = \Delta S_{v,200-120}$ in R (R Core Team, 2017), as it was previously performed (McQuinn et al., 2013). The frequency-differenced values were weighted by the signal amplitude at 120 kHz in order to give more emphasis to the denser concentration of organisms. Finally, density function was applied using the library *ks* to define 70% volume contour with kernel density estimate in order to compare fish shoals acoustic multifrequency response in the same two-dimensional space (Duong, 2014). The Kernel density estimate is a common statistical method for density estimation (Silverman, 1986). The $\Delta S_{v,120-38}$ threshold previously described (McQuinn et al., 2013) of 5 dB for krill and fish discrimination was used for data visualization.

2.4 | eDNA extraction

eDNA extraction was performed using a QIAshredder and DNeasy Blood and Tissue Kit (Qiagen) according to a previously developed protocol (Goldberg, Pilliod, Arkle, & Waits, 2011). The extracted eDNA was stored at -20°C until amplification by quantitative real-time PCR (qPCR) method. For each extraction batch, an extraction negative laboratory control was added to account for possible contamination.

2.5 | Design of primers and probes specific to the five fish species and optimization

In order to identify the five fish species of interest in our study (*Ammodytes* sp., *C. harengus*, *M. villosus*, *O. mordax*, and *Sebastes* sp.), we used DNA sequences from the mitochondrial gene cytochrome *c* oxidase subunit 1 (COI). COI is a gene that has been very successfully used to discriminate between closely related fish species based on sequence divergence (e.g., April, Mayden, Hanner, & Bernatchez, 2011; McCusker, Denti, Van Guelpen, Kenchington, & Bentzen, 2013).

COI sequences from the five species were obtained from the bold system (Barcode of Life Database <http://www.boldsystems.org/index.php/>). The COI sequences from related species present in

TABLE 1 Species-specific qPCR primers used for the amplification of different regions of the mitochondrial COI gene designed to identify targeted shoal species

Species	Forward (5'→3')	Reverse (5'→3')	Probe sequences	Length of the targeted fragment
<i>Ammodytes</i> sp.	GTTGATTTAAACAATCTTCTCACTGCATC	ATTAGCACAGCTCACACAAATAACG	AACTTCATCACCACAATTA	143
<i>Clupea harengus</i>	ACGGTATATCCTCTCTGTGTCAGGA	TAACAAGAACGGATCAGACAAACAGA	CATCAGTTGACCTAACCAT	193
<i>Mallotus villosus</i>	GCAATCTCGCTCACGCG	AAGAAGAACGGCTGTAATTAGCACA	AAACCTCTGCTATTTCTC	185
<i>Osmerus mordax</i>	GCAGGCGCCGGGACT	GCAGGAGGCTTCATATTAATAATGGTT	CACGCGGGAGCTT	167
<i>Sebastes</i> sp.	TTACCACAATTATTAATATGAAGCCACC	GATGCCGCGCAGCAAGAACT	CTGTCTTCTCTCTCTATCT	125

the same environment were also used to design the specific primers (Table S2). Each primer pair and probe was developed to maximize the number of mismatches between the targeted species and the related species. The sequences were aligned using the software Geneious (<https://www.geneious.com/>). From aligned COI gene sequences, specific primers and probes were successfully designed also using Geneious and verified using the software Primer Express 3.0 (Life Technologies). In addition, the Primer-BLAST tool (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>) was used to verify the specificity of the amplification on other species that could be present in the same environment. The sequences of the designed primers and probes are presented in Table 1. Note that for the primers designed for *O. mordax*, there was a polymorphism on the 3' end of the reverse primer for the subspecies *O. m. dentex*, which would likely prevent the use of these primers to target this specific subspecies.

2.6 | Test of primer and probe specificity by qPCR method

Specific primers and probes were tested on DNA tissues of targeted and related species (list of tested species are available in Table S2). Tissues of one sand lance, one herring, and one redfish were obtained from Parks Canada. Tissues of capelin and rainbow smelt and other related species were available in our laboratory at the Institut de Biologie Intégrative et de Systèmes (IBIS), Université Laval, Québec. No tissue for related species of sand lance was available. Genomic DNA was extracted using a modified version of a salt extraction protocol (Aljanabi & Martinez, 1997). In order to identify the species of fish of interest, the COI was amplified by PCR using the FishCOIF primers (forward, F and reverse, R, Table S3) (Lara et al., 2010) and using AccuStart II Master Mix following these PCR conditions: initial incubation at 94°C for 60 s; 35 cycles of denaturation at 94°C for 30 s; annealing at 55°C for 30 s; and extension at 72°C for 45 s. The PCR products were sequenced by Sanger sequencing using the 3,500 Genetic Analyzer (Applied Biosystems®, Life technologies Corporation) at the Genomic Analysis Platform, IBIS, Université Laval, Québec. Sequencing results were aligned and compared to the BOLD fish database using the software Geneious and the BOLD system (<http://www.boldsystems.org>).

The qPCR method was used to test specificity and efficiency of each primer pair on DNA of targeted species and related species

(Table S2). The amplification was performed on the 7500 Fast Real-Time PCR (Life Technologies) in a final volume of reaction of 20 µl including 1.8 µl of each primer (10 µM), 0.5 µl of probe (10 µM), 10 µl of Environmental Master Mix 2.0 (Life Technologies), 3.9 µl of H₂O, and 2 µl of DNA following these conditions: 2 min at 50°C, 10 min at 95°C, 50 cycles of 15 s at 95°C, and 60 s at 60°C.

For the specific primer tests, two methods were used: (a) FAST SYBR Green method for testing specificity and efficiency of primers and (b) TaqMan method for testing specificity of primers and probes. Specificity and efficiency of all primer pairs were first tested with FAST SYBR Green method, and once amplification of targeted species only was achieved, the TaqMan assay was performed with the species-specific probes for further specificity enhancement.

2.7 | Assay sensitivity

A standard curve experiment was performed following the reaction and condition protocol using TaqMan Environmental Master Mix 2.0 as described above. A synthetic DNA template of 500 base pairs including the target amplicon sequence was designed from the COI gene sequence and prepared by Integrated DNA Technologies Inc. From the stock at 1.00E + 10 copies/µl, an eight-level dilution series (2,000, 1,000, 500, 100, 20, 8, 4, 2, and 1 copies per reaction) was prepared in sterile yeast tRNA at 10 µg/µl. Ten replicates of each dilution series were run to determine the amplification efficiency and the limit of detection defined as the lowest copies per reaction with >95% amplification success (Bustin et al., 2009). The threshold of one molecule was detected at 40 C_t for each primer pair. Therefore, all signals exceeding 40 C_t were considered as potential artifact of PCR and were eliminated from further analyses.

2.8 | eDNA samples analysis by qPCR method

To analyze the collected eDNA samples, the TaqMan qPCR method was used with the addition of the SPUD to the reaction, as well as a standard curve. SPUD is a universal system for rapid quality control of nucleic acid templates before qPCR (Nolan, Hands, Ogunkolade, & Bustin, 2006). DNA presence of each targeted species was tested on six technical replicates for each eDNA sample

and the negative laboratory (extraction) controls. Note that the presence of the rainbow smelt *O. mordax* was only tested in shoals of 2018 because the species has very rarely been observed in the area covered in 2017.

The amplification was performed in a final volume reaction of 20 μl including 1.8 μl of each primer (10 μM), 0.5 μl of probe (10 μM), 10 μl of Environmental Master Mix 2.0 (Life Technologies), 3.9 μl of SPUD, and 2 μl of DNA following these conditions: 2 min at 50°C, 10 min at 95°C, 50 cycles of 15 s at 95°C, and 60 s at 60°C.

All qPCR results were quantified using a standard curve of known DNA quantities which allowed us to quantify positive PCR amplification in number of molecules. The average of the number of molecules measured in the six replicates of a given sample was calculated. For each shoal, except for the five shoals for which no upstream location could be sampled, the average number of molecules at one location sampled within the shoal was corrected by subtracting the value obtained at the upstream location. Thus, the average number of molecules obtained at the upstream location was always scaled back to zero. If the corrected average of number of molecules measured for a species was zero or below zero in a given sample, then we concluded that the DNA of this species was not present in that sample. Alternatively, detection of a species was inferred when the corrected average of number of molecules measured for a species at a specific location was above zero (Table S1).

2.9 | Statistical analyses for environmental DNA

Statistical analyses were performed using the R software version 3.5.2 (R Core Team, 2017). First, we used the package lme4 (Bates, Mächler, Bolker, & Walker, 2015) to determine whether the location within a shoal (upstream, T1, T2, T3, or T4) or the location of a shoal compared to other shoals impacted eDNA concentration. Distinct analyses were performed for the shoals with or without the upstream location sampled. We used a linear mixed model including the depth, the temperature, and the salinity of the shoals as fixed effects and the IDs of the shoals as a random effect. We checked for homogeneity of variances using a Levene test and also tested normality of residuals using graphical assessment (Q–Q plot and normal distribution histogram) and a Shapiro–Wilk test. To obtain p -values, we performed pairwise comparisons using locations within/between shoals with the package lmerTest (Kuznetsova, Brockhoff, & Christensen, 2017). Then, we used a nonparametric Mann–Whitney test to compare eDNA concentrations of a specific species between shoals and years.

2.10 | Comparison of hydroacoustics and eDNA data

The comparison between both datasets was examined using principal component analysis (PCA) and clustering methods. PCA was

first conducted on the three ΔS_v values integrated by shoal for each sample from the hydroacoustics dataset. eDNA dataset was then compared using corrected mean concentrations of the target species combined with environmental parameters (depth, salinity, and temperature) of the sampling sites. Then, hierarchical clustering on principal component (HCPC function in FactoMineR package (Lê, Josse, & Husson, 2008)) was performed using Ward's criterion on the selected principal components. The hierarchical tree was cut at the appropriate level (≈ 0.5) of the height which allowed creating distinct clusters of sample similarities. The quantiles of the normal distribution associated with the p -values (v -test) were used to determine the influence of the variables of the clusters. Only significant variables ($p < .05$) that described each cluster were selected. We considered negative v -test values as those below the overall mean of the variable and conversely, the positive v -test values were above.

3 | RESULTS

3.1 | Hydroacoustic approach

The density analysis of the acoustic data (EIUs, 25 \times 2 m) in a two-dimensional space ($\Delta S_{v,120-38}$, $\Delta S_{v,200-120}$) revealed significant differences in the acoustic responses of the different shoals (Figure 3; Figure S1; Table S1) with different CTD profiles (Figure S2). The 70% volume contours for each sample illustrate two main distinct patterns explained by the positive or negative difference between the 200 and the 120 kHz (Figure 3) corresponding to the volume backscattering coefficient of fish without swim bladder (six shoals in 2017 (IDs: 2017_03, 2017_04, 2017_06, 2017_08, 2017_10, and 2017_11) and five shoals in 2018 (IDs: 2018_03, 2018_04, 2018_05, 2018_06, and 2018_08)) and fish with swim bladder (five shoals in 2017 (IDs: 2017_01, 2017_05, 2017_07, 2017_09, and 2017_12) and three shoals in 2018 (IDs: 2018_01, 2018_02, and 2018_07)). Another difference is also observable on the axis $\Delta S_{v,120-38}$. These differences are more easily observable for the 2017 samples, especially for the difference explained by $\Delta S_{v,120-38}$. Based on the $\Delta S_{v,120-38}$ threshold of 5 dB and the 70% volume contours, three shoals in 2017 (IDs: 2017_03, 2017_04, and 2017_06) and two shoals in 2018 (IDs: 2018_05 and 2018_06) also contained euphausiids.

3.2 | Environmental DNA approach

3.2.1 | Specificity of primers and probes

Following sequencing of genomic DNA, the alignment of the COI sequences of sandlance, herring, capelin, redbfish, and rainbow smelt tissues showed the following results using the software BLAST and the database from NCBI (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>): The sandlance was identified as *Ammodytes hexapterus* with 99% of

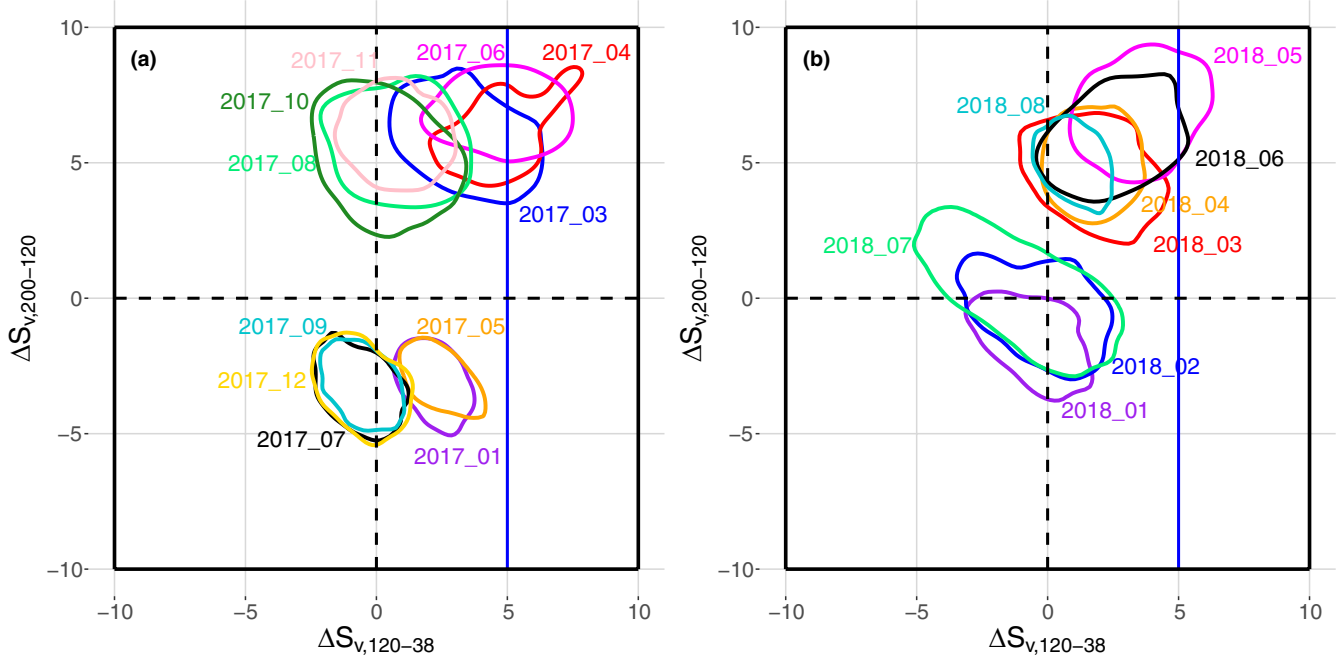


FIGURE 3 Distribution of $\Delta S_{v,200-120}$ versus $\Delta S_{v,120-38}$ of the shoals sampled in (a) 2017 and (b) 2018 using hydroacoustics. Solid color contours represent 70% kernel estimate density of each sampling site. The solid blue line represents the 5 dB threshold between fish and krill. Each shoal is represented by a specific color. Shoals that tend to cluster together are more similar in their fish composition than the other shoals. Shoals that are distributed on both sides of the solid blue line include fish and krills, while shoals that are localized solely on the left side include mainly fish. Shoals with swim bladder fish tend to be on the lower part of the figure

identity. The herring was identified as *C. harengus* with 99% of identity, as for the capelin that was identified as *M. villosus*. The redfish was identified as *Sebastes fasciatus*, *Sebastes mentella*, and *Sebastes norvegicus* with 99% of identity. Therefore, we refer to this taxon as *Sebastes* sp. The rainbow smelt was identified as *O. mordax* with 99% of identity.

Results of the specific amplification by qPCR are presented in Figure S3. Specific primers amplified the desired species, and no amplification was detected for the related species. For the primers specific to *M. villosus*, we observed an amplification with a DNA mix containing three species, but this amplification had a very low efficiency and was under the threshold of detection.

Based on the standard curve experiment, the assays for the targeted species had an amplification efficiency between 95.5% and 114.5% and a limit of detection between 20 mtDNA copies/rxn (*O. mordax*) and 4 mtDNA copies/rxn (*A. hexapterus*) depending on the targeted species (Table 2).

3.2.2 | eDNA samples from shoals

Detection of targeted species in shoals

An environmental DNA-qPCR-based approach was applied to infer the presence of five target fish species in 20 shoals sampled in the Saguenay–St. Lawrence Marine Park. Typically, two or more targeted species were identified in the eDNA samples from all shoals (Table 3).

Only one shoal (ID: 2017_04) had a positive amplification for one species only, namely the capelin *M. villosus* (Table 3 panel A). Furthermore, two species were detected in seven shoals comprising

different assemblages of the following three species: capelin, redfish, and herring (Table 3 panel B). In total, 10 shoals showed a positive amplification for at least three targeted species, including sandlance, herring, capelin, or redfish (Table 3 panels C and D). These four species were all identified in three shoals sampled in 2017 (Table 3 panel D—IDs: 2017_02, 2017_09, and 2017_12). These three shoals were localized in distinct areas (2017_02 in the Saguenay Fjord; 2017_09 and 2017_12 near the mouth of the Saguenay Fjord), illustrating the usefulness of the eDNA method for detecting four species of interest at various locations in the Saguenay–St. Lawrence Marine Park. Finally, none of the species were detected in two shoals (IDs: 2017_10 and 2018_01).

Capelin eDNA detection

The presence of capelin was detected in all shoals but three: only three shoals did not have a positive amplification for the capelin. For the first two shoals (IDs: 2017_10 and 2018_01), none of the five fish species of interest were detected after the averages of number of molecules sampled within the shoal were corrected by subtracting the values obtained at the upstream location. For the third shoal (ID: 2017_01), the presence of three fish species was inferred, namely sandlance, herring, and redfish, but the capelin eDNA signal was not significant after applying the correction with the eDNA detected upstream (Table 3 panel C). For the 2 years, the number of molecules detected for the capelin tended to be the highest compared to the other species. Note that for two shoals sampled in 2018 (IDs: 2018_02 and 2018_07), the capelin eDNA was detected at each position within these shoals and in all qPCR

TABLE 2 Percentage of amplification efficiency (eff%), limit of detection, intercept (y -inter), and r^2 results for each standard curve developed with a synthetic DNA template

Scientific name	r^2	y -inter	eff%	Limit of detection (mtDNA copies by rxn)
<i>Ammodytes</i> sp.	.985	39.8	102.7	4
<i>Clupea harengus</i>	.961	40.5	103.9	8
<i>Mallotus villosus</i>	.970	39.6	100.0	8
<i>Osmerus mordax</i>	.948	41.5	114.5	20
<i>Sebastes</i> sp.	.981	39.9	95.5	8

replicates. Therefore, we decided to tentatively consider these shoals as positive for the presence of capelin (despite negative numbers of eDNA molecules after correction with the upstream location) (Table 3 panel B).

Redfish eDNA detection

In 2017, nine out of the 12 shoals that were sampled included eDNA from the redfish (IDs: 2017_01, 2017_02, 2017_03, 2017_05, 2017_06, 2017_07, 2017_08, 2017_09, and 2017_12), with a total average of number of molecules measured in the shoals of 16.7 (± 18.8) (Table 3 panels B–D). In 2018, the eDNA values that were detected for the redfish were significantly lower as the six shoals that showed positive detection for this species (IDs: 2018_03, 2018_04, 2018_05, 2018_06, 2018_07, and 2018_08) had a total average of eDNA molecules of 0.3 (± 0.3) ($p = .018$) (Table 3 panels B and C).

Sandlance eDNA detection

As reported for the redfish, the identification of the sandlance varied between 2017 and 2018, as this species was detected in five shoals of 2017 (IDs: 2017_01, 2017_02, 2017_09, 2017_11, and 2017_12) but in none of 2018. Furthermore, the quantity of eDNA molecules detected in the shoals of 2017 for the sandlance was systematically lower than the other species (Table 3 panels C and D).

Herring eDNA detection

The herring was detected both in 2017 (IDs: 2017_01, 2017_02, 2017_03, 2017_05, 2017_09, 2017_11, and 2017_12) and 2018 (IDs: 2018_02, 2018_03, 2018_05, and 2018_08), at similar molecular levels between the 2 years. However, herring eDNA apparently never dominated in any shoals compared to other species, namely the capelin or the redfish (Table 3 panels B–D).

Absence of the rainbow smelt eDNA detection

The presence of the rainbow smelt was not found in any of the shoals of 2018, the only year this species was tested for eDNA.

Variations in the level of eDNA detection

DNA quantity and the number of positive detections tended to be variable depending on shoals (Table 3 and Table S4), on the position within a shoal (upstream, T1, T2, T3, or T4) (Figure 4), and on the targeted species (Table 3, Table S4 and Figure 4). However, using a linear mixed model, we did not find any significant effect of the

location within a shoal or of the location of a specific shoal on eDNA concentration. Among the 60 samples coming from 15 shoals for which the presence of all species but rainbow smelt was estimated, 38 of them showed the lowest DNA quantity at the upstream location compared to within the shoal (locations T1, T2, T3, or T4).

Negative controls and positive amplification sequencing

Positive amplifications were detected in thirteen-field negative controls (in six controls from shoals sampled in 2017 and in seven controls from shoals sampled in 2018). However, quantities were much lower than actual shoal samples; therefore, the results can be taken with confidence. All extraction negative controls showed no positive amplification indicating the absence of contamination during sample extraction.

Moreover, to confirm that positive detections represent actual targeted species, several positive amplifications were sequenced by Sanger sequencing. The sequencing results showed that positive amplifications came from targeted species except for the sandlance for which the obtained sequence was identified as *Ammodytes americanus* instead of *A. hexapterus* previously identified from the tissue sample. As *A. americanus* was identified in samples with positive amplification, we can conclude that our developed assay is not specific to *A. hexapterus* and the sandlance species presents in shoals is *A. americanus*. The positive detections of herring and capelin were well identified as *C. harengus* and *M. villosus*, respectively. Positive detections of redfish were identified as *S. fasciatus*, *S. mentella*, or *S. norvegicus* as expected.

3.3 | Combining hydroacoustic and environmental DNA

Principal component analysis and hierarchical clustering on principal component (HCPC) analysis allowed comparing clusters obtained with both datasets (Figure 5 and Figure S4). The variables used were the three frequency differences ($\Delta S_{v,200-120}$, $\Delta S_{v,200-38}$, and $\Delta S_{v,120-38}$) and corrected eDNA molecules with abiotic parameters (depth, salinity, and temperature). HCPC analysis indicated how variables contributed for grouping samples. Clusters of the same color shared similar samples between datasets. V-test values indicated if variables were positively or negatively represented in the clusters. Only significant v-test values were selected. Three clusters were resolved from acoustics data (Figure 5 panel a) and five from eDNA dataset (Figure 5 panel b). Cluster #1 (yellow) appeared to be more associated with fish with a swim bladder as negative values were computed for 200 and 120 kHz frequencies with acoustics dataset, and a positive v-test value was

TABLE 3 Detection of four target species in shoals of the Saguenay–St. Lawrence Marine Park using eDNA

ID shoal	Ammodytes	Clupea	Mallotus	Sebastes
	eDNA mean ± SD	eDNA mean ± SD	eDNA mean ± SD	eDNA mean ± SD
A. One species				
2017_04	-0.1 ± 0.1 (3/24)	-0.1 ± 0.3 (2/24)	7.8 ± 4.2 (24/24)	-0.1 ± 0.4 (10/24)
B. Two species				
2017_06	0.0 ± 0.0 (0/24)	0.0 ± 0.0 (0/24)	5.3 ± 9.6 (24/24)	0.6 ± 0.5 (9/24)
2017_07 ^π	0.0 ± 0.0 (0/24)	0.0 ± 0.0 (0/24)	3.9 ± 1.6 (22/24)	12.7 ± 7.3 (24/24)
2017_08	0.0 ± 0.0 (0/24)	-0.6 ± 0.9 (11/24)	142.4 ± 253.9 (24/24)	1.6 ± 3.7 (22/24)
2018_02	0.0 ± 0.0 (0/24)	0.1 ± 0.2 (1/24)	-42.6 ± 10.3 (24/24)	-0.1 ± 0.1 (2/24)
2018_04	0.0 ± 0.0 (0/24)	0.0 ± 0.0 (0/24)	59.7 ± 64.6 (24/24)	0.3 ± 0.1 (6/24)
2018_06	0.0 ± 0.0 (0/24)	0.0 ± 0.0 (0/24)	55.1 ± 15.4 (24/24)	0.06 ± 0.1 (4/24)
2018_07	0.0 ± 0.0 (0/24)	0.0 ± 0.0 (0/24)	-167.1 ± 48.9 (24/24)	0.8 ± 3.1 (12/24)
C. Three species				
2017_01	0.02 ± 0.2 (2/24)	0.2 ± 0.2 (2/24)	0.0 ± 1.3 (20/24)	24.5 ± 16.2 (24/24)
2017_03	-0.1 ± 0.1 (7/24)	0.2 ± 0.3 (1/24)	49.6 ± 46.4 (24/24)	0.1 ± 0.5 (15/24)
2017_05 ^π	0.0 ± 0.0 (0/24)	0.4 ± 0.3 (3/24)	4.6 ± 1.6 (24/24)	47.5 ± 26.3 (24/24)
2017_11	0.6 ± 0.7 (20/24)	16.6 ± 6.5 (24/24)	29.8 ± 14.2 (23/24)	0.0 ± 0.9 (17/24)
2018_03 ^π	0.0 ± 0.0 (0/24)	0.04 ± 0.1 (1/24)	8.6 ± 3.0 (24/24)	0.1 ± 0.1 (2/24)
2018_05	0.0 ± 0.0 (0/24)	0.1 ± 0.2 (1/24)	203.4 ± 193.5 (24/24)	0.5 ± 0.4 (9/24)
2018_08	0.0 ± 0.0 (0/24)	0.2 ± 0.2 (4/24)	14.5 ± 15.0 (24/24)	0.04 ± 0.1 (1/24)
D. Four species				
2017_02	0.009 ± 0.0 (1/24)	0.1 ± 0.1 (1/24)	1.8 ± 1.8 (13/24)	0.2 ± 0.3 (6/24)
2017_09 ^π	0.02 ± 0.0 (1/24)	0.3 ± 0.4 (2/24)	6.0 ± 1.1 (24/24)	18.4 ± 11.4 (24/24)
2017_12 ^π	0.1 ± 0.1 (2/24)	11.1 ± 11.1 (22/24)	56.9 ± 27.9 (24/24)	44.3 ± 8.6 (24/24)

Note: For each shoal, the number of eDNA molecules detected was corrected by subtracting the eDNA value obtained at the upstream location (except for shoals: 2017_05, 2017_07, 2017_09, 2017_12, and 2018_03 (^π)). For each species detected in a shoal, the mean represents the average number of DNA molecules measured in 24 samples (four sample locations with six qPCR replicates). Standard deviation (SD) is reported. Positive detection of a species was inferred when the corresponding mean eDNA value was above zero. Shoals 2017_10 and 2018_01 are not represented because no eDNA was measured for any of the species.

detected for redfish variable with eDNA data. In the opposite, cluster #2 (green) was more likely associated with fish without swim bladder as samples with a positive response to $\Delta S_{v,200-120}$ and a negative response to redfish were detected in the group. In cluster #3 (gray), all samples overlapped with the 5 dB $\Delta S_{v,120-38}$ threshold (Figure 3) and almost all of them were located near the mouth of the Saguenay Fjord (Figure 1). Clusters #4 (red) and #5 (magenta) computed with eDNA dataset contained only one sample (ID: 2018_07 and 2017_11, respectively). Cluster #4 was attributable to a negative response to capelin after applying the correction for the mean eDNA concentrations. Cluster #5 was associated with a strong positive response for both sandlance and herring. The similarity between clusters (#1, #2, and #3) for both methods ranged from 50% to 75% of the samples.

4 | DISCUSSION

Few studies have used distinct noninvasive methods in combination to estimate the abundance and diversity of fish in marine ecosystems. Here, we applied hydroacoustics and environmental DNA to

identify five pelagic forage fish species in a whale feeding area of the Saguenay–St. Lawrence Marine Park.

The combined hydroacoustics and eDNA results suggest that fish diversity and abundance in shoals of the marine park are variable annually. Hydroacoustic analysis allowed to discriminate shoals mainly composed of fish with a swim bladder (5 in 2017 vs. 3 in 2018) from shoals composed of swim bladderless fish (6 in 2017 vs. 5 in 2018). The interannual variation in shoal composition was further investigated using eDNA. First, eDNA analyses highlighted that capelin was the most predominant species in both 2017 and 2018. This species was detected in all shoals but three, and the levels of eDNA molecules measured tended to be the highest compared to the other species. This result is consistent with previous acoustic studies that indicated that the capelin tends to aggregate in upwelling systems of the marine park (Simard, Lavoie, & Saucier, 2002). The lower SLE and the Saguenay Fjord, a major tributary of the SLE, have been described as an important spawning areas for capelin, and capelin larvae dominate the spring and summer ichthyoplankton communities (Ouellet et al., 2013; Sirois et al., 2009). The three shoals for which eDNA of the capelin was not detected (IDs: 2017_01,

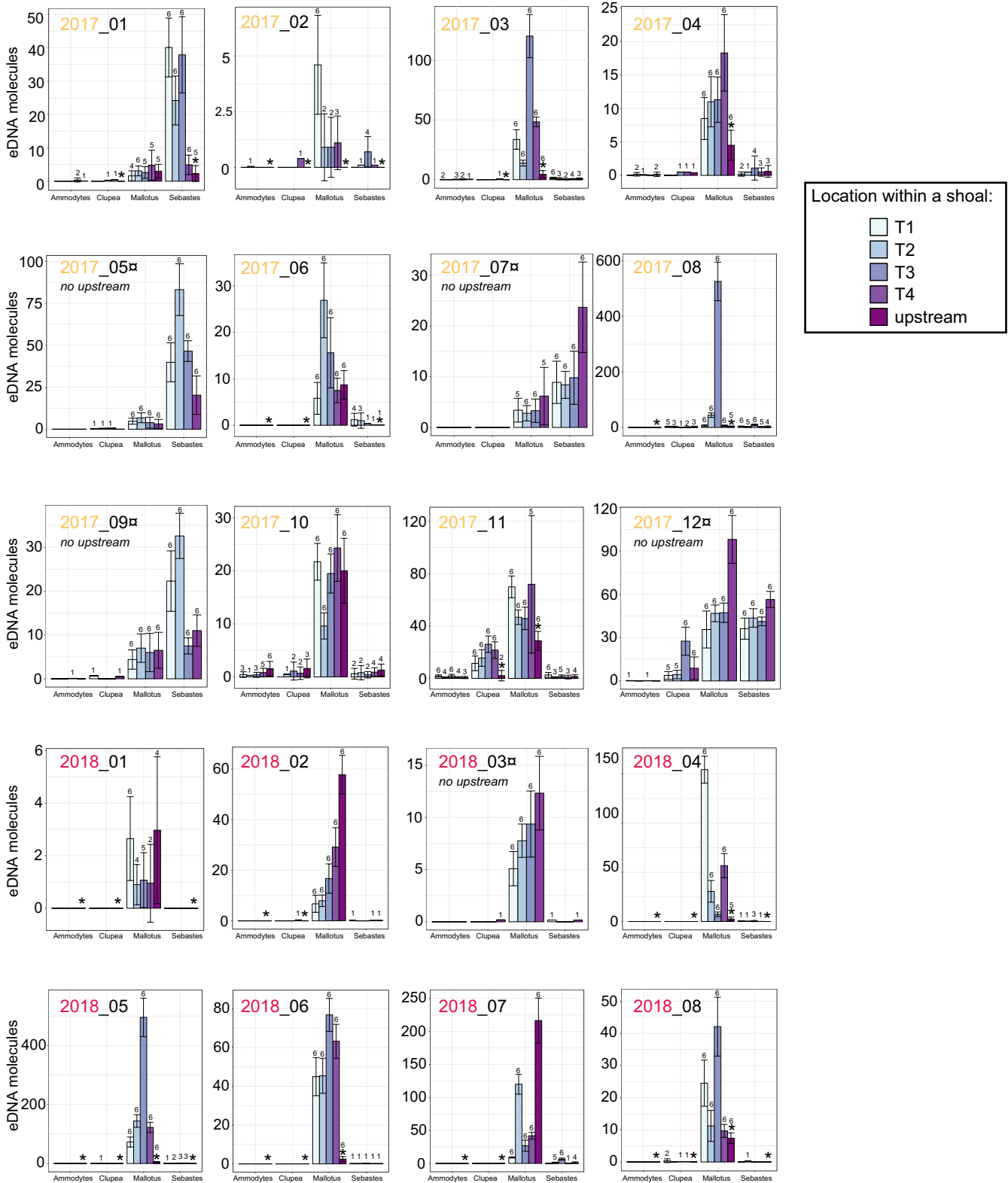


FIGURE 4 eDNA concentrations of four target species depending on locations within shoals of the Saguenay–St. Lawrence Marine Park. For each shoal, the number of eDNA molecules measured at five locations (T1, T2, T3, T4, and upstream) for four species is represented. At each location, the mean represents the average number of DNA molecules of the six qPCR replicates (correction with the upstream location was not realized). The number of positive replicates (maximum of 6) is indicated above each mean bar, and standard deviations on each estimate are also illustrated. The symbol (*) was added when the lowest eDNA concentration of a specific species was measured upstream of the shoal of interest as expected

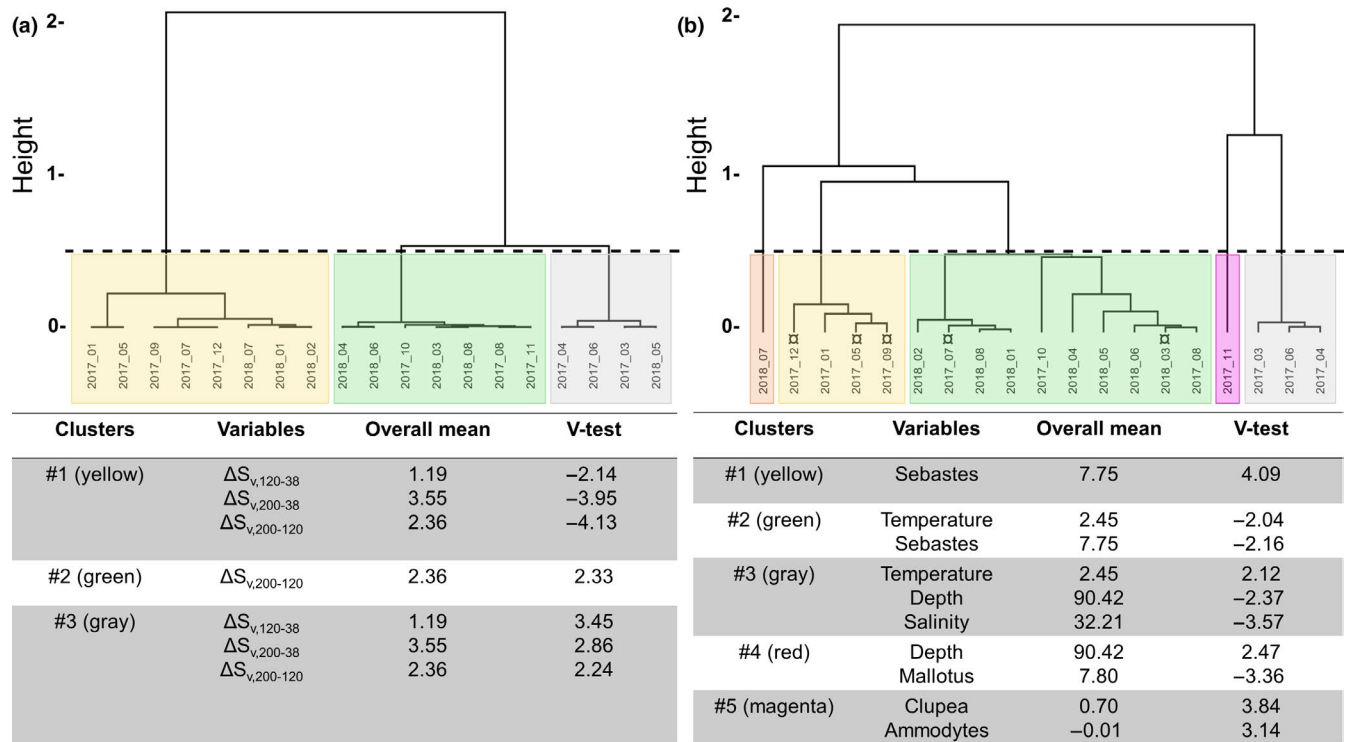


FIGURE 5 Comparison between (a) differences in mean volume backscattering strength (200, 120, and 38 kHz) and (b) eDNA concentrations combined with environmental parameters (depth, salinity, and temperature) of the sampling sites. Hierarchical clustering on principal component (HCPC) was performed for each dataset, allowed in grouping samples into distinct clusters (3 and 5). V-test was computed to determine the significant variables ($p < .05$) for each cluster, and the v-test values were associated with the overall mean of the variable. The hierarchical trees were cut at suggested level (dashed black line). The symbol (π) was added to samples when eDNA concentrations were not corrected by the eDNA value obtained at the upstream location. One shoal (ID: 2017_02) was completely excluded from the analysis

2017_10, and 2018_01) were distributed throughout the sampling location without clear spatial pattern. The absence of eDNA at these stations could however be linked to local hydrodynamics, where water masses being sampled may be influenced by the tidal cycle. For example, the presence at the station of deep upwelled water (2017_01 and 2018_01) or of water outflowing from the Saguenay Fjord (2017_10, sampled late in the season when larval capelin would be reduced in the Saguenay) could explain the absence of eDNA of capelin at these stations. Furthermore, the aggregation of capelin at these stations could directly covary with tidal cycles, as it was previously reported using hydroacoustic surveys (Simard et al., 2002). Second, strong differences between 2017 and 2018 in terms of fish abundance were observed for the redfish *Sebastes* sp. and for the sandlance *Ammodytes* sp. In 2018, detection of eDNA molecules of the redfish was significantly lowered compared to 2017, while the sandlance was no longer detected at all. We can only speculate on the possible causes of such a reduction in the level of detection of the redfish and the sandlance in 2018. Redfish juveniles migrate gradually deeper during their development (Senay et al., 2019), suggesting that older redfish may have migrated to deeper areas in 2018 as compared to 2017. Also, fish presence might be affected by the abundance of food resource as zooplankton, which is known to highly fluctuate over short periods in the Saguenay–St. Lawrence Marine Park (Simard & Roy, 2018). For the sandlance, in addition to

the absence of detection in 2018, the amount of eDNA molecules measured in 2017 was systematically the lowest compared to the other species, even in shoals identified as fish without a swim bladder. While previous studies effectively used eDNA methods to detect *Ammodytes* sp. in the water column (Sigsgaard et al., 2017), it is important to keep in mind that the sandlance alternates between swimming pelagically (for feeding) and lying buried in the sediments (Behrens & Steffensen, 2007; Robards, 1999). This particular behavior could have restricted the detection of eDNA molecules for the sandlance as our study was based on the analysis of samples from the water column. But perhaps more important is the possibility that more than one species of the genus *Ammodytes* coexist in the system and that the primers we developed based on the *A. hexapterus* specimen that was collected in the studied system cannot efficiently amplify DNA from *A. americanus*, which could be the predominant *Ammodytes* species in this system. Clarifying this issue will have to await the collection of more specimens of both species in order to thoroughly compare their sequences and possibly redesign new primers. Finally, while herring was detected in 2017 and 2018, eDNA from rainbow smelt was not found in any of the samples. It may be explained by the oceanographic processes (e.g., tidal upwellings) near the head of the Laurentian Channel that prevent the transport between the rainbow smelt populations of the middle and lower estuary (Bernatchez & Martin, 1996; Ingram & El-Sabh, 1990). In order

to obtain a better estimate of the rainbow smelt distribution, future studies may concentrate sampling efforts on the north and south shores of the SLE, where more suitable habitat for this species is found.

The use of eDNA methods allowed to groundtruth acoustic data, and their combined integration led to specifically define five clusters in the Saguenay–St. Lawrence Marine Park. The first cluster was associated with fish with a swim bladder and was primarily located on the north shore of the marine park. The second cluster was more associated with swim bladderless fish and was generally found in the center of the SLE. The third cluster composition was mixed and was detected near the mouth of the Saguenay. This zone is known for being highly dynamic with localized upwelling currents and tidal mixing (Marchand, Simard, & Gratton, 1999). The fourth cluster, which was solely computed from the eDNA dataset, contained only one shoal (2018_07) which was associated with a negative response to capelin after applying the correction with the upstream location. Capelin eDNA was detected at each position within this shoal and in all qPCR replicates. We thus hypothesize that this species was very abundant at the upstream location of this shoal that was sourced by either schooling capelin or larvae which can be highly abundant in the SLE (with 216.52 mean molecules detected [uncorrected values]), so that the correction was biased toward negative values at the other locations, despite the presence of the species (T1: 9.07; T2: 120.02; T3: 26.84, and T4: 41.94 mean molecules detected [uncorrected values]). The last cluster that was also computed from the eDNA dataset contained one shoal (2017_11). During sampling, this shoal was the southernmost shoal located in the middle estuary. Interestingly, the highest levels of eDNA molecules for sandlance and herring were measured in this shoal (respectively, 20/24 and 24/24 eDNA replicates were positive for these species, with a corrected number of molecules detected of 0.6 and 16.6). The middle estuary therefore appears as a promising place to perform sampling and try to increase the detection levels of the fish species, especially for the sandlance.

Combining hydroacoustics and eDNA helped to obtain a picture of the dynamics of pelagic forage fish species in the Saguenay–St. Lawrence Marine Park over a 2-year period. However, the limitations of these methods, as well as the complexity of this marine ecosystem, have to be taken into account when interpreting the collected data. First, hydroacoustics allowed to discriminate between shoals including fish with swim bladder or fish without swim bladder, mixed shoals, and shoals including euphausiids. Nonetheless, this nonselective method remains inadequate for species identification and for collecting samples near the surface and the bottom of the water column (Scalabrin, Marfia, & Boucher, 2009; Thorne, 1983). Second, the level of eDNA molecules detected by qPCR was used to infer and compare the relative abundance of each species in the shoals of the marine park, as it was previously done in other aquatic systems (Pilliod, Goldberg, Arkle, & Waits, 2013). However, eDNA concentration is influenced by many factors, including the biology of the species of interest and the environmental conditions encountered by the species (Rees et al., 2014). Concerning the species biology,

the rate of release of eDNA by an organism may vary depending on its physiology or developmental state (Pilliod, Goldberg, Arkle, & Waits, 2014). But up to now, no study examined this rate among the five fish species studied here. Beyond the intrinsic limitations for the organisms, environmental factors such as abiotic conditions (temperature, pH...) or biotic conditions (microbial communities) influence the persistence or degradation of eDNA (Barnes et al., 2014), and these conditions were likely to diverge in our study between distant shoals and years. Furthermore, the SLE is a unique environment characterized by strong currents, intense vertical mixing, and upwellings that are likely to have effects on eDNA concentration in the water column, for instance, by promoting the rise to the surface of traces of eDNA from fish that are no longer present. Data from eDNA showed that for seven shoals, eDNA of at least one species was detected in only one qPCR replicate (1/24) (IDs: 2017_02 for sandlance and herring, 2017_09 for sandlance, 2017_03, 2018_02, 2018_03 and 2018_05 for herring, and then 2018_08 for redfish). In these cases, eDNA detection represents probably traces of the species carried at the surface by strong currents rather than direct presence in the shoal.

To get a better estimate of fish abundance, we measured for each shoal, an upstream location for which no or very low levels of species detection were expected, and which could therefore be used as an external reference. Indeed, it has previously been reported that spatial location is an important factor when interpreting eDNA data (Evans et al., 2017). However, for five shoals, we were not able to collect upstream samples to correct the eDNA values measured. Correction with the upstream location primarily allowed obtaining more stringent results: In more than 80% of the samples, the correction with the upstream location simply reduced the levels of eDNA molecules measured for the species detected in a specific shoal, without influencing their presence/absence. In this context, the eDNA values measured for the five uncorrected shoals are less stringent, but may nevertheless represent a good indicator of species presence. Furthermore, for one-third of the samples, the eDNA values measured upstream were higher than in the actual shoal samples, suggesting that the upstream locations were not sampled far enough from the shoals to prevent species detection. In particular, for two shoals sampled in 2018 (IDs: 2018_02 and 2018_07), the concentrations obtained upstream were very high suggesting that fish were still present at high density in this external location. This especially led to underestimate the presence of the capelin inside these two shoals. Interpreting eDNA data in terms of fish presence and abundance therefore requires to choose appropriate external, negative controls, as well as appropriate locations for the sampling sites. Future studies in the SLE could, for example, select control sites more distant to the shoals of interest (at least 1 km from the core of the shoals) and sample water at different depths within the shoals. We chose to sample 1 L of water at each position within each shoal. The choice of this volume was appropriate in regard to other studies that generally use 1 L to maximize the recovery of eDNA (Hinlo, Gleeson, Lintermans, & Furlan, 2017). However, sampling a more important

volume could be an efficient way to increase species detection. For example, it was suggested in river systems that five 1 L water samples may be required to enable the detection of multiple species (Shaw et al., 2016). Also, hydroacoustic data could be used to estimate total fish biomass (Boswell, Wilson, & Wilson, 2007) in relation to eDNA concentrations. Finally, metabarcoding performed on eDNA could offer promising opportunities to simultaneously identify all species present in the shoals of interest (Deiner et al., 2017). This could help refining the estimate of species proportions with or without swim bladder in each shoal, as well as determining whether other pelagic fish species than the ones targeted in this study could contribute to the diversity in the SLE.

To our knowledge, our study is the first to combine hydroacoustics and eDNA to document the occurrence of different whale forage species and that suggested that prey composition may vary annually in this highly dynamic marine environment that represents a major feeding ground for marine mammals. Our study was conducted during a 2-year period, and sampling efforts should be extended over years in order to get a complete picture of the situation in the area. Climatic changes, which already appear to contribute to habitat degradation of St. Lawrence belugas (Plourde et al., 2014), are most likely to exacerbate these fluctuations in fish resources, as it was already described in other systems of the Atlantic (Godo, 2003). Furthermore, our study highlights the necessity to sample appropriate external controls in eDNA studies to get a precise estimation of fish abundance. We hope that our study will ultimately facilitate the implementation of conservation and fisheries management policies that will be appropriate to the ecological variability reported in the SLE. Hopefully also, it will stimulate the interest in performing similar combined studies between eDNA and hydroacoustics in other systems.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

L.B., N.M., and S.T. designed the study. S.T., S.F., and N.M. performed field sampling and analyzed the hydroacoustic results. B.B. performed the eDNA experiments, and C.S.B. analyzed the results. C.S.B. wrote the manuscript with collaboration from all the authors.

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SUPPORTING INFORMATION

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

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