ICES Journal of Marine Science, 2023, **80**, 1529–1538
DOI: 10.1093/icesjms/fsad083
Advance access publication date: 27 May 2023
Original Article

eDNA metabarcoding enriches traditional trawl survey data for monitoring biodiversity in the marine environment

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Marine Protected Areas require comprehensive monitoring to ensure objectives are achieved; however, monitoring natural ecosystems at scale is challenged by the biodiversity it aims to measure. Environmental DNA (eDNA) metabarcoding holds promise to address this monitoring challenge. We conducted paired sampling at 54 sites for fish and invertebrate assemblages in the Northwest Atlantic using groundfish trawls and eDNA metabarcoding of benthic seawater using four genetic markers (*12S rRNA*, *16S rRNA*, *18S rRNA*, and *CO1*). Compared to trawling, eDNA detected similar patterns of species turnover, larger estimates of gamma diversity, and smaller estimates of alpha diversity. A total of 63.6% (42/66) of fish species captured by trawling were detected by eDNA, along with an additional 26 species. Of the 24 missed detections by eDNA, 12 were inevitable as they lacked reference sequences. Excluding taxa assigned to higher than species level and those without a species name, 23.6% (17/72) of invertebrate species captured by trawling were detected by CO1, which detected an additional 98 species. We demonstrate that eDNA is capable of detecting patterns of community assemblage and species turnover in an offshore environment, emphasizing its strong potential for a non-invasive, comprehensive, and scalable tool for biodiversity monitoring supporting marine conservation programmes.

Keywords: Atlantic Ocean, bottom trawl, fish, invertebrates, marine mammals, Marine Protected Area.

Introduction

Conservation of natural ecosystems is a centre point of rapidly growing global efforts to curb biodiversity loss and erosion of ecosystem services. In the marine realm, spatial conservation measures, including Marine Protected Areas (MPAs) are a key tool for biodiversity conservation (Edgar *et al.*, 2014; Gill *et al.*, 2017). Biodiversity is a measure of the variety of life that, by its nature, is complex and perspective-dependent (Rocchini *et al.*, 2018). Estimating biodiversity has been a core challenge for ecologists for generations; it incorporates not only differences between species but variation within species and the environments in which they are found. Unsurprisingly, biodiversity surveys for aquatic metazoans are a challenging task that requires a correspondingly diverse toolbox of sampling approaches (Snelgrove *et al.*, 2012; Corlett, 2017). Traditional sampling (visual surveys, scientific fishing) and technologies (remote underwater vehicles, acoustic surveys) have unique inherent biases and environmentally dependent applications. However, none is universally applicable within the marine environment.

Application of MPAs as a conservation tool requires robust and comprehensive assessments to ensure conservation objectives are being achieved. The need for ongoing assessment of spatial conservation areas (Dunham *et al.*, 2020) is well established (Aichi Target 11), but is generally secondary in emphasis to the establishment of areas (Leverington *et al.*, 2010; Addison *et al.*, 2015). The disconnect between establishment, monitoring, and adaptive management can become problematic, whereby efforts are unduly focused on quantity and not quality of conservation areas (Barnes, 2015; Edgar, 2017). Indeed, at a global scale, the focus on numerical targets for conservation areas (e.g. "30 by 30") has generally abstracted a review of whether biodiversity is appropriately reflected and conserved therein (Barnes *et al.*, 2018), which could lead to inefficient conservation action (Jantke *et al.*, 2018). At regional and local scales, a lack of suitable monitoring and management is a primary reason why MPAs do not achieve their intended objectives (Gill *et al.*, 2017). Thus, there is a clear need for more efficient and scalable monitoring tools for MPAs and MPA networks.

Environmental DNA (eDNA) is DNA extracted directly from environmental samples (Rodriguez-Ezpeleta *et al.*, 2021). eDNA metabarcoding combines eDNA sampling with high-throughput amplicon sequencing and is a powerful biodiversity monitoring tool with several advantages over direct sampling methods, including that it is minimally invasive, relatively easy to sample, independent of taxonomists, and cost-effective (Ji *et al.*, 2013). Furthermore, high-throughput sequence data can be reanalysed to improve taxonomic assignments as reference DNA sequence libraries are updated. Indeed, eDNA metabarcoding can be an effective monitoring tool for MPAs, offering a complementary or alternative approach to traditional sampling (Stat *et al.*, 2019).

The objective of this study is to compare eDNA metabarcoding with a long-term biodiversity sampling programme using trawl surveys spanning a broad geographic region in the Canadian Northwest Atlantic. Using a paired-sampling approach, we compare patterns of fish and invertebrate diversity

Received: 14 December 2022; **Revised:** 26 April 2023; **Accepted:** 27 April 2023
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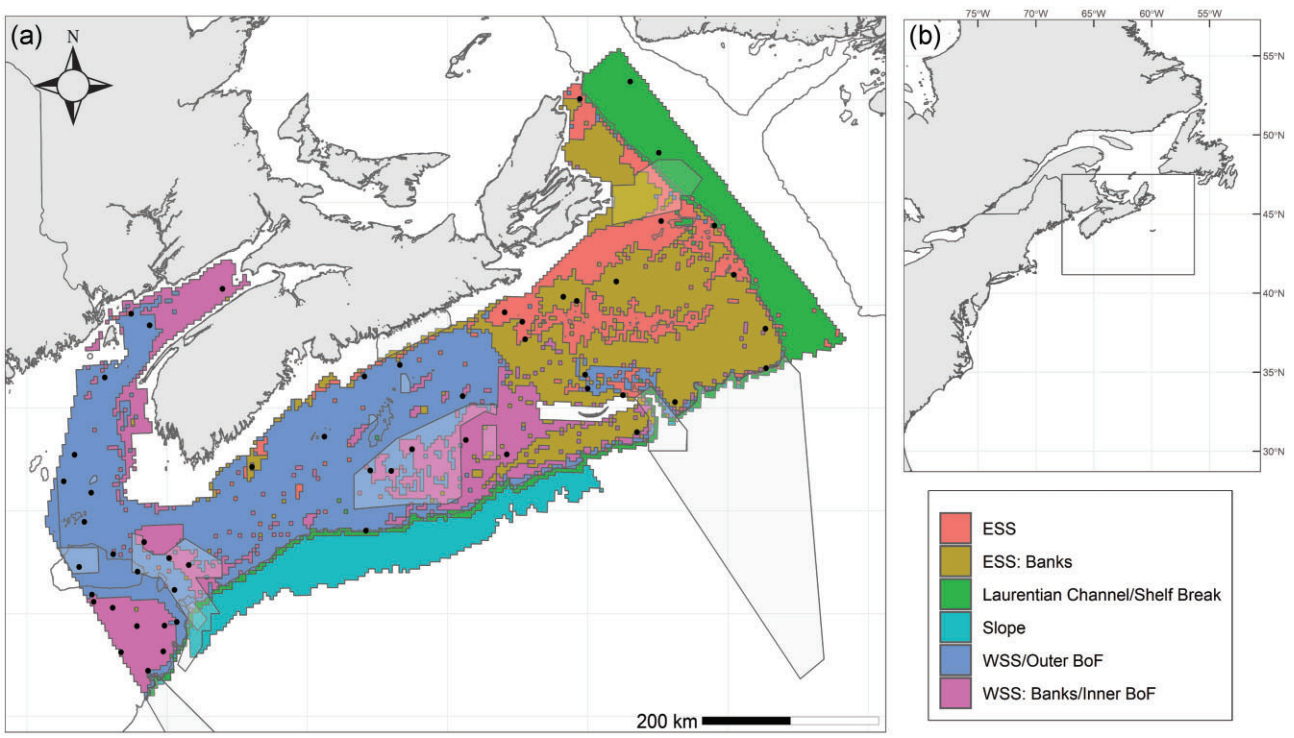

Figure 1. Map of paired-sample sites ($n = 54$) in this study. In panel (a), colours depict bioclassification groups outlined in O'Brien *et al.* (2022) and shaded polygons depict locations of spatial conservation measures, including MPAs, Areas of Interest, and Marine Refuges within the Scotian Shelf and Bay of Fundy Bioregion. The grey line in panel (a) depicts the 250-m isobath. Panel (b) depicts the geographic location of the study area in Atlantic Canada.

between trawling and eDNA metabarcoding of seawater and discuss the potential for metabarcoding to contribute to biodiversity monitoring in offshore areas of conservation concern and where non-invasive approaches are required.

Materials and methods

Trawl survey and eDNA sampling

Fish and invertebrates were captured by a western IIA bottom otter trawl across the Scotian Shelf and Bay of Fundy (NAFO divisions 4VWX5Y) by the Research Vessel *Alfred Needler* between June and August 2020. This annual random stratified trawl survey has followed a standardized protocol since 1970 to assess trends in fish population abundance and diversity (DFO, 2021). Standard trawls have a speed and length of 3 knots and 1.75 nautical miles (~3 km), respectively. Catch from each set is sorted and identified at sea to the lowest taxonomic level following standardized identification keys. The mass and abundance of each species are recorded per trawl set and normalized to a standard tow length of 1.75 nautical miles.

Seawater samples for eDNA sampling were collected at the end of each trawl transect using an SBE-25 profiling CTD and rosette sampler (Sea-Bird Scientific) as part of the survey's standardized protocol. The rosette's Niskin bottles were deployed open and triggered within 2 m of the bottom (based on the vessel sounding). On board the vessel, one litre of seawater was drained from the Niskin bottle spigot into a sterilized, amber Nalgene bottle. Given a limited capacity to filter water in a sterile environment on the vessel, samples were frozen at -25°C. Because freezer space was limited, we opted not to replicate water samples at each site in favour of maximizing the spatial distribution and number of paired samples. Overall, water samples were collected alongside a paired trawl set at 54 sites across a depth range spanning 30–250 m (Figure 1). Environmental covariates, including bottom temperature, salinity, and depth were obtained from the CTD profiles.

Samples were filtered in the laboratory after thawing at 4°C for 48–60 h. For each sample, 800 ml water was filtered through a 0.22 µm Sterivex polyvinylidene difluoride filter using a peristaltic pump. Field blanks consisting of distilled water poured into Nalgene bottles at the time of sample collection (and subsequently frozen), and laboratory blanks of distilled water were filtered in the same way. After filtration, all samples were preserved with Longmire's buffer, sealed, and frozen until DNA extraction. As the water samples were collected after each trawl set, there was a chance of contamination, particularly through disrupted sediments being incidentally captured. However, no samples contained noticeable sediment in the filter, suggesting little bottom contact contamination.

eDNA library preparation and metabarcoding

DNA extraction and library preparation were performed in separate clean labs specifically for eDNA research at the Bedford Institute of Oceanography. eDNA was extracted using DNeasy Blood and Tissue kits (Qiagen) based on Spens *et al.* (2017), with minor modifications listed in He *et al.* (2022). eDNA was extracted from a total of 80 samples (54 eDNA samples, 16 field blanks, six laboratory blanks, and four DNA extraction negative controls) using a final elution volume of 100 µl.

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Table 1. Primer sequences for the four markers used in this study.

Marker	Primer name	Sequence (5'-3')	Amplicon size	Source
12S	12S_248F	CGTGCCAGCCACCGCGGTT	~160 bp	He *et al.*, 2022
	Mifish-U-R	CATAGTGGGGTATCTAATCCCAGTTTG		Miya *et al.*, 2015
16S	16S_Fish_F	AGCGYAATCACTTGTCTYTTAA	~192 bp	McInnes *et al.*, 2017
	16S_Fish_R	CRBGGTCGCCCCAACCRAA		
18S	TAReuk454FWD1	CCAGCASCYGCGGTAATTCC	~380 bp	Stoeck *et al.*, 2010
	TAReukREV3	ACTTTCGTTCTTGATYRA		
CO1	mlCOIintF	GGWACWGGWTGAACWGTWTAYCCYCC	~313 bp	Leray *et al.*, 2013
	jgHCO2198	TAIACYTCIGGRTGICCRAARAAYCA		Geller *et al.*, 2013

For metabarcoding, we used one-step PCR library preparation following Lacoursière-Roussel *et al.* (2018). Libraries were prepared separately for each marker following He *et al.* (2022). Markers from four genes were amplified using a template volume of 3 µl for all 80 samples: *12S rRNA*, *16S rRNA*, *18S rRNA*, and *cytochrome c oxidase subunit I* (*CO1*). Of these, 12S and 16S targeted fish (Miya *et al.*, 2015; McInnes *et al.*, 2017; He *et al.*, 2022); CO1 targeted invertebrates (Geller *et al.*, 2013; Leray *et al.*, 2013); and 18S targeted general eukaryotes (Stoeck *et al.*, 2010). See Table 1 for primer sequences and Supplementary Tables S1 and S2 for PCR thermocycling conditions. Each sample was PCR amplified in triplicate by each marker in 25 µl PCR reaction volumes, plus one PCR negative control. After confirming no amplification in PCR negative controls using a QIAxcel (Qiagen) machine, the three replicate PCR products from each marker for each sample were pooled. Then, PCR products were purified, quantified, and pooled. Pooled PCR products from all samples were purified and quantified again. Libraries for 12S and 16S were sequenced using MiSeq v2 (300 cycles) kits (Illumina); libraries for CO1 and 18S were sequenced separately using a MiSeq v2 (500 cycles) kit and a MiSeq v3 (600 cycles) kit, respectively, at the Pacific Biological Station.

Data analyses

Sequence processing

MiSeq sequences were processed separately for each marker using QIIME2 (Bolyen *et al.*, 2019). Cutadapt was used to trim marker-specific primer regions (Martin, 2011). DADA2 was used to denoise sequences, remove chimeras, and generate an amplicon sequence variant (ASV) table and an ASV sequence file for each marker (Callahan *et al.*, 2016). To reduce potential false positives related to tag jumping (Schnell *et al.*, 2015) and/or contamination, low-frequency ASVs within each sample in 12S and 16S ASV tables were excluded following these thresholds (Hänfling *et al.*, 2016): if the number of reads of an ASV in a sample was <0.1% of the number of reads of that ASV across samples, or <0.1% of the total number of reads in that sample, the number was changed to 0. In the 18S and CO1 ASV tables, if an ASV had fewer than 10 reads in a sample, the number of reads for that ASV in that sample was changed to 0.

Taxonomic assignment and further filtration

Taxonomic assignment for 12S and 16S ASVs was done using the BLAST top hit method with a minimum similarity of 98% applied to custom reference libraries built by He *et al.* (2022), consisting of sequences from Canadian marine bony fish. Unassigned ASVs were then blasted against the NCBI Nucleotide database. ASVs with the highest similarity to more than one species (i.e. ASVs with sequence identical to or equally different from two or more species) were assigned to the lowest level possible (i.e. genus or family). Unassigned ASVs were discarded, and 12S and 16S ASV tables were collapsed to taxon tables. Geographic occurrence of fish taxa detected was checked using FishBase and OBIS databases. Non-target taxa were carefully checked, and most were discarded (Supplementary Tables S3 and S4), except a few (Supplementary Tables S5 and S6). Once non-target taxa were removed, reads in negative controls were addressed by the subtraction method, following Nguyen *et al.* (2015), as follows. For each taxon present in DNA extraction negative controls or laboratory blanks, the number of reads for that taxon in the control was subtracted from the number of reads for that taxon in each water sample. For taxa with reads in field blanks, the number of reads for that taxon in the field blank was subtracted from the number of reads for that taxon in the water sample from that site.

Taxonomic assignment for CO1 ASVs was first performed using the Ribosomal Database Project (RDP) naïve Bayesian classifier (Wang *et al.*, 2007) with the reference database trained by Porter and Hajibabaei (2018). ASVs assigned to Metazoa by the RDP classifier were BLASTed using the NCBI Nucleotide database. ASVs with ≥97% similarity to reference sequences and query cover ≥97% were assigned to species level. ASVs with the greatest similarity to more than one species were assigned to a common genus or family level. ASVs with the greatest similarity to multiple species from different families were discarded. Based on BLAST results, the metazoan ASV table was collapsed into a taxon table. Taxa were verified as marine organisms using the WoRMS database, and non-marine taxa were removed; reads in controls were addressed using the subtraction method (Supplementary Table S7).

The PR^2 database (Guillou *et al.*, 2013) was used for taxonomy assignment for 18S ASVs using two methods. The PR^2 database files were imported into QIIME2 and trained using the feature-classifier plugin. Taxonomic assignment of all 18S ASVs was performed using the q2-feature-classifier (Bokulich *et al.*, 2018). A metazoan ASV table was generated based on taxonomic assignment results by the classifier, and reads in controls were addressed using the subtraction method. ASVs assigned to Metazoa by the classifier were then assigned using the classify-consensus-vsearch (an alignment-based method) (Rognes *et al.*, 2016).

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Species diversity analyses: trawl and eDNA data

Rarefactions were performed using the 12S, 16S, and CO1 taxon tables, respectively, to check if sequencing depths were enough (Supplementary Figure S1). Alpha diversity was analysed separately for fish and invertebrates. Taxon accumulation curves were generated for both fish and invertebrates across samples using the iNEXT R package (Hsieh *et al.*, 2016). Beta diversity was analysed using QIIME2 (Bolyen *et al.*, 2019), as follows: metazoan taxa detected by 12S, 16S, and CO1 were combined to compute a Jaccard dissimilarity distance matrix for eDNA, and all taxa caught at sea were used to compute a Jaccard dissimilarity distance matrix for trawling. Principal coordinate analyses (PCoA) were conducted on the two Jaccard dissimilarity distance matrices. Mantel tests were performed to analyse the correlation between the eDNA and trawl Jaccard dissimilarity distance matrices, and the correlations between Jaccard dissimilarity distance matrices and Euclidean distance matrices computed from each environmental covariate.

Results

Summary of trawling data

Trawling caught 189 taxa (Supplementary Table S8) comprising 118 invertebrates and 71 fish. The most abundant species by count was northern shrimp (*Pandalus borealis*), whereas the most abundant taxon by biomass was redfish (*Sebastes* spp.), for which individuals generally could not be identified to species.

Summary of eDNA metabarcoding data

For 12S, 6.64 million raw paired-end reads were generated. After taxonomy assignment and discarding unassigned reads, 5.76 million reads remained. This number of reads in eDNA samples, field blanks, and laboratory blanks was 5.55 million, 91295, and 118866, respectively; there were no reads in DNA extraction negative controls (Supplementary Table S3). The 98.8% reads in field blanks and 99.5% reads in laboratory blanks were assigned to terrestrial animals. Reads assigned to non-target taxa were removed, and reads in field blanks and laboratory blanks were then addressed using the subtraction method, as described above. There were 1.67 million reads from the 54 eDNA samples in the final 12S taxon table; this marker detected 64 taxa consisting of 45 bony fish, eight cartilaginous fish, seven marine mammals, three invertebrates, and one seabird (Supplementary Table S5).

For 16S, 7.98 million raw paired-end reads were generated. After taxonomic assignment and discarding unassigned reads, 7.59 million reads remained. The number of reads in eDNA samples, field blanks, laboratory blanks, and DNA extraction negative controls was 7.59 million, 241, 124, and 26, respectively (Supplementary Table S4). After non-target taxa were removed, and reads in negative controls were addressed using the subtraction method, 7.59 million reads remained in the final 16S taxon table. This marker detected 55 taxa, consisting of 44 bony fish, 10 cartilaginous fish, and one invertebrate (Supplementary Table S6).

For CO1, 10.19 million raw paired-end reads were generated. There were 7.21 million reads remaining after removing ASVs with fewer than 10 reads. The RDP classifier assigned 6.07 million reads to Eukaryota and 0.26 million to bacteria with a confidence value ≥0.8. Of the Eukaryota reads, 4.38 million could not be assigned to kingdom with a confidence value ≥0.8, and 0.97 million were assigned to Metazoa. After these metazoan ASVs were assigned using the BLAST top hit method, unassigned ASVs were discarded, and a CO1 metazoan table containing 0.82 million reads was generated. The number of reads in eDNA samples, field blanks, and laboratory blanks was 0.80 million, 11326, and 8064, respectively, there were no reads in DNA extraction negative controls (Supplementary Table S7). The 99.4% reads in field blanks and all reads in laboratory blanks were assigned to non-marine taxa. After these non-target taxa were removed and reads in negative controls were addressed using the subtraction method, 0.77 million reads remained in the final CO1 metazoan table. This marker detected 182 taxa, including 150 invertebrates, 20 bony fish, six cartilaginous fish, five marine mammals, and one hagfish (Supplementary Table S9).

For 18S, 14.26 million raw paired-end reads were generated. There were 11.12 million reads in the ASV table after removing ASVs with fewer than 10 reads. The three most abundant taxonomic groups amplified were Dinoflagellata (5.84 million reads), Ochrophyta (2.90 million reads), and Metazoa (1.28 million reads). Metazoan reads comprised 431 ASVs, but only 20 ASVs were assigned to species level with a confidence value ≥0.8 using the alignment-based method (Supplementary Table S10). Given that most 18S metazoan ASVs were not assigned to species, 18S data were excluded from downstream biodiversity analyses.

Comparison between methods for fish taxa

A total of 76 fish taxa were detected by eDNA when 12S, 16S, and CO1 data were combined (Supplementary Table S11), 68 of which were assigned to species level, six to genus level, and two to family level. Of these, 62 were bony fish, 13 were cartilaginous fish, and one was a hagfish. Trawling caught 71 fish taxa, 66 of which were identified to species level, four to genus level, and one to family level. Of these, 62 were bony fish, eight were cartilaginous fish, and one was a hagfish. The five most abundant fish taxa caught by trawling were redfish, northern sand lance (*Ammodytes dubius*), haddock (*Melanogrammus aeglefinus*), silver hake (*Merluccius bilinearis*), and Atlantic herring (*Clupea harengus*). These were among the most abundant taxa detected by 12S and 16S, except Atlantic herring in the 16S data (Supplementary Table S11).

Both eDNA and trawling each detected 61 fish genera, 45 of which were shared (Figure 2). Considering species-level identification only, 42 fish species were detected by both eDNA and trawling; 26 species were detected only by eDNA, and 24 species were detected only by trawling (Figure 2). Taxon accumulation curves using both rarefaction and extrapolation methods showed trawling and eDNA (using all three markers) detected a similar number of fish taxa (Figure 3). At the site level, trawling detected more fish taxa than eDNA (Figure 4a).

For the 24 species captured by trawls and not detected by eDNA, 12 did not have 12S or 16S reference sequences in our reference databases, and seven had reference sequences for one marker only. The 12 fish species with at least one sequence in the database and caught by trawling but not detected by eDNA were netted at an average of 2.7 sites (ranging from 1 to 8), and the average number of individuals caught was 4.6 per site (ranging from 1 to 22). In contrast, the 42 fish species detected by trawling and eDNA were netted at an average of

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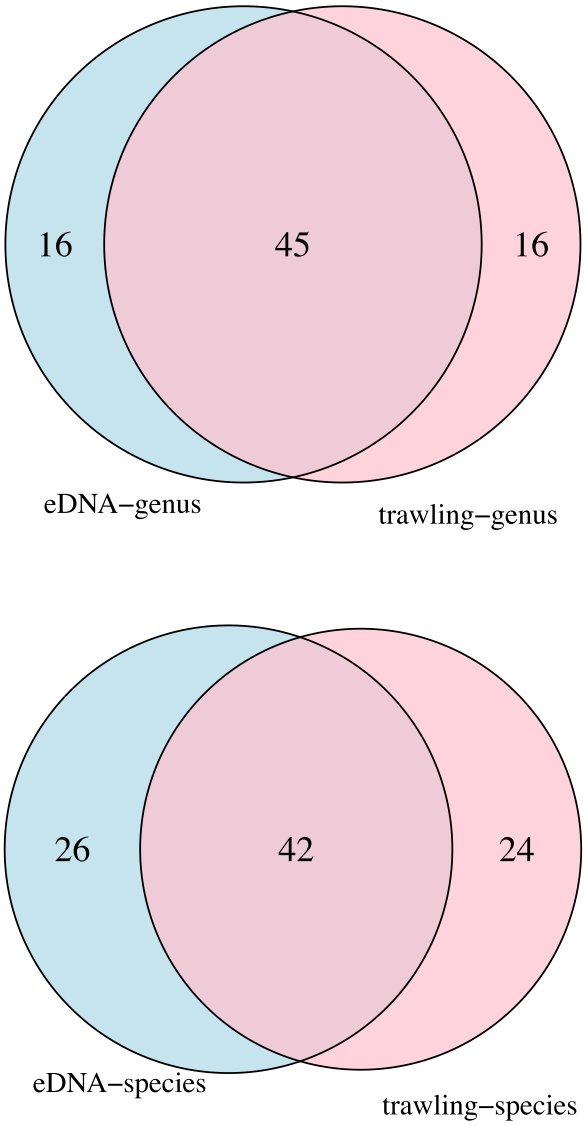

Figure 2. Venn diagrams showing the numbers of fish taxa detected by eDNA and trawling at genus and species levels. For the lower panel, only taxa identified to the species level were considered.

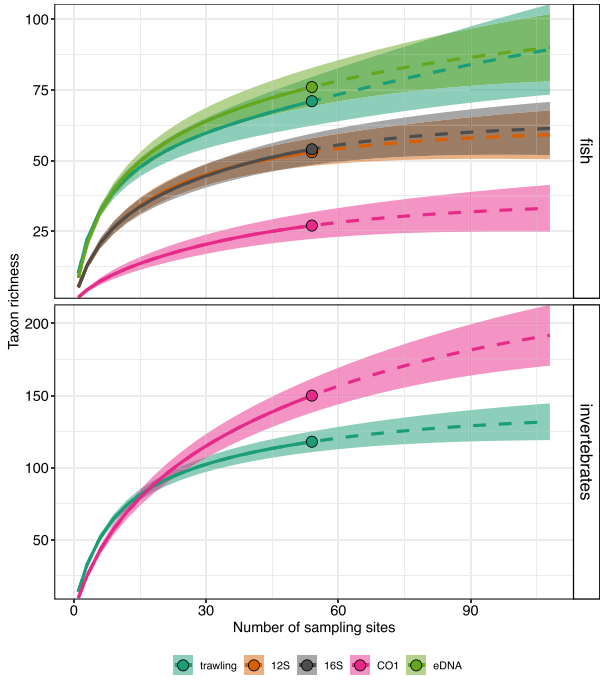

Figure 3. Sample-size-based taxon accumulation curves for fish (upper panel) and invertebrates (lower panel) using interpolation (solid line) and extrapolation (dashed line) methodologies. Fish taxa (including non-species-level taxa) detected by trawling, 12S, 16S, CO1, and the three markers combined eDNA data were used for the analyses, whereas only invertebrates detected by trawling and CO1 were used (as 12S and 16S detected very few invertebrate species).

10.0 sites (ranging from 1 to 43). Considering only sites where these species were caught, the average number of individuals caught per site was 36.9 (ranging from 1 to 598). Hence, low eDNA availability appears to be a factor for the missed eDNA detections.

Other vertebrates

Combining marine mammals detected by 12S and CO1, nine taxa were detected by eDNA, eight of which were assigned to species and one to family (Table 2). One 12S ASV had 100% similarity to reference sequences from four duck species, thus was assigned to Anatidae.

Invertebrates

A total of 118 invertebrate taxa were identified in the trawl survey, 72 of which were identified to species level and 46 to a higher level. The CO1 marker detected 150 invertebrate taxa, 130 of which were assigned to species, 15 of which did not have a species name (Supplementary Table S9). Excluding taxa above species level and those without a species name, 23.6% (17/72) of species caught by trawling were detected by eDNA, whereas 14.8% (17/115) of species detected by eDNA were caught by trawling (Supplementary Figure S2). Species accumulation curves showed eDNA detected more invertebrate taxa than trawling overall (Figure 3); however, similar to our results for fish, at the site level trawling detected more invertebrate taxa than eDNA (Figure 4b).

Spatial patterns

PCoA analyses using all taxa caught by trawling and all metazoan taxa detected by eDNA showed similar spatial patterns: there was a clear difference between samples collected from sites on the eastern Scotian Shelf (ESS) versus the western Scotian Shelf (WSS) (Figure 5). For trawling data, the ESS and WSS were separated along PCoA axis 1, which explained 13.1% of the variation. For the eDNA data, the ESS and WSS were divided along PCoA axis 2 (8.3%), whereas PCoA axis 1 (9.2%) primarily divided the deep and shallow sets in both the ESS and WSS. The Mantel test showed a moderate correlation between the Jaccard dissimilarity matrices computed using trawling and eDNA taxa ($r = 0.449$, $p = 0.001$). Temperature, salinity, and sampling depth had a significant correlation with community composition (Table 3), indicating that environmental gradients within the study region had a significant influence on spatial patterns of species turnover.

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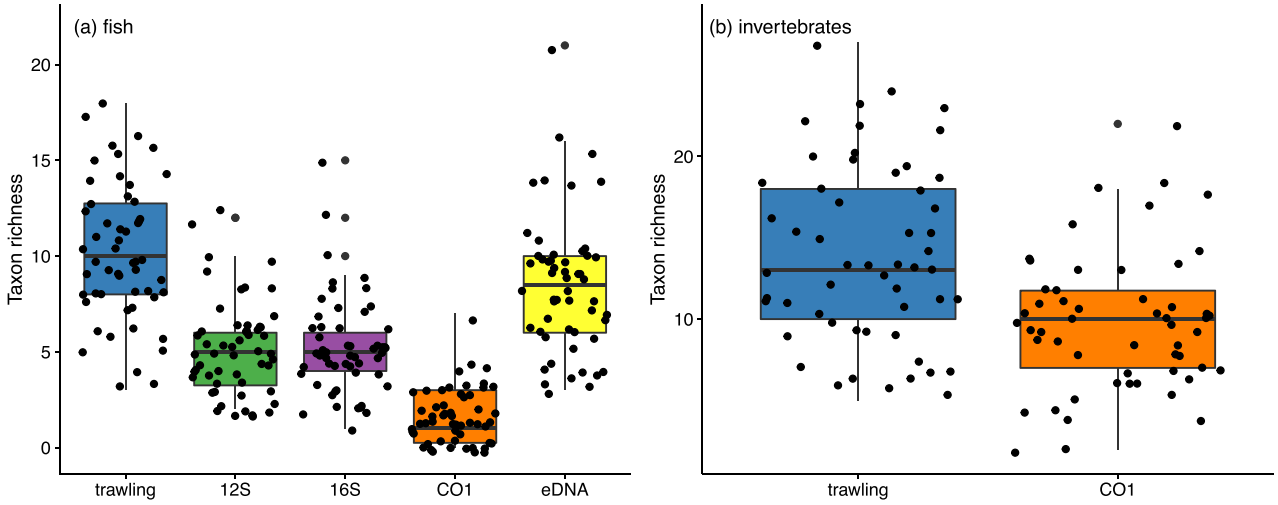

Figure 4. Richness of fish taxa detected by trawling, 12S, 16S, CO1, and the three markers combined eDNA data (a) and of invertebrates detected by trawling and CO1 (b). All taxa detected by eDNA and trawling (including non-species-level taxa) were used for the analyses.

Table 2. Number of reads for marine mammals detected by 12S and CO1 markers.

Taxon	12S		CO1	
	Number of reads	Percentage of reads (%)	Number of reads	Percentage of reads (%)
Balaenoptera acutorostrata	384	0.023	17	0.002
Balaenoptera musculus	–	–	125	0.016
Balaenoptera physalus	715	0.043	–	–
Delphinidae	2234	0.134	82	0.011
Lagenorhynchus acutus	369	0.022	–	–
Megaptera novaeangliae	230	0.014	–	–
Phocoena phocoena	194	0.012	18	0.002
Stenella coeruleoalba	–	–	16	0.002
Tursiops truncatus	67	0.004	–	–

Discussion

Fish diversity has been targeted widely by eDNA metabarcoding studies to compare eDNA-derived estimates with those from conventional methods (Pawlowski *et al.*, 2021). It is important to note results across studies will inevitably vary given the complexity of the eDNA metabarcoding workflow and the absence of a single accepted standardized approach (Larson *et al.*, 2020; Burian *et al.*, 2021). Varying factors include primer choice, filter material, pore size, water volume, number of replicates, DNA extraction method, library preparation protocol, sequencing depth, and bioinformatics pipeline (Deiner *et al.*, 2018; Bailet *et al.*, 2020). Nonetheless, it is still informative to compare our findings with analogous studies with similar aims to aid in the development, refinement, and operationalization of eDNA metabarcoding for monitoring and management.

Relatively few eDNA metabarcoding studies targeting fish compare species composition and diversity between eDNA metabarcoding and trawling. While some studies found a larger number of fish taxa detected by eDNA (Afzali *et al.*, 2021; Zhou *et al.*, 2022), others found the opposite (Thomsen *et al.*, 2016; Stoeckle *et al.*, 2021). Our study also found eDNA identified more fish taxa than trawls (gamma diversity). About two-thirds (64%) of fish species caught by trawls were also detected by eDNA. This percentage was lower than that found by Stoeckle *et al.* (2021) and Afzali *et al.* (2021), but larger than by Zhou *et al.* (2022). We observed 45.6% (42/92) of fish species were caught by both methods. The level of overlap between the two methods was larger than in Zhou *et al.* (2022), who found 20.8% (27/130) of fish species were detected by both methods, but was smaller than in Afzali *et al.* (2021), who found 54% (47/87) of fish species were detected by both methods. Similar to Stoeckle *et al.* (2021), who found eDNA detected all abundant species caught by trawling, we found eDNA availability was an important factor affecting species detection; fish species detected by both methods were much more abundant than those detected by trawling alone. In addition, reference DNA sequence availability was also important: 18.2% of fish species caught by trawling in this study and 10% of fish species caught by Stoeckle *et al.* (2021) lacked reference sequences.

Average taxon richness for fish at the site level (alpha diversity) calculated using eDNA data was 16% smaller than that based on trawling data, contrasting with similar studies showing eDNA indicated greater fish species richness than trawling (Afzali *et al.*, 2021; Stoeckle *et al.*, 2021; Zhou *et al.*, 2022). The main potential reason for the difference in patterns of site-level diversity between our study and others is we used only 0.8 l water and no site replicates due to operational limitations. In contrast, Stoeckle *et al.* (2021) collected both surface and benthic samples, while Zhou *et al.* (2022) collected three

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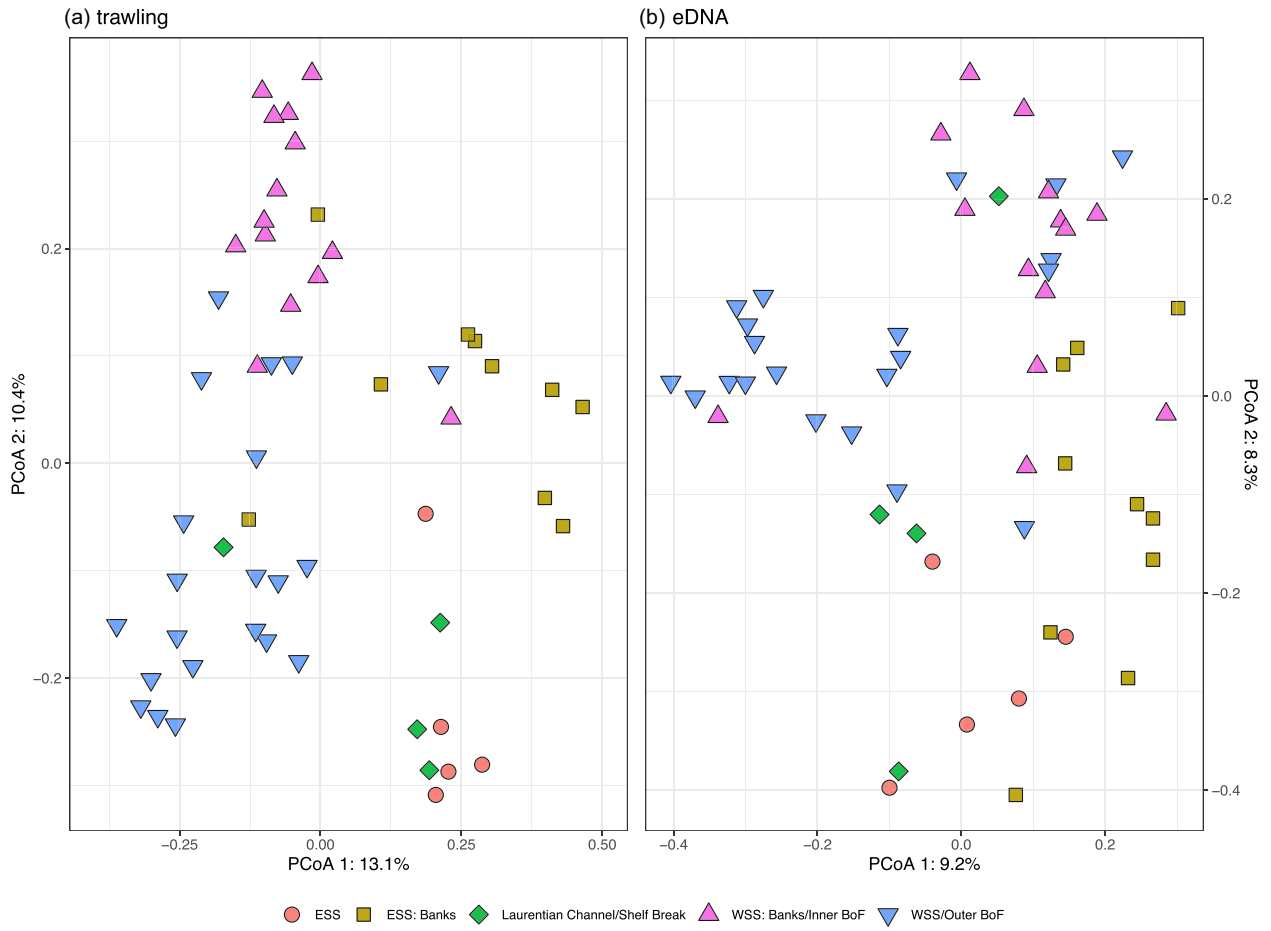

Figure 5. PCoA of Jaccard dissimilarity distances depicting spatial differences based on trawling and eDNA data for all detected metazoan taxa. Sites are coloured according to the bioclassification groups outlined in O'Brien *et al.* (2022).

Table 3. Results of Mantel tests between Jaccard dissimilarity distance matrices and Euclidean distance matrices computed from each environmental parameter.

Parameter	eDNA		Trawling	
	Spearman rho	*p*-value	Spearman rho	*p*-value
Bottom temperature	0.294	0.001	0.468	0.001
Bottom salinity	0.216	0.001	0.284	0.001
Depth	0.218	0.001	0.260	0.001

1 l replicates per site. In the Gulf of St. Lawrence, Afzali *et al.* (2021) also detected more fish taxa than paired trawls, by collecting single 2 l water samples, using a larger filter pore size (1.2 µm), and by sampling various depths at a subset of stations. Thus, sampling a greater water volume, or including eDNA sample replicates, would likely have increased eDNA-based species richness for fish at the site level in our study, and the proportion of species collected by trawls also detected by eDNA. Indeed, eDNA rarity has been reported as the most limiting factor for 12S metabarcoding surveys of marine fish (Stoeckle *et al.*, 2022). This may be particularly important when sampling is restricted to relatively small sample volumes in comparison to a trawl set deployed over several kilometres. Nevertheless, our results highlight the remarkable ability of a 0.8 l water sample to detect most fish taxa captured in millions of litres of swept water volume over a 3 km trawl set. Moreover, eDNA detected the same or more fish taxa at 25 of the 54 sampled sites.

Comparisons focusing on eDNA and trawling for invertebrates are relatively rare. We were not surprised that only a small portion of invertebrate taxa were detected by both methods, as trawls do not reliably capture small-bodied taxa (e.g. zooplankton and meiofauna). Theoretically, metabarcoding can detect any taxon if their eDNA is sampled, extracted, and amplified; however, incompleteness of reference databases, sequencing depth, non-specific amplification, and competition among DNA templates during PCR can impact species detection (Zinger *et al.*, 2019). Despite only small numbers of reads being assigned to marine mammals, our study demonstrates that eDNA contains extra-organismal DNA from marine megafauna, which would be detected more efficiently with specific primers. Overall, we illustrate that by sampling

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0.8 l (or more) of seawater and applying taxon-specific markers, eDNA metabarcoding can broadly survey marine biodiversity and trophic levels.

Our beta diversity analyses show that eDNA can capture known spatial patterns of species turnover in the study region. O'Brien *et al.* (2022) identified six distinct communities of demersal fish and invertebrate in the Scotian Shelf and Bay of Fundy Bioregion using trawling data from 2007 to 2017: the eastern and western Scotian Shelves, each of which is further divided into deep and shallow areas, the continental slope, and the shelf break at the Laurentian Channel. Both our 2020 trawl and metabarcoding data show the same deep and shallow divisions within and between the ESS and WSS, with the Laurentian Channel samples clustering primarily with the ESS deep samples. This demonstrates the remarkable consistency in community structure across this region, even after only one season of sampling. Unfortunately, no samples were collected from the continental slope, which comprised unique fish and invertebrate assemblages (Clark and Emberley, 2011; O'Brien *et al.*, 2022). The utility of eDNA for detecting deep-water species on the Scotian Shelf slope will require further investigation, but has been successful in other regions (McClenaghan *et al.*, 2020).

MPAs are a key tool for biodiversity conservation globally, and effective monitoring is a cornerstone to their success (Edgar *et al.*, 2014). Our findings suggest that even limited sampling for eDNA metabarcoding has great promise for estimating benthic and demersal fishes and invertebrates over a wide spatial scale, particularly when complemented by trawl catch data. Despite contamination being a common problem in eDNA data, careful technique can alleviate this, and negative controls at each step can identify if and when contamination occurred. eDNA metabarcoding is well-suited for MPA monitoring as it enables standardized biodiversity assessments (Bohmann *et al.*, 2014; Schmeller *et al.*, 2017) between biogeographic regions and depth ranges. Further, the cost of adding eDNA sampling to an existing survey is marginal (<$20 k for processing ~90 samples) and will become more cost-efficient over time, and the method has the additional benefit of capturing other vertebrates of conservation interest not sampled by trawls. This might be particularly advantageous for detecting invasive species (Larson *et al.*, 2020) or species undergoing range shifts under climate change. For example, blackbelly rosefish were captured by both eDNA and trawling in this study, corresponding to its rapidly increasing spawning biomass on the Scotian Shelf (DFO, 2021). eDNA metabarcoding presents the opportunity to greatly expand the spatial-temporal coverage of sampling (Gold *et al.*, 2021) and taxonomic scope, while enabling non-destructive monitoring of sensitive bottom habitats (He *et al.*, 2022). It can be a powerful supplement to existing direct sampling approaches, even though the ability of eDNA metabarcoding to reflect patterns of biomass in marine ecosystems requires more study. Although eDNA metabarcoding cannot provide estimates of absolute biomass, developmental stage, and size distribution, our results align with a growing body of evidence demonstrating the potential power of eDNA as a biodiversity monitoring tool in MPAs and sensitive ecosystems (Boulanger *et al.*, 2021; Gold *et al.*, 2021; He *et al.*, 2022).

Acknowledgements

We thank Jeff Spry, Kevin Pauley, and Jamie Emberley (DFO Science) for assistance in collecting samples at sea and providing trawl catch and water chemistry data, and Louise-Marie Roux, Angela Schulze, Kara Aschenbrenner, and Kristi Miller-Saunders for access to and assistance with MiSeq runs. We thank Scott Gilmore for help with data interpretation.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

Conflict of interest

The authors declare no conflict of relevant financial or non-financial interests.

Funding

This work was supported by a Fisheries and Oceans Canada Genomics Research and Development Initiative grant awarded to C.L.A., E.M.R., R.R.E.S., and N.W.J.

Author contributions

C.L.A., E.M.R., R.R.E.S., and N.W.J. conceived this study. N.W.J. filtered all water samples. L.C.H. and X.H. did molecular lab work. X.H., R.R.E.S., and C.L.A. did data analyses. X.H., N.W.J., R.R.E.S., C.L.A., and E.M.R. wrote the first draft. All authors reviewed the final version of the manuscript.

Data availability

Raw MiSeq sequences are deposited in the NCBI Sequence Read Archive (SRA) database under BioProject PRJNA740347 within BioSample accessions SAMN28405505-SAMN28405744 and SAMN28408210-SAMN28408289. R scripts and data used in this study are available online (https://github.com/rystanley/offshore_edna).

References

Addison, P. F. E., Flander, L. B., and Cook, C. N. 2015. Are we missing the boat? Current uses of long-term biological monitoring data in the evaluation and management of marine protected areas. Journal of Environmental Management, 149: 148–156.

Afzali, S. F., Bourdages, H., Laporte, M., Mérot, C., Normandeau, E., Audet, C., and Bernatchez, L. 2021. Comparing environmental metabarcoding and trawling survey of demersal fish communities in the Gulf of St. Lawrence, Canada. Environmental DNA, 3: 22–42.

Bailet, B., Apothéloz-Perret-Gentil, L., Baričević, A., Chonova, T., Franc, A., Frigerio, J.-M., Kelly, M. *et al.* 2020. Diatom DNA metabarcoding for ecological assessment: comparison among bioinformatics pipelines used in six European countries reveals the need for standardization. Science of The Total Environment, 745: 140948.

Barnes, M. 2015. Aichi targets: protect biodiversity, not just area. Nature, 526: 195.

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Barnes, M. D., Glew, L., Wyborn, C., and Craigie, I. D. 2018. Prevent perverse outcomes from global protected area policy. Nature Ecology & Evolution, 2: 759–762.

Bohmann, K., Evans, A., Gilbert, M. T. P., Carvalho, G. R., Creer, S., Knapp, M., Yu, D. W. *et al.* 2014. Environmental DNA for wildlife biology and biodiversity monitoring. Trends in Ecology & Evolution, 29: 358–367.

Bokulich, N. A., Kaehler, B. D., Rideout, J. R., Dillon, M., Bolyen, E., Knight, R., and Huttley, G. A., 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome, 6: 90.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H. *et al.* 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology, 37: 852–857.

Boulanger, E., Loiseau, N., Valentini, A., Arnal, V., Boissery, P., Dejean, T., Deter, J. *et al.* 2021. Environmental DNA metabarcoding reveals and unpacks a biodiversity conservation paradox in Mediterranean marine reserves. Proceedings of the Royal Society B: Biological Sciences, 288: 20210112.

Burian, A., Mauvisseau, Q., Bulling, M., Domisch, S., Qian, S., and Sweet, M. 2021. Improving the reliability of eDNA data interpretation. Molecular Ecology Resources, 21: 1422–1433.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nature Methods, 13: 581–583.

Clark, D. S., and Emberley, J. 2011. Update of the 2010 summer Scotian Shelf and Bay of Fundy research vessel survey. Canadian Data Report of Fisheries and Aquatic Sciences, 1238.

Corlett, R. T. 2017. A bigger toolbox: biotechnology in biodiversity conservation. Trends in Biotechnology, 35: 55–65.

Deiner, K., Lopez, J., Bourne, S., Holman, L. E., Seymour, M., Grey, E. K., Lacoursière-Roussel, A. *et al.* 2018. Optimising the detection of marine taxonomic richness using environmental DNA metabarcoding: the effects of filter material, pore size and extraction method. Metabarcoding and Metagenomics, 2: e28963.

DFO. 2021. Maritimes research vessel survey trends on the Scotian Shelf and Bay of Fundy for 2020. Canadian Science Advisory Secretariat Science Response, 2021/022. Available from https://waves-vagues.dfo-mpo.gc.ca/library-bibliotheque/40977328.pdf

Dunham, A., Dunham, J. S., Rubidge, E., Iacarella, J. C., and Metaxas, A. 2020. Contextualizing ecological performance: rethinking monitoring in marine protected areas. Aquatic Conservation: Marine and Freshwater Ecosystems, 30: 2004–2011.

Edgar, G. J. 2017. Marine protected areas need accountability not wasted dollars. Aquatic Conservation: Marine and Freshwater Ecosystems, 27: 4–9.

Edgar, G. J., Stuart-Smith, R. D., Willis, T. J., Kininmonth, S., Baker, S. C., Banks, S., Barrett, N. S. *et al.* 2014. Global conservation outcomes depend on marine protected areas with five key features. Nature, 506: 216–220.

Geller, J., Meyer, C., Parker, M., and Hawk, H. 2013. Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. Molecular Ecology Resources, 13: 851–861.

Gill, D. A., Mascia, M. B., Ahmadia, G. N., Glew, L., Lester, S. E., Barnes, M., Craigie, I. *et al.* 2017. Capacity shortfalls hinder the performance of marine protected areas globally. Nature, 543: 665–669.

Gold, Z., Sprague, J., Kushner, D. J., Zerecero Marin, E., and Barber, P. H. 2021. eDNA metabarcoding as a biomonitoring tool for marine protected areas. PLoS ONE, 16: e0238557.

Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., Boutte, C. *et al.* 2013. The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. Nucleic Acids Research, 41: D597–D604.

Hänfling, B., Lawson Handley, L., Read, D. S., Hahn, C., Li, J., Nichols, P., Blackman, R. C., *et al.* 2016. Environmental DNA metabarcoding of lake fish communities reflects long-term data from established survey methods. Molecular Ecology, 25: 3101–3119.

He, X., Stanley, R. R. E., Rubidge, E. M., Jeffery, N. W., Hamilton, L. C., Westfall, K. M., Gilmore, S. R. *et al.* 2022. Fish community surveys in eelgrass beds using both eDNA metabarcoding and seining: implications for biodiversity monitoring in the coastal zone. Canadian Journal of Fisheries and Aquatic Sciences, 79: 1335–1346.

Hsieh, T. C., Ma, K. H., and Chao, A. 2016. iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). Methods in Ecology and Evolution, 7: 1451–1456.

Jantke, K., Jones, K. R., Allan, J. R., Chauvenet, A. L. M., Watson, J. E. M., and Possingham, H. P. 2018. Poor ecological representation by an expensive reserve system: evaluating 35 years of marine protected area expansion. Conservation Letters, 11: e12584.

Ji, Y., Ashton, L., Pedley, S. M., Edwards, D. P., Tang, Y., Nakamura, A., Kitching, R. *et al.* 2013. Reliable, verifiable and efficient monitoring of biodiversity via metabarcoding. Ecology Letters, 16: 1245–1257.

Lacoursière-Roussel, A., Howland, K., Normandeau, E., Grey, E. K., Archambault, P., Deiner, K., Lodge, D. M. *et al.* 2018. eDNA metabarcoding as a new surveillance approach for coastal Arctic biodiversity. Ecology and Evolution, 8: 7763–7777.

Larson, E. R., Graham, B. M., Achury, R., Coon, J. J., Daniels, M. K., Gambrell, D. K., Jonasen, K. L. *et al.* 2020. From eDNA to citizen science: emerging tools for the early detection of invasive species. Frontiers in Ecology and the Environment, 18: 194–202.

Leray, M., Yang, J. Y., Meyer, C. P., Mills, S. C., Agudelo, N., Ranwez, V., Boehm, J. T. *et al.* 2013. A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. Frontiers in Zoology, 10: 34.

Leverington, F., Costa, K. L., Pavese, H., Lisle, A., and Hockings, M. 2010. A global analysis of protected area management effectiveness. Environmental Management, 46: 685–698.

Martin, M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal, 17: 10–12.

McClenaghan, B., Fahner, N., Cote, D., Chawarski, J., McCarthy, A., Rajabi, H., Singer, G. *et al.* 2020. Harnessing the power of eDNA metabarcoding for the detection of deep-sea fishes. PLoS ONE, 15: e0236540.

McInnes, J. C., Jarman, S. N., Lea, M.-A., Raymond, B., Deagle, B. E., Phillips, R. A., Catry, P. *et al.* 2017. DNA metabarcoding as a marine conservation and management tool: a circumpolar examination of fishery discards in the diet of threatened albatrosses. Frontiers in Marine Science, 4: 277.

Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J. Y., Sato, K., Minamoto, T. *et al.* 2015. MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. Royal Society Open Science, 2: 150088.

Nguyen, N. H., Smith, D., Peay, K., and Kennedy, P. 2015. Parsing ecological signal from noise in next generation amplicon sequencing. New Phytologist, 205: 1389–1393.

O'Brien, J. M., Stanley, R. R. E., Jeffery, N. W., Heaslip, S. G., DiBacco, C., and Wang, Z. 2022. Modeling demersal fish and benthic invertebrate assemblages in support of marine conservation planning. Ecological Applications, 32: e2546.

Pawlowski, J., Bonin, A., Boyer, F., Cordier, T., and Taberlet, P. 2021. Environmental DNA for biomonitoring. Molecular Ecology, 30: 2931–2936.

Porter, T. M., and Hajibabaei, M. 2018. Automated high throughput animal CO1 metabarcode classification. Scientific Reports, 8: 4226.

Rocchini, D., Luque, S., Pettorelli, N., Bastin, L., Doktor, D., Faedi, N., Feilhauer, H. *et al.* 2018. Measuring β-diversity by remote sensing: a challenge for biodiversity monitoring. Methods in Ecology and Evolution, 9: 1787–1798.

Rodriguez-Ezpeleta, N., Morissette, O., Bean, C. W., Manu, S., Banerjee, P., Lacoursière-Roussel, A., Beng, K. C. *et al.* 2021. Trade-offs between reducing complex terminology and producing accurate interpretations from environmental DNA: comment on "Environmental

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DNA: what's behind the term?" by Pawlowski et al., (2020). Molecular Ecology, 30: 4601–4605.
Rognes, T., Flouri, T., Nichols, B., Quince, C., and Mahé, F. 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ, 4: e2584.
Schmeller, D. S., Böhm, M., Arvanitidis, C., Barber-Meyer, S., Brummitt, N., Chandler, M., Chatzinikolaou, E. *et al.* 2017. Building capacity in biodiversity monitoring at the global scale. Biodiversity and Conservation, 26: 2765–2790.
Schnell, I. B., Bohmann, K., and Gilbert, M. T. P. 2015. Tag jumps illuminated–reducing sequence-to-sample misidentifications in metabarcoding studies. Molecular Ecology Resources, 15: 1289–1303.
Snelgrove, P. V. R., Archambault, P., Juniper, S. K., Lawton, P., Metaxas, A., Pepin, P., Rice, J. C. *et al.* 2012. Canadian Healthy Oceans Network (CHONe): an academic–government partnership to develop scientific guidelines for conservation and sustainable usage of marine biodiversity. Fisheries, 37: 296–304.
Spens, J., Evans, A. R., Halfmaerten, D., Knudsen, S. W., Sengupta, M. E., Mak, S. S. T., Sigsgaard, E. E. *et al.* 2017. Comparison of capture and storage methods for aqueous macrobial eDNA using an optimized extraction protocol: advantage of enclosed filter. Methods in Ecology and Evolution, 8: 635–645.
Stat, M., John, J., DiBattista, J. D., Newman, S. J., Bunce, M., and Harvey, E. S. 2019. Combined use of eDNA metabarcoding and video surveillance for the assessment of fish biodiversity. Conservation Biology, 33: 196–205.
Stoeck, T., Bass, D., Nebel, M., Christen, R., Jones, M. D. M., Breiner, H.-W., and Richards, T. A. 2010. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Molecular Ecology, 19: 21–31.
Stoeckle, M. Y., Adolf, J., Ausubel, J. H., Charlop-Powers, Z., Dunton, K. J., and Hinks, G. 2022. Current laboratory protocols for detecting fish species with environmental DNA optimize sensitivity and reproducibility, especially for more abundant populations. ICES Journal of Marine Science, 79: 403–412.
Stoeckle, M. Y., Adolf, J., Charlop-Powers, Z., Dunton, K. J., Hinks, G., and VanMorter, S. M. 2021. Trawl and eDNA assessment of marine fish diversity, seasonality, and relative abundance in coastal New Jersey, USA. ICES Journal of Marine Science, 78: 293–304.
Thomsen, P. F., Møller, P. R., Sigsgaard, E. E., Knudsen, S. W., Jørgensen, O. A., and Willerslev, E. 2016. Environmental DNA from seawater samples correlate with trawl catches of subarctic, deepwater fishes. PLoS ONE, 11: e0165252.
Wang, Q., Garrity, G. M., Tiedje, J. M., and Cole, J. R. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology, 73: 5261–5267.
Zhou, S., Fan, C., Xia, H., Zhang, J., Yang, W., Ji, D., Wang, L. *et al.* 2022. Combined use of eDNA metabarcoding and bottom trawling for the assessment of fish biodiversity in the Zhoushan Sea. Frontiers in Marine Science, 8: 2056.
Zinger, L., Bonin, A., Alsos, I. G., Bálint, M., Bik, H., Boyer, F., Chariton, A. A. *et al.* 2019. DNA metabarcoding—need for robust experimental designs to draw sound ecological conclusions. Molecular Ecology, 28: 1857–1862.

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Received: 14 December 2022; **Revised:** 26 April 2023; **Accepted:** 27 April 2023
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