





Comparing eDNA metabarcoding and conventional pelagic netting to inform biodiversity monitoring in deep ocean environments

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The performance of environmental DNA (eDNA) metabarcoding has rarely been evaluated against conventional sampling methods in deep ocean mesopelagic environments. We assessed the biodiversity patterns generated with eDNA and two co-located conventional methods, oblique midwater trawls and vertical multineets, to compare regional and sample-level diversity. We then assessed the concordance of ecological patterns across water column habitats and evaluated how DNA markers and the level of sampling effort influenced the inferred community. We found eDNA metabarcoding characterized regional diversity well, detecting more taxa while identifying similar ecological patterns as conventional samples. Within sampling locations, eDNA metabarcoding rarely detected taxa across more than one replicate. While more taxa were found in eDNA than oblique midwater trawls within sample stations, fewer were found compared to vertical multineets. Our simulations show greater eDNA sampling effort would improve concordance with conventional methods. We also observed that using taxonomic data from multiple markers generated ecological patterns most similar to those observed with conventional methods. Patterns observed with Exact Sequence Variants were more stable across markers suggesting they are more powerful for detecting change. eDNA metabarcoding is a valuable tool for identifying and monitoring biological hotspots but some methodological adjustments are recommended for deep ocean environments.

Keywords: biodiversity, conservation, Deep Ocean, eDNA, environmental DNA, marine ecology, mesopelagic, metabarcoding, monitoring, pelagic netting.

Introduction

Oceans are under increasing pressure from expanding resource use and climate change (Martin *et al.*, 2020). This has spurred greater efforts to understand the impacts on ocean ecosystems, incorporate more holistic management approaches, and expand protected areas, all of which heighten the demands for ecological information, particularly biodiversity data.

Meeting the demands for biodiversity data across expanding spatial scales and in challenging and sensitive marine habitats may not be possible without new, less intrusive, more versatile, and cost-effective techniques (He *et al.*, 2022). Environmental DNA (eDNA) metabarcoding is an emerging tool that is being used for marine research, but for which there remains limited uptake in its use to support management decisions. The approach is increasingly popular among researchers as it is extremely versatile, capable of simultaneously detecting organisms across a variety of taxonomic lineages, easily collected over broad depth ranges and substrate types, and is minimally disruptive to sensitive taxa (Valentini *et al.*, 2016; Bani *et al.*, 2020).

Like many countries, Canada has committed to expanding its protected area network in marine environments to meet

global targets (UN Environment Program, 2022). Many newly established protected areas exist in remote regions of deep water and protect epifauna like corals and sponges that are sensitive to disturbance (Neves *et al.*, 2015). Large portions of these areas are beyond the depths accessible to standard research surveys (e.g. Fisheries and Oceans Canada's multi-species surveys extend to depths of 1500 m; Rideout and Ings, 2021), and since these habitats have been protected, sampling the accessible portions of these habitats using intrusive trawl surveys has become undesirable. Identifying, characterizing, monitoring, and managing these new protected areas requires cost-effective, and non-destructive methods that work in remote and challenging habitats (He *et al.*, 2022). Environmental DNA metabarcoding has great potential for this purpose but its performance has yet to be adequately evaluated in these environments.

As with any new sampling method, studies investigating contextual limitations are required before expanding the range of applications. For example, deciding whether to use eDNA as a standalone method or in combination with a suite of conventional methods may depend on the spatial scale or taxonomic resolution of interest. For eDNA tools to be useful for management, they need to be able to reliably detect

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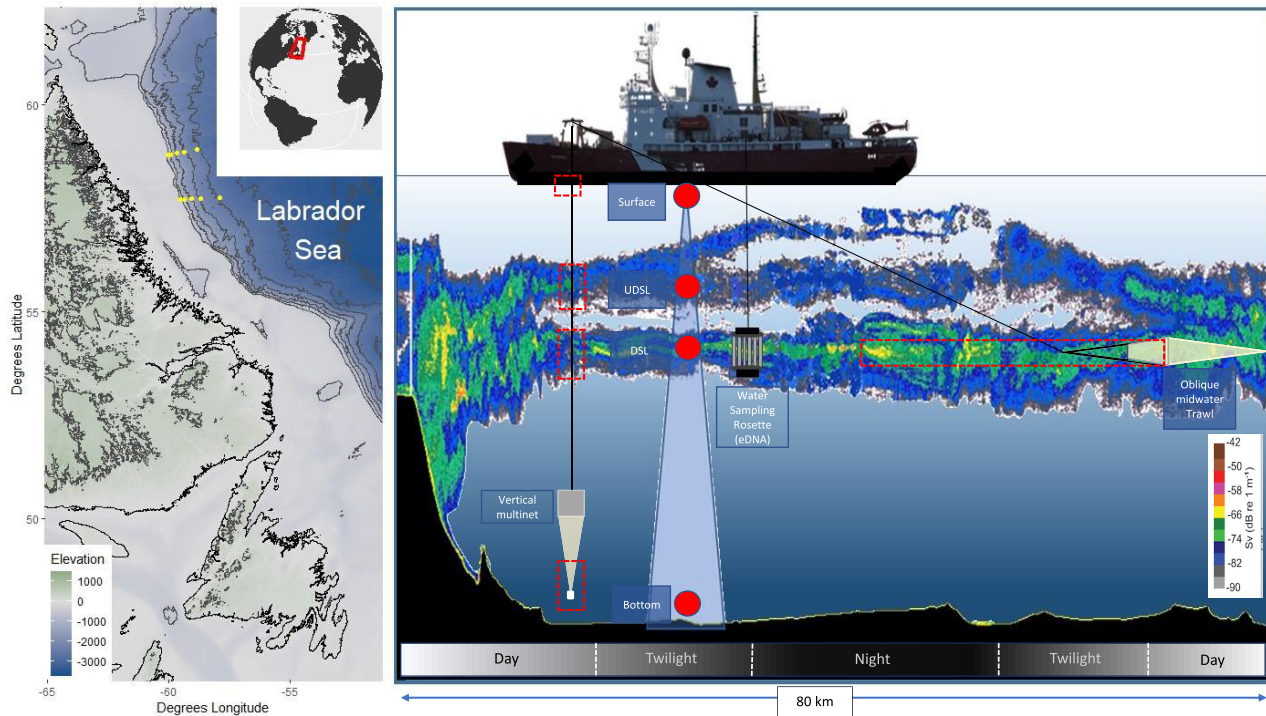


Figure 1. Stations ($n = 10$, 500–2500 m depth, yellow points) sampled across two transects in the Labrador Sea (left panel). Co-located sampling conducted aboard the CCGS *Amundsen* (right panel) is depicted, where red shapes denote sampling zones for eDNA (dots), vertical multinet (vertically elongated rectangles), and oblique midwater trawls (horizontally elongated rectangles). The illustration is overlaid upon a depiction of the diel changes in the acoustic scattering layers detected with the ship's EK80 echosounder near the continental shelf break and a general representation of the bathymetry across the transects.

biodiversity patterns and be sensitive to ecologically meaningful change across spatial and temporal scales of interest. Enhancing our current understanding of eDNA's taxonomic biases and ability to reveal ecological patterns will clarify the utility of this novel technique (Jeunen *et al.*, 2019; Easson *et al.*, 2020) and offer opportunities for methodological refinement (Bucklin *et al.*, 2016).

From co-located samples collected during the Integrated Studies and Ecosystem Characterization of the Labrador Sea Deep Ocean (ISECOLD) programme (Cote *et al.*, 2019), we compared the biodiversity patterns detected using eDNA metabarcoding and conventional sampling methods targeting pelagic fish and zooplankton. Our objectives were to: (i) evaluate regional (gamma), site-level (alpha), and between-site (beta) diversity patterns generated using eDNA and conventional netting methods in the deep ocean; (ii) assess the effects of analytical parameters (e.g. DNA marker choice and taxonomic assignment) on site-level and between-site diversity patterns; and (iii) predict the effects of increased eDNA sampling effort on site-level diversity assessments.

Methods

Study area

We surveyed biological communities in the Labrador Sea, in the Northwest Atlantic Ocean, from 25 to 30 June 2019, aboard the research icebreaker CCGS *Amundsen*. Surveys included eDNA sample collections co-located with oblique midwater trawls and vertical multinetts along two transects with stations at depth intervals of ~ 500 , 1000, 1500, 2000, and 2500 m (Figure 1).

Environmental DNA methods

Field methods

Across the world's oceans, including in the Labrador Sea, mesopelagic organisms concentrate in zones of the water column that can be detected by hull-mounted echosounders (e.g. Figure 1; Proud *et al.*, 2017). This zone is referred to as the deep scattering layer (DSL). We sampled water at each station from the surface (2 m), the upper extent of the deep scattering layer (UDSL, ~ 250 m), the deep scattering layer (DSL, ~ 500 m), and just above the bottom to depths up to 2500 m (Figure 1). In total 38 triplicate samples were taken across all depth zones. Water samples were co-located with vertical multinetts (Surface, UDSL, and DSL only) that targeted zooplankton and oblique midwater trawls that targeted pelagic fish and larger zooplankton (DSL only). We collected triplicate 1.5 L eDNA samples from each depth at each station using a Niskin-style rosette sampler (Seabird Electronic SBE 32). Rosette bottles were assigned to eDNA sampling for the field mission and were decontaminated prior to sampling and between stations using ELIMINase (Decon Labs, Inc., King of Prussia, PA, USA). At each sampling station, a field blank was collected and filtered following the same protocols to assess potential contamination. Conductivity, temperature, and depth profiles were collected for the full water column at each station using a CTD (Seabird SBE911; Supplementary Figure S1).

Lab methods

Filtration on the vessel took place in a dedicated lab space that included a positive pressure ventilation system. Before each

filtration session, surfaces and equipment were all decontaminated with ELIMINase and rinsed with deionized water. Filtering began immediately after sample collection (average volume filtered 1.35 ± 0.15 L) using $0.22 \mu\text{m}$ PVDF Sterivex filters (MilliporeSigma, Burlington, MA, USA) and a peristaltic pump (Cole-Parmer Masterflex MFLX07571-02). The filtration process typically lasted 5–30 min (mean ~ 10 min) per filter, but the sequential filtering of the full set of samples at a station could take up to 4 h. Following filtration, Sterivex filters were capped and stored in Ziploc bags at -18°C for the duration of the mission. On shore, filters were shipped on dry ice to the Centre for Environmental Genomics Applications (St. John's, Canada) for the remaining analysis. There, DNA was extracted from all filter membranes using the DNeasy PowerWater Kit (Qiagen, Hilden, Germany). Negative controls were added during extraction and were carried through to sequencing to screen for contamination. DNA extracts were quantified using the Quant-iT PicoGreen dsDNA assay with a Synergy HTX plate fluorometer (BioTek, Winooski, VT, USA).

Four DNA markers from two gene regions [cytochrome *c* oxidase I (COI) and 18S] were selected to assess metazoan biodiversity and two additional markers from the 12S gene region were included in the analysis to add to the recovery and resolution of bony fish (Table 1). All markers were amplified using PCR following the conditions in Table 1B, where each reaction contained 1X reaction buffer, 2 mM MgCl_2 , 0.2 mM dNTPs, $0.2 \mu\text{M}$ of each of the forward and reverse Illumina-tailed primers, 1.5 U Platinum Taq (Invitrogen, Carlsbad, CA, USA), and $1.2 \mu\text{L}$ of DNA in a total volume of $15 \mu\text{L}$. PCRs were performed using diluted DNA based on a serial dilution assessment for PCR inhibition ($1/10$ and $1/2$ for surface samples and samples collected at depth, respectively). The mean concentration of template DNA used for PCR was $0.88 \pm 1.9 \text{ ng}/\mu\text{L}$. Three PCR replicates were performed for each primer set from each sample and then pooled for a single PCR cleanup with the QIAquick 96 PCR purification kit (Qiagen). Negative controls were added during PCR and were carried through to sequencing to screen for contamination.

Amplicons were visualized using agarose gel (1.5% w/v) electrophoresis to verify amplification of DNA markers and to assess negative controls generated during PCR, extraction, filtration, and field collection. Negative controls were carried through to sequencing as an added level of verification. Amplicons were then indexed using unique dual Nextera indexes (IDT, Coralville, IA, USA; 8-bp index codes). Indexing PCR conditions were initiated for 3 min at 95°C , followed by 12 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 5 min. Amplicons were quantified with Quant-iT PicoGreen dsDNA assay and pooled together in equimolar concentrations by DNA marker. Amplicon pools were cleaned using AMPure XP cleanups, quantified with a Qubit fluorometer (Thermo Fisher, Waltham, MA, USA) and the size distribution of each pool was verified with the DNA 7500 kit on the Agilent 2100 Bioanalyzer. The 12Sm2, 12Sm1, 18Sm1, COIm3, and COIm1 amplicon pools were combined into one library while the COIm2 marker was sequenced as a second library (see Table 1 for DNA markers). The libraries were sequenced with a 300-cycle S1 kit and a 500-cycle SP kit, respectively, on the Illumina NovaSeq 6000 following the NovaSeq standard workflow with a target minimum sequencing depth of 1 million sequences per sample per amplicon. Raw sequence reads are available in NCBI's sequence read archive under project PRJNA643526.

Table 1A. Details of markers and methods used for biodiversity assessments.

DNA marker	Gene region	Forward primer	Reverse primer	Source
COIm2	COI	5'-GGWACWGGWTGAACWGTWTAYCCYCC-3'	5'-TAAACTTCAGGGTGACCAAAAATCA-3'	Leray <i>et al.</i> (2013)
COIm1	COI	5'-ACYAANCAYAAAGAYATNGGCAC-3'	5'-CTTATRTTTRTTTATNCGNGGRAANGC-3'	Shokralla <i>et al.</i> (2015)
COIm3	COI	5'-GGTCAACAAATCATAAGATATTGG-3'	5'-CTTATRTTTRTTTATNCGNGGRAANGC-3'	Gibson <i>et al.</i> (2015)
18Sm1	18S	5'-GTACACACCCGCCGTC-3'	5'-TGATCCTTCTGCAGGTTACACTAC-3'	Stoeck <i>et al.</i> (2010)
12sm2	12S	5'-ACACCGCCCGTCACTCT-3'	5'-CTTCGGGTACACTTACCATG-3'	Valentini <i>et al.</i> (2016)
12Sm1	12S	Blocking: 5'-ACCCTCCTCAAGTATATCTTCAAAGGAC-SPC3I 5'-NNNNNNGTCCGTAATAACTCGTCCAGC-3'	5'-NNNNNNCATAGTGGGGTATCTAATCCCAAGTTTG-3'	Miya <i>et al.</i> (2015)

Table 1B.

(B)							
DNA marker	Amplicon size	Initial	#Cycles	Denaturation	Annealing	Extension	Final extension
COIm2	330	5 min 95°C	35	40 s 94°C	60 s 46°C	30 s 72°C	5 min 72°C
COIm1	226	5 min 95°C	35	40 s 94°C	60 s 46°C	30 s 72°C	5 min 72°C
COIm3	226–235	3 min 95°C	35	30 s 94°C	40 s 46°C	60 s 72°C	10 min 72°C
18Sm1	145	3 min 95°C	35	30 s 94°C	30 s 55°C	60 s 72°C	10 min 72°C
12sm2	100	10 min 95°C	35	30 s 94°C	30 s 55°C	10 s 72°C	5 min 72°C
12Sm1	163–185	3 min 95°C	35	20 s 95°C	15 s 55°C	15 s 72°C	5 min 72°C

Bioinformatics

Base calling and demultiplexing were performed using Illumina's bcl2fastq software (v2.20.0.422). Primers were trimmed from sequences using *cutadapt* v1.168 (Martin, 2011) and then DADA2 v1.8.0.1512 (Callahan *et al.*, 2016) was used for quality filtering, joining paired end reads (maxEE = 2, minQ = 02, truncQ = 20, and maxN = 02), and denoising using default parameters to produce exact sequence variants (ESVs) (see [Supplementary Material for NovaSeq Denoising Validation approach](#)). Singletons were discarded as part of DADA2 processing. Taxonomy was assigned to ESVs using NCBI's megablast tool v2.11.9.0 (Altschul *et al.*, 1990) and the *nt* database (downloaded: 25 November 2020) with an e-value cut-off of 0.001. In cases where a sequence matched multiple taxa with an equally high score, we only assigned taxonomy to the lowest common ancestor of the ambiguous hits using a custom algorithm. The resulting taxonomic hits were filtered using a selection criterion (% sequence similarity multiplied by % overlap between the query sequence and the reference sequence). Family-level matches were reported using a minimum of 95% selection criterion, genus-level matches were reported using a minimum of 97% selection criterion and species-level matches were reported using a 100% or perfect match. The thresholds used here are similar or more stringent than those reported previously in the literature for these markers (Lanzén *et al.*, 2012; Elbrecht *et al.*, 2017; Lamy *et al.*, 2021; Valdivia-Carrillo *et al.*, 2021; Kumar *et al.*, 2022; Macher *et al.*, 2022). All taxa detected were verified using the WoRMS (WoRMS Editorial Board, 2020) and EOL (Encyclopedia of Life, 2014) databases and spurious or irrelevant hits (e.g. terrestrial or domestic species) were omitted. Only ESVs that were attributed to metazoans were retained for subsequent analyses.

Any ESVs detected in lab and field blanks were removed from the associated samples. Most of these ESVs were present at very low read counts indicative of minor cross-contamination (<50 reads). However, one species, *Oithona similis*, was detected in three field blanks and one extraction blank at relatively high read counts (>1000 reads). The ESVs associated with this species were removed from the associated samples as described above, resulting in the species being removed from eight samples.

Net sampling methods

Harvester logbooks and research vessel (RV) surveys using Campelen trawls are typically used to monitor and manage demersal fish communities in Atlantic Canadian waters, but these collections are restricted to demersal habitats <1500 m and are sparse for northern areas (Cote *et al.*, 2019). Much of the data available for fish and zooplankton communities in the Labrador Sea have been collected with the same oblique mid-water trawls (modified Isaac Kidd; 13.5 m² mouth, 11 mm net mesh, 5 mm cod end mesh, 2–3 kts tow speed, ~1 h tow duration, ~54000 m³ volume sampled; Chawarski *et al.*, 2022) and vertical multinetts (Hydrobios; 0.5 m² opening, 200 µm mesh, 11.5–100 m³ volume sampled; Darnis *et al.*, 2022) used in this study. The oblique midwater trawl samples were collected from the mesopelagic DSL. Vertical multinetts targeted zooplankton through the water column from the surface to within 15 m of the bottom (where depths allowed) at depth intervals of 2–24 (Surface), 25–49, 50–99, 100–249, 250–399 (UDSL), 400–599 (DSL), 600–799, and 800–999 m. However, only samples co-located with eDNA collections (Surface, UDSL, DSL, and Bottom) were used in comparative analyses. Conventional net sampling was conducted within hours (average of 5.2 h for oblique midwater trawls and 3.8 h for vertical multinetts) of eDNA sampling; however, due to scheduling constraints, sampling operations sometimes (5 of 10 stations) extended into the adjacent diel period ([Supplementary Table S1](#)). For these stations, sampling in the adjacent diel period occurred on average 1.0 h (max: 3.0 h) and 1.2 h (max: 3.0 h) from the diel transition for oblique midwater trawls and vertical multinetts, respectively ([Supplementary Table S1](#)). At the remaining stations, eDNA sampling was conducted in the same diel period as net sampling. Upon retrieval, oblique midwater trawl samples were photographed and preserved in ethanol. On shore, these samples were further examined through microscopy to identify morphometric features associated with the lowest possible taxonomic level using regionally appropriate keys (e.g. Coad and Reist, 2018; Mecklenburg *et al.*, 2018). Vertical multinet samples were preserved in a borax-buffered formalin solution before being sent to the Université Laval Biology Department, where they were identified using zooplankton keys such as ICES (2014) and Razouls *et al.* (2005–2023).

Comparisons

Biodiversity data can be assessed at multiple spatial scales that increase from local site-level (alpha diversity) to regional (gamma diversity) scales (Whitaker, 1972). Gamma diversity is the accumulation of alpha diversity across many habitat types, whereas the variation of diversity across those habitat types represents beta diversity. The relevant scale(s) of diversity depends on the end-use of the data. For example, alpha diversity might be most useful in understanding local-scale impacts of development or habitat quality, whereas beta diversity can be used to understand changes across environmental gradients (Piazzi and Checcherelli, 2020). In turn, gamma diversity might be most useful for making regional management decisions (Socolar *et al.*, 2016) or to conduct large-scale biodiversity reporting (Andermann *et al.*, 2022). All sampling methods have inherent biases and such biases may have differing influences on each scale of biodiversity assessment. We therefore consider each of these in our comparisons.

Regional (Gamma) diversity assessment

Taxa lists from eDNA metabarcoding were compiled across both transects and all sampling depths and compared with numbers of taxa from oblique midwater trawls and vertical multineets (conventional methods).

Site-level (Alpha) diversity assessments

We assessed the consistency of eDNA detections for individual taxa across replicates from individual sampling stations and depths. This was conducted for individual markers as well as the aggregate lists of all six markers. We further compared taxa lists compiled for co-located samples for eDNA and oblique midwater trawls (multiple stations at DSL depths) and vertical multineets (multiple stations at Surface, UDSL, and DSL depths). The conventional methods included in this study target specific elements of the mesopelagic community and have been used effectively to characterize biogeographic patterns across large regions (e.g. Chawarski *et al.*, 2022; Darnis *et al.*, 2022). We therefore evaluated eDNA's capability of detecting patterns across only those taxa captured by each conventional netting method. Specifically, we compared frequency of detection across all depths and stations for each method and quantified both shared detections within an aggregated eDNA sample and agreement between methods (i.e. shared detections + shared non-detects). We also compared these metrics across levels of taxonomic resolution (species, genus, and family). Taxonomic lists of eDNA replicates within a sample were aggregated since there was poor agreement across replicates and taxonomic accumulation curves did not reach their asymptote (Supplementary Figures S2–S6).

Between-site (Beta) diversity assessment

We assessed eDNA's ability to differentiate the biological communities across pelagic zones. Specifically, we used the Sørensen Index (equivalent to the Bray–Curtis similarity index when used on presence–absence data; Clarke and Warwick, 2001) to compare taxa lists of each pair of samples derived from six eDNA markers (COIm1, COIm2, COIm3, 12Sm1, 12Sm2, and 18S; Table 1) aggregated across replicates within a sample. We visualized habitat-related patterns among samples with non-Metric Dimensional Scaling (nMDS; Kruskal, 1964) ordination plots and tested for statistical differences

with ANOSIM tests (PRIMER-E, v. 7), a non-parametric permutational analogue of ANOVA (Clarke and Green, 1988). ANOSIM post-hoc tests were used to statistically assess differences in communities detected using each method across habitat zones (Surface, UDSL, and DSL). Subsets of focal taxa (fish, corals, and sponges), known to have distinct bottom-associated communities from pelagic habitats, were also examined within ANOSIM to establish if habitat differences were detectable using eDNA methods. Taxa that typified (i.e. contributed most to within-habitat similarity values) and discriminated (i.e. contributed most to dissimilarity values of samples in different habitats) were identified using SIMPER (PRIMER-E, v. 7).

Assessing effects of analytical approaches on site-level and between-site diversity

Considering the potential of eDNA as a monitoring tool in marine environments, we used our data as a case study to identify the impact of methodological approaches and evaluate strategies that improve this technique's effectiveness. We first assessed individual markers for their performance in the Labrador Sea in terms of number of taxa detected as well as their ability to detect species of high conservation value (i.e. taxa targeted for protection by marine refuge initiatives). To further investigate whether habitat-specific patterns in community structure are consistent between conventional methods and eDNA approaches using different markers and taxonomic identification approaches (taxonomy vs. ESVs), we performed a second-stage nMDS analysis (Sommerfield and Clarke, 1995, PRIMER-E v.7). This method uses Spearman rank correlations between pairs of Sørensen resemblance matrices derived for each assessment methods/taxonomy approach (Supplementary Figures S7–S8) as a measure of pattern similarity. The resulting pairwise correlations were combined in a secondary correlation matrix, which was visualized with an nMDS plot, where proximity of points reflects similarity in the associated ecological patterns detected. Community patterns based on three COI markers and the 18S marker were considered for taxonomically identified samples (presence–absence) as well as Exact Sequence Variants (ESVs; presence/absence only). Since ESV datasets were so large and computationally challenging, only randomly selected subsets (10% of ESVs) were included. The 12S (fish-specific) markers were excluded from this analysis as they had few detections in some habitat zones (Surface and UDSL).

Predicting impacts of increased eDNA sampling effort on site-level diversity

We conducted simulations to evaluate whether increasing the number of aggregated eDNA samples (i.e. 3×1.5 L) would improve alpha diversity estimates for taxa known to occur at a sample station and depth based on co-located conventional samples (oblique midwater trawls and vertical multineets). Specifically, we simulated 1000 conventional taxonomic lists for each conventional method (oblique midwater trawl and vertical multinet) by assembling community taxa based on the mean observed detection probability across all our real samples for a given habitat type (i.e. probability of a taxon included in a simulated conventional sample = # of samples in which a taxon was detected/# of samples). We restricted simulated taxa lists to those known to be present from conventional approaches since false-negative detections of these

Table 2. Unique taxa detected across all samples (gamma diversity) using eDNA, vertical multinet, and oblique midwater trawls.

Taxonomic level	eDNA: all depths ¹	Vertical multinet ²	eDNA: co-located with vertical multinet ²	Oblique midwater trawl ³	eDNA: co-located with oblique midwater trawl ³
Species	193	50	87	19	59
Genus	207	54	74	25	47
Family	175	19	65	26	45

¹Sampled zones include Surface, UDSL, DSL, and Bottom.

²Sampled zones include Surface, UDSL, and DSL.

³Sampled zones include DSL only.

taxa at the site-level were observed to be minimal when using conventional sampling (as assessed using eDNA).

Subsequently, we simulated a corresponding eDNA-derived taxa list for each simulated conventional sample taxa list, using the probability that a given taxon was detected by both eDNA and the conventional method, for samples where that taxon was positively detected by the conventional method (i.e. probability of a taxon included in eDNA = # of samples with shared eDNA and conventional positives/# of samples with conventional positives). The resulting simulated data mirrored our real-world datasets and sampling approach and enabled the generation of additional aggregate samples with which to assess the benefits of increasing our sampling effort. We simulated eDNA-derived taxonomic lists for progressively greater numbers of aggregated samples (i.e. $n = 2, 5, 10, 20, 30, 40,$ or 50) and generated resulting taxa lists for each permutation.

Concordance across methods was assessed for each conventional sample and number of aggregated replicates using the Sørensen Index, with higher scores indicating greater similarity. The results were plotted to identify the level of sampling effort after which additional sampling would result in diminishing returns. It is important to note that our method does not account for the fact that additional sampling effort for eDNA would likely detect new taxa that went undetected in these samples and further increase concordance between methods. Nevertheless, our results include taxa that account for the majority of the biomass captured using conventional methods in our surveys.

Results

Profiles of temperature and salinity were variable across stations at depths >250 m but were similar across stations at depths <250 m (Supplementary Figure S1). During our study, the sun never dropped >12° below the horizon. Nevertheless, a DSL was consistently centred at ca. 500 m during both daytime and nighttime. During daytime, the DSL was thicker and extended up to ca. 250 m. However, parts of the assemblage forming the DSL conducted diel vertical migrations and migrated upward to form a distinct upper DSL centred at ca. 250 m during nighttime (Figure 1). A lesser portion of the assemblage migrated further up at night and formed a diffused layer at ca. 100 m.

Regional (Gamma) diversity assessment

Environmental DNA metabarcoding detected 193 species, including 17 species of fish and 3 species of marine mammals. It captured more taxa than conventional methods, increasing the number of species found compared to the vertical multinet and oblique midwater trawls by 3.9 and 10.1 times, respec-

tively (Table 2). When eDNA data was restricted to co-located samples, the number of species detected was still 3.1 times greater than oblique midwater trawls and 1.7 times greater than vertical multinet. Environmental DNA detected more unique genera than species or families over all samples but more species than other taxonomic levels for the co-located subsets (Table 2). In contrast, genus was the most taxonomic-rich level for vertical multinet and family was the richest taxonomic level for oblique midwater trawls (Table 2).

Site-level (Alpha) diversity assessment

When comparing co-located samples, eDNA continued to detect more taxa (species, genus, and family) than oblique midwater trawls (probability of all paired t -tests <0.05) but less than vertical multinet (probability of all paired t -tests $P < 0.001$; Figure 2, Supplementary Figures S9–S10). Overall, shared taxa amongst eDNA and conventional methods made up a small proportion of the generated taxonomic lists (Figure 2, Supplementary Figure S11).

When we isolated those taxa that were captured in conventional sampling, we found that they were detected less frequently across sites by eDNA than conventional methods, and this outcome persisted across taxonomic levels spanning species to family (paired t -test: $P < 0.006$ for all taxonomic and gear type comparisons; Figure 3, Supplementary Figure S12).

Between-site (Beta) diversity assessment

Ecological patterns were consistent across the vertical multinet and eDNA metabarcoding methods (Figure 4). Significant differences were detected in the community across zones of the water column (Figure 4a: ANOSIM R Statistic: 0.544; $P < 0.001$), but not across diel periods within depth zones (Supplementary Table S2). Pairwise tests showed all adjacent zones had significantly different communities, with the most pronounced differences occurring between Surface and UDSL samples (Figures 4 and 5; ANOSIM R Statistic: 0.759; $P < 0.001$) where SIMPER analyses indicated abundant Calanoid and Oithonoid copepods distinguished Surface communities. Differences between UDSL and DSL samples (Figures 4 and 5; R Statistic 0.217; $P = 0.044$), and DSL and Bottom samples (Figures 4 and 5; R Statistic: 0.289; $P = 0.006$) were also significant but less distinct. SIMPER analyses also identified that DSL communities differed from UDSL communities due to a greater prevalence of myctophids such as *Benthosema glaciale* and *Lampanyctus macdonaldi*, crown jellyfish (*Periphylla periphylla*), krill (*Thysanoessa longicaudata*), and flatworms (*Platyhelminthes*). The greater prevalence of myctophids and crown jellyfish also differentiated the DSL from Bottom habitats, whereas the lat-

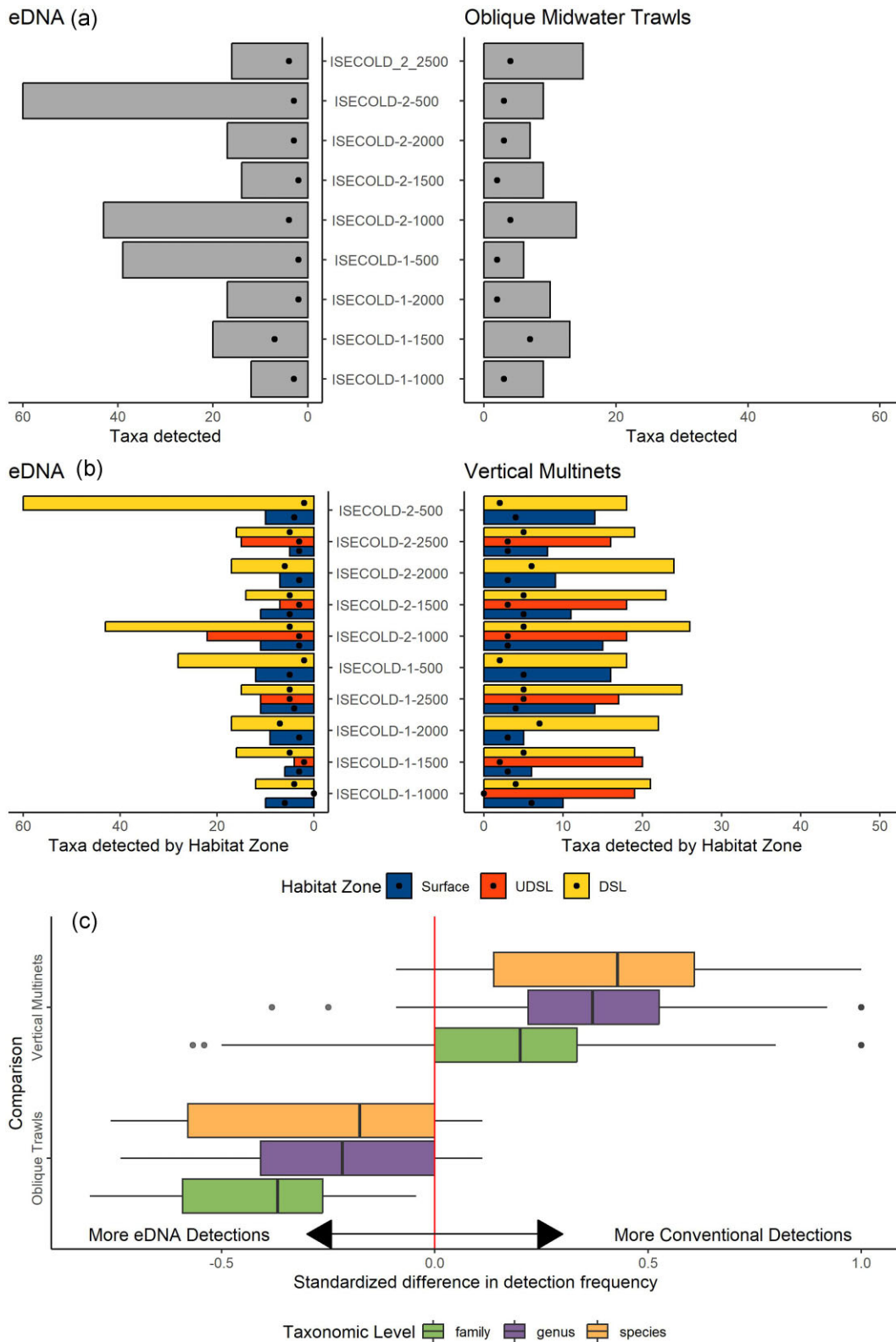


Figure 2. Comparisons of co-located eukaryote taxa (family) detections between eDNA (all markers combined) and conventional sampling methods (a: oblique midwater trawls and b: vertical multinets). Bars depict the number of taxa detected by each method, whereas points indicate the number of taxa shared across methods. Corresponding plots for other taxonomic levels (species and genus) are provided in [Supplementary Figures S9–S10](#). Panel C shows standardized differences in number of taxa detected between eDNA and conventional methods (oblique midwater trawls and vertical multinets) across species, genus, and family-level taxonomy. Boxes represent mid-quartiles, whereas whiskers represent 1.5 times the mid-quartile range. Family-level summaries depicted in C summarize data displayed in panels A and B.

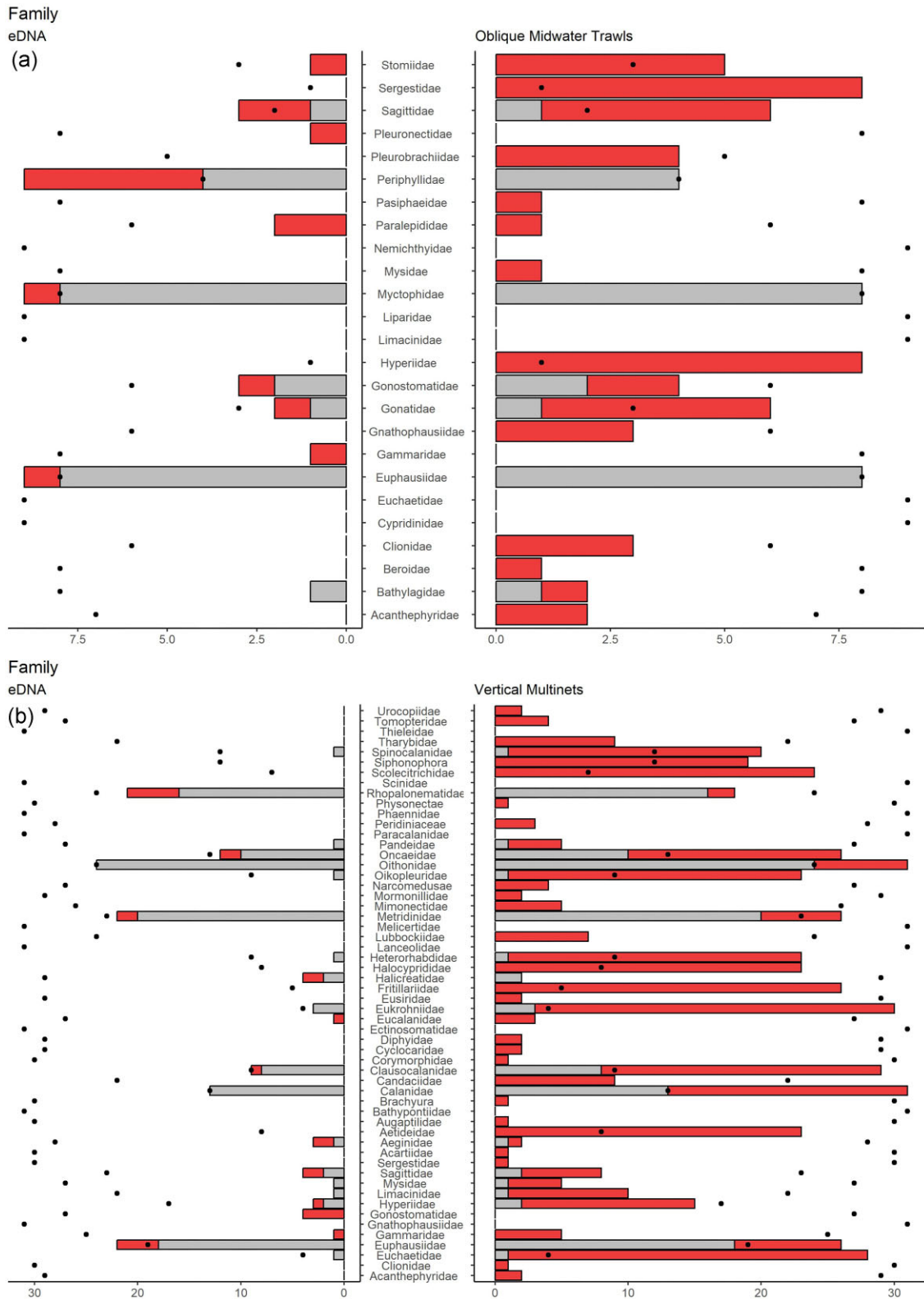


Figure 3. Comparisons of co-located detections of families derived from eDNA (all markers combined) to conventional (a) oblique midwater trawl and (b) vertical multinet methods. Comparisons only consider those taxa detected by conventional methods. Red bars indicate the number of sites where a taxon was detected by a single method, whereas grey bars indicate the number of sites where that taxon was detected by both methods. Points denote the sum of shared positive and negative detections across sampling methods. Other taxonomic levels are displayed in [Supplementary Figure S12](#).

ter had higher occurrences of *Aponemia* siphonophores. Collectively, these patterns qualitatively matched what was observed across depths with the vertical multinet (Figure 4b),

where the greatest separation in the nMDS plot occurred near the surface and was primarily driven by the prevalence of copepods.

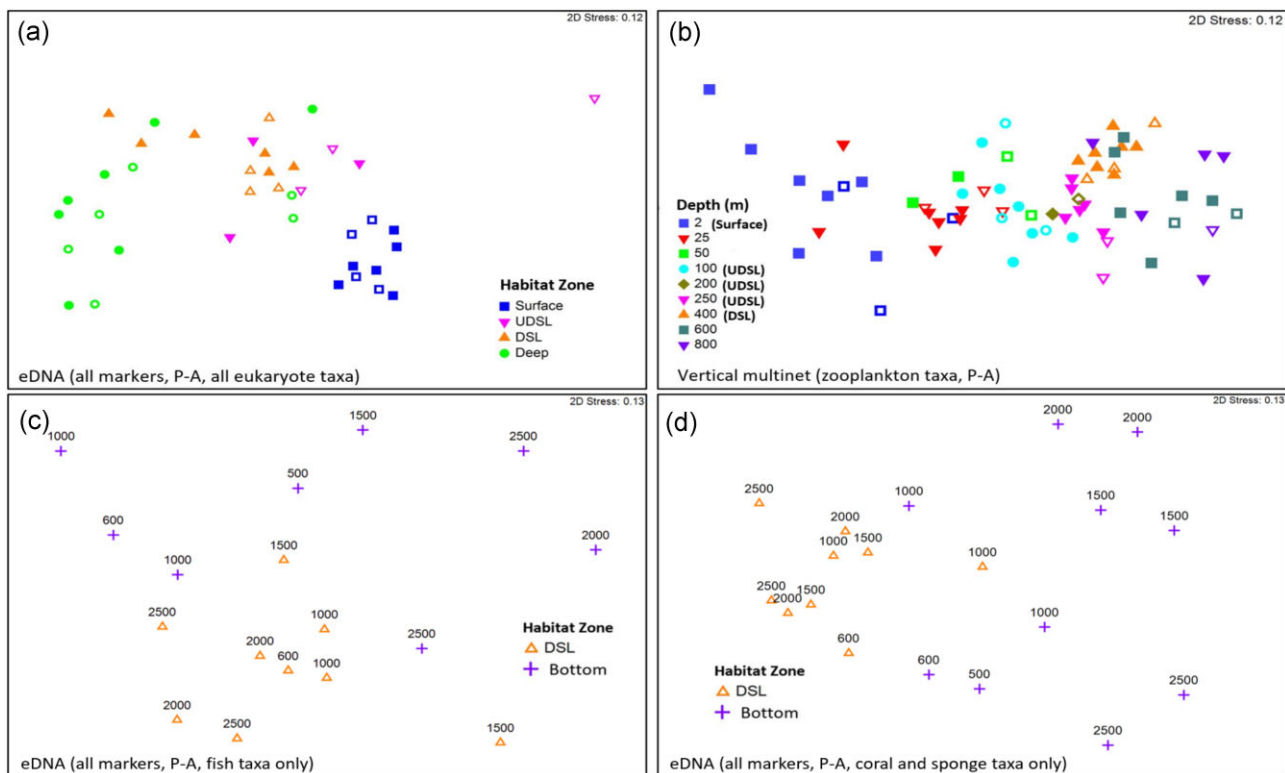


Figure 4. Differentiation of biotic communities in the Labrador Sea by depth zone displayed through nMDS plots of presence–absence data. (a) Eukaryote communities detected using multiple eDNA markers. (b) Zooplankton communities sampled with a vertical multinet, including the co-located samples (Surface, UDSL, and DSL) used for comparative analyses. (c) Fish taxa present using multiple eDNA markers. (d) Coral and sponge taxa using multiple eDNA markers. Distance between pairs of points within a plot is indicative of similarity in their respective taxonomic lists. For panels A and B, closed symbols denote daytime collections, whereas open symbols denote nighttime collections.

Community differences observed with eDNA were slightly more pronounced between Bottom and pelagic DSL habitats when only comparing fish taxa (Figure 4c; ANOSIM R Statistic: 0.299; $P = 0.003$). The top taxa differentiating DSL and bottom communities in SIMPER analyses were myctophids, mainly present in the DSL, and cod-like fishes (Gadiformes) dominating the bottom community. Fish were not widespread across pelagic or bottom samples with *B. glaciale* being the most widespread species at approximately a third of DSL stations. Despite low detection frequencies, and detections in both the DSL pelagic zone and bottom habitats for most taxa, fish were more frequently found in the habitats they are known to associate with (i.e. demersal fish in Bottom samples and pelagic fish in DSL samples).

When eDNA detections were restricted to coral (Anthozoa) and sponges (Porifera), DSL communities showed even greater differentiation from Bottom communities (Figure 4d; ANOSIM R Statistic: 0.543; $P < 0.001$). The top 27 differentiating coral and sponge taxa in SIMPER analyses were more widespread in bottom habitats relative to DSL samples. However, as with fish, few taxa were widespread across bottom stations with only demosponges, such as *Alcyonacea* and *Tectractinellida*, at more than half the bottom samples.

Assessing the effects of analytical approaches on site-level and between-site diversity

Environmental DNA markers also showed variable performance, detecting different numbers of taxa and coverage across the tree of life. As expected, 12S markers, selected for

their ability to discriminate fish captured the lowest phyla diversity and the fewest number of taxa (Figure 6). At the other end of the spectrum, 18S captured the most taxa across the broadest spectrum of phyla (Figure 6). When used to discriminate ecological patterns, choice of marker had the strongest effect on ecological patterns. Similarity in ecological patterns is depicted on the second-stage nMDS plot by proximity of points (Figure 7). The plot depicts COI markers on the periphery of the second-stage plot, indicating that those markers characterized divergent ecological patterns, whereas the ecological pattern generated by the 18S marker is more centrally located and more proximal (i.e. similar) to the patterns created using all markers together and the vertical multinet (Figure 7). The ecological patterns resulting from ESV subsamples were less variable across markers than taxonomically assigned lists (Figure 7).

Predicting impacts of increased eDNA sampling effort on site-level diversity

In our study, eDNA replicates from the same sample rarely captured the same taxa (Figure 8). Across all markers, the average frequency of detecting a taxon a second time within a sample ranged from 0.08 (COIm2) to 0.24 (18S). Using all markers together provided a redetection frequency of 0.20. Except for 18S, no marker had more than half of its taxa re-detected in any other sample replicate.

Given the low repeatability of results across eDNA replicates and the low site-specific frequency of taxa detected by vertical multinetts and oblique midwater trawls, we evaluated

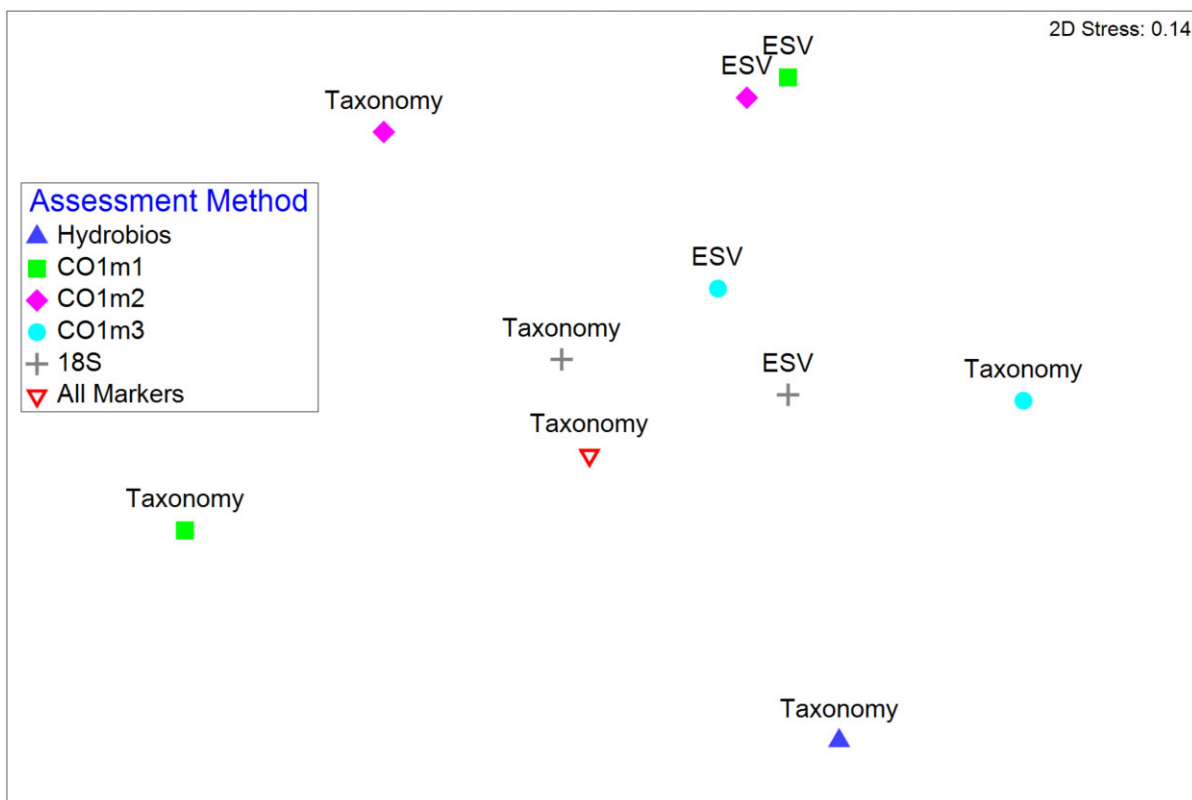


Figure 7. Comparisons of ecological patterns of community structure for co-located pelagic stations across assessment methods and taxonomic assignment methods, where proximity of points in second-stage nMDS indicates similarity in habitat-specific biodiversity patterns. Methods depicted include presence–absence data from the vertical multinet, four eDNA markers, and all markers combined. Datasets include those derived from taxonomic identification and those derived from subsamples of Exact Sequence Variants (ESVs—eDNA markers only). Each point is a representation of a nMDS community pattern for the pelagic sample subsets (Surface, UDSL, and DSL) shown in [Supplementary Figures S7–S8](#).

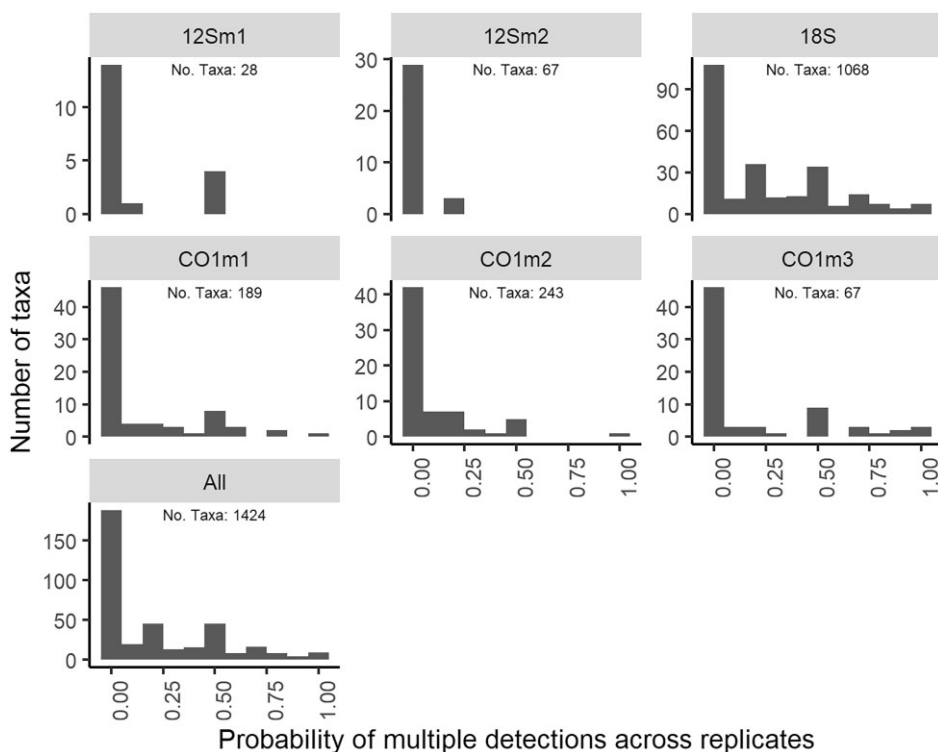


Figure 8. The frequency that taxa (lowest taxonomic level) were detected in multiple replicates within the same sample (by depth and station) as a function of marker. Values of zero denote taxa that were never detected in multiple replicates at any station. Values of one denote taxa that were detected in every replicate each time it was detected in a sample.

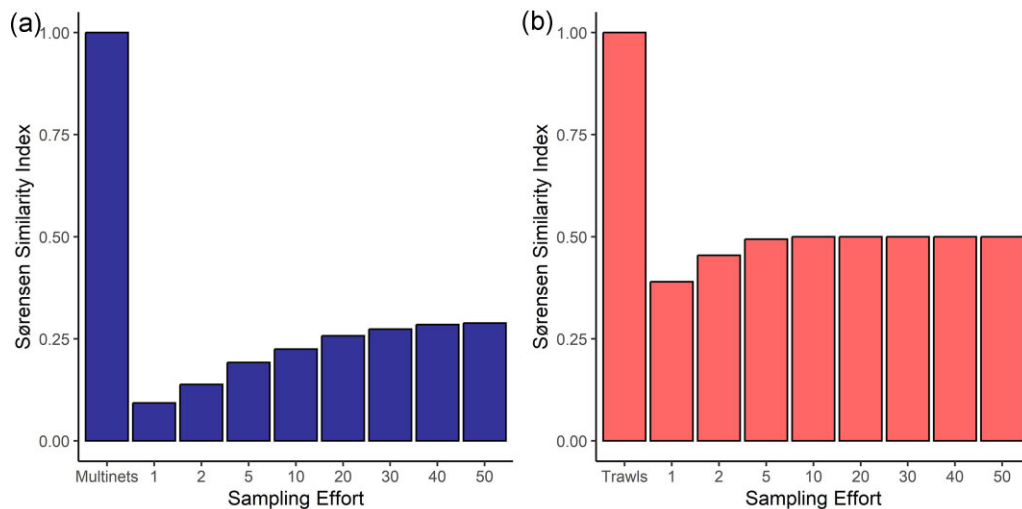


Figure 9. Number of aggregated eDNA water samples (3×1.5 L replicates, all markers combined) required to maximize Sørensen similarity index values between eDNA and vertical multinet (a) and eDNA and oblique midwater trawls (b). Taxa considered are restricted to those captured in the respective conventional sampling methods. Note that simulations did not account for new taxa being detected via eDNA with increased sampling effort (e.g. increased sampling volume or additional replicates). See species accumulation curves in [Supplementary Figures S2–S5](#) for estimates of the taxonomic richness detected with additional replicates.

if collecting more samples would improve concordance at the site level. We determined that the similarity between eDNA and conventional samples reached an asymptote at ~ 20 -fold increase in sampling effort (i.e. 20 aggregate samples each consisting of three 1.5 L replicates) for the vertical multinet taxa simulations and a 5-fold increase for the oblique midwater trawl taxa simulations (Figure 9).

Discussion

The application of eDNA metabarcoding to marine environments is rapidly increasing due to its versatility, holistic, non-invasive, and cost-efficient nature (Thomsen *et al.*, 2016; Stat *et al.*, 2017; He *et al.*, 2022; He *et al.*, 2023). However, because the performance of eDNA approaches can be heavily influenced by environmental conditions (Hansen *et al.*, 2018) and taxa composition, extending this methodology to new environments requires assessment and adaptation to refine techniques and complement existing datasets (Hansen *et al.*, 2018; Jeunen *et al.*, 2019; Miya, 2022). Using metabarcoding to monitor deep ocean environments may prove challenging due to longer eDNA persistence (Collins *et al.*, 2018), strong currents to disperse eDNA (Hansen *et al.*, 2018), underrepresentation of biota in genetic databases (Bucklin *et al.*, 2021; Duhamet *et al.*, 2023), and lower densities of eDNA (McClenaghan *et al.*, 2020). Studies such as ours comparing eDNA metabarcoding to conventional taxonomic methods are rare in deep ocean environments (Miya, 2022), and particularly in mesopelagic habitats (but see Govindarajan *et al.*, 2021; Feng *et al.*, 2022).

Assessing biodiversity

We found that eDNA metabarcoding detected a greater number of taxa than both conventional approaches evaluated in this study (oblique midwater trawls and vertical multinet), providing a more comprehensive description of the taxa inhabiting a region (gamma diversity). Several studies have also shown discrepancies between biodiversity metrics obtained from eDNA and conventional approaches, with eDNA tech-

niques capturing a wider range of taxa than their conventional counterparts (e.g. Evans *et al.*, 2017; Strickland and Roberts, 2019; Easson *et al.*, 2020; Afzali *et al.*, 2021; Fraija-Fernandez *et al.*, 2020; He *et al.*, 2023). However, the ability of eDNA to outperform conventional methods for biodiversity detection at the site level (alpha diversity) was mixed, with eDNA metabarcoding detecting more taxa than oblique midwater trawls and less than vertical multinets. Moreover, those taxa detected by conventional methods in our study were more likely to be detected in a given sample by conventional methods than with eDNA metabarcoding, particularly for less abundant taxa. A similar result was observed by Easson *et al.* (2020) who noted fish were much less abundant in eDNA samples than expected from hydroacoustic estimates. While eDNA primer and reference database biases play a role, discrepancies in gear-specific sampling volumes are an important factor in detection success in deep-sea environments. Depending on variation in tow duration and speed, our oblique midwater trawls sample ~ 12 million times more water than that filtered for eDNA metabarcoding for a given sample station. For vertical multinets, water volume sampled ranged from 2.6 to 22 thousand times more for a given sample-depth stratum. Moreover, each conventional method samples a broader depth range than the point samples acquired with an oceanographic rosette.

Our bottom eDNA samples showed a similar qualitative trend, where they infrequently detected fish species in depth zones where they were consistently detected using conventional methods such as baited cameras and longlines in this region (Cote *et al.*, 2019; Cote *et al.*, 2023). Unlike the pelagic nets examined in this study, baited cameras and longlines are passive gear and are not towed through large volumes of water. The bait they use, however, does attract and concentrate biota from a broader area. Other recent studies examining benthic taxa in shallower (<500 m) ocean environments reported comparable results. For example, He *et al.* (2023) detected lower alpha (site) diversity than co-located trawls, whereas Jensen *et al.* (2023) note that Arctic skate and Greenland halibut were detected much less frequently than would

be expected given results from other conventional surveys in their study area.

We found metabarcoding to be effective at detecting ecological patterns in deep ocean environments of the Labrador Sea, with our study showing strong vertical zonation of biological communities (beta diversity) that correspond to patterns observed with conventional net sampling. For example, surface taxonomic lists, inferred through metabarcoding, were dominated by copepods and were very distinct from mesopelagic ones characterized by lanternfish, jellyfish, and krill despite being separated by only hundreds of metres. When all taxa were viewed together, differences between communities from the bottom and the mesopelagic layer were less obvious with eDNA metabarcoding, also mirroring patterns obtained from our plankton nets, where the primary differences occurred between epipelagic (<200 m) and mesopelagic (>200 m).

The absence of strong differentiation between mesopelagic and bottom samples in eDNA data opposed known differences of organisms occupying benthic and mesopelagic habitats. This might be explained by high relative abundance of zooplankton obscuring the signal of less abundant elements of the community (e.g. fish, corals, and sponges). Certainly, when fish, coral, and sponge taxa were isolated, differences between mesopelagic and benthic metabarcoding communities emerged, although still less distinct than one might expect based on data from conventional sampling methods. For example, demersal species were more often, but not exclusively, found by eDNA in bottom samples, whereas longline and trawl samples from bottom habitats in this region (Cote *et al.*, 2019, 2023) generated fish species lists that are almost mutually exclusive of those detected in our oblique midwater trawls conducted in pelagic habitats.

Taxonomic disparities across methods could arise if eDNA material is transported from its point of origin (Hansen *et al.*, 2018). However, the differences observed could also be driven by the substantial gear-specific limitations of the conventional methods (Andruszkiewicz *et al.*, 2021; Boulanger *et al.*, 2021). Because many benthic organisms have pelagic larvae, it is possible that the blurred communities observed with metabarcoding are accurate representations of habitat use across an organism's full life history (Sommer *et al.*, 2017; Bucklin *et al.*, 2019). While many have expressed concerns about the dispersal of eDNA decreasing the spatial resolution of eDNA data (e.g. Hansen *et al.*, 2018), particularly in open ocean environments, a mechanistic model (Andruszkiewicz *et al.*, 2021) predicted that biological phenomena (e.g. vertical migration) are likely to play a much greater role on distribution patterns than oceanographic influences (i.e. mixing, advection) resulting in eDNA being detected in proximity to where it was shed (i.e. within 10 s of metres in the water column). These predictions have been supported by *in situ* studies that noted the low persistence time of eDNA in marine environments (Murakami *et al.*, 2019) and the ability to detect community change across small vertical (Andruszkiewicz *et al.*, 2017; Lacoursière-Roussel *et al.*, 2018; Easson *et al.*, 2020; Andruszkiewicz *et al.*, 2021; Canals *et al.*, 2021; Feng *et al.*, 2022), horizontal (Port *et al.*, 2016; Bista *et al.*, 2017; O'Donnell *et al.*, 2017; Yamamoto *et al.*, 2017; Jeunen *et al.*, 2019; Stat *et al.*, 2017; Boulanger *et al.*, 2021; Gold *et al.*, 2021), and temporal scales. For example, two recent studies could detect diel changes in distribution of organisms in deep ocean environments (Easson *et al.*, 2020; Canals *et al.*, 2021).

Accordingly, it is possible that diel vertical migration could explain the absence of some vertically migrating taxa in eDNA samples at some depths and stations in our study. Due to logistical limitations, co-located sampling operations extended into adjacent diel periods at five stations. Because (i) these samples were collected shortly after the diel transition (Supplementary Table S1); (ii) community structure as a whole was not significantly affected by diel period (Figure 4, Supplementary Table S2); (iii) our hydroacoustics show vertical migrations to be restricted to the DSL and UDSL (Figure 1); and (iv) not all individuals of a given species undertake diel vertical migration (Pearre, 2003; Pepin, 2013; Sommer *et al.*, 2017; Feng *et al.*, 2022), we do not believe these biases to be strong enough to affect our conclusions related to site level comparisons.

Optimizing sampling effort and markers for biodiversity monitoring

Establishing appropriate sampling effort is important for any monitoring programme to enable managers to assess ecological trends and condition of areas based on aggregations of site-level assessments. High frequencies of false negatives at the site level will erode confidence in eDNA data for management applications that rely on site-specific detections. Our current eDNA metabarcoding sampling effort resulted in a low degree of repeatability across replicates from the same depth and station. We infrequently detected the same taxa more than once across three 1.5 L replicates extracted from the same Niskin bottle. Andruszkiewicz *et al.* (2017) found similar results and suggested that this is the result of eDNA being heterogeneously mixed throughout the environment. Our simulations showed that a 5–20-fold increase in sampled water would maximize the potential agreement with the biological communities delineated with conventional methods. These conclusions align with other studies that recommended increasing replication and sampling effort to provide more reliable presence–absence data (Jeunen *et al.*, 2019; Gold *et al.*, 2021), especially in open ocean and deep-sea environments where organisms might be scarce (Ficetola *et al.*, 2014; Miya *et al.*, 2016; McClenaghan *et al.*, 2020; Stauffer *et al.*, 2021; Yoshida *et al.*, 2023). Increasing sampling effort can overcome the sporadic distribution of eDNA in seawater (Bessey *et al.*, 2020; Yoshida *et al.*, 2023); however, the manner and degree of increased effort necessary will be dependent on the community being studied, and the methodological approach being used. For example, filtering larger volumes of water captures more organisms per sample (Bessey *et al.*, 2020; McClenaghan *et al.*, 2020; Govindarajan *et al.*, 2022; Yoshida *et al.*, 2023). While samples 1–2 L are generally recommended for marine eDNA sampling (Patin and Goodwin, 2023), collecting samples of 30+ L across ocean depths is becoming more achievable (Boulanger *et al.*, 2021; Govindarajan *et al.*, 2022; Maiello *et al.*, 2022) and may reduce variability in eDNA replicates by capturing a larger proportion of the biodiversity in each sample. Sampling effort can also be increased in the lab by increasing the amount of DNA included in PCR reactions (Krehenwinkel *et al.*, 2017) or by increasing sequencing depth, resulting in more taxa detections per sample (Singer *et al.*, 2019). Rarefaction curves showed that saturation was not reached for several primer sets even at the sequencing depth used here (1 million reads per sample; Supplementary Figure S6). While increasing sampling effort

in the lab cannot overcome the heterogeneity of eDNA in the environment, it can increase the taxonomic coverage within a sample. Improving our understanding of the effects of increasing sampling effort at various stages in the workflow will enable the development of optimized sampling designs that balance effort in the lab and field. Establishing appropriate sampling effort that reduces false negatives of common species will be a critical step to fulfilling the promise of using this method for tracking endangered or rare species (Thomsen *et al.*, 2012; Bohmann *et al.*, 2014) or managing commercial fish stocks (Hansen *et al.*, 2018) in deep ocean environments, including potential mesopelagic fisheries. More research is needed to understand the interactions between number of replicates, sample volume, and sequencing depth to optimize efforts across these three factors.

Marker selection is an important study design consideration as it can have a strong influence on metabarcoding results. In this study, we used general biodiversity markers and those proven for focal taxa such as fish and corals. Despite elevated laboratory costs, several studies recommend using multiple markers to mitigate biases associated with single markers (Lacoursière-Roussel *et al.*, 2018; Jeunen *et al.*, 2019; Easson *et al.*, 2020; McClenaghan *et al.*, 2020). We noted the benefits of this approach as the patterns observed using vertical multinetts were most similar to those observed with the taxa lists derived from all markers combined. Interestingly, subsets of ESV data reduced marker-specific bias in terms of delineating patterns in habitat-specific communities. While ESVs may be limited by the fact that they are more difficult to interpret, they may be an efficient and statistically powerful way to detect change in marine ecosystems while incomplete genomic databases (Cordier *et al.*, 2017) are improved.

Applying eDNA metabarcoding to protected area monitoring in remote and challenging environments

The absence of knowledge on biodiversity is a significant barrier to the conservation and sustainable management of the world's least accessible habitats (Martin *et al.*, 2020; Jensen *et al.*, 2023). Environmental DNA metabarcoding has the potential to accelerate our understanding of these environments, including the deep ocean (McClenaghan *et al.*, 2020). Once refined for these environments, the cost-effectiveness, ease of sampling, and versatility of this method will help researchers identify biodiversity hotspots (Laroche *et al.*, 2020) and provide holistic information that can be applied to understanding ecosystem processes and advancing management beyond single species to ecosystem-based management (Link *et al.*, 2011; Bohmann *et al.*, 2014; Hansen *et al.*, 2018; Miya, 2022). Importantly, with the development of automated samplers that can be installed on moorings, eDNA will provide new insights on temporal variability (Hansen *et al.*, 2018; Gold *et al.*, 2021), particularly for seasonally ice-covered environments like the Labrador Sea, where sea ice limits the use of most conventional sampling methods to summer and fall.

eDNA metabarcoding methods have recently been explored for use in monitoring protected areas in coastal environments (Boulanger *et al.*, 2021; Gelis *et al.*, 2021; Gold *et al.*, 2021). In Canada marine protected areas and marine refuges are predominantly large (on average ~ 3500 km²), deep (area-weighted depth: 1400 m), and remote. Environmental DNA can play a key role in monitoring such challenging environ-

ments. Conservation objectives for these areas are currently focused on specific taxa—many of which are sensitive to, or poorly sampled with, existing standardized research trawling methods. For example, the Laurentian Channel MPA was established to protect sea pens, leatherback turtles, porbeagle sharks, and three species of demersal fish (black dogfish, smooth skate, and wolffish; Muntoni *et al.*, 2019). Sea pen habitats are damaged by bottom trawls, whereas the leatherback turtles and porbeagle sharks are rarely sampled by trawls. For the three demersal fish species captured by the bottom trawls, trend assessments are plagued by poor statistical power due to their tendency to be highly variable and/or in low abundance in bottom trawls (Cote *et al.* unpubl. data). Similarly, many of Atlantic Canada's marine refuges were established to protect deep-water corals that are sensitive to trawling (Sherwood *et al.*, 2006; Sherwood and Edinger, 2009; Neves *et al.*, 2015) and take decades or longer to recover (Neves *et al.*, 2015). Tracking the fortunes of these individual taxa will likely require more data than eDNA can provide (Hansen *et al.*, 2018; Jeunen *et al.*, 2019), as information on demographics, size distributions, fecundity, and condition is necessary. However, eDNA offers the potential to provide a relatively low-cost, complementary screening tool that can be applied at higher temporal resolution to simultaneously assess the supporting food web and potential changes related to climate (e.g. Jensen *et al.*, 2023) or other anthropogenic effects (Miya, 2022). Moreover, the holistic nature provides the opportunity to unveil unexpected benefits resulting from protected areas (Boulanger *et al.*, 2021).

The versatility of eDNA across different environments provides a means for standardized comparison and aggregation of data across protected areas and other marine environments. The choice of eDNA primer sets and reference database biases play a role in determining what taxa can be identified from eDNA samples (see Weigand *et al.*, 2019; Bucklin *et al.*, 2021; Duhamet *et al.*, 2023). Therefore, standardizing eDNA metabarcoding approaches across protected areas will require alignment on several methodological fronts (Lacoursière-Roussel *et al.*, 2018; Gold *et al.*, 2021), not least of which is marker selection. A customized suite of markers will be needed to address the data needs related to area-specific conservation objectives. For example, the best markers to detect and differentiate bony fish taxa are different from those optimized to cartilaginous fish and deepwater corals. However, an effective general biodiversity marker should be identified for these programmes to track broader spatio-temporal trends. Tools like GAPeDNA can help identify markers with the best taxonomic and geographic representation (Marques *et al.*, 2021), but considerable work is needed to fill out taxonomic databases, even for fish taxa (Gold *et al.*, 2021; He *et al.*, 2023). For example, in this study, of the 174 taxa (order, family, genus, and species-level identifications) that were identified with conventional methods but not with eDNA, there were 31 taxa that had no reference sequences present in the reference database for any of the target gene regions (Supplementary Table S3). Filling gaps in reference databases will improve our ability to detect and resolve a broader range of species. Also, it should be noted that the taxonomic assignment used for this study was conducted in 2020 based on the reference sequences available at that time. Four taxa that were missed by eDNA but detected with conventional methods now have reference sequences available for at least one gene region. The potential addition of these identifi-

cations to the eDNA data would not change the main conclusions of this study, but this highlights the continuous updates occurring in reference databases and the challenges of applying standardized approaches to long-term monitoring data.

Limitations of eDNA metabarcoding include those that will be difficult to overcome (e.g. inability to measure life stage and condition) and those that will be rectified or will improve as the technology advances (e.g. improved taxonomic assignment with more complete genetic databases) (Bucklin *et al.*, 2016). Our study and others show that even in its current form, eDNA metabarcoding is a powerful tool that can be used to understand, compare, and monitor marine ecosystems from the coast to the deep ocean (Miya, 2022). Environmental DNA metabarcoding reveals gamma and beta biodiversity patterns comparable or better than conventional methods, but site-specific (alpha) biodiversity assessments in deep ocean environments require optimization to improve performance. Understanding methodological limits and potential areas of improvement will allow researchers to apply this method in a way that complements existing methods (Evans *et al.*, 2017; Leduc *et al.*, 2019) and maximizes the deployment of scientific resources in this era of unprecedented technological advancement and ecological threats.

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Supplementary data

[Supplementary material](#) is available at the *ICESJMS* online version of the manuscript.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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Conflict of interest statement

BM, NF, and GS are employees of eDNAtec Inc. MH is the founder and Chief Scientific Officer. This does not alter our adherence to ICES policies on sharing data and materials.

Author contributions

Conceptualization: DC, BM, NF, MH, MG, and JC; Experimental design: DC, JC, MG, NF, and BM; Data collection and processing: DC, SR, JC, CA, BM, NF, and GS; Statistical Analysis and Interpretation: DC, BM, GS, NF, and JD, Manuscript Preparation: All Authors

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