Jeunen, GJ, Lamare, M, Cummings, V, Treece, J, Ferreira, S, Massuger, J, Pryor Rodgers, L, Tait, L, Lust, B, Wilkinson, S, Mariani, S, Mills, S and Gemmell, N

Unveiling the hidden diversity of marine eukaryotes in the Ross Sea: A comparative analysis of seawater and sponge eDNA surveys

Received: 2 September 2023 | Revised: 16 November 2023 | Accepted: 24 November 2023

DOI: 10.1002/edn3.500

Environmental DNA
Open Access
Dedicated to the study and use of environmental DNA for basic and applied sciences

WILEY

ORIGINAL ARTICLE

Unveiling the hidden diversity of marine eukaryotes in the Ross Sea: A comparative analysis of seawater and sponge eDNA surveys

Gert-Jan Jeunen[1,2] | Miles Lamare[1] | Vonda Cummings[3] | Jackson Treece[2] | Sara Ferreira[2] | Jack Massuger[4] | Lily Pryor Rodgers[5] | Leigh Tait[5] | Bobby Lust[6] | Shaun Wilkinson[6] | Stefano Mariani[7] | Sadie Mills[3] | Neil Gemmell[2]

[1]Marine Science Department, University of Otago, Dunedin, New Zealand

[2]Department of Anatomy, School of Biomedical Sciences, University of Otago, Dunedin, New Zealand

[3]National Institute of Water and Atmospheric Research (NIWA), Wellington, New Zealand

[4]National Institute of Water and Atmospheric Research (NIWA), Hamilton, New Zealand

[5]National Institute of Water and Atmospheric Research (NIWA), Christchurch, New Zealand

[6]Wilderlab NZ Ltd, Wellington, New Zealand

[7]School of Biological and Environmental Sciences, Liverpool John Moores University, Liverpool, UK

Correspondence
Gert-Jan Jeunen, Marine Science Department, University of Otago, Dunedin 9016, New Zealand.
Email: gjeunen@gmail.com

Funding information
Marsden Fund, Grant/Award Number: MFP-UOO2116; Ministry of Business, Innovation and Employment, Grant/Award Number: MBIE ANTA1801

Abstract

The Ross Sea, Antarctica, while largely pristine, is experiencing increased anthropogenic pressures, necessitating enhanced biomonitoring efforts for conservation purposes. Environmental DNA (eDNA) extracted from marine sponges provides a promising approach for biodiversity monitoring in remote areas by circumventing the need for time-consuming water filtration. Investigations into the efficacy of eDNA signal detection across the tree of life from marine sponges have yet to be fully explored. Here, we conducted a seawater and sponge eDNA metabarcoding survey at seven coastal locations in the Ross Sea to assess spatial eukaryote biodiversity patterns and investigate eDNA signal differences between both substrates. In total, we detected 1450 operational taxonomic units (OTUs) across 30 phyla. Significant differences in water and sponge eDNA signal richness and composition were observed, with a partial overlap in OTU detection between both substrates and, thereby, underscoring the crucial role of substrate selection in eDNA metabarcoding surveys. Furthermore, alpha and beta diversity analyses revealed distinct eDNA signals among sampling locations, which were corroborated by known species distributions. However, only 135 OTUs (9%) could be successfully assigned to species level, and 574 OTUs (40%) were unable to be taxonomically classified, due to limitations in the reference database. Our results provide evidence for the potential of eDNA monitoring in remote areas, demonstrate the need to consider more sophisticated sampling strategies whereby multiple eDNA substrates are incorporated, and highlight the importance of complete reference databases for robust taxonomy assignment of eDNA signals.

KEYWORDS
Antarctica, biodiversity detection, COI, metabarcoding, Southern Ocean

1 | INTRODUCTION

The Ross Sea is the southernmost sea on Earth, extending to 76° S latitude and encompassing a total area of 637,000 km^2. This high-latitude location has the coldest sea temperatures on the planet and significant ice coverage (Smith Jr. et al., 2014). Despite these potential ecological constraints, the Ross Sea contains some of the most productive waters globally, sustaining the largest phytoplankton biomass in the Southern Ocean (Smith Jr. et al., 2014). The Ross Sea also sustains high secondary productivity (Sala et al., 2002), including a diverse benthic community with distinct characteristics (Clarke & Johnston, 2003), such as a high biomass of sponges, lack of reptantian crustaceans, and reduced benthic fish diversity and abundance (Clarke & Johnston, 2003). Logistical difficulties in accessing remote nearshore Antarctic regions using traditional sampling and observational methods, and the likelihood that many taxonomic groups and cryptic species are under-described means that the biodiversities of these ecosystems are significantly underestimated (Clarke, 2008; Stark et al., 2014). A quantitative understanding of the spatial and temporal patterns in biodiversity around the coastal Ross Sea exists in some areas (Cummings et al., 2018), particularly around research stations, but remains understudied elsewhere (Clarke, 2008). Given the significant effects of environmental change now being seen in the Southern Ocean (Siegert et al., 2023), quantifying present-day biodiversity patterns is critical in detecting and understanding the implications for Antarctic ecosystems.

Environmental DNA (eDNA) surveys have been proposed as an innovative monitoring method (Ficetola et al., 2008; Thomsen & Willerslev, 2015), whereby species are detected indirectly through DNA signals obtained from environmental samples, such as water (Ficetola et al., 2008), sediment (Turner et al., 2015), or air (Lynggaard et al., 2022). Species detections through eDNA surveys can augment and extend other sampling methods (e.g., visual species observations) and, ultimately, facilitate large-scale monitoring across the tree of life with limited required field time (Bessey et al., 2021; Stat et al., 2017), a major benefit for remote or difficult-to-sample areas (Olmedo-Rojas et al., 2023). Within the marine biome, water is the most-used substrate (Bowers et al., 2021), and the accuracies of such eDNA surveys have been proven, by identifying high spatial (Jeunen et al., 2019) and temporal (Jensen et al., 2022; Minamoto et al., 2017) resolutions.

While eDNA metabarcoding surveys have enabled the transformation of how the marine biome can be monitored (Takahashi et al., 2023), the need for immediate sample processing, i.e., water filtration, to halt DNA degradation (Sales et al., 2019) is a time-consuming process. This can limit the practicality of the eDNA monitoring method in remote areas and reduces the number of samples incorporated into eDNA surveys (Bessey et al., 2021; Jeunen, von Ammon, et al., 2022). Further exacerbating the time commitment for water filtration is the increased water volume required to be processed in marine eDNA studies compared to freshwater ecosystems (Bowers et al., 2021). To circumvent the need for water filtration, multiple alternative methods have been successfully trialed, including passive filtration (Bessey et al., 2021; Jeunen, von Ammon, et al., 2022; Kirtane et al., 2020; Verdier et al., 2021), autonomous sampling (Hansen et al., 2020; Yamahara et al., 2019), and direct sampling of filter-feeding organisms such as marine sponges (Brodnicke et al., 2023; Cai et al., 2022; Harper et al., 2023; Jeunen, Cane, et al., 2023; Jeunen, Lamare, et al., 2023; Mariani et al., 2019; Neave et al., 2023; Turon et al., 2020).

The use of marine sponges to investigate eDNA signals commenced after the observation of natural eDNA accumulation through their filter-feeding strategy (Mariani et al., 2019). Thus far, optimal DNA extraction protocols have been investigated (Harper et al., 2023), as well as eDNA retention time in sponges through a mesocosm experiment to gain insight into temporal detection (Cai et al., 2022). Furthermore, high spatial resolutions have been observed, with different eDNA signals on small spatial scales representing the local biological community (i.e., fish and vertebrates; Jeunen, Cane, et al., 2023; Turon et al., 2020). Additionally, no significant differences in vertebrate eDNA signals were reported between marine sponges and water eDNA when collected from the same location simultaneously (Jeunen, Cane, et al., 2023). Finally, variability in the efficiency of eDNA accumulation between different marine sponge species has been observed (Cai et al., 2022; Turon et al., 2020) and potentially linked to filtration rates and microbial activity (Brodnicke et al., 2023).

Filter-feeding organisms for conducting eDNA surveys may hold great potential in the Ross Sea region, as (i) sponges are frequently caught as bycatch in benthic trawls (Hanchet et al., 2008) and onboard fishing vessels (Jeunen, Lamare, et al., 2023), thereby facilitating expansive sampling opportunities; (ii) the Porifera community in the Ross Sea is abundant and diverse (Vargas et al., 2015), thereby limiting the potential negative impact when collecting tissue biopsies from abundant species; and (iii) unlike passive eDNA filtration methods, filter-feeding organisms do not require extended deployment times to capture eDNA (Bessey et al., 2021; Jeunen, von Ammon, et al., 2022).

In this study, we explore the use of two eDNA substrates, that is, water and living sponges, to describe the eukaryotic biogeographical patterns at seven locations around the McMurdo Sound, Ross Sea, coastline (Figure 1; Table 1). Water eDNA samples were collected at each location to determine if small-volume water samples can describe spatial patterns in marine communities. Sponges were collected at three locations to enable a direct comparison between near-bottom seawater and benthic sponge eDNA signals. Two questions were specifically addressed: (1) does the detected biodiversity, using a "universal" COI metabarcoding survey from small water volumes, distinguish different coastal sampling locations, and (2) are eukaryote eDNA signals obtained from living sponges indistinguishable from or complementary to water eDNA signals collected at the same location and time?

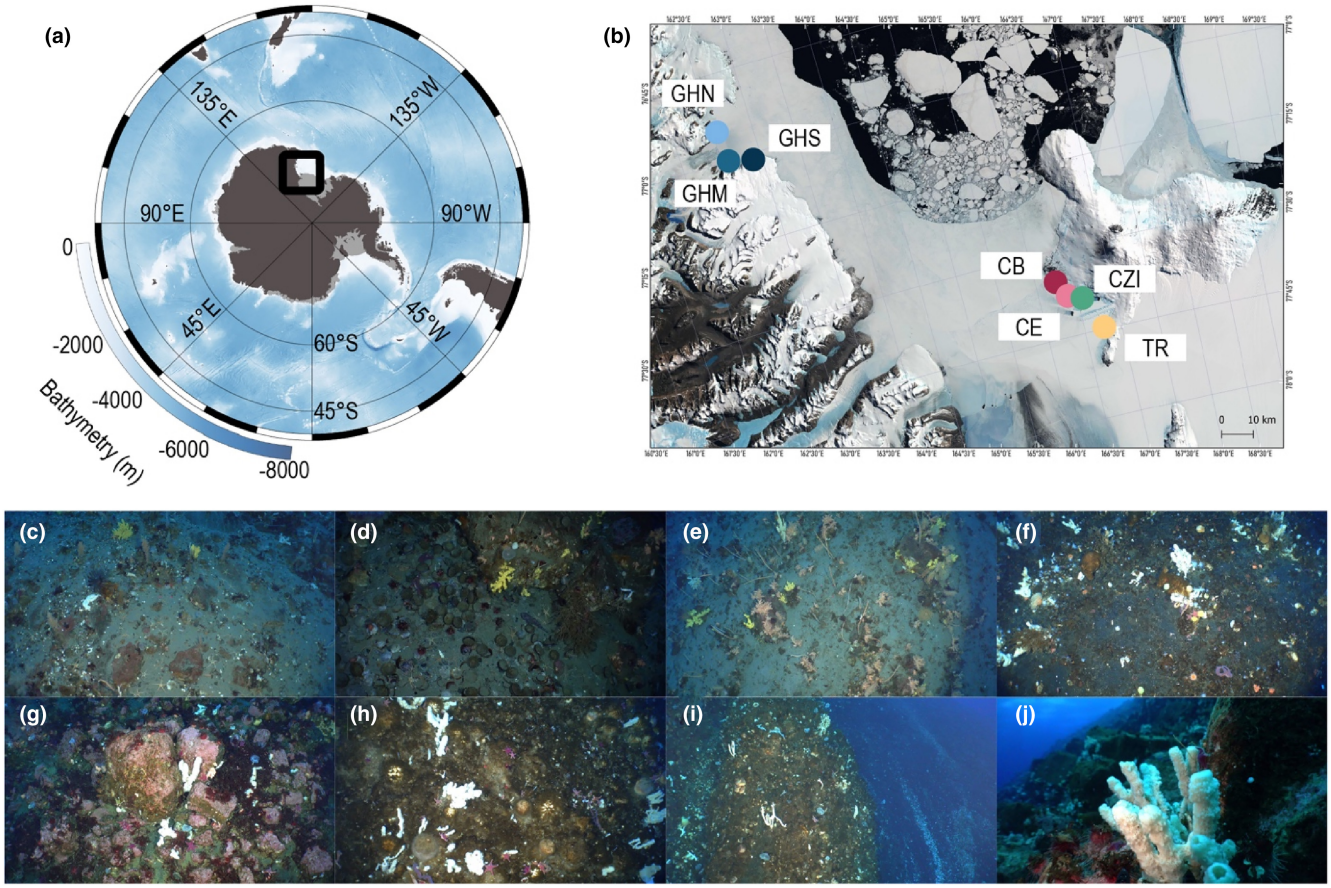

FIGURE 1 (a) Map of Antarctica and the Southern Ocean with the sampling locations along the Ross Sea coastline indicated by a black square. Bathymetry of the Southern Ocean floor is color coded from light blue (shallow) to dark blue (deep sea). Bathymetry information was gathered from Quantarctica inside QGIS (https://www.scar.org/resources/quantarctica). (b) Map of the Ross Sea coastline with the seven sampling locations indicated by colored dots, including Granite Harbor North (GHN; light blue), Granite Harbor Middle (GHM; blue), Granite Harbor South (GHS; dark blue), Cape Barne (CB; dark red), Cape Evans (CE; light red), Cziko Seamount (CZI; green), and Turtle Rock (TR; yellow). Images of the benthic diversity collected by ROV, including (c) GHN, (d) GHM, (e) GHS, (f) CB, (g) CE, (h) CZI, and (i) TR. (j) The target sponge, Haliclona scotti, prior to biopsy by ROV. Photo credit: Leigh Tait.

TABLE 1 Sampling site information, including site identification, name, latitude, longitude, and eDNA sample number and depth of collection.

Site ID	Site name	Latitude	Longitude	Seafloor depth (m)[a]	Sponge sampling depth (m)
CB	Cape Barne	77°34.955′ S	166°15.822′ E	20	~30
CE	Cape Evans	77°38.095′ S	166°31.843′ E	19	19–34
CZI	Cziko Seamount	77°38.927′ S	166°31.873′ E	18	22–30
GHM	Granite Harbor Middle	77°00.2425′ S	162°35.2964′ E	17	NA
GHN	Granite Harbor North	77°54.9510′ S	162°35.4300′ E	20	NA
GHS	Granite Harbor South	77°00.9604′ S	162°52.5822′ E	20	NA
TR	Turtle Rock	77°44.639′ S	166°46.175′ E	19	NA

[a]Water samples were collected <2 m above the seafloor, immediately below the access hole through the sea ice.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Coastal biodiversity monitoring consisted of water and sponge eDNA sampling. Sample collection was conducted between October 21 and 30, 2021 along the Ross Sea coastline (Figure 1; Table 1). Five replicate 500 mL water samples were collected at each of seven locations within 2 m of the ocean floor using a Niskin bottle. Water samples were filtered on site using standard eDNA syringe mini kits (Wilderlab, New Zealand). DNA/RNA Shield (*Cat # R1100-50*; Zymo Research, US) was added to the enclosed filter housing to preserve eDNA during transportation to the University of Otago's PCR-free eDNA facilities at Portobello Marine Laboratory (PML). At the same

time, five sponge specimens were collected by ROV at a depth range of 18–30 m from three out of the seven locations, thereby enabling sponge and near-bottom water eDNA signal comparison. The sponge *Haliclona scotti* (Kirkpatrick, 1907) (Porifera; Demospongiae; Haplosclerida; Chalinidae) was targeted for this study, due to its regional abundance, morphological characteristics facilitating identification by ROV footage prior to collection, and a form suited for obtaining tissue biopsies using an ROV with minimal disturbance (V. Cummings, pers. obs.). Specimens were placed in separate 50 mL falcon tubes filled with 99.8% molecular-grade ethanol (Cat # BP2818500; Fisher BioReagents™, Fisher Scientific, US) and stored in the dark on ice during shipment to the University of Otago.

2.2 | Laboratory processing of eDNA samples

Environmental DNA sample processing was conducted in a designated PCR-free clean room. Benches and equipment were decontaminated using a 10-min exposure to 10% bleach solution and wiped with ultrapure water (UltraPure™ DNase/RNase-Free Distilled Water, Invitrogen™) to reduce contamination risk prior to laboratory work. To identify external contaminating eDNA signals and potential cross-contamination, negative control samples were processed alongside eDNA samples and consisted of 50 µL ultrapure water for DNA extraction negatives and 2 µL ultrapure water for PCR no-template controls.

DNA extraction of water eDNA samples was performed on the DNA Shield buffer (David et al., 2021) using the Qiagen DNeasy Blood & Tissue Kit (Qiagen GmbH) according to the manufacturer's recommendations with slight modifications (Supplement S1). eDNA from marine sponge specimens was obtained through DNA extraction from tissue biopsies using the Qiagen DNeasy Blood & Tissue Kit with slight modifications (Jeunen et al., 2023; Supplement S2).

Input DNA of each sample for qPCR amplification was optimized using a 10-fold dilution series to identify inhibitors and low-template samples prior to library preparation (Murray et al., 2015). Amplification was carried out in 25 µL duplicate reactions. The qPCR Mastermix consisted of 1× SensiMix SYBR Lo-ROX Mix (Cat # QT625-05; Meridian Bioscience, UK), 0.4 µmol/L of the forward and reverse primer (Integrated DNA Technologies), 2 µL of template DNA, and ultrapure water as required. The thermal profile included an initial denaturation step of 95°C for 10 min, followed by 50 cycles of 30 s at 95°C, 30 s at 51°C, and 45 s at 72°C, and a final melt-curve analysis.

Library preparation followed a one-step amplification protocol using fusion primers (Berry et al., 2017). Fusion primers consisted of an Illumina adapter, a modified sequencing primer, a 6–8 bp barcode tag, and the template-specific primer (Leray et al., 2013; mlCOIintF: 5′-GGWACWGGWTGAACWGTWTAYCCYCC-3′; jgHCO2198: 5′-TAIACYTCIGGRTGICCRAARAAYCA-3′) amplifying a ~313 bp fragment of the cytochrome c oxidase subunit I (COI) gene region. Each sample was amplified in duplicate and assigned a unique barcode combination, whereby forward and reverse barcodes differed from each other in a single sample. The qPCR conditions followed the protocol as described above. Sample duplicates were pooled to reduce stochastic effects from PCR amplification (Alberdi et al., 2018; Leray & Knowlton, 2015). As an intermediary step to reduce the number of Qubit measurements and simplify equimolar pooling of the library, pooled duplicate PCR replicates were further pooled into mini-pools based on end-point qPCR fluorescence, Ct values, and melt-curve analysis. Mini-pools were visualized using gel electrophoresis to determine the presence of a single band, and molarity of mini-pools was measured on Qubit (Cat # Q32854; Qubit™ dsDNA HS Assay Kit, ThermoFisher Scientific, US). The mini-pools were pooled at an equimolar concentration to produce a single DNA library. Due to differences in cycle number between samples and negative controls, which contained a reduced amount of DNA, the latter were spiked into the library to ensure the library achieved an optimal concentration for sequencing. Size selection was performed using Pippin Prep (Cat # PIP0001; Sage Science, US). The size-selected library was purified with Qiagen's QIAquick PCR Purification Kit (Cat # 28104; Qiagen GmbH) prior to final library quantitation on qubit. Sequencing was performed on an Illumina MiSeq® v2 2×250 bp kit following the manufacturer's protocols, with 5% PhiX to minimize issues associated with low-complexity libraries.

2.3 | Bioinformatic analysis and taxonomy assignment

Raw sequencing files were checked for quality using FastQC *v* 0.11.5 (Andrews, 2010). Forward and reverse reads were merged using default settings in PEAR *v* 0.9.20 (Zhang et al., 2014). Sequencing data were demultiplexed using cutadapt *v* 4.0 (Martin, 2011). Quality filtering in VSEARCH *v* 2.13.3 (Rognes et al., 2016) was conducted on the following parameters: a maximum expected error of 1.0 ('*--fastq_maxee*'), a minimum length of 311 bp ('*--fastq_minlen*'), a maximum length of 315 bp ('*--fastq_maxlen*'), and a maximum number of ambiguous base calls of 0 ('*--fastq_maxns*'). Filtered sequencing data were dereplicated (function: '*vsearch --derep_fulllength*'), and singleton unique sequences were discarded ('*--minuniquesize* 2'). Chimeric sequences were removed from the dataset by the UCHIME algorithm (Edgar et al., 2011) implemented in VSEARCH. Remaining reads were clustered into Operational Taxonomic Units (OTUs) using a 97% similarity threshold (function: '*vsearch --cluster_size*'), and a frequency table was generated using the '*--usearch_global*' function implemented in VSEARCH (Edgar, 2010). Further data filtering was conducted using default settings in the LULU algorithm *v* 0.1.0 (Frøslev et al., 2017).

A custom-curated reference database was generated using CRABS *v* 0.1.4 (Jeunen, Dowle, et al., 2022). The custom-curated reference database was built from COI reference sequences obtained from NCBI (Federhen, 2012) and BOLD (Ratnasingham & Hebert, 2007). Amplicon regions were extracted from downloaded sequences through in silico PCR analysis (function: '*insilico_pcr*')

and pairwise global alignments (function: '*pga*'). Finally, the curated reference database was filtered (function: '*seq_cleanup*') and dereplicated (function: '*dereplicate*'). The final reference database was formatted using BLAST+ *v* 2.10.1+ (Camacho et al., 2009; function: '*makeblastdb*').

BLAST results obtained from the curated reference database for each OTU were parsed using an in-house Python script (LCA_BLAST_calculator.py; https://github.com/gjeunen/LCA_BLAST_calculator). Finally, the taxonomic level of the assignment was determined based on similarity thresholds, with species-level assignments requiring a similarity threshold higher than 99%, genus-level assignments when similarity was observed between 97% and 99%, family-level assignments for similarity between 95% and 97%, order-level assignments for similarity between 90% and 95%, class-level assignments for similarity between 85% and 90%, and phylum-level assignments for similarity between 80% and 85%. After taxonomy assignment, the OTU table underwent final processing prior to statistical analysis, whereby (i) detections per sample were set to zero if the number of reads was below the 0.1% threshold of the total number of reads across all samples to avoid issues related to tag jumping (Schnell et al., 2015), (ii) OTUs with a positive detection in negative control samples were removed from the analysis, (iii) OTUs which could not be assigned a taxonomy were removed, (iv) non-marine eDNA signals were removed from the final dataset, and (v) the data were transformed to presence–absence, as the correlation between eDNA signal strength and biomass/abundance of an organism has not been established thus far (Ushio et al., 2017).

2.4 | Statistical analysis

All statistical analyses were conducted in R *v* 4.0.5 (R; http://R-project.org). Rarefaction curves were generated from the unfiltered OTU table to assess sequencing coverage using the vegan *v* 2.5-7 package. For the spatial analysis based on near-bottom water and sponge eDNA signals, OTU richness was compared between sampling locations through Welch's ANOVA, followed by the post hoc Games–Howell test to determine significant differences between locations. eDNA signal accumulation curves were generated in the *BiodiversityR v* 2.13-1 package to assess differences in total diversity between sampling locations. A permutational multivariate analysis of variance (PERMANOVA) was conducted to determine whether eDNA signal composition differed between locations using the vegan package. Significant differences in dispersion between groups were tested (PERMDISP) to aid in the interpretation of PERMANOVA results. A Principal Coordinates Analysis (PCoA) was performed using the vegan package to visualize patterns of sample dissimilarity using the Jaccard index. For the comparison between near-bottom water and sponge eDNA signals, analyses were conducted per location to eliminate the impact of spatial variation observed in the spatial analysis. OTU richness was compared between substrates using a Student's two-sample *t*-test. A PERMANOVA was used to determine whether eDNA signal composition differed between substrates, while PERMDISP evaluated differences in dispersion between substrates to the centroid. A PcoA analysis was performed to visualize patterns of sample dissimilarity using the Jaccard index, while Venn diagrams were drawn to visualize overlap in eDNA signal detection between water and sponge samples. To determine the eDNA signals driving the difference observed between eDNA substrates in the ordination analysis, an indicator species analysis (ISA) was conducted using the indicspecies *v* 1.7-14 package.

3 | RESULTS

3.1 | Sequencing and diversity observations

After stringent quality filtering, we retained 898,234 sequences across 49 samples. Samples achieved an average of 18,331 ± 7523 reads. Overall, eDNA samples achieved sufficient sequencing coverage based on the plateauing of rarefaction curves, except for water and sponge samples collected at Cape Evans and sponges collected at Cape Barne (Supplement S3). Fifteen OTUs (1478 reads) were removed, due to a positive signal in negative control samples (Supplement S4). Taxonomy filtering removed an additional 209,666 (17.64%) reads assigned to 574 (35.45%) OTUs, as no taxonomy could be assigned to these sequences due to an incomplete reference database (Figure 2). Furthermore, only 135 (8.33%) OTUs could be assigned a species ID across all samples. While the proportion of OTUs assigned to species was similar between eDNA substrates (water: 9.24% ± 1.59%; sponge: 10.83% ± 1.82%), the proportion of reads assigned to species was markedly different between water (36.03% ± 15.85%) and sponge samples (68.85% ± 9.61%) due to the co-amplification of host DNA (*Haliclona scotti*) in the marine sponge samples (water: No Detection; sponge: 25.46% ± 14.91%; Figure 2). The final frequency table contained a total of 898,234 reads assigned to 876 OTUs (Supplement S5).

Overall, we detected 30 phyla across the kingdoms Animalia (15 phyla), Chromista (nine phyla), Protozoa (four phyla), and Plantae (two phyla; Figure 3). Highest sequence diversity was observed within the phylum Arthropoda (158 OTUs), followed by Bacillariophyta (131 OTUs), and Oomycota (103 OTUs). Highest read abundance, on the other hand, was observed within the phylum Annelida (17.82%), followed by Cnidaria (16.19%) and Bacillariophyta (14.56%). The haptophyte *Phaeocystis antarctica* Karsten, 1905 was the most frequently detected OTU containing a species ID (sample detection: 91.84%; read abundance: 2.53%), followed by the sea star *Odontaster validus* Koehler, 1906 (sample detection: 83.67%; read abundance: 1.98%), the dinoflagellate *Margalefidinium polykrikoides* (Margalef) FGómez Richlen & D.M. Anderson, 2017 (sample detection: 81.63%; read abundance: 0.08%), and the sea urchin *Sterechinus neumayeri* (Meissner, 1900) (sample detection: 69.39%; read abundance: 2.85%). The

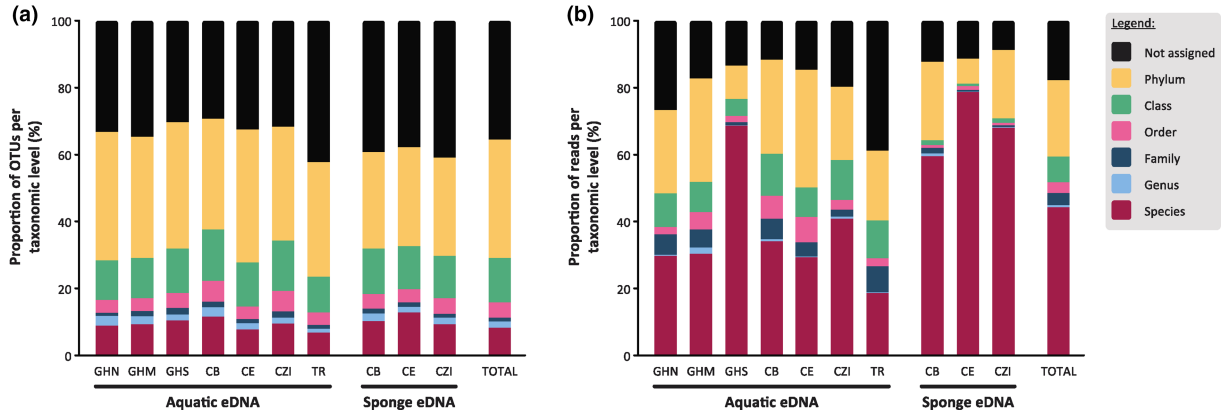

FIGURE 2 Proportion of (a) OTUs and (b) reads assigned to seven taxonomic levels for eDNA samples collected at Granite Harbor North (GHN), Granite Harbor Middle (GHM), Granite Harbor South (GHS), Cape Barne (CB), Cape Evans (CE), Cziko Seamount (CZI), Turtle Rock (TR), and all samples combined. Samples are organized per substrate, i.e., near-bottom water eDNA and sponge eDNA.

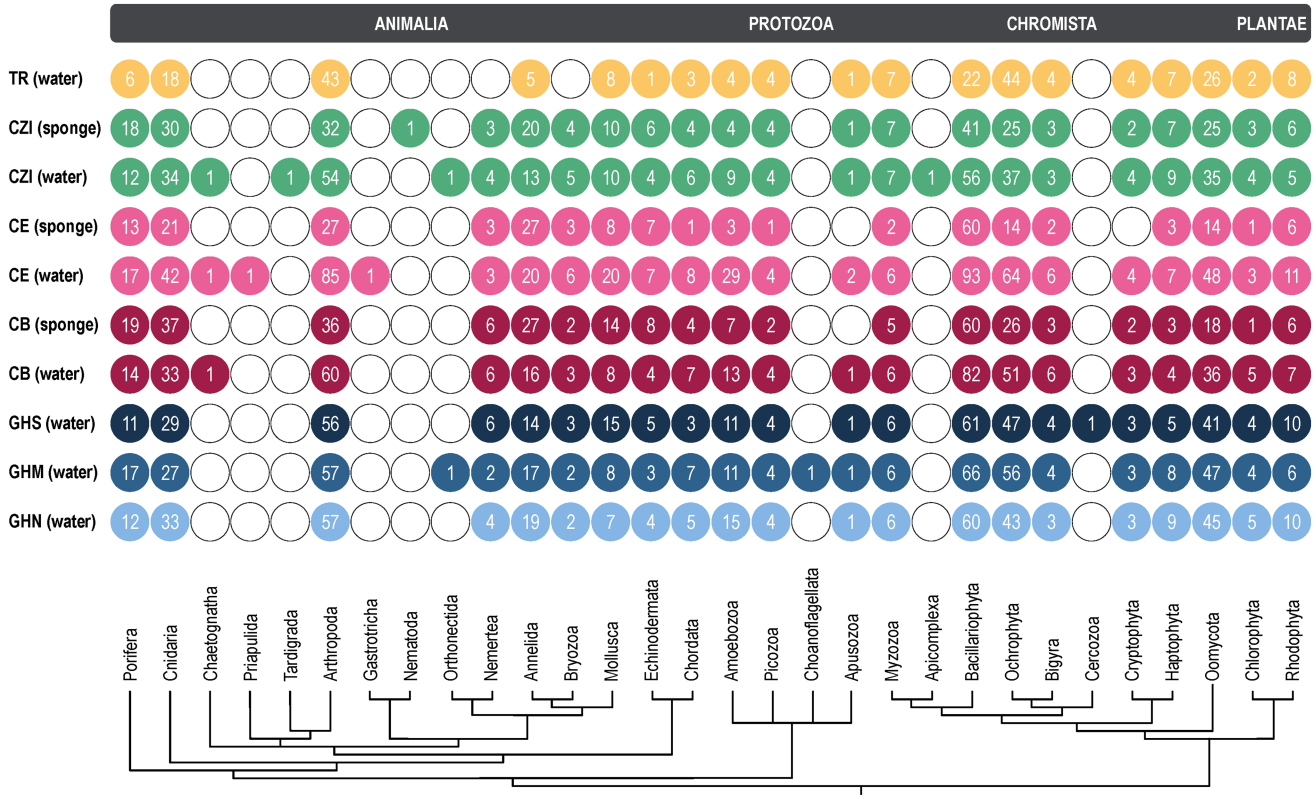

FIGURE 3 Observed OTU diversity within each sampling location (Granite Harbor North [GHN]; Granite Harbor Middle [GHM]; Granite Harbor South [GHS]; Cape Barne [CB]; Cape Evans [CE]; Cziko Seamount [CZI]; Turtle Rock [TR]) and sampling substrate (water; marine sponge) per phylum. Color coding follows location color of Figure 1. Number within colored circles indicates number of OTUs assigned to the phylum. Unfilled circles indicate OTU absence.

highest read abundance for an OTU containing a species ID was observed for the octocoral *Alcyonium haddoni* Wright & Studer, 1889 (sample detection: 40.82%; read abundance: 14.08%), followed by the sponge *Haliclona scotti* (sample detection: 30.61%; read abundance: 8.19%), *Sterechinus neumayeri*, *Odontaster validus*, *Phaeocystis antarctica*, the annelid *Barrukia cristata* (Willey, 1902) (sample detection: 61.22%; read abundance: 1.55%), the diatom *Pseudo-nitzschia subcurvata* (G.R. Hasle) G.A. Fryxell, 1993 (sample detection: 55.10%; read abundance: 1.46%), the seal *Leptonychotes weddellii* (Lesson, 1826) (sample detection: 59.18%; read abundance: 1.38%), and the annelid *Harmothoe crosetensis* (McIntosh, 1885; sample detection: 48.98%; read abundance: 1.20%).

3.2 | Near-bottom water eDNA spatial analysis

We detected a total of 831 OTUs after stringent quality filtering across the seven sampling sites along the Ross Sea coastline

(Figure 4). Significant differences in OTU richness were observed across locations according to Welch's ANOVA ($F_{6,11}=21.933$; $p=1.22\times10^{-5}$***). Samples collected at Cape Evans detected a significantly larger number of OTUs compared to Granite Harbor North, Granite Harbor Middle, Cziko Seamount, and Turtle Rock according to the post hoc Games–Howell test (Figure 4a). While samples collected at Turtle Rock detected the lowest number of OTUs on average, the reduction in OTU number was not significantly different from the remainder of the study sites. Species accumulation curves revealed a similar pattern to OTU richness, whereby Cape Evans samples detected the largest diversity and Turtle Rock samples detected the lowest diversity (Figure 4b).

Significant differences in community composition among sampling locations were also observed (PERMANOVA: $F_{6,27}=3.34$; $p<0.001$***), while no significant differences in dispersion were detected (PERMDISP: $F_{6,27}=1.7654$; $p=0.123$). Community differences among sampling locations were confirmed by ordination analysis (PCoA analysis; Figure 4c), whereby Turtle Rock and Cape Evans separated from the remaining locations along the primary axis, which explained 12.9% of the variation in the dataset. Samples collected at the three Granite Harbor sites (GHN, GHM, and GHS) separated from the remaining locations along the secondary axis that explained 8.6% of the variation.

3.3 | Sponge eDNA spatial analysis

We detected a total of 423 OTUs after stringent quality filtering across the three sampling sites along the Ross Sea coastline where sponges were collected by ROV (Figure 4). Unlike near-bottom water eDNA, no significant differences in OTU richness were observed across locations according to Welch's ANOVA ($F_{2,8}=1.153$; $p=0.365$; Figure 4d). Species accumulation curves revealed similar results between the three sampling locations (Figure 4e), with near-identical number of OTUs detected between locations.

Significant differences in community composition among the three sampling locations were also observed (PERMANOVA: $F_{2,12}=2.94$; $p<0.001$***), while no significant differences in dispersion were detected (PERMDISP: $F_{2,12}=0.1816$; $p=0.857$). Community differences among sampling locations were further confirmed by ordination analysis (PCoA analysis; Figure 4f), whereby Cziko Seamount and Cape Evans separated from each other along the primary axis, explaining 16.7% of the variation in the dataset. Sponges from Cape Barne, on the other hand, separated from the other two sites along the secondary axis that explained 11.5% of the variation.

3.4 | Near-bottom water and sponge eDNA substrate analysis

We detected a total of 725 OTUs after stringent quality filtering across the three sampling sites (Cape Barne, Cape Evans, and Cziko Seamount) and two substrates (water and sponge). On average, water samples contained a higher OTU diversity compared to the *Haliclona scotti* sponge. Significant differences in OTU richness were observed between the substrates within Cape Barne ($t=2.42$; d.f.$=8$; $p<0.05$*) and Cape Evans ($t=11.63$; d.f.$=8$; $p<0.001$***), while no significant difference in OTU richness was observed between substrates at Cziko Seamount ($t=2.00$; d.f.$=8$; $p>0.05$) according to Student's two-sample *t*-tests (Figure 5a–c).

Significant differences in community composition were also observed between substrates within each location (PERMANOVA; Cape Barne: $F_{1,8}=5.5992$, $p<0.01$**; Cape Evans: $F_{1,8}=6.436$, $p<0.01$**; Cziko Seamount: $F_{1,8}=3.5033$, $p<0.01$**). Community differences between substrates within each location were confirmed by ordination analysis (PCoA analysis; Figure 5d–f), whereby substrates separated from each other along the primary axis, explaining 32.2%, 34.9%, and 27.4% of the variation in the dataset for Cape Barne, Cape Evans, and Cziko Seamount, respectively. The differences in community composition resulted in a partial OTU overlap between eDNA substrates within each sampling location, ranging from 30.0% at Cape Evans to 41.8% at Cziko Seamount (Figure 5g–i). Besides community composition differences between substrates, ordination analysis identified increased variation in sponge eDNA signals within a sampling location compared to water eDNA signals (Figure 5d–f), which was further corroborated by PERMDISP analysis (Cape Barne: $F_{1,8}=3.7409$, $p=0.084$; Cape Evans: $F_{1,8}=9.8741$, $p<0.001$***; Cziko Seamount: $F_{1,8}=7.0022$, $p<0.01$**).

To determine the eDNA signals driving the difference observed between substrates in the ordination analysis, an indicator species analysis (ISA) was conducted for each sampling location separately (Supplement S6). Rhodophyte and polychaete eDNA signals were indicative of sponge samples at Cziko Seamount, while mollusk and polychaete eDNA signals were indicative of sponge samples at Cape Barne. Due to the increased OTU diversity detected within water eDNA samples, the number of indicator species identified for water samples was larger than marine sponge samples and ranged from bacillariophytes to vertebrates, such as the Weddell seal (*Leptonychotes weddellii*; Supplement S6).

4 | DISCUSSION

This study provides new insights into the potential of eDNA surveys to aid in the monitoring of biodiversity and community composition in coastal Antarctica. Our results show distinct bottom water and sponge eDNA signals when investigated through a "universal" COI metabarcoding approach, thereby demonstrating that eDNA substrate choice critically influences the detected marine community and highlighting the need to establish more sophisticated sampling strategies beyond the standard surface water sampling used to date in marine environments (Jeunen et al., 2019; Koziol et al., 2019). Additionally, the ability to describe community structures along the Ross Sea coastline from low-volume water

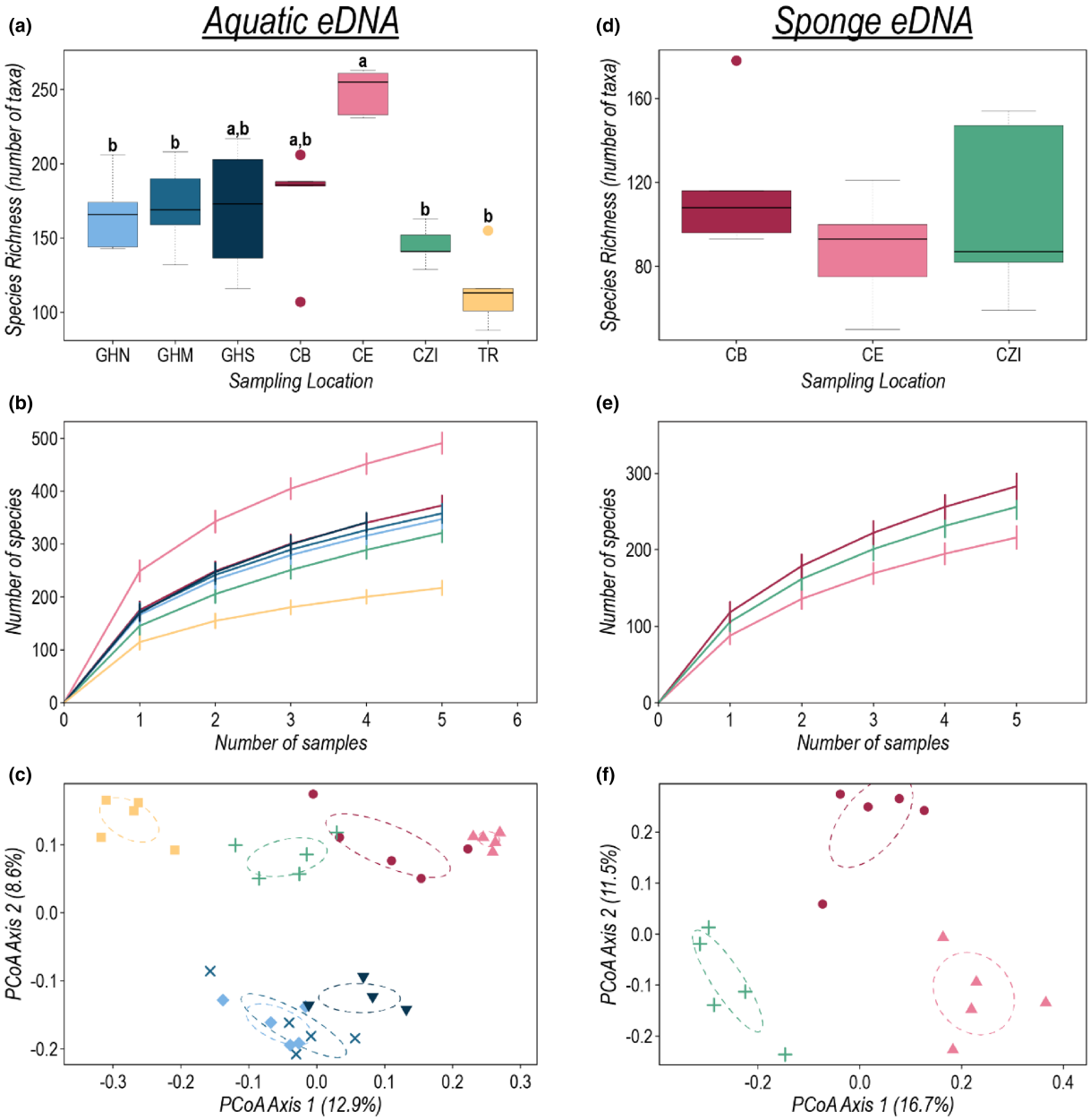

FIGURE 4 Boxplots representing average (a) water OTU richness among the seven sampling locations sorted from north to south and (d) sponge OTU richness. Outliers are indicated by colored circles. The median is indicated by a black line within each boxplot. Significant differences in OTU richness among locations according to Welch's ANOVA and Games–Howell post hoc test are indicated by a different lowercase letter. (b) Water eDNA and (e) sponge eDNA species accumulation curves for each location, with the number of samples on *x*-axis and number of OTUs on *y*-axis. Vertical lines depict standard error values. (c) Water eDNA and (f) sponge eDNA Principal Coordinates Analysis (PCoA) depicting similarity in community composition based on OTU incidence (Jaccard index; presence–absence). Ellipses surrounding each group of samples represent 95% confidence intervals. Granite Harbor North (GHN), Granite Harbor Middle (GHM), Granite Harbor South (GHS), Cape Barne (CB), Cape Evans (CE), Cziko Seamount (CZ), and Turtle Rock (TR). Color coding follows location color of Figure 1.

samples, as well as marine sponges facilitates more comprehensive monitoring in isolated regions where the efficiency of traditional survey techniques is hampered (Cecchetto et al., 2021; Fediajevaite et al., 2021). Furthermore, our data showcase the importance of complete reference databases for robust taxonomy assignment, an element exacerbated for remote and understudied areas (Jackman et al., 2021).

Environmental DNA signals obtained from water samples and *Haliclona scotti* tissue biopsies revealed distinct eukaryote community compositions. While water is the most-used substrate in marine eDNA studies (Bowers et al., 2021; Takahashi et al., 2023), only a partial overlap with sponge eDNA signals was observed, ranging from 30.0% at Cape Evans to 41.8% at Cziko Seamount. Indicator species analysis revealed a higher diversity and frequency of benthic

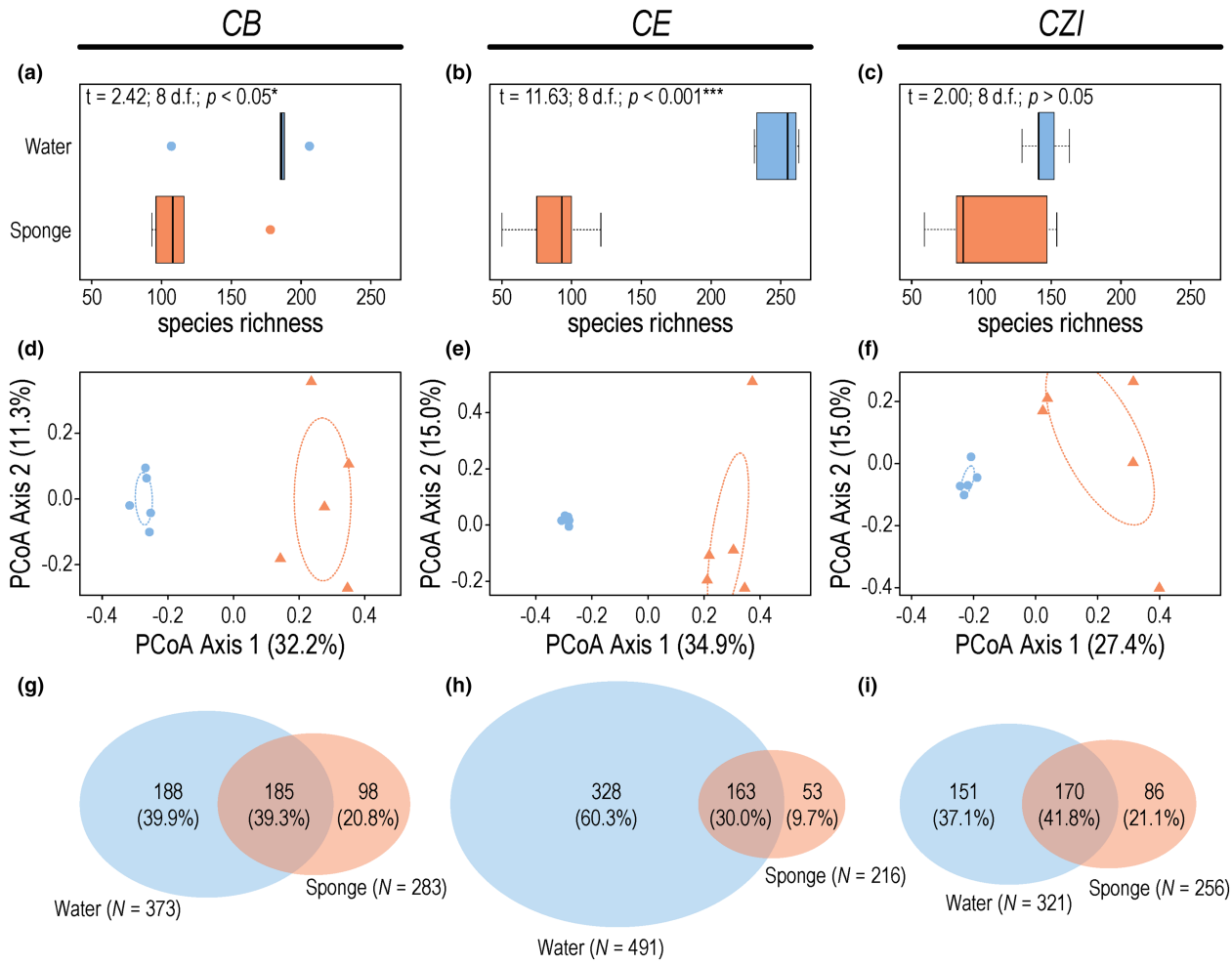

FIGURE 5 Boxplots representing average OTU richness between near-bottom water (blue) and sponge (orange) eDNA signals for the three sampling locations, including (a) Cape Barne (CB), (b) Cape Evans (CE), and (c) Cziko Seamount (CZI). Outliers are indicated by colored circles. The median is indicated by a black line within each boxplot. Student's two-sample *t*-test statistics are provided at the top left-hand corner for each comparison. Principal Coordinates Analysis (PCoA) depicting similarity in community composition based on OTU incidence (Jaccard index; presence–absence) for the three sampling locations, including (d) CB, (e) CE, and (f) CZI. Water and sponge eDNA signals are depicted in blue circles and orange triangles, respectively. Ellipses surrounding each group of samples represent 95% confidence intervals. Venn diagrams depicting OTU overlap between water (blue) and sponge (orange) eDNA signals for the three sampling locations, including (g) CB, (h) CE, and (i) CZI. Total number of OTUs for each eDNA substrate is represented between brackets. Venn diagram size is proportional to the number of detected OTUs.

and sediment-living organisms from *H. scotti* eDNA, while water eDNA indicator species were mostly composed of planktonic and nektonic organisms (Supplement S6). The difference in eukaryote eDNA signals between substrates is, therefore, potentially influenced by ecological factors such as species habitat preference, a known contributing factor to observed differences between water and sediment eDNA (Holman et al., 2019; Koziol et al., 2019; Shaw et al., 2016). Our results provide further evidence for the inability of single-substrate eDNA surveys to uncover the total biodiversity within a region (Holman et al., 2019; Koziol et al., 2019; Shaw et al., 2016). Hence, future eDNA surveys might incorporate a sampling strategy that moves beyond the current standard of surface water sampling used to date and that is more tailored toward the aims of the study. For example, investigations into Antarctica's dominant benthos could benefit from sponge or sediment sampling, while eDNA monitoring of plankton and nekton might require water sampling to increase species detection probability (Holman et al., 2019; Koziol et al., 2019).

Interestingly, the observed difference between water and sponge eDNA signals in this study is in stark contrast with the highly similar vertebrate eDNA signals found in water and sponges by Jeunen et al. (2023). eDNA obtained from *Haliclona scotti* in the present study detected only three benthic fish species, all within the *Trematomus* genus (rockcods; Boulenger, 1902), and failed to detect five other pelagic fish species and Weddell Seals (*Leptonychotes weddellii*) that were observed in the water eDNA samples. The discrepancy with Jeunen et al. (2023) could potentially be due to technical limitations in our experimental design. First, unlike the targeted metabarcoding approach employed by Jeunen et al. (2023), we opted for a "universal" metabarcoding

approach through COI amplification (Leray et al., 2013) to uncover a broader range of taxonomic groups, thereby increasing false-negative detection rates through highly variable amplification efficiencies. Second, *H. scotti* host DNA constituted on average 32.76% of reads (CBSP1: 3.00%; CZSP2: 79.46%) within sponge samples and failed to be detected in our water eDNA survey. The presence of host DNA most likely increased the risk of false-negative detections in our sponge eDNA survey. To alleviate this issue, the use of blocking primers could be considered in future studies (Rojahn et al., 2021; Wilcox et al., 2014). The false-negative detections of *H. scotti* in the water eDNA samples, on the other hand, most likely originated from low amplification efficiency for this target organism, due to the presence of multiple mismatches in the forward and reverse primer-binding region (Supplement S7). Third, missing taxa in near-bottom water eDNA samples could have been induced by the processing of smaller water volumes (i.e., 500 mL) rather than the standard vacuum filtration of ~2000 mL in the marine environment (Bowers et al., 2021; Takahashi et al., 2023), a consideration made based on logistics of in situ sampling in Antarctic field conditions. A fourth potential influencing factor for the observed differences between sponges and water samples could be the preservation technique used for each treatment (i.e., sponges were stored in ethanol and water eDNA in DNA Shield), whereby different preservation methods have been shown to influence downstream results in eDNA comparative studies (Minamoto et al., 2016; Sales et al., 2019).

The efficiency of sponge eDNA metabarcoding surveys could be further enhanced by gaining a better understanding of how sponges accumulate eDNA and how to optimally obtain eDNA from sponge tissue biopsies (Harper et al., 2023; Mariani et al., 2019). The increased variability observed in eDNA composition between *Haliclona scotti* samples within a site compared to water eDNA signals demonstrates the need for protocol optimization (Figure 5d–f). Unlike the myriad of studies on protocol optimization for water eDNA (Sanches & Schreier, 2020; Spens et al., 2016), only a single study has investigated the impact of various DNA extraction protocols on eDNA signal recovery from sponge tissues thus far (Harper et al., 2023). Since the use of optimized protocols is linked to increased DNA concentration, diversity detection consistency, and probability of rare taxa (Spens et al., 2016), additional research into protocol optimization for sponge eDNA surveys is required. Furthermore, differences in eDNA accumulation efficiency have been observed for various sponge species (Cai et al., 2022; Turon et al., 2020) and linked to filtration rates and microbial activity (Brodnicke et al., 2023). While our survey targeted a sponge species with a form suited for obtaining tissue biopsies using an ROV with minimal disturbance, to limit negative impacts on the benthic community, increasing our knowledge on which species are most suitable for eDNA monitoring would enhance species detection through sponge eDNA metabarcoding surveys.

The eDNA diversity we recorded from water at the seven sampling locations showed strong spatial structuring based on known species distributions in the Ross Sea region (Cummings et al., 2018; Thrush et al., 2006). The distinct water eDNA signals we recovered at each location were significantly different when analyzed for richness (Figure 4a) and composition (Figure 4c). Highest diversity was observed at Cape Evans, a relatively exposed bay with seafloor bathymetry providing protection from icebergs in the nearshore area. The site has a high proportion of rocky substrata and a high abundance of sessile organisms (Figure 1g). Lowest diversity, on the other hand, was observed at Turtle Rock, a relatively sheltered site south of the Erebus Ice Tongue. The seafloor is composed largely of soft sediment and the benthos by sea stars, sea urchins, and infaunal bivalves (Figure 1i). The spatially discrepant eDNA signals observed in this study and elsewhere (Beentjes et al., 2019; Minamoto et al., 2017; Murphy & Jenkins, 2010; O'Donnell et al., 2017) demonstrate the potential of eDNA surveys as a non-invasive method for species detection and monitoring. Indeed, our ability to recover 1450 genetic signals across 30 phyla and identify 135 species from a limited number of low-volume samples improves our capability of biodiversity monitoring across the tree of life in logistically difficult-to-sample regions (Cecchetto et al., 2021).

The power of eDNA metabarcoding stems from species detection based on mathematical criteria applied to sequences obtained from environmental samples (Alberdi & Gilbert, 2019). However, the reliance on reference databases for taxonomy assignment is limiting the robustness of taxonomy assignment in remote regions and for understudied taxonomic groups (Ammon et al., 2018). Only 135 (8.33%) OTUs could be assigned a species ID, even though the cytochrome b oxidase subunit I (COI) amplicon fragment used in this study (Leray et al., 2013) provides species-level taxonomic resolution (Bucklin et al., 2010). Furthermore, the proportion of OTUs assigned to a higher taxonomic resolution differed among taxonomic groups and was correlated to the extensiveness of molecular research published on the phylum. For example, a large proportion of OTUs assigned to Porifera (58.33%), Chordata (81.82%), and Echinodermata (92.86%) phyla achieved a taxonomic resolution of genus or species, while Ochrophyta (76.70%), Oomycota (82.18%), and Arthropoda (89.87%) were mostly assigned to phylum level. Hence, effort should be directed to filling these gaps in the sequence database where possible.

In conclusion, our near-bottom water and marine sponge eDNA survey along the Ross Sea coastline provide evidence for the potential of eDNA metabarcoding for biomonitoring in isolated areas using two low-tech solutions, that is, 500 mL manual water filtration through a syringe setup and marine sponge biopsies. While spatial eDNA patterns distinguished sampling locations, comparisons with visually analyzed benthic community composition require further research. Additionally, the distinct eDNA signals recovered from both substrates indicate a need to incorporate more sophisticated sampling strategies beyond the standard surface water sampling used to date to capture the full diversity at a particular site.

AUTHOR CONTRIBUTIONS

The study design was conceptualized by GJJ, SM, SM, ML, and NJG. Coastal sampling was conducted by VC, JM, LPR, and LT. Laboratory

work was performed by GJJ, JT, SF, BL, and SW. The bioinformatic analysis was conducted by GJJ. GJJ performed the statistical analysis, with input from SM, SM, ML, VC, and NJG. GJJ wrote the manuscript with significant input from ML, VC, and NJG. All co-authors contributed to the writing of the manuscript and approved of the submission.

ACKNOWLEDGMENTS

A Marsden Fast-Start MFP-UOO2116 and University of Otago Research Grant to GJJ funded sample processing and sequencing costs. Ministry of Business, Innovation, and Employment Grant/Award Number MBIE ANTA1801 covered sample collection and transport to and from the eDNA facilities at PML, University of Otago. We thank Antarctica New Zealand for logistical support, and especially Jon Tyler for the sea ice holes. We thank Steve Rupp and Rob Robbins (United States Antarctic Program) for the use of their dive huts for sample collection at CZI and TR. We thank Otago Genomics Facility for their sequencing assistance and Erica Spain (NIWA) for Figure 1b. Open access publishing facilitated by University of Otago, as part of the Wiley - University of Otago agreement via the Council of Australian University Librarians.

CONFLICT OF INTEREST STATEMENT

No conflicts of interest.

DATA AVAILABILITY STATEMENT

Raw sequencing data, as well as the barcode metadata file for demultiplexing have been deposited on FigShare (https://figshare.com/projects/Unveiling_the_Hidden_Diversity_of_Marine_Eukaryotes_in_the_Ross_Sea_A_Comparative_Analysis_of_Seawater_and_Sponge_eDNA_Surveys/186127).

ORCID

Gert-Jan Jeunen https://orcid.org/0000-0001-7458-3550
Stefano Mariani https://orcid.org/0000-0002-5329-0553

REFERENCES

Alberdi, A., Aizpurua, O., Gilbert, M. T. P., & Bohmann, K. (2018). Scrutinizing key steps for reliable metabarcoding of environmental samples. *Methods in Ecology and Evolution*, *9*(1), 134–147. https://doi.org/10.1111/2041-210X.12849

Alberdi, A., & Gilbert, M. T. P. (2019). A guide to the application of hill numbers to DNA-based diversity analyses. *Molecular Ecology Resources*, *19*(4), 804–817. https://doi.org/10.1111/1755-0998.13014

Ammon, U., Wood, S. A., Laroche, O., Zaiko, A., Tait, L., Lavery, S., Inglis, G. J., & Pochon, X. (2018). Combining morpho-taxonomy and metabarcoding enhances the detection of non-indigenous marine pests in biofouling communities. *Scientific Reports*, *8*(1), 16290. https://doi.org/10.1038/s41598-018-34541-1

Andrews, S. (2010). *FastQC: A quality control tool for high throughput sequence data*. Babraham Bioinformatics, Babraham Institute.

Beentjes, K. K., Speksnijder, A. G. C. L., Schilthuizen, M., Hoogeveen, M., & van der Hoorn, B. B. (2019). The effects of spatial and temporal replicate sampling on eDNA metabarcoding. *PeerJ*, *7*, e7335. https://doi.org/10.7717/peerj.7335

Berry, T. E., Osterrieder, S. K., Murray, D. C., Coghlan, M. L., Richardson, A. J., Grealy, A. K., Stat, M., Bejder, L., & Bunce, M. (2017). DNA metabarcoding for diet analysis and biodiversity: A case study using the endangered Australian sea lion (*Neophoca cinerea*). *Ecology and Evolution*, *7*(14), 5435–5453. https://doi.org/10.1002/ece3.3123

Bessey, C., Neil Jarman, S., Simpson, T., Miller, H., Stewart, T., Kenneth Keesing, J., & Berry, O. (2021). Passive eDNA collection enhances aquatic biodiversity analysis. *Communications Biology*, *4*(1), 236. https://doi.org/10.1038/s42003-021-01760-8

Bowers, H. A., Pochon, X., von Ammon, U., Gemmell, N., Stanton, J.-A. L., Jeunen, G.-J., Sherman, C. D. H., & Zaiko, A. (2021). *Towards the optimization of eDNA/eRNA sampling Technologies for Marine Biosecurity Surveillance*. Water (vol. 13, issue 8). https://doi.org/10.3390/w13081113

Brodnicke, O. B., Meyer, H. K., Busch, K., Xavier, J. R., Knudsen, S. W., Møller, P. R., Hentschel, U. H., & Sweet, M. J. (2023). Deep-sea sponge derived environmental DNA analysis reveals demersal fish biodiversity of a remote Arctic ecosystem. *Environmental DNA*, *3*, 471–508. https://doi.org/10.1002/edn3.451

Bucklin, A., Steinke, D., & Blanco-Bercial, L. (2010). DNA barcoding of marine Metazoa. *Annual Review of Marine Science*, *3*(1), 471–508. https://doi.org/10.1146/annurev-marine-120308-080950

Cai, W., Harper, L. R., Neave, E. F., Shum, P., Craggs, J., Arias, M. B., Riesgo, A., & Mariani, S. (2022). Environmental DNA persistence and fish detection in captive sponges. *Molecular Ecology Resources*, *22*, 2956–2966. https://doi.org/10.1111/1755-0998.13677

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L. (2009). BLAST+: Architecture and applications. *BMC Bioinformatics*, *10*(1), 421. https://doi.org/10.1186/1471-2105-10-421

Cecchetto, M., Di Cesare, A., Eckert, E., Fassio, G., Fontaneto, D., Moro, I., Oliverio, M., Sciuto, K., Tassistro, G., Vezzulli, L., & Schiaparelli, S. (2021). Antarctic coastal nanoplankton dynamics revealed by metabarcoding of desalination plant filters: Detection of short-term events and implications for routine monitoring. *Science of the Total Environment*, *757*, 143809. https://doi.org/10.1016/j.scitotenv.2020.143809

Clarke, A. (2008). Antarctic marine benthic diversity: Patterns and processes. *Journal of Experimental Marine Biology and Ecology*, *366*(1), 48–55. https://doi.org/10.1016/j.jembe.2008.07.008

Clarke, A., & Johnston, N. M. (2003). Antarctic marine benthic diversity. *Oceanography and Marine Biology*, *41*, 47–114.

Cummings, V. J., Hewitt, J. E., Thrush, S. F., Marriott, P. M., Halliday, N. J., & Norkko, A. (2018). Linking Ross Sea coastal benthic communities to environmental conditions: Documenting baselines in a spatially variable and changing world. *Frontiers in Marine Science*, *5*, 232. https://doi.org/10.3389/fmars.2018.00232

David, B. O., Fake, D. R., Hicks, A. S., Wilkinson, S. P., Bunce, M., Smith, J. S., West, D. W., Collins, K. E., & Gleeson, D. M. (2021). Sucked in by eDNA—A promising tool for complementing riverine assessment of freshwater fish communities in Aotearoa New Zealand. *New Zealand Journal of Zoology*, *48*(3–4), 217–244. https://doi.org/10.1080/03014223.2021.1905672

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, *26*(19), 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., & Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, *27*(16), 2194–2200. https://doi.org/10.1093/bioinformatics/btr381

Federhen, S. (2012). The NCBI taxonomy database. *Nucleic Acids Research*, *40*(D1), D136–D143.

Fediajevaite, J., Priestley, V., Arnold, R., & Savolainen, V. (2021). Meta-analysis shows that environmental DNA outperforms traditional

surveys, but warrants better reporting standards. *Ecology and Evolution*, *11*(9), 4803–4815. https://doi.org/10.1002/ece3.7382

Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. (2008). Species detection using environmental DNA from water samples. *Biology Letters*, *4*(4), 423–425.

Frøslev, T. G., Kjøller, R., Bruun, H. H., Ejrnæs, R., Brunbjerg, A. K., Pietroni, C., & Hansen, A. J. (2017). Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. *Nature Communications*, *8*(1), 1188. https://doi.org/10.1038/s41467-017-01312-x

Hanchet, S. M., Mitchell, J., Bowden, D., Clark, M., Hall, J., O'Driscoll, R., Pinkerton, M., & Robertson, D. (2008). *Preliminary report of the New Zealand RV Tangaroa IPY-CAML survey of the Ross Sea region, Antarctic*. February–March.

Hansen, B. K., Jacobsen, M. W., Middelboe, A. L., Preston, C. M., Marin, R., Bekkevold, D., Knudsen, S. W., Møller, P. R., & Nielsen, E. E. (2020). Remote, autonomous real-time monitoring of environmental DNA from commercial fish. *Scientific Reports*, *10*(1), 13272. https://doi.org/10.1038/s41598-020-70206-8

Harper, L. R., Neave, E. F., Sellers, G. S., Cunnington, A. V., Arias, M. B., Craggs, J., MacDonald, B., Riesgo, A., & Mariani, S. (2023). Optimized DNA isolation from marine sponges for natural sampler DNA metabarcoding. *Environmental DNA*, *5*, 438–461. https://doi.org/10.1002/edn3.392

Holman, L. E., de Bruyn, M., Creer, S., Carvalho, G., Robidart, J., & Rius, M. (2019). Detection of introduced and resident marine species using environmental DNA metabarcoding of sediment and water. *Scientific Reports*, *9*(1), 11559.

Jackman, J. M., Benvenuto, C., Coscia, I., Oliveira Carvalho, C., Ready, J. S., Boubli, J. P., Magnusson, W. E., McDevitt, A. D., & Guimarães Sales, N. (2021). eDNA in a bottleneck: Obstacles to fish metabarcoding studies in megadiverse freshwater systems. *Environmental DNA*, *3*(4), 837–849. https://doi.org/10.1002/edn3.191

Jensen, M. R., Sigsgaard, E. E., Ávila, M. P., Agersnap, S., Brenner-Larsen, W., Sengupta, M. E., Xing, Y., Krag, M. A., Knudsen, S. W., Carl, H., Møller, P. R., & Thomsen, P. F. (2022). Short-term temporal variation of coastal marine eDNA. *Environmental DNA*, *4*, 747–762. https://doi.org/10.1002/edn3.285

Jeunen, G.-J., Cane, J. S., Ferreira, S., Strano, F., von Ammon, U., Cross, H., Day, R., Hesseltine, S., Ellis, K., Urban, L., Pearson, N., Olmedo-Rojas, P., Kardailsky, A., Gemmell, N. J., & Lamare, M. (2023). Assessing the utility of marine filter feeders for environmental DNA (eDNA) biodiversity monitoring. *Molecular Ecology Resources*, *23*(4), 771–786. https://doi.org/10.1111/1755-0998.13754

Jeunen, G.-J., Dowle, E., Edgecombe, J., von Ammon, U., Gemmell, N., & Cross, H. (2022). CRABS—A software program to generate curated reference databases for metabarcoding sequencing data. *Molecular Ecology Resources*, *23*, 725–738. https://doi.org/10.1111/1755-0998.13741

Jeunen, G.-J., Lamare, M., Devine, J., Mariani, S., Mills, S., Treece, J., Ferreira, S., & Gemmell, N. J. (2023). Characterizing Antarctic fish assemblages using eDNA obtained from marine sponge bycatch specimens. *Reviews in Fish Biology and Fisheries*. https://doi.org/10.1007/s11160-023-09805-3

Jeunen, G. J., Lamare, M. D., Knapp, M., Spencer, H. G., Taylor, H. R., Stat, M., Bunce, M., & Gemmell, N. J. (2019). Water stratification in the marine biome restricts vertical environmental DNA (eDNA) signal dispersal. *Environmental DNA*, *2*, 99–111. https://doi.org/10.1002/edn3.49

Jeunen, G.-J., von Ammon, U., Cross, H., Ferreira, S., Lamare, M., Day, R., Treece, J., Pochon, X., Zaiko, A., Gemmell, N. J., & Stanton, J.-A. L. (2022). Moving environmental DNA (eDNA) technologies from benchtop to the field using passive sampling and PDQeX extraction. *Environmental DNA*, *4*, 1420–1433. https://doi.org/10.1002/edn3.356

Kirtane, A., Atkinson, J. D., & Sassoubre, L. (2020). Design and validation of passive environmental DNA samplers using granular activated carbon and montmorillonite clay. *Environmental Science & Technology*, *54*(19), 11961–11970. https://doi.org/10.1021/acs.est.0c01863

Koziol, A., Stat, M., Simpson, T., Jarman, S., DiBattista, J. D., Harvey, E. S., Marnane, M., McDonald, J., & Bunce, M. (2019). Environmental DNA metabarcoding studies are critically affected by substrate selection. *Molecular Ecology Resources*, *19*(2), 366–376. https://doi.org/10.1111/1755-0998.12971

Leray, M., & Knowlton, N. (2015). DNA barcoding and metabarcoding of standardized samples reveal patterns of marine benthic diversity. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(7), 2076–2081. https://doi.org/10.1073/pnas.1424997112

Leray, M., Yang, J. Y., Meyer, C. P., Mills, S. C., Agudelo, N., Ranwez, V., Boehm, J. T., & Machida, R. J. (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*(1), 1–14. https://doi.org/10.1186/1742-9994-10-34

Lynggaard, C., Bertelsen, M. F., Jensen, C. V., Johnson, M. S., Frøslev, T. G., Olsen, M. T., & Bohmann, K. (2022). Airborne environmental DNA for terrestrial vertebrate community monitoring. *Current Biology*, *32*(3), 701–707.e5. https://doi.org/10.1016/j.cub.2021.12.014

Mariani, S., Baillie, C., Colosimo, G., & Riesgo, A. (2019). Sponges as natural environmental DNA samplers. *Current Biology*, *29*(11), R401–R402. https://doi.org/10.1016/j.cub.2019.04.031

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. Journal*, *17*(1), 10–12.

Minamoto, T., Fukuda, M., Katsuhara, K. R., Fujiwara, A., Hidaka, S., Yamamoto, S., Takahashi, K., & Masuda, R. (2017). Environmental DNA reflects spatial and temporal jellyfish distribution. *PLoS One*, *12*(2), 1–15. https://doi.org/10.1371/journal.pone.0173073

Minamoto, T., Naka, T., Moji, K., & Maruyama, A. (2016). Techniques for the practical collection of environmental DNA: Filter selection, preservation, and extraction. *Limnology*, *17*(1), 23–32. https://doi.org/10.1007/s10201-015-0457-4

Murphy, H., & Jenkins, G. (2010). Observational methods used in marine spatial monitoring of fishes and associated habitats: A review. *Marine and Freshwater Research*, *61*, 236. https://doi.org/10.1071/MF09068

Murray, D. C., Coghlan, M. L., & Bunce, M. (2015). From benchtop to desktop: Important considerations when designing amplicon sequencing workflows. *PLoS One*, *10*(4), 1–21. https://doi.org/10.1371/journal.pone.0124671

Neave, E. F., Cai, W., Arias, M. B., Harper, L. R., Riesgo, A., & Mariani, S. (2023). Trapped DNA fragments in marine sponge specimens unveil North Atlantic deep-sea fish diversity. *Proceedings of the Royal Society B: Biological Sciences*, *290*(2005), 20230771. https://doi.org/10.1098/rspb.2023.0771

O'Donnell, J. L., Kelly, R. P., Shelton, A. O., Samhouri, J. F., Lowell, N. C., & Williams, G. D. (2017). Spatial distribution of environmental DNA in a nearshore marine habitat. *PeerJ*, *5*, e3044. https://doi.org/10.7717/peerj.3044

Olmedo-Rojas, P., Jeunen, G.-J., Lamare, M., Turnbull, J., Terauds, A., Gemmell, N., & Fraser, C. I. (2023). Soil environmental DNA metabarcoding in low-biomass regions requires protocol optimization: A case study in Antarctica. *Antarctic Science*, *35*(1), 15–30.

Ratnasingham, S., & Hebert, P. D. N. (2007). BOLD: The barcode of life data system (http://www.Barcodinglife.Org). *Molecular Ecology Notes*, *7*(3), 355–364.

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: A versatile open source tool for metagenomics. *PeerJ*, *4*, e2584.

Rojahn, J., Gleeson, D. M., Furlan, E., Haeusler, T., & Bylemans, J. (2021). Improving the detection of rare native fish species in environmental DNA metabarcoding surveys. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *31*, 990–997. https://doi.org/10.1002/aqc.3514

Sala, A., Azzali, M., & Russo, A. (2002). Krill of the Ross Sea: Distribution, abundance and demography of Euphausia superba and Euphausia crystallorophias during the Italian Antarctic expedition (January-February 2000). *Scientia Marina*, *66*(2), 123–133.

Sales, N. G., Wangensteen, O. S., Carvalho, D. C., & Mariani, S. (2019). Influence of preservation methods, sample medium and sampling time on eDNA recovery in a neotropical river. *Environmental DNA*, 119–130. https://doi.org/10.1002/edn3.14

Sanches, T. M., & Schreier, A. D. (2020). Optimizing an eDNA protocol for estuarine environments: Balancing sensitivity, cost and time. *PLoS One*, *15*(5), e0233522. https://doi.org/10.1371/journal.pone.0233522

Schnell, I. B., Bohmann, K., & Gilbert, M. T. P. (2015). Tag jumps illuminated—Reducing sequence-to-sample misidentifications in metabarcoding studies. *Molecular Ecology Resources*, *15*(6), 1289–1303. https://doi.org/10.1111/1755-0998.12402

Shaw, J. L. A., Clarke, L. J., Wedderburn, S. D., Barnes, T. C., Weyrich, L. S., & Cooper, A. (2016). Comparison of environmental DNA metabarcoding and conventional fish survey methods in a river system. *Biological Conservation*, *197*, 131–138. https://doi.org/10.1016/j.biocon.2016.03.010

Siegert, M. J., Bentley, M. J., Atkinson, A., Bracegirdle, T. J., Convey, P., Davies, B., Downie, R., Hogg, A. E., Holmes, C., Hughes, K. A., Meredith, M. P., Ross, N., Rumble, J., & Wilkinson, J. (2023). Antarctic extreme events. *Frontiers in Environmental Science*, *11*, 1229283. https://doi.org/10.3389/fenvs.2023.1229283

Smith, W. O., Jr., Dinniman, M. S., Hofmann, E. E., & Klinck, J. M. (2014). The effects of changing winds and temperatures on the oceanography of the Ross Sea in the 21st century. *Geophysical Research Letters*, *41*(5), 1624–1631. https://doi.org/10.1002/2014GL059311

Spens, J., Evans, A. R., Halfmaerten, D., Knudsen, S. W., Sengupta, M. E., Mak, S. S. T., Sigsgaard, E. E., Hellström, M., & Yu, D. (2016). 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*(5), 635–645. https://doi.org/10.1111/2041-210X.12683

Stark, J. S., Kim, S. L., & Oliver, J. S. (2014). Anthropogenic disturbance and biodiversity of marine benthic communities in Antarctica: A regional comparison. *PLoS One*, *9*(6), e98802. https://doi.org/10.1371/journal.pone.0098802

Stat, M., Huggett, M. J., Bernasconi, R., DiBattista, J. D., Berry, T. E., Newman, S. J., Harvey, E. S., & Bunce, M. (2017). Ecosystem biomonitoring with eDNA: Metabarcoding across the tree of life in a tropical marine environment. *Scientific Reports*, *7*(1), 12240. https://doi.org/10.1038/s41598-017-12501-5

Takahashi, M., Saccò, M., Kestel, J. H., Nester, G., Campbell, M. A., van der Heyde, M., Heydenrych, M. J., Juszkiewicz, D. J., Nevill, P., Dawkins, K. L., Bessey, C., Fernandes, K., Miller, H., Power, M., Mousavi-Derazmahalleh, M., Newton, J. P., White, N. E., Richards, Z. T., & Allentoft, M. E. (2023). Aquatic environmental DNA: A review of the macro-organismal biomonitoring revolution. *Science of the Total Environment*, *873*, 162322. https://doi.org/10.1016/j.scitotenv.2023.162322

Thomsen, P. F., & Willerslev, E. (2015). Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation*, *183*, 4–18. https://doi.org/10.1016/J.BIOCON.2014.11.019

Thrush, S., Dayton, P., Cattaneo-Vietti, R., Chiantore, M., Cummings, V., Andrew, N., Hawes, I., Kim, S., Kvitek, R., & Schwarz, A.-M. (2006). Broad-scale factors influencing the biodiversity of coastal benthic communities of the Ross Sea. *Deep Sea Research Part II: Topical Studies in Oceanography*, *53*(8), 959–971. https://doi.org/10.1016/j.dsr2.2006.02.006

Turner, C., Uy, K., & Everhart, R. (2015). Fish environmental DNA is more concentrated in aquatic sediments than surface water. *Biological Conservation*, *183*, 93–102. https://doi.org/10.1016/j.biocon.2014.11.017

Turon, M., Angulo-Preckler, C., Antich, A., Præbel, K., & Wangensteen, O. S. (2020). More than expected from old sponge samples: A natural sampler DNA metabarcoding assessment of marine fish diversity in Nha Trang Bay (Vietnam). *Frontiers in marine science*, *7*, 605148. https://doi.org/10.3389/fmars.2020.605148

Ushio, M., Murakami, H., Masuda, R., Sado, T., Miya, M., Sakurai, S., Yamanaka, H., Minamoto, T., & Kondoh, M. (2017). *Quantitative monitoring of multispecies fish environmental DNA using high-throughput sequencing*. BioRxiv. http://biorxiv.org/content/early/2017/03/05/113472.abstract

Vargas, S., Kelly, M., Schnabel, K., Mills, S., Bowden, D., & Wörheide, G. (2015). Diversity in a cold hot-spot: DNA-barcoding reveals patterns of evolution among Antarctic demosponges (class Demospongiae, phylum porifera). *PLoS One*, *10*(6), e0127573-. https://doi.org/10.1371/journal.pone.0127573

Verdier, H., Konecny, L., Marquette, C., Reveron, H., Tadier, S., Grémillard, L., Barthès, A., Datry, T., Bouchez, A., & Lefébure, T. (2021). *Passive sampling of environmental DNA in aquatic environments using 3D-printed hydroxyapatite samplers*. BioRxiv https://doi.org/10.1101/2021.05.12.443744

Wilcox, T. M., Schwartz, M. K., McKelvey, K. S., Young, M. K., & Lowe, W. H. (2014). A blocking primer increases specificity in environmental DNA detection of bull trout (*Salvelinus confluentus*). *Conservation Genetics Resources*, *6*(2), 283–284. https://doi.org/10.1007/s12686-013-0113-4

Yamahara, K. M., Preston, C. M., Birch, J., Walz, K., Marin, R. III, Jensen, S., Pargett, D., Roman, B., Ussler, W. III, Zhang, Y., Ryan, J., Hobson, B., Kieft, B., Raanan, B., Goodwin, K. D., Chavez, F. P., & Scholin, C. (2019). In situ autonomous acquisition and preservation of marine environmental DNA using an autonomous underwater vehicle. *Frontiers in Marine Science*, *6*, 373. https://doi.org/10.3389/fmars.2019.00373

Zhang, J., Kobert, K., Flouri, T., & Stamatakis, A. (2014). PEAR: A fast and accurate Illumina paired-end reAd mergeR. *Bioinformatics*, *30*(5), 614–620. https://doi.org/10.1093/bioinformatics/btt593

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Jeunen, G.-J., Lamare, M., Cummings, V., Treece, J., Ferreira, S., Massuger, J., Pryor Rodgers, L., Tait, L., Lust, B., Wilkinson, S., Mariani, S., Mills, S., & Gemmell, N. (2023). Unveiling the hidden diversity of marine eukaryotes in the Ross Sea: A comparative analysis of seawater and sponge eDNA surveys. *Environmental DNA*, *5*, 1780–1792. https://doi.org/10.1002/edn3.500