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ORIGINAL RESEARCH

Characterizing Antarctic fish assemblages using eDNA obtained from marine sponge bycatch specimens

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Abstract International conservation goals have been set to mitigate Southern Ocean ecosystem deterioration, with multiple monitoring programs evaluating progress towards those goals. The scale of continuous monitoring through visual observations, however, is challenged by the remoteness of the area and logistical constraints. Given the ecological and economic importance of the Southern Ocean, it is imperative that additional biological monitoring approaches are explored. Recently, marine sponges, which are frequently caught and discarded in Southern Ocean fisheries, have been shown to naturally accumulate environmental DNA (eDNA). Here, we compare fish eDNA signals from marine sponge bycatch specimens to fish catch records for nine locations on the continental shelf (523.5–709 m) and 17 from the continental slope (887.5–1611.5 m) within the Ross Sea, Antarctica. We recorded a total of 20 fishes, with 12 fishes reported as catch, 18 observed by eDNA, and ten detected by both methods. While sampling location was the largest contributor to the variation observed in the dataset, eDNA obtained significantly higher species richness and displayed a significantly different species composition compared to fish catch records. Overall, eDNA read count correlated more strongly with fish abundance over biomass. Species composition correlated on a regional scale between methods, however eDNA signal strength was a low predictor of catch numbers at the species level. Our results highlight the potential of sponge eDNA monitoring in the Southern Ocean by detecting a larger fraction of the fish community compared to catch recordings, thereby increasing our knowledge of this understudied ecosystem and, ultimately, aiding conservation efforts.

Keywords Metabarcoding · Passive eDNA sampling · Ross Sea · Southern Ocean · Fisheries · Biodiversity

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Introduction

Marine ecosystems are currently undergoing dramatic shifts in structure and functioning, due to increased anthropogenic impacts and rapid climate change (Allison et al. 2009). Antarctic ecosystems represent some of the least modified marine ecosystems on the planet (Aronson et al. 2011) and the largest marine protected area (Ballard et al. 2012). The Southern Ocean is, however, already being impacted by climate change, notably along the Antarctic Peninsula (Clarke et al. 2006), as well as being exposed to increased fishing and tourism pressures (Aronson et al. 2011; Tejedo et al. 2022). Antarctic-wide impacts of warming, loss of sea ice, and ocean acidification are predicted through modelling over the coming decades (Koerich et al. 2022). While multiple national research programs are currently being undertaken to determine the effects of such a raft of pressures on Antarctica's marine ecosystem (CCAMLR 2022a, b), Southern Ocean research is by its nature logistically and financially demanding (Xavier et al. 2016). Hence, most regions are understudied and necessary biological information for successful conservation is incomplete (Griffiths 2010; Xavier et al. 2016).

One such data-deprived region is the Ross Sea, a large embayment of the Southern Ocean in Antarctica, between Victoria Land and Marie Byrd Land (74.5487° S, 166.3074° W). The Ross Sea is the southernmost sea on Earth with a total area of 637,000 km^2 and exhibits substantial variations in physical forcing, ice cover, and biological processes on a variety of temporal and spatial scales (Smith et al. 2014). The Ross Sea contains some of the most productive waters globally, sustaining the largest phytoplankton biomass in the Southern Ocean (Smith et al. 2014), highly abundant zooplankton (Sala et al. 2002), the most diverse benthos in the Southern Ocean (Clarke and Johnston 2003), and exceptional abundances of apex predators (Ballard et al. 2012). The fish fauna exhibits low diversity and is dominated by notothenioids (cod icefishes), liparids (snailfishes), and zoarcids (eelpouts; Ainley and Pauly 2014; Eastman 2005). The abundance of notothenioids, specifically the Antarctic toothfish (*Dissostichus mawsoni* Norman, 1937, Nototheniidae), has supported a commercial longline fishery since 1997 (Fisheries New Zealand 2022). While important aspects of the Ross Sea ecosystem are yet to be explored (Griffiths 2010), an international consensus was reached to establish the world's largest MPA in 2016 to provide protection to previously fished areas (Ballard et al. 2012).

Due to the logistical constraints for routine monitoring in the Southern Ocean, much of the data used to increase our understanding on continental shelf and slope fish population distributions and abundances is generated in association with commercial fishing activities, including catch and bycatch information (Polanowski et al. 2018). These data are integrated into management of fishing activity through the CCAMLR system (Trathan and Agnew 2010). In addition to direct observations of fish catches, the catches provide other opportunities to obtain important biological information associated with the fishery and wider pelagic ecosystems. For example, analysis of gut samples from commercially caught Antarctic toothfish provided new information on their cephalopod prey (Stevens et al. 2014). While essential to our current understanding of the Ross Sea ecosystem, hook-and-line fishing is known to be selective and biased towards the target organism (Løkkeborg and Bjordal 1992; Moreno 1991). Hence, it is imperative that additional monitoring approaches are explored to enable the gathering of essential ecological data.

Environmental DNA (eDNA) monitoring has been proposed as an innovative method with great potential (Ficetola et al. 2008; Thomsen and Willerslev 2015), whereby species are detected indirectly through DNA signals obtained from environmental samples, such as water (Bowers et al. 2021), sediment (Koziol et al. 2019), or air (Lynggaard et al. 2022). Within the marine biome, water is the most frequently used substrate in eDNA surveys (Bowers et al. 2021). Aquatic eDNA surveys have shown to be highly accurate, due to high spatial (Jeunen et al. 2019a, b) and temporal (Berry et al. 2019) resolutions, as well as sensitive, by facilitating early detection of invasive species (Bowers et al. 2021). Furthermore, aquatic eDNA metabarcoding surveys have compared favorably to a variety of traditional monitoring methods with regards to diversity detection in a time-efficient and cost-effective manner (Fediajevaite et al. 2021), including baited remote underwater videos (Jeunen et al. 2020; Stat et al. 2019), trawling (Salter et al. 2019; Stoeckle et al. 2021; Thomsen et al. 2016), and underwater visual census (Polanco Fernández et al. 2021).

Several studies have compared aquatic eDNA metabarcoding to trawling catch records (Salter et al.

2019; Stoeckle et al. 2021; Thomsen et al. 2016). In general, aquatic eDNA tends to recover a larger portion of the fish diversity. While false-negative detections are inherent to all survey methods deployed thus far, false-negative eDNA detections compared to catch records are a frequent occurrence, due to missing reference barcodes (Weigand et al. 2019), low taxonomic resolution in the amplicon region for specific taxonomic groups (Zhang et al. 2020), or amplification bias induced through mismatches in primer-binding regions (Hansen et al. 1998). The partial overlap in species detection, as well as difficulties in obtaining abundance, sex, and size information from eDNA surveys, has led to a proposed combined approach to gather as much information possible (Zhou et al. 2022). Routine implementation of eDNA analysis into existing monitoring programs has so far been hampered by the need for immediate, careful, and time-consuming sample collection, DNA preservation and storage (Bessey et al. 2021).

To circumvent the need for water filtration, passive eDNA collection has been trialed with success (Bessey et al. 2021; Jeunen et al. 2022b; Maiello et al. 2022), whereby filter membranes (Bessey et al. 2021; Jeunen et al. 2022b), artificial sponges (Jeunen et al. 2022b), or other substrates (Maiello et al. 2022; Verdier et al. 2021) are submerged to capture eDNA from the water column. Besides achieving comparable results to active filtration (Jeunen et al. 2022b), passive filtration devices allow for increased sampling and replication by omitting the time-consuming active filtration step (Bessey et al. 2021). An alternative to using artificial substrates for passive eDNA collection is to exploit the natural eDNA accumulation in filter-feeding organisms, such as marine sponges (Cai et al. 2022; Harper et al. 2023; Jeunen et al. 2021; Mariani et al. 2019; Turon et al. 2020). Similar to artificial substrates, sponge eDNA displays high similarity with aquatic eDNA surveys (Jeunen et al. 2021) and might be preferred over using artificial substrates, as they are frequently caught as bycatch and omit the need to attach passive samplers to fishing gear.

In this study, we explore the use of novel eDNA methods to describe the biogeographical patterns of fish on the continental shelf (9 locations; depth range: 523.5–709 m) and slope (17 locations; depth range: 887.5–1611.5 m) in the Ross Sea, Antarctica. Environmental DNA was obtained from marine sponges caught as bycatch on the demersal longline fishing vessel, *FV* San Aotea II. Catch records enabled us to validate eDNA signals, as well as determine the utility of eDNA biodiversity monitoring in the Southern Ocean. Furthermore, catch biomass and abundance measures were correlated to eDNA signal strength to investigate the quantitative value of eDNA metabarcoding on a regional and local scale. Two questions were specifically addressed: (1) Does the eDNA accumulated in sponges caught as bycatch in longline fisheries detect all caught species and provide additional information to fish biodiversity patterns in the Ross Sea, and (2) can we estimate catch and bycatch biomass/abundance from eDNA obtained from marine sponge bycatch specimens?

Materials and methods

Study area and sample collection

Data between catch records and eDNA detections were compared across a total of 26 sites located on the continental slope (17 locations) and shelf in the Ross Sea (9 locations; Fig. 1; Supplemental Table 1). The exact location of sampling sites has not been disclosed to preserve commercial interest of the fishing vessel.

Sponge specimens were collected during longline fishing by *FV* San Aotea II on the continental shelf and slope regions of the Ross Sea between the 13th of December 2021 and 17th of January 2022 (Fig. 1; Supplemental Table 1). During the deployment of the longlines, sponges are accidentally hooked off the seafloor and brought to the surface as reported bycatch when the lines are retrieved. A total of 30 marine sponge specimens on 26 fishing lines were sampled for this experiment, with 23 fishing lines represented by a single marine sponge specimen, two fishing lines represented by two marine sponge specimens, and one fishing line represented by three marine sponge specimens. Marine sponges were taxonomically identified to class level (22 Demospongiae [Demosponges]; 8 Hexactinellida [Glass sponges]) on the fishing vessel by fishery observers and each placed in a separate 50 ml falcon tube filled with 99.8% molecular-grade ethanol (Fisher BioReagents™, Fisher Scientific). Specimens were stored in the dark on ice during shipment to the University of Otago's PCR-free eDNA facilities at Portobello

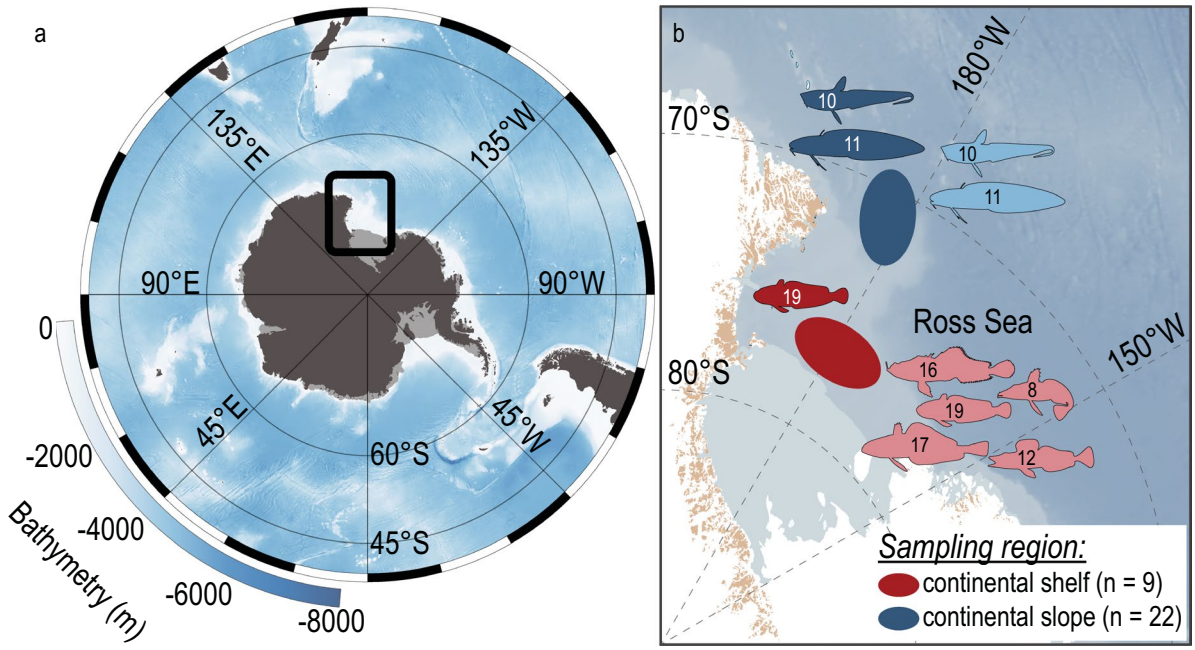

Fig. 1 **a** Map of Antarctica and the Southern Ocean with sample collection sites in the Ross Sea indicated by a black square. Bathymetry of the Southern Ocean floor is color coded from light blue (shallow) to dark blue (deep sea). Bathymetry information was gathered from Quantarctica inside QGIS (https://www.scar.org/resources/quantarctica/). **b** Map of Ross Sea and the Ross Sea ice shelf (grey) with the continental shelf sampling region indicated in red and the continental slope sampling region indicated in blue. Fish silhouettes represent the results of the indicator species analysis, with continental shelf eDNA and catch indicator species depicted in light red and dark red, respectively. Continental slope eDNA and catch indicator species are depicted in light blue and dark blue, respectively. Number inside fish silhouettes indicates species name as found in Supplemental Table 8

Marine Laboratory (PML). The ethanol-stored specimens were stored at 4 °C in the dark until further sample processing. Due to logistical difficulties of working onboard a commercial fishing vessel in the Southern Ocean, no negative field controls were collected.

Fish catch recordings

For each of the 26 fishing lines where sponges were caught as bycatch, catch composition was recorded by fisheries observers, as per governmental regulations. Although taxon identification is usually conducted at a level coarser than the species—except for toothfish—observers were asked by CCAMLR to identify catches to the lowest taxonomic level possible and measure up to ten individual bycatch species per longline set. Fish bycatch measurements followed standard practices according to the Ross Sea data collection plan (Hanchet et al. 2015) and CCAMLR observer protocols (CCAMLR 2023), which consisted of length, weight, sex, and maturity stage for each recorded specimen. A tissue sample from each species caught on the 26 fishing lines within the taxonomic group Actinopterygii was dissected and shipped frozen to the University of Otago for barcoding purposes. Tissue samples from Chondrichthyes, which are tagged and released after capture, were not obtained for this study.

Fish reference barcodes

A tissue biopsy of ~ 25 mg was dissected from each Actinopterygii species caught as bycatch and extracted using the Qiagen DNeasy Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's recommendation, except for an overnight lysis step. Total DNA was quantified using qubit (Qubit™ dsDNA HS Assay Kit, ThermoFisher Scientific) and visualized with gel electrophoresis to

determine high molecular weight DNA was present. DNA was amplified using the 16SarL/16SbrH primer set (Palumbi 1991; Supplemental Table 2) to generate reference barcodes for the fish metabarcoding assay used in this experiment. PCR was carried out in 20 µl reactions using BIOTAQ™ DNA Polymerase (Meridian Bioscience®) according to the manufacturer's instructions, with a 0.2 mM final concentration of each primer and dNTPs, as well as a 2 mM final concentration of $MgCl_2$. The thermal cycling profile included an initial denaturation step of 94 °C for 2 min; followed by 30 cycles of 1 min at 94 °C, 90 s at 53 °C, 90 s at 72 °C; and a final extension step for 10 min at 72 °C. PCR products were checked for amplification through gel electrophoresis, and upon successful amplification the reaction was cleaned using a QIAquick PCR purification Kit (Qiagen, Cat. No. 28104). Cleaned up products were then quantified by spectrophotometry using a DeNovix® DS-11 FX+, and Sanger sequenced in both the forward and reverse direction by submitting 6 ng of each product with 3.2 pmol of either primer in a total volume of 5 µl through the Genetic Analysis Service of the University of Otago (https://gas.otago.ac.nz). Forward and reverse sequences from each tissue sample were imported into Geneious Prime® *v* 2022.0.1 (Kearse et al. 2012). Sequences were checked for accuracy based on the electropherogram. Reverse sequences were reverse complemented, and a full barcode sequence was generated through pairwise alignment using the '*Geneious Alignment*' with standard settings. The full barcode sequence was exported in.fasta format and imported into CRABS *v* 0.1.3 (Jeunen et al. 2022a) to generate a custom curated reference database (see "Bioinformatic analysis and taxonomy assignment" section for more information).

Laboratory processing of eDNA samples

Pre-PCR laboratory work was conducted in a designated PCR-free clean room. Prior to laboratory work, bench spaces and equipment were sterilized using a 10-min exposure to 10% bleach dilution (0.5% hypochlorite final concentration) and wiped with ultrapure water (UltraPure™ DNase/RNase-Free Distilled Water, Invitrogen™) to reduce contamination risk (Prince and Andrus 1992). Additionally, negative control samples were processed alongside samples to investigate issues with cross-contamination. Negative control samples consisted of 50 µl ultrapure water for DNA extraction negatives and 2 µl ultrapure water for PCR no-template controls. Field controls were not collected on the fishing vessel due to extreme environmental conditions and sample collection being undertaken by fishery observers.

One tissue biopsy of ~ 0.5 cm^3 was dissected from each sponge specimen for DNA extraction. DNA extraction followed the protocol described in (Jeunen et al. 2021). Briefly, DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit according to the manufacturer's recommendations, with slight modifications (Supplemental Table 3). DNA extracts were stored at − 20 °C until further processing.

Library preparation followed the protocol described in (Jeunen et al. 2018). Briefly, eDNA samples were analyzed for fish diversity using the fish (16S) metabarcoding assay (Berry et al. 2017), targeting a ~ 200 bp fragment of the 16S rDNA gene region. Prior to library preparation, input DNA for each sample was optimized using a dilution series (undiluted, tenfold dilution, 100-fold dilution) to identify inhibitors and low-template samples (Murray et al. 2015). Amplification was carried out in duplicate in 25 µl reactions. The qPCR mastermix consisted of 1 × SensiFAST SYBR Lo-ROX Mix (Bioline, London, UK), 0.4 µmol/l of each primer (Integrated DNA Technologies, Australia), 2 µl of template DNA, and ultrapure water as required. The thermal cycling 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 54 °C, 45 s at 72 °C; and a final melt-curve analysis. A one-step amplification protocol using fusion primers was employed for library building (Berry et al. 2017). Fusion primers contained an Illumina adapter, a modified sequencing primer, a barcode tag (6–8 bp in length) and the template specific primer (Murray et al. 2015). Each sample was assigned a unique barcode combination (different forward and reverse barcodes). qPCR conditions followed the protocol as described above. Post qPCR, sample duplicates were pooled to reduce stochastic effects from PCR amplification (Alberdi et al. 2018; Leray and Knowlton 2015). Samples were then pooled into mini-pools based on end-point qPCR fluorescence, Ct-values, and melt-curve analysis (Murray et al. 2015). Size selection and qPCR clean-up followed the AMPure XP (Beckman Coulter, US) standard protocol. Mini-pools were visualized using gel electrophoresis to

determine the presence of a single band and molarity of mini-pools was measured on Qubit. Pooling occurred equimolarly to produce a single DNA library. Due to differences in cycle number between samples and negative controls, the latter were spiked into the library to allow for optimal concentration of the library (Jeunen et al. 2019a). The resultant library was size selected once more using Pippin Prep (Cat # PIP0001; Sage Science, USA) and purified with Qiagen's QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany) prior to final library quantitation on QIAxcel Advanced System (Qiagen GmbH, Hilden, Germany) and Qubit. Sequencing was performed on an Illumina MiSeq® using a 1 × 300 bp V2 Nano Illumina sequencing kit, following the manufacturer's protocols, with 5% PhiX to minimize issues associated with low-complexity libraries.

Bioinformatic analysis and taxonomy assignment

Prior to the bioinformatic processing of sequencing data, raw fastq files were checked for quality using FastQC *v* 0.11.5 (Andrews 2010). Reads were demultiplexed and assigned to samples using cutadapt *v* 4.1 (Martin 2011), allowing for a single mismatch in the barcode and primer region. The assigned amplicons were filtered using '*-fastq_filter*' function in USEARCH *v* 11.0.667 (Edgar 2010) based on a maximum expected error of 1.0 and minimum length of 150 bp. The success of quality filtering was checked in FastQC by comparing reports of FASTQ files before and after quality filtering. Remaining reads were dereplicated using the '*-fastx_uniques*' function in USEARCH. Chimeric sequences were removed and ZOTUs (Zero-radius Operational Taxonomic Units) were generated using the '*-unoise3*' function in USEARCH (Edgar 2016b). Finally, a ZOTU table was generated using the '*-otutab*' function in USEARCH.

A custom curated reference database was generated using CRABS *v* 0.1.3 (Jeunen et al. 2022a). The custom curated reference database consisted of sequences downloaded from multiple online repositories (('*db_download*' function) and *in-house* generated barcodes of Southern Ocean fish species (See "Fish reference barcodes" section; Supplemental File 1; '*db_import*' function). Amplicon regions were extracted from sequences through in silico PCR analysis ('*insilico_pcr*' function) and pairwise global alignments ('*pga*' function). The '*visualization*' function in CRABS was used to explore the reference database for missing barcodes ('*-method db_completeness*'), mismatches in primer-binding regions ('*-method primer_efficiency*'), and taxonomic resolution of the amplicon region ('*-method phylo*').

Taxonomy of ZOTUs were assigned using the '*-sintax*' function in USEARCH (Edgar 2016a), with the custom curated reference databases generated by CRABS as input for the '*-db*' parameter. ZOTUs were assigned to species level when a confidence of 1.00 was observed for the SINTAX algorithm and assigned to genus level for a confidence level between 0.97 and 0.99. The final taxonomic resolution of each taxonomic group was lowered to the lowest resolution observed between catch recordings and eDNA signals to enable accurate comparisons. For example, individuals within the Zoarcidae (eelpouts) family caught as bycatch are recorded as Zoarcidae, while CRABS identified no base pair mismatches in the amplicon regions for the genera *Macrourus* (rattails), *Pogonophryne* (barbeled plunderfishes), and certain species within the *Trematomus* genus (cod icefishes; CRABS '*-method phylo*' function). After taxonomy assignment, the ZOTU table underwent final processing prior to statistical analysis, whereby (1) single read detections within each sample were removed to avoid issues related to tag jumping (Schnell et al. 2015), (2) reads were averaged between multiple sponges caught on a single line, and (3) the ZOTU table was transformed to relative abundance.

Statistical analysis and visualization

Rarefaction curves were generated from the unfiltered ZOTU table to assess sequencing coverage using the *vegan v* 2.5-7 package in R *v* 4.0.5 (R; http://www.R-project.org). Species richness was calculated for each sample and compared between eDNA and bycatch through multiple pairwise t-tests with Bonferroni correction to adjust for multiple comparisons using the *rstatix v* 0.7.0 package. Species accumulation curves were generated in the *BiodiversityR v* 2.13-1 package to assess differences in total number of fish species between eDNA and bycatch. Prior to beta diversity analyses, data tables were transformed to presence-absence. A permutational multivariate analysis of variance (PERMANOVA) was used to determine whether fish assemblage composition differed between eDNA

and bycatch. Significant differences in dispersion between groups was tested (PERMDISP) to assess the reliability of PERMANOVA. A principal coordinate analysis (PCoA) was performed to visualize patterns of sample dissimilarity using the Jaccard index. Indicator values were calculated for each species using the *labdsv v* 2.0-1 package. Upper limits were set for indicator species, that is, species driving the difference in eDNA signal between sampling regions, to an indicator value index > 0.70 and a p value < 0.025 (Dufrêne and Legendre 1997). In this study, indicator values were used to determine the taxa driving the partitioning of samples between the two sampling regions found in the ordination analysis. Habitat preference of indicator species was used as biological validation of the difference found between sampling regions. Prior to Pearson correlation analysis, data tables were log-transformed to account for non-normal distributions. Pearson correlation was calculated using the '*cor.test*' function within the native *stats v* 4.2.1 package. All bioinformatic and statistical scripts can be found in Supplemental Files 2 and 3.

Results

Fish catch recordings

Across all 26 fishing lines, a total weight of 22,834 kg of fish were recorded by fishery observers, with 16,479 kg caught on the continental slope and 6356 kg caught on the continental shelf (Supplemental Table 4a, b). Besides the target Antarctic toothfish, which constituted the highest abundance (22,151.5 kg; 97.0%) and count (955 individuals; 65.1%), an additional 14 fish species were recorded as bycatch. Taxonomic IDs within the *Macrourus* (Rattail fish) and *Pogonophryne* (Barbeled Plunderfish) genera were combined, as eDNA taxonomic resolution was set to genus level for both groups. Therefore, a total of 12 unique taxonomic IDs were caught by the fishing vessel, covering 9 families, 4 orders, and 2 classes. *Macrourus* sp. was the most by-caught taxon (1.3%), followed by the Antarctic starry skate (*Amblyraja georgiana* [Norman, 1938], Rajidae; 1.0%), and eel cod *Muraenolepis* sp. (0.3%).

Sequencing results

Demultiplexing of raw sequencing data resulted in assigning 156,854 sequences to eDNA samples (Supplemental Table 5). Filtering and quality control returned 144,671 (92.2%) sequences. Although PCR products of negative controls were spiked into the library, no sequences were returned after quality control. Denoising resulted in 27 ZOTUs, with 153,724 (98.0%) raw sequences matching to ZOTUs to create the unfiltered ZOTU table. After final quality filtration and taxonomy assignment, 153,695 (98.0%) sequences were incorporated for statistical analysis. Overall, eDNA samples achieved sufficient sequencing coverage based on the plateauing of rarefaction curves (Supplemental File 4) and mean number of reads per sample ± *SD*: 5123 ± 1443.

Taxonomy assignment returned 20 unique taxonomic IDs. After combining the taxonomic IDs of the Zoarcidae family (eelpouts), as bycatch recordings are limited to family level for this taxonomic group, a total of 18 unique taxonomic IDs were observed within eDNA samples, covering 11 families, 5 orders, and 2 classes (Supplemental Table 6). Overall, the Antarctic toothfish achieved the highest abundant eDNA signal (61.3%), followed by *Macrourus* sp. (15.0%), *Chionobathyscus dewitti* Andriashev & Neyelov, 1978 (Channichthyidae; 6.8%), and *Trematomus* sp. (6.5%).

Alpha and beta diversity comparison

A total of 20 fish taxa were detected across all sampling regions and monitoring methods, with a large overlap in species detection between methods across all samples and within each sampling region (Fig. 2; Table 1). While an eDNA signal for the skate genus, *Bathyraja*, was detected, we were unable to resolve the ZOTU to species level, despite the potential species-level taxonomic resolution reported during the in silico PCR analysis (Supplemental File 5). Thus, eDNA failed to distinguish two species recorded as bycatch, i.e., Eaton's skate (*Bathyraja eatonii* [Günther, 1876]; Arhynchobatidae) and McCain's skate (*Bathyraja maccaini* Springer, 1971; Arhynchobatidae).

Overall, species were detected more frequently with eDNA than recorded as catch, except for *Pogonophryne* sp., *Macrourus* sp., and *Dissostichus*

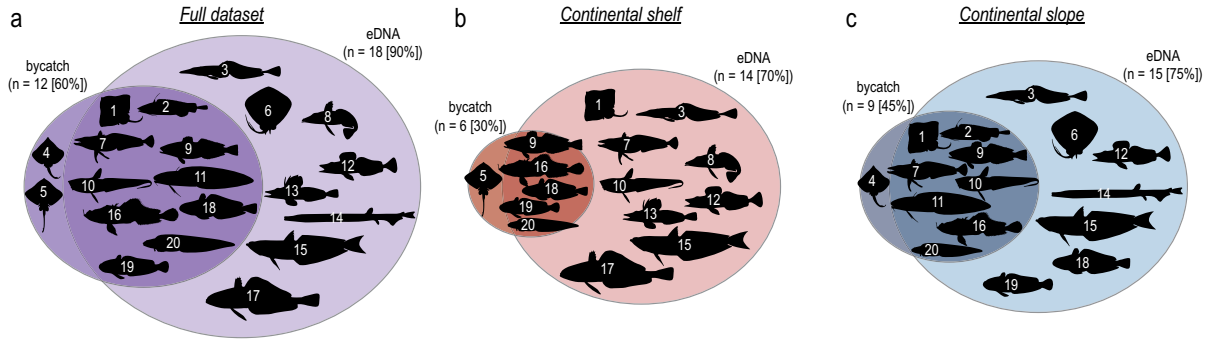

Fig. 2 Venn diagrams depicting species overlap between our eDNA survey and bycatch recordings for **a** all datapoints combined in purple, **b** the continental shelf sampling region in red, and **c** the continental slope sampling region in blue. Total number of species per monitoring method and proportion of species is represented between brackets. Venn diagram size is proportional to the number of detected species. Fish silhouette size is not representative of actual fish size. Number within silhouettes indicates species name as found in Supplemental Table 8

Table 1 Number of fish taxa detected in the continental shelf and continental slope sampling regions based on eDNA metabarcoding and catch records

Values in bold indicate the total number of taxa detected across both sampling locations

	Sampling location	Order	Family	Genus	Species
eDNA metabarcoding	Continental shelf	4	7	11	9
	Continental slope	5	11	12	7
	Total	**5**	**11**	**14**	**10**
Demersal longlining	Continental shelf	3	5	4	3
	Continental slope	4	9	8	5
	Total	**4**	**9**	**9**	**7**
Combined surveys	Continental shelf	4	8	12	10
	Continental slope	5	11	12	8
	Total	**5**	**11**	**14**	**12**

mawsoni, which were detected in the same number of samples (Fig. 3a). Significant differences in species richness were observed between monitoring methods within each sampling region according to multiple pairwise t-test with Bonferroni correction (continental shelf: $t[8]=8.4$, $p<<0.001$; continental slope: $t[16]=3.9$, $p<0.01$), with eDNA significantly detecting a greater number of species on average compared to catch records (Fig. 3b). This result was further corroborated by species accumulation curves (Fig. 3c). Additionally, eDNA signals differed significantly in species richness between sampling regions according to Welch's t-test ($t[24]=4.2$, $p<0.001$), with the continental slope containing a greater fish diversity over the continental shelf. Catch records, on the other hand, revealed no significant difference between sampling regions ($t[24]=0.7$, $p<0.5$).

Significant differences were also observed in community composition between sampling regions and monitoring methods according to PERMANOVA (sampling region: $F_{1,48}=86.6$, $p<0.001$; monitoring method: $F_{1,48}=19.6$, $p<0.001$), while no significant differences in dispersion were detected according to PERMDISP ($F_{3,48}=0.29$; $p<1.0$). PERMANOVA revealed sampling region ($R^2=0.54$) to be the largest explanatory variable for the variation observed in the dataset, followed by monitoring method ($R^2=0.12$). Community differences between sampling regions and monitoring methods were confirmed by ordination analysis (PCoA analysis; Jaccard index; presence-absence transformation; Fig. 3d), whereby sampling regions separated along the primary axis explaining 43.4% of the variation and monitoring methods separated along the secondary axis explaining 15.5% of the variation.

Due to differences in fish community detection between monitoring methods (alpha and beta diversity analyses), the indicator species analysis was

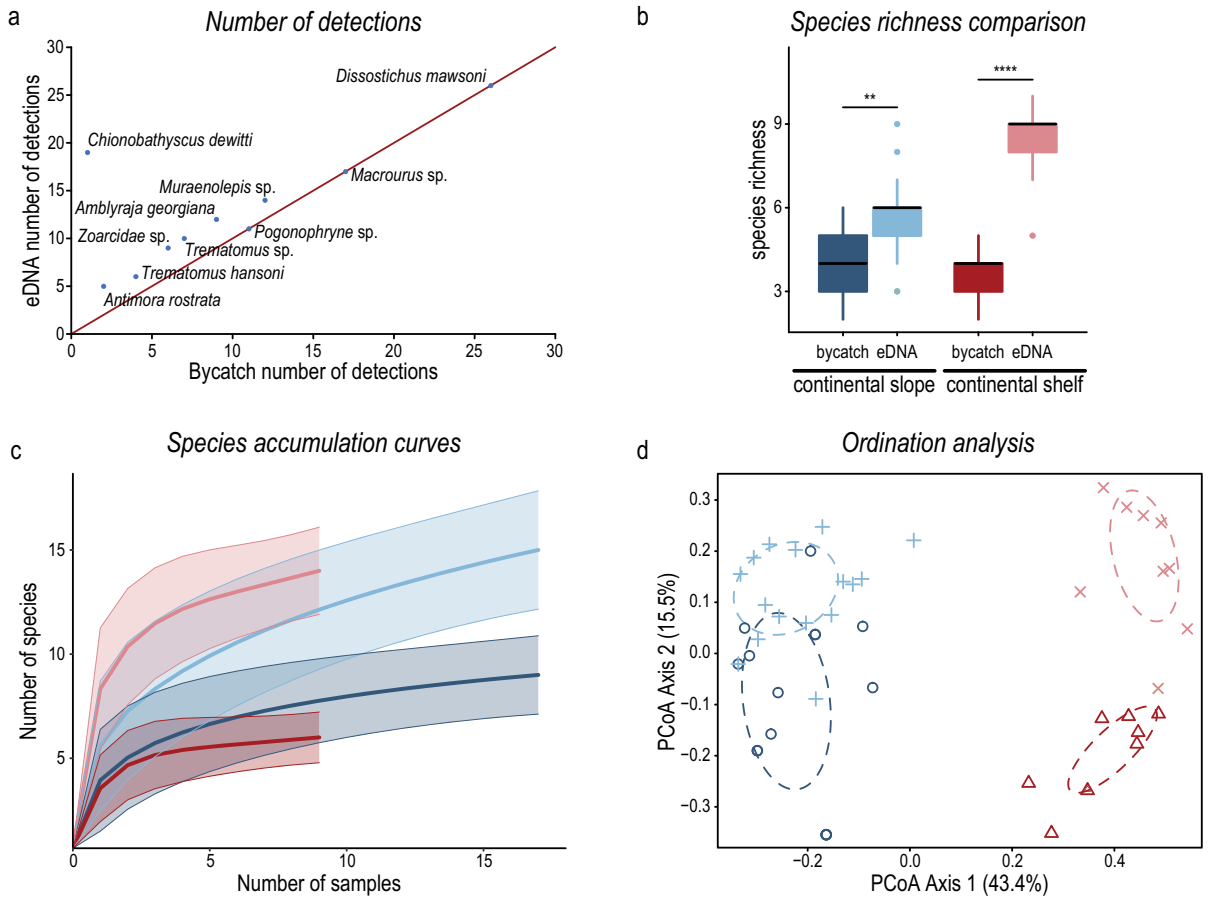

Fig. 3 **a** Frequency of taxon detection between eDNA and bycatch recordings. Maximum number of detections is 26. The red line (y = x) separates the taxa between those more frequently detected by eDNA (above) and those more frequently detected by catch (below). **b** Boxplots representing average species richness between eDNA and catch for the continental shelf (red) and continental slope (blue) sampling regions. Outliers are indicated by colored circles. The median is indicated by a black line within each boxplot. Significant differences, as indicated by multiple pairwise t-test with Bonferroni correction, are visualized with $^{**}p < 0.001$ and $^{****}p < 0.00005$. **c** Species accumulation curves per sampling region (continental shelf [red] and continental slope [blue]) and monitoring method (eDNA [light color] and catch [dark color]). Number of samples are represented on x-axis and number of taxa on y-axis. The solid line indicates the average value, while shaded area depicts the standard error. **d** Principal Coordinates Analysis (PCoA) depicting similarity in community composition based on taxonomic incidence (Jaccard index; presence-absence), with the primary x-axis explaining 43.4% of the variation seen in the dataset and secondary y-axis explaining 15.5% of variation. Catch and eDNA data from the continental shelf are depicted in dark red triangles and light red crosses, respectively. Catch and eDNA data from the continental slope are depicted in dark blue circles and light blue plusses, respectively. Ellipses surrounding each group of samples represent 95% confidence intervals

conducted per monitoring method. The eDNA monitoring method identified two indicator species for the continental slope region, including *Macrourus* sp., and *Muraenolepis* sp., and five indicator species for the continental shelf region, including Blunt scalyhead (*Trematomus eulepidotus* Regan, 1914; Nototheniidae), *Trematomus* sp., *Pogonophryne* sp., Myers' icefish (*Chionodraco myersi* DWitt & Tyler, 1960; Channichthyidae), and Jonah's icefish (*Neopagetopsis ionah* Nybelin, 1947; Channichthyidae; Fig. 1b). Catch records identified the same two indicator species for the continental slope region, however, only identified a single indicator species for the continental shelf region, i.e., *Trematomus* sp. (Fig. 1b; Supplemental Table 7). Ecological descriptions from all indicator species identified in our dataset showed

strong habitat preference in concordance with the spatial trend of the detections made either by eDNA or recorded as fish catch.

Catch abundance and biomass correlation to eDNA signal strength

Overall, eDNA signal strength and catch records showed significant correlation on a regional scale (Fig. 4). Across all samples, eDNA signal strength correlated better with catch abundance ($R^2 = 0.69$; $p < 0.005$) compared to biomass ($R^2 = 0.41$; $p < 0.05$). Additionally, correlation between eDNA and catch abundance was higher for the continental shelf region ($R^2 = 0.96$; $p << 0.001$) compared to the continental slope region ($R^2 = 0.75$; $p < 0.005$).

While regional fish diversity patterns correlated significantly between eDNA signal strength and catch abundance, single species correlation between monitoring methods displayed variable significance (Fig. 5). On average, low R^2 values were obtained, indicating low predictive success for eDNA signal strength to estimate catch records, with the lowest R^2 value of 0.0541 reported for biomass correlation of Zoarcidae and the highest R^2 value of 0.8454 reported for abundance correlation of Trematomus *sp*. While the target fish, *D. mawsoni*, obtained a significant correlation between eDNA signal strength and catch abundance ($p < 0.005$), an R^2 value of 0.32 was recorded due to the consistent number of specimens recorded and highly variable eDNA signal strength. Highly significant correlation and high R^2 values were observed for *Trematomus* sp. ($p << 0.001$; $R^2 = 0.845$), followed by Striped rockcod (*Trematomus hansoni* Boulenger, 1902; Nototheniidae; $p << 0.001$; $R^2 = 0.788$), *Macrourus* sp. ($p << 0.001$; $R^2 = 0.523$), and *Muraenolepis* sp. ($p << 0.001$; $R^2 = 0.493$). Non-significant correlation was observed for *Chionobathyscus dewitti* ($p < 0.1$; $R^2 = 0.116$) and *Zoarcidae* sp. ($p < 0.1$; $R^2 = 0.135$).

Discussion

This study provides evidence for the application of using the naturally accumulated eDNA obtained from marine sponges (Cai et al. 2022; Jeunen et al. 2021; Mariani et al. 2019; Turon et al. 2020) caught as bycatch in demersal longlining fisheries to describe fish diversity patterns in the Ross Sea, Antarctica. Compared to fish catch records, eDNA metabarcoding allows for a more comprehensive investigation into fish biodiversity by detecting a larger proportion of the fish community. In addition, while our results show eDNA signal strength to be a suitable measurement for regional fish community composition (Salter et al. 2019; Stoeckle et al. 2021; Thomsen et al. 2016), the metabarcoding application is currently unable to predict catch numbers at the species level.

Our eDNA survey exceeded fish catch records in total number of fishes detected, as well as average species diversity per sample, thereby enabling us to gather additional biogeographical information on the data-limited Ross Sea ecosystem (Ainley 2002).

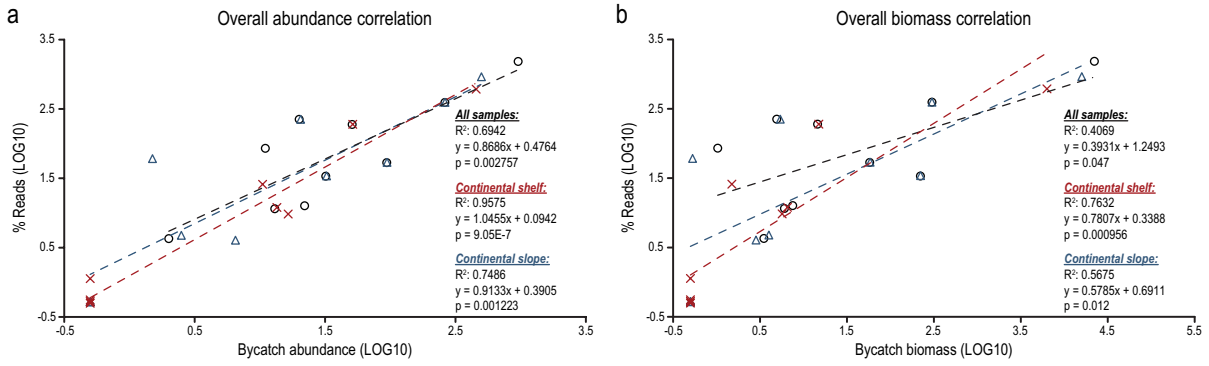

Fig. 4 Correlation between relative eDNA signal strength and **a** catch abundance and **b** catch biomass. Linear regressions are shown for the full dataset (black; circles), the continental shelf sampling region (red; cross), and the continental slope sampling region (blue; triangle). Data was log–log transformed prior to analysis. Results are shown for all taxa detected using both methods

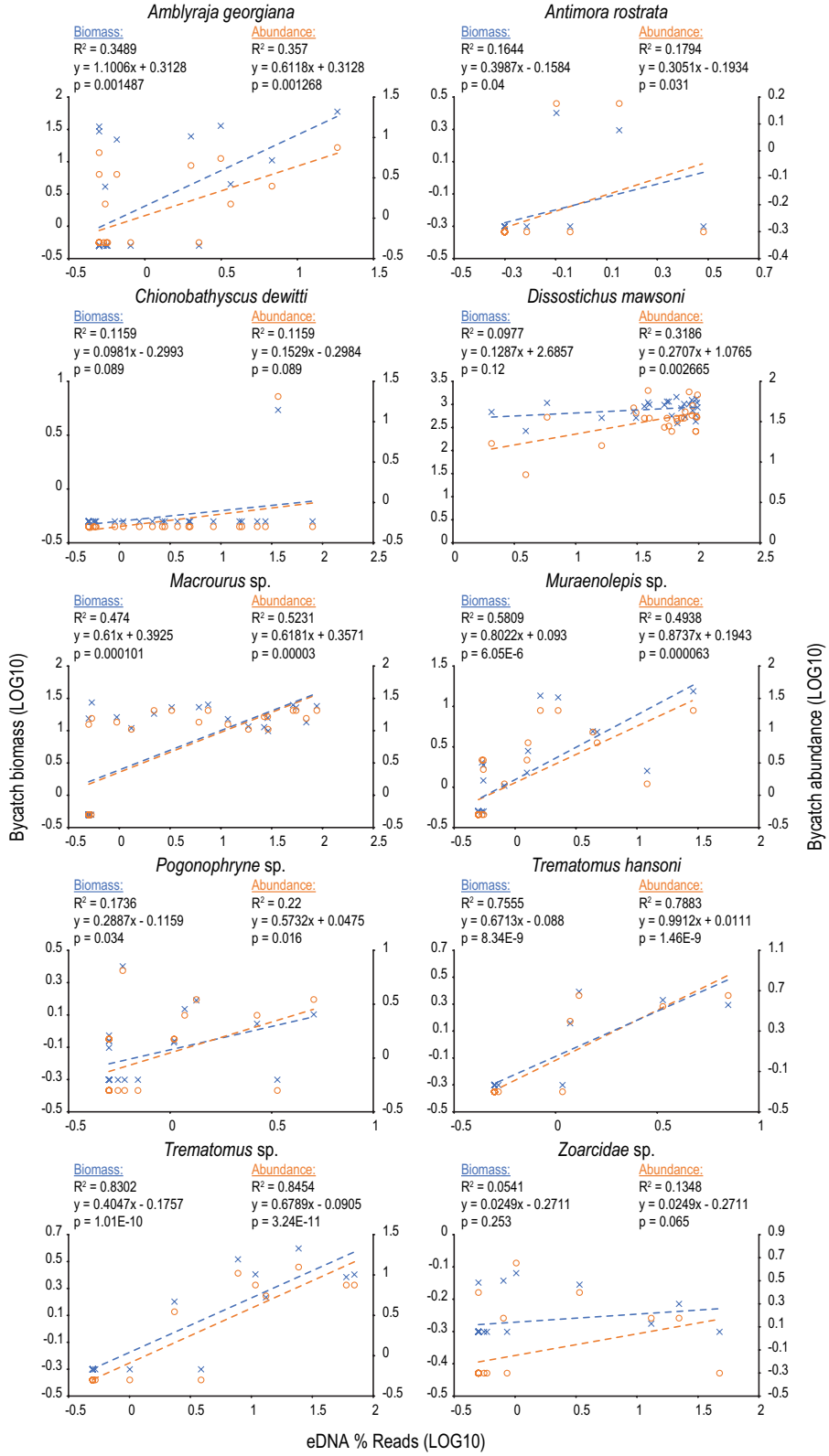

Fig. 5 Correlation between relative eDNA signal strength (x-axis) and catch biomass (blue; primary y-axis) and catch abundance (orange; secondary y-axis) for each taxon detected using both methods. Data was log–log transformed prior to analysis. Linear regression is indicated by a dashed line. The *p*-value, R^2-value, and equation is provided above the graph for biomass and abundance for each species

This discrepancy between monitoring methods could have resulted from the high selectivity and bias of the hook-and-line fishing method endorsed by CCAMLR in the Ross Sea Antarctic toothfish fishery (Løkkeborg and Bjordal 1992; Moreno 1991). While selectivity and bias towards the target fish reduces bycatch, the utilization of such catch records to describe fish diversity patterns could potentially be hampered. Aquatic and natural sampler eDNA metabarcoding surveys, on the other hand, have previously been successfully implemented to describe biodiversity patterns in the marine environment (Berry et al. 2019; Jeunen et al. 2021; Nguyen et al. 2020; O'Donnell et al. 2017). Furthermore, aquatic eDNA metabarcoding surveys are frequently reported to outperform traditional monitoring surveys with regards to number of species detected (Afzali et al. 2020; Salter et al. 2019; Stat et al. 2019; Stoeckle et al. 2021; Thomsen et al. 2016). These previous findings agree with this study. The increase in number of species lies mainly in the manner of detection, whereby eDNA surveys do not rely on visual observations, but rather detect species indirectly through DNA released in the environment by inhabiting organisms, thereby increasing detection accuracy for low-abundant or elusive organisms (Mauvisseau et al. 2017; Simpfendorfer et al. 2016; Uthicke et al. 2022). Detection probability for eDNA surveys, however, might still be impacted through, e.g., (1) amplification bias (Kelly et al. 2019), (2) varying DNA shedding rates (Sassoubre et al. 2016; Wood et al. 2020), or (3) incomplete reference databases (Hestetun et al. 2020), potentially causing the reported false-negative detections in eDNA surveys in this study and elsewhere (Maiello et al. 2022; Stoeckle et al. 2021; Thomsen et al. 2016).

The only two species that could not be detected by our eDNA metabarcoding survey were the skates, *Bathyraja eatonii* and *Bathyraja maccaini*. While one eDNA signal for *Bathyraja* sp. was recovered, we were unable to resolve the ZOTU to species level. To date, 55 species have been described in this genus, with six species occurring in Antarctic and sub-Antarctic waters where they represent the dominant group of Chondrichthyan fauna (Long 1994; Smith et al. 2008). Only 12 species (21.8%) were represented with a reference barcode in our database, including three (50%) Antarctic and sub-Antarctic species, i.e., *Bathyraja eatonii*, *Bathyraja maccaini*, and Dark-belly skate (*Bathyraja meridionalis* Stehmann, 1987; Arhynchobatidae; Supplemental File 5). Given the multiple mismatches between the ZOTU and all reference barcodes and the species-level resolution of the amplicon region for the *Bathyraja* genus (based on the 12 available reference barcodes), it is likely the detected eDNA signal is from a described species lacking a reference barcode for the 16S rDNA gene or an undescribed species, as this taxonomic group is understudied with new species being described in recent years (Smith et al. 2008; Stehmann et al. 2021). Incomplete reference databases are, hence, a major limitation to eDNA surveys (Hestetun et al. 2020). It should be noted, however, that the reference barcodes used in this study for *Bathyraja* were obtained from the online data repository NCBI, known to contain erroneous sequences (Bagheri et al. 2020). Reference barcode validation could, therefore, lead to a reclassification to one of the two species recorded as bycatch. The reliance of eDNA to assign taxonomy based on online databases is powerful, because the entire community contributes to the completion of the reference database; but it is also a downside, due to lack of stringent curation potentially leading to misclassification (Bagheri et al. 2020). Additionally, only a single tissue biopsy was collected from each sponge specimens in our experiment. The inclusion of multiple replicate biopsies per sponge specimen could have increased the detection likelihood for rarer species due to the abundance distribution observed in eDNA metabarcoding data between high and low abundant DNA signals, thereby increasing the probability of detecting both skate species (Skelton et al. 2022). While eDNA most likely failed to detect both species, we cannot exclude the possibility of misidentification by fisheries observers, due to the understudied and poorly described nature of this taxonomic group (Smith et al. 2008; Stehmann and Bürkel 1990).

Highest taxonomic resolution was not consistently obtained for one survey method over the other. For example, taxonomic resolution of *Macrourus* sp., *Pogonophryne* sp., and *Trematomus* sp. had to be reduced to genus level for catch recordings, while taxonomic resolution of Zoarcidae sp. was reduced to family level for eDNA detection (Supplemental Table 8). While both monitoring methods achieved highest resolution for certain taxonomic groups, eDNA shows the highest potential for accurate, high-resolution taxonomy assignment by not

relying on morphology-based identification (Seymour et al. 2021). To achieve this potential, reference databases will need to be completed and curated (Bagheri et al. 2020; Hestetun et al. 2020). Additionally, short amplicon primers with high taxonomic resolution and without amplification bias will need to be developed (Kelly et al. 2019). Alternatively, innovative sequencing technologies, such as Oxford Nanopore Technologies (ONT), enable longer DNA fragments to be sequenced, thereby potentially increasing the taxonomic resolution of eDNA metabarcoding data (Ames et al. 2021; Doorenspleet et al. 2021). Finally, the use of species-specific assays has the potential to increase the taxonomic resolution, as well as detection probability due to increased sensitivity over eDNA metabarcoding (Yu et al. 2022). However, the cost and time associated with the design of multiple specific assays is likely a hindrance for high diverse community monitoring (Yu et al. 2022).

Our natural sampler eDNA survey correlated highly to regional fish abundance and biomass observations from fish catch records, indicating quantitative information to be gathered from eDNA on a regional scale (Salter et al. 2019; Stoeckle et al. 2021; Thomsen et al. 2016). Similar observations were made for comparative experiments between aquatic eDNA and trawling (Salter et al. 2019; Stoeckle et al. 2021; Thomsen et al. 2016). However, the metric to which eDNA best correlated differed among studies, whereby Thomsen et al (2016) described variable results dependent on taxonomic resolution, Salter et al. (2019) found the highest correlation between eDNA and biomass, and Stoeckle et al (2021) identified an allometric index calculated from biomass to obtain the highest correlation. Further investigations into quantitative eDNA metabarcoding are, therefore, needed to tease out the current discrepancies between studies. While regional abundance information was obtained for our eDNA survey, we could not estimate fish catch numbers from a single line using eDNA metabarcoding. The lack of predictive power for our eDNA survey could be due to the bias of the longlining fishing method. For example, target species *Dissostichus mawsoni* biomass/abundance catch records were consistent across sampling regions, while eDNA signal strength varied. The consistent catch records could have been induced by the attraction of *D. mawsoni* to bait, thereby masking local abundance patterns (Kuriyama et al. 2018). For bycatch specimens, abundance/biomass estimates from catch records might be biased due to the selectivity of the gear against these organisms (Løkkeborg and Bjordal 1992). Hence, eDNA metabarcoding might be a better predictor of local fish abundance compared to longlining records. On the other hand, difficulties in obtaining abundance information from eDNA metabarcoding data are a well-known limitation of the methodology (Kelly et al. 2019). Environmental DNA signal strength can be influenced by biological (e.g., species-specific DNA shedding rates Kirtane et al. 2021), physical (e.g., environmental parameters Rourke et al. 2022), and technical (e.g., PCR amplification Kelly et al. 2019) factors, thereby potentially reducing the correlation between eDNA signal strength and taxon biomass/abundance.

Our results show the potential of using marine sponge bycatch specimens as a low-tech and cost-effective eDNA survey method to monitor the fish diversity in the Southern Ocean (Mariani et al. 2019). Marine sponges as natural eDNA samplers have been shown to achieve comparable results to aquatic eDNA surveys (Jeunen et al. 2021) and considered an innovative application of passive eDNA sampling (Bessey et al. 2021; Jeunen et al. 2022b), an effort to increase sample number in eDNA surveys by circumventing the need for the time-consuming step of active filtration (Bessey et al. 2021; Jeunen et al. 2022b). The use of marine sponge bycatch specimens in eDNA surveys will, furthermore, enable additional data gathering of the Porifera taxonomic group, which is among the less-studied benthic invertebrates with regards to extinction risk and conservation status (Bell et al. 2015). Additionally, yearly sponge bycatch sampling from the annual fishing season will enable us to investigate temporal biodiversity patterns associated with anthropogenic and climate impacts (Berry et al. 2019). Obtaining eDNA signals from previously collected sponge specimens stored in museums will also allow us to infer past ecosystem states and further our understanding of this understudied ecosystem to aid conservation efforts, such as the world's largest marine protected area (Ballard et al. 2012).

Relying on bycatch specimens, however, could potentially hinder robust experimental design and consistent monitoring, as marine sponges are caught only when entangled on fishing lines dragging over the seafloor (Parker et al. 2009). For example, during the voyage underpinning this study, the San Aotea

II fishing vessel recorded marine sponge specimens as bycatch in 26 (19.0%) out of the 137 fishing lines deployed. Additionally, the collection of specimens onboard commercial fishing vessels could potentially limit the opportunity for robust in-field contamination control, the standard approach within aquatic eDNA surveys (Takahashi et al. 2023). The upside of using bycatch specimens, on the other hand, is the lack of additional destructive sampling to gather data. An alternative consideration to marine sponges is the deployment of passive filtration devices, such as the metaprobe (Maiello et al. 2022) or artificial sponges (Jeunen et al. 2022b). Prior to deployment, however, it is imperative to investigate the impact of attached passive samplers to fishing lines to ensure commercial interests are not hindered. Autonomous sampling devices are another avenue currently being explored for eDNA surveys (Hansen et al. 2020; Yamahara et al. 2019). While initial success has been reported, deployment costs in remote areas, such as the Southern Ocean, and initial acquisition cost could hinder large-scale monitoring.

Conclusion

Effective conservation relies on detailed and extensive knowledge of the ecosystem. While international conservation goals have been put in place to limit the effect of detrimental anthropogenic pressures (Ballard et al. 2012), continuous monitoring is hindered by logistical constraints brought on from the remoteness of the Southern Ocean. In this experiment, we provide evidence for using marine sponge bycatch specimens as natural eDNA samplers to gain additional information on fish diversity patterns in the Southern Ocean. These passive samplers enabled us to survey a larger proportion of the fish community compared to fish catch records. Furthermore, proper curation of specimens and eDNA extracts will enable the re-examination of results when technological advances might allow for accurate abundance estimates and population genetic structure investigations. Finally, annual sponge bycatch collection and museum-stored sponges have the potential to let us uncover long-term temporal biodiversity patterns in relation to anthropogenic and climate impacts, thereby expanding our knowledge of this understudied ecosystem and aid conservation efforts.

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Author contributions The study design was conceptualized by GJJ, SM, SM, ML, and NJG. Sample collection was conducted by the FV San Aotea II fishing vessel. Laboratory work was performed by GJJ, JT, and SF. The bioinformatic analysis was conducted by GJJ. GJJ performed the statistical analysis, with input from SM, SM, ML, and NJG. GJJ wrote the manuscript with significant input from ML and NJG. All co-authors contributed to the writing of the manuscript and approve of the submission.

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Data availability Raw and demultiplexed sequencing data have been deposited on Sequence Read Archive (SRA) and can be accessed through BioProject ID: PRJNA1022078. Bioinformatic and statistical scripts to analyze the demultiplexed sequencing data are made available in Supplemental File 2 and Supplemental File 3, respectively.

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