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Streamlining large-scale oceanic biomonitoring using passive eDNA samplers integrated into vessel's continuous pump underway seawater systems

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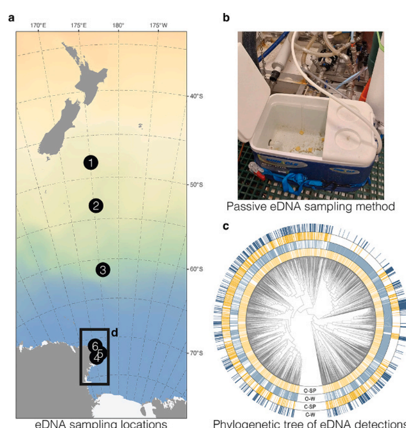
HIGHLIGHTS

- Passive samplers scale eDNA biomonitoring in our oceans, bypassing water filtration.
- Novel sampler design reduces handling time, connects to vessels seamlessly, without interfering with onboard activities.
- Passive eDNA sampling shows potential during a voyage from New Zealand to Antarctica when compared to water filtration.
- Passive samplers detect more phyla and recover more ZOTUs than water filtration.
- Passive eDNA sampler enables large-scale biomonitoring when deployed onboard the world's oceanic fleet.

GRAPHICAL ABSTRACT



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ABSTRACT

Passive samplers are enabling the scaling of environmental DNA (eDNA) biomonitoring in our oceans, by circumventing the time-consuming process of water filtration. Designing a novel passive sampler that does not require extensive sample handling time and can be connected to ocean-going vessels without impeding normal underway activities has potential to rapidly upscale global biomonitoring efforts onboard the world's oceanic fleet. Here, we demonstrate the utility of an artificial sponge sampler connected to the continuous pump underway seawater system as a means to enable oceanic biomonitoring. We compared the performance of this passive sampling protocol with standard water filtration at six locations during a research voyage from New Zealand to Antarctica in early 2023. Eukaryote metabarcoding of the mitochondrial COI gene revealed no significant difference in phylogenetic α-diversity between sampling methods and both methods delineated a

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progressive reduction in number of Zero-Radius Operational Taxonomic Units (ZOTUs) with increased latitudes. While both sampling methods revealed comparable trends in geographical community compositions, distinct clusters were identified for passive samplers and water filtration at each location. Additionally, greater variability between replicates was observed for passive samplers, resulting in an increased estimated level of replication needed to recover 90 % of the biodiversity. Furthermore, traditional water filtration failed to detect three phyla observed by passive samplers and extrapolation analysis estimated passive samplers recover a larger number of ZOTUs compared to water filtration for all six locations. Our results demonstrate the potential of this passive eDNA sampler protocol and highlight areas where this emerging technology could be improved, thereby enabling large-scale offshore marine eDNA biomonitoring by leveraging the world's oceanic fleet without interfering with onboard activities.

1. Introduction

The effectiveness of marine conservation efforts is hampered, due to a lack of detailed knowledge on current biodiversity trends (Katsanevakis et al., 2020; Pendleton et al., 2018). This missing information stems from barriers to marine biodiversity monitoring across the tree of life (Luypaert et al., 2020; Stat et al., 2017). The lack of information on ecosystem functioning is further exacerbated for the open ocean, deep sea, and remote regions, such as the Southern Ocean, as the added logistical and expense constraints, as well as the inhospitable environment, limit research opportunities (Danis et al., 2020; Glover and Smith, 2003; Liu et al., 2022; Ramírez et al., 2022).

Traditionally, marine biomonitoring has relied on morphological characteristics for species identification through direct human observation. Frequently-used monitoring techniques for the marine environment include underwater visual censuses by diver, video, or acoustic sensors (Mallet and Pelletier, 2014; Rajan et al., 2019; Steenweg et al., 2017), trawling (Pusceddu et al., 2014), and plankton tows (Reid et al., 2003). However, these biomonitoring methods may often be biased, invasive, and require taxonomic expertise to identify species (Ayma et al., 2016; Kwong et al., 2018; de Mendonça and Metaxas, 2021; Trebitz et al., 2017). Moreover, traditional monitoring methods can be time-consuming, expensive, and fail to detect the true biodiversity present at the site of interest due to a focus on a limited number of taxonomic groups during the survey and an inability to identify juveniles, cryptic species, and damaged specimens (Hernández-Becerril et al., 2023; Zhang et al., 2020). With the increased urgency of obtaining accurate marine biodiversity data for conservation purposes, scientists are currently exploring novel tools to aid marine biodiversity monitoring efforts (Danovaro et al., 2016).

One biomonitoring technique that offers great potential is environmental DNA (eDNA) metabarcoding (Ficetola et al., 2008). Through the examination of the DNA signature present in environmental samples, which stems from genetic material released by all organisms into their surroundings (Thomsen and Willerslev, 2015), eDNA metabarcoding circumvents the need for invasive sampling procedures (Stat et al., 2019) and automates taxonomic identification by matching DNA fragments to online reference databases (Jeunen et al., 2022b). This approach obviates the necessity for direct visual identification of species to infer presence (Afzali et al., 2020; Fernández et al., 2021; Stat et al., 2019) and quantify abundance (Rourke et al., 2022; Stoeckle et al., 2017; Uthicke et al., 2018).

While aquatic eDNA has become a pivotal data source for marine ecosystem assessment (Takahashi et al., 2023), the eDNA biomonitoring workflow is still undergoing innovation to overcome current challenges (Rishan et al., 2023; Takahashi et al., 2023). One of the major hurdles to scale eDNA for global monitoring is the need to collect large volumes of water to increase detection probabilities (Govindarajan et al., 2022), while filtering and processing of samples upon collection is also required to halt DNA degradation (Hunter et al., 2019). The time-consuming process of water filtration is currently limiting the number of samples included in eDNA surveys (Bessey et al., 2021) and immediate filtration is not always logistically feasible (Jeunen et al., 2024). Additionally, this in-field sample handling step in the eDNA workflow increases the risk of contamination, either between samples when gear is not properly sterilized or from outside sources (Sepulveda et al., 2020b).

A promising recently explored approach to circumvent the necessity for water filtration in eDNA biomonitoring are passive samplers (Bessey et al., 2021), a process whereby various substrates are placed in the water to passively accumulate eDNA on or within the surfaces of the submerged matrix (Bessey et al., 2021; Jeunen et al., 2022a; Verdier et al., 2021). Inspired by the action of powerful filtering organisms such as sponges (Phylum Porifera) (Mariani et al., 2019), mesocosm and in situ experiments have indicated that artificial sponges can serve as suitable passive samplers (Jeunen et al., 2022a), with a deployment time of minutes to hours deemed sufficient for passive samplers to collect the eDNA signals of the residing marine community (Bessey et al., 2022). By eliminating the need for the time-consuming process of water filtration, passive samplers enable increased replication within a location (Jeunen et al., 2022a). Furthermore, with effective submergence times for passive eDNA samplers reported as low as several minutes, passive eDNA samplers enable an increased number of locations to be incorporated in eDNA biomonitoring surveys (Bessey et al., 2021). Nevertheless, passive eDNA sample collection thus far has still either restricted a vessel's movement or required specific circumstances for sample deployment. For example, the use of modified plankton samplers limits a vessel's speed to ≤5 knots (Pochon et al., 2024) and the metaprobe passive eDNA sampler used to monitor fish catch and bycatch has been deployed inside trawling nets (Maiello et al., 2023, 2022). Such limitations may have slowed the adoption of these developments.

With the aim of enabling large-scale eDNA biomonitoring across a greater proportion of the marine biome, this study investigated the deployment of passive eDNA samplers without impeding a vessel's movement or requiring specialised gear that limits implementation of eDNA collection to a subset of ocean-going vessels. By connecting artificial sponges to the continuous pump underway seawater system, we monitored the eukaryote biodiversity during a voyage from New Zealand to Antarctica in early 2023. Such continuous pump underway seawater systems are present on a majority of ocean-going vessels to cool a vessel's engine, as well as separate secondary systems for research purposes. Simultaneous eDNA sampling through standard water filtration approaches (Jeunen et al., 2023) enabled us to compare metabarcoding results between both methods and answer the following questions: (i) can passive eDNA samplers be deployed without hindering a boat's manoeuvrability and/or requiring extensive handling time to limit the impact of the day-to-day activity of crew members, (ii) are eDNA metabarcoding results between passive and active sampling comparable, and (iii) are observed biodiversity patterns with eDNA reflective of known large-scale ecological patterns and physical features in the Southern Ocean (i.e., water masses and oceanic fronts).

2. Materials and methods

2.1. Flow through system for passive eDNA collection

New Zealand's research vessel, RV *Tangaroa's* Underway Flow Through System (TUFTS) continuously collects seawater at ~25 l min^{-1} through a variable speed mono pump via an intake at about 5 m water

depth in the vessel's hull from the keel of the ship. Water was diverted into a 50 l insulated flow-through tank for environmental DNA sampling by connecting a hose to a fitting drilled into one side near the top of the insulated tank. To ensure the tank remained near-full of seawater with a continuous flow of ~1.5 l min^{-1}, an overflow tube covered with a 2 mm mesh was fitted to the plumbing at the base of the tank on the opposite side of the water entry point (Fig. 1c).

2.2. Sample collection

Sixty eDNA samples were collected onboard the RV *Tangaroa* during the TAN2023 Antarctic voyage between 17 January and 14 February 2023 at six locations, including three open ocean sites and three coastal regions (Fig. 1; Supplement 1). At each location, five passive eDNA samplers in the form of 1cm^3 excised subsamples from artificial Whirl-Pak® Speci-Sponges® (henceforward referred to as “artificial sponges”) were submerged for 24 h in the insulated flow-through tank while the vessel was moving. A 24 h deployment was chosen to limit the impact of the crew collecting eDNA samples, whereby retrieval and deployment could be achieved at a single time during the day on consecutive sampling days. After the 24 h deployment, artificial sponges were placed into 5 ml Eppendorf tubes prefilled with ethanol. Upon retrieval of the artificial sponges, five 500 ml water samples were collected from the insulated flow-through tank and filtered using eDNA syringe mini kits (1.2 μm cellulose acetate encapsulated syringe filter, Sartorius Cat # 17953; Wilderlab, New Zealand; henceforward referred to as “aquatic

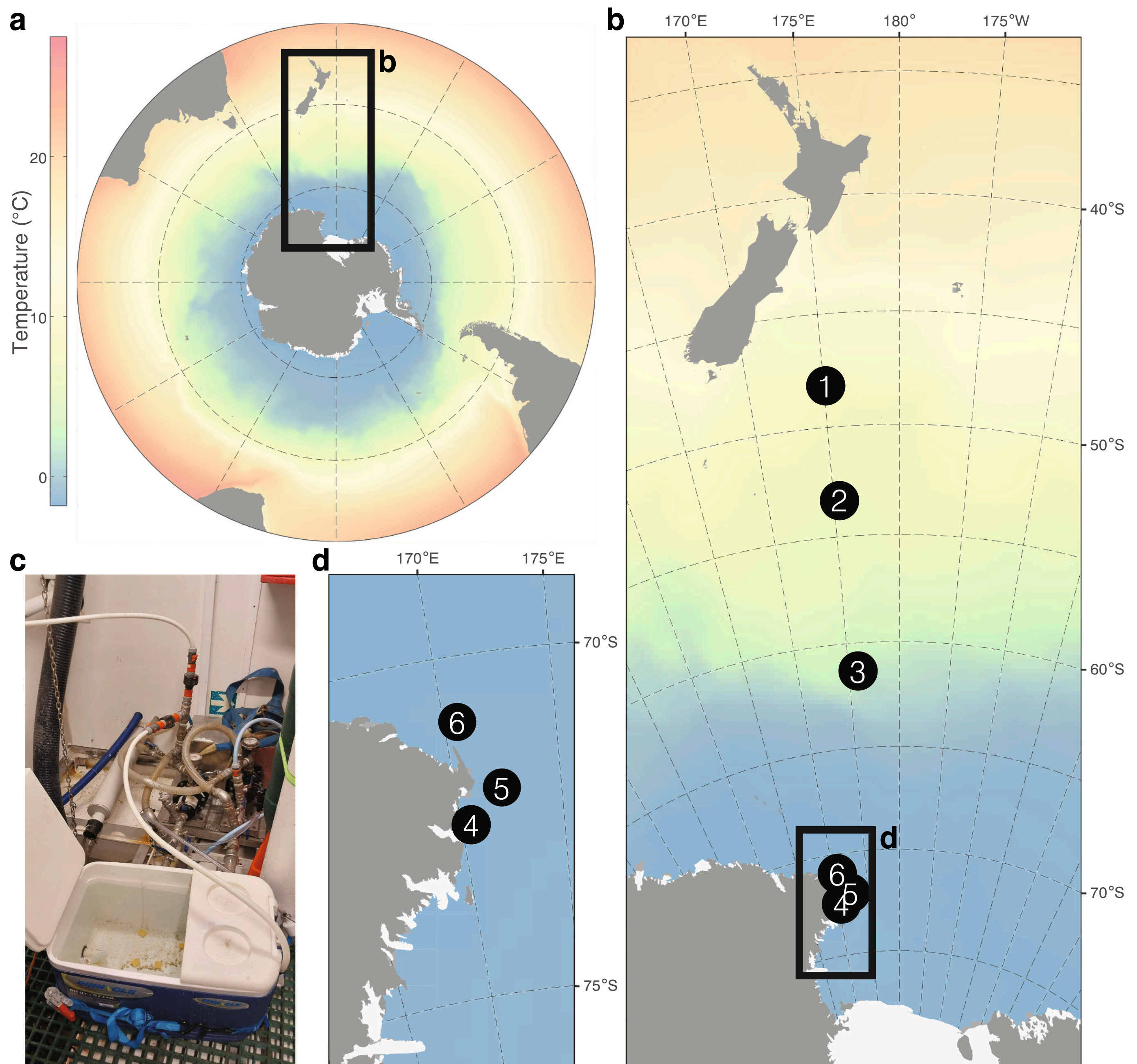

Fig. 1. (**a**) Map of the Southern Ocean displaying average sea surface temperature between 2010 and 2020 (data source: https://www.bio-oracle.org/). The sampling region is indicated by a black rectangle. (**b**) Sampling region in the Ross Sea showing sampling locations as black circles. Numbers inside black circles denote location ID used throughout the document. The coastal sampling region is indicated by a black rectangle (**c**) Photograph of passive eDNA sampler set up onboard the RV Tangaroa during the 2023 Antarctic voyage. Photo credit @Miles Lamare. (**d**) Coastal sampling region in the Ross Sea showing sampling locations as black circles.

eDNA"), following the methodology of Jeunen et al. (2023). Wilderlab collection filters were submerged in DNA/RNA Shield (Zymo Research, US) to halt DNA degradation. Hence, total water volume per method differed between methods, with 500 ml for water filtration and 2160 l for passive eDNA samples (1.5 l min^{-1} continuous flow x 24 h). Passive eDNA samples and filters were stored in the dark at −20 °C during the Antarctic voyage and transported to the designated PCR-free eDNA facilities at Portobello Marine Laboratories (PML), University of Otago, New Zealand at the completion of the voyage. Upon arrival, samples were kept in the dark at −20 °C until further processing.

2.3. Laboratory processing

Before commencing laboratory processing of eDNA samples, bench spaces and equipment were sterilized using a 10-min exposure to 10 % bleach solution (0.5 % hypochlorite final concentration) and wiped with ultrapure water (UltraPure™ DNase/RNase-Free Distilled Water, Invitrogen™) to reduce the risk of contamination(Prince and Andrus, 1992). Additionally, six negative field controls (Whirl-Pak® Speci-Sponges® subsampled to 1 cm^3 stored in 500 ml ultrapure water; one control at each sampling location), eight negative extraction control samples (50 μl ultrapure water; one control during each DNA extraction round), and two PCR no-template controls (2 μl ultrapure water; one control per 96-well PCR plate) were processed and sequenced alongside eDNA samples to investigate cross-contamination at various stages of the laboratory processing workflow.

As detailed in Supplement 2, seventy-six samples (sixty field samples and sixteen negative controls), including five biological replicates per site and per method, were extracted using Qiagen's DNeasy Blood & Tissue Kit (Cat # 69506; Qiagen GmbH, Germany) with slight modifications from the manufacturer's specifications. DNA extracts were stored at −20 °C until further processing. Before library preparation, each sample was investigated for optimal input DNA, low-template samples, and inhibitors through quantitative PCR (qPCR) screening of a dilution series (undiluted, 10-fold dilution, 100-fold dilution) (Murray et al., 2015). Quantitative PCRs were carried out on a QuantStudio™ 3 (ThermoFisher Scientific, USA). 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. Samples were amplified using the mlCOIintF/jgHCO2198 primer set (Geller et al., 2013; Leray et al., 2013), targeting a ~313 bp fragment of the cytochrome *c* oxidase subunit I (COI) gene region (mlCOIintF: 5′-GGWACWGGWTGAACWGTWTAYCCYCC-3′; jgHCO2198: 5′-TAIACYTCIGGRTGICCRAARAAYCA-3′). The thermal cycling profile included an initial denaturation step of 95 °C for 3 min; followed by 50 cycles of 30 s at 95 °C, 30 s at 51 °C, 45 s at 72 °C; and a final meltcurve analysis. We opted to investigate eukaryotic eDNA signals for this experiment over standard eDNA fish monitoring, as the shifts in phyto- and zooplankton communities are well-described in the sampling region (Pinkerton et al., 2020).

Library preparation followed a one-step amplification protocol using fusion primers, containing an Illumina adapter, a modified sequencing primer, a 6–8 bp barcode tag and the template-specific primer (Murray et al., 2015). Quantitative PCR conditions followed the protocol as described above, with each sample amplified in duplicate and assigned a unique barcode combination with differing forward and reverse barcodes. PCR replicates, assigned identical barcode combinations, were pooled to reduce stochastic effects from amplification (Alberdi et al., 2018; Leray and Knowlton, 2015). Size selection and qPCR clean-up followed the AMPure XP (Beckman Coulter, USA) standard protocol. Samples were visualized using gel electrophoresis to determine the presence of a single band and molarity was measured on Qubit (Qubit™ dsDNA HS Assay Kit, ThermoFisher Scientific, USA). Samples were pooled in equimolar concentrations to produce a single DNA library. The resultant library was size selected using Pippin Prep (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 at the Otago Genomics sequencing facility on an Illumina MiSeq® using a 2 × 250 bp V2 Illumina sequencing kit, following the manufacturer's recommendations, with 5 % PhiX to minimize issues associated with low-complexity libraries.

2.4. Bioinformatic analysis and taxonomy assignment

Upon quality investigation of raw sequence files using FastQC *v* 0.12.1 (Andrews, 2010), forward and reverse reads were merged (function: '*–fastq_mergepairs*') using default settings in VSEARCH *v* 2.23.0 (Rognes et al., 2016). Once the forward and reverse reads were merged, data was demultiplexed, i.e., reads assigned to samples, by allowing for a maximum of 2 errors (parameter: '*-e*') and no indels (parameter: '*–no-indels*') in the barcode and primer binding region using cutadapt *v* 4.4 (Martin, 2011). Demultiplexed reads were quality filtered using the '*–fastq_filter*' function in VSEARCH based on a maximum expected error of 1.0 (parameter: '*–fastq_maxee*'), an amplicon length of 313 bp (parameters: '*–fastq_minlen*'; '*–fastq_maxlen*'), and without allowing for ambiguous base calls (parameter: '*–fastq_maxns 0*'). To determine only high-quality reads remained after filtering, FastQC reports between raw and processed reads were compared. Reads passing the quality filtering thresholds were dereplicated using the '*–derep_fulllength*' function in VSEARCH with default parameters. Unique sequences were denoised using the unoise3 algorithm (Edgar, 2016) as implemented in the '*–cluster_unoise*' function in VSEARCH with default parameters. Chimeric sequences were removed using the uchime3 algorithm (Edgar, 2010) as implemented in the '*–uchime3_denovo*' function in VSEARCH with default parameters, thereby retaining ZOTU sequences (Zero-Radius Operational Taxonomic Unit) used to generate a count table (function: '*–usearch_global*') using VSEARCH.

A custom curated reference database for the mlCOIintF/jgHCO2198 primer set was generated using CRABS *v* 0.1.8 (Jeunen et al., 2022b). COI gene fragments were downloaded from the NCBI (Federhen, 2012) and BOLD (Ratnasingham and Hebert, 2007) online repositories using the `*db_download*` function. Data from both sources were merged using the '*db_merge*' function. Amplicons were retrieved through an in silico PCR step (function: '*insilico_pcr*'), while amplicons with missing primer-binding information were retrieved through pairwise global alignments (function: '*pga*') by using the amplicons extracted by the in silico PCR step as seed sequences. The database was further curated through dereplication (function: '*dereplicate*') and various filtering parameters (function: '*seq_cleanup*'), including (i) removal of amplicons containing ambiguous base calls, (ii) amplicon length restrictions, and (iii) exclusion of environmental sequences and sequences without species-level taxonomic information. Finally, the local curated reference database was formatted to BLAST specifications using the '*tax_format*' function within CRABS and the '*makeblastdb*' function within the BLAST 2.10.1+ command-line tool suite (Altschul et al., 1990).

The taxonomic identification of all ZOTU sequences was accomplished by employing a locally executed BLASTn analysis with default settings against the curated reference database. To gain access to the private reference barcode sequences on the BOLD repository, a secondary taxonomic ID was generated using the *v*3 BOLD Identification Engine and automated through the BOLDigger *v* 2.2.1 command-line tool (Buchner and Leese, 2020) with default settings. The final taxonomic ID for each sequence was set to the BLAST identification and only switched to the BOLD ID when a private sequence on the BOLD repository obtained an improved classification over the local reference database.

Once a taxonomic ID was assigned to each ZOTU, artefact sequences were filtered by merging child-parent reads based on taxon-dependent co-occurrence patterns as implemented in tombRaider *v* 0.1.0 using default settings. Negative control samples were treated for potential

contamination using the microDecon *v* 1.0.2 R package (McKnight et al., 2019) with default settings. Finally, samples that neither reached the rarefaction plateau when plotting ZOTU richness against sequencing depth, nor showed a curvature index above 0.40 were discarded, whereby rarefaction curves and curvature indices were generated using the DivE *v* 1.3 R package (Laydon et al., 2014) to assess sufficient sequencing coverage was obtained. A custom python script was used (https://github.com/gjeunen/ALEX) to parse and update all output files, as well as generate a taxonomic lineage for each taxonomic ID.

2.5. Statistical analysis and visualization

All statistical analyses were conducted in R *v* 4.3.1 and RStudio *v* 2023.06.1 + 524. Due to the limited number of available reference barcodes for Southern Ocean eukaryotes (Jeunen et al., 2023), statistical analyses were based on ZOTU sequence diversity, henceforward referred to as taxonomic diversity (TD) and phylogenetic diversity (PD). Bayesian phylogenetic trees were generated using BEAST *v* 2.7.6 (Bouckaert et al., 2019) after aligning ZOTU sequences using the '*AlignSeqs*' function of the DECIPHER *v* 2.28.0 R package (Wright, 2020). Phylogenetic tree construction was performed with a Markov chain Monte Carlo (MCMC) chain length of 10^8 iterations, sampling trees every 1000. Convergence of the MCMC chains and effective sample size was checked using TRACER *v* 1.7.2 (Rambaut et al., 2018). The maximum credibility tree from the posterior sample of phylogenetic time-trees with a burn-in percentage of 85 % was identified through TreeAnnotator *v* 2.7.6 (Bouckaert et al., 2019) and used for subsequent analyses. As the correlation between eDNA signal strength and species abundance has not yet been established for metabarcoding approaches using universal primer sets (Fonseca, 2018), the count table was transformed to presence-absence prior to statistical analysis.

Differences in α-diversity were assessed through Faith's PD using a two-way ANOVA with eDNA sample type and location as factors, followed by post hoc Tukey multiple comparisons of means to assess significant differences between factors and interactions. Venn diagrams and phylogenetic trees were drawn to visualise differences in α-diversity. Additionally, the total number of observed ZOTUs were compared between eDNA sampling methods using a Wilcoxon signed-rank test. Furthermore, after transforming the count table to an incidence-frequency data type, the total taxonomic diversity for each sampling method and location was estimated for Hill order $q = 0$ through inter- and extrapolation calculations in the iNEXT.3D *v* 1.0.1 R package (Chao et al., 2021) (function: '*estimate3D*'). A Wilcoxon signed-rank test was used to assess differences in α-diversity under optimal replication conditions within each sampling method. Phylogenetic diversity (PD) estimates for Hill order $q = 0$ were also calculated from the incidence-frequency data table (function: '*estimate3D*'; package: iNEXT.3D) to assess the required replication at 90 % eDNA signal coverage. Significant differences in estimated replication between eDNA sample types was tested through a paired *t-test*. Differences in β-diversity were examined through PERMANOVA and PERMDISP analyses on an unweighted unifrac (PD) distance matrix using the '*adonis2*' and '*betadisper*' functions in the vegan *v* 2.6–4 R package (Dixon, 2003), respectively. Non-metric Multi-Dimensional Scaling (NMDS) ordination plots were drawn using the '*ordinate*' function in the phyloseq *v* 1.44.0 R package (McMurdie and Holmes, 2013) to visualise β-diversity patterns. The raw sequence data files, as well as bioinformatic and R scripts are available on figshare (https://figshare.com/projects/Marsden_Obj3_Tangaroa_Underway/189474).

3. Results

3.1. Sequencing results

After quality filtration, our dataset included a total of 4,414,466 reads, with an average (±s.e.) of 73,574 reads (±4168.23) across 60 samples (30 aquatic eDNA samples: 70,135 ± 4871.59; 30 artificial sponge eDNA samples: 77,013 ± 6793.14). The plateauing of rarefaction curves and curvature indices reaching the threshold indicated sufficient sequencing coverage was obtained for all samples (Supplement 3). Spearman rank correlation between sequencing depth and detected number of ZOTUs was not significant (ρ (58) = 0.24; $p = 0.06$), hence data was not rarefied prior to statistical analysis (Supplement 3).

3.2. ZOTU and species overlap

We detected a total of 3051 ZOTUs (Fig. 2a). Across all locations, number of ZOTUs detected was similar between artificial sponges (2393; 78.4 %) and aquatic eDNA (2318; 76.0 %). A total of 1660 ZOTUs (54.4 %) overlapped between sample types across all sites, while 733 (24.0 %) and 658 (21.6 %) ZOTUs were detected only by artificial sponges and aquatic eDNA, respectively. ZOTU overlap between sample types within each sampling location ranged from 26.6 % for location 4 to 43.6 % for location 5 (Supplement 4).

Due to the limited availability of reference barcodes for Southern Ocean organisms, only 673 (22.06 %) ZOTUs could be taxonomically identified to phylum level when employing an 85 % sequence similarity threshold (Fig. 2b). Non-assigned ZOTUs comprised 1,797,447 (40.72 %) reads in the total data set and ranged between 28.75 % and 56.36 % within each sampling location for artificial sponges and between 33.28 % and 69.73 % for aquatic eDNA. A total of 25 phyla were detected across all samples. The most abundant phylum, based on read count, was Haptophyta (total: 24.17 %; artificial sponges: 19.79 %; aquatic eDNA: 28.97 %), followed by Bacillariophyta (total: 9.80 %; artificial sponge: 8.26 %; aquatic eDNA: 11.5 %), Arthropoda (total: 9.70 %; artificial sponge: 17.09 %; aquatic eDNA: 1.58 %), and Ochrophyta (total: 4.09 %; artificial sponge: 1.89 %; aquatic eDNA: 6.51 %). Bacillariophyta consisted of the most amount of ZOTUs (total: 194; artificial sponge: 180; aquatic eDNA: 161), followed by Ochrophyta (total: 95; artificial sponge: 77; aquatic eDNA: 78), Amoebozoa (total: 80; artificial sponge: 77; aquatic eDNA: 34), and Arthopoda (total: 58; artificial sponge: 49; aquatic eDNA: 39). All 25 phyla were detected by artificial sponges, while aquatic eDNA failed to detect ZOTUs assigned to Nemertea, Annelida, and Placozoa (Fig. 2b). A total of 48 (1.57 %) ZOTUs matched perfectly to a reference barcode, with 41 ZOTUs detected by artificial sponges and 36 ZOTUs by aquatic eDNA (Supplement 5). The most abundant ZOTU with a perfect similarity score matched to *Phaeocystis antarctica* (Haptophyte, relative read abundance: 13.30 %), followed by *Pseudo-nitzschia subcurvata* (Diatom, relative read abundance: 4.15 %), *Clausocalanus brevipes* (Copepod, relative read abundance: 2.51 %), *Calanus simillimus/propinquus* (Copepod, relative read abundance: 1.81 %), and *Fragilariopsis kerguelensis* (Diatom, relative read abundance: 1.09 %).

3.3. Alpha and beta diversity comparison

The total number of observed ZOTUs between eDNA sampling methods within each location was not significantly different according to a Wilcoxon signed-rank test ($W = 9$, $p > 0.2$; Fig. 3a). However, the estimated total number of ZOTUs detectable with each method, calculated through inter- and extrapolation, was found to be significantly higher for artificial sponges compared to aquatic eDNA ($W = 0$, $p < 0.001$**; Fig. 3b). Furthermore, a two-way ANOVA revealed significant differences in α-diversity for Faith's PD ($F_{5,48} = 7.24$, $p < 0.0001$***; Fig. 3c) within the interaction between location and sample type. The post hoc Tukey HSD revealed this difference to be driven by sample location, rather than sample type (Supplement 6). For all α-diversity measures (observed ZOTUs, estimated total ZOTUs, Faith's PD), a general pattern of a reduced number of ZOTUs with increasing latitudes was observed for both eDNA sampling methods (Fig. 3).

Significant differences were also observed in β-diversity among sampling locations and sample types according to PERMANOVA

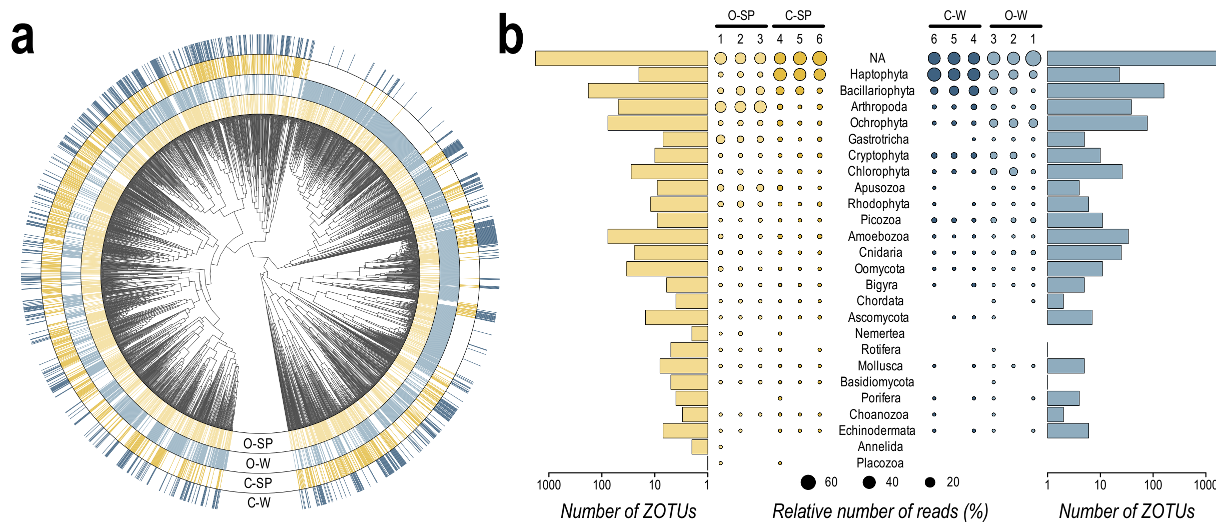

Fig. 2. (**a**) Bayesian phylogenetic tree of 3051 ZOTU sequences detected using the mlCOIintF/jgHCO2198 primer set and the occurrence within O-SP (light yellow; artificial sponge eDNA data grouped for location 1, 2, and 3), O-W (light blue; aquatic eDNA data grouped for location 1, 2, and 3), C-SP (dark yellow; artificial sponge eDNA data grouped for location 4, 5, and 6), and C-W (dark blue, aquatic eDNA data grouped for location 4, 5, and 6). Samples were pooled by region to visualise differences between open ocean and coastal sites, while also showing the similarity in ZOTU detection between sample types within each region. (**b**) Dot plot displaying relative read abundance for each phylum separated by sample type and location. NA denotes ZOTUs not obtaining a taxonomic identification at 90 % threshold. Bar plot displaying ZOTUs detected by artificial sponges (yellow) and aquatic eDNA (blue). X-axes are log10 transformed. Phyla are sorted by total read count.

(sampling location: pseudo-$F_{5,48}$ = 13.55, p < 0.001; sample type: pseudo-$F_{1,48}$ = 17.49, p < 0.001). PERMANOVA revealed sampling location (R^2 = 0.43) to be the largest explanatory variable for the observed variation in the dataset, while sample type had a more limited effect on community composition (R^2 = 0.11, p < 0.001). Significant differences in dispersion were also observed according to PERMDISP ($F_{11,48}$ = 7.62, p < 0.001). PERMANOVA and PERMDISP results were corroborated by ordination analysis (Fig. 4a). The NMDS plot revealed sample location to separate along the primary axis and sample type separating along the secondary axis (stress = 0.086). Variation in dispersion between sample type was also observed in the NMDS plot, with filtered biological replicates within a sampling location clustering more closely together compared to artificial sponge biological replicates (Fig. 4a).

The increased variability between biological replicates within a sampling location for artificial sponges compared to aquatic eDNA resulted in a significant difference in estimated required level of replication to detect 90 % of the diversity within a site according to a paired *t-test* (t [5] = 4.65, p < 0.01). Inter- and extrapolation calculations estimated 3.17 ± 0.60 and 8.14 ± 0.68 biological replicates per site for aquatic eDNA and artificial sponges, respectively (Fig. 4b).

Differences in relative read abundance and proportional ZOTU count for specific taxonomic groups were observed between geographical regions, with artificial sponges and aquatic eDNA displaying similar patterns (Fig. 2b; Supplement 7). Both eDNA sampling methods identified an increased relative read abundance of Haptophyta for coastal locations compared to open ocean sampling sites. Additionally, artificial sponges identified a higher relative read abundance for Arthropoda and Gastrotricha at open ocean locations compared to coastal regions (Fig. 2b; Supplement 7). A similar pattern was observed for Ochrophyta, however, this pattern was more apparent with aquatic eDNA compared to artificial sponges (Fig. 2b; Supplement 7).

3.4. Diversity per unit effort and cost

In our study design, passive samplers required less in-field handling time (~1 min to place an artificial sponge in a prefilled ethanol tube vs ~30 min of manual filtration per sample for aquatic eDNA), were more cost-effective (aquatic eDNA cost per sample: $20 NZD; artificial sponge cost per sample: $0.13 NZD), and has the potential to reduce the risk for contamination by fewer in situ handling steps. When factoring in the unit effort on α-diversity measures (eDNA α-diversity/time), we observed a significant difference between passive samplers and aquatic eDNA for observed total α-diversity (artificial sponge: 853.0 ± 310.8; aquatic eDNA: 26.9 ± 8.7), estimated total α-diversity (artificial sponge: 1234.0 ± 426.3; aquatic eDNA: 32.0 ± 9.6) and average observed α-diversity (artificial sponge: 45.2 ± 17.3; aquatic eDNA: 1.7 ± 0.5). A similar observation was made when factoring in the cost of a sample (eDNA α-diversity/cost) for the observed total α-diversity (artificial sponge: 6561.5 ± 2391.0; aquatic eDNA: 40.3 ± 13.1), estimated total α-diversity (artificial sponge: 9492.6 ± 3279.1; aquatic eDNA: 48.1 ± 14.4), and average observed α-diversity (artificial sponge: 347.6 ± 133.1; aquatic eDNA: 2.5 ± 0.8).

4. Discussion

Aquatic eDNA has transformed how scientists monitor the biodiversity of marine ecosystems (Takahashi et al., 2023), providing essential evidence, from populations to ecosystems, to inform conservation efforts (Aglieri et al., 2021; Pochon et al., 2017; Sigsgaard et al., 2016). However, the implementation of large-scale eDNA monitoring to assess global patterns is hindered by current methodological approaches, such as time-consuming water filtration processes (Bessey et al., 2022; Hunter et al., 2019), automated samplers that can be cost-prohibitive (Hendricks et al., 2023; Sepulveda et al., 2020a), or passive sampling approaches that require specific deployment conditions (Jeunen et al., 2024; Maiello et al., 2022) or limit the day-to-day activity of either the vessel and/or the crew (Pochon et al., 2024).

When connected to the continuous pump underway seawater system, artificial sponges can be used as a cost-effective passive eDNA sampler without interfering with a boat's manoeuvrability or day-to-day activities. Furthermore, passive eDNA samplers, as implemented in this study, require less sample handling time and expertise to acquire eDNA samples. During sampling, we made use of the continuous pump underway seawater system dedicated for research purposes on the RV *Tangaroa*. To further expand upon the application of the method, we

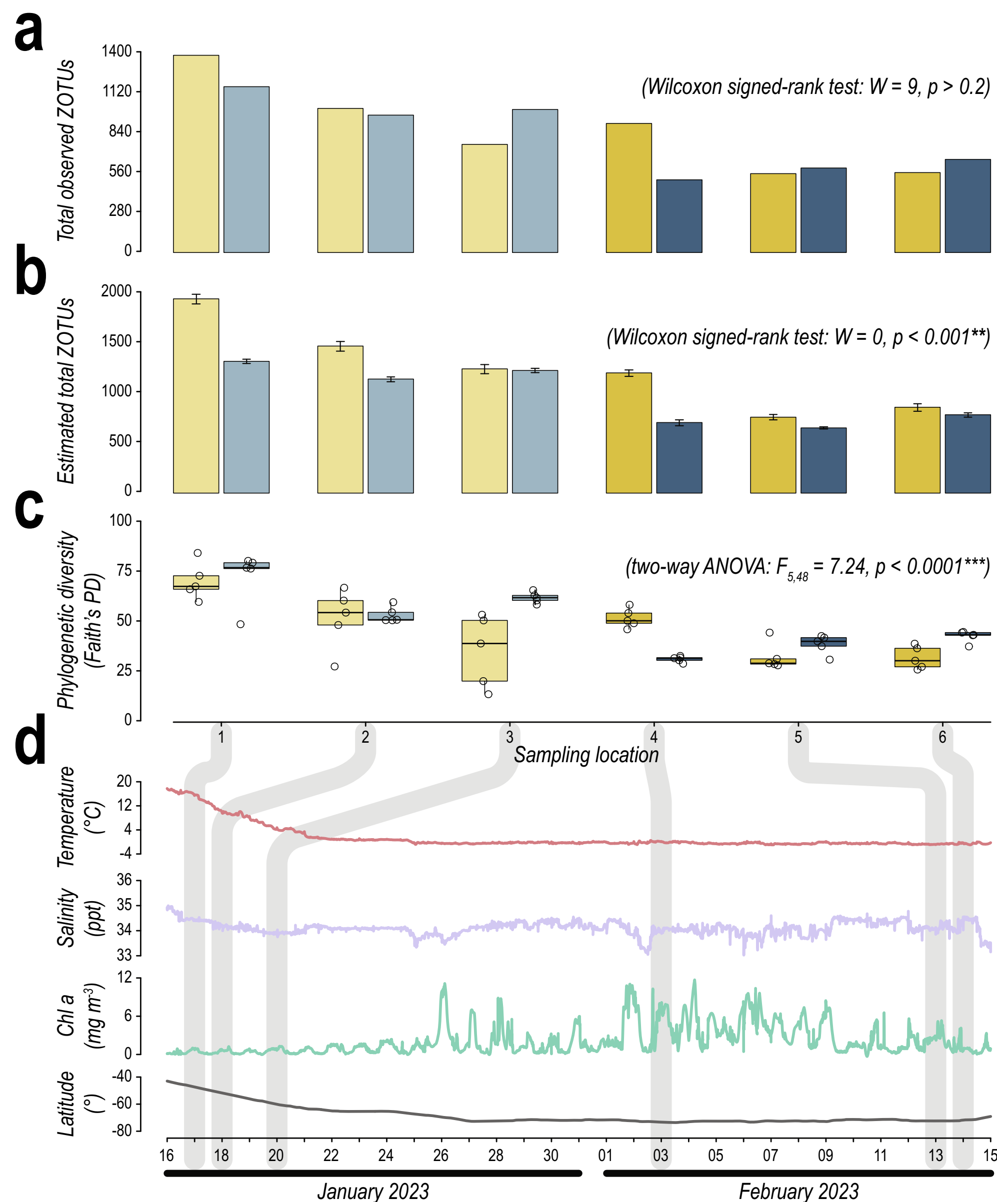

Fig. 3. Bar plots depicting (**a**) total number of observed ZOTUs across all replicates within each sample type and location and (**b**) average estimated total number of ZOTUs per replicate between artificial sponges (yellow) and aquatic (blue) eDNA samples at each location (open ocean: light; coastal: dark). Error bars represent standard error of the diversity estimation. (**c**) Boxplot depicting phylogenetic α-diversity comparisons (Faith's PD). (**d**) Metadata measurements collected every minute during the 2023 RV Tangaroa expedition, with date on the x-axis and latitude (grey), chlorophyll *a* (green), salinity (purple), temperature (red) on the y-axis. Shaded grey areas link date and metadata measurements to sampling location and are not representative of the 24 h sampling window for passive eDNA samplers.

recommend future studies to implement this approach by by-passing a small volume of water from the intake of continuous pump systems to cool a vessel's engine, thereby enabling passive eDNA sampling onboard most ocean-going vessels, including commercial ships. Additionally, improving on our insulated flow-through tank by developing smaller individual flow-through sampler that can be easily swapped out will facilitate standardised sampling.

Our passive sampler captured large-scale biogeographical diversity patterns, such as a reduced total diversity with increased latitudes and shifts in krill species and abundance between latitudes 48° to 61°, previously described in global studies of marine biodiversity (Chaudhary et al., 2021; Edgar et al., 2017). While metabarcoding results between artificial sponges and aquatic eDNA revealed similar spatial biodiversity patterns (Fig. 2) (Jeunen et al., 2023), passive eDNA sampling required less in-field handling time (~10 min to process all sponge eDNA samples vs ~30 min of manual filtration per sample for aquatic eDNA) and was more cost-effective (aquatic eDNA cost per sample: $20 NZD; artificial sponge cost per sample: $0.13 NZD). The reduced cost and handling time for passive eDNA sampling facilitate implementation of eDNA biomonitoring over larger scales, with higher replication, and an increased number of locations (Bessey et al., 2021, 2022). With the advent of robotic assistance for DNA extraction (e.g., QIAcube Qiagen GmbH, Germany) (Guthrie et al., 2023), ultra-high-throughput sequencing systems (e.g., Illumina NovaSeq® X Plus), and scalable bioinformatic pipelines (Buchner et al., 2022; Mathon et al., 2021), passive eDNA sampling could be the solution to overcome a major hurdle to global scale-up of eDNA biomonitoring.

While the observed biogeographical patterns in this study match known records (Chaudhary et al., 2021; Edgar et al., 2017), only a small subset of ZOTUs could be assigned a species ID due to the lack of

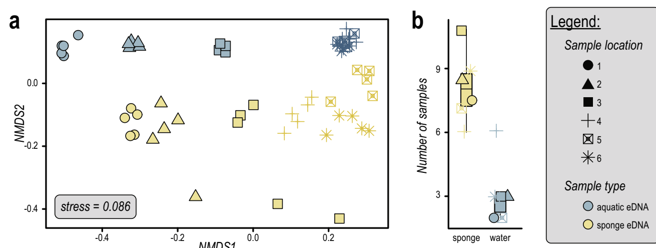

Fig. 4. (**a**) Non-metric multi-dimensional scaling (NMDS) plot depicting similarity in community composition based on an unweighted UniFrac (presence-absence) distance matrix. The stress value is reported in the lower left-hand corner. (**b**) Boxplot depicting the estimated required level of replication within each site to recover 90 % of the phylogenetic diversity calculated through inter- and extrapolation. Points and boxplots are coloured according to sample type, with artificial sponges in yellow and aquatic eDNA in blue. Point shape is dictated by sampling location, with site 1 represented as circles, site 2 as triangles, site 3 as squares, site 4 as plusses, site 5 as outlined squares, and site 6 as stars.

reference barcodes (Weigand et al., 2019). Barcoding efforts are being undertaken globally (Grant et al., 2021), but focussing these efforts on understudied and relatively pristine environments, such as the Southern Ocean, will strengthen the use of eDNA biomonitoring to provide baseline data against expected community shifts influenced by increasing anthropogenic impacts and climate change (He and Silliman, 2019), with passive eDNA samplers aiding in the simplification and scaling of monitoring efforts.

Although the spatial biodiversity patterns obtained were similar between both eDNA sampling techniques, artificial sponges were estimated to detect a significantly higher fraction of the biodiversity (Fig. 3b) and detected three phyla not observed by aquatic eDNA (Fig. 2), two of which (Nemertea, Annelida) are important taxa often used in the characterisation of marine ecosystems (Capa and Hutchings, 2021). The increased total estimated biodiversity for artificial sponges could have been influenced by the 24 h submergence time, enabling passive samplers to detect organisms from a larger geographical area, as compared to the point sampling of water filtration. With the high spatial resolution previously reported for aquatic eDNA in the marine environment, capturing eDNA from a larger geographical area could have resulted in the increased biodiversity estimated for passive samplers (Jeunen et al., 2019a; Minamoto et al., 2017; O'Donnell et al., 2017; Port et al., 2016). The higher water volume processed by passive samplers could also have influenced total biodiversity estimates. Our manual filtration protocol, adapted from (Jeunen et al., 2023), filtered 2.5 l every five samples, while passive samplers captured DNA from ~2160 l of seawater (~1.5 l min^{-1} × 24 h). While the sample processing effort is considerably less for passive samplers, the variation in water volume could plausibly account for the observed differences in estimated total biodiversity, given the recognised impact of water volume on eDNA detection (Hunter et al., 2019; Li et al., 2018; Sepulveda et al., 2019). Therefore, we recommend future studies to investigate the temporal aspect of eDNA accumulation in passive samplers (Bessey et al., 2022) to disentangle the scenarios of eDNA accumulation saturation and eDNA signal turnover, i.e., optimal submergence time and the possibility of eDNA replacement within the passive sampler substrate for prolonged submergence times.

The prolonged sampling time of passive monitoring likely increased the detection probability of low-abundance, highly motile organisms, which are difficult to accurately detect with water filtration (Eble et al., 2020; Jensen et al., 2022; Leray and Knowlton, 2017). For example, metazoans were detected more frequently and consistently with passive samplers compared to aquatic eDNA in our data. Longer submergence times may also take advantage of diel vertical migration patterns (Bandara et al., 2021; Brierley, 2014; Hays, 2003), facilitating the capture of DNA from diurnal and nocturnal organisms (Canals et al., 2021; Easson et al., 2020; Feng et al., 2022). Due to the vertical structuring of eDNA in the water column (Canals et al., 2021; Jeunen et al., 2019b) and the passive sampler's use of a continuous flow of surface water, this methodology is especially well-suited to detect biogeographical patterns of epi- and mesopelagic communities. For the detection of organisms residing deeper in the water column and benthic species, we suggest future studies to investigate alternative strategies of passive eDNA sampler placement, such as remotely operated vehicles, CTD casts, and fishing gear (Maiello et al., 2022).

Even though the estimated total diversity of artificial sponges was higher compared to aquatic eDNA (Fig. 3), passive samplers detected a lower number of ZOTUs on a per sample basis, as well as displayed a larger variation between replicates within a sampling location. Both observations suggest that passive eDNA sampling would benefit from optimisation of sampling protocols and laboratory techniques. During the last decade, a multitude of studies have optimised aquatic eDNA workflows and investigated the impact of eDNA capture substrates (i.e., filter membrane choice) and extraction protocols on downstream detection probabilities (Cowart et al., 2022; Deiner et al., 2018; Djurhuus et al., 2017; Hinlo et al., 2017; Jeunen et al., 2018; Kawato et al., 2021; Minamoto et al., 2016; Spens et al., 2016; Turner et al., 2014). Such optimisation for passive samplers is still in its infancy, with several studies having investigated the effects of substrate choice (Bessey et al., 2021, 2022; Jeunen et al., 2022a) and submergence times (Bessey et al., 2022). Based on the increased detection efficiencies observed with optimised methods for aquatic eDNA, we predict the current variability in biodiversity detection between passive eDNA samplers within a location to reduce in the future as methodologies improve. With ongoing efforts towards augmenting DNA sequence repositories globally, it is reasonable to expect that coordinated campaigns to leverage existing underway facilities from a variety of vessels will open up huge opportunities for large scale ocean biodiversity assessment.

CRediT authorship contribution statement

Gert-Jan Jeunen: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Sadie Mills:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Stefano Mariani:** Writing – review & editing, Writing – original draft, Visualization, Funding acquisition, Conceptualization. **Jackson Treece:** Writing – review & editing, Validation, Methodology. **Sara Ferreira:** Writing – review & editing, Methodology. **Jo-Ann L. Stanton:** Writing – review & editing, Funding acquisition. **Benjamín Durán-Vinet:** Writing – review & editing, Writing – original draft, Visualization. **Grant A. Duffy:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Neil J. Gemmell:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Miles Lamare:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The raw sequence data files, as well as bioinformatic and R scripts are available on figshare (https://figshare.com/projects/Marsden_Obj3_Tangaroa_Underway/189474).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.174354.

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