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Environmental DNA for measuring offshore marine biodiversity: what can DNA in water collected from the RV *Investigator* tell us?

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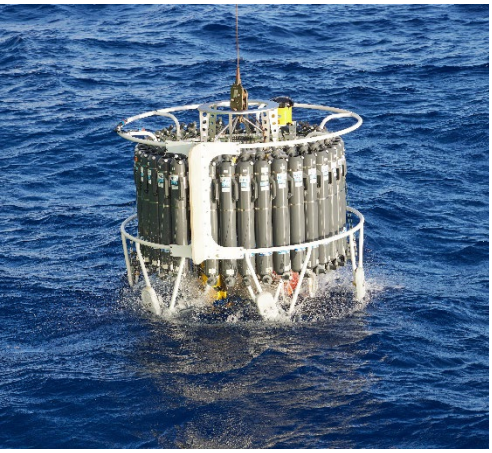

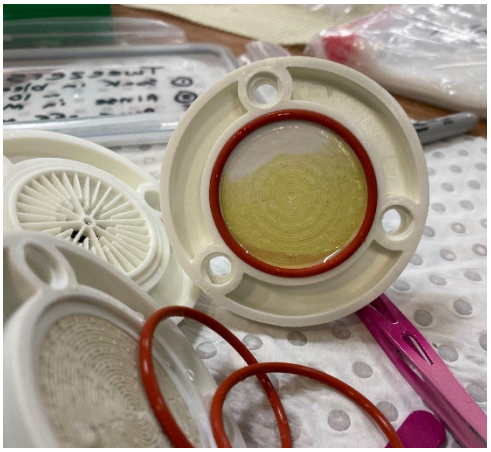

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Cover images: Left: CTD being lowered into the water – Ben Healley, Museums Victoria.
Centre: eDNA filter – Bruce Deagle/CSIRO
Right: RV Investigator off the east cost of Tasmania – Wild Pacific Media.

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Contents

List of figures

List of tables

1. Executive summary

Environmental DNA (eDNA) in seawater comes from all organisms in marine ecosystems, from bacteria to vertebrates. We can recover this eDNA by filtering water samples and use the information in it to characterise biodiversity. The approach allows for non-invasive surveys of communities of organisms and can be used to maximise the information being obtained from locations that are difficult or expensive to sample. There is significant interest amongst ecologists and conservation managers in applying standardised methods to collect rapid, scalable and comprehensive biodiversity information from eDNA.

Surveying biodiversity using eDNA is an emerging focus in Australia for monitoring marine parks, marine biosecurity, fisheries management, and environmental reporting for offshore oil, gas and renewable energy developments. The approach relies on relatively new technology – therefore, it is a priority to carry out research to allow evaluation of how well eDNA-based approaches can address questions relevant for various environmental management applications.

The objectives of this project were:

(i) To generate a set of high-quality eDNA-based biodiversity datasets from samples collected at broadly distributed offshore sites across Southeast Australia and to conduct an initial analysis of these datasets. The eDNA data were collected in parallel with traditional ship-based biodiversity survey methods allowing direct comparisons.

(ii) To assess key technical aspects of eDNA data collection to refine sampling methods and provide guidance for best practices in future offshore eDNA research.

Ultimately, this study seeks to support the integration of eDNA data into established biodiversity monitoring efforts and contribute to the development of a reliable and well-understood set of eDNA techniques. This will help empower researchers and managers to leverage this rich source of biodiversity information.

We collected eDNA samples from the CSIRO research vessel (RV) *Investigator* as part of the South-East Australian Marine Ecosystem Survey (SEA-MES). The SEA-MES sampling occurred at sites along the edge of the continental shelf (~80 to 500 m) stretching from the east coast of Tasmania to Batemans Bay (NSW). On the voyage, a variety of biodiversity surveying techniques were used, including fish trawl sampling, zooplankton sampling, acoustics and deployment of a deep tow camera. The eDNA sampling was conducted in conjunction with these conventional sampling techniques during two voyages: IN2023_V05 (July 2023) and IN2024_V03 (May 2024). The eDNA samples primarily came from water collected from Niskin bottles on a CTD rosette. We also deployed an eDNA sampler on the deep tow camera system to collect eDNA when this device was towed along transects near the bottom.

During the two voyages, water was collected from over 100 CTD deployments, with most samples collected from near the surface and bottom. We used eDNA metabarcoding to obtain information from the samples. In this method DNA barcode markers are amplified from a target group of organisms. The taxonomically informative eDNA sequences that are recovered can be matched to marine taxa represented in DNA reference databases. This project used several different eDNA markers (also referred to as assays) allowing detection of a broad range of marine species. In total our sequencing of eDNA produced over 180 million sequences.

Characterising eDNA from fish was a focus since their diversity and biomass was being measured by other methods in the SEA-MES voyages (e.g. bottom trawls). We also sequenced a DNA marker which captures the full diversity of eukaryotes, from single-celled phytoplankton to zooplankton. Finally, we investigated the use of a panel of eDNA markers targeting a range of marine taxonomic groups (from bacteria to crustaceans and mammals). Technical considerations associated with eDNA sampling are also discussed.

eDNA derived fish diversity and comparison with trawl catches

Fish eDNA (mtDNA 16S assay) was sequenced from >250 water samples collected at 91 survey sites during the two SEA-MES voyages. In these samples we identified 230 fish taxa, including 138 at the species level and 77 at the genus level. Multivariate analysis of the eDNA community composition highlighted variation between the voyages. Differences in community composition were also observed between surface and bottom collected eDNA; on average samples collected near the seafloor detected twice as many fish species as those from surface water. When other components of variation were removed, patterns emerged separating fish communities by depth of the bottom and by latitude.

At sites where both trawling and eDNA sampling occurred, trawling yielded 170 fish taxa (145 at the species level) from 8,407 kg of catch, while eDNA analysis identified 165 taxa (99 at the species level) from over 12 million sequences. Comparing percentage by mass of the total trawl catch versus the mean percentage of eDNA sequences recovered, of the top 20 taxa in each dataset, 9 were common to both. A notable difference, however, was the dominance of Chondrichthyes (sharks and rays) in the trawl catch biomass, while none of these species appeared in the top 20 taxa by eDNA sequence proportion. The eDNA data had a large proportion of sequence reads from several small schooling fish that were not dominant in trawl biomass.

Deep tow camera eDNA sampling

The CSIRO developed a passive eDNA sampler that is deployed alongside the deep tow camera system enabling an integrated near-bottom eDNA sample over the length of a transect. We were able to investigate how this new technology compares to eDNA water sampling from CTD deployments using a universal fish primer assay. We found there was no significant difference in the number of fish taxa detected between the two methods. While this indicates the passive eDNA sampler is collecting as much information as our existing sampling approach, our findings suggest this new technology is not yet optimized to take advantage of the larger water volumes and spatial extent of the sampling. We have suggested some changes which we hope will improve its performance.

eDNA derived plankton biodiversity survey

We sequenced 199 water samples collected from 69 survey sites during the two SEA-MES voyages, targeting plankton eDNA using the 18S V4 assay. This yielded over a thousand unique zOTUs (zero-radius operational taxonomic units, representing distinct sequence reads for the 18S V4 region). While assigning zOTUs to specific species is challenging, the dataset likely captures information from more than 1,000 species, as a single zOTU can correspond to multiple species. Multivariate statistical analyses highlighted major differences between the plankton communities between the two voyages (July 2023 and May 2024), as well as between communities in surface and deep chlorophyll maximum layers compared to

those in bottom waters. During the July 2023 voyage when the water column was well mixed, the depth difference was relatively subtle. During the May 2024 voyage, however, when oceanic processes moved up nutrient rich waters to the continental shelf, depth differences were clear. The deeper bottom waters (>100m), characterized by high concentrations of nitrate, phosphate and silicate, were dominated by Radiolaria and contained very little phytoplankton and copepods. In contrast, bottom waters from the shallower part of the continental shelf (up to 100m deep), where the fish eDNA work identified higher fish diversity compared to the July 2023 voyage, were associated with high relative abundance of copepods. We suggest a Radiolaria dominated plankton assembly, could negatively impact the diversity of fish present on the deeper (>100m) continental slope.

Tree of life eDNA metabarcoding

Rather than focussing on a single DNA marker, it is possible to collect information from eDNA by combining several metabarcoding assays – this is often referred to as Tree of Life (or ToL) eDNA metabarcoding. The approach potentially provides rapid characterisation of the broad biological community with good taxonomic resolution. We sent a subset of 150 eDNA samples to a commercial laboratory (Wilderlab) where sequence data from 12 different assays were generated (targets include bacteria, crustaceans, fish, etc.). While most of the recovered eDNA sequences were not assigned taxonomy, the sequences that could be assigned identified 1,439 unique taxa across the 12 markers. Joining together data from these different eDNA assays produces a skewed view of overall diversity, shaped by assay choice and differences in coverage of target taxa in reference databases. One of the ToL assays targeting fish identified 84 fish species compared to the 79 our Fish 16S sequencing assay identified in the same eDNA samples. Surprisingly only 29 of these fish species were found in both datasets. Overall, characterizing eDNA with multiple assays that capture informative DNA regions from across the Tree of Life is a promising approach to maximise the information which can be collected from eDNA.

Summary of fish eDNA methodological findings

Our standard fish eDNA sampling approach (10 L, 0.45 µm filter) did not effectively capture the full diversity of fish present at the sampled sites, as evidenced by the low taxonomic overlap—less than 30% on average—among sample replicates. The Australian Microbiome Initiative’s standard plankton eDNA protocol (2 L, 0.22 µm Sterivex filters) performed even less effectively for fish detection, recovering approximately half as many taxa per litre of water filtered. Different fish eDNA assays applied to the same eDNA sample recovered distinct subsets of the fish community, and a shorter DNA marker (~80 bp) identified more taxa. Furthermore, differences observed between surface and bottom water samples reflect the localization of eDNA in the water column, underscoring the importance of sampling at depths appropriate to the study objectives.

Conclusions

There has been a rapid rise in the use of eDNA approaches to assess and monitor biodiversity for natural resource management. This technology offers the potential to revolutionize environmental monitoring, but widespread adoption still depends on user acceptance and a solid understanding of the insights eDNA data can provide. Our analysis is an initial exploration of several eDNA datasets, we hope these datasets will form the basis of detailed case studies and provide reference points for future longitudinal research. We have only touched the surface of answering the question "What can DNA in water collected from the RV Investigator tell us?" but hopefully have demonstrated the exciting potential of eDNA-derived biodiversity information.

2. Introduction

2.1. Context for the study

Environmental DNA (eDNA) is genetic material that is found in the environment. In marine ecosystems eDNA is ubiquitous in seawater, and it comes from everything from bacteria to invertebrates and marine vertebrates. We can recover eDNA by filtering water samples and then using it to characterise biodiversity.

Surveying biodiversity using the information from eDNA is an emerging area of focus in marine environmental management (Takahashi *et al*. 2023). The approach allows for surveys of almost any organism and can be used in locations that are difficult or expensive to sample. There is a huge amount of interest in applying standardised methods to collect rapid, scalable and comprehensive biodiversity information from eDNA. There are numerous examples where eDNA has been used to provide biodiversity baselines on a regional scale and where eDNA has been used to document temporal/spatial changes in species distributions. Still, the approach relies on relatively new technology – therefore, it is a priority to carry out focussed research to evaluate how well eDNA-based approaches can address questions relevant for environmental management end-users and to optimise methods for specific applications.

The term ‘eDNA’ is often used to refer to a technique, but it is more correctly used to describe the physical object. In this view ‘eDNA techniques’ are methods used to collect biodiversity data from eDNA, in the same way that fish survey techniques are used to collect biodiversity data from fish. Much like fish survey methods, there are many approaches to characterise eDNA, for example, those which exhaustively characterise the sample, or methods which only look at a small component of information from the sample (e.g. specific species). Conventional ways to measure marine biodiversity (e.g. different nets or underwater video approaches) and eDNA methods all provide a biased view of true biodiversity. The use of eDNA is unique in that for larger biota (e.g. fish, large zooplankton) it is detecting a proxy of an organism rather than the organism itself and we need to understand the relationship between the proxy and organism (e.g. is eDNA collected at the surface telling us only about biodiversity at the surface, or also about benthic biodiversity?). The diversity of eDNA methods and the complexity of the resulting DNA sequence datasets can make eDNA-derived data inaccessible. Building a reliable suite of well understood eDNA techniques and integrating this form of biodiversity data into conventional biodiversity data streams will increase understanding and allow researchers/managers to make the most of this extremely rich source of biodiversity information.

One significant application of eDNA methods is for biodiversity surveys in Australia’s offshore Marine Park network (De Brauwer *et al*. 2023). Australian Commonwealth Marine Parks (those over 5.5 kms from the coast) cover 4.0 million square kilometres square kilometres and depths down to >6000 meters. Monitoring these large diverse ecosystems requires a range of innovative approaches. The use of eDNA is particularly appealing for surveys in marine protected areas since it can provide non-extractive information on biodiversity. Parks Australia has already started investing in eDNA methods to collect information on presence of important species and to collect data on composition and change in biological communities. This data will help with understanding the current state of marine parks, allow evaluation of management strategy effectiveness and identify opportunities for

future adaptive improvements. Surveys using eDNA are also commonly used in marine biosecurity monitoring and are being increasingly applied in other areas of marine environmental management (e.g. fisheries and environmental reporting for offshore oil, gas and renewable energy developments).

2.2. Main objectives of the project

The first objective is to produce a set of high quality eDNA-based biodiversity datasets from samples collected throughout the water column at offshore sites broadly distributed in southeast Australia. To do this eDNA samples were collected from the CSIRO research vessel (RV) *Investigator* as part of the South-East Australian Marine Ecosystem Survey (SEA-MES). The SEA-MES voyages were designed to replicate fishery and ecosystem assessments that were conducted in this region 25 years ago and the voyages also included some survey sites in the South-East Marine Park Network. The marine waters of southeast Australia are a warming hotspot where the ocean surface is warming at a rate four times the global average. Many species have already extended their distributions southward and there have been changes in commercial fisheries productivity in the region. Our project adds eDNA biodiversity data into this marine science voyage, which is focussed on waters of the outer continental shelf and upper continental slope (~80 to 500 m depth). The SEA-MES voyage is surveying biodiversity using conventional trawl sampling, zooplankton sampling, acoustics for mid-water marine organisms and deployment of a deep towed camera. The integration of eDNA data collection into the SEA-MES voyages allows for a direct comparison of eDNA results with a diverse range of alternative biodiversity survey methods, provides insight into the nature of eDNA data for a range of end-users, and will deliver a detailed eDNA baseline knowledge in the southeast temperate Australian marine region.

The eDNA datasets that were collected during the project are large and complex, so we have only done an initial investigation of what could potentially be explored. As such, analyses of the data are ongoing, and the results presented in this report should be treated as initial findings. The main questions we focused on here are:

- What are the patterns of eDNA biodiversity that we see in the fish data across the survey area?
- How does the fish eDNA data compare to the fish identified in the trawl survey?
- What patterns of biodiversity do we see using a broad assay targeting all eukaryotes?

The second objective of the project was to evaluate several technical aspects of data collection from eDNA samples. The rationale behind this was to help improve the sampling methodology and inform future studies on best practice. Our focus was to address questions relevant for offshore surveys collecting biodiversity data from a scientific research vessel and to provide information to help refine approaches that have been used in Australia for eDNA data collection.

Some of the key technical questions we set out to address here include:

- How different are data from surface collected and bottom collected eDNA in these relatively shallow offshore sites – do more easily collected surface water samples provide biodiversity information on benthic taxa?
- CSIRO has developed an eDNA sampler that can be deployed on the deep towed camera in conjunction with benthic habitat surveys. How well does this sampler capture eDNA-based biodiversity compared to conventional water filtration approaches?

- An emerging approach to collect marine eDNA data is to collect data from a large panel of assays covering many groups across the “Tree of Life” (ToL; from bacteria to vertebrates). What does this type of data look like when collected at offshore sites and how does it compare to more detailed eDNA data collected with a single assay.
- Many 2 L marine water eDNA samples have been collected at sites around Australia following the standard protocol of the Australian Microbiome Initiative. How well do these samples capture diversity of fish, compared to 10 L eDNA samples?

Some of the conclusions are likely to be specific to temperate Australian regions, but the data will add to a growing literature on the use of eDNA in biodiversity surveys and our findings compliment similar work being carried out throughout the world’s oceans.

2.3. Project overview: sampling and eDNA datasets

Sampling of eDNA was conducted on two voyages of the RV *Investigator* that were part of the SEA-MES series of research voyages throughout a large area of the marine ecosystem of southeast Australia. The first voyage was in July 2023 and the second in May 2024 (Figure 2-1). The focus of the voyages was to repeat and update fishery and ecosystem assessments. The area being surveyed corresponds to habitats at historical commercial fisheries depths (approx. 80 to 500 m deep). In biogeographic terms the offshore survey area spans the Tasmanian Province in the south into the South-Eastern Transition (Last *et al*. 2005). These depths correspond to the outer continental shelf (90-220 m) and upper slope (310-520 m) fish biomes (Last *et al*. 2005).

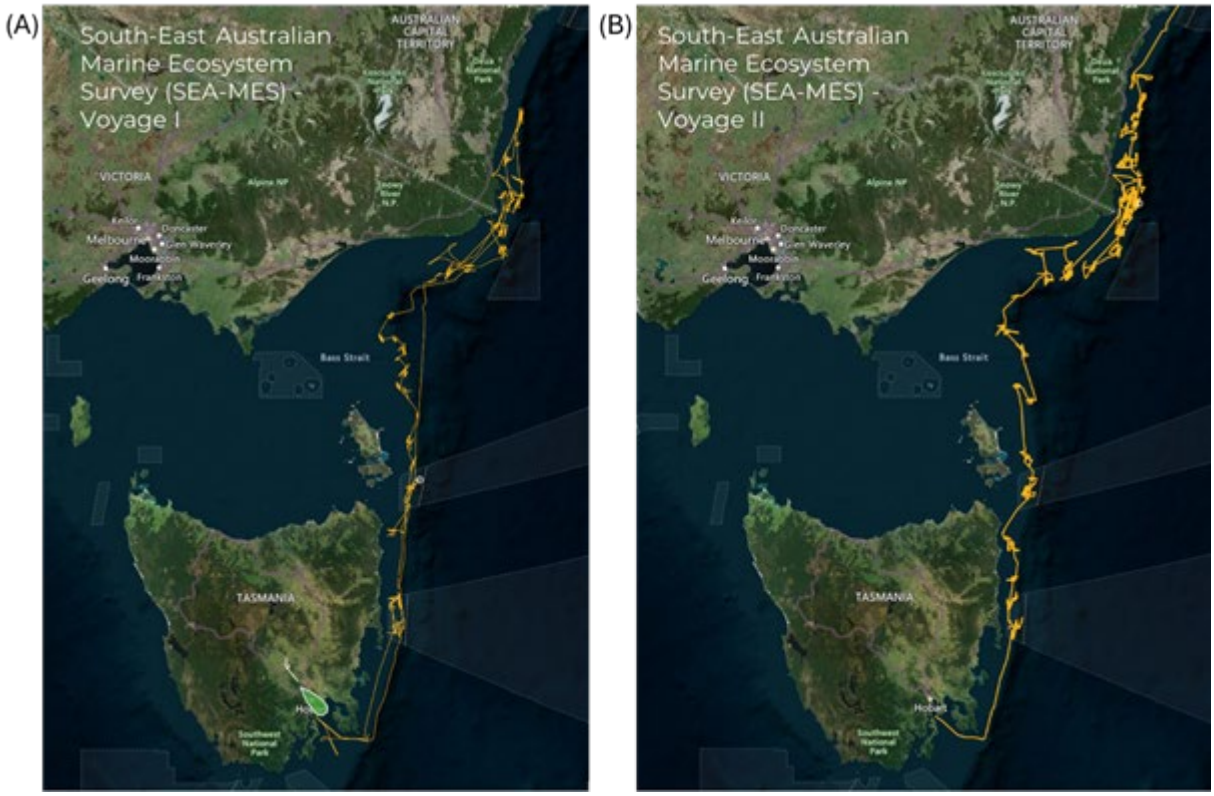


Figure 2-1 Voyage tracks of SEA-MES in south-east Australia on which eDNA samples were collected. (A) IN2023_V05, July 2023 (B) IN2024_V03, May 2024. Images from Voyage catalogue - CSIRO.

The eDNA samples were primarily collected from water obtained using Niskin bottles mounted on a CTD rosette, which was deployed through the water column to collect oceanographic data at sampling sites throughout the voyages (sampling locations are shown in Figure 2-2; an image of the CTD rosette with attached Niskin bottles appears on the cover of this report). In the two voyages water was collected from over 100 CTD deployments. Most eDNA samples were taken from near the surface and the seafloor, although some mid-water samples were also collected.

The eDNA samples came from 2 L water volumes filtered through fine 0.2 µm pore filters—consistent with the standard method used by the Australian Microbiome Initiative for marine plankton sampling—and 10 L volumes filtered through 0.45 µm pore filters, a method previously applied in fish eDNA surveys aboard the RV *Investigator* (Table 2-1).

During Voyage 2, a CSIRO-developed auto-sampler was also deployed on the deep-tow camera system to collect eDNA while the camera was towed just above the seafloor to capture video footage (Bessey *et al*. 2025). This enabled eDNA collection along transects spanning several kilometres.

To collect information from the eDNA samples we used a metabarcoding approach which involves amplifying a DNA marker from a target group of organisms and sequencing the marker (Takahashi *et al*. 2023). The recovered DNA barcode sequence allows us to match eDNA with marine species in DNA reference databases (e.g. Appleyard *et al*. 2025). The project used several different eDNA markers, also referred to as assays, which are outlined in the individual sections.

Table 2-1 Summary of eDNA samples analysed in this report. Additional genetic markers were also sequenced from some of these samples (see Appendix A for extra data not discussed in this report and Table 9-2 for a list of samples from Commonwealth Marine Parks).

Voyage^	Volume (L)	Filter	Marker	Number***	Report Section
V1	10	0.45	Fish (16S mtDNA)**	133	3
V2	10	0.45	Fish (16S mtDNA)	155	3
V2	Variable *	mix	Fish (16S mtDNA) **	96	4
V1	2	0.22	Eukaryote (18S rRNA) **	99	5
V2	2	0.22	Eukaryote (18S rRNA)	100	5

^V1 refers to IN2023_V05 (July 2023) and V2 to IN2024_V03 (May 2024).

* Variable volume - deep tow camera transect integrated samples.

** A subset of these samples was run with Tree of Life (ToL) 12 assay panel. The ToL results from the V1 10L samples (n=90) are presented in Section 6 of the report.

*** This includes all laboratory negative controls as well as field collected samples.

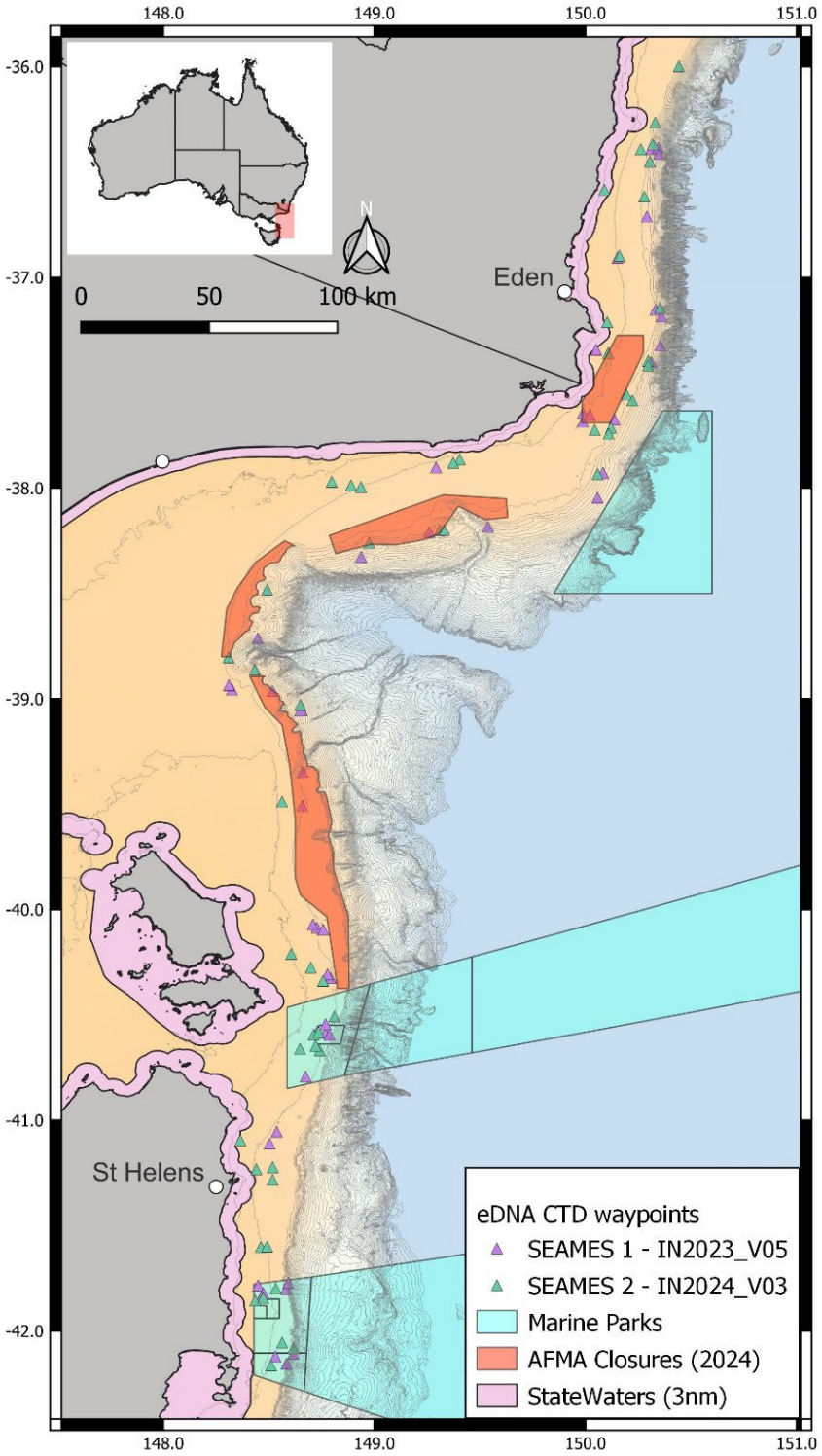


Figure 2-2 SEA-MES survey region in south-east Australia. Collection of eDNA occurred during CTD deployments shown for Voyage 1 (IN2023_V05, July 2023) as purple triangles and Voyage 2 (IN2024_V03, May 2024) as green triangles. The two southern marine parks that were sampled (shaded light blue) are Flinders Marine Park (top) and Freycinet Marine Park (bottom). Map created by Franzis Althaus, CSIRO.

2.4. Report structure

The report is divided into four main sections and a technical summary:

Section 3 focuses on results obtained from fish eDNA (Fish 16S mtDNA marker) obtained from 10 L samples. This includes a comparison between fish eDNA data, and the fish biodiversity data obtained from trawls carried out on the voyages.

Section 4 describes the eDNA results from the sampler attached to the deep tow camera, providing an evaluation of the performance of this sampler compared to filtering of CTD water samples.

Section 5 provides a view of the biodiversity in the region from the perspective of the broad Eukaryote 18S eDNA marker which amplifies DNA from a range of plankton groups from single celled protists to crustaceans.

Section 6 reports on SEA-MES voyage 1 eDNA data collected using the 'Tree of Life' metabarcoding approach. This data combines eDNA information from 12 assays, covering organisms from bacteria to marine mammals.

Section 7 gives a summary of technical findings relevant to fish eDNA sampling.

Appendices provide additional technical information:

Appendix A Outlines the different eDNA datasets produced during the project and provides links to these data in public repositories.

Appendix B Compares data from the 2 L versus 10 L eDNA samples.

Appendix C Lists all fish species identified in 10 L eDNA samples.

Appendix D Summarises environmental indices generated from 18S metabarcoding data.

General comments on report structure

The report was written by several authors, and the sections have different styles reflecting this.

While we have made efforts to clarify sample numbers throughout the report, there may still be some ambiguity around how specific sample counts were derived. For example, the number of field-collected samples differs from the number of samples with eDNA data for several reasons: not all collected samples were processed; additional laboratory negative controls were added; and some controls and samples were excluded after failing quality checks. In addition, some eDNA assays were only run on a subset of samples and in many analyses, only subsets of the full dataset were used.

3. SEA-MES eDNA derived fish diversity and comparison with trawl catches

3.1. Background

While environmental DNA (eDNA) can be used to characterize biodiversity across all organisms, this project focused primarily on fishes. One key reason for this focus is that the SEA-MES voyages aimed to investigate the drivers behind changes in commercial fisheries productivity in the region—naturally directing much of the sampling effort toward fish communities. This included extensive bottom trawling to assess species composition and biomass, enabling direct comparisons between traditional catch data and fish eDNA results

Fish were also prioritised because their distributions are comparatively well studied, and detailed biodiversity records have contributed to the development of bioregionalization maps used in designing Australia's network of Marine Protected Areas (Last *et al*. 2005). As a result, fish are a taxonomic group that many marine scientists and resource managers are already familiar with, making eDNA data on fish more likely to be evaluated and applied in management contexts.

From a technical standpoint, fish are also an ideal target group for eDNA research. They have one of the most comprehensive DNA reference libraries, allowing for higher taxonomic resolution in sequence identification. Additionally, a substantial body of literature—comprising hundreds of eDNA studies over the past decade—provides guidance specific to fish eDNA sampling and analysis (Takahashi *et al*. 2023).

In this section of the report, we present results from sequencing a commonly used fish eDNA marker (mitochondrial 16S rRNA). The eDNA was extracted from 10 L water samples collected throughout the voyage. We provide an initial overview of fish biodiversity patterns observed in the eDNA dataset and compare results from surface and bottom water samples. As noted above, we also make a direct comparison between the eDNA data and fish trawl catch data.

3.2. Methods

3.2.1. CTD water sampling

Samples of eDNA were collected from 62 CTDs during Voyage 1 and 80 CTDs during Voyage 2. For each sample, 10 L of water was collected from a 12 L Niskin bottle attached to a 36-bottle CTD rosette.

Due to limitations in the number of water samples that could be processed, and to obtain samples relevant to questions being addressed, three combinations of the water column were sampled depending on the local timing of CTD deployment:

(i) Night: Two replicate 10 L water samples were collected from the seafloor at night to coincide with the deployment of the deep tow camera. These samples were primarily used in Section 4 of the report for comparison with eDNA collected by the deep tow camera mounted sampler.

(ii) Morning: One 10 L sample from the bottom depth and one 10 L sample from the surface in the morning before trawl operations.
(iii) Afternoon: Two replicate 10 L samples from the bottom depth, one 10 L sample from the deep-chlorophyll maximum (DCM) layer (identified on the downward CTD trace) and a 10 L sample from the surface in the afternoon. This provided more samples for comparison with the afternoon trawl operation.

Collected water samples were stored at 4°C and filtered within 12 hours of collection. Water samples were filtered using a Masterflex L/S console pump system (Cole-Parmer, USA) through 47 mm, 0.45 µm pore size mixed cellulose ester (MCE) filter membranes (Merck-Millipore, USA), and immediately stored at -80°C. Filtration and sampling equipment was soaked in 10% bleach solution for at least 6 hours between each filtration run or sampling. Prior to filtering, filtration lines were primed with 10% bleach solution for 15 minutes and then flushed thrice with Mili-Q water. Field filtration controls consisted of 5 L samples of Mili-Q water. The total number of samples collected is provided in Appendix 1.

3.2.2. Laboratory Processing of eDNA

For the 10 L filtered water samples DNA extraction and PCR amplification were conducted following methods previously outlined in West *et al*. (2024). DNA was extracted in a dedicated pre-PCR room (CSIRO, Hobart) from filter membranes using the DNeasy Blood and Tissue Kit (Qiagen) with a slightly modified protocol. The filter paper was cut into small pieces, then digested in 540 µL of ATL lysis buffer and 60 µL of Proteinase K for 3 hours at 56°C with gentle shaking of the tubes. After digestion 300 µL of the solution was combined with 300 µL of buffer AL and 300 µL of ethanol, and the standard protocol followed. The eDNA was eluted in 100 µL of buffer AE. Extraction blanks were processed in parallel to detect any cross-contamination.

A 16S mtDNA metabarcoding assay (see Table 3-1) was employed to amplify a marker from fish (teleosts and elasmobranchs) eDNA in the samples. We also processed the same eDNA samples using a broader metazoan marker (COI Leray). Only results from results the fish marker are reported here; data from the COI marker is available for future investigations (Appendix 1).

Quantitative PCR (qPCR) was performed in a two-step library preparation protocol. In the first-stage qPCR, respective primer sequences were flanked by a unique (dual) index (6 bp in length) and a Nextera-style overhang sequence (Forward overhang P5-tag:5′ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-[locus-specific sequence] 3′; Reverse overhang P7-tag:5′ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-[locus-specific sequence] 3′). First-stage qPCR reactions were prepared in a dedicated pre-PCR room (CSIRO, Hobart) in 25 µL volumes containing: 1× QIAGEN Multiplex PCR MasterMix, 0.4 µM of each primers (Integrated DNA Technologies),1× EvaGreen Dye (Biotium), 2 µL of eDNA template (neat), and made to volume with Ultrapure Distilled Water (LifeTechnologies). Each qPCR was performed on a QuantStudio5 Real-Time PCR System (Thermo Fisher Scientific) with the following protocol: initial denaturation at 95°C for 15 min, followed by 40 cycles of 95°C for 30 s, 54°C for 1 min 30 s, and 72 °C for 1 min 30 s, with a final extension for 10 min at 72 °C. First-stage amplicons were sent to the Australian Genome Research Facility (AGRF) for second-stage PCR. In this step Nextera-style index primers (containing overhang, dual 8 bp index tag [i5 and i7], and P5 and P7 regions that bind to the Illumina flowcell) were added. Second-stage amplicons were then cleaned,

pooled, and normalized prior to sequencing on an Illumina NextSeq 2000 platform (PI flow cell: 600 cycle, 2 × 300 bp) at AGRF.

Table 3-1 PCR assay information for eDNA metabarcoding of the 10L samples. These assays were also used to process samples from the deep-towed camera sampler described in Section 4.

PCR assay	*Target taxa*	*Primer name*	*Sequence (5'-3')*	*Target length*	*Annealing (°C)*	*Reference*
16S Fish	Fish	16SF/D	GACCCTATGGAGCTTTAGAC	~180–230 bp	54	Berry *et al.* (2017)
		16S2R-degenerate	CGCTGTTATCCCTADRGTAACT			Deagle *et al.* (2007)
COI Leray	Metazoa	mlCOIintF	GGWACWGGWTGAACWGTWTAYCCYCC	313 bp	46	Leray *et al.* (2013)
		jgHCO2198	TAIACYTCIGGRTGICCRAARAAYCA			

3.2.3. Bioinformatic analysis

Sequencing reads were demultiplexed into their respective samples and trimmed using a custom R script. Sample data were then quality filtered (minimum sequence length = 100 bp, maximum expected errors = 2, no ambiguous nucleotides), denoised, filtered for chimeras, and dereplicated (pool = “pseudo”) using the DADA2 package (Callahan *et al*. 2016) in R (v4.2.1; R Core Team 2024). This generated an amplicon sequence variant (ASV) fasta file and count table; the former was queried against NCBI's GenBank nucleotide database (accessed in August 2023) using BLASTn (minimum percentage identity of 85%, query coverage (qCov) of 96%, maximum target sequences of 20) using Petrichor, a HPC cluster based at the CSIRO. Taxonomic assignment of ASVs was performed using the lowest common ancestor (LCA) Python script in eDNAFlow (Mousavi-Derazmahalleh *et al*. 2021). In this pipeline, BLAST matches required a threshold qCov of 96%, a minimum percentage identity of 85%, and a difference (Diff) of 1. A species-level assignment required a minimum percentage identity of 98% with a qCov of 100%. In cases where an ASV had multiple top hits within 1% similarity of each other, the assigned taxonomy was collapsed back to the lowest common ancestor.

In metabarcoding analyses, the taxonomic assignment is a crucial step but is challenging to fully automate. We carried out several manual curation steps of the taxonomically assigned fish ASV data to check accuracy of the assignments. First the geographic distribution of species was checked (https://fishesofaustralia.net.au/) to ensure that the species potentially occurred in the Southeast Australia – if not the taxa was assigned at genus level or higher based on related species in the area. BLAST matches with < 98.5% identity and assignment to species level were reviewed and changed to genus level if congeneric species with no reference sequence were present in the region. BLAST matches with a good match (>97%) and poor taxonomic resolution were checked to see if errors in the reference database was the cause. In some cases, there were large numbers of ASVs assigned at family-level (e.g. Myctophidae) or to a genus (e.g. *Urolophus*). Rather than collapsing these, we extracted all sequences from the group and constructed a distance-based tree to see if multiple unnamed clusters that differed by >2% were present. If so, each distinct cluster was assigned a distinct name (e.g. *Urolophus* Sp1 and *Urolophus* Sp2) to capture the ASV diversity that likely corresponded to distinct fish species. In our curation process no fish taxonomic

assignments were nonsensical (i.e. we had no assignments to fish outside the region that could not be explained by a potential match to a local related species). The only ASVs that were removed were chimeric sequences or rare variants close to common ASVs. Some curation was subjective and relied on expert knowledge of fish in the region and DNA reference database coverage. Once taxonomic assignment was completed the data was collapsed based on scientific name using the summarise function in the R package dplyr.

3.2.4. Statistical Analyses

Tests for differences in number of species detected per sample between different sampling layers, depths and CTD deployment time using one-way ANOVA in R. Community dissimilarity measurements were conducted on raw and square-root transformed sequence counts using Bray-Curtis dissimilarity, and on presence-absence data using binary Jaccard. Analysis was done using the R package *vegan* (Oksanen *et al*. 2019). Community composition was visualised by nonmetric multidimensional scaling (nMDS). Correlations between environmental variables (Temperature, pressure, salinity, oxygen, phosphate, ammonia, silicate and NOx) were explored using the *envfit* function (*vegan*) and the community response to all environmental variables were tested with PERMANOVA using the *adonis* function in *vegan*. Distance-based ReDundancy Analysis (dbRDA) analyses were carried out via the Primer-E software following the methodology outlined in Section 5.

3.2.5. Comparison between trawl and eDNA sampling

We made comparisons of fish biodiversity data obtained from SEA-MES sites where both trawl and eDNA sampling was carried out.

Trawl data (mass and number of fish species caught at each site) analysed here was made available by the CSIRO team carrying out this research on the SEA-MES voyages. Demersal trawls were conducted at >160 sites during the two voyages using a McKenna semi V-wing fish net. We only used trawl data where eDNA data was collected on the first two voyages (SEA-MES will span four voyages). The trawl data presented here should not be considered representative of the overall SEA-MES project.

To compare the fish taxa observed in trawl versus eDNA sampling we considered only data from afternoon samples collected at sites with paired results from trawl catch and 2 x 10 L eDNA bottom samples. Sequences from the replicate 10 L eDNA samples were combined.

To allow comparison of fish data from the two different sampling techniques we analysed data at the lowest possible shared taxonomic assignment. When species-level identifications were present in both datasets, these were retained. In cases where species-level identification was available in one dataset and this species could not be identified to species-level with the other, the data were collapsed at a higher taxonomic level. An example of this was two species of mackerel (*T. novaezelandiae* and *T. declivis*) identified to species in the trawl data but only to the genus *Trachurus* with the eDNA (due to sequence similarities). Most of the taxonomic adjustments occurred in cases like this, where the eDNA marker provided genus-level identifications and data was collapsed to that level for trawl data. A few uncommon fish taxa were collapsed at higher taxonomic levels (family or order) if they were only identified to that level in one dataset.

There were some situations where shared species-level identification occurred for some species in the genus, and the remaining species were collapsed since they were unresolved in one or both datasets. For example, the stingaree *Urolophus cruciatus* was identified and retained in both datasets, but eDNA could not differentiate other species of *Urolophus*,

therefore other *Urolophus* species in the trawl were collapsed to genus level. We retained taxa that did not have an obvious counterpart in the other dataset (e.g. those fish detected by only one method).

For the summaries presented in this section we have not done any formal statistical testing of the differences between paired trawl and eDNA data.

3.3. Results

3.3.1. Overview of eDNA sequencing results

In total, DNA was sequenced from 285 of the 10 L filtered water samples collected during the two voyages. The two metabarcoding assays (16S Fish and COI Metazoan) applied across these samples yielded 81,616,249 sequencing reads. Here, we report only on the results from the Fish16S marker.

For the Fish 16S marker, we obtained 40,835,971 sequences. A total of 19 negative controls were included (10 field filtration controls, 6 extraction controls and 3 PCR controls). Of these only 5 of the field filtration controls retained more than 30 sequences after bioinformatic processing. The 5 negative controls that did test positive only contained sequences from 1 or 2 fish species, but the number or reads in some cases was similar to numbers seen in samples. All negative controls showed late amplification in the first-round qPCR with a cycle threshold (Ct) value of 37 or higher (Figure 3-1A). This indicates that the concentration of DNA in these controls was low despite the high number of sequences recovered in a few cases. All samples with Ct values ≥ 37 were excluded from the analysis. Following the removal of negative controls and high-Ct samples, a total of 262 samples remained. The mean number of quality-filtered reads per sample for the 16S Fish assay was 152,358 ± 5,822 (SE).

We chose not to remove the six fish species detected in a negative control from all samples, these species (including *Trachurus* sp., *Scomber australasicus*, *Urolophus sp3*) were among the most common fish in the dataset and their removal would produce a clearly biased view by excluding abundant fish. These species were only detected in a small percentage of the negative controls indicating sporadic contamination of DNA from fish commonly caught on the voyage. It is also worth noting the detections occurred in samples with no other DNA present, the sensitivity of the eDNA assays means any trace DNA present is amplified. A more realistic negative control would have DNA at low concentration (similar concentration to eDNA) from exotic fish species (e.g. tropical freshwater fish) and any local marine fish detections seen in these samples would indicate contamination was at a level that would show up in eDNA samples.

The Fish 16S metabarcoding detected 2,675 ASVs (unique assigned sequence variants) across 91 survey sites, corresponding to 230 fish taxa, including 138 species-level taxa, 77 genera-level, 11 family-level, and 4 order-level classifications (Figure 3-2; a table providing a list of all fish taxa is given in Appendix C). The number of species detected per sample approached an asymptote across all samples, suggesting that the sequencing depth was sufficient to capture all fish amplified by the marker (Figure 3-1B). Individual replicates were not subsampled to an equal read depth. The number of species detected per sample ranged from 1 to 31, with a mean of 10.1 ± 0.3 (SE).

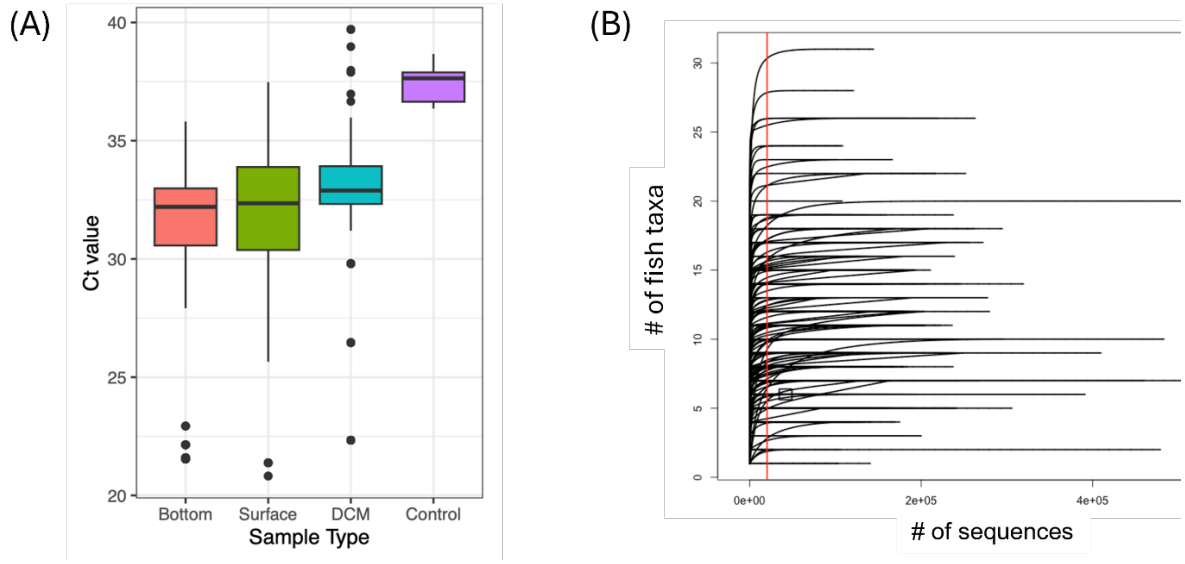


Figure 3-1 (A) Boxplot showing when samples amplified (cycle threshold of samples; n = 285) from fish 16S assay. Samples are grouped by layer sampled from CTD deployment. (B) Species accumulation curves for 10 L eDNA samples (n = 262) from fish 16S assay. Red vertical line indicates minimum read number. Maximum number of reads was truncated at 500,000.

3.3.2. Fish taxa in the eDNA data

In the 230 fish taxa identified with eDNA, the most common sequences recovered came from pelagic schooling species. Mackerel (*Trachurus* sp.) accounted for the highest proportion of total eDNA sequence reads (14.73%) and was the only taxon detected in over half of the samples (59.9%). The Australian sardine (*Sardinops sagax*) had the second highest proportion of total reads and exhibited the highest mean read proportion per sample, averaging 13.06%. Other small, pelagic species frequently detected included redbait (*Emmelichthys nitidus*), blue mackerel (*Scomber australasicus*), and Australian anchovy (*Engraulis australis*). Species that are more bottom associated which featured among the top ten taxa, including gurnard (*Lepidotrigla* sp.), morwong (*Cheilodactylidae* sp.), perch (*Caesioperca* sp.), and tiger flathead (*Platycephalus richardsoni*).

Of the fish identified, 30 were members of the class *Chondrichthyes* (cartilaginous fishes, including sharks and rays). Collectively, Chondrichthyans comprised only 1.31% of total reads, but some were detected in many samples. Based on sequence reads, Melbourne skate *(Spiniraja whitleyi*) was the most abundant, contributing 0.33%. Stingarees were the most frequently detected chondrichthyan group, with *Urolophus* sp4, *Urolophus cruciatus*, *Urolophus* sp3, and *Urolophus viridis* detected in 76, 40, 43, and 46 samples, respectively. Despite their widespread detection, these four stingaree taxa accounted for just 0.49% of total reads.

There were many fish that were uncommon; of the 230 fish taxa detected, 75 (33%) were only detected in a single sample (Figure 3-3). A further 37 (16%) were only detected twice.

The eDNA included a sequence recovered from a handfish (Brachionichthyidae) collected from a site approximately 25 km east of Mallacoota at a depth of 135 m. There were 792 handfish sequences in the sample (for context, 137,000 sequences in total were recovered from this eDNA sample, matching 16 fish species).

The handfish family comprises five genera and 14 extant species; due to limited reference sequences species-level identification is challenging. A search of publicly available sequences in GenBank showed the closest match as the spotted handfish (*Brachionichthys hirsutus*), but with a 12.9% sequence divergence—suggesting it originated from a distantly related handfish. Additional reference sequences from an unpublished Honours thesis (Lawler 1999) showed closer matches (~7% divergence) to the red handfish (*Thymichthys politus*), warty handfish (*T. verrucosus*), and pink handfish (*Brachiopsilus dianthus*). A species-level match typically requires less than 2% divergence indicating the species we recovered does not match any of these species. No further reference sequences are currently available for this family.

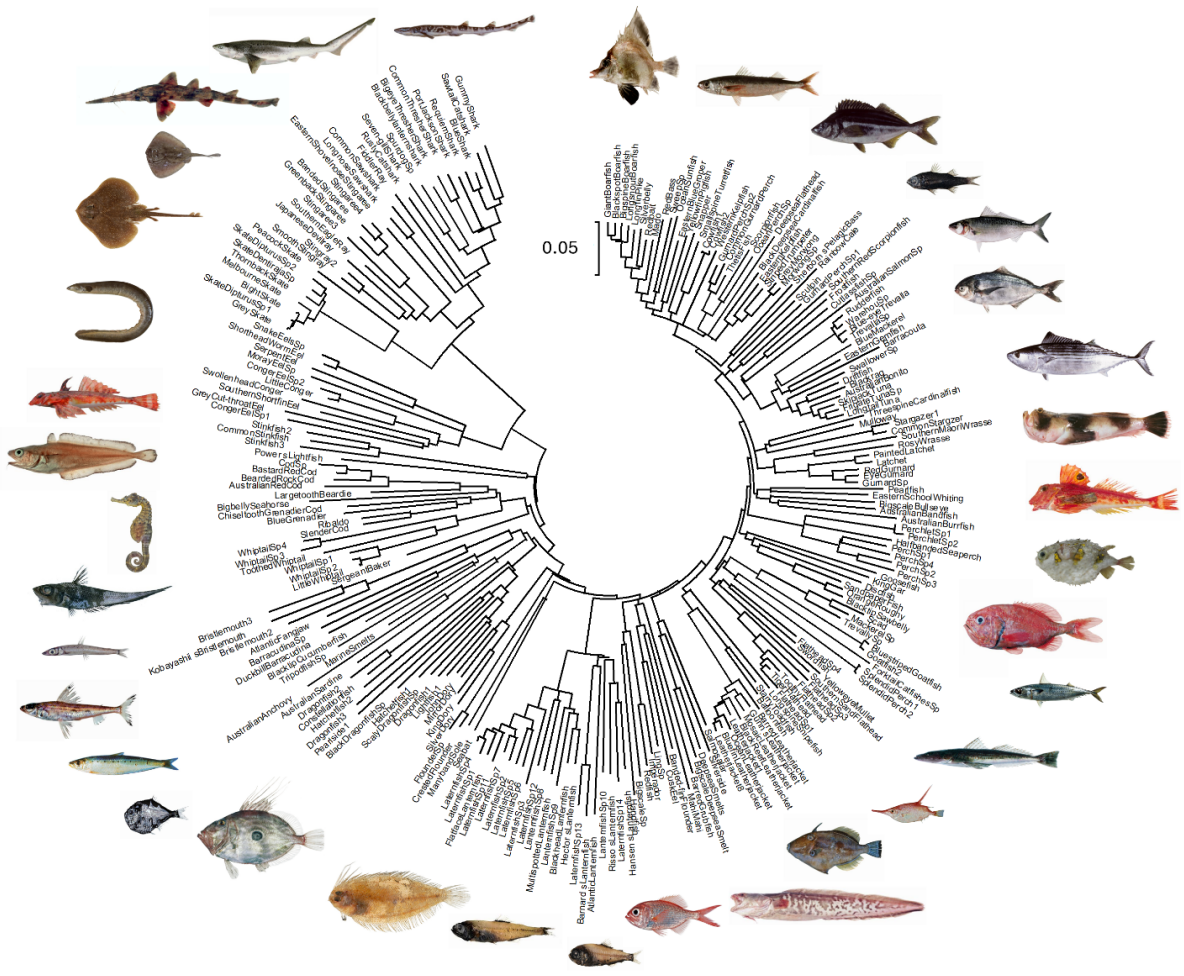


Figure 3-2 The diversity of fish eDNA detected in the 10 L samples shown as a tree based on genetic distance of recovered sequences (mtDNA Fish16S marker). In this Minimum Evolution tree (based on p-distance) all positions with less than 60% site coverage were eliminated, leaving a total of 206 base pairs in the final dataset. Photos show representative taxa (Images: Australian National Fish Collection, CSIRO).

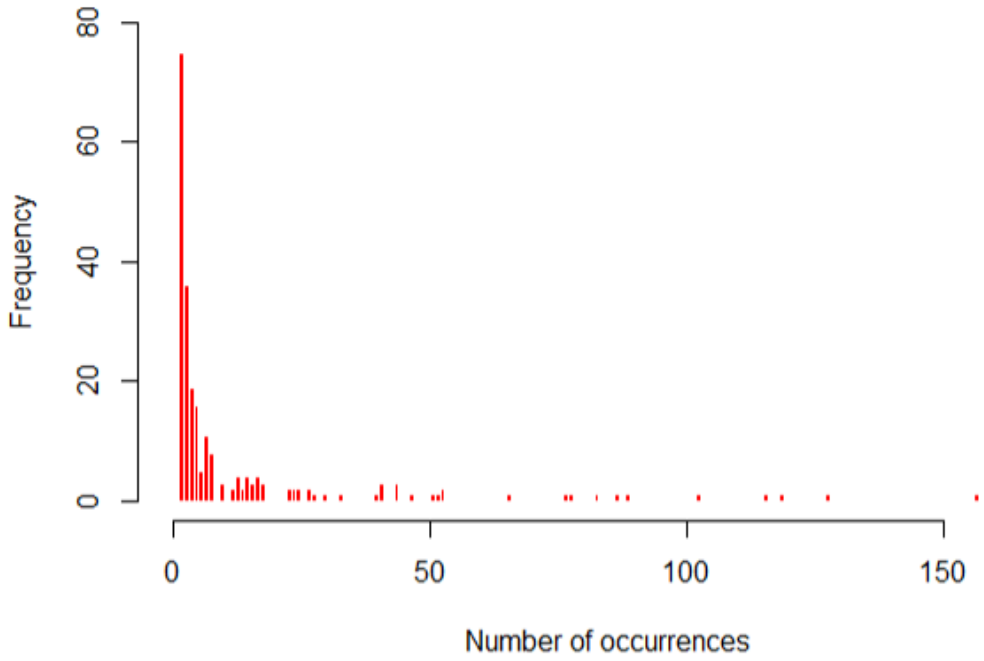


Figure 3-3 Histogram showing number of occurrences of all 230 fish taxa in the 262 eDNA samples. The highest occurrence is *Trachurus* sp. which was found in 157 samples; 75 fish are only observed in a single eDNA sample.

Table 3-2 The 25 most common fish taxa in the eDNA data (ordered by the percentage of total sequence reads of the 230 fish taxa). The complete list of fish is in Appendix C Table 11-1.

	Taxa	Common name	% of reads	Mean % reads per sample	% Occurrence
1	*Trachurus Sp*	Mackerel Sp	14.7	12.0	59.9
2	*Sardinops sagax*	Australian Sardine	14.6	13.0	43.9
3	*Emmelichthys nitidus*	Redbait	9.4	10.2	45.0
4	*Scomber australasicus*	Blue Mackerel	5.5	5.7	48.5
5	*Engraulis australis*	Australian Anchovy	5.4	3.2	19.8
6	*Lepidotrigla Sp*	Gurnard Sp	4.0	4.5	33.6
7	*Cheilodactylidae Sp*	Morwong Sp	3.5	4.0	38.9
8	*Caesioperca Sp*	Perch Sp1	3.3	3.7	19.5
9	*Platycephalus richardsoni*	Tiger Flathead	3.2	3.7	31.3
10	*Maurolicus Sp1*	Pearlside Sp	2.8	3.0	24.8
11	*Seriolella Sp*	Warehou Sp	2.6	3.5	15.3
12	*Paraulopus nigripinnis*	Blacktip Cucumberfish	2.4	2.1	29.4
13	*Parapercis allporti*	Barred Grubfish	1.5	1.5	19.8
14	*Thyrsites atun*	Barracouta	1.3	1.1	6.1
15	*Gnathophis Sp*	Conger Eel Sp2	1.3	1.5	19.1
16	*Verilus anomalus*	Threespine Cardinalfish	1.2	1.2	14.9
17	*Sillago flindersi*	Eastern School Whiting	1.0	0.9	11.1
18	*Lampanyctodes hectoris*	Hector's Lanterfish	0.9	0.9	32.8
19	*Pseudophycis breviuscula*	Bastard Red Cod	0.9	0.6	8.4
20	*Argentina australiae*	Silverside	0.9	1.1	9.2
21	*Helicolenus Sp*	Ocean Perch Sp	0.8	1.0	16.4
22	*Genypterus Sp*	Ling Sp	0.8	0.8	8.8
23	*Nemadactylus douglasii*	Grey Morwong	0.8	0.6	12.2
24	*Foetorepus calauropomus*	Common Stinkfish	0.8	0.9	16.4
25	*Rexea solandri*	Eastern Gemfish	0.7	0.6	10.3

3.3.3. Temporal differences in fish diversity and environmental influences

Nonmetric multidimensional scaling (nMDS) ordination plots of fish eDNA data revealed that samples were primarily grouped by voyage (Figure 3-4), indicating that voyage was the largest source of variation in the dataset. This variation was likely driven by the distinct environmental conditions between the IN2023_V05 (July 2023) and IN2024_V03 (May 2024) voyages (Table 3-3; Figure 3-5). Compared to the 2023 voyage, samples from 2024 had an average temperature 1.5°C higher, with mean concentrations of phosphate, silicate, NOx, and ammonia increasing by 33%, 59%, 156%, and 300%, respectively. In contrast, salinity and dissolved oxygen were 10% and 11% lower in 2024 samples. Additionally, during the 2023 voyage eDNA samples were taken at an average depth 54.6 m greater than the 2024 voyage. PERMANOVA analysis confirmed that samples grouped by voyage, with Bray-Curtis dissimilarity based on raw sequence counts ($F_{261,262} = 7.32$, $R^2 = 0.027$, $p < 0.001$), square-root transformed sequence counts ($F_{261,262} = 9.95$, $R^2 = 0.037$, $p < 0.001$), and Jaccard distances on presence-absence data ($F_{261,262} = 13.903$, $R^2 = 0.051$, $p < 0.001$).

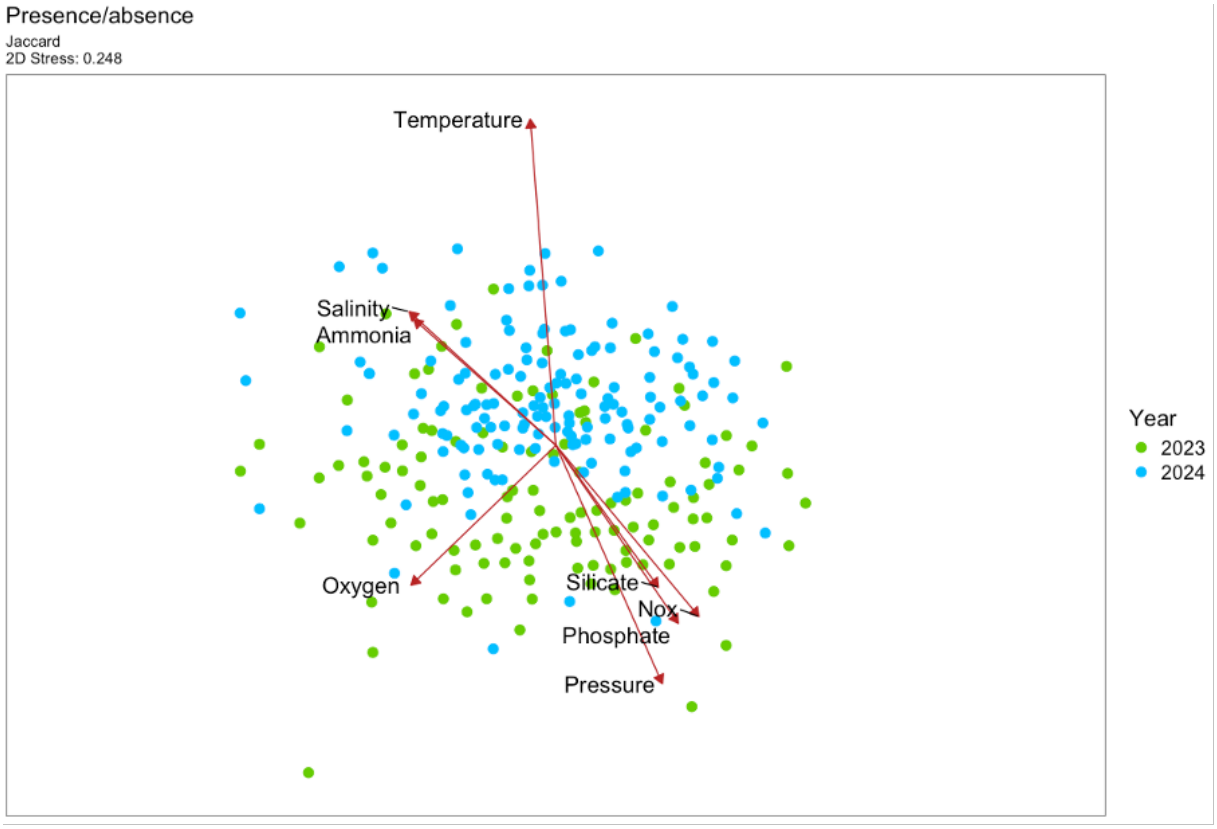


Figure 3-4 Nonmetric multidimensional scaling ordination plots of presence-absence transformed fish eDNA data. Samples are grouped by voyage IN2023_V05 (green) and IN2024_V03 (blue).

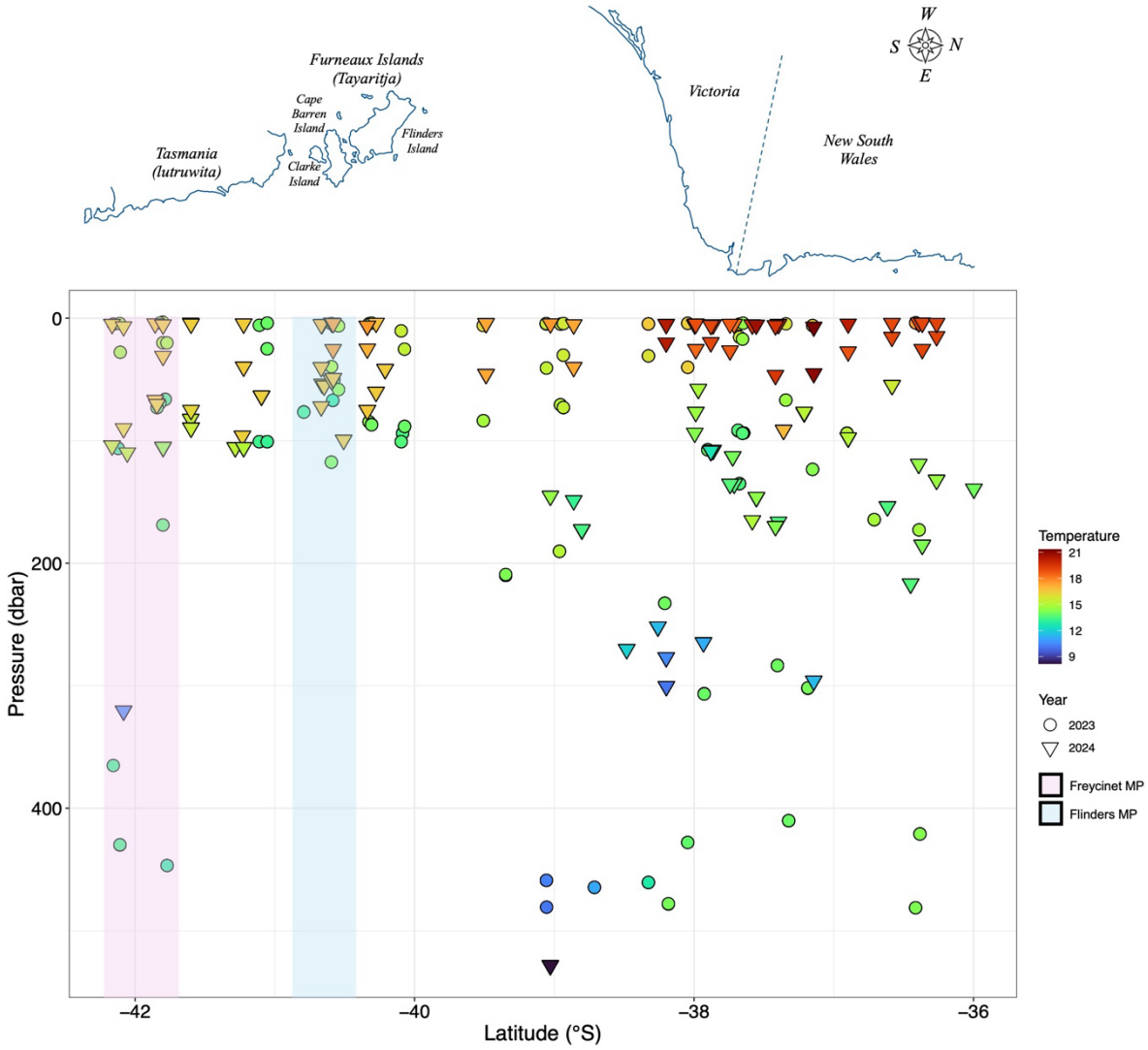


Figure 3-5 Temperature, depth and latitudinal profile of the 10 L eDNA samples (n = 262) analysed in this study. Circles represent samples collected in 2023 and triangles represent samples collected in 2024. Temperature is coloured by a gradient from 9°C (blue) to 21°C (red). Pink shaded area represents the latitudinal boundaries of the Freycinet Marine Park and the blue shaded area represents the same for Flinders Marine Park.

Table 3-3 Summary of environmental conditions for IN2023_V05 (July 2023) and IN2024_V03 (May 2024). Mean values and standard error reported from CTD-based measurements taken from the water where eDNA was collected.

Voyage	Pressure (dBar)	Temperature (°C)	Salinity (PSU)	Dissolved Oxygen (mM)	Phosphate (mM)	Silicate (mM)	Ammonia (mM)	NOx (mM)
IN2023_V05	151.43 ± 14.66	14.19 ± 0.11	35.51 ± 0.02	246.33 ± 0.97	0.40 ± 0.02	1.47 ± 0.10	0.04 ± 0.01	3.90 ± 0.28
IN2024_V03	96.85 ± 8.14	15.68 ± 0.22	35.40 ± 0.02	221.81 ± 1.28	0.53 ± 0.03	2.34 ± 0.15	0.12 ± 0.02	6.09 ± 0.44

3.3.4. Sampling depth and latitudinal differences in fish diversity

To remove the overarching influence of voyage, we continued analysis of the fish eDNA occurrence data for each voyage separately. nMDS plots and PERMANOVA analyses revealed a significant, albeit weak, correlation in samples grouped by depth (Figure 3-6). Samples from 2024 exhibited slightly stronger depth-related clustering ($F_{139,143} = 5.42$, $R^2 = 0.13$, $p < 0.001$) compared to those from 2023 ($F_{113,117} = 2.92$, $R^2 = 0.09$, $p < 0.001$).

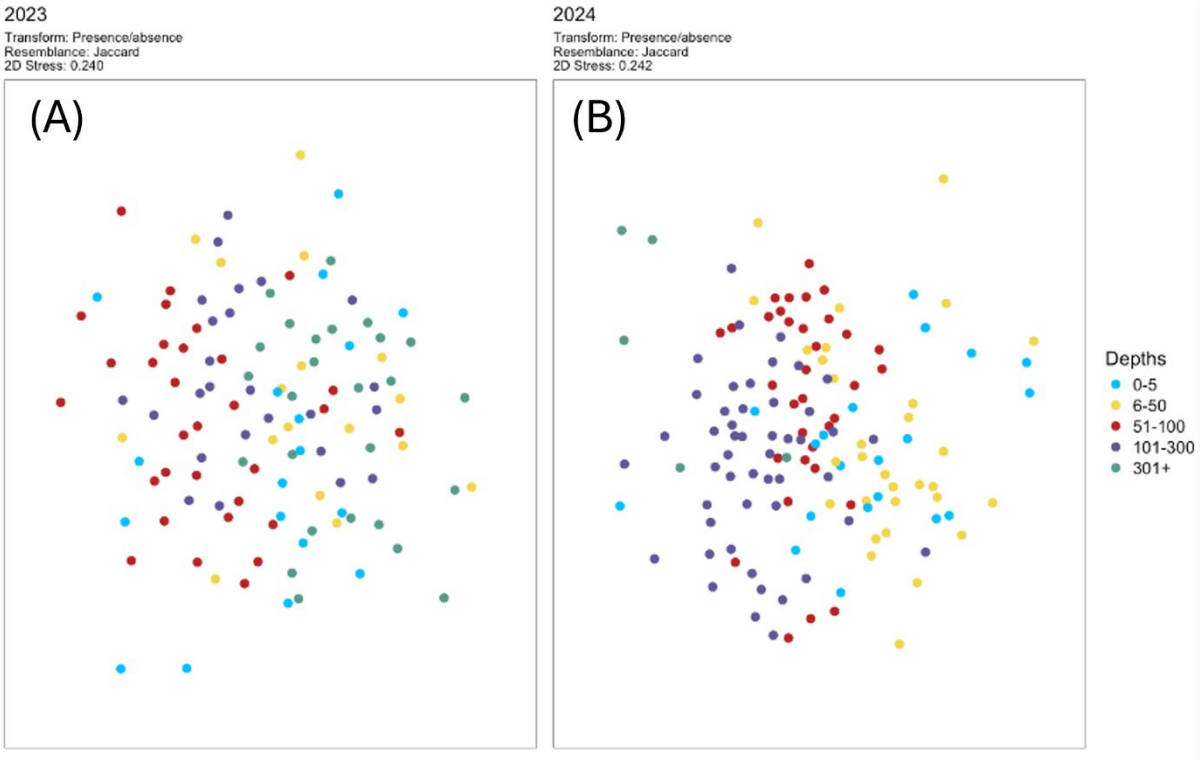


Figure 3-6 Nonmetric multidimensional scaling ordination plots of presence-absence transformed data. Samples are grouped by binned survey depths 0-5 m (blue), 6-50 m (yellow), 51-100 m (red), 101-300 m (purple), and 300+ m (green) in (A) IN2023_V05 and (B) IN2024_V03.

A significant difference was observed in the number of fish species detected between surface and bottom samples across all 10 L eDNA samples (Figure 3-7). This comparison contains more observations from the bottom since it includes night samples, for which replicate 10L samples were taken only at the bottom. We investigated the relationship in more detail below with more balanced sampling.

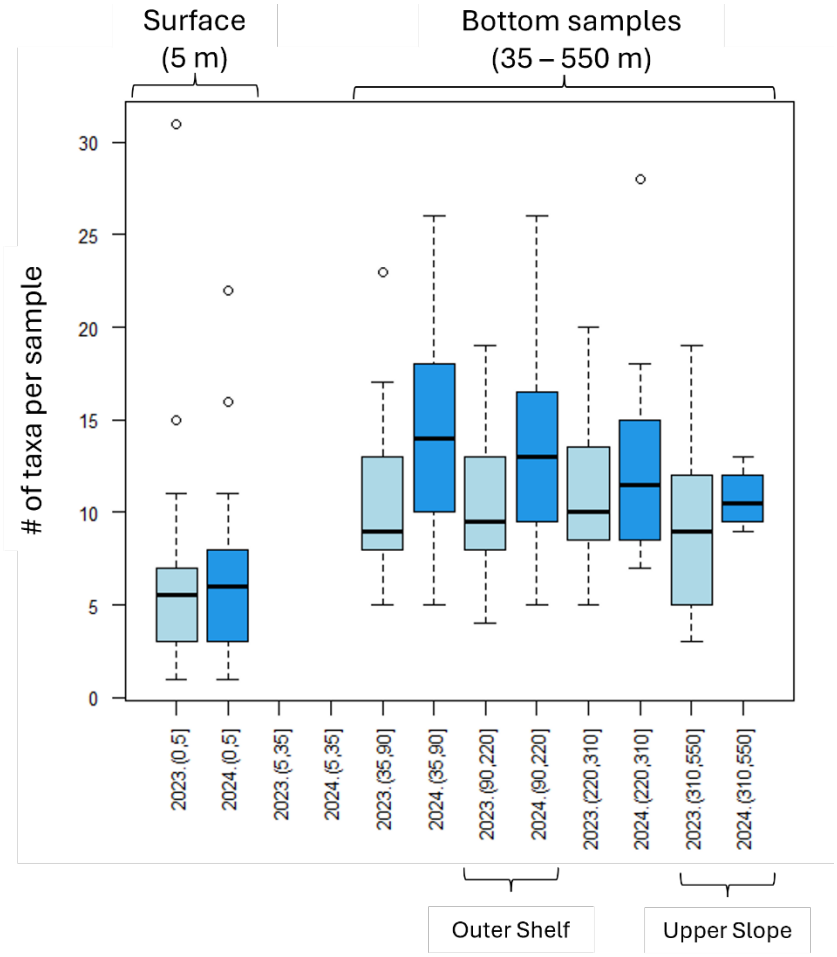


Figure 3-7 Boxplots showing the number of fish taxa observed at different sampling depths. All 10 L surface and bottom samples were included. Surface samples were occasionally taken slightly below 5 m but pooled here for clarity. Bottom samples are shown at different depths to highlight fish diversity at depth bands corresponding to biomes (Outer Shelf and Upper Slope) based on previous records of fish distributions (Last *et al*. 2005).

To further examine species detection across different sampling depths, we restricted our analysis to samples collected from morning and afternoon CTD deployments when we had paired samples from different depth layers (Figure 3-8). In the morning CTD deployments, species detection significantly differed between bottom and surface samples in both 2023 ($F_{1,25} = 12.14$, $p < 0.01$) and 2024 ($F_{1,25} = 7.75$, $p < 0.05$). Morning bottom samples detected 10.2 ± 1.3 species in 2023 and 11.5 ± 1.2 species in 2024, while morning surface samples detected 4.8 ± 0.7 species in 2023 and 6.6 ± 1.3 species in 2024.

In the afternoon CTD deployments, species detection varied significantly between sampling layers in 2024 ($F_{2,76} = 28.48$, $p < 0.001$) but not in 2023 ($F_{2,45} = 1.22$, $p = 0.30$). Afternoon

bottom samples detected 10.6 ± 0.8 species in 2023 and 13.6 ± 0.9 species in 2024, while DCM samples detected 7.8 ± 1 species in 2023 and 5.5 ± 0.4 species in 2024, and surface samples detected 9.9 ± 2.3 species in 2023 and 6.25 ± 0.8 species in 2024.

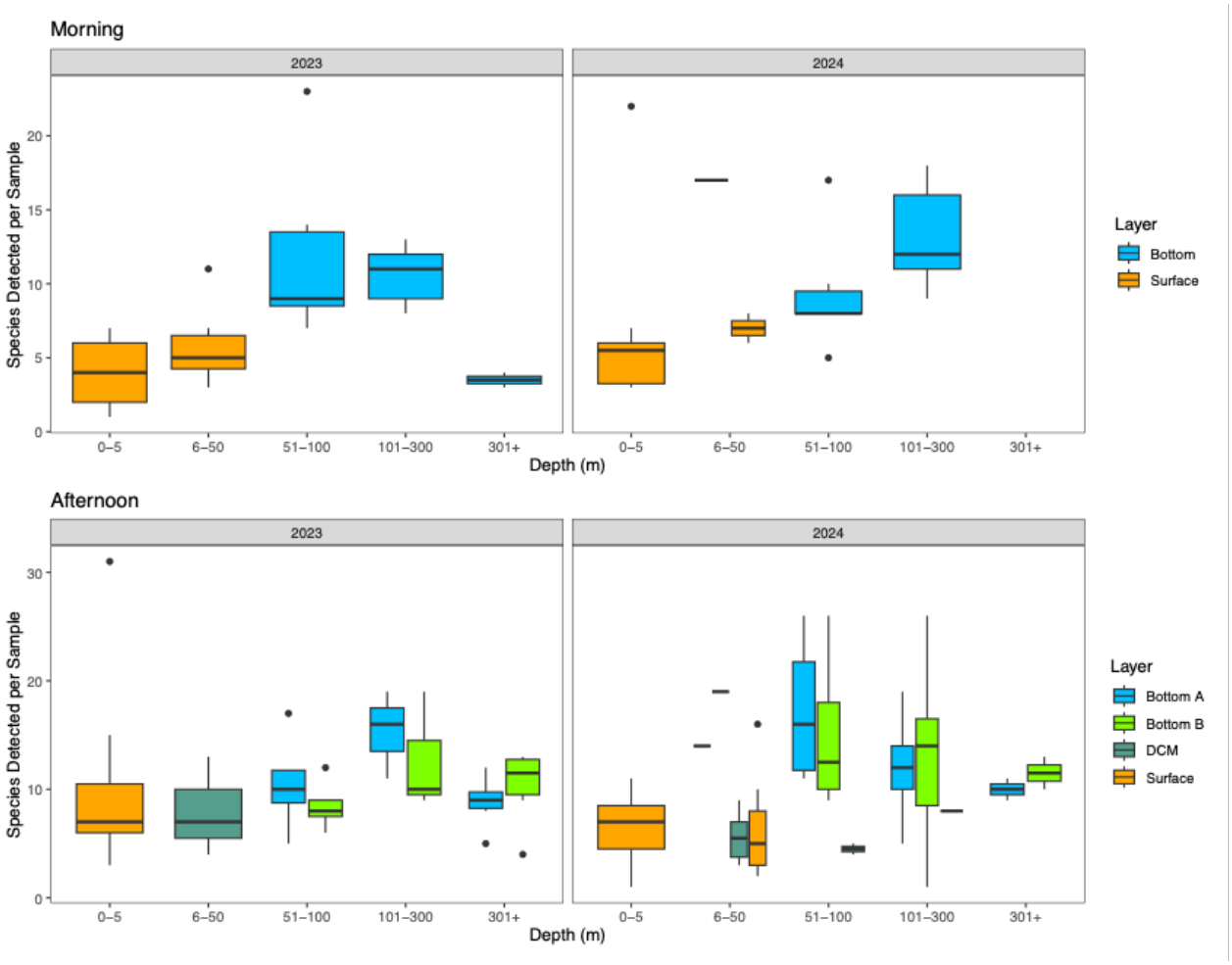


Figure 3-8 Boxplots showing species detected per sample across binned survey depths (0-5, 6-50, 51-100, 101-300, 300 + m) grouped by sampling layer and voyage Top plot shows data from morning samples (paired surface and bottom samples taken) and bottom plots shows afternoon samples (paired surface, DCM and replicate bottom samples taken).

Using the paired sampling design, we also compared overall fish diversity detected by eDNA across the two 10 L replicate samples and among different depth strata (Surface, DCM, and Bottom).

In the night CTD deployments, a total of 155 fish taxa were detected (Figure 3-9). Two replicate bottom samples were collected during each deployment, detecting 120 and 122 taxa, respectively. Despite similar taxon counts, the proportion of taxa shared between the two groupings was only 56.1%, meaning 43.9% of taxa were unique to either replicate. Differences in 10 L bottom samples were further investigated in Section 4 of the report and show a median overlap of ~28% in shared taxa between paired bottom samples (Figure 4-3).

Morning CTD deployments yielded 99 fish taxa in total (Figure 3-9). Bottom samples (n = 28) accounted for 88.9% of the total taxa. Of these 42.4% were unique – nearly four times more

unique taxa than surface samples. Surface samples (n = 27) detected 57.6% of the total taxa observed during morning sampling.

In the afternoon CTD deployments, comparisons between the 10 L replicate samples (Bottom A and B) showed similar results to the night samples, so we chose to only results from Bottom B to compare to other depth layers. In the three layers 158 fish taxa were identified (Figure 3-9). Bottom samples detected 78.5% of the taxa and three times as many unique taxa as surface samples, and nearly six times as many as DCM samples. Surface (n = 31) and DCM (n = 31) samples detected 49.0% and 41.0% of total afternoon taxa, respectively.

Figure 3-9 Venn diagrams showing the proportion of taxa detected in the respective sampling layers for CTD deployments at night, morning and afternoon.

Preliminary analysis was conducted to further examine environmental drivers influencing fish community composition. In this case the dataset was divided by the two voyage and two depth category (surface and DCM or bottom) and analysed using dbRDA – following the approach used for the plankton community analysis in Section 5. Most of the measured environmental variables (Table 3-3) significantly influenced fish community structure across

all four groups. Additionally, latitude had a significant effect on fish communities at both depth categories in each year (Figure 3-10 shows an example dbRDA plot).

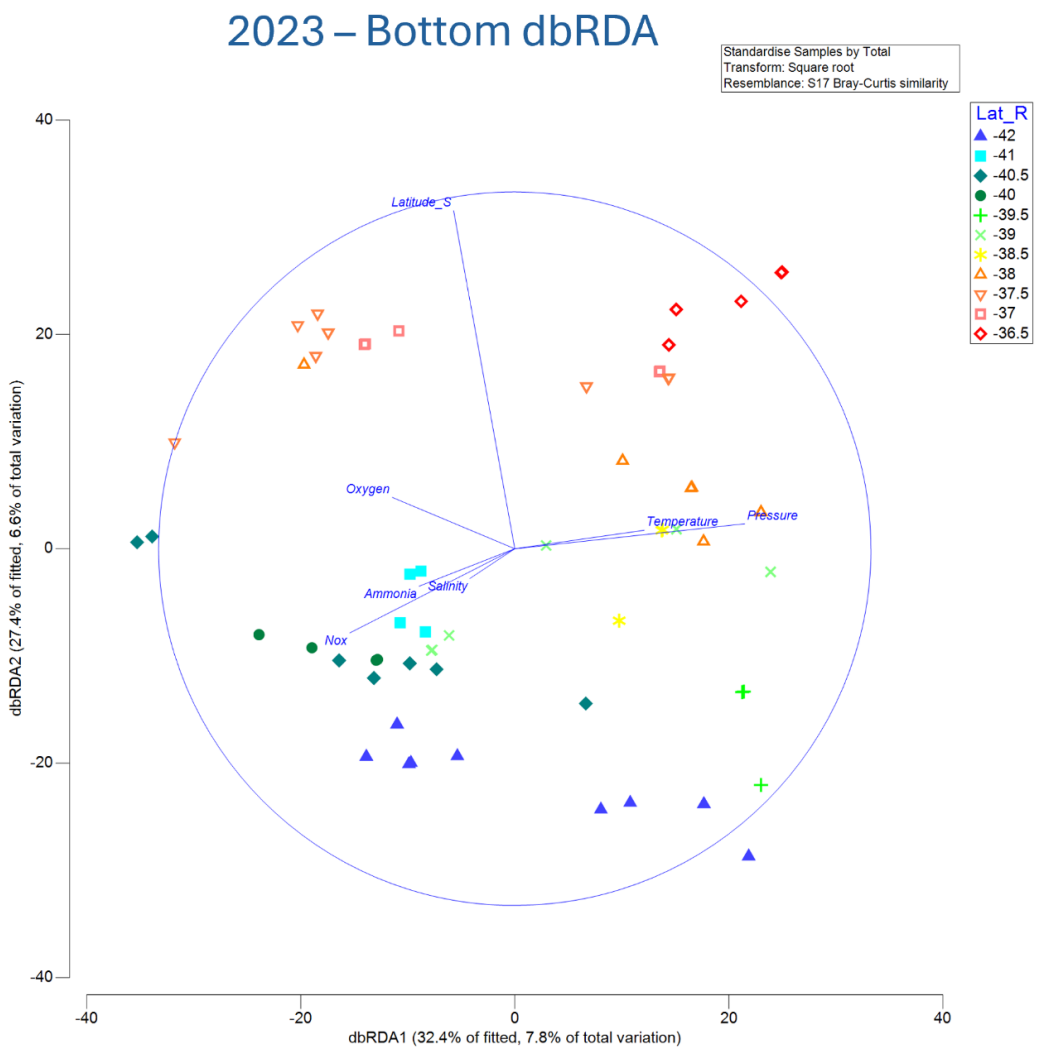


Figure 3-10 Distance based redundancy analysis (dbRDA) plots of fish communities, in the bottom sampled eDNA from the first voyage, showing the spread with location (latitude) highlighted and the environmental drivers indicated by vectors (NOx = Nitrate. Pressure = depth).

3.3.5. Comparison of fish eDNA results with trawl catches

The comparison between these datasets was done at sites where trawls were carried out and 2 x 10 L eDNA samples were collected near the seafloor. This includes a total of 38 sites where we have paired samples across the two SEA-MES voyages (18 sites in 2023 and 20 in 2024). At these sites, the total trawl catch was 8407 kg, from which 170 fish taxa (145 to species-level) were identified. Identifications were based on morphology and COI DNA barcoding of tissue samples taken from voucher specimens. The eDNA samples from the 38 sites produced 12 million sequences which were assigned to 165 fish taxa (99 to species-level). Despite similar number of fish taxa being identified in trawl and eDNA samples (170

versus 165) the overlap in the recorded fish species was quite low. With both methods together there were 182 species-level identifications at the 38 sites; only 62 (34%) of these species were detected by both techniques, 83 were detected in the trawl data but not the eDNA, and 37 were detected in the eDNA data but not the trawl.

Some of the difference in taxa being detected between methods was due to inability of eDNA to provide species-level identifications for some fish. To remedy this and make the data sets more comparable taxonomic data were collapsed (i.e. in cases where a match is likely but at different taxonomic resolution, data were converted to have a shared taxonomic rank; see methods for details). After this step, 200 fish taxa (most at genus- or species-level) were identified in total; 93 (46.5%) were in both trawl and eDNA samples. In this adjusted data, the number of unique fish taxa detections was 55 in the trawl data and the 52 in the eDNA.

Using the collapsed data the mean number of fish taxa identified at a site was higher in trawls compared to eDNA (Figure 3-11A) and there was no clear relationship between diversity measured by the two methods at a particular site (Figure 3-11B). The overlap in the list of taxa being identified at a particular site by trawl and eDNA was quite low at only 20%.

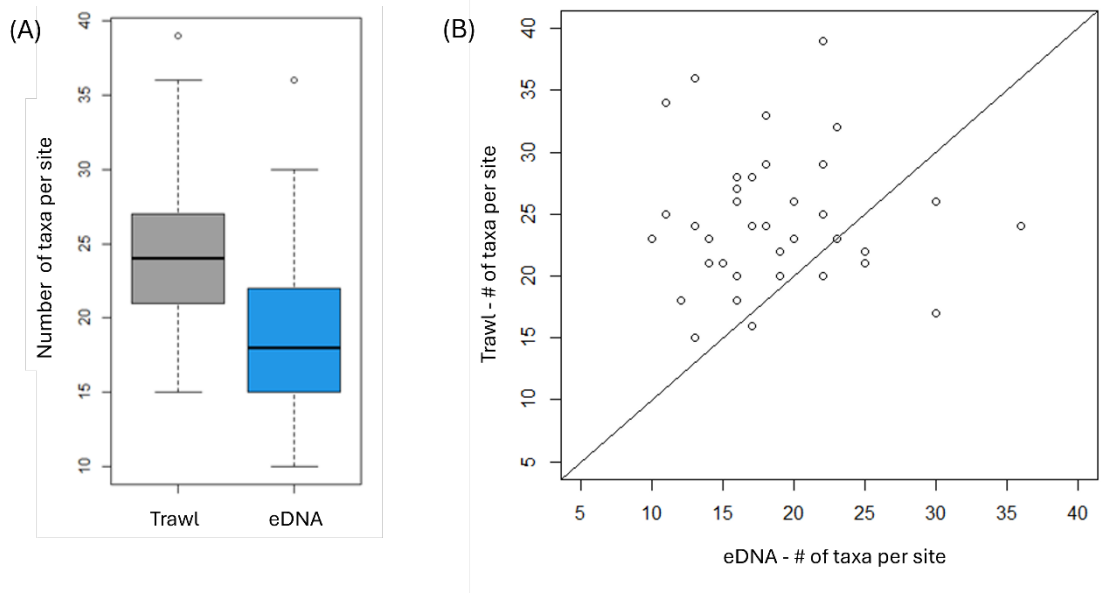


Figure 3-11 Comparison of the number of fish taxa observed in trawl versus eDNA sampling at 38 sites with paired sampling. Only sites with 2 x 10 L eDNA bottom samples were included and eDNA counts were pooled for the analysis. (A) boxplot showing diversity in the two data sets (B) scatter plot showing no correlation between diversity measured at sites (line of equality is plotted).

Another way to look at the datasets is to compare biomass measurements from the trawl data (percentage by mass of the total trawl catch) with the proportions of eDNA recovered (the mean percentage of sequence counts for each fish taxa at the 38 sites). Both methods had mackerel as the top species (Table 3-4). The top 20 taxa make up 84% of the trawl biomass and 78% of the eDNA sequence reads. Across the top 20 taxa, 9 fish taxa were share between the methods. This included blacktip cucumberfish (*Paraulopus nigripinnis*),

redbait (*Emmelichthys nitidus*) and familiar fisheries species such as tiger flathead (*Platycephalus richardson*i) and warehou (*Seriolella* sp.).

One of the clear differences between the datasets was that the Chondrichthyes (sharks and rays) were prevalent in the high biomass species in trawls – none of these were in the top 20 of eDNA sequence proportions Table 3-4). Example large biomass trawl species include: stingaree (*Urolophus* sp.), skate (*Dentiraja* sp.) and swellshark (*Cephaloscyllium* sp.). In contrast, the eDNA data had a large proportion of sequence reads from several small baitfish that were not dominant in trawl biomass. Examples are blue mackerel (*Scomber australasicus*), Australian sardine (*Sardinops sagax*), Pearlside (*Maurolicus* sp.) and Australian anchovy (*Engraulis australis*).

Table 3-4 Comparison of the percentage by mass of the total trawl catch versus the mean percentage of fish eDNA for 38 sites were paired sampling occurred. The top 20 taxa for each method are shown. The Chondrichthyes are highlighted in purple; this group is dominant in the mass of trawl catch but was not found in high abundance in the eDNA.

	Top 20 Trawl	%	Top 20 DNA	%
	Trachurus	23.1	*Trachurus*	*14.3*
	Paraulopus nigripinnis	7.9	*Emmelichthys nitidus*	*10.1*
	Macruronus novaezelandiae	5.9	*Caesioperca*	*6.3*
Stingaree	*Urolophus*	5.5	*Scomber australasicus*	*5.7*
	Seriolella	4.9	*Lepidotrigla*	*5.5*
	Emmelichthys nitidus	4.1	*Sardinops sagax*	*3.9*
	Uranoscopidae	4.1	*Nemadactylus*	*3.9*
Skate	*Dentiraja*	4.0	*Platycephalus richardsoni*	*3.6*
	Lepidotrigla	3.8	*Maurolicus*	*3.6*
	Platycephalus richardsoni	2.8	*Paraulopus nigripinnis*	*3.3*
	Nemadactylus	2.6	*Seriolella*	*2.9*
Swellshark	*Cephaloscyllium*	2.2	*Engraulis australis*	*2.2*
	Kathetostoma	2.0	*Parapercis allporti*	*2.2*
	Zenopsis nebulosa	1.9	*Argentina australiae*	*2.1*
Skate	*Spiniraja whitleyi*	1.7	*Thyrsites atun*	*2.1*
	Helicolenus	1.6	*Lampanyctodes hectoris*	*1.5*
	Genypterus	1.5	*Genypterus*	*1.4*
Catshark	*Asymbolus*	1.4	*Verilus anomalus*	*1.3*
Eagle Ray	*Myliobatis tenuicaudatus*	1.4	*Myctophidae*	*1.1*
Shark	*Squalus*	1.2	*Gnathophis*	*1.1*
		83.6 %		78.1%

Shared

Chondrichthyes

When the proportion by mass of the total trawl catch is plotted against the mean proportion of fish eDNA at the 38 sites there is not a strong overall correlation (Figure 3-12) and the plot is dominated by rare fish taxa, many of which were only identified in one dataset.

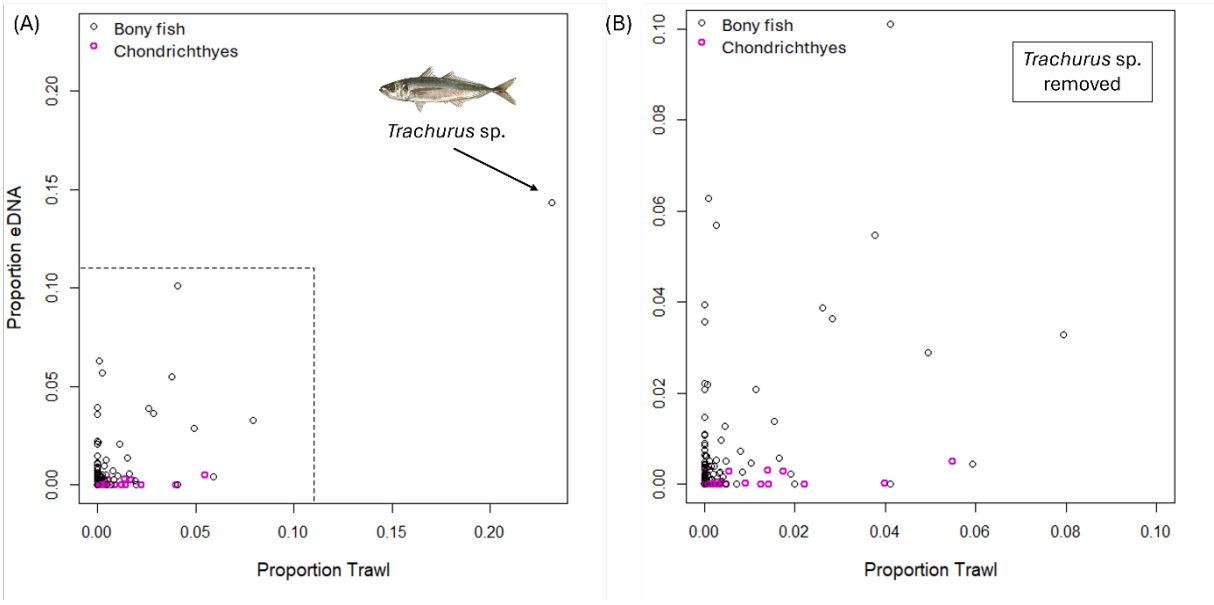


Figure 3-12 Comparison of fish taxa showing the proportion by mass of the total trawl catch versus the mean proportion of fish eDNA across samples at the 38 sites that had paired sampling. The top 20 species detected by each method is shown in Table 3-4. The Chondrichthyes (sharks and rays) are highlighted in purple. Plot (A) shows all fish taxa, plot (B) excludes *Trachurus* to spread out remaining points.

We can also look at how similar abundance estimates are between trawling and eDNA by comparing frequency of occurrence data. This comparison provides detections out of 38 shared sites. The resulting relationship between trawl and eDNA data shows a modest improvement (Figure 3-13), indicating the occurrence data is smoothing some of the technique specific biases (e.g. some of the Chondrichthyes were detected at many sites even though the number of sequence reads was small).

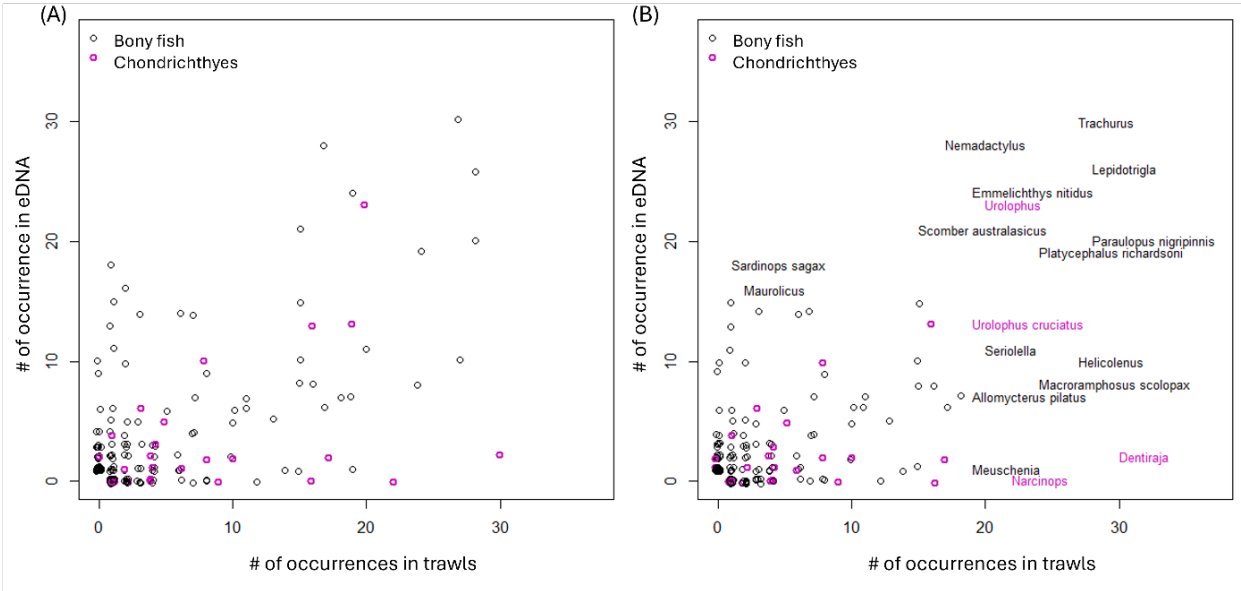


Figure 3-13 Comparison of fish taxa showing the number of occurrences in trawl versus eDNA across samples collected at the 38 sites with paired sampling. The Chondrichthyes (sharks and rays) are highlighted in purple. Plot (A) shows all fish taxa, plot (B) replaces points with names for taxa observed in >18 sites for trawl or >15 sites for eDNA.

3.4. Discussion

In this section of the report, we present the fish eDNA data collected from 10 L samples with the mtDNA 16S marker. We manually curated the fish eDNA taxonomic assignments to improve the accuracy. These steps (outlined in the methods section) were time consuming and somewhat subjective (e.g. reliance on expert knowledge of the regional fish fauna). This curation of data is a challenge that makes the adoption of eDNA data more difficult, but this is similar the effort required for taxonomic experts to accurately identify the diverse range of fish in a trawl catch (using morphological features and tissue DNA barcoding). The process of identifying Australian fish via eDNA is expected to become significantly easier with the upcoming release of marine vertebrate mitochondrial DNA reference sequences from CSIRO's National Biodiversity DNA Library, scheduled for late 2025. Additionally, the accuracy of automated taxonomic assignment tools continues to improve (Bayer *et al*. 2025, Polanco *et al*. 2025).

With eDNA we detected a total of 230 fish taxa, with the majority of sequences originating from small schooling species such as mackerel, sardine, redbait, and anchovy. Other species in the top 10 fish represented by the eDNA included bottom-associated fish like gurnard, morwong, perch (*Caesioperca* sp.), and flathead. The total included 30 taxa from the class Chondrichthyes (cartilaginous fishes, including sharks and rays).

Approximately one-third of the detected taxa were recorded only in a single sample, reflecting the long-tail of the species abundance distribution and the fact that our sampling was spread across several different habitats (i.e. different depths and geographic locations). This suggests that the current eDNA sampling effort is sufficient to reliably detect common fish, but increased number of samples (or larger water volume) would capture more information on the rare fish species.

Many interesting fish were recovered in the eDNA data, this included several large pelagic species – swordfish (*Xiphias gladius*), mahi mahi (*Coryphaena hippurus*), Blue Shark (*Prionace glauca*) and two species of thresher shark (*Alopias* sp.). At the opposite end of the spectrum, one of the eDNA sequences that was recovered came from a handfish (family Brachionichthyidae). The species could not be determined due to lack of reference sequences; however, we could discount the 5 handfish species with reference sequences since matches were between 88-94% similarity (too different to be species matches). The family has 14 described species, so the eDNA could come from any of the 9 species without reference data, or an undescribed species. Interestingly in the two SEA-MES voyages covered in our eDNA project only a single handfish was identified using other methods. This one was seen in the deep tow camera images (Figure 3-14) a few hundred kilometres south of where we detected the eDNA. It is believed to be an image of the narrowbody handfish (*Pezichthys compressus*) known from only two specimens and with no genetic data available.

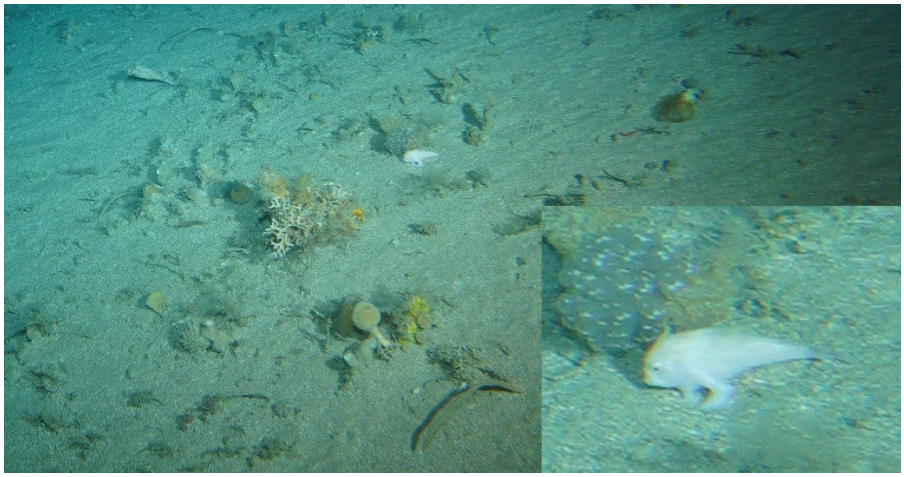


Figure 3-14 This handfish was seen on footage from the RV Investigator's deep tow camera it is thought to be a narrowbody handfish (*Pezichthys compressus*), a species known only from two specimens. One eDNA sequence matching handfish was collected but currently cannot be matched to a species due to lack of reference sequences (Image: CSIRO).

The primary source of variation observed in the fish eDNA data was the difference between the two SEA-MES voyages. These voyages differed in several key aspects: the first took place in winter (July 2023), while the second occurred in autumn (May 2024) with seasonal differences in environmental variables such as water temperature. Sampling locations also varied, with the first voyage having more deep-water sites and the second more shallow northern sites (see Figure 3-5). The latitudinal range of the voyages does not intersect any major biogeographic breaks in offshore demersal fish communities; the study area spans the Tasmanian Province in the south and extends into the South-Eastern Transition zone (Last et al. 2005). Accordingly, the latitudinal signal in the eDNA data was relatively weak. Bottom samples ranged from approximately 80 to 500 m in depth, covering both the outer continental shelf (90–220 m) and upper slope (310–520 m) fish biomes (Last *et al*. 2005). While there was some differentiation of eDNA-derived fish communities by bottom depth, traditional indicator species—primarily Chondrichthyans—were not well represented in the eDNA data, so differentiation focussed on different fish in the community. Previous research has shown that eDNA data is effective at detecting biogeographic breaks (e.g. West *et al*. 2021), especially when analyses incorporate the full range of species detectable through this method (such as the datasets presented in Section 5 and Section 6 of this report).

3.4.1. Fish diversity in bottom versus surface collected eDNA

When we compared surface versus bottom collected eDNA we see a significant differences in the number of taxa detected. Our findings indicate a near twofold increase in the number of taxa detected per sample in bottom samples compared to those collected at the surface. This follows expectation if eDNA is not mixed well through the water column since demersal fish, which live on or near the seabed, generally exhibit higher species diversity compared to pelagic fish. This difference is largely due to the greater habitat complexity found near the bottom, offering a wider range of niches for demersal species to occupy.

Several additional factors may explain the differences in the diversity of fish eDNA detection between benthic and surface water samples. In marine ecosystems, key sources of eDNA include biological excretions and propagules such as scales, eggs, sperm, and faeces

(Barnes & Turner 2014; Diaz *et al*. 2020). Much of this eDNA-rich material is expected to sink, potentially leading to higher species detection rates at greater depths, where both benthic and pelagic species may be represented (Sakata *et al*. 2020). However, our study found a peak in species detection at depths of 100–300 m, with deeper samples showing detection rates similar to those at the surface. A detailed analysis of which species are detected at each depth stratum has not yet been conducted, but such an analysis could clarify whether pelagic species are being detected in bottom samples and vice versa.

Another contributing factor may be the influence of environmental variables on DNA persistence in the water. Water temperatures, UV radiation, and microbial activity at the surface are higher than at greater depths and these factors are known to accelerate the degradation of DNA molecules (Eichmiller *et al* 2016; Andruszkiewicz *et al.* 2020), whereas colder temperatures tend to promote their preservation (Barnes *et al.* 2014). Consequently, the lower species detection rate at the surface may be attributable to a reduced quantity and quality of amplifiable DNA. Therefore, the discrepancies in detection rates across the water column could potentially be mitigated by employing DNA extraction methodologies optimized for the recovery of degraded or damaged genetic material (Wilcox *et al.* 2018), and mini-metabarcoding primers designed to amplify shorter, fragmented DNA sequences (Miya *et al.* 2015). We did observe a higher number of fish species being recovered with a shorter marker used in the in the ToL metabarcoding approach, suggesting the fish eDNA is quite degraded (see Figure 6-8).

Regardless of the exact drivers of differences in eDNA-based diversity between surface and bottom, biodiversity assessments should aim for a vertically integrated sampling. Even though some taxa can be detected distant from their known habitat (e.g. Martino *et al*. 2025) our data show sampling near the bottom at offshore marine sites is essential to capture the full extent of fish biodiversity. In pelagic layers sampling may not need to be on a very fine scale, we noted that there was a negligible difference in metazoan taxonomic richness detected between surface and deep chlorophyll maximum (DCM) water samples. This finding implies that the sampling of both layers may not be a prerequisite for comprehensive biodiversity assessments of marine metazoans, particularly in studies focused on fish. While the DCM layer is frequently targeted in oceanographic research due to its characteristically elevated levels of microbial productivity, which can, in turn, influence the spatial distribution and activity of certain fish species (Sabatés *et al*. 2007), our results demonstrate that at least 90% of the taxa we identified were represented by taking only surface and bottom water samples.

Comparable levels of species richness were observed across replicate 10 L bottom water samples collected concurrently at identical locations. However, despite the similar overall richness, the taxonomic overlap between these replicates was surprisingly low (56 %). Section 4 of the report shows a median overlap of ~28% in shared taxa between replicate 10 L bottom samples (Figure 4-3).This low repeatability is likely attributed to the presence of rare or low-abundance taxa that may be inconsistently captured across individual replicates (McGill *et al.* 2007; Stauffer *et al.* 2021). We can investigate this further by looking to see if the fish being missed in the replicates have low read counts when they are detected. This inter-replicate variation underscores the inherent heterogeneity of the aquatic environment at fine spatial scales (Bessey *et al.* 2020; Harrison *et al.* 2019) and reinforces the principle that increased sample volume generally correlates with enhanced species detection (Stauffer *et al.* 2021).

3.4.2. Comparison of fish eDNA results with trawl catches

Numerous studies have now compared fish eDNA datasets with traditional trawl catch data. (Thomsen *et al*. 2016, Stoeckle *et al*. 2021, Afzali *et al*. 2021, *He et al*. 2023). While some have found a strong correlation between eDNA results and trawl catches, outcomes vary across studies depending on methods used and the ecological context. Nonetheless, several general conclusions have emerged, which are mirrored in our findings.

One general point is that it is not straightforward to make a direct comparison between eDNA datasets and fish trawl catches. This is partly because the recorded taxonomic resolution of fish taxa differs between methods (mostly due to incomplete DNA reference database and the limits of taxonomic information present in short eDNA markers). Because of this, comparisons usually only include a subset of the fish taxa; in our case the two data sets were converted to have a shared taxonomic rank, limiting the resolution of the comparison. Deciding on what summary data from each survey type to use is also not simple. Looking at diversity of fish in each dataset is one possibility (again this can be limited by differences in taxonomic resolution), but looking at a measure of fish abundance is usually what is of interest. For eDNA abundance this can be frequency of occurrence or relative number of sequences recovered (Deagle *et al*. 2019) and for trawl catch it can be frequency of occurrence, biomass or surface area of fish (Skelton *et al*. 2023).

Another point to make is that the specimens collected by trawl net during the SEA-MES voyages were also being used to provide direct information on fish size, age structure and diet. We acknowledge that the eDNA data we collected does not provide this information and our comparison is limited to the diversity and relative amounts of fish in the survey area.

The diversity of fish we detected by trawl and eDNA was similar, but neither survey method captured the full community of fish. This is a common finding and often leads to the general conclusion that trawl data and eDNA provide complementary information and using both survey methods is beneficial (*He et al*. 2023). However, one of the main benefits of eDNA biodiversity data is that that it can be produced without the negative impacts of bottom trawling – so it is important to ask: is eDNA data on its own is suitable for marine management applications? The answer to the question is application-specific, so needs a focussed investigation in collaboration with marine ecologists and managers. We envision that the eDNA data on fish diversity, when paired with conventional biodiversity datasets being used in management applications, will be useful as a source of information for this type of future collaborative work.

Another benefit of eDNA sampling compared to trawl derived data is that water can be sampled from any bottom type (e.g. rocky reefs, steep slopes) whereas sites suitable for trawling are much more limited. In our comparison of trawl versus eDNA, this advantage is nullified, but future eDNA sampling will undoubtedly focus on areas where net sampling is not possible (highly structured bottoms or protected areas). As mentioned in several sections of the report the eDNA data could be improved in the future is by increasing the volume of water sampled at each site, which would increase the number of fish being detected. Increasing the number of separate samples is also useful since this provides a basis for calculating occurrence-based estimates of fish abundance.

Comparison of the biomass of fish captured in trawl with eDNA showed that there was general agreement between methods on which were the most common fish in the region. However, there were some noticeable differences. The eDNA provided lower estimates of biomass for Chondrichthyes (sharks and rays) compared to the trawl catch and higher estimates of several small baitfish species. Getting an accurate measure of “true” fish biomass is difficult since all methods have different biases or are targeted towards a subset of the fish community (think of mid-water trawl versus bottom trawl nets). Comparing the biomass of the full diversity of fish between methods is also made difficult by having few common taxa and many rare taxa (i.e. a typical species abundance distribution) in both eDNA and trawl data. With so many taxa at very low abundance, making meaningful comparisons is challenging (i.e. does it matter if a fish is 0.2% or 0.02% of the catch, and can any method provide that level of accuracy needed to differentiate these?). Focussing on getting accurate information on abundance of a few common species is the most realistic option. In most cases the eDNA data tend to be proportional rather than absolute, so conventional biomass estimates (e.g. kilograms of catch) cannot be easily estimated. Nonetheless, this is an area of active research, and some exciting recent work allows prediction of absolute abundance of fish in trawl catches based entirely from eDNA data (Guri *et al*. 2024). Once again, our comparative dataset provides a starting point for evaluating eDNA-based measures of fish abundance within this unique marine environment.

Plenty of scope remains for further analyses using this fish eDNA dataset. For instance, we have not looked in detail at the fish species composition differences between surface and bottom collected eDNA. We also have yet to compare the fish eDNA results with species identified in video footage and still images collected by the deep tow camera during transects conducted throughout the voyage. Comparisons between the eDNA data from this section and other fish eDNA data collected from SEA-MES samples are presented in the following section and in Section 6 of the report.

Trachurus sp. specimens from a SEA-MES trawl catch. Photo CSIRO-Rich Little

4. SEA-MES Deep tow camera eDNA sampling

4.1. Background

The use of environmental DNA (eDNA) methodologies for biomonitoring of aquatic systems has been rapidly evolving from active filtration of collected water samples to less labour intensive, automated, collection techniques (Takahashi *et al*. 2023, McQuillan and Robidart 2017, Scholin *et al*. 2017). For example, a diverse array of passive collection devices have been developed that use natural water movement or vessel movement to capture eDNA samples (Bessey *et al*. 2021, Kirtane *et al*. 2020, Jeunen *et al*. 2024). Autonomous in-situ samplers have also been developed, of which some have inbuilt analytical platforms (Scholin *et al*. 2017), or use permanently networked pumping systems that offer a continuous flow for water samples, some of which can come from deep waters due to fixed water column cables (Sepulveda *et al*. 2020, Aguzzi *et al*. 2019). These diverse methods potentially offer cost savings, time savings, high-frequency sampling, and access to difficult to reach environments. Furthermore, eDNA samplers that are deployed alongside existing technologies, can offer complementary data which can include both biotic and abiotic monitoring capabilities.

One such recently designed and validated eDNA sampler is the Open-Close Device (OCD), which is attached to the frame of a preexisting deep tow camera system and can capture an integrated eDNA sample over the length of a bottom water transect (Bessey *et al*. 2025). The sampler is a 300 x 100 x 100mm mountable box that can be opened upon reaching depth for the duration of the transect and closed before accent using on-board communication capabilities. The internal compartment of the OCD can hold a variety of eDNA capture configurations accommodating the most optimal material for the target of interest. The deep tow camera system is equipped with lighting and offers corresponding live video and still imagining capture capabilities and can be deployed to depths of 4000m. The advantage of using such newly developed sampling technologies is in its ability to passively capture eDNA from a large volume of water with little post-deployment processing, whereas active filtering of large volume water samples is time consuming and laborious. Furthermore, large volume sampling is known to yield higher diversity estimates allowing for a more thorough understanding of the sampled environment (Bessey *et al*. 2020). Although the OCD eDNA sampler has been validated against fish species identifications obtained from paired trawl catches, it is not currently known how this device compares to more conventional eDNA active filtration methods.

The goal of this section of our study was to evaluate how the OCD eDNA sampler compares to conventional active eDNA sampling of different water volume quantities. We conducted paired sampling at 15 sites where we filtered water obtained at depth using a Niskin bottle attached to a CTD to those obtained from the same site using the OCD eDNA sampler. Using fish as our example organism, we used the mtDNA16S fish assay (same as Section 3) to look at taxa detections in 1L triplicate and 10L duplicate CTD water samples, and those obtained from the OCD eDNA sampler. We also conducted suitable in-situ field controls to determine if the OCD eDNA sampler suffered from unrecognized contamination issues by deploying the sampler without activating the opening of the device. We also included

appropriate vessel-based laboratory controls to ensure our on-board decontamination procedures and sterile deionized water source were sufficient.

4.2. Methods

4.2.1. Sample collection

Paired sampling was conducted at 15 sites in the southeast waters of Australia from the research vessel *RSV Investigator* (IN2024_V03) during the month of May 2024 (Figure 4-1). Two deployment methods were used to obtain water samples at each site; Niskin bottles in a CTD rosette opened approximately 10m above the seafloor, and the Open-Close Device (OCD) eDNA sampler (Bessey *et al*. 2025) attached to the deep tow camera system which was opened for the duration of a transect at approximately one meter above the sea floor (Figure 4-2). Since CTD water sampling gear and the OCD deep tow camera system are deployed at different times, the paired sites were conducted as close as possible given logistical constraints at the time of sampling for each site. Therefore, paired sites were approximately 1000m apart (minimum=236m and maximum=3541m). Full details regarding CTD deployment are outlined in Section 3.2.1 and we provide a comprehensive description of the OCD deployment following the description of the water samples obtained by both deployment methods.

Water samples obtained from CTD deployments (“Active” samples) included three replicate 1L samples and duplicate 10L samples. The corresponding OCD samples combined the 1L of water remaining in the OCD sampler with 1L of deionized water used to dislodge any remaining eDNA that could be embedded on the 100µm mesh net used within the internal component of the OCD sampler. All water samples were filtered using a peristaltic pump. All small volume samples (1L) were filtered using a 0.45µm nylon filter, while large volume samples (10L) were filtered using an 0.45µm cellulose ester filter. Nylon filter membranes were used to potentially increase eDNA capture efficiency (Jeunen *et al*. 2022, Zaiko *et al*. 2022), while cellulose ester membranes were used with large volume samples to follow standard, previously used, operating protocols.

The OCD sampler was designed and tested during a previous *RV Investigator* voyage in the northwest of Australia (Bessey *et al*. 2025). The sampler is a 300 x 100 x 100 mm mountable open-ended box that attaches to the frame of a preexisting deep tow camera system. The sampler was developed to leverage existing survey technology and capture an integrated eDNA sample over the length of a transect. Theoretically, the idea is that towing the open sampler over the length of the transect would result in eDNA becoming enmeshed in the collection material used within the internal chamber. We used a 100µm mesh net within the internal chamber of the sampler, which was in the shape of a mini-bongo net held open by a plastic filter holder cartridge (Figure 4-2). The large (100 µm) mesh size was used to ensure the continual flow of water through the system without clogging up the capture material, which can happen in waters that contain high rates of particulate matter. The OCD sampler was loaded with a sterile mesh net and plastic cartridge prior to each deployment, filled with deionized water ensuring no air bubbles remained, and the lid was then secured. The OCD sampler was then installed on the back of the deep tow camera frame and the power cable attached. The vessel crew deployed the deep tow camera system using the vessel A-frame, winch system, and lowered the system down to approximately 1m above the

sea floor where it was piloted along the transect. Upon reaching the seafloor, the OCD was then opened for the duration of the tow which ranged from 1.3km to 4km depending on the site. Upon completion of the transect, the OCD was closed prior to ascent, and the deep tow camera system retrieved. The OCD was stored in a 4°C fridge upon retrieval and the sample was filtered within four hours of retrieval. All equipment was sterilized in 10% bleach water for 30 minutes and rinsed with deionized water between deployments.

4.2.2. OCD and Laboratory Controls

In-situ field controls of the OCD sampler were deployed in both the central (Site N8; 4km tow to a maximum depth of 550m) and northern sampling area (Site N30; 4km tow to a maximum depth of 190m) to evaluate contamination or leakage issues during the deployment process (Figure 4-1; red waypoints). The OCD was deployed as outlined above except the sampler remained closed for the duration of the deployment. We found no evidence of any contamination with both deployments resulting in zero fish reads.

Laboratory controls throughout the voyage consisted of filtered 1 L of the 10% bleach water being used to sterilize all the equipment and was conducted three times over the trip (20/05/2024, 24/05/2025 and at the end of the trip 30/05/2024). As well, 1 L of the de-ionized water used to prepare the OCD sampler was also filtered to test for contamination. All samples resulted in zero fish reads, except for the final bleach water which contained 12950 reads of an unidentified Ariidae species which was not found in any other sample used for our deep tow camera sampling comparisons. A PCR plate control was also included during molecular laboratory processing of OCD samples which also resulted in zero fish reads.

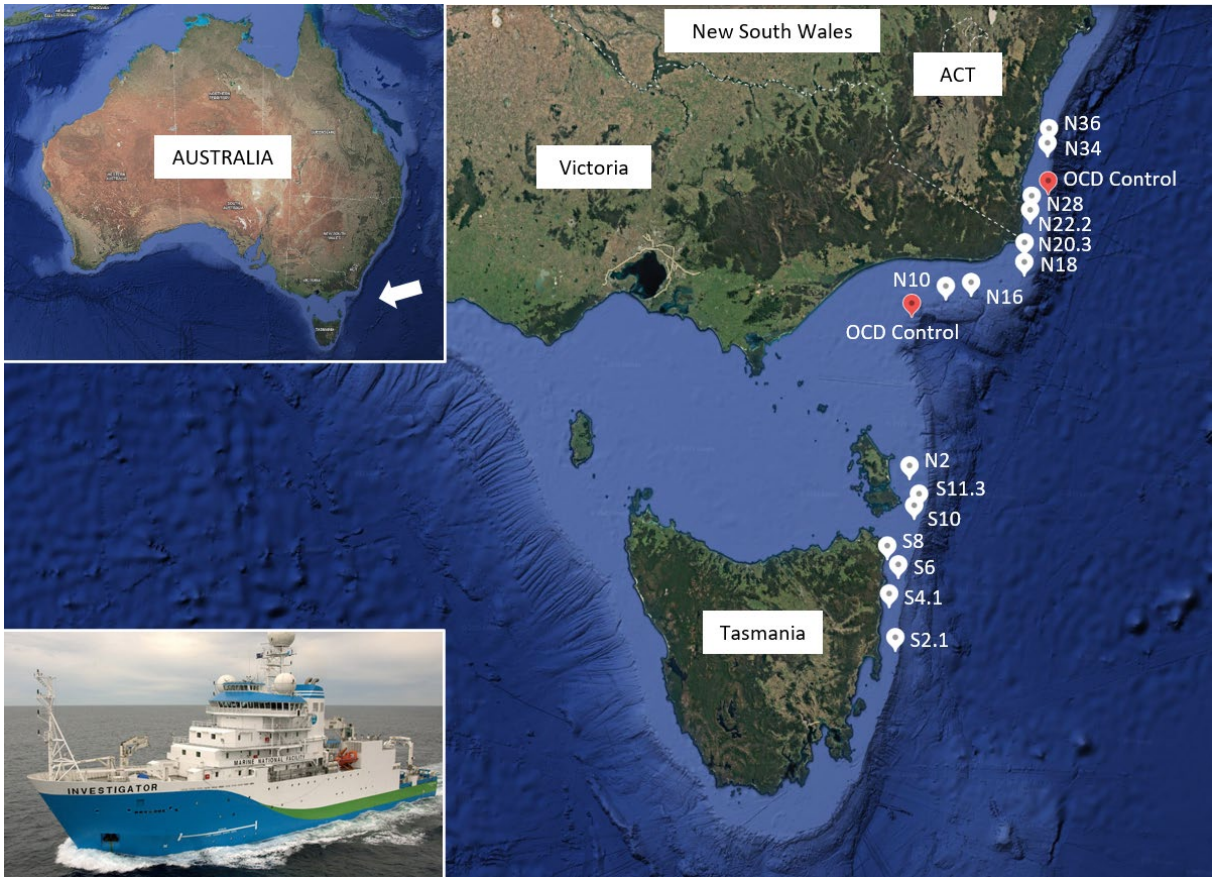


Figure 4-1 The 15 sites where paired samples from Niskin bottles in a CTD rosette and from the OCD eDNA sampler attached to the deep tow camera system were obtained. Sample sites are denoted by white waypoints while OCD control sites are demarked by red waypoints. Inlay pictures depict the general location of the sites in the southeast corner of Australia and the vessel used for deployment, the RV *Investigator*, is also shown.

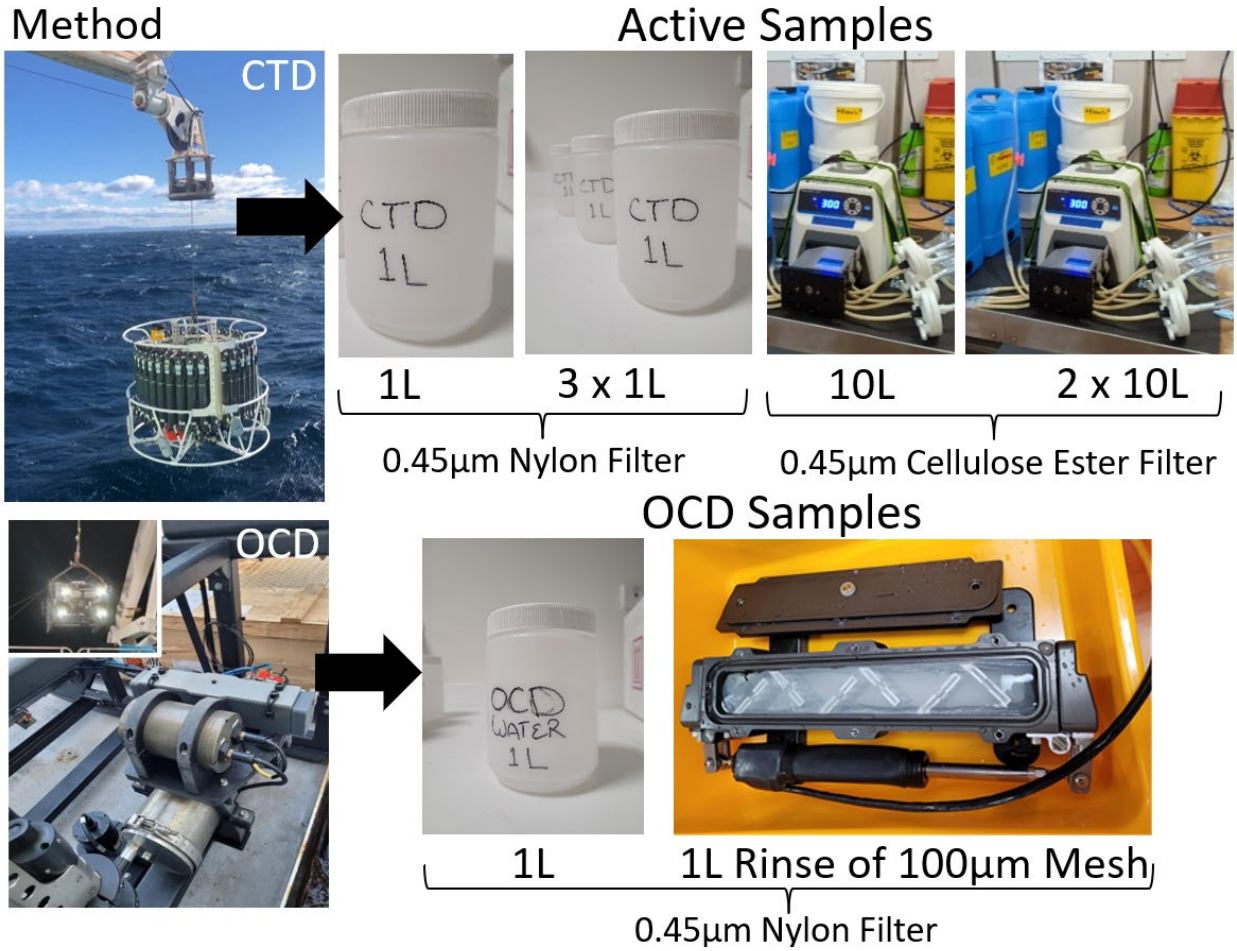


Figure 4-2 Two deployment methods were used to obtain water samples; Niskin bottles in a CTD rosette opened approximately 10m above the seafloor, and the OCD eDNA sampler attached to the deep tow camera system which was opened for the duration of a transect at approximately one meter above the sea floor. Water samples collected from the Niskin bottles included three replicate 1L samples and duplicate 10L samples, while an OCD sample combined the 1L of water remaining in the closed device with 1L of deionized water used to dislodge any remaining eDNA that could be embedded on the 100µm mesh net. The material and size of the filter membranes are indicated for each sample.

4.2.3. Sample processing

Sample processing and bioinformatics are detailed in Section 3.2.2 and 3.2.3. Briefly, DNA was extracted from all filters using a QIAGEN DNeasy Blood and Tissue kit following standard protocols outlined by the manufacturer, with the exception that filters were digested in 540 µL of ATL lysis buffer and 60µL of Proteinase K for 3 hours at 56°C on rotation (300rpm). These extractions were performed in dedicated eDNA laboratories of the CSIRO in both Perth and Hobart. All resulting DNA was shipped to the CSIRO Hobart laboratory where it was amplified using a 16S mtDNA metabarcoding assay for fish (Section 3.2.2). DNA libraries were prepared and sequenced on an Illumina NextSeq 2000 platform (Section 3.2.2). The resulting sequencing data was demultiplexed into their respective samples, quality controlled, and assigned to taxa as outlined in Section 3.2.3. Taxa with only one read in a sample (singletons) were removed prior to any statistical analyses.

4.2.4. Statistical analyses

Due to the asymmetrical skewness within some groups of data, non-parametric statistics were used to compare across the different sampling categories (Active 1L, Active 3L, Active 10L, Active 20L and OCD Samples). We used a Kruskal Wallis Test, followed by a post-hoc Dunn's test, to compare the number of fish taxa detected between sampling categories where outliers were included, a Bonferroni correction was applied, and the level of significance was 0.05. We also calculated the proportion of taxa overlap between replicate samples to investigate sample heterogeneity, as well as the overlap between the OCD samples with all CTD samples and the total taxa detected at a site. Proportions were calculated as follows:

$$3\ replicates\ x\ 1L_i = \frac{number\ of\ taxa\ in\ common\ between\ all\ 3\ replicates}{total\ number\ of\ taxa\ detected\ in\ the\ 3\ replicate\ samples}$$

$$2\ replicates\ x\ 10L_i = \frac{number\ of\ taxa\ in\ common\ between\ the\ 2\ replicates}{total\ number\ of\ taxa\ detected\ in\ the\ 2\ replicate\ samples}$$

$$OCD\ Samples\ x\ CTD\ Taxa_i = \frac{number\ of\ taxa\ in\ common\ between\ the\ OCD\ and\ CTD\ samples}{total\ number\ of\ taxa\ detected\ in\ all\ CTD\ samples}$$

$$OCD\ Samples\ x\ Site\ Taxa_i = \frac{number\ of\ taxa\ detected\ with\ OCD\ samples}{total\ number\ of\ taxa\ detected\ in\ all\ samples}$$

where *i* is sampling site.

The forementioned nonparametric statistics were also used to determine significant differences between taxa overlap. All associated statistics and graphics were produced using Excel and Statistics Kingdom.

Statistical analyses to investigate the influence of collection method and depth on the number of taxa detected were undertaken using R Studio (version 2021.09.0+351 "Ghost Orchid" Release (2021-09-20). We used 'adonis2' (to partition distance matrices among sources of variation to fit linear models), 'pairwise.adonis' (to perform pairwise multilevel comparisons), and 'metaMDS' (to perform nonmetric multidimensional scaling) from the R package vegan), as well as 'ggplot' functions. A permutation multivariate ANOVA, i.e., PERMANOVA (adonis2), was used to test for differences between collection method categories and depth. Collection method and depth were treated as fixed factors (distance = 'jaccard' which ignores joint absences and focusses on proportion of shared species, permutations = 999, and by='margin' to include an assessment of significance against a model that includes all variables so that the order of variables does not affect the outcome). We used 'metaMDS' to produce a visual representation of the similarities of species communities by both collection method and depth with data transformed to presence/absence (permutations=999 and method='jaccard'). Statistical ellipses ('stat_ellipse' in 'ggplot) were drawn for collection method based on a 95% confidence interval assuming a t-distribution. Fish taxa are also overlayed in nonmetric multidimensional scaling (NMDS) space to investigate how taxa relate to the depth gradient.

4.3. Results

4.3.1. Effect of water volume on fish taxa detections

There was an increase in fish detections with quantity of water filtered (Figure 4-3i); replicate water samples were not significantly different in number of fish taxa detected. The OCD samples had a median detection of 7 taxa, as compared to 0, 2, 10 and 17 for the Active 1L, Active 3L, Active 10L and Active 20L samples, respectively. Nevertheless, no statistical differences were found between the number of taxa detected in the OCD samples and the Active 3L, Active 10L, nor Active 20L samples. The data indicated strong heterogeneity in the composition of fish taxa detected, even between replicates. Therefore, we compared the overlap between replicates and the overlap between OCD detections with all filtered CTD samples, and with all detections at a site (Figure 4-3ii). The median overlap of taxa between 1L triplicates was 0%, with a maximum of 25% excluding outliers. Outliers of 100% and 50% overlap occurred when one taxon was in common between all replicates when only one or two taxa were detected within all triplicates. In contrast, a median overlap of 27.6% was observed in 10L duplicates with a maximum of 50% and a minimum of 0%, which was significantly greater than the overlap in 1L samples yet clearly still displaying great variation. The median overlap of the OCD samples with all filtered CTD samples was 13% with a maximum of 44% and a minimum of 0%, while the overlap of the OCD samples with all taxa at a site was 29.2%, with a minimum of 8.8% and a maximum of 60%. The overlap in taxa for the 1L samples was significantly less than that of the overlap in taxa between 10L replicates and the OCD samples compared to all taxa detected at a site.

4.3.2. Other factors affecting fish taxa detections

Both sample collection method and depth were significant variables in fish taxa detections (Method df=2, F=18.549, p<0.001; Depth df=1, F-3.233, p=0.026, Figure 4-4). The active 1L samples detected significantly less taxa than the active 10L samples (F=3.655, p=0.01) and the OCD samples (F=1.594, p=0.43). However, there was no significant difference in detections between the active 10L samples compared to the OCD samples (F=1.216, p=0.175). Despite the overlap in detections, the 95% confidence ellipses became increasingly smaller from the active 1L samples to the OCD samples, to the 10L samples (Figure 4-4i). The depth gradient in detections could also be visualized in NMDS space with the deepest samples being most prominent in the second quadrant of the graph followed by the third quadrant, and the shallowest samples being most prominent in the first and fourth quadrants (Figure 4-4ii).

When fish taxa were overlaid on NMDS space there were patterns revealed in the species caught at shallower versus deeper sites (Figure 4-5). For example, shallow water species such as leatherjackets and kelpfish (*Eubalichthys gunnii* and *Chironemus marmoratus*), that are typically found at depths of 50m or less, were evident in the first quadrant of the NMDS plot. In contrast, a Cottidae species was evident in the second quadrant, which are a family of sculpin, with the two known Australian species (*Antipodocottus elegans* and *A. galatheae*) both found in deeper waters (150m-735m) on the southeast waters of Australia in the survey zone. Other deeper or mesopelagic species, such as a *Maurolicus* species (most likely *Maurolicus* australis), Stomiiformes species, and *Diaphus* species, were also evident in the second and third quadrants of the NMDS plot. Also detected (6 reads) from Site N34 in

150m of water from an Active 1L samples was the IUCN listed endangered species, *Alopias superciliosus*, the Bigeye Thresher shark.

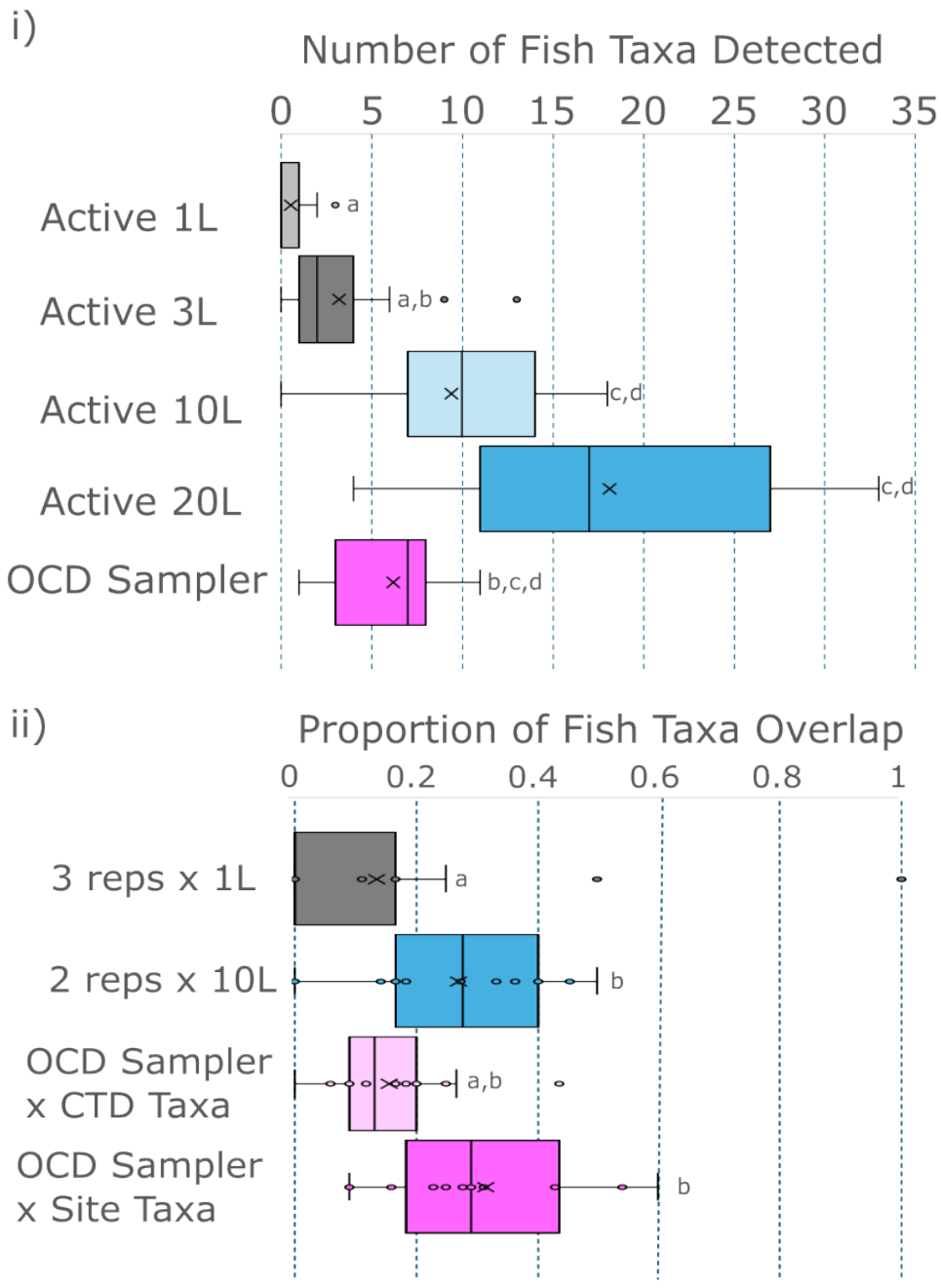


Figure 4-3 The number of fish taxa detected in cumulative water samples collected from the Niskin bottles compared to those detected using the OCD sampler. Paired comparisons were obtained at 15 sites (n=15). ii) The proportion of fish taxa overlap between 1L replicates, 10L replicates, the OCD sampler and compared to active samples from the CTD, and the OCD sampler compared to all taxa detected at each site. Letters indicate statistical differences as determined by a Kruskal-Wallis, followed by a post-hoc Dunn's Test. (α=0.05). Dots indicate outliers and the x denotes the average.

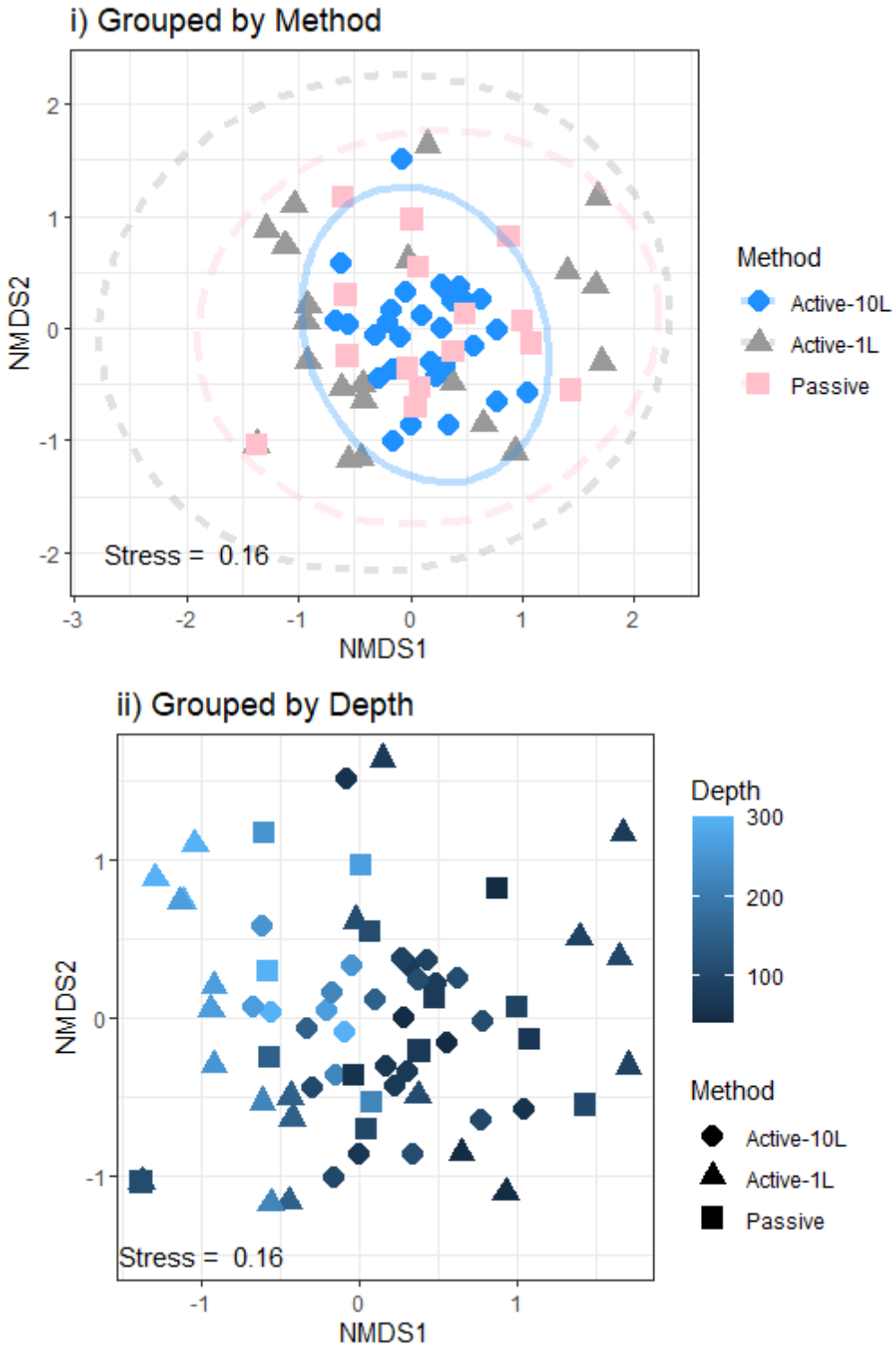


Figure 4-4 Nonmetric multidimensional scaling plot of the presence/absence data (distance=jaccard) for fish taxa grouped by i) collection method and ii) depth.

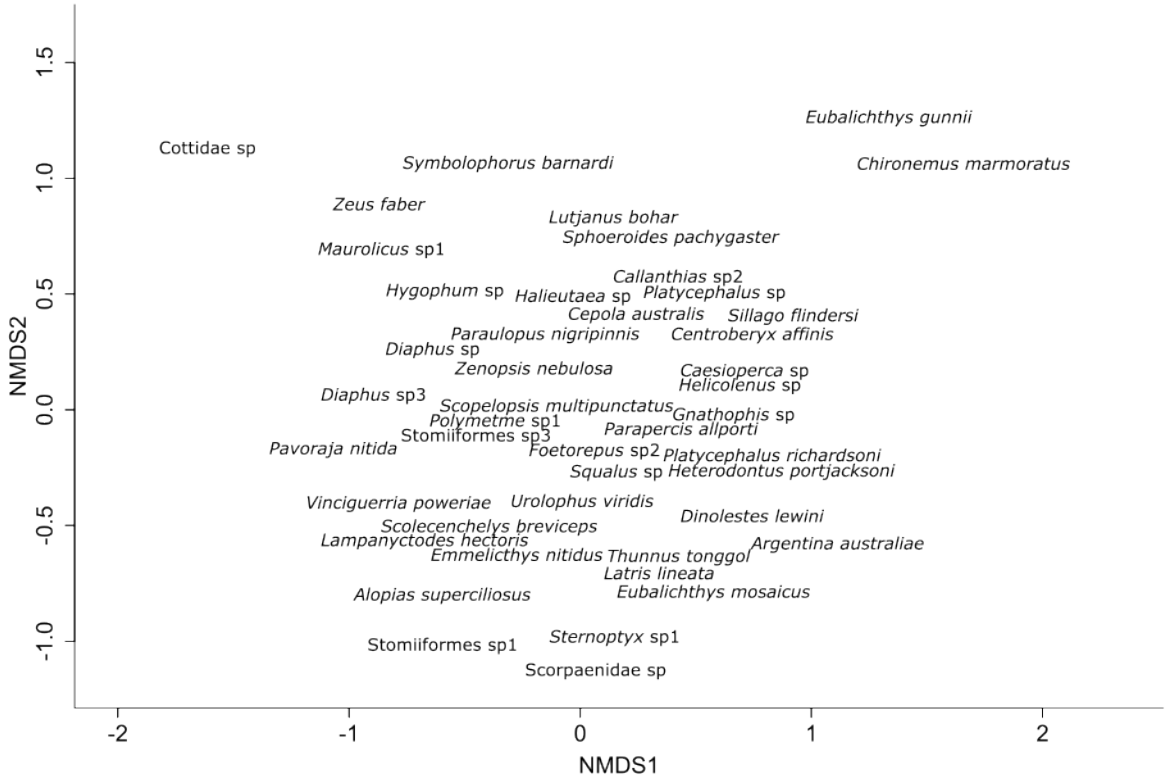


Figure 4-5 Fish taxa overlaid on nonmetric multidimensional space.

4.4. Discussion

This study shows that passive sampling using the OCD is not significantly different in fish taxa detections than the conventional eDNA sampling technique of active filtering 10L water samples. Furthermore, we found no evidence of contamination during the deployment process that could be caused from water ingression or leakage of the device. We found no credible evidence of contamination from onboard vessel processing which indicates that our sterilization procedures were adequate, and vessel based deionized water sources were reliable for eDNA studies. The resulting data revealed depth gradients in fish taxa detections which is consistent with fish distribution patterns and existing studies. Nevertheless, the number of taxa detected by the OCD sampler is less than would be theoretically predicted given the amount of water passing through the device, indicating that optimisation of eDNA capture efficiency has not yet been achieved, at least when using the 16S fish primer assay. Furthermore, the lack of fish taxa detection overlaps in replicate water samples, as well as with the OCD sampler at each site, indicates that the heterogeneity of eDNA in the water is extremely high and needs to be considered when making ecological interpretations based on eDNA sampling alone.

4.4.1. Optimization of OCD sampler

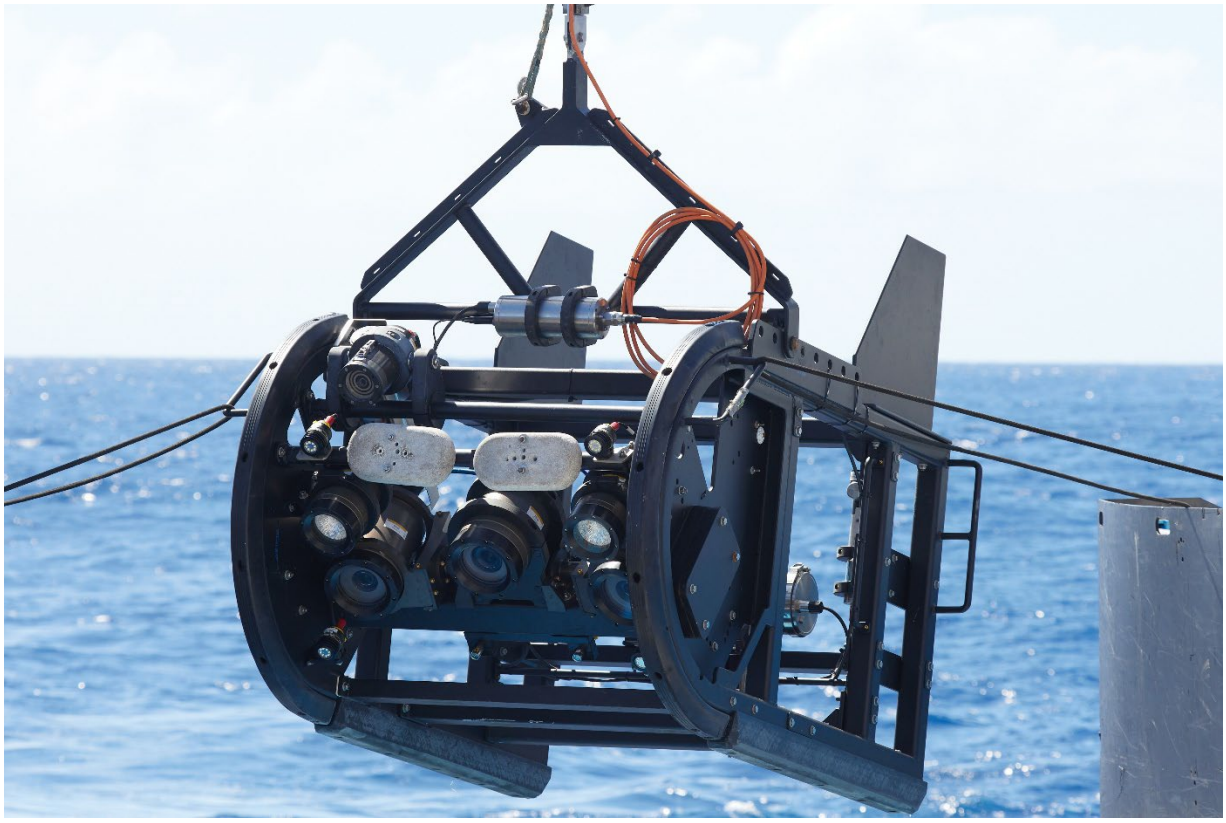
Despite the lack of statistical difference in fish taxa detections between the OCD and 10L actively filtered water samples, there was a clear trend that filtering larger volumes of water led to increased taxa detections. Given that the OCD remains open for the duration of a

transect which is pulled through the water column at a speed of about two knots (~ 3.7km/hr) for as long as 4km, it would be reasonable to estimate that approximately 500L of water is moving through the OCD per minute. Given such a large water volume passing through the device, we would have expected the OCD to detect significantly more fish than the 10L duplicate samples. As this was not the case, it's evident that the eDNA capture efficiency has not yet been optimized. We used a 100um mesh net to capture eDNA because we didn't want the capture material to clog and impede water flow through the device. However, we did not see any evidence of the nylon net clogging, so it is possible that the large mesh size was not sufficient to retain fish eDNA. Rather, this capture material may have been more efficient at capturing a representative sample for other target taxa. It would be possible to test this idea by amplifying the extracted DNA with other primer assays. Indeed, this was investigated, with a select few samples, in Section 6 of this report. It is possible that the optimization of the OCD sampler may be taxa specific, and that different size capture material within the internal compartment is needed dependent upon the intended target of the study. Given the open design of the internal compartment of the OCD, several components could be made to accommodate any number of collections materials of various sizes. It is known that eDNA exists in a broad spectrum of physical states, such as extracellular DNA fragments, whole cells, tissue fragments, and even whole organisms, so the choice of filter size and material can target specific components of this spectrum (Power *et al*. 2023). Furthermore, an understanding of the turbidity of the system being sampled will aid in knowing which collection material to use, as membrane clogging was not an issue given the open ocean, low particulate, waters that were sampled during our study. Additionally, other possible areas for optimization occur at the molecular level of the sample amplification stage. Given the theoretical increase in DNA capture using the OCD, optimization at the polymerase chain reaction (PCR) step warrants further investigation. For example, the concentration of DNA in a 1L and 10L sample, compared to that obtained from the OCD sampler, may require different conditions for amplification. We used the same conditions for all samples, however, failure to amplify samples under their required optimal conditions can lead to the exclusion of the desired product (Cold Spring Harb Protocols, 2009). Possible PCR optimization strategies could include reamplification of template DNA using a 10-fold dilution series at a fixed annealing temperature, incorporating technical PCR replicates, and adding enhancers to the PCR mix.

4.4.2. Heterogeneity of overlapping samples

Our results provide evidence of the extent of heterogeneity in fish taxa detections, with our large volume (10L) water samples displaying a median overlap of <30% in shared taxa. It is widely known and accepted that one of the main characteristics of eDNA is the heterogeneity of extracts obtained from environmental samples due to the wide range in DNA quality and/or PCR inhibition. This heterogeneity needs to be taken into consideration when providing ecological interpretations from eDNA data. The depth-related patterns seen in this study are consistent with previous studies (McClenaghan *et al*. 2020) and the known behaviour of fish and does provide an increased understanding of the environments being sampled. Furthermore, eDNA studies do provide valuable insights into the taxa present in an area using non-invasive and non-lethal methods, which is particularly important in marine protected areas or when sampling for rare and vulnerable species. For example, this study found evidence of the endangered Bigeye Thresher (*Alopias superciliosus*) which is an endangered species of shark that is commonly caught by offshore fisheries and is an

important by-catch shark species for tuna longline fisheries (Tsai *et al*. 2020). This species is highly susceptible to overexploitation and is listed as endangered on the International Union for Conservation of Nature Red List. Regional fisheries management organizations prohibit retention of this species for commercial use (Indian Ocean Tuna Commission, Working Party on Ecosystems and Bycatch, 2010). The use of eDNA provides evidence of the distribution of such species using non-destructive methodologies.



Deep Towed Camera used to collect underwater imagery and sample eDNA. Photo: Museums Victoria-Benjamin Healley

5. SEA-MES eDNA derived plankton biodiversity survey

5.1. Background

In this part of the study, we applied one of the broadly used eukaryote markers, the V4 region of the 18S rRNA gene, to analyse the diversity and composition of the plankton communities. We collected 2L water samples and analysed them following the standardised protocols of the Australian Microbiome to enable direct comparison of our results with Australian Microbiome dataset, this extensive dataset contains thousands of pelagic samples from Australian waters. This approach delivers representative and reliable information on the relative abundance, diversity and taxonomic composition of most of the pelagic eukaryote community, from phytoplankton to small gelatinous zooplankton. While it also delivers data on fishes and mammals, those observations are sparse and were thus not considered representative of the fishes and mammals present.

The aim of the study was to assess the ability of the eDNA approach to measure the diversity and composition of the pelagic plankton communities and its major drivers. More broadly we wanted to assess the suitability of the approach to monitor the health of the pelagic ecosystem. Finally, we wanted to collect data enabling causal modelling to explicitly link the status of the lower levels of the food chain to those above.

5.2. Methods

5.2.1. CTD water sampling

Samples of eDNA for plankton work were collected from 99 CTDs during IN2023_V05 and 100 CTDs during IN2024_V03. For each sample, 2 L of water was collected from a 12 L Niskin bottle (Ocean Test Equipment, USA) attached to a 36-bottle CTD rosette. Samples were collected from surface (SFC), from the location of the deep chlorophyll maximum (DCM) and from the bottom of the water column (BTM) (without disturbing the sediment). Collected water samples were stored at 4°C and filtered within 12 hours of collection. Water samples were filtered using a Masterflex L/S console pump system (Cole-Parmer, USA) onto 0.22 mM pore size Sterivex GP filters (Millipore), and immediately stored at -80°C. Filtration and sampling equipment was soaked in 10% bleach solution for at least 6 hours between each filtration run or sampling. Prior to filtering, filtration lines were primed with 10% bleach solution for 15 minutes and then flushed thrice with Mili-Q water.

5.2.2. Laboratory processing of eDNA

Environmental DNA was extracted from the 2L water samples following the standardised protocol of the Australian Microbiome (https://www.australianmicrobiome.com/protocols/, meth_3.1.9.). Briefly, Sterivex filters were shaken for 60 minutes with a lysis buffer containing lysozyme, SDS and CTAB, extracted with phenol, incubated for 120 minutes at 60C with proteinase K, extracted twice with chlorophorm-isoamyl alcohol mixture, then purified on Quiagen DNeasy Powerwater filter columns. DNA was eluted into 80uL 0.1xTE buffer.

Tag sequencing of the V4 region of the 18S rRNA gene was carried out at the Ramaciotti Centre for Genomics (UNSW), following the standardised protocols of the Australian Microbiome (https://www.australianmicrobiome.com/protocols/, meth_5.1,), amplified using the TAReuk454FWD1 (CCAGCASCY- GCGGTAATTCC) (Piredda *et al*., 2016) and a modified TAReuk-Rev3 (ACTTTCGTTCT- TGATYRATGA) primers (Stoeck *et al*., 2010).

5.2.3. Bioinformatic analysis

Paired reads were trimmed using the trimfq function of Seqtk (https://github.com/lh3/seqtk) and merged using FLASH (Magoc and Salzberg, 2011). The screen.seqs command in Mothur (Schloss *et al*., 2009) was used to remove reads with ambiguous bases and homopolymers > 8. Quality filtered sequences were mapped to biologically correct, chimera-free zOTUs using USEARCH 64 bit v10.0.240 (Edgar, 2010) and a sample by read abundance matrix is generated. Zero-radius operational taxonomic unit (zOTU) data with single nucleotide variation between zOTUs were used to enable data analysis at the highest possible phylogenetic variation. zOTUs were taxonomically identified with the SILVA database (v138.1) (Quast *et al*., 2013) and the PR2 Database (v 5.0.0) (Guillou *et al*., 2013) using the Wang classifier with a 60% Bayesian probability cut-off (Callahan *et al*., 2016; Wang *et al*., 2007). Reads not classified as eukaryotes at the Phylum (Silva) or Domain (PR2) level were removed from the dataset.

5.2.4. Statistical analyses

The zOTU table was rarefied to 26739 reads, excluding 9 samples with very low reads (61 or below). Based on the very low read numbers for the excluded samples, we are confident that those samples failed amplification during the tag sequencing process.

Statistical analyses were carried out via the Primer-E software (Anderson 2001). Diversity indices (Richness as total number of zOTUs, Margalef richness, Pielou's evenness, Shannon diversity, Simpson diversity) were calculated using the rarefied zOTU table. Multivariate analyses were based on Bray-Curtis distances calculated from rarefied and square root transformed zOTU table. nMDS plots were used to visualise overall beta diversity. Distance based linear models (DistLM) were used to identify environmental and biological variables of significant influence and results were visualised on distance-based redundancy analysis (dbRDA) plots.

5.3. Results

5.3.1. Overview of eDNA sequencing results

A total of 202 samples were sequenced in the study. These resulted in over 16 million sequencing reads. After removing 34807 reads belonging to 860 zOTUs that were not classified as Eukaryotes, the final dataset contained 16,053,313 reads, belonging to 16,954 ASVs identified as Eukaryotes.

The average richness was 1564 eukaryote zOTUs per sample. Bottom samples had the lowest average richness during both voyages, accompanied by the highest variation (Figure 5-1).

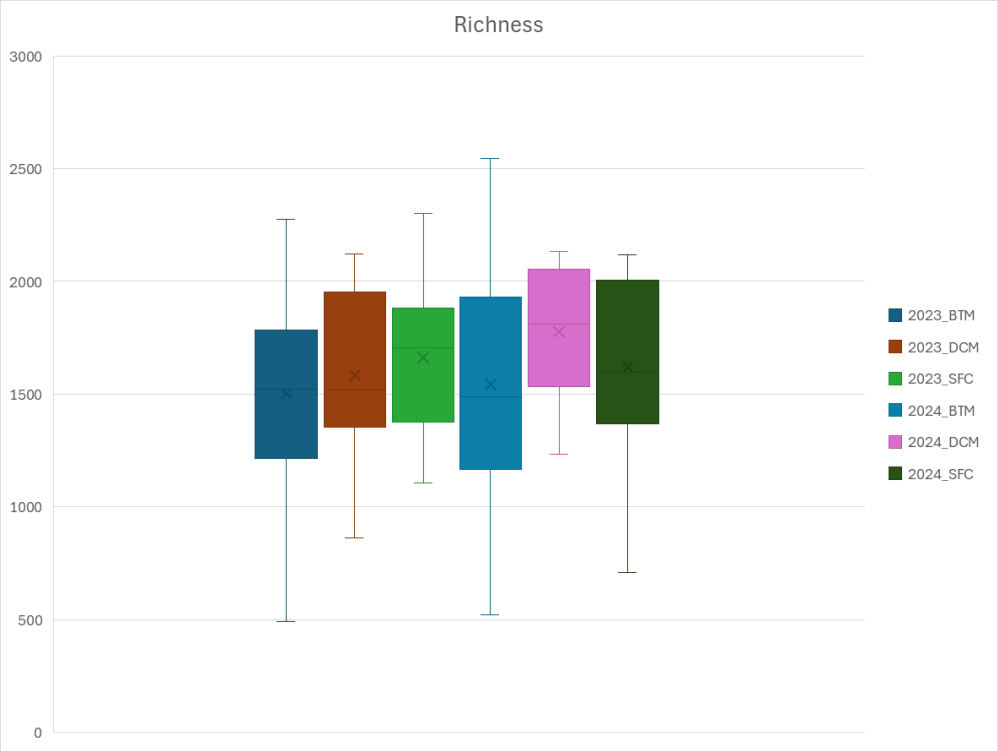


Figure 5-1 Richness of eukaryote plankton expressed as the number of eukaryote ASVs detected. Separate box plots representing groups of samples per voyage and layer of the water column. BTM – bottom, DCM – deep chlorophyll maximum, SFC – surface.

5.3.2. Environmental drivers of the plankton assemblages

As detailed in section 3.3.2, the environmental conditions differed significantly between IN2023_V05 (July 2023) and IN2024_V03 (May 2024). The 2023 voyage was characterized by a relatively well mixed, cold water column. In contrast, the water column was fairly stratified during the 2024 voyage with significantly warmer surface temperatures and a significant supply of nutrients (nitrate, phosphate, silicate) in the deeper layers (Figure 5-2).

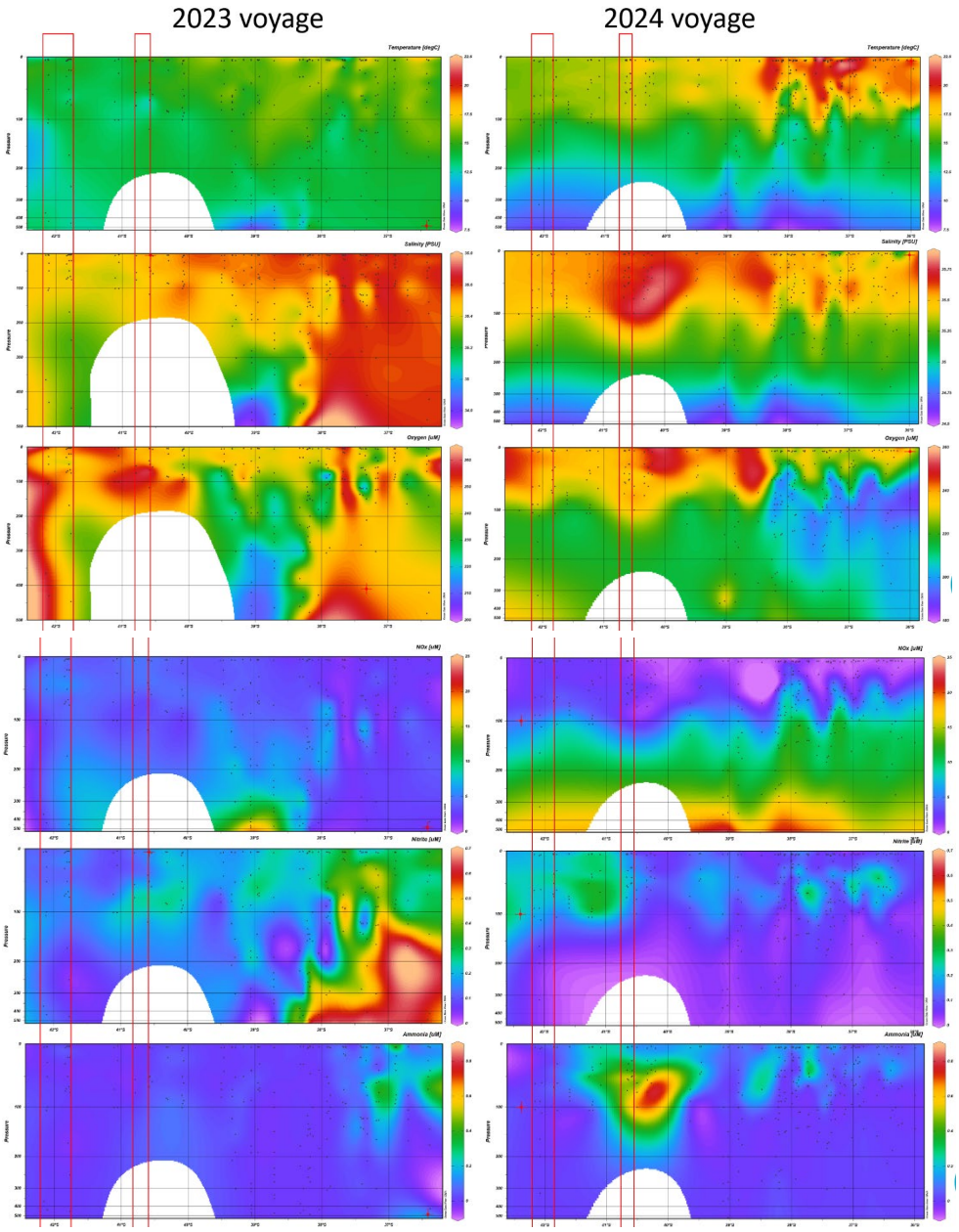


Figure 5-2 Oceanography plots of the two SEA-MES voyages. Plots from top to bottom: temperature, salinity, dissolved oxygen, nitrate, nitrite, ammonia. Phosphate and silicate concentrations correlated strongly with nitrate, resulting in very similar plots.

These differences were reflected in the plankton community composition as well. This was supported by Permanova analysis (P<0.001) and clearly shown on nMDS plots (Figure 5-3).

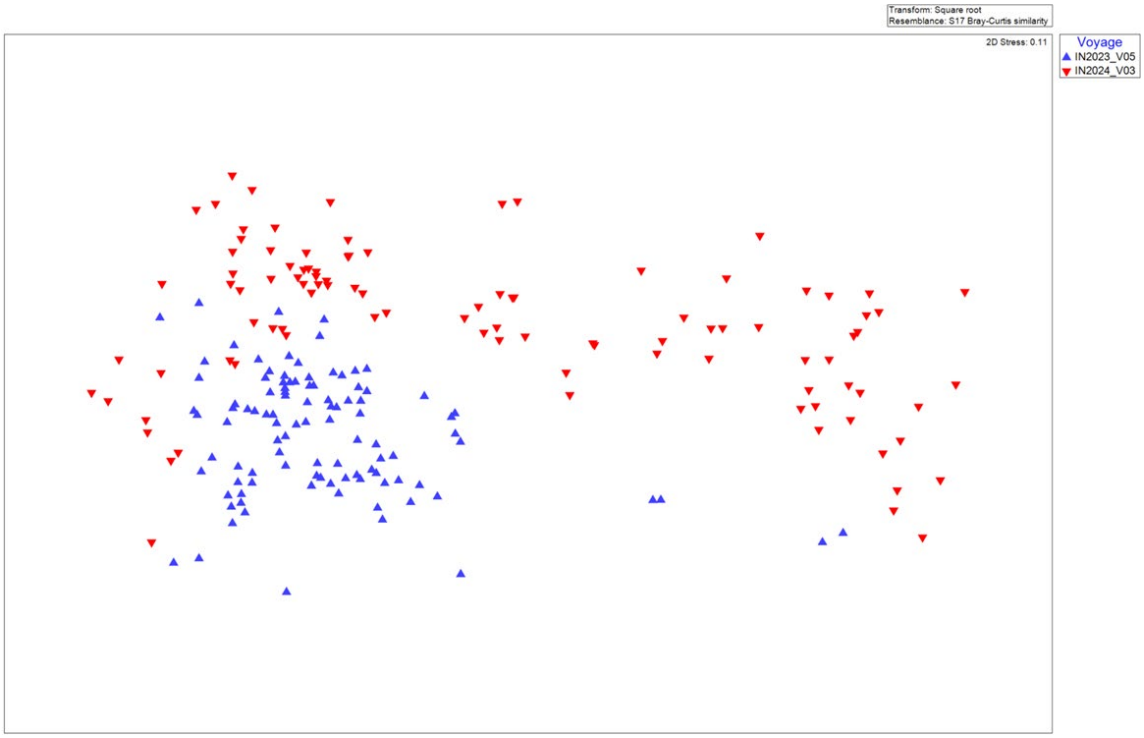


Figure 5-3 nMDS plot highlighting the difference in the plankton communities between the two voyages.

Pairwise Permanova testing showed that bottom samples were significantly different from surface and deep chlorophyll maximum samples (P<0.0001), but there was no significant difference between surface (depth=5m) and deep chlorophyll maximum samples (average depth=36.8m) (P=0.2274). These findings were also clearly reflected on nMDS plots (Figure 5-4)

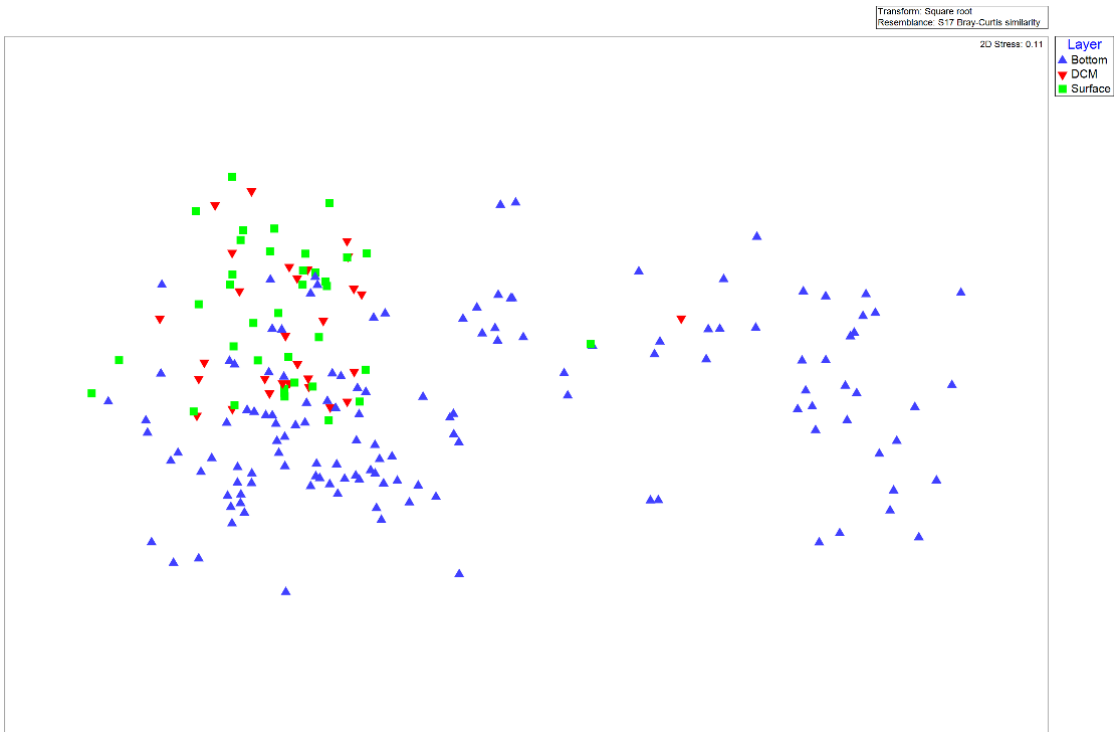


Figure 5-4 nMDS plot highlighting the difference in the plankton communities between surface, deep chlorophyll maximum (DCM) and bottom samples.

Based on the above findings, we separated the dataset into four groups and carried out further analyses separately across each group:

- **2023 bottom** Voyage 2023, bottom samples
- **2023 SFC+DCM** Voyage 2023, surface + deep chlorophyll maximum samples
- **2024 bottom** Voyage 2024, bottom samples
- **2024 SFC+DCM** Voyage 2024, surface + deep chlorophyll maximum samples

Distance based redundancy analysis plots and distance based linear modelling identified different environmental drivers influencing the plankton community across the four separate groups (Figure 5-5).

Most of the measured environmental variables had significant influence on the plankton communities across all the four groups ($P<0.005$). These variables grouped into 4 major categories: location (latitude and depth; physical oceanography (temperature, salinity and dissolved oxygen); nutrients (nitrite, nitrate and ammonia); and biology (richness and diversity of the eukaryote community). We note that phosphate and silicate concentrations showed very strong correlation with nitrate, and thus nitrate was used as a proxy for all three in the analysis. The time of sampling had no significant effect on the plankton community. Depth (pressure used as a proxy) had a significant effect only on the bottom communities, but not on the surface and deep chlorophyll maximum communities.

Location was one of the most important drivers: Latitude across all four groups and depth for the bottom samples. Depth was not a significant driver for the surface and deep chlorophyll maximum samples suggesting that the water and the ecosystem was well mixed at those depths during both voyages.

The surface plankton community during the 2023 voyage was influenced by all the physical, chemical and biological variables to a similar extent (as shown by similar SS(trace) and Pseudo_F values, see Table 5-1).Salinity showed a strong correlation with latitude (Table 5-2).

The environmental drivers of surface plankton community during the 2024 voyage were generally stronger with temperature and richness standing out. Temperature was strongly correlated with latitude (Table 5-1 and Table 5-2).

The plankton communities near the bottom were influenced by different environmental drivers. In 2023 they were oxygen and nitrate (plus phosphate and silicate) which showed a strong negative correlation with each other. In 2024 they were salinity, temperature and nitrate (plus phosphate and silicate), with a strong positive correlation between salinity and temperature and a strong negative autocorrelation of both with nitrate (Table 5-1 and Table 5-2).

Table 5-1 DistLM marginal test results of statistically significant environmental drivers. S – richness (number of eukaryotic zOTUs per sample). 1- λ ' – Simpson's evenness.

2023 SFC & DCM

Variable	SS(trace)	Pseudo-F	P	Prop.
Latitude	4677.4	3.9972	0.0001	0.14277
Temperature	3315.5	2.7023	0.0001	0.1012
Salinity	4624	3.944	0.0001	0.14114
S	4528.3	3.8493	0.0001	0.13822
1-λ'	2696.3	2.1523	0.0039	0.082299
Oxygen	2803.9	2.2462	0.0015	0.085583
Nitrate	4725.8	4.0455	0.0001	0.14425
Ammonia	4039.8	3.3756	0.0001	0.12331
Nitrite	5123	4.4485	0.0001	0.15637

2024 SFC & DCM

Variable	SS(trace)	Pseudo-F	P	Prop.
Latitude	9376.5	5.7867	0.0001	0.15314
Temperature	10279	6.4561	0.0001	0.16788
Salinity	4166.8	2.3367	0.0048	0.068053
S	8774.1	5.3527	0.0001	0.1433
1- λ '	5573.6	3.2047	0.0002	0.09103
Oxygen	6113.3	3.5494	0.0002	0.099845
Nitrate	6319.9	3.6832	0.0001	0.10322
Ammonia	4769	2.703	0.001	0.077888
Nitrite	7166.2	4.2418	0.0001	0.11704

2023 bottom

Variable	SS(trace)	Pseudo-F	P	Prop.
Latitude	12516	7.3628	0.0001	0.10615
Depth	10225	5.8874	0.0001	0.086723
Temperature	10539	6.0861	0.0001	0.089388
Salinity	15394	9.3103	0.0001	0.13056
S	6690.3	3.7297	0.0001	0.056743
1- λ '	7021.4	3.926	0.0001	0.059551
Oxygen	15540	9.4125	0.0001	0.1318
Nitrate	16214	9.8856	0.0001	0.13752
Ammonia	4530	2.4773	0.0035	0.038421
Nitrite	12221	7.1698	0.0001	0.10365

2024 bottom

Variable	SS(trace)	Pseudo-F	P	Prop.
Latitude	29263	12.829	0.0001	0.16698
Depth	31246	13.887	0.0001	0.17829
Temperature	38711	18.145	0.0001	0.22089
Salinity	41741	20.009	0.0001	0.23818
S	10879	4.2357	0.0006	0.062075
1- λ '	20428	8.4443	0.0001	0.11656
Oxygen	32037	14.317	0.0001	0.18281
Nitrate	43576	21.18	0.0001	0.24865
Ammonia	35289	16.136	0.0001	0.20136
Nitrite	37220	17.258	0.0001	0.21238

Table 5-2 Correlations between environmental variables per group. R>0.75 and R<-0.75 values are highlighted. S – zOTU richness. 1- λ' – Simpson's evenness.

2024 SFC&DCM

	Latitude	Depth	Temperature	Salinity	S	1-λ'	Oxygen	Nitrate	Ammonia
Depth	-0.1327								
Temperature	0.824011	-0.41377							
Salinity	-0.32555	-0.41247	-0.05094						
S	-0.3285	0.304476	-0.4133	-0.18346					
1-Lambda'	-0.04684	0.278661	-0.15352	-0.38867	0.760665				
Oxygen	-0.60481	-0.40565	-0.51247	0.583945	0.065011	-0.16601			
Nitrate	-0.42526	0.793294	-0.6933	-0.47027	0.395955	0.317627	-0.24752		
Ammonia	-0.04196	-0.1355	0.063676	0.426518	-0.50067	-0.34597	0.064872	-0.15873	
Nitrite	-0.73058	0.149683	-0.65018	0.034398	0.594668	0.42073	0.407721	0.3845	-0.02179

2023 SFC&DCM

	Latitude	Depth	Temperature	Salinity	S	1- λ'	Oxygen	Nitrate	Ammonia
Depth	0.207398								
Temperature	0.68571	0.02707							
Salinity	0.863441	0.078779	0.623892						
S	-0.4905	-0.16485	-0.31629	-0.59792					
1-Lambda'	-0.24903	-0.12546	-0.01251	-0.20457	0.545074				
Oxygen	-0.19134	0.027183	-0.78067	-0.09825	0.130459	-0.12058			
Nitrate	-0.88107	-0.09632	-0.46096	-0.83681	0.395765	0.246235	-0.14631		
Ammonia	0.662036	0.400829	0.446029	0.55001	-0.36715	-0.23178	-0.08323	-0.60904	
Nitrite	0.87131	0.090541	0.537528	0.936029	-0.61592	-0.33974	-0.01754	-0.89885	0.574693

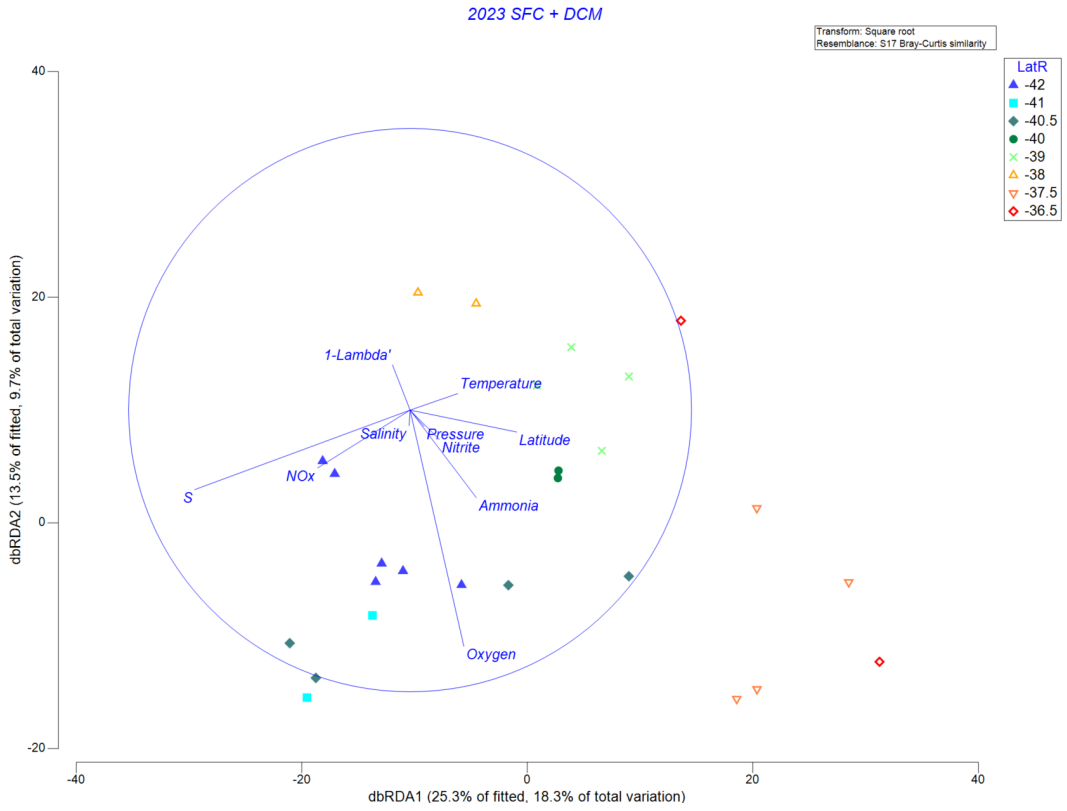
2024 BTM

	Latitude	Depth	Temperature	Salinity	S	1- λ'	Oxygen	Nitrate	Ammonia
Depth	0.291375								
Temperature	-0.3426	-0.93461							
Salinity	-0.3806	-0.89235	0.961581						
S	-0.04308	-0.22125	0.200667	0.071025					
1-Lambda'	0.289081	0.306897	-0.37492	-0.47052	0.418399				
Oxygen	-0.74566	-0.25761	0.319098	0.488432	-0.28957	-0.54896			
Nitrate	0.497337	0.90215	-0.95915	-0.98064	-0.11435	0.464841	-0.55342		
Ammonia	-0.33915	-0.44051	0.539771	0.722923	-0.33472	-0.63637	0.750337	-0.67842	
Nitrite	-0.61958	-0.65921	0.743718	0.662063	0.178364	-0.41157	0.433767	-0.73785	0.360383

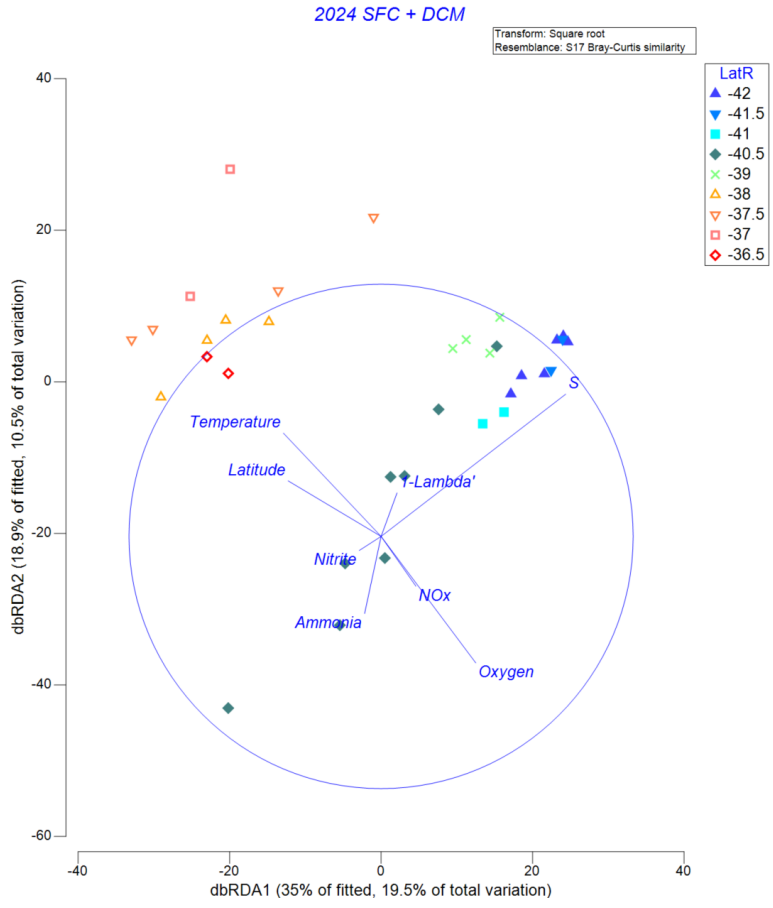
2023 BTM

	Latitude	Depth	Temperature	Salinity	S	1- λ'	Oxygen	Nitrate	Ammonia
Depth	0.294271								
Temperature	0.275346	-0.3606							
Salinity	0.411509	-0.31772	0.79612						
S	-0.1173	0.109884	0.146584	-0.00755					
1-Lambda'	-0.01996	0.253881	-0.01439	-0.24869	0.452399				
Oxygen	-0.18632	-0.45918	0.386135	0.700766	0.044058	-0.3617			
Nitrate	-0.131	0.402814	-0.70602	-0.93767	-0.06403	0.283444	-0.88453		
Ammonia	0.157843	-0.23024	0.196033	0.183305	-0.19815	-0.09181	0.175319	-0.18468	
Nitrite	0.741671	0.144844	0.424885	0.728935	-0.02779	-0.16785	0.368324	-0.59019	0.026215

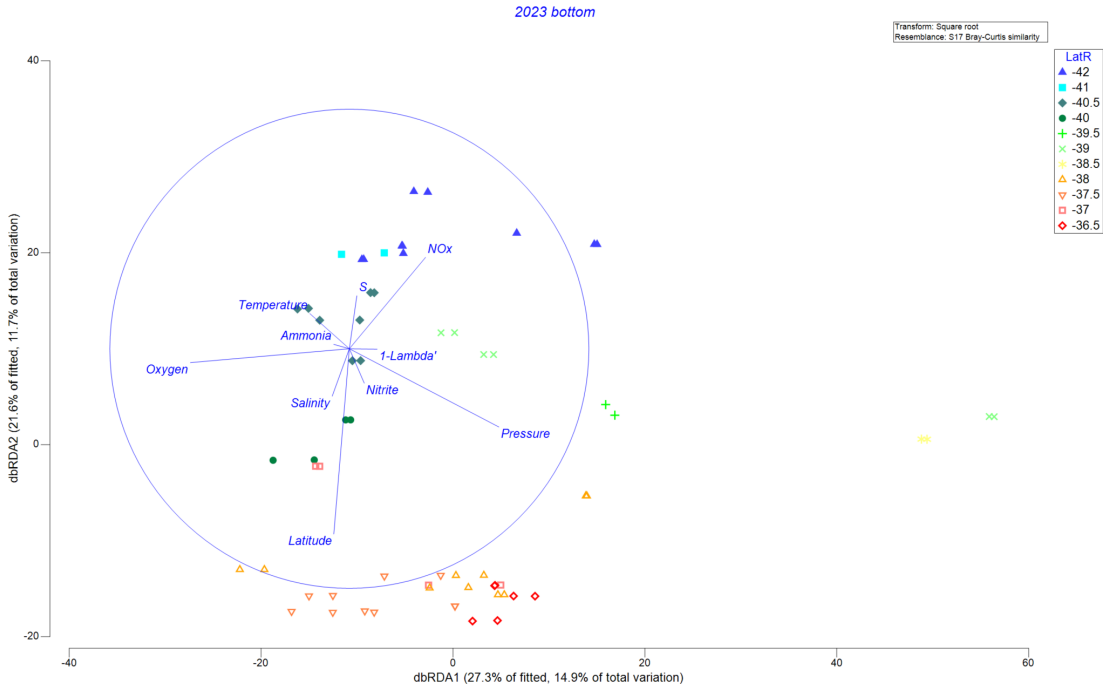
(A)


2023 SFC + DCM
Transform: Square root
Resemblance: S17 Bray-Curtis similarity
LatR
-42
-41
-40.5
-40
-39
-38
-37.5
-36.5
1-Lambda'
Temperature
Salinity
Pressure
Nitrite
Latitude
NOx
S
Ammonia
Oxygen
dbRDA2 (13.5% of fitted, 9.7% of total variation)
dbRDA1 (25.3% of fitted, 18.3% of total variation)

(B)


2024 SFC + DCM
Transform: Square root
Resemblance: S17 Bray-Curtis similarity
LatR
-42
-41.5
-41
-40.5
-39
-38
-37.5
-37
-36.5
S
Temperature
Latitude
1-Lambda'
Nitrite
NOx
Ammonia
Oxygen
dbRDA2 (18.9% of fitted, 10.5% of total variation)
dbRDA1 (35% of fitted, 19.5% of total variation)

(C)



(D)

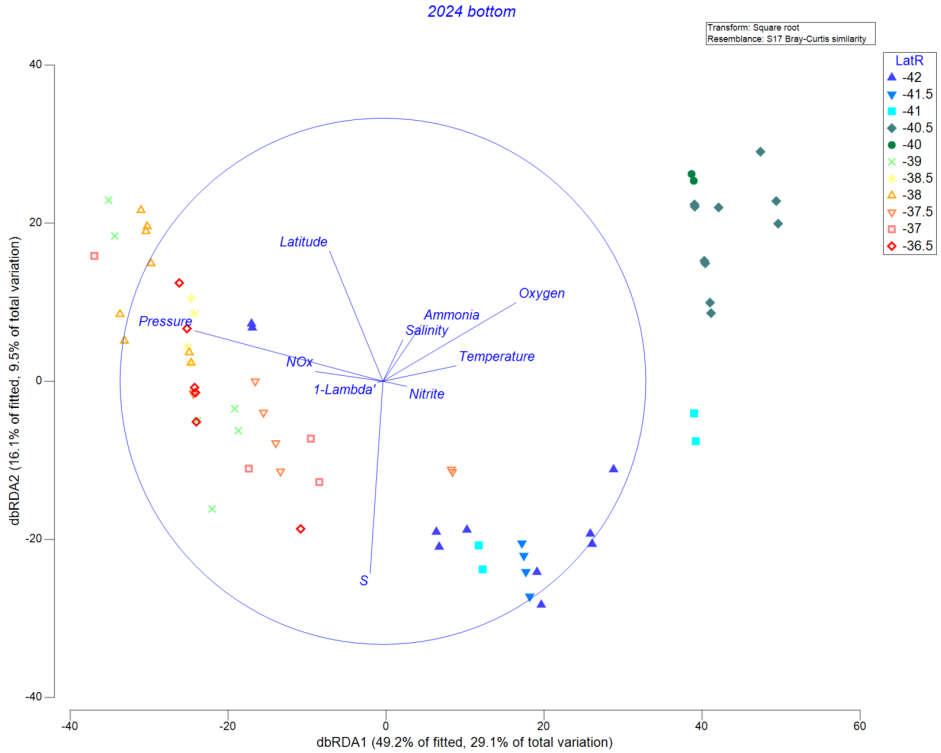


Figure 5-5 Distance based redundancy analysis (dbRDA) plots of the plankton communities, showing the spread with location (latitude) highlighted and the environmental drivers indicated by vectors. (A) Surface and DCM 2023 (B) Surface and DCM 2023 (C) Bottom 2023 (D) Bottom 2024. Note, that the length of the vectors shows their weight in the 2 coordinates shown on the graphs only. NOx = Nitrate. Pressure = Depth. S = Richness. 1-Lambda' = Simpson's evenness.

5.3.3. Status of the main plankton functional groups.

Main components

The 18S based ASV table was split into subsets representing the major plankton functional groups. The 5 most abundant functional groups were arthropods, photosynthetic phytoplankton, (parasitic) syndiniales, (mostly mixotrophic) dinoflagellates and heterotrophic flagellates, making up 90%of all the observations (sequence reads) (Figure 5-6).

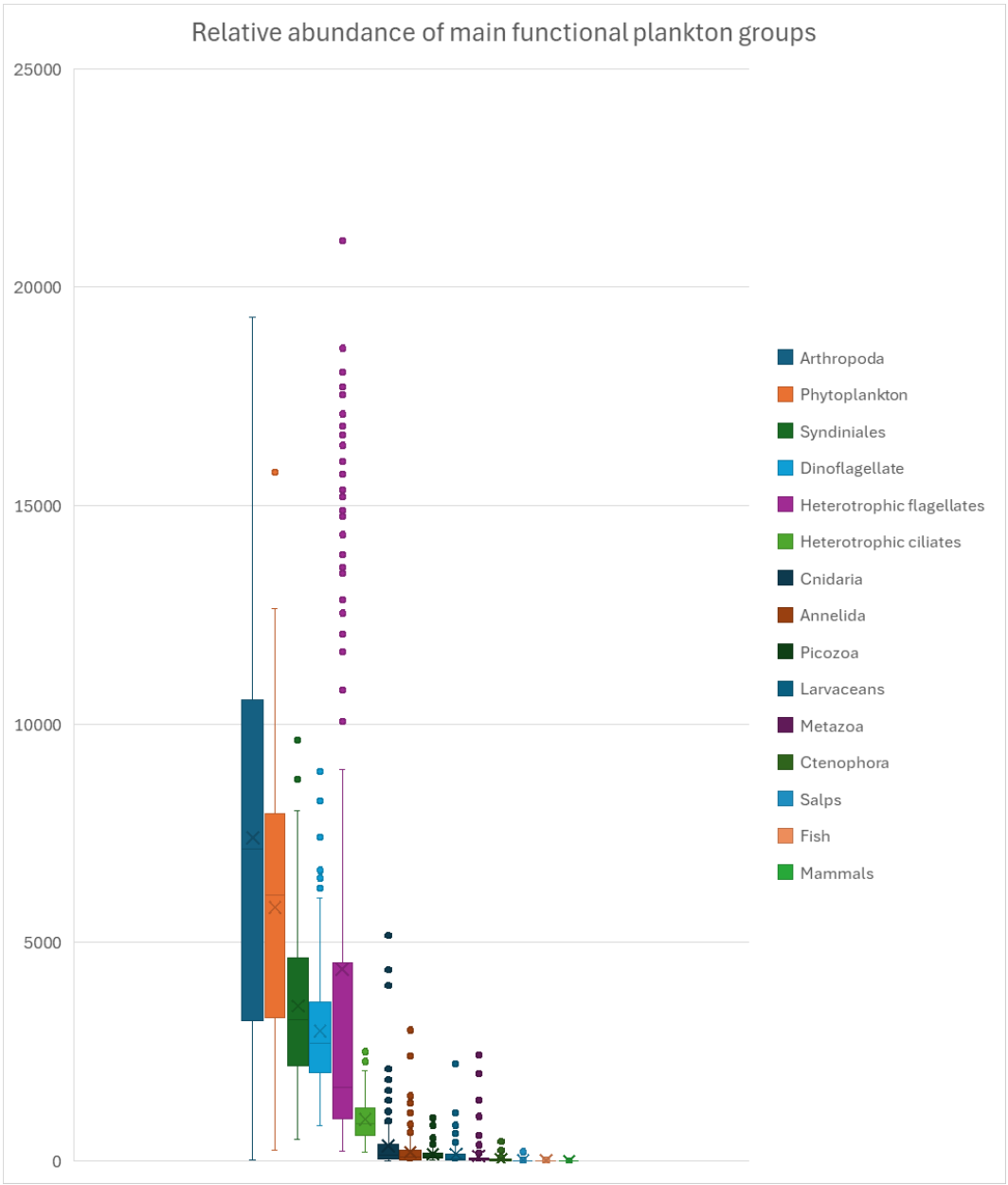


Figure 5-6 Whisker plot showing the relative abundance of each of the major functional groups across the two SEA-MES voyages analysed.

The 2023 voyage observed phytoplankton and arthropods at similar relative abundances overall, across the water column. During the 2024 voyage, however, we observed more arthropods than phytoplankton in surface, deep chlorophyll maximum and in shallow (<100m) bottom samples. Interestingly, most of the deep (>100m) bottom samples indicated a fundamentally different composition of the plankton community, dominated by heterotrophic flagellates (made up almost exclusively by Radiolaria) with hardly any arthropods or phytoplankton present (Figure 5-7).

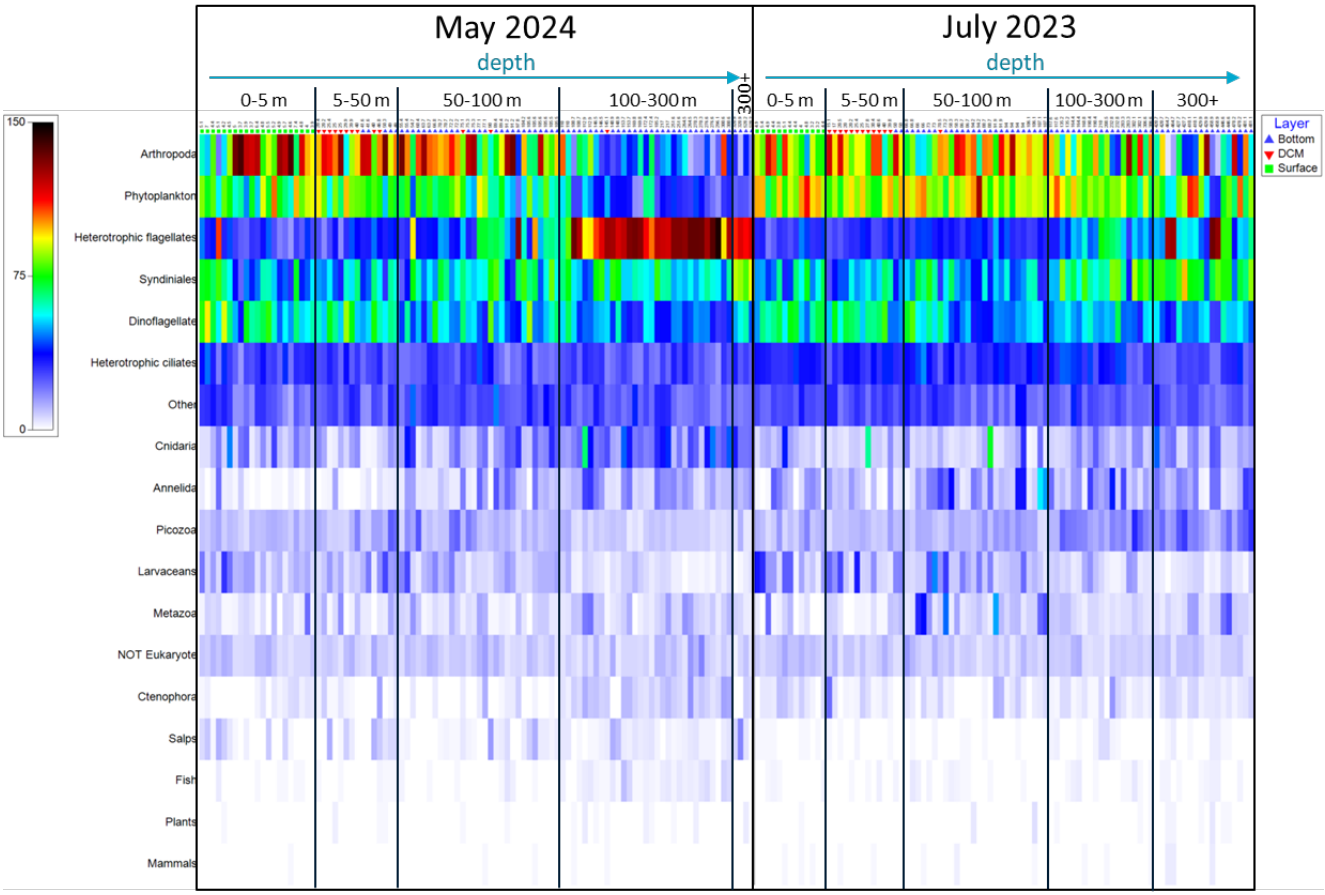


Figure 5-7. Shade plot of the plankton community across the two SEA-MES voyages analysed. Samples were rarefied to 26,739 reads. Colours and the scale bar refer to the square root of reads. If a sample consisted solely of a single functional group, the number on the plot for that group would be 163.5 (the square root of 26,739).

The negative correlation between the relative abundance of arthropods and heterotrophic flagellates was confirmed by direct analysis. Positive correlations were identified between the relative abundances of heterotrophic ciliates and phytoplankton and between syndiniales and heterotrophic flagellates, respectively (Figure 5-8).

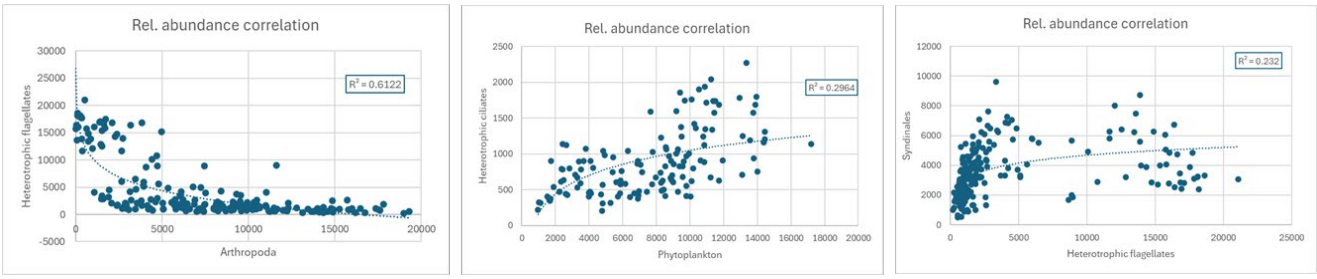


Figure 5-8 Correlations of relative abundances between functional plankton groups.

Arthropods were overwhelmingly made up by copepods.The most abundant copepod genera were *Paracalanus*, *Oithona*, *Clausocalanus* and *Calocalanus*, together making up 73% of all copepod observations. The phytoplankton was more diverse, with green algae, diatoms, pelagomonads, chryptomonads and haptophytes making up most of the total observations (34%, 15%, 7%, 7% and 7%, respectively).

Diversity data

Our data enabled the calculation of diversity indices for the separate functional groups. Syndiniales were the most diverse group, followed by (photoshynthetic) phytoplankton, dinoflagellates, heterotrophic ciliates, heterotrophic flagellates, then arthropods and finally gelatinous zooplankton (Figure 5-9).

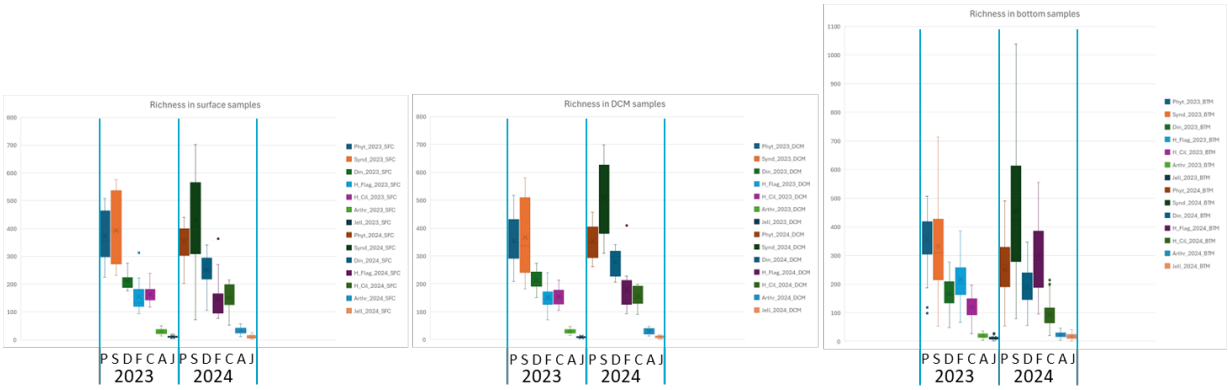


Figure 5-9 Richness of the major plankton groups for surface, deep chlorophyll maximum and bottom samples from the 2023 and the 2024 voyages. P – Phytoplankton, S – Syndiniales, D – Dinoflagellates, F – Heterotrophic Flagellates, C – Heterotrophic Ciliates, A – Arthropoda, J – Gelatinous zooplankton.

Initial analyses show two significant correlations in richness between major groups: heterotrophic ciliates show a strong correlation with phytoplankton and heterotrophic flagellates with syndiniales. Both of these correlations are more pronounced in the bottom samples (Figure 5-10) and less clear in surface and deep chlorophyll samples (data not shown).

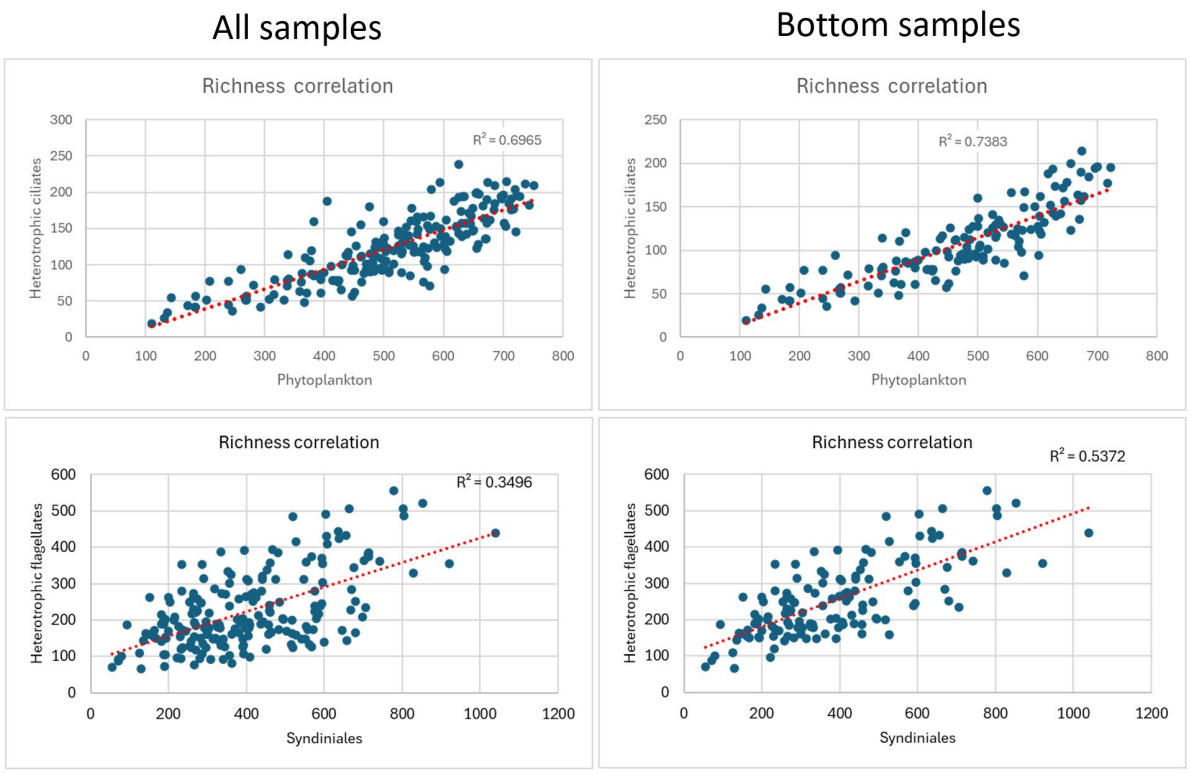


Figure 5-10 Richness correlation between major plankton groups.

Potential to derive bespoke environmental health indicators

The scientific literature contains dozens of environmental health indicators proposed for pelagic ecosystems with many of them in use for environmental management (McQuatters-Gollop *et al.*, 2019; Tett *et al.*, 2007). As the definition of environmental health varies depending on priorities and location, it is hard for this study to define the ideal indicator for the pelagic ecosystem in the area studied by the SEA-MES project.

Our data allows the easy generation of a wide range of bespoke environmental health indicators. We demonstrated this by generating two of the more broadly used indicators: overall species richness and diatom:dinoflagellate ratios (Figures Figure 5-6, Figure 5-9 and Appendix D).

5.4. Discussion

The metabarcoding data based on the 18S V4 region provided a detailed overview of the pelagic ecosystem along the two SEA-MES voyages analysed. The dataset highlighted major differences between the two voyages, aligned with the differences found in the differences in the physical and chemical oceanography. The data also showed a significant difference between the pelagic communities from the surface and deep chlorophyll maximum compared to those from the bottom of the water column. The major drivers of the eukaryote community were different between the pelagic layers: latitude was the main driver for the

surface communities while nutrients in the bottom layers. Additionally, dissolved oxygen was a major driver of the pelagic ecosystem in the bottom layer during the 2023 voyage when the water column was well mixed. In contrast, temperature and salinity were major drivers in the bottom layer during the 2024 voyage when the water column was strongly stratified.

The bottom samples of the May 2024 voyage showed a fundamentally different plankton community, dominated by Radiolaria and parasitic Syndiniales and containing very little phytoplankton or copepods. The samples showing this type of plankton community were deeper samples with high nutrient concentrations (>100uM nitrate). We only found two cases of similar communities during the July 2023 voyage. Initial analysis of the oceanographic conditions between the two voyages suggested that a spatially broad upwelling brought up nutrient rich waters along the East coast from 800-1000m depths to the continental shelf. There are several indications that the abundant presence of Radiolaria is not preferable for fish diversity and growth. Radiolaria are not a preferred food source for fish; they compete for inorganic and organic nutrients with phytoplankton; and some have been shown to pray on copepods (Suzuki and Not, 2015).

A deeper analysis of these results in the context of the fish data observed during the SEA-MES voyages would clarify whether Radiolaria dominated plankton communities compromise diversity and abundance of keystone or commercially important fish species in the deeper waters. Such analysis is beyond the scope of this study, but it will be carried out as part of the broader SEA-MES project portfolio.

We have shown that the dataset can be divided into subsets corresponding to the major groups of the pelagic foodweb: phytoplankton, Syndiniales (parasites), heterotrophic ciliates, heterotrophic flagellates, arthropods and gelatinous zooplankton. Through this, the dataset lends itself to the easy generation of diversity data for the major groups of the pelagic food web. Ultimately this enables the generation of bespoke indicators for ecosystem health monitoring.

The derived datasets of major food web groups are also suitable for structural equation modelling (SEM) (Eisenhauer *et al*., 2015) work that will establish causality in plankton food dynamics (which groups underpin the proliferation of the others). Initial analysis highlighted interesting correlations between some of the major groups, supporting the expectation that SEM analysis will provide valuable insights into plankton dynamics, towards better informed ecosystem modelling and forecasting.

6. SEA-MES Tree of life eDNA metabarcoding

6.1. Background

Tree of Life (or ToL) metabarcoding is an approach that combines several metabarcoding assays to characterise the broad range of taxonomic groups present in eDNA (Stat *et al*. 2017). One of the trade-offs faced when choosing metabarcoding markers is that those with high taxonomic breadth generally have lower taxonomic resolution, and the reverse is also true. So, when you choose only a few markers, you tend to get good taxonomic resolution for a few focus groups (e.g. mtDNA 16S fish marker in Section 3) or wide coverage but lower taxonomic resolution (e.g. 18S eukaryotic marker in Section 4). By combining data from many markers, the ToL metabarcoding approach provides the ability to rapidly characterised a complete biological community, potentially with good taxonomic resolution for many groups. It also allows the use of several markers in a particular group of interest (e.g. fish) to reduce marker specific biases. One limitation is that it is a more technically complex approach compared to applying standard assays focusing on defined groups and there is little scientific consensus on how to combine metabarcoding data from multiple markers. ToL metabarcoding has recently become a feasible option for environmental monitoring due to the development of user-friendly sample processing and data delivery platforms from commercial service providers (e.g. DiBattista *et al*. 2024).

As part of the current project, we sent a subset of eDNA samples (n=150) collected in the first SEA-MES voyage to Wilderlab for ToL analysis. This company can provide rapid characterisation of eDNA samples using a panel of metabarcoding assays and provides customers with tables of results summarising detection of a huge range of species in the sample - from bacteria to mammals.

In this section we describe the first ToL metabarcoding characterisation of the southeastern Australian region and have an initial look at ToL data collected in offshore marine sampling. Since multiple eDNA assays are included, you might expect less detailed information on individual taxonomic groups (e.g. fishes) compared to more focussed single marker datasets. To evaluate this trade-off, we compare the data obtained from the ToL metabarcoding approach with data from our fish-specific assay using a subset of the 10 L eDNA samples (i.e. the samples described in Section 3 of this report).

Much more detailed analysis could be carried out on this dataset than was possible in the timeframe of this NESP project. The samples we sent to Wilderlab included a number of 2 L samples (Section 3) and samples collected by the deep tow camera OCD sampler (Section 4). We compare the ToL data from 2 L samples with 10L in Appendix B but have not carried out further analysis of these samples. The full dataset will be made available as part of the publicly available eDNA data accessible from Wilderlab, as well as archived in public repositories.

6.2. Methods

6.2.1. Samples and NGS library preparation

A total of 150 eDNA samples were sent to Wilderlab for analysis. These included some representative samples from each of the previous three sections of the report:

(i) 90 of the 10 L eDNA samples from SEA-MES Voyage 1(including 7 negative controls).

(ii) 42 of the 2L samples, also from SEA-MES Voyage 1.

(iii) 18 samples from the OCD sampler study. These included samples from the OCD device and CTD water collected from the same location. All these samples were from Voyage 2.

Sample collection and DNA extraction methods are detailed in previous sections of the report. The DNA extracts (35 µl) were sent to Wilderlab where they were PCR-amplified using fusion-tag mitochondrial and nuclear rRNA assays for the detection of target DNAs (see Table 6-1 for primer sequences and associated taxon targets).

Table 6-1 The 12 assays used by Wilderlab for ToL metabarcoding of our eDNA samples. Locus-specific sections of fusion-tag primers used are shown. Asterisks signify long-amplicon assays that are run with a single index, reading through only the first 150 bp of the amplicon. References for primer pairs are provided; an M indicates the primer has been modified from original.

Assay code	Gene region	Primer sequences	Target	Reference
WV	mt16S	GACGAGAAGACCCTWTGGAGC CCRYGGTCGCCCCAAC	Vertebrates	(Nester *et al*. 2020)
RV	mt12S	TTAGATACCCCACTATGC TAGAACAGGCTCCTCTAG	Vertebrates	(Riaz *et al*. 2011)
LG	mt12S	CGGCGTAAAGWGTGGTTAGG CATAGTGGGGTATCTAATCCCAGTTTG	Fish	Wilderlab in house (Miya *et al*. 2015)
RJ	mt12S	TTAGATACCCTACTATG AAGCTAGCGCTTGTAGT	Sharks and rays	(Riaz *et al*. 2011) (M) Wilderlab in house
BE	18S-V9	CCCTGCCHTTTGTACACAC CCTTCYGCAGGTTCACCTAC	Eukaryotes	(Amaral-Zettler *et al*. 2009)
BU	18S-V9	TTGTACACACCGCCC CCTTCYGCAGGTTCACCTAC	Eukaryotes and bacteria	(Amaral-Zettler *et al*. 2009)
BX*	18S	GCCAGTAGTCATATGCTTGTCT GCCTGCTGCCTTCCTT	General eukaryote	(Pochon *et al*. 2013)
CI	COI	DACWGGWTGAACWGTWTAYCCHCC GTTGTAATAAAATTAAYDGCYCCTARAA TDGA	Invertebrates	(Leray *et al*. 2013) (M) (Wilkinson *et al*. 2024)
GD*	ITS2	GARTCTTTGAACGCAAATGGC GCTTATTAATATGCTTAAATTCAGCG	Coral	(Brian *et al*. 2019)
HD*	mt16S	GGACGATAAGACCCTATAAA ACGCTGTTATCCCTAAAGT	Crustaceans	(Komai *et al*. 2019)
UM	16S-V5	GGATTAGATACCCTGGTA CCGTCAATTCMTTTRAGTTT	Bacteria	(Morey *et al*. 2006) (Lane *et al*. 1985)
XN*	COI	DACWGGWTGAACWGTWTAYCCHCC TANACYTCNGGRTGNCCRAARAAYCA	Invertebrates	(Leray *et al*. 2013) (M) (Geller *et al*. 2013) (M)

The methods for laboratory processing and bioinformatics outline were provided by Wilderlab. Fusion tag primers include Illumina P5 and P7 adapter sequences, Illumina TruSeq™ sequencing primer bind site (forward primer only), unique 8 or 9bp index

sequences, and locus specific primers, respectively. All index sequences differ from each other by at least 3 bp.

All PCR reactions are carried out in duplicate. Each PCR reaction contains 3 µl MyTaq 2x Red Mix (Bioline) with 2 mg ml-1 BSA (Sigma Aldrich), 0.5 µl forward primer (10 µM), 0.5 µl reverse primer (10 µM) and 1.5 µl template DNA. PCR cycling conditions include an initial denaturation step of 3 min at 95 °C, followed by 38 cycles of 5 s at 95 °C, 10 s at the assay-specific annealing temperature, and 15 s at 72 °C.

Sequencing libraries including negative controls are pooled, cleaned and double-end size selected using AMPure XP magnetic beads (0.9x and 1.2x for lower and upper size bounds, respectively). The final pooled library concentration is determined using a Qubit 4 Fluorometer (ThermoFisher Scientific) and the concentration adjusted to 50 pM (basic panel) or 650 pM (comprehensive panel) in sterile DNAse/RNAse free water (IDT). For basic panel analysis, the library is loaded onto an iSeq i1 V2 reagent cartridge with 5% Phi X and run for 200 cycles in a single direction on an Illumina iSeq 100 instrument. For the comprehensive panel, the library is loaded onto an Illumina NextSeq 1000/2000 P1 XLEAP-SBS Reagent cartridge with 15% Phi X and run for 200 cycles in a single direction on an Illumina NextSeq 1000 instrument.

6.2.2. ASV generation and taxonomic assignment

The sequence fastq files are de-multiplexed in R (R_Core_Team 2024) using the insect package (v 1.4.0) (Wilkinson *et al*. 2018) and trimmed sequences are filtered to produce a table of exact amplicon sequence variants (ASVs) using the DADA2 package.

ASVs are identified to the lowest possible taxonomic rank using a global reference sequence database primarily compiled of trimmed reference sequences downloaded from GenBank, BOLD and the RDP reference database (v18; accessed 15 June 2022; used for UM assay only). Any ASV matching with 100% identity and 100% coverage to at least one reference sequence is assigned at the lowest common ancestor level (LCA; i.e assigned to genus level if matched to more than one species, or to family level if matched to more than one genus). For the low-resolution BU, BE, BX and UM markers, taxon assignment is restricted to genus level or above. Unmatched sequences > 50 bp in length are queried against the reference database using the SINTAX classification algorithm (Edgar 2016) with a conservative assignment threshold of > 0.99 and taxonomic assignment restricted to genus level or above.

6.2.3. Data analysis

The full data table provided by Wilderlab was imported into R (R_Core_Team 2024) and used to produce the summaries outlined below. The data table shows all sequences that were recovered, and it identifies what assay (i.e. which of the 12 primer sets) the sequence came from. Information is also provided on taxonomic assignment (including flags for pre-defined taxonomic groups, e.g. bacteria, fish) and sequence read numbers from each of the samples. No curation of the taxonomic data was performed. The data was collapsed based on scientific name using the *summarise* function in the R package *dplyr*. The Shiny Wheels app (https://wilderlab.shinyapps.io/ShinyWheel/) was used to visualise the phylogenetic tree for example files.

6.3. Summary of ToL data

The ToL metabarcoding dataset included 76.9 million sequences from the 150 SEA MES eDNA samples with a relatively even coverage across samples (Figure 6-1 shows coverage for the 2L and 10L samples). For the purposes of this report, we will focus on the 10 L samples (n=90 including negative controls); 51 million sequences were recovered from these samples with a mean of 608388 sequences per eDNA sample. The seven negative control samples had a mean read depth of 73786 sequences (mean value 12% compared to the eDNA samples; Figure 6-1). Based on low number of sequences recovered in the negatives, they were removed from downstream analysis along with one failed 10 L sample. The full dataset for the remaining 82 10 L samples had been clustered into 39040 unique sequences (ASVs).

Most sequence reads and most of the ASVs could not be assigned to taxonomy below Kingdom level (Figure 6-2). The majority of ASVs were not assigned any taxonomy (58%; 22763/39040), a further 19% (7304/39040) were assigned only to Kingdom level and 19% were classified with a taxonomic rank of Class or better (Figure 6-2). When the ASVs assigned to the same taxonomy (regardless of marker) are merged, 1439 unique taxa remain (Figure 6-2C). This merging step combines many sequences that have the same broad-level taxonomy but retains the distinctive taxonomy in genus and species level assignments, so there is large drop in number of taxa and a shift in distribution toward genus and species level assignments.

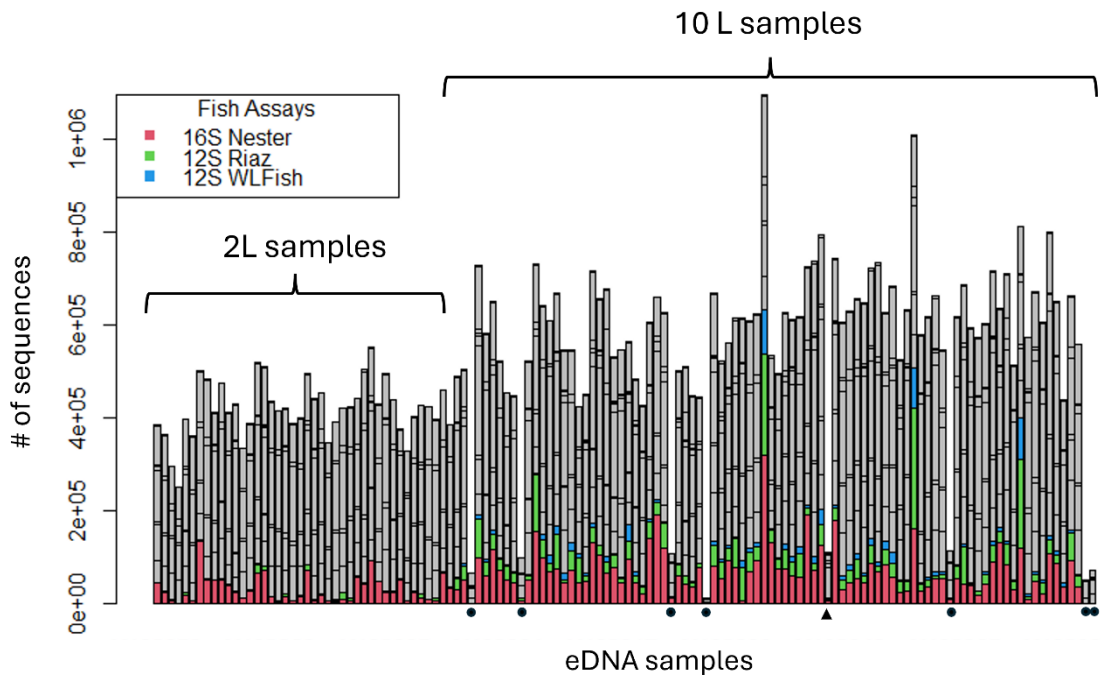


Figure 6-1 Bar plots showing number of sequences recovered from 2 L (n=42) and 10 L (n=90) eDNA samples from SEA-MES Voyage 1. The reads from 12 different assays are stacked in each column. The three assay targeting fish/vertebrates (WV 16S Nester, RV 12S Riaz, LG 12S WLFish) and are coloured, remaining assays are in grey. The black dots indicate the 7 negative controls (included field filtration and laboratory controls), the black triangle indicates one sample with low reads.

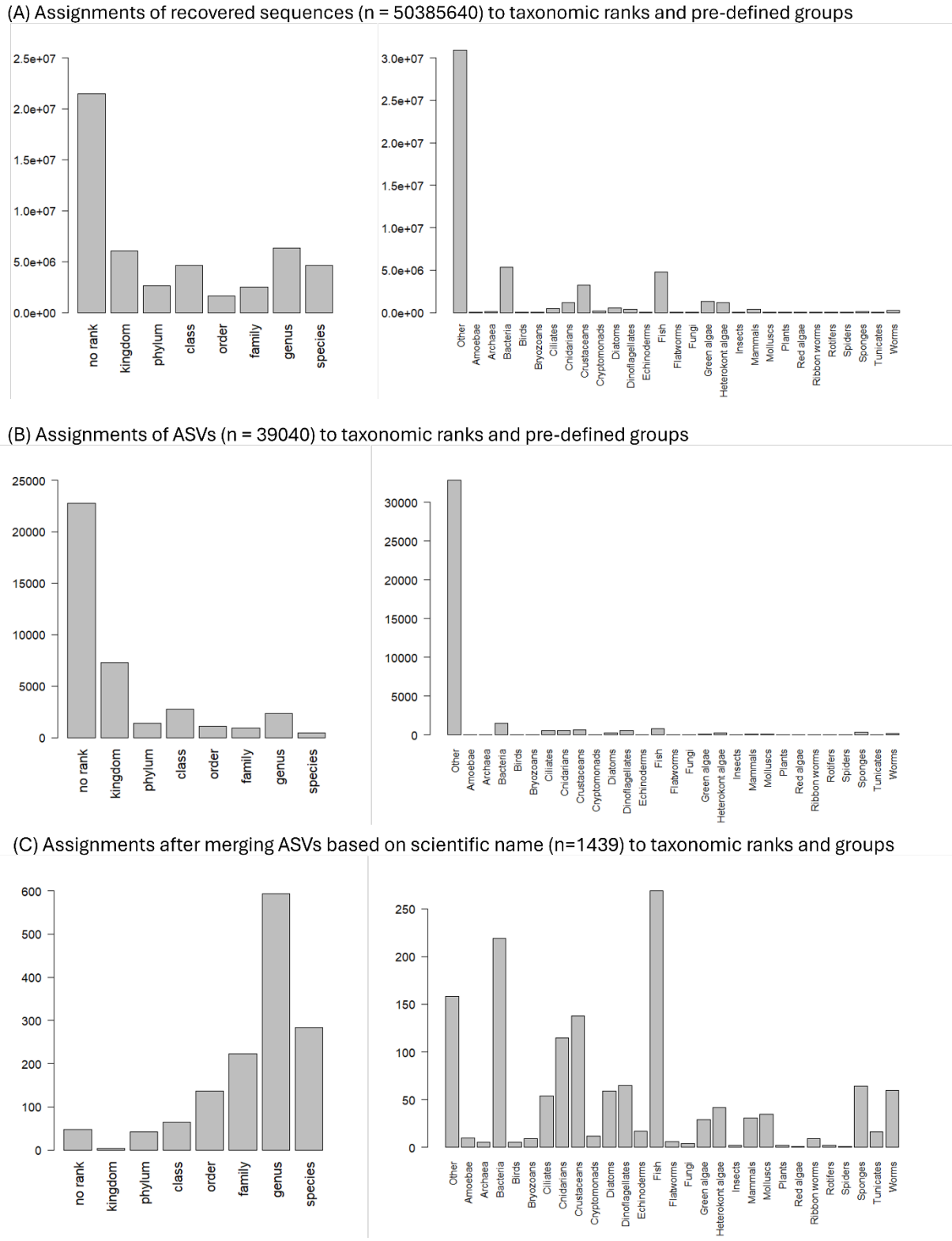


Figure 6-2 Bar plots showing how DNA sequence data from ToL metabarcoding is assigned to various taxonomic ranks and pre-defined groups of taxa (based on sequences recovered from 82 of the 10 L eDNA samples from Voyage 1). (A) number of sequences assigned to each taxonomic rank and group. (B) number of sequence variants (ASVs) assigned to each taxonomic rank and group. (C) assignments after merging sequences with same scientific name and group.

The ToL metadata barcoding data combines several assays to get a broad overview of the phylogenetic diversity of taxa in the samples. In the ‘groups of taxa’ categories ToL metabarcoding the group ‘fish’ had the most taxa, followed by ‘bacteria’, ‘crustaceans’ and ‘cnidarians’ (Figure 6-2C). The recovered sequences and the taxonomic assignments can be visualised in “Wheel of Life” software available from Wilderlab. The tree with all 1439 unique taxa in the 82 10L samples is very crowded, so we provide an example based on taxa identified in two samples (Figure 6-3).

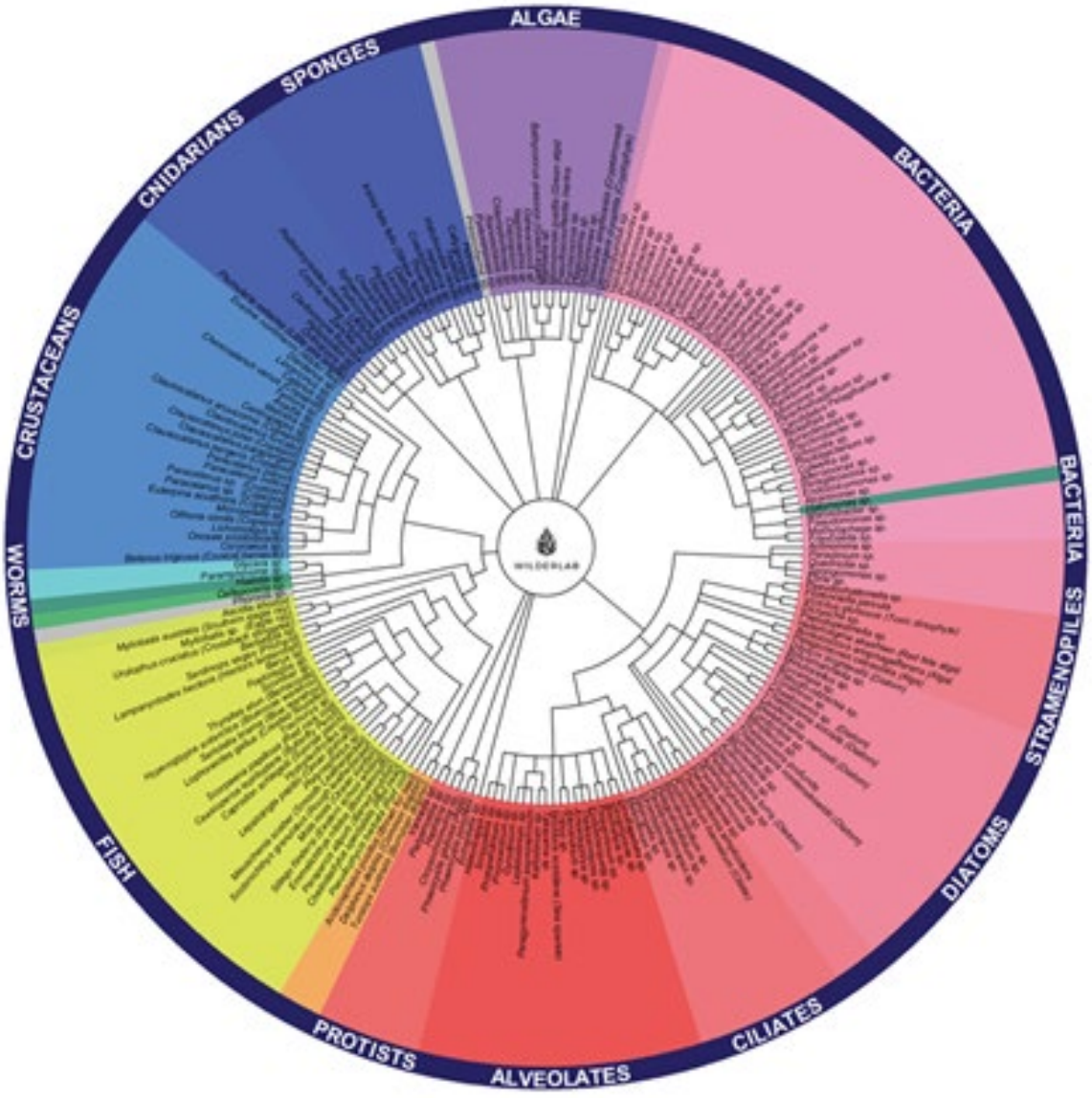


Figure 6-3 Wheel of Life visualization of ToL metabarcoding data from two 10 litre eDNA samples (M158 and M159) taken at a single site in Flinders Marine Park (water collected at 65 meters depth near seafloor).

To highlight the power of the approach and type of information present in the data we pulled out the mammal sequences (Table 6-2). This identifies 10 marine mammals to species level, with detection of the common dolphin at 61 of the 82 sites. The mammal species list also includes several terrestrial mammals, highlighting the sensitivity of the approach and the essential need for data curation steps.

Table 6-2 Mammals detected using ToL metabarcoding and identified to genus or species level in our 82 eDNA samples (10 L) from SEA-MES voyage 1.

Scientific Name	Rank	Common Name	Occurrences
Marine mammals			
Delphinus delphis	species	Common dolphin	61
Tursiops truncatus* or *D. delphis	species	Common or bottlenose dolphin	18
Arctocephalus pusillus	species	Cape fur seal	17
Megaptera novaeangliae	species	Humpback whale	15
Tursiops australis	species	Burrunan dolphin	6
Tasmacetus shepherdi	species	Shepherd's beaked whale	2
Orcinus orca	species	Orca	2
Balaenoptera edeni	species	Pygmy Bryde's whale	1
Tursiops truncatus	species	Common bottlenose dolphin	1
Stenella coeruleoalba	species	Striped dolphin	1
Arctocephalus forsteri	species	Long-nosed fur seal	1
Mesoplodon	genus	Beaked whale	1
Arctocephalus	genus	Southern fur seals	1
Balaenoptera	genus	Baleen Whale	1
Megaptera	genus	Humpback whale	1
Terrestrial mammals			
Bos taurus	species	Cattle	13
Ovis aries	species	Sheep	9
Felis catus	species	Cat	1
Canis lupus familiaris	subspecies	Dog	1
Osphranter rufus	species	Red kangaroo	1
Ovis	genus	Sheep; Ovine	4
Felis	genus	Cats	1

6.4. Comparison of ToL fish data with single marker metabarcoding of fish eDNA

In this project there are several examples where we analysed the same eDNA sample with multiple approaches. The 82 eDNA samples (10L) that were analysed with ToL metabarcoding were also processed using our fish-specific assay (Section 3 of this report). Here we provide a brief comparison of the fish datasets produced with these separate approaches (single marker versus ToL).

There were three markers in the ToL metabarcoding data that targeted fish and one additional marker which targeted a subset of fish (sharks and rays). Variable number of reads came from each marker in different samples (Figure 6-4). The number of taxa (any taxonomic rank) that came identified in the assays were WV=324, RV= 118, LG = 72, RJ = 16. The percentage of these taxa that were fish ranged from 55% (WV) to 85% (LG); data from WV shown in Figure 6-5.

The RJ (shark/ray) marker had a relatively small number of sequence reads (mean = 3132; Figure 6-4); 11 samples had no reads, and 37 more samples had less than 1500 reads. With the RJ marker 13 taxa were identified. Four sharks/rays were identified to species level: Smooth Stingray, *Bathytoshia brevicaudata*, Slender Lanternshark *Etmopterus pusillus*, Longnose spurdog *Squalus Blainville* and Silver chimaera *Chimaera phantasma*. The first 2 species are present in the Southeast Australian waters, but the other 2 are from the Atlantic and North Pacific Oceans respectively (both have close relatives in Southeast Australian waters). The remaining 9 shark/ray taxa are identified to genus (*Mustelus*, *Dipturus*, *Cephaloscyllium*, *Carcharhinus, Bathytoshia*), family (Rajidae, Dasyatidae) or order (Myliobatiformes, Lamniformes). It is possible many species within these higher taxonomic groups are present in the eDNA data, but they can't be differentiated. The RJ (shark/ray) data highlights the need for careful curation of taxonomy in all eDNA metabarcoding pipeline outputs and the need for higher resolution markers for sharks/rays. The other vertebrate/fish markers identified several shark/ray taxa – the use of multiple markers targeting the same group can help characterise all taxa present in eDNA samples.

The WV 16S Nester assay used in ToL metabarcoding amplifies the same mtDNA gene as the 16S fish marker in Section 3. The WV marker is shorter, covering the first ~80 bp of the ~200 bp region covered by the16S fish marker (exact length of the gene region varies between species). We make a direct comparison of data from these two markers; we will refer to these as 'WV 16S Nester' assay used in ToL metabarcoding compared to the longer '16S fish' assay we used in Section 3 of this report. It should also be noted that in the ToL barcoding approach, sequences from groups outside the primary target of the assay are retained. Counts of non-fish sequences from the WV 16S Nester assay are shown in Figure 6-5. With the16S fish assay, we only retained sequences that were identified as coming from fish. To allow comparisons we consider only the fish sequences from each marker.

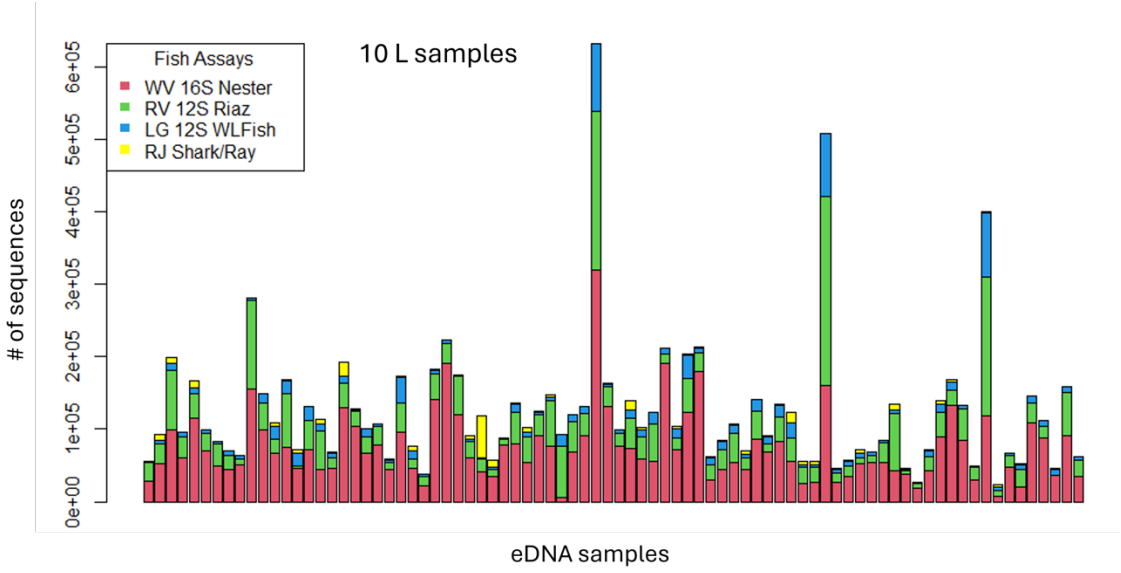


Figure 6-4 Bar plots showing number of sequences recovered from the fish assays in 10 L (n=82) eDNA samples from SEA-MES Voyage 1 using ToL metabarcoding. The reads from 4 different assays are stacked in each column.

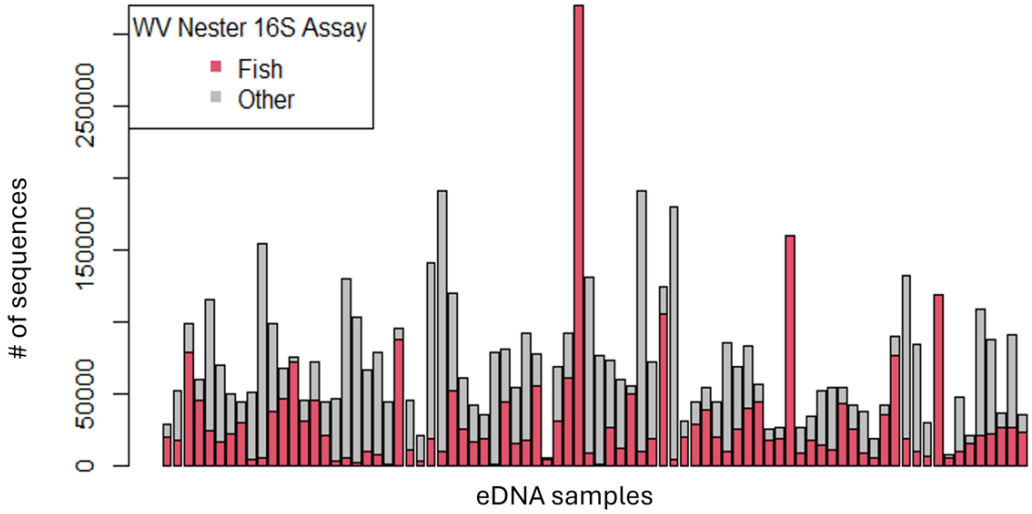


Figure 6-5 Bar plots showing number of sequences coming the primary group targeted by the WV 16S Nester assay (sequences expected to be from fish) compared to sequences coming from other groups. Data from ToL metabarcoding of 10 L (n=82) eDNA samples from SEA-MES Voyage 1.

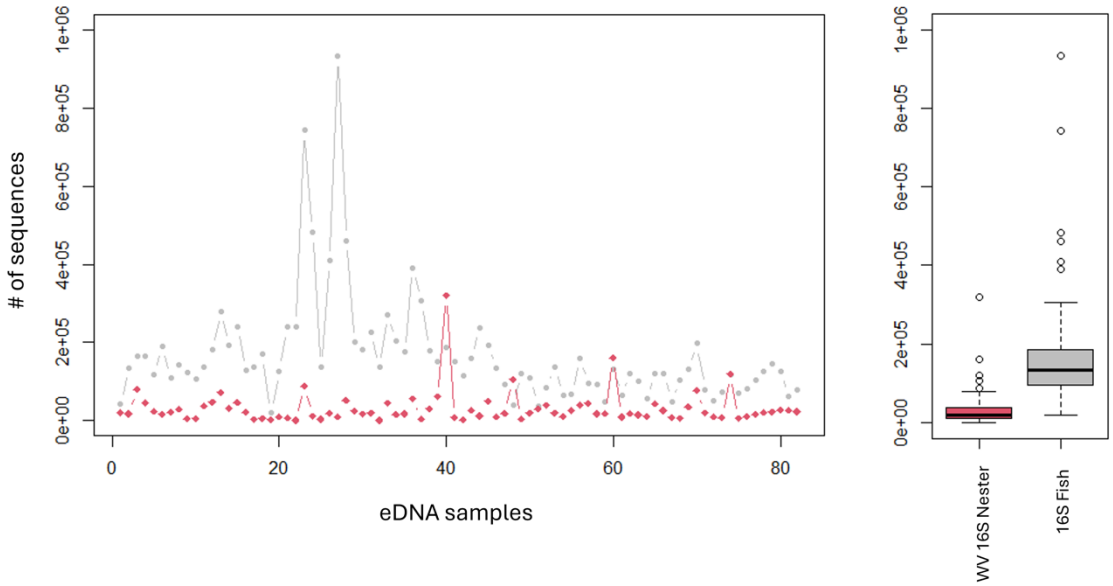


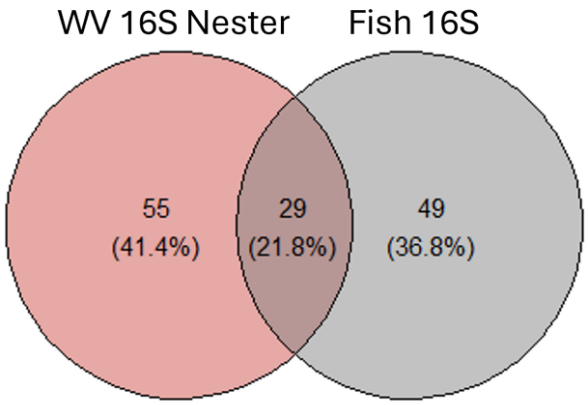
Figure 6-6 Plots showing number of fish sequences in the same 82 eDNA for WV 16S Nester (red) and Fish 16S (grey). Counts of all sequences assigned to the broad group fish and identified to genus or species-level were counted.

The mean number of reads from Fish 16S was 165872 and from WV 16S Nester it was 30856 (about 5 time more sequence data from Fish 16S; Figure 6-6). At the species level, there were 84 fish identified from WV 16S Nester sequences and 78 from Fish 16S sequences, only 29 of these species were found in both datasets (Figure 6-7). Given the number of species detected by each marker is similar, it is surprising that the species make-up is so different. Some of these differences is due to assignment at different taxonomic levels and uncurated data from the WV 16S assay. Still, the use of both markers together provides a much more comprehensive view of fish in the region (133 species in the combined list).

The taxonomic richness measured by the two markers is highly correlated, indicating they each capture trends in species richness (Figure 6-8). When we consider taxa identified in the 82 eDNA samples including both genus-level and species-level identifications, the higher number of fish being identified by the shorter WV 16S Nester marker becomes apparent compared to species level identifications only shown above (Figure 6-8; number of fish detected was 155 for WV 16S Nester and 119 with Fish 16S).

WV 16S Nester
Allothunnus fallai
Alopias vulpinus
Antimora rostrata
Atypichthys strigatus
Caesioperca lepidoptera
Callorhinchus milii
Centroberyx affinis
Centrolophus niger
Centrophorus squamosus
Centropogon australis
Cephaloscyllium umbratile
Chelidonichthys kumu
Conger verreauxi
Crossorhombus howensis
Cubiceps capensis
Cyttus novaezealandiae
Diodon nicthemerus
Galeorhinus galeus
Genypterus blacodes
Gonorynchus greyi
Gymnapistes marmoratus
Helicolenus avius
Helicolenus sp. KP-2015
Hippocampus abdominalis
Hypoplectrodes nigroruber
Katsuwonus pelamis
Lampanyctus australis
Latridopsis forsteri
Lepidotrigla argus
Lepidotrigla grandis
Lepidotrigla papilio
Lotella rhacina
Meuschenia scaber
*Mustelus lenticulatus**
Myctophum punctatum
Myliobatis australis
Myliobatis sp. KP-2015
Nelusetta ayraudi
Neosebastes pandus
Paratrachichthys trailli
Platycephalus grandispinis
Plectranthias kelloggi
*Pseudolabrus miles**
*Pseudophycis bachus**
Scolecenchelys breviceps
Scorpaenodes evides
Seriolella brama
Sphoeroides pachygaster
*Squalus blainville**
Sternoptyx diaphana
Trygonorrhina guanerius
Upeneichthys vlamingii
Valenciennellus tripunctulatus
Zebrias scalaris
*Zenopsis nebulosus**

Identified in Both
Allomycterus pilatus
Bathytoshia brevicaudata
Emmelichthys nitidus
Engraulis australis
Etmopterus lucifer
Foetorepus calauropomus
Hoplostethus atlanticus
Hygophum hanseni
Hypoplectrodes maccullochi
Lampanyctodes hectoris
Lampichthys procerus
Lepidopus caudatus
Lophonectes gallus
Macruronus novaezelandiae
Nemadactylus douglasii
Parapercis allporti
Parequula melbournensis
Pristiophorus cirratus
Pseudophycis barbata
Pseudophycis breviuscula
Salmo salar
Sardinops sagax
Scopelopsis multipunctatus
Scorpaena papillosa
Thamnaconus degeni
Thyrsites atun
Urolophus cruciatus
Urolophus viridis
Zeus faber



Fish 16S
Alopias superciliosus
Argentina australiae
Azygopus pinnifasciatus
Bassanago bulbiceps
Beryx decadactylus
Chironemus georgianus
Coelorinchus gormani
Cubiceps caeruleus
Dipturus canutus
Dipturus gudgeri
Diretmus argenteus
Eubalichthys bucephalus
Eubalichthys gunnii
Heterodontus portjacksoni
Hoplichthys haswelli
Macroramphosus scolopax
Melanolagus bericoides
Mola mola
Mora moro
Mustelus antarcticus
Myliobatis tenuicaudatus
Nelusetta ayraud
Neosebastes scorpaenoides
Neosebastes thetidis
Ophisurus serpens
Parascyllium ferrugineum
Paratrachichthys macleayi
Paraulopus nigripinnis
Pavoraja nitida
Pempheris multiradiata
Platycephalus aurimaculatus
Platycephalus bassensis
Platycephalus richardsoni
Psenes pellucidus
Pseudolabrus rubicundus
Pseudophycis palmata
Pterygotrigla and ertoni
Pterygotrigla polyommata
Rexea solandri
Scomber australasicus
Scomberesox saurus
Sillago flindersi
Spiniraja whitleyi
Symbolophorus barnardi
Thunnus tonggol
Verilus anomalus
Xiphias gladius
Zanclistius elevatus
Zenopsis nebulosa

Figure 6-7 Venn diagram showing overlap in species-level identifications between the two markers in the 82 eDNA samples and list of fish species in each group. In the uncurated WV 16S Nester data a few species are out of range or have invalid taxonomy (marked with asterisk).

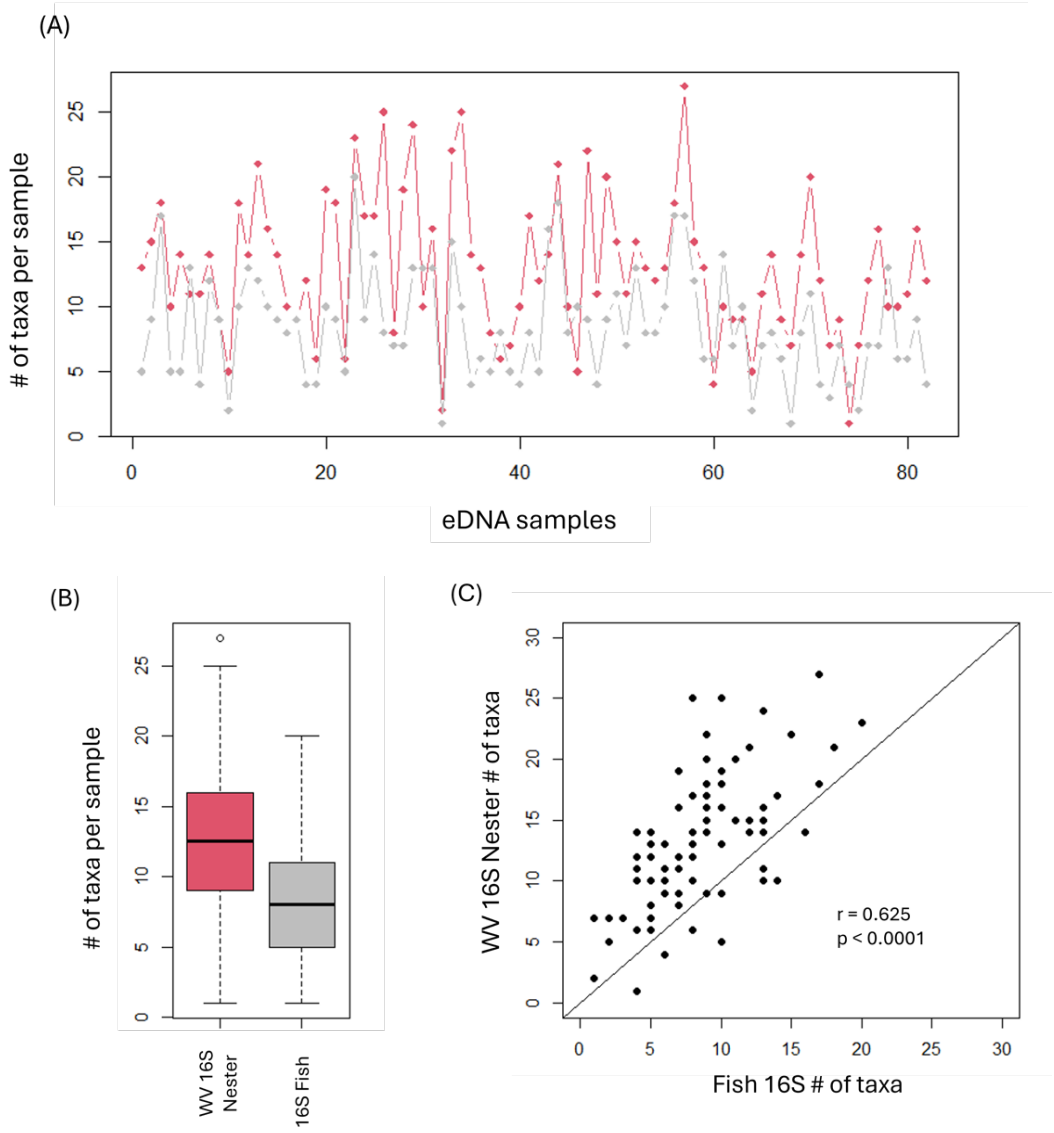


Figure 6-8 Fish richness in the 82 eDNA samples for WV 16S Nester (red) and Fish 16S (grey) assays based on taxa identified to genus-level or species-level. (A) boxplot showing higher diversity in the WV 16S Nester assay (B) correlation in richness between the two assays (line of equality is plotted; p-value is for Pearson correlation coefficient).

We compared the proportion of sequences recovered from fish taxa for each of the assays in cases where there are shared detections. To do this we collapsed all genus or species-level detections to the level of genus. The number of shared genera detected was 65; >85% of the sequences from each assay were assigned to these 65 genera (indicating that the most common fish DNA is being picked up by both assays). The sequence proportions in data from each of the assays show there is general agreement (Table 6-3); 14 of the top 18 species are the same. One species of myctophid fish (*Lampanyctodes* sp.) stands out as unusual (Figure 6-9A), this genus was the most common detected in terms of sequence proportion (26%) with WV 16S Nester but had few reads in the 16S Fish data (presumably due to primer mismatch in the later assay – although *Lampanyctodes* sp. was still detected in 33% of the overall samples with 16S Fish see Table 11-1). If we remove this genus (and adjust proportions to add to 1) there is a reasonable correlation between proportions of reads recovered by the two assays (Figure 6-9B).

Table 6-3 The 18 most common fish genera detected in the two assays based on the proportion of fish sequence reads. Green fill in the table indicates fish genera that are in both lists.

WV 16S Nester	Proportion of Reads	16S Fish	Proportion of Reads
Lampanyctodes	0.256	*Trachurus*	0.164
Trachurus	0.146	*Emmelichthys*	0.097
Engraulis	0.072	*Sardinops*	0.081
Emmelichthys	0.066	*Seriolella*	0.077
Lepidotrigla	0.060	*Caesioperca*	0.057
Seriolella	0.051	*Engraulis*	0.051
Sardinops	0.042	*Maurolicus*	0.049
Platycephalus	0.038	*Platycephalus*	0.044
Scomber	0.034	*Lepidotrigla*	0.040
Caesioperca	0.025	*Hygophum*	0.026
Genypterus	0.021	*Parapercis*	0.024
Myliobatis	0.020	*Helicolenus*	0.019
Pseudophycis	0.017	*Allomycterus*	0.018
Parapercis	0.014	*Scomber*	0.018
Helicolenus	0.012	*Genypterus*	0.016
Urolophus	0.012	*Neosebastes*	0.015
Thyrsites	0.010	*Foetorepus*	0.013
Foetorepus	0.007	*Urolophus*	0.013

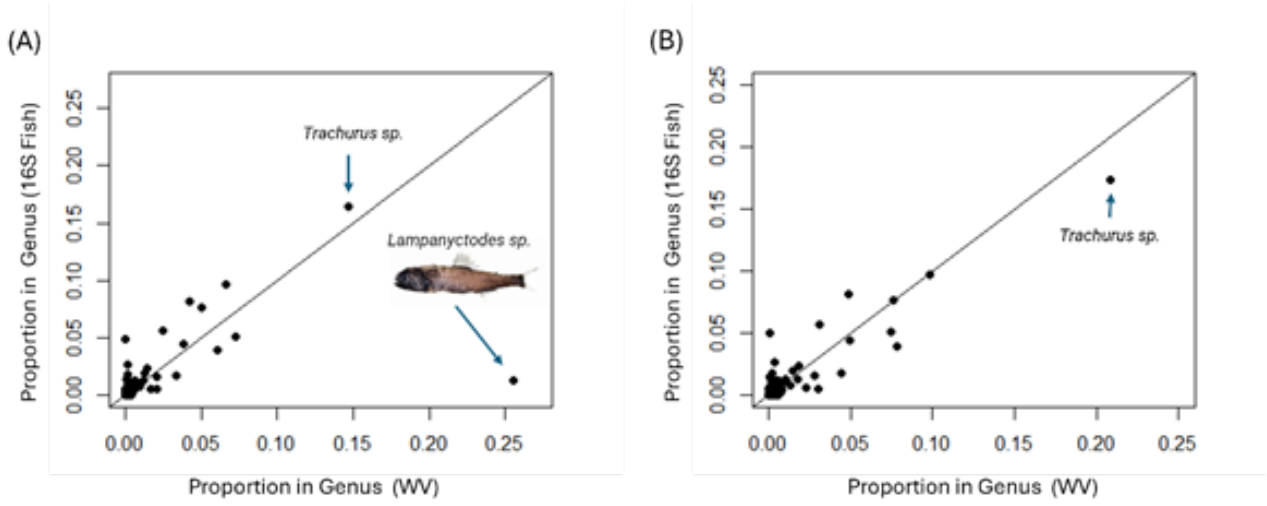


Figure 6-9 Plots showing correlation in the proportion of sequences recovered from fish taxa for Fish 16S and WV 16S Nester assays. (A) Plot highlighting the myctophid fish *(Lampanyctodes* sp.) with very abundant detections from WV 16S Nester but not common in the 16S Fish data. (B) Plot with *Lampanyctodes* sp. removed from the data and proportions adjusted to sum to 1. Image: Australian National Fish Collection, CSIRO.

6.5. Discussion of the ToL approach for marine eDNA surveys

6.5.1. Overall impressions of ToL approach

The very broad coverage of taxa recovered by the ToL approach is easily seen in the phylogenetic plot of data recovered from a few of our eDNA samples (Figure 6-3). Even though there are many unclassified sequences returned in the dataset, well over 1000 taxa were identified. Matching this coverage using conventional biodiversity survey methods would require a very large effort. To enable timely analysis of the ToL dataset for this report we used the taxonomy as provided (i.e. no manual curation). Most of our analysis focussed on fish taxa being detected by ToL metabarcoding and carrying out a preliminary comparison to what we had found in our own analysis of fish16S eDNA (Section 3 of the report). The discussion here is by no means a comprehensive assessment of the ToL metabarcoding approach, it simply reflects a few ideas that came from our initial analysis of this data.

Interpreting the overall diversity presented by the ToL data is challenging due to the huge amount of metabarcoding information being summarised. As was outlined in Section 3 of the report, automated taxonomic assignment is imperfect and manual curation of the assignments is required. With so many assays from a diverse range of taxa this step becomes very difficult. This is compounded by limitations in the reference database coverage for several different gene regions from these diverse taxa.

In some ways, combining several assays in the ToL metabarcoding approach is analogous to combining several conventional biodiversity assessments. For example, joining together voyage biodiversity data from different trawl nets (benthic and mid-water), towed video, zooplankton nets and phytoplankton microscopy into a single dataset. In the conventional assessments the choice of methods will strongly impact what taxa are detected and the availability of taxonomists for each group will impact taxonomic certainty (e.g. copepod species-level identification in plankton is going to be higher with a copepod specialist team member). The choice of assays in ToL metabarcoding are similarly influential – which taxonomic groups are recovered and how well they are identified is dependent on choice of eDNA assays and on completeness of reference databases for different groups.

Just like combining multiple conventional biodiversity datasets, combining eDNA assays from microbes with higher organisms in a joint analysis is not standard practice. Joining together different datasets produces a view of overall diversity that is skewed and not quantitative. We can see this in our data; the most diverse group in our ToL data was the fishes, even though many other groups of organisms in this environment are expected to be more diverse. The eDNA samples likely contain the diversity of small microorganisms (e.g. phytoplankton) in the environment, but the large number of these species may mean that the low sequencing skimming approach misses some of the diversity. This is something that could be examined by comparing our 18S eukaryotic data in Section 4 of this report with similar markers in the ToL assays. Larger organisms such as fish are not as well sampled in a small eDNA sample of water (see Section 3 of report). However, the diversity of fish that are in the samples seem to be captured well in our ToL data (as seen in the ToL fish data captured by the WV 16S Nester assay).

There is potential to double count taxa when using ToL metabarcoding since sequences from different markers can be assigned to different taxonomic levels. This can be illustrated by looking at the mammals recovered in our samples from all 12 ToL assays (Table 6-2). For the humpback whale (*Megaptera novaeangliae*) there are species level detections and a genus level identification. In this case, *Megaptera* is a monotypic genus, and the genus level assignment is presumably detecting the same DNA that has been counted at species level – but comes from a marker with lower taxonomic resolution. This type of duplication could inflate the number of taxa in ToL metabarcoding datasets, in some cases additional taxonomic levels could also be counted (e.g. the order Artiodactyla to which humpback whale belongs was also included in the dataset).

Another challenge (in common with all metabarcoding approaches) is the detection of DNA that does not come from the surveyed environment. In our marine samples, eDNA from several terrestrial mammals was detected and has presumably come from coastal runoff. In this case the data can be safely removed from the analysis, but it is not clear how these types of detection impact the rest of the data. Are all the marine mammal detections from species close to the survey area? If we are removing the terrestrial mammals because they are not in the area, how can we curate out inaccurate detections of species that are potentially present in the area? This example illustrates a general challenge for eDNA data that needs more consideration.

6.5.2. Insights from comparison of ToL fish data with single marker metabarcoding

Our comparison between the fish taxa detected by the ToL WV 16S Nester assay (~80 bp and ~30K sequences per sample) versus the longer 16S fish we used in Section 3 of the report (~200 bp and ~165k sequences per sample) revealed several interesting findings.

- The different levels of sequencing coverage did not impact the diversity of fish detected, indicating a sequence coverage of 30K per sample is sufficient to detect all fish the markers have amplified.
- The WV 16S Nester marker detected a higher diversity of fishes to genus- or species-level. This could be due to an increase in sensitivity because the WV 16S Nester is a shorter marker and can detect degraded DNA missed by the longer marker.
- Increased sensitivity of the short marker likely explains some of the additional species detected by the WV 16S Nester assay, since some of these extra species were detected by 16S Fish in the additional eDNA samples described in Section 3 of the report – so these fish are detectable by both markers.
- The fact the short marker did not detect all the species (or genera) detected by the long marker suggests this is not the full story. Some of the differences may be due to variations in data processing and assignments of some of these taxa to genus level in one marker but not the other.
- Large differences in fish species were detected with different assays from the same eDNA samples (Figure 6-7). This may come down to variation in the assay efficiency for different fish species (i.e. primer binding) and this was seen in the data. It is also possible that stochastic differences in PCR mean some rare fish species are missed even though they are represented in the eDNA. The ToL assays were carried out in duplicate compared to a single PCR for 16S fish and it would be worth testing the impact of PCR replicates.

- The RJ (shark/ray) marker in the ToL assay panel did not perform well but each of the multiple fish assays did add to the total species diversity, suggesting this multi-assay approach can provide the most information from each eDNA sample.

6.5.3. ToL conclusions

Characterising eDNA using multiple assays that recover informative DNA regions from across the Tree of Life is a very promising approach. The complexity of the data makes it challenging to interpret, but there are many different levels it can be looked at. Ironically, even though the ToL eDNA data is very rich in information it was the easiest to collect due to efficient processing by a dedicated eDNA metabarcoding team. The choice of assays for the Australian temperate marine environment could be improved to better capture key marine groups. Having a large range of assays would allow the dataset to be customised depending on the specific marine environment and taxonomic groups are of interest. One of the challenges with the larger number of assays is the amount of eDNA that is required; we used a third of our eDNA sample (35 µl out of 100 µl) to run the ToL approach with 12 assays. If eDNA samples are to be saved for potential future analysis, the amount being used needs to be minimised.

We have in many ways considered the ToL approach as a tool to produce a conventional biodiversity dataset. If you extend this into the future for marine science voyages, you could imagine teams of molecular curators continuing work to build more comprehensive reference databases (like CSIRO's National Biodiversity DNA Library) and optimising approaches allowing the assignment of eDNA sequences to Linnaean species. This is not unlike the curation of physical voucher specimens and current post-voyage identification of specimens by researchers from CSIRO and Australian museums. Given the large numbers of unassigned sequences, better databases would make a large difference to the number of species detected and improve the uptake to the approach. Another future vision would be to focus less on taxonomy and more on what useful information is in the biogeographic distribution of sequences, regardless of which species the DNA came from. This type of approach has been used to produce indices of aquatic health in the freshwater environment (Wilkinson *et al*. 2024). Regardless of how the sequence data is initially used it can be archived and re-analysed into the future using different methods. It is likely that these two visions (with and without a focus on taxonomy) will both proceed and eventually merge once taxonomic assignments improve enough.

7. Summary of technical findings relevant to fish eDNA sampling

We employed several methods to capture information from the fish component of the eDNA collected during the SEA-MES voyages. Since technical insights are distributed throughout this report, we provide a non-exhaustive summary of these findings here for ease of reference.

7.1. Filtration of 20 L of water and a single fish-specific eDNA assay did not capture full diversity of fish at a location

Because previous studies have emphasized the need for large water volumes to improve the detection of fish eDNA we collected 10 L of water for fish eDNA analysis, in addition to the typical 2 L collected for marine plankton sampling. We also took replicate 10 L samples at many sites near the seafloor. The 10 L replicates revealed distinct subsets of the fish community (Figure 3-9), with a median taxonomic overlap of less than 30% (Figure 4-3), indicating that even with 20 L of water filtered we were not capturing full fish biodiversity.

Our results also show that two eDNA assays, each designed to detect all fish taxa, recovered different components of the fish community (Figure 6-7). This underscores the value of using multiple assays, such as in the Tree of Life (ToL) approach, to maximise taxonomic coverage of fish within each eDNA sample.

In addition, a shorter DNA fragment (~80 bp), targeting a sequence within the longer ~200 bp fragment amplified by the 16S Fish assay, detected a greater number of fish taxa (Figure 6-8). This is likely due to the higher abundance of shorter DNA fragments in the degraded eDNA, as well as differences in the fish taxa detected by each assay (due to characteristics of the PCR primers). It should also be noted that replicate PCR amplifications were done with the shorter marker only, and this may also have increased the diversity of eDNA recovered too. Finally, while shorter markers do allow recovery of more eDNA, the trade-off is that there may be less taxonomic information in the DNA sequence.

The number of sequences and the taxonomic resolution obtained from Chondrichthyes (sharks and rays) was generally lower than for bony fish. The development of optimised eDNA assays targeting this group would be useful.

Identifying optimal eDNA assays (i.e. PCR primers which are conserved across Australian fish groups and amplify short, informative markers) will be made possible with the release of a comprehensive fish mtDNA genome sequence database from CSIRO's National Biodiversity DNA Library.

To achieve comprehensive detection of fish diversity, an ideal approach would involve a combination of larger sample volumes, more replication, and multiple complementary eDNA assays. The large volume of water sampled by the OCD eDNA sampler (Section 4) should be able to capture more fish diversity if eDNA can be recovered effectively.

7.2. Bottom eDNA samples contained more fish diversity compared to surface samples at the same site

We observed nearly twice as many fish taxa per eDNA sample in bottom samples compared to surface samples (Figure 3-7; Figure 3-8). At most collection sites the bottom depth was less than 200 m in depth (range from ~80 to 500 m). This observation is consistent with current eDNA literature, which generally indicates that eDNA signals are relatively localized. The finding highlights the need for vertically integrated sampling to accurately characterise full fish communities in waters of this depth.

7.3. High sequencing depth is not essential for characterising fish eDNA in a sample

Fish diversity detected in our eDNA samples was relatively low, with an average of 10 taxa per sample from 10 L of water. Most of the fish taxonomic diversity in each sample was recoverable with fewer than 30,000 reads (Figure 3-1). We sequenced the assay at a higher depth (mean of 150,000 reads per sample), which increased the number of artifactual sequences, complicating downstream data processing for a modest gain in the number of fish species recovered (see Section 6 for comparison with the lower number of sequences recovered in Fish ToL approach). Sampling eDNA from larger water volumes and including more replicates will add more value to a dataset than more sequencing; however, the low cost of DNA sequencing makes it tempting to focus on getting more out of each sample. It is important to note that the optimal sequencing depth per sample is context-dependent and will vary with the fish diversity of the sampled environment.

7.4. Standard marine plankton eDNA sampling reduces detection of fish

Standard marine plankton eDNA sampling used by the Australian Microbiome Initiative involves sampling 2 L of water and filtering it filtered through a 0.22 µm pore Sterivex filter. The finer filter is ideal for collection of bacterial cells but reduces the relative proportion of fish eDNA being detected compared to 0.45 µm pore-size membranes (Figure 10-2). We also found the diversity of fish detected with the Australian Microbiome method was lower – when we account for differences in volume about half the diversity of fish was detected compared to our 0.45 µm filter sampling. There are a few possible causes, the DNA extraction methods were different, and this could have had an impact. The increase in filter pore size allows a greater proportion of microbial cells to pass through, minimizing filter blockage and theoretically maximizing the recovery of metazoan DNA. Certainly, larger filter sizes allow increased volume of water to be filtered and screened (e.g. Nester *et al*. 2024).

8. References

Afzali, S. F., Bourdages, H., Laporte, M., Mérot, C., Normandeau, E., Audet, C., & Bernatchez, L. (2021). Comparing environmental metabarcoding and trawling survey of demersal fish communities in the Gulf of St. Lawrence, Canada. *Environmental DNA*, 3, 22–42. https://doi.org/10.1002/edn3.111 UQAR

Aguzzi, J., Chatzievangelou, D., Marini, S., Fanelli, E., Danovaro, R., *et al*. (2019). New high-tech flexible networks for the monitoring of deep-sea ecosystems. *Environmental Science & Technology*, 53, 6616–6631. https://doi.org/10.1021/acs.est.9b00494

Amaral-Zettler, L. A., E. A. McCliment, H. W. Ducklow, and S. M. Huse. 2009. A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of small-subunit ribosomal RNA genes. *PloS ONE* 4:e6372. https://doi.org/10.1371/journal.pone.0006372

Anderson, M.J. (2001), A new method for non-parametric multivariate analysis of variance. Austral Ecology, 26: 32-46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

Andruszkiewicz, E. A., Sassoubre, L. M., & Boehm, A. B. (2020). Persistence of marine fish environmental DNA and the influence of sunlight. *PLoS ONE*, *12*, e0185043. https://doi.org/10.1371/journal.pone.0185043

Appleyard, S.A., Ward, R.D., Pogonoski, J.J. *et al.* (2025) Australia's marine fishes DNA barcode reference library for integrated taxonomy, metabarcoding & eDNA research. *Scientific Data* 12, 21 https://doi.org/10.1038/s41597-025-04375-4

Barnes, M. A., Turner, C. R., Jerde, C. L., Renshaw, M. A., Chadderton, W. L., & Lodge, D. M. (2014). Environmental conditions influence eDNA persistence in aquatic systems. *Environmental Science & Technology*, *48*, 1819-1827. https://doi.org/10.1021/es404734p

Barnes, M. A., Turner, C. R. (2016) The ecology of environmental DNA and implications for conservation genetics. *Conservation Genetics* 17, 1–17 (2016). https://doi.org/10.1007/s10592-015-0775-4

Bayer, P. E., Bennett, A., Nester, G., Corrigan, S., Raes, E. J. *et al*. (2025). A comprehensive evaluation of taxonomic classifiers in marine vertebrate eDNA studies. *Molecular Ecology Resources*, e14107 https://doi.org/10.1111/1755-0998.14107

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

Bessey, C., Martini, A., Currie, A., Ponsonby, W., Tyndall, A., Crossing, R., Dawkins, K. L., Pogonoski, J. J., Moore, G., Mortimer, N., & Keesing, J. K. (2025). Design and Validation of an Open–Close Device for Integrated Environmental DNA Sampling Detects a Depth Gradient in Indian Ocean Deep-Sea Fish Assemblages. *Ecology and Evolution*, 15, e70902. https://doi.org/10.1002/ece3.70902

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

Bessey, C., Jarman, S. N., Berry, O., Olsen, Y. S., Bunce, M., Simpson, T., Power, M., McLaughlin, J., Edgar, G. J., & Keesing, J. (2020). Maximizing fish detection with eDNA metabarcoding. *Environmental DNA*, 2(4), 493–504. https://doi.org/10.1002/edn3.74

Brian, J. I., Davy, S. K., & Wilkinson, S. P. (2019). Elevated Symbiodiniaceae richness at Atauro Island (Timor-Leste): a highly biodiverse reef system. *Coral Reefs*, 38, 123–136. https://doi.org/10.1007/s00338-018-01762-9

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, 13, 581–583. https://doi.org/10.1038/nmeth.3869

Deagle, B. E., Gales, N. J., Evans, K., Jarman, S. N., Robinson, S., Trebilco, R., & Hindell, M. A. (2007). Studying seabird diet through genetic analysis of faeces: a case study on macaroni penguins (*Eudyptes chrysolophus*). *PLoS ONE*, 2(9), e831. https://doi.org/10.1371/journal.pone.0000831 PLOS

Deagle, B. E., A. C. Thomas, J. C. McInnes, L. J. Clarke, E. J. Vesterinen, E. L. Clare, T. R. Kartzinel, and J. P. Eveson. 2019. Counting with DNA in metabarcoding studies: How should we convert sequence reads to dietary data? *Molecular Ecology* 28:391-406. https://doi.org/10.1111/mec.14734

DiBattista, J. D., Fowler, A. M., Shalders, T. C., Williams, R. J., & Wilkinson, S. (2024). Tree of life metabarcoding can serve as a biotic benchmark for shifting baselines in urbanized estuaries. *Environmental Research*, 258, 119454. https://doi.org/10.1016/j.envres.2024.119454

Edgar, R. C. (2016). UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. *bioRxiv*, 081257. https://doi.org/10.1101/081257

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

Eichmiller, J. J., Sorensen, P. W., & Li, W. (2016). Effects of temperature and trophic state on degradation of environmental DNA in lake water. *Environmental Science & Technology Letters*, 3(1), 60–64. https://doi.org/10.1021/acs.estlett.5b00224

Eisenhauer, N., Bowker, M. A., Grace, J. B., & Powell, J. R. (2015). From patterns to causal understanding: Structural equation modeling (SEM) in soil ecology. *Pedobiologia*, 58(2–3), 65–72. https://doi.org/10.1016/j.pedobi.2015.03.002

Geller, J., Meyer, C., Parker, M., & Hawk, H. (2013). Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Molecular Ecology Resources*, 13, 851–861. https://doi.org/10.1111/1755-0998.12138

Guri, G., Shelton, A. O., Kelly, R. P., Yoccoz, N. G., Johansen, T., Præbel, K., Hanebrekke, T., Ray, J. L., Fall, J., & Westgaard, J. I. (2024). Predicting trawl catches using environmental DNA. *ICES Journal of Marine Science*, 81, 1536–1548. https://doi.org/10.1093/icesjms/fsad105

Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., Boutte, C., Burgaud, G., de Vargas, C., Decelle, J., *et al*. (2013). The Protist Ribosomal Reference database (PR2): A catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. *Nucleic Acids Research*, 41(D1), D597–D604. https://doi.org/10.1093/nar/gks1160del Campo Lab

Harrison, J. B., Sunday, J. M., & Rogers, S. M. (2019). Predicting the fate of eDNA in the environment and implications for studying biodiversity. *Proceedings of the Royal Society B-Biological Sciences*, 286, 20191409. https://doi.org/10.1098/rspb.2019.1409

He, X., N. Jeffery, R. Stanley, L. Hamilton, E. Rubidge, and C. Abbott. 2023. eDNA metabarcoding enriches traditional trawl survey data for monitoring biodiversity in the marine environment. *ICES Journal of Marine Science* 80:1529-1538. https://doi.org/10.1093/icesjms/fsad083

Hinlo, R., Gleeson, D., Lintermans, M., & Furlan, E. (2017). Methods to maximise recovery of environmental DNA from water samples. *PLoS One*, 12(6), e0179251. https://doi.org/10.1371/journal.pone.0179251

Jeunen, G.-J., Mills, S., Mariani, S., Treece, J., Ferreira, S., Stanton, J.-A. L., Duran-Vinet, B., Duffy, G. A., Gemmell, N. J., & Lamare, M. (2024). Streamlining large-scale oceanic biomonitoring using passive eDNA samplers integrated into vessel's

continuous pump underway seawater systems. *Science of the Total Environment*, 946, 174354. https://doi.org/10.1016/j.scitotenv.2024.174354

Kirtane, A., Atkinson, J. D., & Sassoubre, L. (2020). Design and validation of passive environmental DNA samplers using granular activated carbon and montmorillonite clay. *Environmental Science & Technology*, 54, 13000–13008. https://doi.org/10.1021/acs.est.0c01863

Komai, T., Gotoh, R. O., Sado, T., & Miya, M. (2019). Development of a new set of PCR primers for eDNA metabarcoding decapod crustaceans. *Metabarcoding and Metagenomics*, 3, e33835. https://doi.org/10.3897/mbmg.3.33835

Kumar, G., Farrell, E., Reaume, A. M., Eble, J. A., & Gaither, M. R. (2022). One size does not fit all: Tuning eDNA protocols for high- and low-turbidity water sampling. *Environmental DNA*, 4(1), 167–180. https://doi.org/10.1002/edn3.235

Lane, D. J., Pace, B., Olsen, G. J., Stahl, D. A., Sogin, M. L., & Pace, N. R. (1985). Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proceedings of the National Academy of Sciences*, 82(20), 6955–6959. https://doi.org/10.1073/pnas.82.20.6955

Lawler, M. M. (1999). Conservation genetics of handfishes: Family Brachionichthyidae (Honours thesis, University of Tasmania). https://doi.org/10.25959/23249756.v1 ePrints

Leray, M., Yang, J. Y., Meyer, C. P., Mills, S. C., Agudelo, N., Ranwez, V., Boehm, J. T., & Machida, R. J. (2013). A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: Application for characterizing coral reef fish gut contents. *Frontiers in Zoology*, 10, 34. https://doi.org/10.1186/1742-9994-10-34

Magoc, T., & Salzberg, S. L. (2011). FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, 27, 2957–2963. https://doi.org/10.1093/bioinformatics/btr507

Martino, F., Cananzi, G., Gregori, I., *et al*. (2025). Linking water to the bottom: eDNA study of benthic invertebrates and invasive species in the Venice Lagoon. *Environmental DNA*, 7, e70093. https://doi.org/10.1002/edn3.70093

McClenaghan, B., Fahner, N., Cote, D., Chawarski, J., McCarthy, A., Rajabi, H., ... & Hajibabaei, M. (2020). Harnessing the power of eDNA metabarcoding for the detection of deep-sea fishes. *PLOS ONE*, 15, e0236540. https://doi.org/10.1371/journal.pone.0236540

McGill, B. J., Etienne, R. S., Gray, J. S., Alonso, D., Anderson, M. J., Benecha, H. K., ... & White, E. P. (2007). Species abundance distributions: Moving beyond single prediction theories to integration within an ecological framework. *Ecology Letters*, 10(10), 995–1015. https://doi.org/10.1111/j.1461-0248.2007.01094.x

McQuillan, J. S., & Robidart, J. C. (2017). Molecular-biological sensing in aquatic environments: Recent developments and emerging capabilities. *Current Opinion in Biotechnology*, 45, 43–50. https://doi.org/10.1016/j.copbio.2016.11.020

Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J. Y., Sato, K., ... & Nishida, M. (2015). MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: Detection of more than 230 subtropical marine species. *Royal Society Open Science*, 2:150088. https://doi.org/10.1098/rsos.150088

Morey, R. E., Galloway, R. L., Bragg, S. L., Steigerwalt, A. G., Mayer, L. W., & Levett, P. N. (2006). Species-specific identification of Leptospiraceae by 16S rRNA gene sequencing. *Journal of Clinical Microbiology*, 44(10), 3510–3516. https://doi.org/10.1128/JCM.00670-06

Nester, G. M., De Brauwer, M., Koziol, A., West, K. M., DiBattista, J. D., White, N. E., ... & Bunce, M. (2020). Development and evaluation of fish eDNA metabarcoding assays facilitate the detection of cryptic seahorse taxa (family: Syngnathidae). *Environmental DNA*, 2, 614–626. https://doi.org/10.1002/edn3.93

Nester, G. M., Suter, L., Kitchener, J. A., Bunce, M., Polanowski, A. M., Wasserman, J., & Deagle, B. (2024). Long-distance Southern Ocean environmental DNA (eDNA) transect provides insights into spatial marine biota and invasion pathways for non-native species. *Science of The Total Environment*, 951, 175657. https://doi.org/10.1016/j.scitotenv.2024.175657

Oksanen J, Simpson G, Blanchet F, *et al*. (2025). *vegan: Community Ecology Package*. R package version 2.7-0, https://github.com/vegandevs/vegan, https://vegandevs.github.io/vegan/.

Piredda, R., Tomasino, M.P., D'Erchia, A.M., Manzari, C., Pesole, G., Montresor, M., Kooistra, W.H.C.F., Sarno, D. & Zingone, A. 2016. Diversity and temporal patterns of planktonic protist assemblages at a Mediterranean Long Term Ecological Research site. *FEMS Microbiology Ecology*, 93: fiw200. https://doi.org/10.1093/femsec/fiw200

Pochon, X., N. J. Bott, K. F. Smith, and S. A. Wood. 2013. Evaluating detection limits of next-generation sequencing for the surveillance and monitoring of international marine pests. *PloS one* 8:e73935. https://doi.org/10.1371/journal.pone.0073935

Polanco F, A., R. Rozanski, V. Marques, M. Helmkampf, D. Mouillot, S. Manel, C. Albouy, O. Puebla, and L. Pellissier. 2025. A Confidence Scoring Procedure for eDNA Metabarcoding Records and Its Application to a Global Marine Fish Dataset. *Environmental DNA* 7:e70077. https://doi.org/10.1002/edn3.70077

Power, H., Takahashi, M., Jarman, S., & Berry, O. (2023). What is environmental DNA? *Environmental DNA*, *5*, 1743-1758. https://doi.org/10.1002/edn3.353

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J. & Glöckner, F.O. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, 41: D590–D596. https://doi.org/10.1093/nar/gks1219

R_Core_Team. 2024. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Riaz, T., W. Shehzad, A. Viari, F. Pompanon, P. Taberlet, and E. Coissac. 2011. ecoPrimers: inference of new DNA barcode markers from whole genome sequence analysis. *Nucleic acids research* 39:e145-e145. https://doi.org/10.1093/nar/gkr732

Sabatés, A., Olivar, M.P., Salat, J., Palomera, I., Alemany, F. (2007). Physical and biological processes controlling the distribution of fish larvae in the NW Mediterranean. *Progress in Oceanography*, 74:355-376. https://doi.org/10.1016/j.pocean.2007.04.017.

Sakata MK, Yamamoto S, Gotoh RO, Miya M, Yamanaka H, Minamoto T. Sedimentary eDNA provides different information on timescale and fish species composition compared with aqueous eDNA. *Environmental DNA*. 2020; 2: 505–518. https://doi.org/10.1002/edn3.75

Sepulveda AJ, Birch JM, Barnhart EP, Merkes CM, Yamahara KM, Marin R, Kinsey SM, Wright PR, Schmidt C. Robotic environmental DNA bio-surveillance of freshwater health. *Scientific Reports*. 2020; 10: 14389. https://doi.org/10.1038/s41598-020-71304-3

Scholin CA, Birch J, Jensen S, Marin III R, Massion E, Pargett D, Preston C, Roman B, Ussler III W. The quest to develop ecogenomic sensors: a 25-year history of the Environmental Sample Processor (ESP) as a case study. *Oceanography*. 2017; 30: 100–113. https://doi.org/10.5670/oceanog.2017.427

Skelton J, Cauvin A, Hunter ME. Environmental DNA metabarcoding read numbers and their variability predict species abundance, but weakly in non-dominant species. *Environmental DNA*. 2023; 5: 1092–1104. https://doi.org/10.1002/edn3.355

Stat M, Huggett MJ, Bernasconi R, DiBattista JD, Berry TE, Newman SJ, Harvey ES, Bunce M. Ecosystem biomonitoring with eDNA: metabarcoding across the tree of life in a tropical marine environment. *Scientific Reports*. 2017; 12240. https://doi.org/10.1038/s41598-017-12501-5

Stauffer, S., Jucker, M., Keggin *et al*. (2021). How many replicates to accurately estimate fish biodiversity using environmental DNA on coral reefs? *Ecology and Evolution*, 11, 14630–14643. https://doi.org/10.1002/ece3.8150

Stoeck, T., Bass, D., Nebel, M., Christen, R., Jones, M.D.M., Breiner, H.W. & Richards, T.A. 2010. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. *Molecular Ecology*, 19: 21–31. https://doi.org/10.1111/j.1365-294X.2009.04480.x

Stoeckle, M. Y., J. Adolf, Z. Charlop-Powers, K. J. Dunton, G. Hinks, and S. M. VanMorter. 2021. Trawl and eDNA assessment of marine fish diversity, seasonality, and relative abundance in coastal New Jersey, USA. *ICES Journal of Marine Science* 78:293-304. https://doi.org/10.1093/icesjms/fsaa225

Suzuki, N., Not, F., 2015. Biology and Ecology of Radiolaria, in: S. Ohtsuka, T. Suzaki, T. Horiguchi, N. Suzuki, F. Not (Eds.), *Marine Protists: Diversity and Dynamics*. Springer Japan, Tokyo, 179-222. https://doi.org/10.1007/978-4-431-55130-0_8

Takahashi, M., M. Sacco, J.H. Kestel *et al*. (2023) Aquatic environmental DNA: a review of the macro-organismal biomonitoring revolution. *Science of the Total Environment*, 873, 162322. https://doi.org/10.1016/j.scitotenv.2023.162322

Tett, P., Gowen, R., Mills, D., Fernandes, T., Gilpin, L., Huxham, M., Kennington, K., Read, P., Service, M., Wilkinson, M. & Malcolm, S. 2007. Defining and detecting undesirable disturbance in the context of marine eutrophication. *Marine Pollution Bulletin*, 55(1–6): 282–297. https://doi.org/10.1016/j.marpolbul.2006.08.028

Thomsen, P. F., J. Kielgast, L. L. Iversen, P. R. Møller, M. Rasmussen, and E. Willerslev. 2012. Detection of a diverse marine fish fauna using environmental DNA from seawater samples. *PLoS ONE* 7: e41732. https://doi.org/10.1371/journal.pone.0041732

Thomsen, P. F., P. R. Møller, E. E. Sigsgaard, S. W. Knudsen, O. A. Jørgensen, and E. Willerslev. 2016. Environmental DNA from seawater samples correlate with trawl catches of subarctic, deepwater fishes. *PloS ONE* 11:e0165252. https://doi.org/10.1371/journal.pone.0165252

Tsai, W. P., Liu, K. M., & Chang, Y. J. (2020). Evaluation of biological reference points for conservation and management of the bigeye thresher shark, *Alopias superciliosus*, in the Northwest Pacific. *Sustainability*, *12*, 8646. https://doi.org/10.3390/su12208646

Wang, Q., Garrity, G.M., Tiedje, J.M. & Cole, J.R. 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and *Environmental Microbiology*, 73: 5261–5267. https://doi.org/10.1128/AEM.00062-07

West K, Travers MJ, Stat M, *et al*. (2021) Large-scale eDNA metabarcoding survey reveals marine biogeographic break and transitions over tropical north-western Australia. *Diversity and Distrib*utions 27: 1942–1957. https://doi.org/10.1111/ddi.13228

West, K. M., T. R. Jones, L. Denis-Roy, O. J. Johnson, E. Clausius, G. Edgar, and B. Deagle (2024) Continual Day–Night eDNA Detectability Amidst Diel Reef Species Fluctuations on Diver Transects. *Environmental DNA* 6:e70018. https://doi.org/10.1002/edn3.70018

Wilcox TM, Zarn KE, Piggott MP, Young MK, McKelvey KS, Schwartz MK. (2018) Capture enrichment of aquatic environmental DNA: A first proof of concept. *Molecular Ecology Resources* 18: 1392–1401. https://doi.org/10.1111/1755-0998.12928

Wilkinson, S., S. Davy, M. Bunce, and M. Stat. 2018. Taxonomic identification of environmental DNA with informatic sequence classification trees. *PeerJ Preprints* 6:e26812v1 https://doi.org/10.7287/peerj.preprints.26812v1

Wilkinson, S. P., A. A. Gault, S. A. Welsh, J. P. Smith, B. O. David, A. S. Hicks, D. R. Fake, A. M. Suren, M. R. Shaffer, and S. N. Jarman. 2024. TICI: a taxon-independent community index for eDNA-based ecological health assessment. *PeerJ* 12:e16963. https://doi.org/10.7717/peerj.16963

9. Appendix A: DNA samples and eDNA datasets

9.1. eDNA data deposited in public repositories

The data used for analyses presented in the report (i.e. eDNA species occurrences tables and sample metadata) have been uploaded to the CSIRO Data Access Portal: https://doi.org/10.25919/ngqx-3557 (Figure 9-1). This data will be made publicly available in December 2025 and can be obtained before this date by request.

The eDNA sequences (>180 million sequences in >2000 fastq files) along with sample metadata have been uploaded to the National Center for Biotechnology Information Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra/). The data will be released December 2025 under BioProject PRJNA1263580: “Environmental DNA measuring offshore marine biodiversity in southeast Australia”.

We are also working with the Minderoo Foundation to link our NESP project data with other eDNA datasets collected on the RV *Investigator* and eDNA data from a Parks Australia and Minderoo Foundation project funded through the Ocean Discovery and Restoration Program.

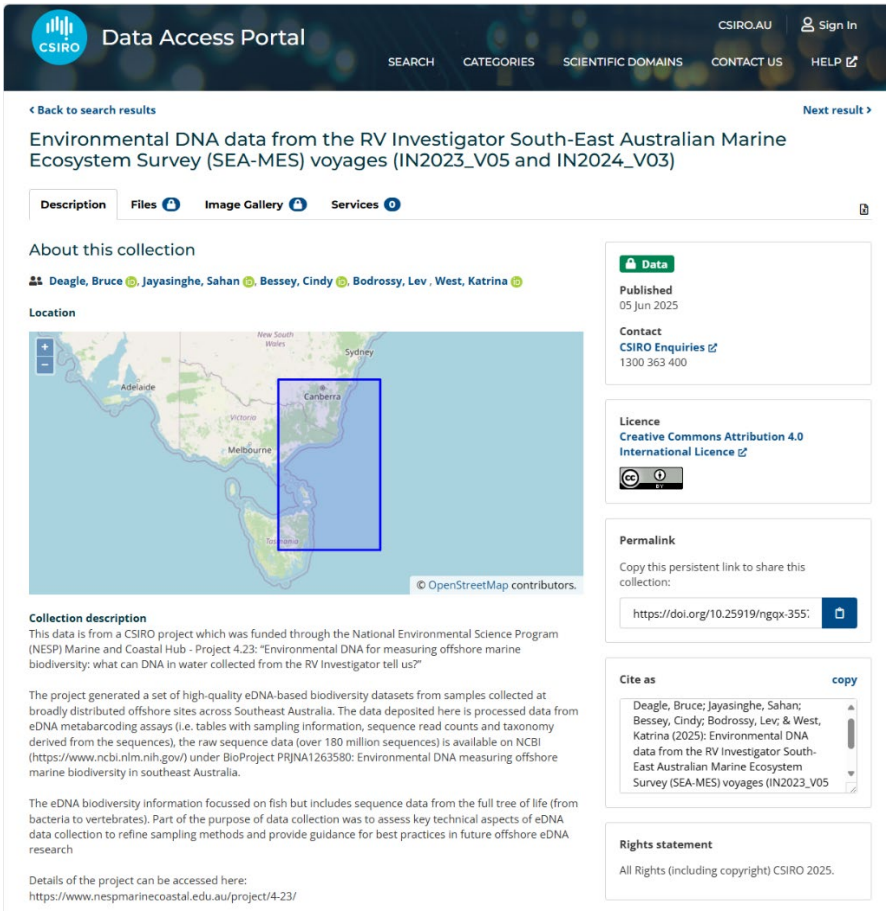


Figure 9-1 Screenshot showing project data record on the CSIRO Data Access Portal

9.2. Samples of eDNA collected during the project

Table 9-1 List of 2 L and 10 L eDNA samples collected on the two SEA-MES voyages and numbers of eDNA samples that were used in the 18S and mtDNA 16S Fish sequencing (sequencing was also done on laboratory negative control samples for 10 L samples).

Sample Type	Filter Type	Volume filtered per Sample	Samples collected		Samples sequenced & analysed	
			IN2023_V05	**IN2024_V03**	**IN2023_V05**	**IN2024_V03**
Microbial eDNA	Sterivex 0.22 µm	2 L	129	148	99	100
Metazoan eDNA	MCE 0.45 µm	10 L	193	210	130	151

9.3. Samples of eDNA from Commonwealth Marine Parks

Table 9-2 Numbers of eDNA sample from Commonwealth Marine Parks (10 L, 2 L & OCD eDNA samples) that were collected, sequenced and analysed on the two SEA-MES voyages.

Marine Park	IN2023_V05*			IN2024_V03			Total
	10 L	2 L	OCD	10 L	2 L	OCD	
Flinders	12	8	*NA*	16	12	10	58
Freycinet	21	17	*NA*	16	16	10	80

* All 10 L 2023 samples were run with the ToL assays; for 2 L only 2 from Flinders and 6 from Freycinet have ToL data.

9.4. DNA sequence data produced during the project

(1) **SEA-MES eDNA data set 1**: Australian Genome Research Facility (AGRF) Illumina sequencing, December 2024

Sequencing: NextSeq 2000, P1 flow cell, 300 cycles PE; 102 million sequences; 61.09 gigabases (Gb) data

Amplicons: 16S mtDNA fish and COI Leray

Samples: 10 L samples from both voyages (n= 288; Section 3 of report); deep towed camera related samples from voyage 2 (n= 96; Section 4 of report)

(2) **SEA-MES eDNA data set 2:** Ramaciotti Centre for Genomics Illumina sequencing, February 2025

Sequencing: MySeq v2 2x250 bp run; 16 million sequences, 12.64 gigabases of data.

Amplicons: 18S v4

Samples: 2L samples from both voyages (n=199; Section 5 of report); plus select 10L samples (n=12; Section 3 of report)

(3) **SEA-MES eDNA data set 3**: Wilderlab Illumina sequencing, December 2024

Sequencing: NextSeq; 76.9 million sequences

Amplicons: 12 amplicons (see Section 6 of the report).

Samples: 10 L (n=90) and 2 L (n=42) samples from voyage 1; deep towed camera comparison samples (n=16) from voyage 2 (Section 6 of the report)

Additional DNA sequencing from the SEA-MES samples outside of the NESP project

(4) **SEA-MES eDNA pilot data set**: AGRF Illumina sequencing, December 2023

Sequencing: MiSeq, 300 cycle; 11 million sequences; 3.29 Gb of data

Amplicons: 16S mtDNA fish

Samples: 10L, 2L and deep towed camera test samples from voyage 1 (n=285 total; the 10L and 2L samples were re-run in SEA-MES eDNA data set 1 and 2).

(5) **SEA-MES fish mtDNA reference sequences**: Ramaciotti Centre for Genomics Sanger sequencing of DNA from voucher specimens (Australian National Fish Collection, CSIRO)

Sequencing: Sanger dideoxy; 550 to 600 bp

Amplicons: 16S mtDNA fish – longer fragment

Samples: Fish specimens (n=199). Sequences have been made available GenBank (https://www.ncbi.nlm.nih.gov/genbank/) 15-APR-2024.

10. Appendix B: Comparison between 2 L and 10 L sampling

10.1. Overview of the comparison

Here we compare eDNA data collected from the same water sampling event but processed with different protocols. Both the 2 L and 10 L water samples were filtered using a Masterflex L/S console pump system (Figure 10-1). The 10 L water samples were filtered through 47 mm, 0.45 µM pore size mixed cellulose ester (MCE) filter membranes. The 2 L waters were filtered through a Sterivex, 0.22 µm pore size polyethersulfone membrane. The DNA extraction methods for the two filter types differed, see Section 3 methods for 10 L and Section 2 for the 2 L water samples.

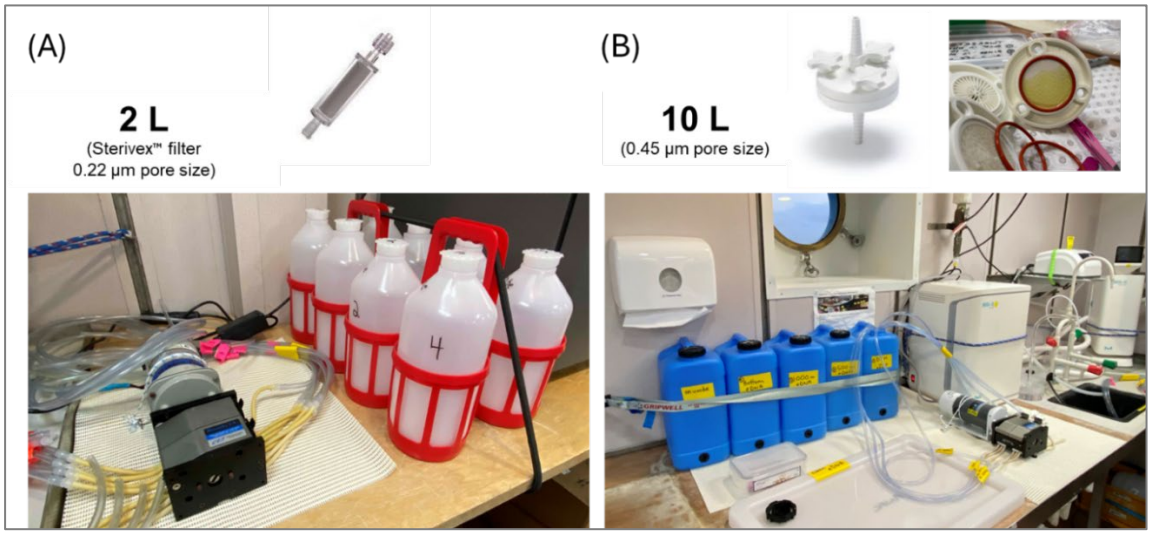


Figure 10-1 Filtration of eDNA from CTD water on the RV *Investigator* (A) 2 L and (B) 10 L

10.2. Fish eDNA in the 2 L versus 10 L samples

We compared ToL metabarcoding fish eDNA data from 41 paired 2 L and 10 L samples collected on SEA-MES Voyage 1 (see Section 6 of this report for description of the ToL data). Each paired sample was filtered from water collected on the same CTD rosette (n= 82 samples in total). The number and proportion of fish sequences recovered was higher in the 10 L samples (Figure 10-2). The mean number of fish taxa detected in the 10 L samples is 20.5 and for the 2 L samples the mean number of fish is 2.4; this includes data from all ToL assays and counts fish taxa identified to genus- or species-level (Figure 10-2).

The difference in fish taxa detection is not accounted for by water volume differences alone. It appears that a larger pore size increases the proportion of fish eDNA and this boosts the fish diversity detected. It is possible that different DNA extraction methods used in 10L versus 2L also impacted the amount of fish DNA being recovered relative to other groups. Looking at the proportion of sequences assigned to other groups (Figure 10-3), we can see the proportion of “Bacteria” and “Crustaceans” is higher in the 2L samples.

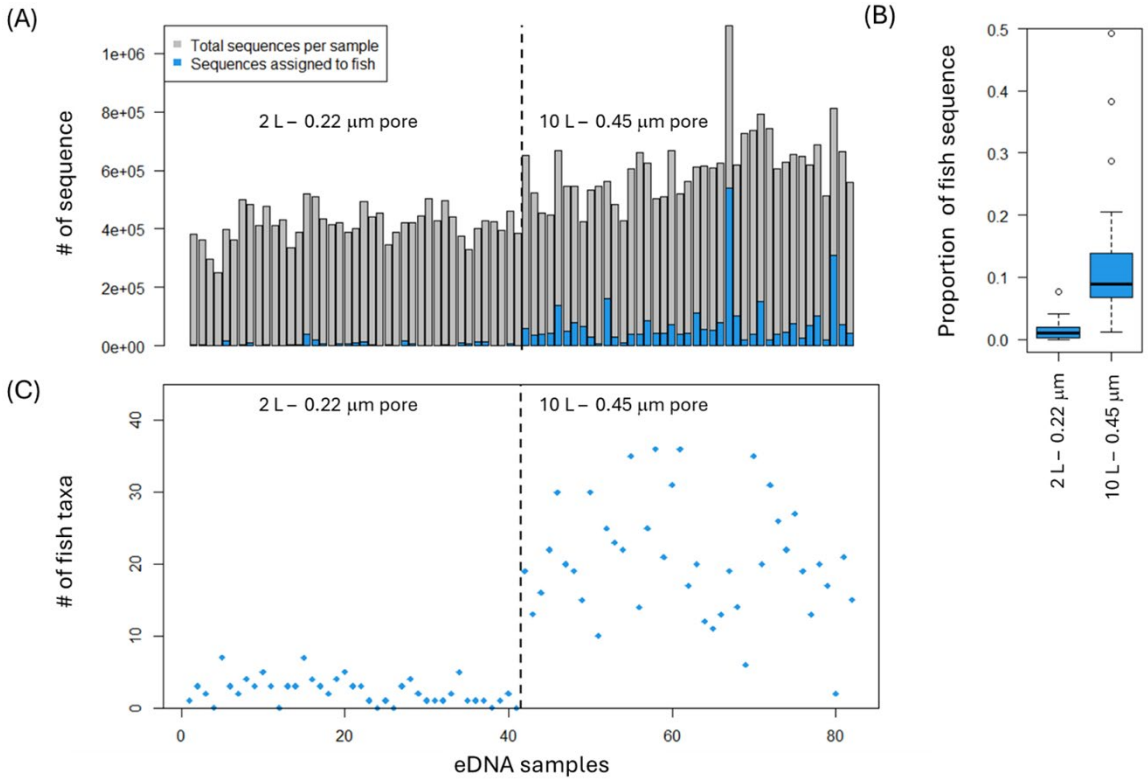


Figure 10-2 Comparison of ToL metabarcoding fish eDNA data from 41 paired 2 L and 10 L samples (A) Bar plot showing total number of sequences and number assigned to the group “Fish” in each sample (includes data from all assays). (B) Boxplot showing fish sequences as a proportion of total reads (C) Fish richness in the 2 L and 10 L eDNA samples (includes data from all assays; only taxa identified to genus- or species-level were counted.

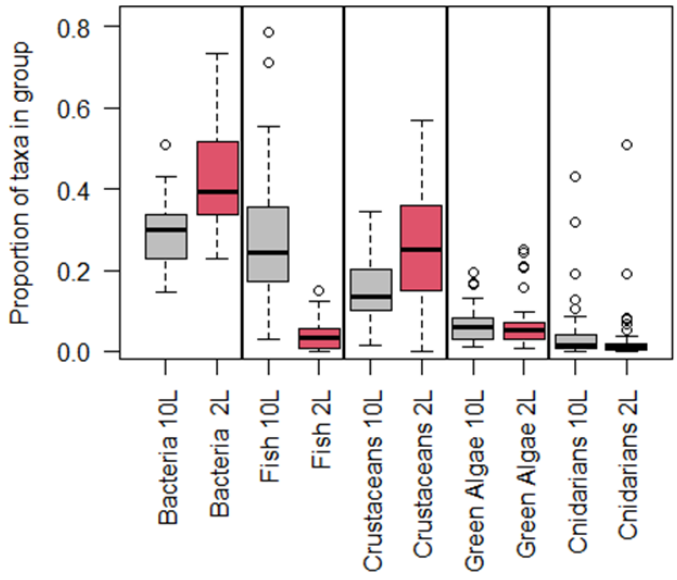


Figure 10-3 Comparison of ToL metabarcoding sequence reads recovered in different taxonomic groups for 10 L and 2 L eDNA samples. The five groups with most sequences assigned are shown, the group “Other” which includes all sequences not in these groups (64% of total) was excluded from calculations.

10.3. Plankton eDNA in the 2 L versus 10 L samples

We compared 18S (region V4) tag sequencing from 10 paired 2L and 10L samples. As outlined above, these samples differed not only in the volume collected, but also in the filter pore size (0.22 µm vs. 0.45 µm) and in the DNA extraction method. We were interested to know whether this would affect the community level view of broad 18S eukaryotic data.

An nMDS analysis (Figure 10-4) indicated that the 2L and 10L samples, despite the significant differences in the methods, showed very similar results, with samples from the same location and depth always clustering together. This suggests that mixing 2L and 10L samples together in a single analysis would lead to only minimal compromise of the statistical power of the broad eukaryotic dataset targeted by the 18S marker.

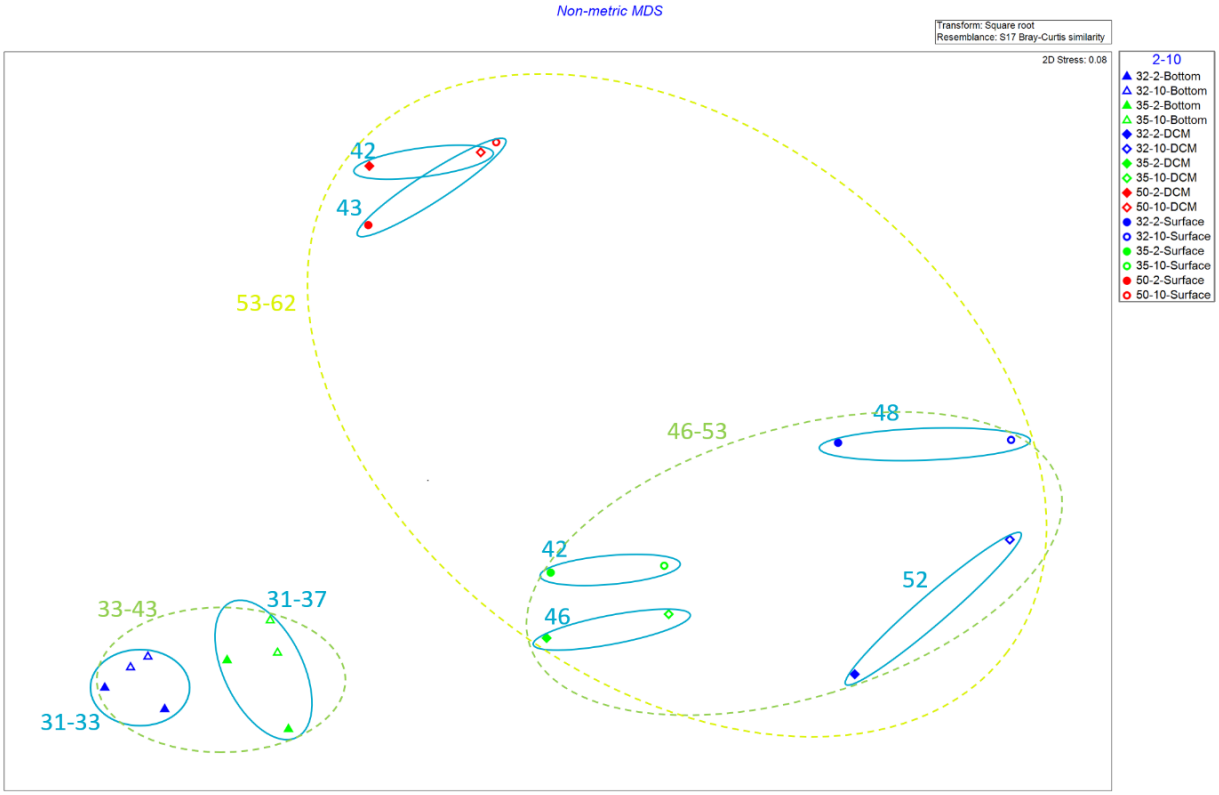


Figure 10-4 Comparison of paired 2L and 10L samples in plankton eDNA results. Full symbols = 2L samples. Empty symbols = 10L samples. Colour and shape of the symbols denote sampling location and sampling depth, respectively.

11. Appendix C Fish identified in the 10 L eDNA samples

Table 11-1 The fish taxa identified with the mtDNA 16S Fish assay eDNA data (ordered by the percentage of total sequence reads that were assigned to each of the 230 taxa).

	Taxa	Common name	% total reads	Mean % reads per sample	Occurrence (# out of 262 samples)	% Presence
1	*Trachurus Sp*	Mackerel Sp	14.733	12.037	157	59.9
2	*Sardinops sagax*	Australian Sardine	14.569	13.063	115	43.9
3	*Emmelichthys nitidus*	Redbait	9.396	10.196	118	45.0
4	*Scomber australasicus*	Blue Mackerel	5.479	5.691	127	48.5
5	*Engraulis australis*	Australian Anchovy	5.400	3.185	52	19.8
6	*Lepidotrigla Sp*	Gurnard Sp	3.961	4.479	88	33.6
7	*Cheilodactylidae Sp*	Morwong Sp	3.450	3.962	102	38.9
8	*Caesioperca Sp*	Perch Sp1	3.318	3.724	51	19.5
9	*Platycephalus richardsoni*	Tiger Flathead	3.182	3.728	82	31.3
10	*Maurolicus Sp1*	Pearlside Sp	2.791	2.997	65	24.8
11	*Seriolella Sp*	Warehou Sp	2.586	3.518	40	15.3
12	*Paraulopus nigripinnis*	Blacktip Cucumberfish	2.425	2.074	77	29.4
13	*Parapercis allporti*	Barred Grubfish	1.505	1.483	52	19.8
14	*Thyrsites atun*	Barracouta	1.348	1.135	16	6.1
15	*Gnathophis Sp*	Conger Eel Sp2	1.298	1.525	50	19.1
16	*Verilus anomalus*	Threespine Cardinalfish	1.227	1.219	39	14.9
17	*Sillago flindersi*	Eastern School Whiting	0.992	0.875	29	11.1
18	*Lampanyctodes hectoris*	Hector's Lanterfish	0.940	0.928	86	32.8
19	*Pseudophycis breviuscula*	Bastard Red Cod	0.886	0.633	22	8.4
20	*Argentina australiae*	Silverside	0.884	1.094	24	9.2
21	*Helicolenus Sp*	Ocean Perch Sp	0.842	0.974	43	16.4
22	*Genypterus Sp*	Ling Sp	0.810	0.830	23	8.8
23	*Nemadactylus douglasii*	Grey Morwong	0.759	0.634	32	12.2
24	*Foetorepus calauropomus*	Common Stinkfish	0.759	0.934	43	16.4
25	*Rexea solandri*	Eastern Gemfish	0.700	0.629	27	10.3
26	*Scomberesox saurus*	King Gar	0.697	0.946	26	9.9
27	*Allomycterus pilatus*	Australian Burrfish	0.598	0.768	24	9.2
28	*Lepidopus caudatus*	Frostfish	0.542	0.568	12	4.6
29	*Pterygotrigla polyommata*	Latchet	0.541	0.776	23	8.8
30	*Diaphus Sp1*	Lanternfish Sp1	0.492	0.498	16	6.1
31	*Neosebastes scorpaenoides*	Common Gurnard Perch	0.479	0.558	14	5.3
32	*Stomiiformes Sp1*	Dragonfish Sp1	0.426	0.566	14	5.3
33	*Mola mola*	Ocean Sunfish	0.418	0.378	9	3.4
34	*Spiniraja whitleyi*	Melbourne Skate	0.331	0.381	22	8.4
35	*Hygophum hanseni*	Hansen's Lanterfish	0.323	0.545	12	4.6
36	*Myctophidae Sp4*	Lanternfish Sp7	0.320	0.470	9	3.4
37	*Neosebastes thetidis*	Thetis Fish	0.295	0.282	12	4.6
38	*Scorpaena papillosa*	Southern Red Scorpionfish	0.292	0.329	13	5.0
39	*Parequula melbournensis*	Silverbelly	0.291	0.262	7	2.7
40	*Coelorinchus gormani*	Little Whiptail	0.264	0.271	14	5.3

41	*Centrolophus niger*	Rudderfish	0.252	0.281	4	1.5
42	*Lepidoperca Sp*	Perch Sp2	0.247	0.180	11	4.2
43	*Chelidonichthys kumu*	Red Gurnard	0.245	0.235	11	4.2
44	*Hygophum Sp*	Lanternfish Sp14	0.228	0.169	7	2.7
45	*Pseudophycis barbata*	Bearded Rock Cod	0.224	0.291	17	6.5
46	*Auxis Sp*	Frigate Tuna Sp	0.222	0.453	6	2.3
47	*Cyclothone Sp2*	Bristlemouth Sp2	0.222	0.185	3	1.1
48	*Urolophus Sp4*	Stingaree Sp4	0.209	0.220	76	29.0
49	*Foetorepus Sp2*	Stinkfish Sp2	0.204	0.277	16	6.1
50	*Myctophidae Sp1*	Lanternfish Sp3	0.200	0.212	7	2.7
51	*Macroramphosus scolopax*	Longspine Snipefish	0.198	0.298	40	15.3
52	*Maxillicosta Sp*	Gurnard Perch Sp1	0.196	0.127	4	1.5
53	*Hoplichthys haswelli*	Deepsea Flathead	0.189	0.238	6	2.3
54	*Stomias Sp*	Scaly Dragonfish Sp	0.185	0.186	2	0.8
55	*Bathylagidae Sp*	Deepsea Smelts	0.182	0.245	7	2.7
56	*Myctophum Sp*	Lanternfish Sp10	0.180	0.215	3	1.1
57	*Xiphias gladius*	Swordfish	0.180	0.184	3	1.1
58	*Zeus faber*	John Dorry	0.179	0.211	15	5.7
59	*Polymetme Sp1*	Lightfish Sp1	0.167	0.184	5	1.9
60	*Trichiuridae Sp*	Cutlassfish Sp	0.161	0.183	1	0.4
61	*Zenopsis nebulosa*	Mirror Dory	0.153	0.161	16	6.1
62	*Zanclistius elevatus*	Blackspot Boarfish	0.140	0.127	6	2.3
63	*Urolophus cruciatus*	Banded Stingaree	0.139	0.160	40	15.3
64	*Macruronus novaezelandiae*	Blue Grenadier	0.137	0.130	17	6.5
65	*Pristiophorus cirratus*	Longnose Sawshark	0.122	0.131	7	2.7
66	*Notoscopelus Sp*	Lanternfish Sp9	0.118	0.120	3	1.1
67	*Platycephalus aurimaculatus*	Toothy Flathead	0.116	0.170	5	1.9
68	*Myliobatis tenuicaudatus*	Southern Eagle Ray	0.115	0.167	15	5.7
69	*Urolophus Sp3*	Stingaree Sp3	0.114	0.157	43	16.4
70	*Arripis Sp*	Australian Salmon Sp	0.113	0.136	4	1.5
71	*Dinolestes lewini*	Longfin Pike	0.098	0.109	6	2.3
72	*Diretmus argenteus*	Discfish	0.098	0.187	2	0.8
73	*Diaphus Sp3*	Lanternfish Sp4	0.097	0.077	4	1.5
74	*Mustelus antarcticus*	Gummy Shark	0.093	0.103	13	5.0
75	*Polyipnus Sp*	Hatchetfish Sp1	0.092	0.094	2	0.8
76	*Lepidotrigla argus*	Eye Gurnard	0.091	0.101	3	1.1
77	*Pseudophycis palmata*	Australian Red Cod	0.084	0.108	3	1.1
78	*Lophonectes gallus*	Crested Flounder	0.078	0.081	26	9.9
79	*Upeneichthys Sp2*	Goatfish Sp2	0.076	0.085	4	1.5
80	*Platycephalus bassensis*	Southern Sand Flathead	0.075	0.077	5	1.9
81	*Pristiophorus nudipinnis*	Common Sawshark	0.072	0.089	3	1.1
82	*Sarda australis*	Australian Bonito	0.071	0.088	1	0.4
83	*Scolecenchelys breviceps*	Shorthead Worm Eel	0.069	0.069	15	5.7
84	*Hypoplectrodes maccullochi*	Halfbanded Seaperch	0.069	0.086	5	1.9
85	*Melanolagus bericoides*	Bigscale Deepsea Smelt	0.069	0.068	4	1.5
86	*Pempheris multiradiata*	Bigscale Bullseye	0.065	0.060	3	1.1
87	*Tetrosomus reipublicae*	Smallspine Turretfish	0.061	0.075	1	0.4
88	*Kathetostoma Sp*	Stargazer Sp1	0.059	0.116	6	2.3
89	*Argentiniformes Sp*	Marine Smelts	0.059	0.047	2	0.8
90	*Hyperoglyphe antarctica*	Blue-eye Trevalla	0.058	0.062	4	1.5

91	*Stomiiformes Sp3*	Dragonfish Sp3	0.056	0.069	14	5.3
92	*Hoplostethus atlanticus*	Orange Roughy	0.056	0.096	2	0.8
93	*Cubiceps caeruleus*	Driftfish	0.055	0.088	3	1.1
94	*Dipturus canutus*	Grey Skate	0.054	0.052	7	2.7
95	*Idiacanthus Sp*	Black Dragonfish Sp	0.054	0.058	2	0.8
96	*Lampadena Sp*	Lanternfish Sp8	0.054	0.066	2	0.8
97	*Thamnaconus degeni*	Bluefin Leatherjacket	0.052	0.088	7	2.7
98	*Nelusetta ayraud*	Ocean Leatherjacket	0.052	0.060	6	2.3
99	*Cepola australis*	Australian Bandfish	0.052	0.045	6	2.3
100	*Bothidae Sp*	Flounder Sp	0.049	0.049	2	0.8
101	*Centrolophidae Sp*	Trevalla Sp	0.048	0.057	2	0.8
102	*Decapterus macrosoma*	Scad	0.048	0.038	3	1.1
103	*Thunnus tonggol*	Longtail Tuna	0.046	0.053	4	1.5
104	*Plectranthias Sp1*	Perchlet Sp1	0.044	0.035	3	1.1
105	*Pseudolabrus rubicundus*	Rosy Wrasse	0.044	0.040	3	1.1
106	*Upeneichthys lineatus*	Bluestriped Goatfish	0.043	0.035	6	2.3
107	*Paratrachichthys macleayi*	Sandpaper Fish	0.043	0.082	3	1.1
108	*Beryx decadactylus*	Imperador	0.041	0.046	2	0.8
109	*Aldrichetta forsteri*	Yelloweye Mullet	0.040	0.031	1	0.4
110	*Ophisurus serpens*	Serpent Eel	0.039	0.035	3	1.1
111	*Centroberyx affinis*	Redfish	0.039	0.035	6	2.3
112	*Magnisudis prionosa*	Duckbill Barracudina	0.039	0.035	1	0.4
113	*Paralepididae Sp*	Barracudina Sp	0.039	0.047	1	0.4
114	*Bodianus flavipinnis*	Yellowfin Pigfish	0.038	0.043	1	0.4
115	*Ventrifossa Sp1*	Whiptail Sp3	0.038	0.046	1	0.4
116	*Atypichthys strigatus*	Mado	0.037	0.036	1	0.4
117	*Lotella rhacina*	Largetooth Beardie	0.037	0.061	2	0.8
118	*Symbolophorus barnardi*	Barnard's Lanternfish	0.037	0.046	17	6.5
119	*Cyclothone Sp3*	Bristlemouth Sp3	0.036	0.028	1	0.4
120	*Psenes pellucidus*	Blackrag	0.035	0.046	1	0.4
121	*Diogenichthys atlanticus*	Atlantic Lanternfish	0.034	0.028	1	0.4
122	*Zebrias scalaris*	Manyband Sole	0.034	0.037	1	0.4
123	*Diaphus Sp2*	Lanternfish Sp2	0.034	0.031	4	1.5
124	*Ophidiidae Sp*	Cusk Eel	0.034	0.020	1	0.4
125	*Pseudocaranx Sp*	Trevally Sp	0.033	0.080	2	0.8
126	*Aracana Sp1*	Cowfish Sp1	0.031	0.037	3	1.1
127	*Chironemus georgianus*	Western Kelpfish	0.031	0.065	1	0.4
128	*Sphoeroides pachygaster*	Balloonfish	0.030	0.032	3	1.1
129	*Urolophus viridis*	Geenback Stingaree	0.030	0.038	46	17.6
130	*Dipturus gudgeri*	Bight Skate	0.029	0.029	4	1.5
131	*Scorpis Sp*	Sweep Sp	0.028	0.025	4	1.5
132	*Serranidae Sp2*	Perch Sp4	0.028	0.025	2	0.8
133	*Paristiopterus labiosus*	Giant Boarfish	0.027	0.039	1	0.4
134	*Arothron firmamentum*	Starry Toadfish	0.025	0.026	2	0.8
135	*Lophiodes Sp*	Goosefish	0.025	0.042	1	0.4
136	*Lampichthys procerus*	Blackhead Lanternfish	0.025	0.037	2	0.8
137	*Callanthias Sp1*	Splendid Perch Sp1	0.025	0.023	2	0.8
138	*Cyclothone kobayashii*	Kobayashii's Bristelemouth	0.024	0.023	4	1.5
139	*Pterygotrigla andertoni*	Painted Latchet	0.023	0.022	1	0.4
140	*Chiasmodontidae Sp*	Swallower Sp	0.023	0.022	2	0.8
141	*Aracana Sp2*	Cowfish Sp2	0.022	0.014	1	0.4
142	*Sternoptyx Sp1*	Hatchetfish Sp2	0.021	0.041	3	1.1
143	*Ventrifossa Sp2*	Whiptail Sp4	0.021	0.020	1	0.4

144	*Moridae Sp*	Cod Sp	0.020	0.059	1	0.4
145	*Bassanago bulbiceps*	Swollenhead Conger	0.018	0.021	1	0.4
146	*Tripterophycis gilchristi*	Chiseltooth Grenadier Cod	0.018	0.024	1	0.4
147	*Callanthias Sp2*	Splendid Perch Sp2	0.018	0.015	6	2.3
148	*Argyrosomus japonicus*	Mulloway	0.018	0.019	1	0.4
149	*Kathetostoma laeve*	Common Stargazer	0.017	0.034	2	0.8
150	*Ipnopidae Sp*	Tripodfish Sp	0.016	0.020	1	0.4
151	*Chrysophrys auratus*	Snapper	0.016	0.009	1	0.4
152	*Stomiiformes Sp2*	Dragonfish Sp2	0.016	0.009	1	0.4
153	*Myctophidae Sp2*	Lanternfish Sp5	0.015	0.012	2	0.8
154	*Achoerodus viridis*	Eastern Blue Groper	0.015	0.011	1	0.4
155	*Coryphaena hippurus*	Mahi Mahi	0.014	0.016	1	0.4
156	*Chironemus marmoratus*	Eastern Kelpfish	0.014	0.013	1	0.4
157	*Salmo salar*	Atlantic Salmon	0.014	0.024	1	0.4
158	*Synaphobranchus affinis*	Grey Cutthroat Eel	0.014	0.013	2	0.8
159	*Notorynchus cepedianus*	Seven-gill Shark	0.014	0.018	2	0.8
160	*Foetorepus Sp3*	Stinkfish Sp3	0.013	0.007	1	0.4
161	*Myctophidae Sp3*	Lanternfish Sp6	0.013	0.038	1	0.4
162	*Coelorinchus Sp1*	Whiptail Sp1	0.012	0.011	5	1.9
163	*Pentaceros decacanthus*	Bigspine Boarfish	0.012	0.013	1	0.4
164	*Ophichthidae Sp*	Snake Eel Sp	0.012	0.012	4	1.5
165	*Pentaceropsis recurvirostris*	Longsnout Boarfish	0.012	0.014	2	0.8
166	*Eubalichthys gunnii*	Gunn's Leatherjacket	0.012	0.019	2	0.8
167	*Conger Sp*	Conger Eel Sp1	0.012	0.009	2	0.8
168	*Scopelopsis multipunctatus*	Multispotted Lanternfish	0.011	0.009	12	4.6
169	*Anguilla australis*	Southern Shortfin Eel	0.011	0.021	2	0.8
170	*Diaphus perspicillatus*	Flatface Lanternfish	0.011	0.007	1	0.4
171	*Epigonus telescopus*	Black Deepsea Cardinalfish	0.010	0.019	1	0.4
172	*Coelorinchus Sp2*	Whiptail Sp2	0.010	0.013	2	0.8
173	*Bathytoshia brevicaudata*	Smooth Stingray	0.010	0.013	7	2.7
174	*Serranidae Sp1*	Perch Sp3	0.010	0.015	1	0.4
175	*Muraenidae Sp*	Moray Eel Sp	0.010	0.006	1	0.4
176	*Valenciennellus tripunctulatus*	Constellationfish	0.009	0.006	2	0.8
177	*Melamphaidae Sp*	Bigscale Sp	0.008	0.006	1	0.4
178	*Katsuwonus pelamis*	Skipjack Tuna	0.008	0.005	1	0.4
179	*Bathylagidae Sp1*	Deepsea Smelts	0.008	0.009	2	0.8
180	*Eubalichthys bucephalus*	Black Reef Leatherjacket	0.008	0.005	1	0.4
181	*Latris lineata*	Striped Trumpeter	0.007	0.010	1	0.4
182	*Myctophidae Sp6*	Laternfish Sp13	0.007	0.006	1	0.4
183	*Latropiscis purpurissatus*	Sergeant Baker	0.007	0.005	1	0.4
184	*Ophthalmolepis lineolata*	Southern Maori Wrasse	0.007	0.006	1	0.4
185	*Gnathophis longicauda*	Little Conger	0.006	0.005	2	0.8
186	*Dentiraja Sp*	Skate Dentiraja Sp	0.006	0.006	2	0.8
187	*Dentiraja lemprieri*	Thornback Skate	0.005	0.003	1	0.4
188	*Myctophidae Sp5*	Laternfish Sp12	0.005	0.005	3	1.1
189	*Cyttus australis*	Silver Dory	0.005	0.008	2	0.8
190	*Neosebastes Sp*	Gurnard Perch Sp2	0.005	0.009	1	0.4

191	*Ratabulus Sp*	Flathead Sp4	0.005	0.005	1	0.4
192	*Halargyreus johnsonii*	Slender Cod	0.004	0.010	1	0.4
193	*Pavoraja nitida*	Peacock Skate	0.004	0.005	2	0.8
194	*Gonostoma atlanticum*	Atlantic Fangjaw	0.004	0.002	1	0.4
195	*Platycephalus Sp1*	Flathead Sp1	0.004	0.006	1	0.4
196	*Dipturus Sp2*	Skate Dipturus Sp2	0.004	0.007	1	0.4
197	*Dipturus Sp1*	Skate Dipturus Sp1	0.003	0.003	4	1.5
198	*Acanthaluteres Sp*	Leatherjacket Sp1	0.003	0.009	1	0.4
199	*Cantherhines dumerilii*	Barred Leatherjacket	0.003	0.005	1	0.4
200	*Hippocampus abdominalis*	Bigbelly Seahorse	0.003	0.008	1	0.4
201	*Cyttus traversi*	King Dory	0.003	0.004	1	0.4
202	*Parascyllium ferrugineum*	Rusty Catshark	0.002	0.003	2	0.8
203	*Brachionichthys Sp*	Handfish	0.002	0.002	2	0.8
204	*Bathytoshia Sp*	Stingray Sp2	0.002	0.003	1	0.4
205	*Meuschenia Sp*	Leatherjacket Sp8	0.002	0.002	1	0.4
206	*Scorpaenidae Sp*	Scorpionfish	0.002	0.003	1	0.4
207	*Platycephalus Sp2*	Flathead Sp2	0.002	0.002	1	0.4
208	*Squalus Sp*	Spurdog Sp	0.002	0.002	9	3.4
209	*Trygonorrhina Sp*	Fiddler Ray	0.001	0.001	3	1.1
210	*Mobula mobular*	Japanese Devilray	0.001	0.001	1	0.4
211	*Sternoptyx Sp2*	Hatchetfish Sp3	0.001	0.001	1	0.4
212	*Azygopus pinnifasciatus*	Banded-fin Flounder	0.001	<0.001	1	0.4
213	*Mora moro*	Ribaldo	0.001	0.001	1	0.4
214	*Lepidorhynchus denticulatus*	Toothed Whiptail	0.001	0.001	4	1.5
215	*Heteroscarus acroptilus*	Rainbow Cale	0.001	0.001	1	0.4
216	*Carcharhinus Sp*	Requiem Shark	0.001	<0.001	6	2.3
217	*Plectranthias Sp2*	Perchlet Sp2	<0.001	<0.001	1	0.4
218	*Etmopterus lucifer*	Blackbelly Lanternshark	<0.001	<0.001	2	0.8
219	*Platycephalus Sp3*	Flathead Sp3	<0.001	<0.001	1	0.4
220	*Hoplostethus mediterraneus*	Blacktip Sawbelly	<0.001	<0.001	1	0.4
221	*Heterodontus portjacksoni*	Port Jackson Shark	<0.001	<0.001	4	1.5
222	*Halieutaea Sp*	Seabat	<0.001	<0.001	1	0.4
223	*Howella sherborni*	Sherbon's Pelagic Bass	<0.001	<0.001	2	0.8
224	*Trygonoptera imitata*	Eastern Shovelnose Stingaree	<0.001	<0.001	1	0.4
225	*Carapidae Sp*	Pearlfish	<0.001	<0.001	1	0.4
226	*Alopias vulpinus*	Common Thresher Shark	<0.001	<0.001	2	0.8
227	*Electrona risso*	Risso's Lanternfish	<0.001	<0.001	1	0.4
228	*Alopias superciliosus*	Bigeye Thresher Shark	<0.001	<0.001	1	0.4
229	*Prionace glauca*	Blue Shark	<0.001	<0.001	1	0.4
230	*Figaro boardmani*	Sawtail Catshark	<0.001	<0.001	1	0.4

12. Appendix D: Environmental health indices generated from 18S metabarcoding data

Table 12-1 Percentage relative abundances of main plankton functional groups P – Phytoplankton (photosynthetic). A – Arthropods. HF – Heterotrophic flagellates. S – Syndiniales. G – Gelatinous zooplankton. D – Dinoflagellates. P:D Phytoplankton:Dinoflagellate ratio.

Sample No.	%_P	%_A	%_HF	%_S	%_HC	%_G	%_D	P:D
S001	25.5	51.9	2.6	4.7	3.6	0.2	7.7	3.3
S002	36.5	33.3	4.0	8.9	3.4	0.2	8.7	4.2
S003	28.9	45.3	2.9	7.2	3.6	0.1	7.9	3.6
S004	33.9	36.7	4.5	8.7	2.3	0.1	10.1	3.4
S005	19.6	51.6	2.2	6.9	2.8	0.1	12.1	1.6
S006	36.7	29.9	3.8	8.7	4.6	0.1	8.6	4.3
S007	37.8	41.1	6.1	4.9	2.5	0.1	4.4	8.6
S008	59.0	14.3	6.3	5.0	4.3	0.2	5.5	10.6
S009	29.3	39.6	5.3	12.5	3.8	0.6	5.3	5.5
S010	20.9	38.3	3.3	5.6	3.4	19.3	5.0	4.2
S011	26.7	32.3	3.5	8.6	4.7	0.3	15.5	1.7
S012	36.0	24.7	4.1	9.1	4.3	0.4	15.8	2.3
S017	27.4	18.0	21.0	15.1	5.1	1.0	8.7	3.2
S018	22.5	27.1	18.5	13.8	4.6	0.6	9.8	2.3
S025	3.5	1.4	66.2	18.1	1.4	2.3	3.0	1.1
S026	3.8	0.2	61.3	24.9	1.4	2.0	3.0	1.3
S027	16.5	53.7	1.3	6.1	4.0	0.6	13.3	1.2
S028	20.0	43.2	2.6	10.6	3.3	0.2	17.0	1.2
S029	24.5	37.2	8.1	12.1	2.3	2.1	9.3	2.6
S030	27.9	22.6	7.1	14.4	6.4	0.7	13.2	2.1
S031	27.4	15.5	6.3	22.2	5.0	0.3	15.1	1.8
S032	25.2	26.9	4.2	14.5	3.4	0.2	10.9	2.3
S033	20.5	42.8	2.5	8.6	5.3	0.2	15.3	1.3
S034	23.1	42.0	2.1	7.5	3.4	0.3	15.5	1.5
S035	11.0	0.0	58.9	18.5	1.7	1.5	4.9	2.3
S036	11.4	0.6	60.0	17.9	1.5	1.8	3.9	2.9
S037	22.4	21.5	10.3	28.3	2.1	1.1	7.1	3.2
S038	14.1	46.1	7.8	20.7	1.6	1.2	4.0	3.5
S041	35.5	12.0	9.9	17.9	6.3	0.7	9.1	3.9
S042	27.8	30.7	8.5	11.7	6.0	1.3	7.8	3.5
S043	25.8	14.3	9.4	22.6	2.1	7.3	11.2	2.3
S044	19.8	34.1	8.5	23.2	1.8	0.8	6.8	2.9
S045	16.9	39.2	9.8	16.2	1.7	0.5	9.0	1.9
S046	27.0	11.7	15.5	27.1	2.4	0.9	6.9	3.9
S047	27.9	37.9	5.0	3.3	1.8	0.7	16.0	1.7
S048	34.8	18.9	3.5	15.3	3.4	0.8	18.3	1.9
S049	30.5	33.7	3.2	7.3	3.4	0.9	9.4	3.3
S050	29.6	30.8	3.6	8.6	3.0	3.6	8.6	3.5
S055	31.2	23.2	10.1	14.7	6.6	0.6	7.3	4.3
S056	20.1	59.7	3.2	4.3	1.8	0.1	7.7	2.6
S061	33.0	10.7	7.9	26.5	7.3	0.2	9.7	3.4
S062	25.6	31.9	5.8	14.0	6.9	0.3	12.1	2.1
S063	22.2	53.0	2.1	2.4	1.8	1.8	9.9	2.2
S064	21.7	44.9	3.0	4.0	3.2	5.8	11.3	1.9
S065	31.4	21.4	9.4	10.5	5.3	0.6	8.7	3.6
S066	22.3	47.9	4.7	8.0	6.0	0.5	7.9	2.8
S067	28.9	35.1	7.0	12.7	4.6	0.3	7.6	3.8
S068	19.7	57.1	3.8	6.1	3.2	0.3	7.3	2.7
S069	28.1	33.7	2.8	5.2	3.8	0.2	20.1	1.4
S070	29.9	30.8	2.4	6.0	3.6	0.8	20.4	1.5
S071	30.6	16.4	10.2	24.8	3.3	0.2	10.6	2.9

S072	17.7	39.2	6.9	18.3	3.2	0.3	11.7	1.5
S073	24.9	17.7	10.9	24.1	3.1	0.2	12.8	1.9
S074	28.8	5.7	12.6	35.9	2.5	0.1	7.7	3.7
S075	33.2	12.9	3.5	16.9	5.7	0.2	23.3	1.4
S076	25.5	26.6	3.4	16.3	6.4	0.4	19.0	1.3
S077	16.5	53.6	4.8	7.6	3.9	0.2	9.9	1.7
S078	41.3	5.6	10.7	18.9	5.9	0.7	11.6	3.6
S083	29.8	30.0	4.7	3.8	4.2	1.7	11.1	2.7
S084	26.7	43.6	3.8	3.5	3.1	0.1	5.4	5.0
S085	45.4	5.9	10.7	20.7	2.8	1.0	8.0	5.7
S086	42.6	7.1	12.0	20.0	2.7	1.5	7.5	5.7
S093	26.5	7.3	16.9	26.1	4.0	1.1	9.0	2.9
S094	25.9	4.1	15.2	25.5	6.5	0.6	18.0	1.4
S103	34.8	31.7	5.8	10.0	3.3	0.3	10.2	3.4
S104	38.1	20.9	8.0	11.8	4.7	0.3	10.1	3.8
S105	35.0	19.1	3.7	10.7	4.9	1.4	19.4	1.8
S106	29.9	26.7	2.8	8.0	4.3	0.1	24.2	1.2
S107	33.0	35.7	4.2	9.7	4.6	0.2	7.6	4.4
S108	42.1	27.4	4.2	9.6	3.5	0.1	9.7	4.4
S109	36.7	23.4	5.7	14.7	5.7	1.0	7.6	4.8
S110	47.3	9.0	6.3	20.1	4.6	0.2	7.4	6.4
S111	34.2	23.2	4.6	17.1	5.0	0.1	6.7	5.1
S112	25.5	50.3	3.9	9.3	2.2	0.1	5.4	4.7
S113	22.7	22.5	12.8	23.6	3.2	0.4	11.3	2.0
S114	29.7	34.2	3.1	6.9	4.6	0.1	18.6	1.6
S115	34.1	30.3	3.4	8.1	4.8	0.2	14.5	2.4
S116	36.7	10.6	6.1	19.0	6.7	0.2	11.7	3.1
S117	39.3	11.1	6.4	15.4	8.5	0.3	10.8	3.6
S118	19.6	14.8	22.4	21.6	3.4	0.2	13.8	1.4
S119	33.8	24.7	3.6	12.2	4.5	0.2	17.2	2.0
S120	25.2	10.0	22.4	21.5	3.6	0.3	10.1	2.5
S121	36.7	21.6	3.6	12.1	4.2	0.0	18.1	2.0
S122	28.3	37.0	2.8	13.4	5.1	0.0	10.6	2.7
S123	30.9	30.9	3.1	13.9	5.9	0.0	11.9	2.6
S124	25.6	31.4	7.3	19.1	3.1	0.2	10.2	2.5
S125	11.0	61.6	4.1	7.1	2.8	0.2	11.2	1.0
S126	24.8	22.0	4.5	12.6	2.8	15.1	16.0	1.6
S127	28.3	21.5	6.4	17.9	2.9	0.5	18.6	1.5
S129	18.2	36.1	3.7	11.1	3.8	0.1	11.0	1.7
S130	11.0	35.8	13.1	22.9	2.3	0.3	11.4	1.0
S131	26.2	13.4	7.8	19.5	5.3	0.2	22.5	1.2
S132	2.0	3.7	45.1	29.3	4.2	4.3	9.1	0.2
S133	2.4	0.4	50.8	27.3	2.2	2.6	12.0	0.2
S134	14.3	13.1	17.2	21.3	5.0	0.8	24.9	0.6
S135	19.9	10.1	4.2	20.3	7.7	0.4	33.3	0.6
S136	10.8	41.2	15.0	15.9	2.3	0.9	10.6	1.0
S137	16.6	12.9	24.2	20.3	2.6	0.8	17.3	1.0
S138	15.4	39.1	5.0	8.7	3.8	0.1	21.7	0.7
S139	24.5	23.9	5.5	17.1	4.1	0.4	18.4	1.3
S140	26.3	16.2	4.3	15.5	4.5	0.4	27.9	0.9
S141	10.5	16.6	37.6	18.3	1.2	2.6	7.8	1.4
S142	35.9	11.5	3.5	13.2	4.7	0.3	27.7	1.3
S143	11.3	1.5	43.6	23.1	1.3	1.8	11.4	1.0
S144	18.9	35.6	9.0	19.2	2.5	0.1	11.2	1.7
S145	19.2	38.6	8.3	15.6	2.8	0.1	11.7	1.6
S146	14.5	20.1	18.1	24.0	2.2	0.7	8.9	1.6
S147	20.5	31.5	15.8	14.1	1.5	0.9	11.5	1.8
S148	22.0	25.9	10.0	16.1	3.6	0.4	16.4	1.3
S149	24.7	11.0	3.9	15.3	5.9	0.4	30.8	0.8
S150	14.8	55.5	9.6	6.9	1.7	1.0	7.2	2.0
S151	25.0	28.1	15.0	12.2	1.8	1.2	10.0	2.5
S152	12.8	22.9	15.8	25.2	1.4	3.6	13.5	1.0
S153	14.6	34.8	13.8	12.3	1.8	2.0	16.7	0.9
S154	25.9	28.0	6.8	11.0	2.1	0.7	20.4	1.3

S155	20.6	27.9	6.7	11.9	3.1	8.1	16.2	1.3
S156	17.9	59.8	4.3	7.6	2.1	0.2	4.2	4.3
S157	22.8	41.2	5.4	11.3	4.0	0.6	9.3	2.4
S158	23.3	46.0	4.5	8.7	2.3	1.0	8.3	2.8
S159	29.2	37.6	5.0	11.8	1.5	1.8	7.7	3.8
S160	24.7	34.4	5.1	16.0	3.2	1.0	10.2	2.4
S161	12.9	65.9	2.9	4.9	2.0	0.1	9.5	1.4
S162	17.9	65.6	3.1	2.0	1.5	0.6	6.3	2.8
S163	22.9	39.0	3.6	5.2	1.5	5.5	14.8	1.5
S164	10.0	64.9	4.0	3.3	1.5	0.3	11.4	0.9
S165	15.1	72.2	2.0	1.8	0.8	1.5	4.7	3.2
S166	22.5	30.9	5.8	14.8	2.3	1.0	15.6	1.4
S167	19.2	39.8	5.2	12.4	2.5	0.7	14.2	1.3
S168	11.6	45.7	2.6	11.2	4.1	0.6	20.1	0.6
S169	8.3	54.5	1.9	8.0	2.7	1.9	20.1	0.4
S172	22.7	42.9	4.6	13.4	1.9	0.0	9.6	2.4
S173	19.8	52.3	3.3	5.6	2.4	0.0	14.4	1.4
S174	11.2	55.2	4.0	12.4	2.8	0.2	10.2	1.1
S175	9.3	59.0	3.3	10.2	3.7	0.2	11.0	0.8
S176	26.1	39.8	4.6	6.8	3.0	0.0	15.2	1.7
S177	10.2	55.9	3.1	7.7	3.2	0.2	15.7	0.7
S178	2.2	5.7	46.9	23.7	3.0	1.7	14.3	0.2
S179	1.4	1.3	51.9	32.2	2.0	1.8	6.8	0.2
S186	9.4	4.0	59.9	10.2	1.2	2.7	10.6	0.9
S187	31.4	22.0	4.6	13.8	4.7	1.1	18.0	1.7
S188	24.2	53.4	4.1	5.8	1.7	0.0	9.1	2.7
S189	20.2	52.3	4.2	7.3	2.6	0.0	10.7	1.9
S190	15.6	5.4	48.0	11.8	2.8	1.4	11.7	1.3
S191	14.9	17.6	40.3	10.6	1.6	1.6	11.2	1.3
S192	4.6	2.4	58.8	22.3	1.6	1.1	6.2	0.7
S193	4.9	3.3	51.9	20.6	2.1	0.8	13.5	0.4
S194	23.6	24.1	7.5	16.1	2.9	0.5	21.3	1.1
S195	19.9	30.4	7.2	13.6	2.7	0.4	22.3	0.9
S196	6.5	1.0	68.1	8.9	1.8	2.0	8.9	0.7
S197	4.7	1.1	67.5	11.8	2.5	0.5	8.0	0.6
S202	6.2	5.2	63.3	9.0	4.0	0.5	8.2	0.8
S203	3.7	7.9	63.0	10.7	2.7	1.5	8.4	0.4
S210	2.8	1.1	66.7	11.2	3.4	1.4	10.5	0.3
S211	2.8	18.6	56.9	9.9	1.8	2.0	6.0	0.5
S212	33.3	35.9	2.5	8.8	2.5	1.1	13.4	2.5
S213	40.6	23.9	2.5	10.1	3.1	2.9	13.2	3.1
S216	2.4	6.4	59.1	14.9	1.7	4.6	8.1	0.3
S217	1.6	0.5	69.6	12.2	1.5	8.0	4.5	0.3
S218	24.9	49.1	1.1	8.1	2.7	0.7	10.1	2.5
S219	20.4	60.6	1.2	5.8	1.9	0.4	7.1	2.9
S220	3.2	43.5	33.5	6.9	3.4	3.0	3.5	0.9
S221	3.8	5.7	57.4	14.7	2.4	7.6	5.9	0.6
S222	32.8	14.7	19.2	11.7	7.1	1.2	7.1	4.6
S223	28.1	25.4	19.1	12.2	4.1	2.0	4.2	6.7
S232	1.0	6.5	65.6	14.1	1.2	1.7	3.5	0.3
S233	0.9	14.3	62.9	12.8	1.2	1.8	3.8	0.2
S234	21.2	39.4	4.9	11.4	4.0	1.0	13.9	1.5
S235	16.0	55.7	1.7	7.1	6.0	0.3	11.7	1.4
S240	3.4	11.9	61.4	9.4	4.2	0.4	5.9	0.6
S241	3.0	0.4	67.8	11.9	2.9	3.6	6.6	0.5
S242	10.9	15.3	32.4	6.1	3.9	16.9	7.5	1.5
S243	8.5	27.9	33.3	7.2	3.4	5.5	7.3	1.2
S244	11.2	58.7	9.8	4.9	0.8	2.4	6.8	1.6
S245	9.5	66.6	6.9	3.7	1.7	1.9	5.6	1.7
S246	5.3	10.0	43.6	20.7	2.9	1.7	9.3	0.6
S247	5.2	2.8	50.3	22.2	3.4	2.0	9.9	0.5
S248	16.3	62.7	1.0	4.2	1.8	0.5	10.1	1.6
S249	12.8	71.0	0.8	3.6	1.5	0.5	8.6	1.5
S252	1.0	2.0	78.8	11.4	0.8	1.6	3.0	0.3

S253	36.1	16.7	2.9	19.4	3.6	1.6	16.2	2.2
S260	1.8	6.1	62.2	17.3	1.4	3.6	5.1	0.4
S261	24.8	51.1	2.1	5.6	3.3	0.7	10.3	2.4
S262	4.9	9.0	55.2	10.4	3.0	7.7	6.3	0.8
S263	4.4	5.4	64.0	10.4	2.3	4.7	4.4	1.0
S264	6.8	17.7	33.3	20.7	2.0	3.2	6.3	1.1
S265	30.1	36.3	6.2	7.1	3.9	0.9	11.5	2.6
S266	4.9	8.6	53.6	14.3	2.9	4.8	4.9	1.0
S267	4.0	10.2	52.2	14.7	1.8	6.4	4.9	0.8
S268	2.4	2.8	55.7	22.7	1.3	3.7	5.2	0.5
S269	34.0	18.9	7.6	14.6	8.7	0.5	8.1	4.2

Table 12-2 Diversity measures of main plankton functional groups P – Phytoplankton (photosynthetic). A – Arthropods. HF – Heterotrophic flagellates. S – Syndiniales. G – Gelatinous zooplankton. D – Dinoflagellates.

Sample No.	S_All	S_P	S_HF	S_HC	S_A	S_G	S_S	S_D	1-λ' All	1-λ' P	1-λ' A	1-λ' HF	1-λ' HC	1-λ' G	1-λ' D	1-λ' S
S001	1185	342	151	128	29	7	193	156	0.89	0.86	0.62	0.98	0.98	0.53	0.94	0.96
S002	1204	333	155	115	25	8	230	149	0.95	0.86	0.71	0.98	0.98	0.77	0.95	0.96
S003	1529	404	179	123	36	6	333	176	0.94	0.88	0.76	0.98	0.94	0.83	0.95	0.98
S004	1480	413	178	110	36	6	293	164	0.93	0.88	0.56	0.98	0.97	0.67	0.92	0.97
S005	1348	353	145	126	37	7	326	202	0.86	0.93	0.50	0.98	0.95	0.69	0.93	0.99
S006	1749	466	191	157	38	8	385	175	0.96	0.91	0.77	0.98	0.97	0.87	0.94	0.98
S007	492	194	70	36	12	1	54	53	0.89	0.94	0.44	0.97	0.94	0.00	0.93	0.91
S008	660	259	87	51	14	5	71	75	0.96	0.91	0.61	0.97	0.95	0.59	0.96	0.94
S009	1133	292	151	85	22	10	281	102	0.91	0.95	0.45	0.98	0.96	0.58	0.93	0.98
S010	1149	315	182	126	28	5	225	145	0.92	0.94	0.71	0.99	0.96	0.01	0.97	0.98
S011	1371	373	161	166	25	9	299	204	0.97	0.96	0.78	0.98	0.98	0.63	0.95	0.98
S012	1326	385	136	142	29	10	288	176	0.98	0.95	0.80	0.98	0.97	0.70	0.95	0.98
S017	665	187	109	51	19	11	125	87	0.99	0.98	0.71	0.95	0.97	0.81	0.97	0.97
S018	881	233	172	71	24	8	164	110	0.98	0.97	0.80	0.95	0.97	0.83	0.97	0.98
S025	1054	118	275	42	8	20	418	72	0.97	0.98	0.54	0.93	0.93	0.82	0.97	0.99
S026	867	98	239	26	17	16	356	49	0.97	0.98	0.76	0.93	0.91	0.82	0.95	0.99
S027	862	227	72	105	16	5	189	151	0.94	0.95	0.70	0.97	0.98	0.69	0.96	0.98
S028	1105	262	94	146	22	8	232	187	0.93	0.94	0.76	0.98	0.98	0.75	0.94	0.98
S029	1659	419	202	174	32	10	376	219	0.99	0.95	0.88	0.97	0.98	0.62	0.97	0.98
S030	1710	430	194	139	35	14	406	210	0.97	0.96	0.85	0.96	0.98	0.29	0.97	0.98
S031	1794	389	199	188	22	9	517	241	0.99	0.95	0.77	0.97	0.99	0.79	0.97	0.99
S032	1712	379	187	167	28	7	456	211	0.97	0.94	0.69	0.97	0.99	0.71	0.96	0.99
S033	1439	343	131	178	30	5	335	207	0.94	0.92	0.69	0.96	0.98	0.15	0.96	0.98
S034	1274	307	107	147	38	9	285	221	0.97	0.94	0.83	0.97	0.98	0.72	0.94	0.98
S035	1508	272	312	88	3	20	475	111	0.96	0.98	0.56	0.90	0.98	0.86	0.88	0.99
S036	1383	268	320	63	9	23	441	108	0.97	0.98	0.66	0.92	0.97	0.87	0.84	0.99
S037	1514	356	264	89	14	13	399	162	0.98	0.96	0.57	0.98	0.96	0.80	0.95	0.97
S038	1626	342	263	99	28	19	425	156	0.87	0.98	0.39	0.98	0.96	0.77	0.95	0.97
S041	1955	470	258	123	14	11	380	203	0.98	0.95	0.58	0.98	0.86	0.47	0.97	0.96

S042	1604	393	224	115	26	14	271	149	0.98	0.96	0.78	0.98	0.96	0.53	0.97	0.96
S043	1523	386	257	85	12	15	274	167	0.99	0.97	0.71	0.98	0.95	0.50	0.92	0.96
S044	1727	394	271	119	30	14	413	171	0.96	0.95	0.68	0.98	0.96	0.69	0.93	0.96
S045	797	193	150	42	25	8	168	104	0.94	0.97	0.64	0.98	0.95	0.77	0.95	0.95
S046	1204	317	226	76	13	14	273	112	0.99	0.97	0.74	0.97	0.97	0.78	0.95	0.95
S047	1263	308	142	109	27	14	182	193	0.95	0.87	0.76	0.91	0.97	0.78	0.81	0.96
S048	1535	333	130	140	29	22	392	222	0.98	0.92	0.78	0.96	0.98	0.86	0.94	0.96
S049	1464	361	154	129	26	16	265	175	0.96	0.91	0.76	0.98	0.97	0.83	0.95	0.95
S050	1556	339	162	137	26	12	300	166	0.96	0.87	0.71	0.98	0.97	0.40	0.94	0.95
S055	1705	418	287	125	17	23	318	154	0.96	0.96	0.29	0.98	0.85	0.77	0.94	0.97
S056	1223	313	119	96	27	10	232	169	0.86	0.90	0.62	0.97	0.97	0.84	0.95	0.98
S061	1231	325	209	100	14	9	215	107	0.98	0.97	0.14	0.98	0.93	0.76	0.91	0.95
S062	1216	303	202	99	7	12	191	148	0.92	0.96	0.26	0.98	0.94	0.80	0.91	0.95
S063	1199	253	106	112	32	11	187	190	0.86	0.79	0.53	0.92	0.98	0.13	0.93	0.99
S064	1586	344	118	161	43	20	269	211	0.96	0.91	0.82	0.91	0.98	0.41	0.88	0.99
S065	1410	366	216	92	16	10	181	133	0.96	0.95	0.51	0.93	0.89	0.53	0.90	0.95
S066	1216	332	199	104	11	12	188	151	0.85	0.96	0.35	0.98	0.92	0.61	0.91	0.96
S067	1430	361	229	92	14	11	264	125	0.90	0.96	0.22	0.98	0.88	0.76	0.90	0.97
S068	1132	327	189	88	22	9	181	143	0.86	0.96	0.57	0.98	0.91	0.66	0.93	0.97
S069	1389	305	123	130	24	7	234	192	0.96	0.89	0.77	0.98	0.95	0.56	0.92	0.98
S070	1370	293	129	131	22	11	253	199	0.95	0.86	0.70	0.98	0.97	0.49	0.92	0.98
S071	1339	367	217	91	21	7	259	126	0.98	0.96	0.80	0.97	0.88	0.66	0.78	0.91
S072	1304	290	194	111	15	12	265	176	0.94	0.96	0.64	0.97	0.95	0.80	0.90	0.93
S073	1867	427	311	119	15	14	400	191	0.98	0.98	0.61	0.98	0.97	0.88	0.89	0.95
S074	1829	432	311	104	17	8	440	152	0.99	0.98	0.73	0.97	0.95	0.62	0.90	0.95
S075	1954	336	149	214	21	7	552	274	0.98	0.92	0.71	0.97	0.98	0.81	0.93	0.99
S076	1769	368	127	239	14	13	567	276	0.97	0.95	0.68	0.97	0.98	0.85	0.95	0.99
S077	1233	300	188	117	27	11	187	144	0.92	0.96	0.74	0.98	0.96	0.79	0.87	0.93
S078	1519	392	222	126	12	17	270	141	0.99	0.96	0.59	0.98	0.96	0.71	0.86	0.93
S083	1076	304	164	112	14	9	141	160	0.93	0.90	0.38	0.97	0.90	0.18	0.96	0.98
S084	1164	296	160	98	18	6	150	117	0.89	0.90	0.50	0.97	0.93	0.38	0.94	0.97
S085	1779	436	324	94	12	20	360	181	0.97	0.88	0.71	0.99	0.90	0.90	0.95	0.96
S086	1873	430	333	98	8	28	353	150	0.97	0.88	0.70	0.99	0.88	0.80	0.95	0.96
S093	2059	435	359	152	11	15	554	209	0.99	0.95	0.72	0.96	0.97	0.70	0.97	0.97
S094	2163	442	356	196	13	14	596	277	0.99	0.92	0.69	0.96	0.97	0.84	0.96	0.98
S103	1947	445	221	147	23	12	460	212	0.96	0.92	0.72	0.98	0.97	0.75	0.95	0.98
S104	2301	508	313	182	22	13	548	248	0.98	0.94	0.76	0.98	0.97	0.85	0.96	0.98
S105	2188	467	202	195	28	11	494	269	0.98	0.94	0.74	0.99	0.98	0.32	0.94	0.99
S106	1727	394	161	193	20	7	362	240	0.95	0.94	0.47	0.98	0.98	0.74	0.93	0.99
S107	1910	438	212	178	37	7	430	223	0.97	0.92	0.85	0.98	0.98	0.61	0.97	0.97
S108	2019	507	226	177	23	8	458	218	0.97	0.94	0.73	0.99	0.98	0.66	0.96	0.99
S109	2122	485	239	184	29	11	575	241	0.98	0.93	0.80	0.97	0.97	0.14	0.98	0.97
S110	2116	498	223	176	27	14	576	200	0.98	0.93	0.80	0.98	0.98	0.88	0.97	0.96

S111	1778	370	178	166	20	6	397	191	0.97	0.90	0.71	0.97	0.98	0.79	0.98	0.93
S112	607	187	66	51	11	4	130	83	0.88	0.94	0.53	0.97	0.96	0.62	0.96	0.95
S113	1877	380	252	139	17	13	679	243	0.97	0.97	0.48	0.97	0.98	0.83	0.98	0.99
S114	1590	386	143	153	38	8	338	244	0.96	0.90	0.79	0.97	0.98	0.87	0.96	0.99
S115	1791	431	179	167	37	7	407	224	0.97	0.91	0.77	0.98	0.97	0.72	0.94	0.99
S116	1796	423	186	172	16	9	408	225	0.99	0.95	0.66	0.98	0.97	0.78	0.96	0.97
S117	1516	402	162	150	9	6	300	199	0.98	0.96	0.22	0.98	0.95	0.60	0.97	0.97
S118	2139	402	382	156	20	16	711	263	0.99	0.97	0.77	0.96	0.98	0.86	0.98	0.99
S119	2277	474	385	162	15	17	713	220	0.99	0.98	0.50	0.95	0.98	0.90	0.98	0.99
S120	1983	471	163	176	27	6	518	256	0.98	0.93	0.83	0.97	0.98	0.41	0.95	0.99
S121	1910	470	170	157	23	4	503	243	0.98	0.93	0.77	0.98	0.98	0.71	0.95	0.99
S122	1423	356	148	135	15	1	314	185	0.96	0.95	0.72	0.99	0.97	0.00	0.97	0.95
S123	1521	406	149	143	22	3	340	223	0.96	0.95	0.64	0.98	0.97	0.59	0.98	0.98
S124	1984	475	246	136	13	11	593	205	0.91	0.97	0.16	0.98	0.97	0.80	0.97	0.99
S125	1295	288	178	120	27	6	304	235	0.92	0.97	0.79	0.98	0.97	0.79	0.98	0.98
S126	1728	371	204	139	21	11	579	237	0.96	0.94	0.76	0.98	0.97	0.03	0.93	0.99
S127	1682	338	182	117	17	7	553	223	0.98	0.93	0.79	0.98	0.98	0.57	0.95	0.99
S129	1582	298	162	160	22	7	457	209	0.95	0.94	0.66	0.98	0.98	0.81	0.97	0.99
S130	1258	221	188	87	22	12	436	191	0.97	0.97	0.75	0.97	0.98	0.60	0.98	0.98
S131	2027	439	140	211	16	8	599	312	0.99	0.95	0.79	0.82	0.99	0.87	0.98	0.99
S132	795	58	181	55	4	17	326	99	0.97	0.97	0.22	0.88	0.96	0.53	0.96	0.99
S133	850	66	188	44	17	11	325	128	0.97	0.97	0.60	0.89	0.96	0.76	0.97	0.99
S134	2058	376	208	191	17	7	697	340	0.99	0.97	0.70	0.84	0.98	0.91	0.96	0.99
S135	2022	367	144	209	22	7	657	342	0.99	0.93	0.79	0.94	0.97	0.82	0.93	0.99
S136	1777	342	280	117	28	11	575	245	0.94	0.97	0.67	0.97	0.98	0.58	0.97	0.99
S137	2280	401	374	157	26	16	714	301	0.99	0.97	0.83	0.98	0.99	0.70	0.98	0.99
S138	1828	333	148	214	32	8	391	346	0.94	0.95	0.63	0.87	0.99	0.78	0.97	0.99
S139	2118	401	174	204	38	6	571	337	0.99	0.95	0.87	0.91	0.98	0.24	0.97	0.99
S140	1918	343	159	200	14	7	527	326	0.98	0.91	0.73	0.91	0.99	0.68	0.95	0.99
S141	2129	355	361	71	18	19	742	253	0.97	0.98	0.08	0.96	0.96	0.64	0.97	0.99
S142	1816	322	133	194	12	8	516	325	0.96	0.80	0.57	0.92	0.98	0.80	0.95	0.99
S143	1990	337	363	76	11	18	701	265	0.99	0.98	0.33	0.97	0.98	0.85	0.98	0.99
S144	2116	391	235	164	32	6	706	298	0.98	0.95	0.83	0.96	0.99	0.80	0.98	0.99
S145	1969	406	239	194	39	9	590	312	0.98	0.96	0.87	0.95	0.99	0.86	0.98	0.99
S146	2547	346	354	162	21	11	920	293	0.98	0.93	0.60	0.98	0.99	0.75	0.98	0.99
S147	2183	360	345	142	30	14	675	303	0.97	0.83	0.75	0.97	0.99	0.81	0.98	0.99
S148	2054	362	218	198	28	14	586	306	0.98	0.89	0.89	0.85	0.99	0.85	0.97	0.99
S149	2108	398	148	215	22	6	541	321	0.99	0.94	0.81	0.95	0.98	0.69	0.94	0.99
S150	1492	313	274	96	36	10	336	200	0.93	0.84	0.78	0.98	0.97	0.33	0.97	0.98
S151	1676	328	279	89	30	12	438	223	0.94	0.72	0.43	0.98	0.97	0.34	0.97	0.98
S152	2238	288	329	127	25	18	828	292	0.98	0.84	0.79	0.98	0.99	0.58	0.97	0.94
S153	1884	300	304	124	36	10	596	320	0.97	0.80	0.82	0.98	0.98	0.41	0.98	0.99
S154	2007	384	167	153	33	9	463	330	0.97	0.89	0.69	0.82	0.99	0.53	0.97	0.98

S155	1836	352	166	157	28	14	463	288	0.97	0.92	0.78	0.84	0.95	0.07	0.97	0.97
S156	1209	258	142	89	30	8	258	150	0.84	0.82	0.57	0.87	0.93	0.79	0.97	0.93
S157	1675	342	186	130	46	11	331	204	0.96	0.86	0.85	0.86	0.94	0.74	0.90	0.93
S158	1426	344	184	101	32	8	270	179	0.94	0.78	0.79	0.98	0.90	0.10	0.96	0.96
S159	1452	333	196	93	24	7	297	183	0.92	0.72	0.61	0.98	0.96	0.17	0.96	0.96
S160	1688	342	196	149	39	7	443	227	0.97	0.76	0.88	0.97	0.98	0.46	0.95	0.97
S161	1381	325	150	139	33	3	321	220	0.79	0.88	0.51	0.97	0.97	0.56	0.95	0.99
S162	802	246	100	61	26	10	79	128	0.86	0.68	0.71	0.90	0.94	0.20	0.96	0.88
S163	1171	282	153	76	19	6	192	184	0.94	0.70	0.76	0.98	0.96	0.18	0.97	0.96
S164	1025	243	96	78	25	4	222	182	0.86	0.90	0.66	0.83	0.92	0.65	0.94	0.98
S165	710	204	92	52	25	6	72	106	0.85	0.59	0.73	0.95	0.96	0.18	0.96	0.86
S166	1729	353	205	122	21	13	492	264	0.97	0.85	0.74	0.96	0.97	0.72	0.96	0.98
S167	1561	290	205	118	21	12	390	251	0.94	0.84	0.64	0.97	0.97	0.74	0.97	0.98
S168	1754	348	124	194	24	4	519	297	0.94	0.96	0.74	0.94	0.99	0.87	0.97	0.99
S169	1446	284	98	160	31	5	409	278	0.90	0.94	0.66	0.95	0.98	0.15	0.96	0.99
S172	1018	260	169	48	19	2	199	111	0.93	0.91	0.64	0.98	0.87	0.29	0.90	0.96
S173	1059	274	105	92	21	3	192	175	0.86	0.90	0.50	0.94	0.96	0.61	0.96	0.97
S174	1137	280	175	78	24	3	234	150	0.88	0.95	0.61	0.98	0.86	0.18	0.95	0.95
S175	1171	259	170	78	30	5	243	162	0.88	0.94	0.67	0.97	0.81	0.58	0.94	0.96
S176	1454	261	127	141	40	7	390	283	0.97	0.90	0.87	0.91	0.86	0.91	0.94	0.98
S177	1356	296	82	152	38	10	362	251	0.92	0.97	0.76	0.79	0.97	0.82	0.96	0.99
S178	993	300	154	62	23	2	162	156	0.88	0.78	0.63	0.98	0.93	0.67	0.95	0.96
S179	916	274	144	65	29	1	135	160	0.93	0.86	0.76	0.97	0.92		0.94	0.79
S186	883	55	219	57	12	18	305	145	0.99	0.95	0.39	0.95	0.97	0.90	0.96	0.98
S187	1401	89	285	77	16	28	669	150	0.98	0.98	0.44	0.94	0.96	0.84	0.96	0.99
S188	1811	285	409	91	12	15	607	231	0.98	0.93	0.63	0.95	0.98	0.71	0.95	0.99
S189	2020	441	134	194	31	7	561	300	0.98	0.91	0.87	0.91	0.98	0.25	0.97	0.99
S190	2085	380	431	128	23	20	605	253	0.99	0.94	0.81	0.95	0.97	0.64	0.97	0.99
S191	1761	316	374	103	16	26	567	207	0.98	0.92	0.66	0.95	0.98	0.82	0.97	0.99
S192	2412	298	439	112	17	16	1039	239	0.98	0.99	0.39	0.95	0.97	0.83	0.98	0.99
S193	1127	94	252	55	31	11	417	186	0.98	0.95	0.83	0.94	0.96	0.89	0.97	0.99
S194	2133	425	171	145	22	1	645	310	0.98	0.94	0.77	0.97	0.98	0.00	0.98	0.99
S195	1899	390	175	128	27	2	558	278	0.98	0.95	0.80	0.97	0.97	0.04	0.97	0.99
S196	1485	284	388	78	10	15	334	145	0.97	0.98	0.69	0.94	0.94	0.60	0.81	0.97
S197	1718	234	415	110	8	16	528	160	0.96	0.99	0.34	0.92	0.96	0.82	0.83	0.99
S202	1221	198	315	59	16	18	290	121	0.98	0.98	0.48	0.96	0.94	0.89	0.95	0.99
S203	1431	196	338	81	18	16	453	158	0.97	0.98	0.61	0.92	0.96	0.68	0.96	0.99
S210	1410	170	391	78	10	22	394	198	0.98	0.99	0.57	0.96	0.96	0.80	0.96	0.97
S211	1559	235	385	87	23	21	481	188	0.98	0.99	0.65	0.96	0.95	0.68	0.94	0.98
S212	1609	456	177	136	36	7	370	224	0.98	0.95	0.87	0.98	0.98	0.62	0.97	0.98
S213	1669	424	165	148	38	12	381	236	0.98	0.93	0.85	0.98	0.98	0.54	0.97	0.98
S216	1617	155	371	79	35	24	593	195	0.97	0.98	0.81	0.91	0.95	0.75	0.96	0.99
S217	1143	96	308	51	20	20	404	128	0.95	0.97	0.50	0.91	0.95	0.51	0.95	0.99

S218	1348	303	103	118	46	19	356	205	0.95	0.81	0.85	0.98	0.92	0.64	0.96	0.98
S219	1170	310	94	124	40	12	280	188	0.91	0.82	0.77	0.97	0.98	0.78	0.97	0.97
S220	1077	146	248	77	34	25	281	101	0.84	0.98	0.17	0.98	0.97	0.83	0.97	0.99
S221	912	100	248	42	24	24	283	96	0.98	0.97	0.75	0.95	0.93	0.71	0.97	0.99
S222	1373	350	263	94	33	20	264	135	0.99	0.96	0.74	0.97	0.89	0.83	0.96	0.98
S223	1982	490	393	132	15	24	466	137	0.94	0.96	0.19	0.95	0.91	0.85	0.95	0.99
S232	1641	115	423	94	14	28	638	188	0.98	0.99	0.22	0.96	0.97	0.89	0.98	0.98
S233	1629	111	444	72	25	23	635	203	0.97	0.98	0.29	0.96	0.96	0.81	0.98	0.99
S234	2127	439	228	191	45	14	668	300	0.97	0.89	0.85	0.98	0.98	0.44	0.98	0.99
S235	1591	346	121	204	51	17	451	243	0.96	0.86	0.88	0.97	0.98	0.91	0.96	0.99
S240	1486	205	357	114	14	25	461	149	0.98	0.98	0.52	0.96	0.94	0.90	0.96	0.99
S241	1873	225	432	120	13	28	655	187	0.98	0.99	0.64	0.96	0.93	0.72	0.97	0.99
S242	1352	263	352	80	29	33	234	139	0.98	0.95	0.79	0.97	0.95	0.70	0.96	0.98
S243	889	153	262	45	35	20	151	94	0.97	0.96	0.71	0.98	0.88	0.78	0.97	0.98
S244	1267	299	274	57	41	17	263	155	0.95	0.96	0.85	0.97	0.96	0.86	0.96	0.98
S245	1287	308	279	92	28	20	233	149	0.91	0.98	0.81	0.98	0.93	0.86	0.97	0.98
S246	2389	300	555	141	27	26	778	265	0.99	0.99	0.70	0.98	0.95	0.79	0.96	0.99
S247	1976	231	507	113	19	25	663	241	0.99	0.98	0.32	0.98	0.95	0.77	0.97	0.98
S248	1233	285	93	123	40	13	309	227	0.93	0.86	0.83	0.97	0.98	0.68	0.97	0.98
S249	1097	277	78	101	58	21	266	202	0.91	0.82	0.83	0.98	0.96	0.88	0.97	0.98
S252	973	53	302	34	15	23	357	104	0.96	0.98	0.43	0.94	0.95	0.84	0.97	0.99
S253	1957	422	166	181	25	27	680	262	0.98	0.90	0.80	0.98	0.98	0.78	0.98	0.99
S260	2080	196	521	87	22	26	853	230	0.99	0.98	0.73	0.97	0.95	0.80	0.98	0.99
S261	1447	374	93	153	47	11	340	219	0.94	0.86	0.81	0.95	0.98	0.83	0.98	0.98
S262	1184	163	353	57	14	33	286	122	0.99	0.98	0.41	0.97	0.96	0.84	0.97	0.99
S263	520	56	187	19	17	22	94	56	0.99	0.95	0.61	0.97	0.91	0.78	0.96	0.98
S264	2360	308	487	90	14	25	804	213	0.97	0.98	0.13	0.97	0.96	0.82	0.97	0.99
S265	1596	365	270	154	44	17	346	216	0.95	0.91	0.70	0.99	0.97	0.78	0.97	0.97
S266	1908	259	485	85	12	29	519	158	0.99	0.98	0.57	0.97	0.95	0.82	0.97	0.99
S267	1982	268	490	61	19	40	604	155	0.99	0.99	0.66	0.98	0.90	0.80	0.98	0.99
S268	2128	198	507	76	9	33	803	215	0.99	0.98	0.63	0.98	0.94	0.74	0.98	0.99
S269	1491	362	124	130	45	9	297	233	0.97	0.89	0.85	0.97	0.96	0.68	0.97	0.94

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