Environmental DNA metabarcoding describes biodiversity across marine gradients

Article *in* ICES Journal of Marine Science · February 2023

DOI: 10.1093/icesjms/fsad017

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ICES Journal of Marine Science, 2023, **0**, 1–19
DOI: 10.1093/icesjms/fsad017
Original Article

Environmental DNA metabarcoding describes biodiversity across marine gradients

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In response to climate change, biodiversity patterns in the oceans are predicted to shift rapidly, thus increasing the need for efficient monitoring methods. Environmental DNA (eDNA) metabarcoding recently emerged as a potent and cost-effective candidate to answer this challenge. We targeted three molecular markers to determine multicellular metazoan communities from two timepoints across a long-standing transect in the Southern Hemisphere, the Munida Observational Time Series. We detected four community types across the successive water masses—neritic, sub-tropical, frontal, and sub-Antarctic—crossed by the transect, together with important community differences between the two sampling points. From indicator species analysis, we found diversity patterns were mostly driven by planktonic organisms. Mesopelagic communities differed from surface-water communities in the sub-Antarctic water mass, with at-depth communities dominated by single-cellular organisms. We evaluate the ability of eDNA to detect species-compositional changes across surface and depth gradients and lay the foundations for using this technique in multi-trophic environmental monitoring efforts across long time series. We observed community differences across time and space. More intensive sampling will be critical to fully capture diversity across marine gradients, but this multi-trophic method represents an invaluable opportunity to understand shifts in marine biota.

Keywords: community biodiversity, eDNA, environmental DNA, monitoring, Munida transect, spatial heterogeneity, temporal heterogeneity.

Background

Climate change is causing rapid shifts in biodiversity patterns within and across marine environments (Perry *et al.*, 2005; Chen *et al.*, 2011; Donelson *et al.*, 2019), increasing the need to efficiently monitor and document our changing ecosystems (Pecl *et al.*, 2017; Berry *et al.*, 2019; Overland *et al.*, 2019). Community biodiversity patterns have tangible impacts on ecosystems and fisheries worldwide (Stewart-Sinclair *et al.*, 2020). The sustainable management of these fisheries; however, and—more broadly—the monitoring of these marine ecosystems, requires the acquisition and analysis of data that are complex and difficult to collect. Developing and testing new, cost-effective, and time-efficient biodiversity monitoring methods and analytical approaches are thus key steps in improving our ability to gather ecological information, with direct consequences for fundamental ecology (for a deeper understanding of biological community structures and dynamics over time and space) and applied ecology (to better match ecological legislations and ecosystem-based management agendas).

Effective fisheries management relies on the accurate characterization of the biodiversity in the targeted area (in terms of species assemblages, typically), and on the ability to follow up changes in this initial assemblage over time. The marine environment, by being complex, stratified, and difficult to access by human means, poses important sampling challenges. To date, there is no single method sufficient for monitoring all aspects of complex oceanic change, as each method has its own set of limitations. For example, net capture monitoring may harm fish during the capture process and are designed

Received: November 17, 2022. **Revised:** January 18, 2023. **Accepted:** January 23, 2023

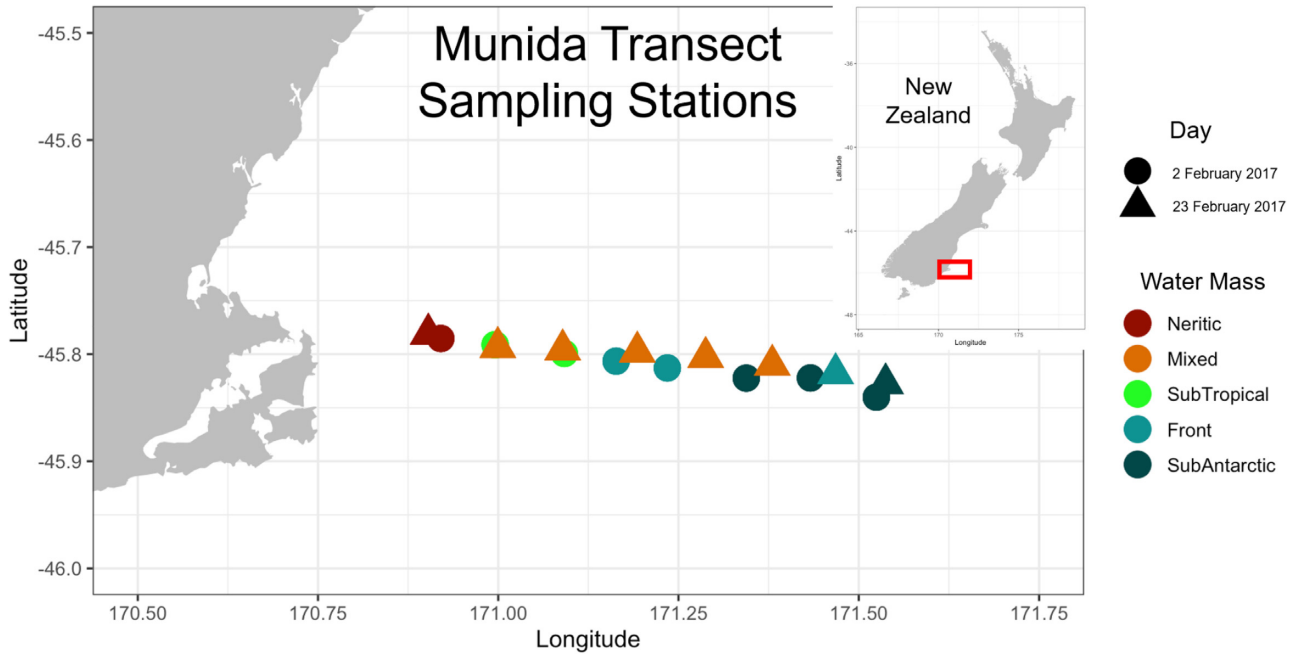

Figure 1. Sampling stations along the Munida transect. Sampling stations for 2 February 2017 are in circles and 23 February 2017 are in triangles. We sampled off the coast of the Otago Peninsula and traverse four different water masses: neritic, subtropical, the Southland Front, and sub-Antarctic waters, indicated by colour. Five replicate surface samples were taken at all stations, while five replicate at-depth samples were only taken at the station farthest from the coast in sub-Antarctic waters.

to capture certain sizes of fishes, giving an incomplete picture of the ecosystem (McClanahan and Mangi, 2004; von Szalay and Somerton, 2005). Non-invasive underwater visual census may bias results due to difficulty in accurately estimating fish size, abundance, or identity, especially if fish are small and far away (Edgar *et al.*, 2004; Ward-Paige *et al.*, 2010; Bozec *et al.*, 2011). Additionally, fish behaviour may vary or be changed by the presence of a diver (Buxton and Smale, 1989; Lindfield *et al.*, 2014; Langlois *et al.*, 2015). Due to capture and non-invasive sampling each having their own biases, different but complementary conclusions may be reached to paint a full picture (Harmelin-Vivien and Francour, 1992). In the last decade, the emergence of molecular techniques for biological monitoring offer new ways to fill in knowledge gaps left by both invasive and non-invasive monitoring methods. Used in conjunction with traditional sampling, molecular methods could eliminate drawbacks associated with traditional approaches, such as the identification of cryptic species and larval forms (Marshall and Stepien, 2019; Mason *et al.*, 2020; Zaiko *et al.*, 2020).

Environmental DNA (eDNA) surveys are gaining interest as a non-invasive monitoring method for megafaunal biodiversity (Fediajevaite *et al.*, 2021; Jerde, 2021), with applications in invasion ecology (Zaiko *et al.*, 2015; Suarez-Menendez *et al.*, 2020), species conservation (Parsons *et al.*, 2018; Adams *et al.*, 2019), functional ecology and biogeochemical cycles (Wakelin *et al.*, 2016), prey–predator interactions and other trophic links (Djurhuus *et al.*, 2020), environmental pollution assessment (Kavehei *et al.*, 2021), and aquaculture impacts (Stoeck *et al.*, 2018). eDNA has been shown to increase detection sensitivity compared to traditional monitoring approaches (Closek *et al.*, 2019; Nester *et al.*, 2020; Afzali *et al.*, 2021), as well as being cost-effective and time-efficient (Thomsen and Willerslev, 2015; Stoeckle *et al.*, 2016; Evans *et al.*, 2017; Fediajevaite *et al.*, 2021). Additionally, previous studies have reported high spatial and temporal resolution for aquatic eDNA in the coastal marine environment (Jeunen *et al.*, 2018; Berry *et al.*, 2019; Jeunen *et al.*, 2020; West *et al.*, 2020). Precise information about the spatial and temporal resolution of eDNA metabarcoding [see (Bálint *et al.*, 2018)] is, however, still lacking for open-oceanic transects spanning across multiple water masses [but see (Port *et al.*, 2016; O'Donnell *et al.*, 2017)].

To address this issue, we used the Munida Time Series, which is a long-term dataset consisting of a series of transects taken every 2 months in the southern Pacific Ocean located off the coast of Otago, *Aotearoa* New Zealand (extending from the coast, −45.77°N 170.72°W to 65 km offshore −45.82°N 171.54°W) (Figure 1). The Munida Time Series, established in 1998, crosses three different marine water masses (neritic, sub-tropical, and sub-Antarctic), and a sub-tropical front (the Southland Front) between the sub-tropical and sub-Antarctic water masses (Bates *et al.*, 2014). Repeated transect monitoring can identify seascape changes across multiple marine water masses. Differences in salinity, temperature, and oceanic microbiome across different water masses can be observed along the transect in seasonal cycles based on water mass properties (Morales *et al.*, 2018; Bagnaro *et al.*, 2020). Water samples, usually taken once every 2 months since 1998, continue to facilitate the study of ocean acidification under climate change (Currie and Hunter, 1999; Bates *et al.*, 2014), as well as the evolution of other limiting macro- and micronutrients, such as iron, copper, and cadmium over time (Croot and Hunter, 1998). Although the chemical properties, microbial communities, and planktonic communities of these transects are well known (Currie and Hunter, 1999; Currie *et al.*, 2011; Bates *et al.*, 2014; Baltar *et al.*, 2015; Morales *et al.*, 2018; Robertson *et al.*, 1978), sampling difficulties prevented the collection of a consistent ecological baseline for marine metazoan megafauna, such as fish.

We set out to overlay a multi-assay eDNA metabarcoding survey on top of the well-studied Munida Time Series. We collated eukaryotes assemblages twice during the month of February 2017 along the ∼65-km transect (Figure 1). These voyages provide snapshots of metazoan biodiversity, which we leveraged to answer the following questions:

1. Does the metazoan eDNA signal differ between the different water masses (neritic, sub-tropical, front, and sub-Antarctic)?
2. Do metazoan communities differ between sampling voyages on different days?
3. How does the eDNA signal differ between surface and deeper water samples in the sub-Antarctic waters?

Methods

Study area

The Munida transect is a 65-km transect running along a gradient of three water masses: neritic, sub-tropical, and sub-Antarctic (Figure 1). Here, we use the term water mass to identify bodies of water with similar salinity and temperature across the Munida transect. The Southland Front separates the sub-tropical and sub-Antarctic water masses (Jillett, 1969; Currie and Hunter, 1999; Jones *et al.*, 2013; Baltar *et al.*, 2015, 2016; Morales *et al.*, 2018). The neritic water mass is located closest to shore (typically 0–15 km offshore) and characterized by warm coastal waters with lower salinity due to freshwater runoff and rivers (Currie *et al.*, 2011). Sub-tropical waters (typically 15–40 km offshore) over the continental shelf are characterized by higher levels of salinity and decreasing temperature, and form part of the northward flowing Southland Front (Currie *et al.*, 2011). Between the sub-tropical and sub-Antarctic waters, the Southland Front (front), also called the sub-tropical front, flows northwards (Sutton, 2003; Currie *et al.*, 2011; Stevens *et al.*, 2019). This front can be recognized from an abrupt decrease in temperature and salinity. Farthest from shore is the sub-Antarctic surface water (typically >45 km offshore), characterized by stable, low salinities, and low temperatures similar to circumpolar sub-Antarctic water (Jillett, 1969). At the easternmost sub-Antarctic station of the transect (station 8), we aimed to compare biodiversity at the surface and at mesopelagic depth [956 metres (m) on Day 1, 900 m on Day 2]. Deeper waters are usually characterized by stable, cold temperatures (e.g. 4.0°C) and relatively low (34.4 parts per thousand) salinity throughout the year (Jillett, 1969).

Water mass delineation

Water masses, water bodies we identified with similar salinity and temperature, were determined by measuring temperature and salinity with a Sea-Bird SBE 38 and Sea-Bird SBE 45 along the transect and averaged into 0.5 km bins (Figure 2). A horizontal temperature and salinity profile of each voyage was drawn with R 4.0.3 (R Core Team, 2020). To determine water mass boundaries for each voyage, using R 4.0.3 and the packages *EcotoneFinder* with fuzzy clustering and four *a priori* groups (neritic, sub-tropical, front, and sub-Antarctic) (R Core Team, 2020; Bagnaro, 2021). Based on the delineation of the water masses from clustering, we assigned water mass to the data, then determined how biodiversity varies between water mass regions. The "mixed" water mass occurs when there is no apparent difference between the three water masses, probably due to a mixing process (Jones *et al.*, 2013). Differences between each voyage were examined with permutational analysis of variance (PERMANOVA) and principal coordinate analysis (PCoA) visualization.

eDNA sampling

Prior to field and laboratory work, we sterilized all equipment and bench spaces with a 10-min exposure to 10% bleach solution (Goldberg *et al.*, 2016). We rinsed all sampling bottles (2 L, HDPE Natural, EPI Plastics) twice with ultrapure water, submerged in 10% bleach for 10 min, and rinsed twice again with ultrapure water. All laboratory work prior to amplification was performed in a dedicated eDNA PCR-free clean laboratory at the University of Otago, Department of Zoology.

We monitored contamination by including negative controls at each step. Sampling controls consisted of two 2 litre (L) bottles containing 500 mL ultrapure water (UltraPureTM Distilled Water, Invitrogen), DNA capture controls were added by filtering 500 mL ultrapure water, and DNA extraction controls consisted of 500 µL ultrapure water. We processed all negative controls alongside the samples.

The Munida transect was sampled twice for eDNA analysis, during voyages on the 2nd and on the 23rd of February 2017. We refer to each voyage by date for analyses. For each date, we collected five 2 L replicate samples at eight stations covering four marine water masses (neritic, sub-tropical, front, and sub-Antarctic). Samples were collected from the ship scientific water supply at a depth of about 2 m. At the easternmost station of the transect (station 8), we took an additional five 2 L replicate water samples from Nisken bottles at a depth of 956 and 900 m (2 February and 23 February, respectively) resulting in a total of 90 samples (Figure 1). Abiotic measurements (pH, temperature, salinity—measured with the SeaBird SBE 38 and SeaBird SBE 45) were collected along the entire Munida transect on the same voyages.

eDNA filtration and extraction

Sample processing followed the recommendations of Jeunen *et al.* (2019): briefly, we filtered water samples on the same day as sample collection over a 1.2 µm cellulose-nitrate filter (CN, WhatmanTM) using a vacuum filtration pump (Laboport®, KNF Neuberger, Inc.) and an in-house made filtration manifold. This filter was chosen to capture metazoan animal cells without excessive clogging (Jeunen *et al.*, 2019). We rolled filters, cut them into two, placed them in two 2 mL LoBind Eppendorf tubes, and stored at −20°C. We extracted DNA from the filters using a standard phenol–chloroform–isoamyl DNA extraction protocol (Renshaw *et al.*, 2015), with modifications described in Jeunen *et al.* (2019). DNA extracts were stored at −20°C until further processing.

Library preparation and sequencing

Library preparation followed the protocol described in Berry *et al.* (2017). We used three metabarcoding assays (Table 1) to describe the fish community [16S-Fish; targeting a region of the 16S rRNA gene (Berry *et al.*, 2017)], the crustacean community [16S-Crustacean; targeting a region of the 16S rRNA gene (Berry *et al.*, 2017)], and the eukaryotic community [COI-eukaryote; targeting a region of the cytochrome c oxidase subunit I gene (2013)].

Prior to library preparation, we measured DNA concentrations for each sample on a Qubit fluorometer (ThermoFisher

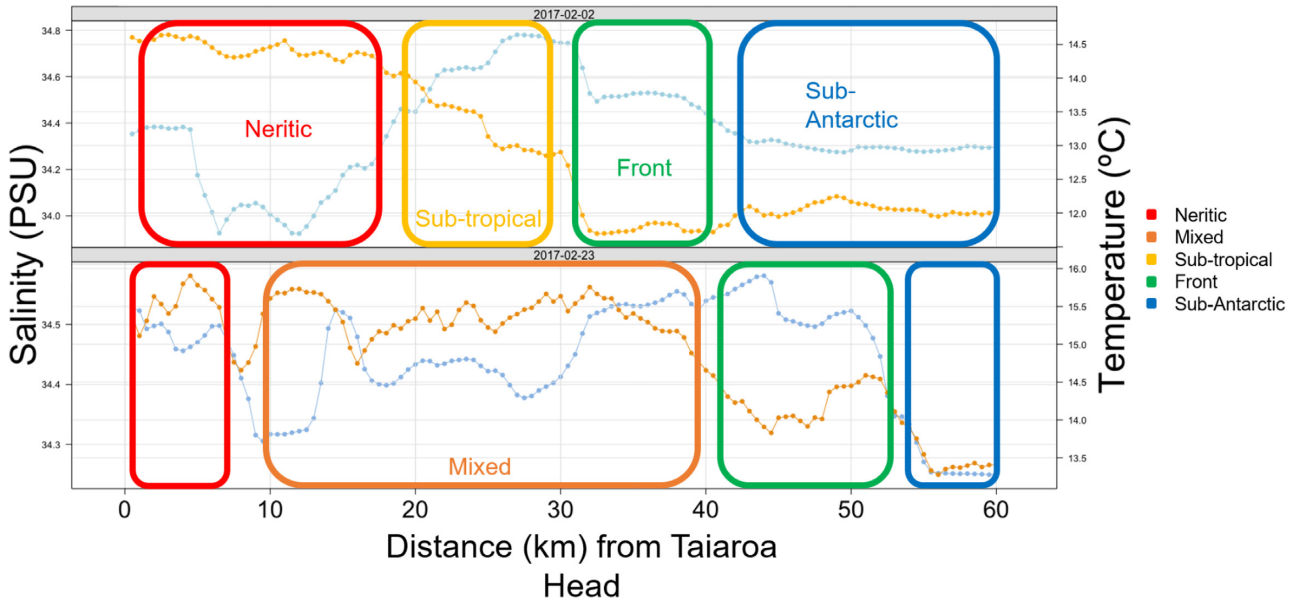

Figure 2. Salinity and temperature delineate water mass for each day along the Munida transect. Salinity (PSU) (in blue lines) and temperature (°C) (in orange lines) mapped as distance from shore during the two different sampling days. Each water mass is boxed according to water mass assignment; red is neritic, orange is mixed, yellow is sub-tropical, green is the Southland Front, and blue is sub-Antarctic. Mixed water is a mix between neritic and sub-tropical water. 2 February 2017 presents a more "typical" profile with low inshore salinity and lower temperatures further offshore, especially a drop at the front, whereas 23 February 2017 presents a more complex profile.

Table 1. Metabarcoding primers used to identify fish, crustacean, and metazoan organisms.

Metabarcoding assay	Primer set	Primer sequence	Length (bp)	Assay T_m (°C)	Reference
Fish (16S)	Fish16SF/D	5′-GACCCTATGGAGCTTTAGAC-3′	~200	54	Berry *et al.* (2017)
	16S2R	5′-CGCTGTTATCCCTADRGTAATC-3′			
Crustacean (16S)	Crust16S_F	5′-GGGACGATAAGACCCTATA-3′	~170	51	Berry *et al.* (2017)
	Crust16S_R	5′-ATTACGCTGTTATCCCTAAAG-3′			
Eukaryote (COI)	mlCOIintF	5′-GGWACWGGWTGAACWGTWTAYCCYCC-3′	~313	51	Leray *et al.* (2013)
	jgHCO2198	5′-TAIACYTCIGGRTGICCRAARAAYCA-3′			

Scientific), and input DNA for each sample was optimized using a dilution series (neat, 1/10, 1/20) to identify inhibitors and low-template samples (Murray *et al.*, 2015). Amplification was carried out in 25 μL reactions, prepared with 1× Taq Gold buffer (Applied Biosystems [ABI], USA), 2 mmol L^{-1} MgCl2 (ABI, USA), 0.4 mg mL^{-1} BSA (Fisher Biotec, Australia), 0.25 mmol L^{-1} dNTPs (Astral Scientific, Australia), 0.4 μmol L^{-1} of each primer (Integrated DNA Technologies, Australia), 0.6 μL of 1/10 000 SYBR Green dye (Life Technologies, USA), 1 U of Taq polymerase Gold (ABI, USA), and 2 μL of DNA. qPCR conditions included an initial denaturation step at 95°C for 5 min, followed by 50 cycles of 30 s at 95°C, 30 s at 51–54°C (see annealing temperatures in Table 1), 45 s at 72°C, and a final extension of 10 min at 72°C.

We used a one-step amplification protocol for library building using fusion primers, which contained a modified Illumina sequencing adapter, a barcode tag (6–8 bp in length) and the template-specific primer. We amplified each sample in duplicate and assigned a unique barcode combination to allow pooling of samples post-qPCR. Cycling conditions for qPCR followed the amplification protocol described above. We pooled qPCR duplicates of each sample together to reduce stochastic effects from PCR amplification. Then, we pooled samples to approximately equimolar concentrations based on end-point qPCR fluorescence and quantification on a LabChip GX Touch 24 (PerkinElmer, USA) to produce a single DNA library. We spiked negatives into the library to allow for optimal concentration of the library. We size-selected the resulting library using a Pippin Prep (Sage Science, USA) and purified with Qiagen's QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany) prior to final library quantitation on a LabChip and Qubit. We sequenced the library on an Illumina MiSeq® (300 cycle, single-end for both 16S assays; and 500 cycle, paired-end for the COI assay), following the manufacturer's protocols, and spiked with PhiX to minimize issues associated with low-complexity libraries.

Sequence analysis

We paired the COI-eukaryote sequencing reads with PEAR v 0.9.11 (Zhang *et al.*, 2014). The single-end 16S-Fish and 16S-Crustacean reads did not require pairing. We used *Obitools* v1.01.22 (Boyer *et al.*, 2016) to demultiplex reads with *ngsfil-*

ter allowing for only one error for matching primers and then used *obigrep* to perform length filtering. 16S-Crustacean reads were filtered for 155–175 bp, 16S-Fish reads were filtered for 190–230 bp, and COI reads were filtered for 290–320 bp. Samples were filtered for a maximum estimated error rate of 1 per 100 bases (maxEE = 1) and a minimum operational taxonomic unit (OTU) size of 10 with USEARCH 11.0.667 (Edgar, 2010). The USEARCH tool unoise3 was used to perform error-correcting denoising of all sequences and produce OTUs and built a frequency table by mapping reads of each sample to the OTUs (Edgar, 2010, 2016). OTU sequences and the OTU table were imported into Qiime2 v 2020.8 (Bolyen *et al.*, 2019) for taxonomy classification. We chose the curated Midori databases (version GB239) for assigning COI, 16S-Crustacean, and 16S-Fish sequences (Machida *et al.*, 2017; Leray *et al.*, 2018).

Taxonomy

To construct the database for each gene region, the Qiime-formatted UNIQ sequences for each gene were downloaded from the Midori Reference 2 mitochondrial database (version GB239), then the fasta and taxonomy files were imported into Qiime2 v 2020.8 (Leray *et al.*, 2018; Bolyen *et al.*, 2019). The extract-reads tool of the feature classifier plugin (Bokulich *et al.*, 2018) was first used to subset each unique haplotype around the primer sequences (once for COI, and separately for each primer set of lrRNA). After extracting, reads were dereplicated by taxon using the DerepByTaxonomy.py script of the Metacurator Software (Richardson *et al.*, 2020). For machine learning taxonomy classification, the sequences for each gene region were then trained using the data with the *fit-classifier-naïve-bayes* tool.

Statistics for community characterization

We analysed community composition and spatial distribution obtained from eDNA metabarcoding qualitative presence–absence (Jaccard) distances for species-level taxonomy datasets using the *phyloseq*, *vegan*, *ggplot2*, and *dplyr* packages for R v4.0.2 (R-project.org). While there is discussion about whether the signal obtained from metabarcoding datasets reflects true organismal abundance (Yates *et al.*, 2019), we attempted to minimize this distortion with a one-step amplification approach when building libraries and using Jaccard distance measurements for ordinating biodiversity (Sickel *et al.*, 2015; Kelly *et al.*, 2019). We characterized community differences using OTUs and genus-level taxonomic assignments with a PERMANOVA, followed by a test of multivariate dispersions (PERMDISP) to understand if significance from the PERMANOVA resulted from heterogeneity instead of location. In cases where heterogeneity of community was significant, i.e. the water mass's community composition spread from the centroid were unequal between communities, PCoA plots using binary Jaccard distance were made to visualize community dispersion. The PERMANOVA and PERMDISP tests were run to find differences in community composition between 2 February and 23 February, surface water communities according to water mass, and sub-Antarctic surface-depth comparison.

An indicator species analysis was run using the R package *indicspecies* for each sampling day (Cáceres *et al.*, 2020; Cáceres and Legendre, 2009). We used the *multipatt* "IndVal.g" function over 10 000 permutations, specifying water masses and not using combinations of water masses. *p*-values for species were adjusted with a Bonferroni correction.

Results

Sequencing results and taxonomic diversity

After quality filtering and taxonomic assignment, the final dataset yielded 11 957 208 reads and 1 262 OTUs for all molecular assays, comprising 4 659 418 reads and 33 OTUs for the fish (16S) assay; 3 093 889 reads and 19 OTUs for the crustacean (16S) assay; and 4 799 341 reads and 1211 OTUs for the Leray COI assay. Due to paucity of databases, OTUs were only assigned to genus level taxonomy, although species-level taxonomy is acknowledged in some cases. A total of 77 genera (genus-level taxonomy was assigned as databases do not always allow for species specificity in New Zealand) (Supplementary Table S1), covering 66 families and 14 different phyla were identified, including Arthropoda (16 genera, 20.8% of total), Chordata (20 genera, 26%, including 19 fishes), and Cnidaria (3 genera, 3.9%). The remaining organisms belonged to the phytoplankton, among which diatoms (7 genera, 9.1%) and dinoflagellates (11 genera, 14.3%) were the most dominant groups. Overall, across all genera, 58 were planktonic (phytoplankton, zooplanktons, or larvae, accounting for 75.3% of all genera), and 19 were active swimmers (fishes, 24.7%). The mean ± *SD* number of reads per sample was 132 858 ± 224 253, with a median of 66 083 reads per sample for the combined dataset. For the separate datasets, the COI dataset had a median of 54 176 reads per sample with mean ± *SD* of 53 326 ± 14 263 reads. The 16S crustacean dataset had a median of 8 837 reads per sample with mean ± *SD* of 37 730 ± 199 438 reads. The 16S fish dataset had a median of 20 852 reads per sample with a mean ± *SD* of 77 657 ± 143 938 reads. A final OTU table encompassing detections from all three assays was created, and used for all downstream analyses. A single read of *Bathycoccus prasinos*, a common picoplankton found in all but one non-negative sample, appeared in both negative controls.

Delineation of waters and the difference between voyages

We delineated the different water masses along the Munida transect for each day. Given the patterns of temperature and salinity for each time point on the first day, 2 February 2017, our clustering identified neritic, sub-tropical, Southland Front (front), and sub-Antarctic water (Supplementary Figure S1). Water mass assignments were, however, less certain during the second sampling date, 23 February 2017, leading us to consider stations two through six (inclusive) as a "mixed" water type, i.e. as a mix between neritic and sub-tropical water masses (Supplementary Figure S2). This mixed water mass was additionally supported by concentrations of nitrate (mmol m^{-3}) and chlorophyll a (mg m^{-3}), which showed little change at these stations until the front water boundary was reached (Supplementary Figures S3 and S4, respectively).

Temporal differences were examined by PERMANOVA, showing each voyage had different surface biodiversity overall (adonis: $F_{1,79} = 10.402$; $p < 0.001$), which was not due to heterogeneity within each biological community (permdisp betadisper: $F_{1,79} = 0.0099$, $p = 0.9212$). This was also supported

by a PCoA visualization, with the x-axis variation likely being driven by distance from shore and the y-axis variation likely being driven by sampling day (Figure 3). The percentage of variance explained by the x-axis was, however, much higher (35%) than the percentage of variance explained by the y-axis (6.7%), suggesting that the water mass structuration played a bigger role in explaining community variation than time in our data. The significance of the PERMANOVA across each day nevertheless caused us to analyse biological community structure separately for each day.

We analysed biological community structure for surface water masses separately for each voyage, 2 and 23 February 2017. Water mass communities were analysed at the level of individual OTUs and then again at the genus level.

2 February 2017 community biodiversity

The composition of biological communities varied according to water mass, following a shore to sub-Antarctic gradient. The differences between water masses were more pronounced at the OTU level than at the genus level. At the OTU level, a gradient of community structure could be seen from a PCoA ordination plot showing different water masses mostly grouping together (especially for the neritic communities close to shore, and the front communities and sub-Antarctic communities offshore, Figure 4). The main community gradient appeared between the two stations located at both ends of the sub-tropical waters, thus breaking this water mass in two distinct community types. This break also corresponded to the main gradient of nitrate concentrations (Supplementary Figure S3), and the end of the main chlorophyll a peak (Supplementary Figure S4). Water mass likely had a significant effect on community structure (adonis: $F_{3,39} = 37.53$, $p < 0.001$), although this difference could be attributed to heterogeneous dispersal within the community (betadisper: $F_{3,39} = 37.53$, $P < 0.0001$).

Although less distinct, communities assigned to genus level presented similar patterns (Figure 4, adonis: $F_{3,39} = 10.62$, $p < 0.001$; betadisper: $F_{3,39} = 5.39$, $p < 0.004$). Interestingly, the loss of community distinctiveness at the genus level resulted in the ambiguous positioning of the front communities across both the sub-tropical and sub-Antarctic communities.

23 February 2017 community biodiversity

A gradient of biological community structure across surface water masses in both OTU-level diversity and genus-level taxonomic diversity was also evident for the second sampling day. At the OTU level, the neritic water mass communities could be distinguished from the mixed water mass communities, of which the latter grouped together according to distance from coast (Figure 5). This time, the main community gradient was located between the mixed water communities and the group formed by the front and sub-Antarctic communities, which again coincided with the main gradient in nitrate concentrations (Supplementary Figure S3). A peak in chlorophyll a concentrations was recorded at the junction between the front and sub-Antarctic communities (Supplementary Figure S4), but it did not translate in the separation of these two water masses communities. As with our first sampling day, a difference between community was found (adonis: $F_{3,39} = 10.545$, $p < 0.001$), with dispersal heterogeneity (betadisper: $F_{3,39} = 51.211$, $p < 0.001$). Communities at the genus level showed similar—but less pronounced—patterns in community structure (Figure 5, adonis: $F_{3,39} = 12.886$; $p < 0.001$; betadisper: $F_{3,39} = 4.72$, $p < 0.01$). Although these genus-level patterns still mirrored those at OTU level, the gap between the neritic and mixed waters on the one side, and the front and sub-Antarctic waters on the other side, mostly disappeared. It thus rendered a more gradual community differentiation from the near-shore to offshore systems, without fully loosing the previously observed (i.e. during the first sampling day) water mass structuration. Overall, despite uncertain water mass assignment and disturbed marine conditions on the second sampling day (Supplementary Figure S2), the sampled communities seemed to still retain some spatial structure along the Munida transect.

Community variability at depth compared to surface waters

We found eDNA signal differences between sub-Antarctic surface water and mesopelagic water for both days. A PCoA showed little overlap of OTU diversity between surface and depth samples (Figure 6). Communities in sub-Antarctic surface water differed significantly from those in deep water at OTU (adonis: $F_{1,19} = 10.02$, $p < 0.001$; permdisp: $F_{1,19} = 35.704$, $p < 0.0001$) and genus levels (adonis: $F_{1,19} = 12.69$, $p < 0.001$; permdisp: $F_{1,19} = 43.51$, $p < 0.0001$), with no overlap between the two different water masses despite heterogenous biological community variance within water mass.

Thirty seven genera (48% of total) were recorded in the mesopelagic waters, the majority of which were also found in surface waters (32 genera). The strictly mesopelagic genera were assigned to an ostracoda (*Obtusoecia* spp.), hydrozoans (*Sphaeronectes* spp.), and fishes (the snaggletooth *Rhadinesthes* spp. and moki *Latridopsis* spp.). The remaining—and shared with the surface—genera mostly belonged to members of the phytoplankton and to a number of fish species (Supplementary Table S1).

Indicator species and taxa of interest

Most species identified as indicators of water mass were planktonic taxa, as targeted by the COI assay (Table 2). Only a few multicellular metazoans were identified as indicator species. One indicator species in the neritic water was a krill (*Nyctiphanes* spp.), while another krill (*Euphausia* spp.) were more indicative of sub-tropical waters (Brewin, 1951; Bartle, 1976; McClatchie *et al.*, 1991). Copepods (*Oithona* spp.) were detected in sub-tropical and mixed water masses (Jillett, 1976). On day two, the mantis shrimp (*Heterosquilla* spp.) was kept as an indicator species of the neritic community, while the medusa *Sphaeronectes* spp. was kept as an indicator species for at-depth samples (Table 2).

No fish species were identified as indicator species for any particular water mass. However, we did find evidence of fish taxa throughout the Munida transect (Table 3). Our assays detected species such as the porbeagle shark (*Lamna* spp.) and the ocean sunfish (*Mola* spp.), although their presence was only detected in a few replicates (one for porbeagle shark and two for ocean sunfish). Some mesopelagic sample and some sub-Antarctic surface water samples identified deep-sea taxa such as lanternfish (*Protomyctophum* spp., *Lampanyctodes* spp.), snaggletooth (*Rhadinesthes* spp.), and smooth-tongue (*Leuroglossus* spp.) fishes. The greatest number of fish reads were assigned to the slender tuna (*Allothunnus* spp.),

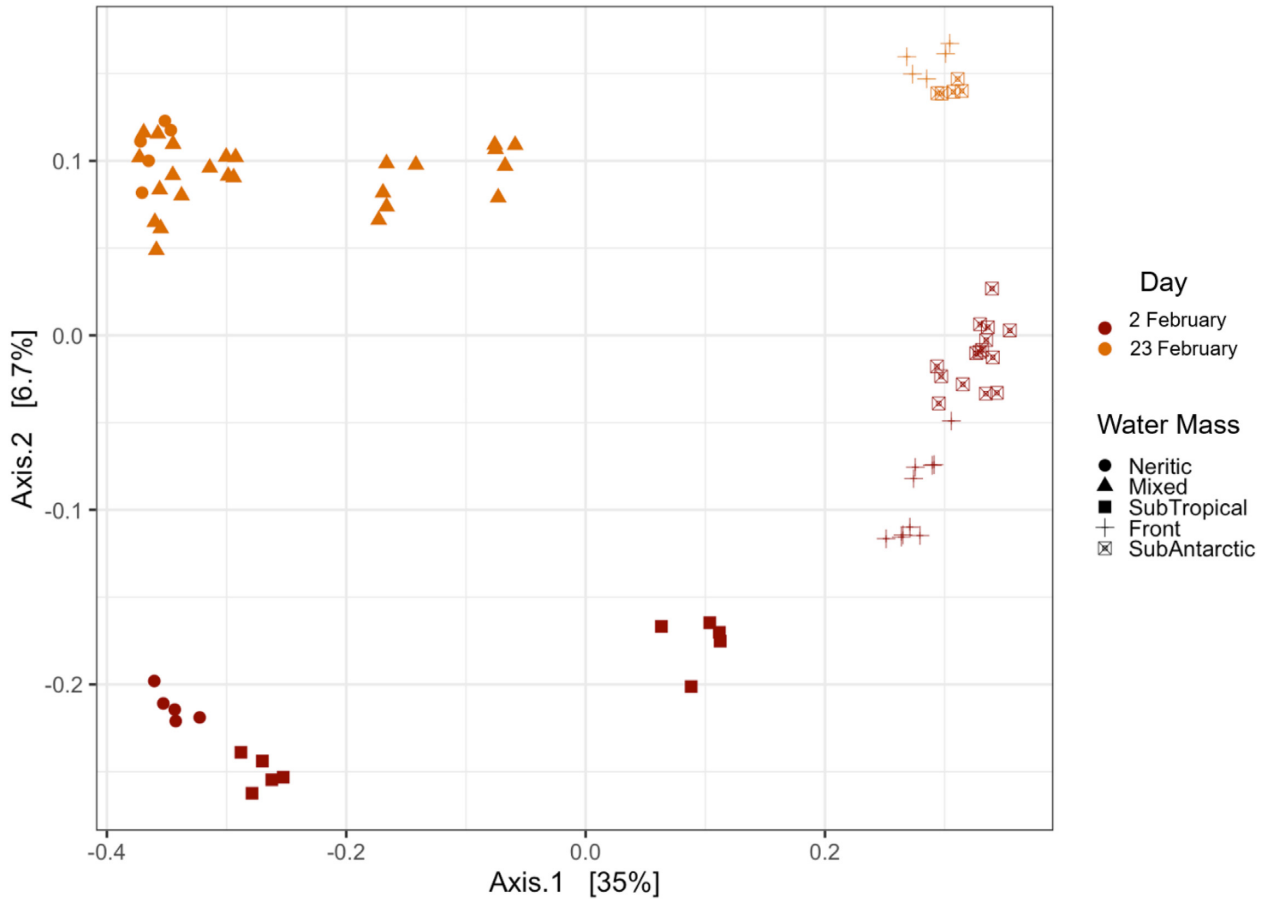

Figure 3. PCoA visualization showing the differences in community OTUs between sampling days. Sampling days were 2 and 23 February 2017 and points reflect replicate samples taken ($n = 5$) for each surface marine station ($n = 8$). Different colours indicate day, whereas different shapes indicate water mass.

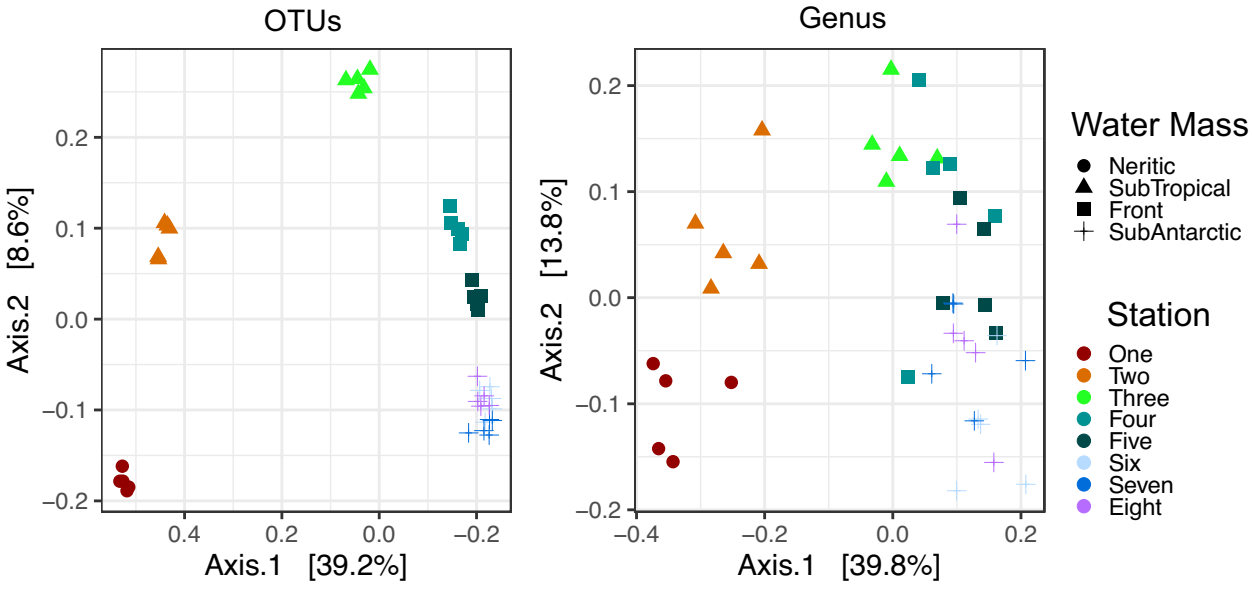

Figure 4. First sampling day community diversity plots. PCoA plots using Jaccard distance of OTU diversity (left) and genus-level diversity (right) by sample for the first day of sampling, 2 February 2017. Colours indicate station sampled whereas shape indicates water type. Water types generally group together.

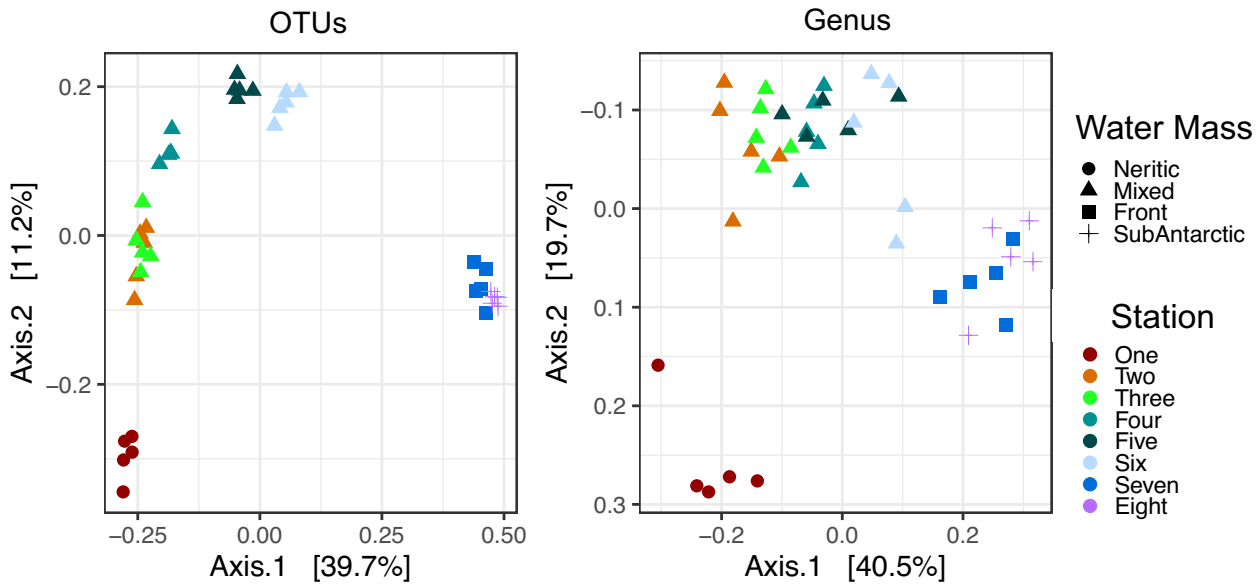

Figure 5. Second sampling day community diversity plots. PCoA plots using Jaccard distance of OTU diversity (left) and genus-level diversity (right) by sample for the second day of sampling, 23 February 2017. Colours indicate station sampled whereas shape indicates water type. Water types generally group together.

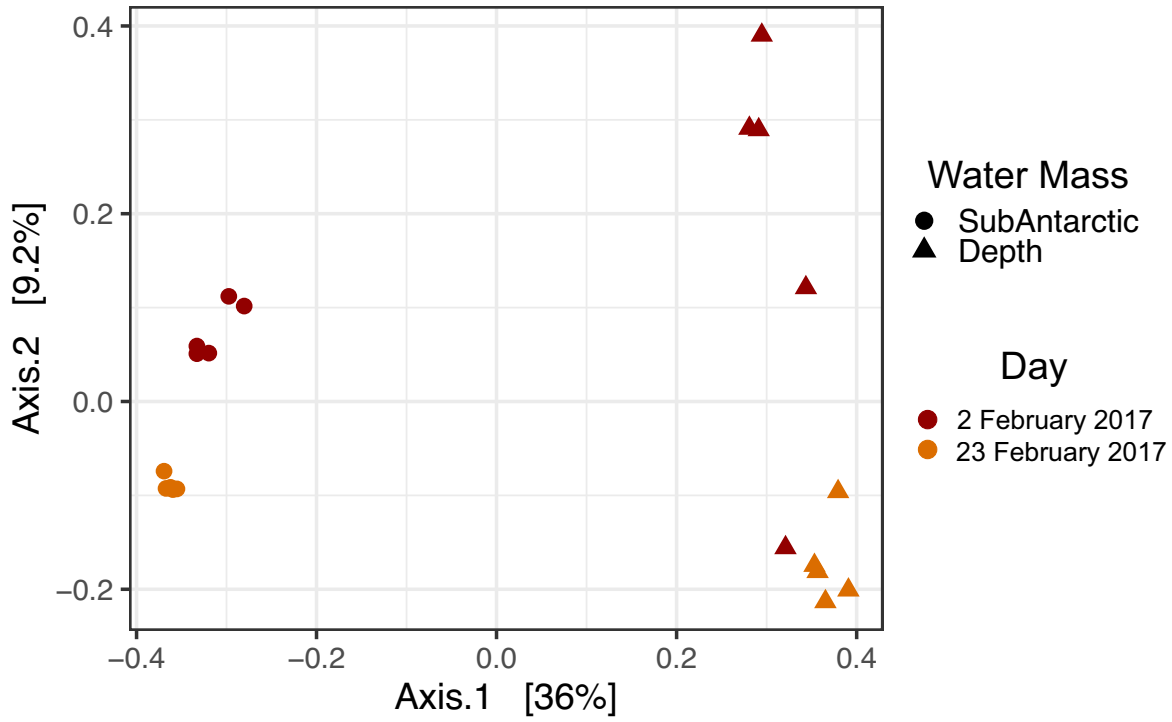

Figure 6. A PCoA plot of OTU-level diversity comparing sub-Antarctic surface and depth. The sub-Antarctic surface (circles) and mesopelagic depth (triangles) are shown disaggregated by sampling day, 2 February 2017 (light blue) and 23 February 2017 (purple).

which was found across all water masses on the first day (Table 3).

Discussion

Our spatial eDNA metabarcoding survey, making use of three eDNA assays, successfully detected different marine communities along the four successive water masses of the Munida transect, on two different dates. The multi-assay approach used in this study further allowed for the identification of multiple trophic levels at once, spanning from unicellular phytoplankton to large pelagic fish, which confirms the potential eDNA has to lift one of the major limitations of more traditional sampling techniques in the pelagic and coastal envi-

Table 2. Eukaryotic indicator species for each water type.

Day	Water type	Species	Common name (multicellular metazoans)	Adjusted *p*-value	Notes
Day 1	Neritic	*Micromonas pusilla*		0.0249	Costal water nanoflagellate (Thomsen and Buck, 1998; Not *et al.*, 2004)
		Pycnococcus provasolii		0.0083	Coastal seawater (Guillard *et al.*, 2004)
		Ascidia ahodori	Tunicate	0.0083	Larvae in neritic waters (Jillett, 1976)
		Heterocapsa rotundata		0.0083	Coastal estuaries (Ansotegui *et al.*, 2003)
		Dolichomastix tenuilepis		0.0083	Costal marine environments (Rockwell *et al.*, 2014)
		Cymbomonas tetramitiformis		0.0083	Coastal marine environments (Backe-Hansen and Throndsen, 2002)
		Rhodospirillaceae bacterium		0.0083	Coastal waters (Li *et al.*, 2011); neritic waters (Baltar *et al.*, 2016)
		Candidatus Pelagibacter sp.		0.0083	Coastal waters (Li *et al.*, 2011)
		Heterocapsa triquetra		0.0083	Neritic waters (Balkis, 2003)
		Mantoniella squamata		0.0083	Wide ranging, including Antarctic waters (Marchant *et al.*, 1989)
		Nyctiphanes australis	Krill	0.0332	Neritic krill (Jillett, 1976)
	Sub-tropical	*Oithona similis*	Copepod	0.0083	Usually neritic copepod (Jillett, 1976)
		Emiliania huxleyi		0.0498	Found in coastal waters (Rhodes *et al.*, 1995)
		Dictyocha speculum		0.0166	Coastal waters (Li *et al.*, 2011)
		Pseudochattonella farcimen		0.0083	Coastal waters (Naustvoll, 2010)
		Teleaulax amphioxeia		0.0166	Coastal waters (Yoo *et al.*, 2017)
		Pseudonitzschia bipertita		0.0083	Coastal waters (Teng *et al.*, 2016)
		Phaeocystis sp.		0.0166	Coastal estuaries (Ansotegui *et al.*, 2003)
		Skeletonema sp.		0.0083	Temperate coastal waters (Kooistra *et al.*, 2008)
		Euphausia lucens	Krill	0.0083	Oceanic krill (Bartle, 1976)
	Front	*Cylindrotheca closterium*		0.0083	Found in sub-Antarctic waters (Hoe Chang *et al.*, 2013)
	Sub-Antarctic	*Emiliania* sp.		0.0083	Found in sub-Antarctic waters (Rigual-Hernández *et al.*, 2020)
		Pseudonitzschia sp.		0.0083	Found in sub-Antarctic waters (Hoe Chang *et al.*, 2013)
		Phaeocystis antarctica		0.0083	Found in sub-Antarctic waters (Hoe Chang *et al.*, 2013), found in Front and sub-Antarctic waters previously in the Munida transect (Allen, 2019)
	Depth	*Hematodinium* sp.		0.0083	Parasitic dinoflagellate (Small *et al.*, 2012)
		Kareniaceae dinoflagellates		0.0083	Has been found at-depth but typically surface-dwelling (Cohen *et al.*, 2021)
Day 2	Neritic	*B. prasinos*		0.0072	Wide-ranging, but ususally at-depth (Eikrem and Throndsen, 1990)
		M. pusilla		0.0072	Costal water nanoflagellate (Thomsen and Buck, 1998; Not *et al.*, 2004)
		P. provasolii		0.0072	Coastal seawater (Guillard *et al.*, 2004)
		A. ahodori	Tunicate	0.0072	Larvae in neritic waters (Jillett, 1976)
		Phaeocystis globosa		0.0072	Coastal waters (Schapira *et al.*, 2008)
		N. australis	Krill	0.0072	Neritic krill (Jillett, 1976)
		Heterosquilla tricarinata	Mantis shrimp	0.0216	Neritic waters (Paavo *et al.*, 2012)

Table 2. Continued

Day	Water type	Species	Common name (multicellular metazoans)	Adjusted *p*-value	Notes
	Mixed	*O. similis*	Copepod	0.0072	Usually neritic copepod (Jillett, 1976)
		E. huxleyi		0.0072	Found in coastal waters (Rhodes *et al.*, 1995)
		Minutocellus polymorphus		0.0072	Everywhere but sub-Antarctic waters (Allen, 2019)
		D. speculum		0.0072	Coastal waters (Li *et al.*, 2011)
		P. farcimen		0.0072	Coastal waters (Eikrem *et al.*, 2009; Hoe Chang *et al.*, 2013)
		Paracalanus sp.	Copepod	0.0072	Subtropical waters (Robertson *et al.*, 1988)
		Heterocapsa sp.		0.0072	Coastal estuaries (Ansotegui *et al.*, 2003)
		Cymbomonas sp.		0.0072	Coastal marine environments (Backe-Hansen and Throndsen, 2002)
		Chaetoceros sp.		0.0216	Coastal, neritic waters (Balkis, 2003)
		C. Pelagibacter sp.		0.0072	Coastal waters (Li *et al.*, 2011)
	Front	*Emiliania* sp.		0.0072	Found in sub-Antarctic waters (Rigual-Hernández *et al.*, 2020)
		P. antarctica		0.0072	Found in sub-Antarctic waters (Hoe Chang *et al.*, 2013)
	Sub-Antarctic	none			
	Depth	*Sphaeronectes gracilis*	Nectophore	0.0072	Generally epipelagic (Palma and Silva, 2004)
		Hematodinium sp.		0.0072	Parasitic dinoflagellate (Small *et al.*, 2012)
		Gymnodinium sp.		0.0072	Generally found near coasts (Chang, 1996)

Taxa were constrained to fit into each water type. Each day was analysed separately.

Table 3. Fishes identified during the Munida transect, disaggregated by day and water type.

Scientific name ID'd from database	Likely taxa common name	2 February 2017					23 February 2017				
		Neritic ($n = 5$)	Sub-tropical ($n = 10$)	Front ($n = 10$)	Sub-Antarctic ($n = 15$)	Depth ($n = 5$)	Neritic ($n = 5$)	Mixed ($n = 25$)	Front ($n = 5$)	Sub-Antarctic ($n = 5$)	Depth ($n = 5$)
Allothunnus fallai	Slender tuna	100.00%	100.00%	40.00%	46.67%	20.00%		12.00%			
Thyrsites atun	Barracouta	40.00%	20.00%		13.33%	20.00%	20.00%	4.00%			
Mola mola	Ocean sunfish								40.00%		
Leuroglossus schmidti	Smoothtongue				13.33%	40.00%		4.00%		20.00%	
Rhadinesthes decimus	Slender snaggletooth										60.00%
Brama australis	Southern bream				20.00%	20.00%					
Notolabrus fucicola	Banded wrasse	20.00%				40.00%					
Pseudophycis barbata	Bastard cod			10.00%				4.00%			
Brama brama	Ray's bream					20.00%		4.00%			
Trachurus japonicus	Mackerel, not Japanese mackerel	60.00%									
Sprattus antipodum	Sprat, *S. mulleri* more common	40.00%									
Trachurus symmetricus	Mackerel, unclear which	40.00%									
Gnathophis bathytopos	Conger eel	20.00%	40.00%								
Seriolella brama	Blue warehou		10.00%	20.00%							
Lampanyctodes hectoris	Hector's lanternfish		10.00%		33.33%	20.00%		12.00%			
Forsterygion lapillum	Triplefin	60.00%	20.00%			20.00%					
Parapercis allporti	Likely blue cod (*P. colias*)		10.00%								
Lamna nasus	Porbeagle shark				6.67%						
Latridopsis forsteri	Moki, likely blue (common) or copper (rare)					60.00%					

Likely taxa indicates which species may have been present given taxonomic identification and ecology. Percentages indicate how many replicates from each water type came back positive for the day.

ronments (Gilbey *et al.*, 2021). Although our results only provide snapshots of biodiversity for each day, and do not allow for the robust exploration of the trophic links shaping the described communities, they still shed light on eDNA method adequacy for marine ecosystem monitoring and spatiotemporal community characterization in the oceans.

Surface level communities across water masses

Water mass specific ecological communities along the Munida transect have been described elsewhere, mainly for prokaryotic organisms [bacterioplankton, using 16S (Allen *et al.*, 2020; Bagnaro *et al.*, 2020)]. Here, we extend these findings to eukaryotic communities, and add to the growing body of literature demonstrating the ability of eDNA to discriminate spatial community patterns in the ocean at a relatively small scale [e.g. (Gelis *et al.*, 2021; García-Machado *et al.*, 2022; Larson *et al.*, 2022)]. Planktonic organisms (both phytoplankton and zooplankton) were the main drivers of community differentiation, reflecting their inability to swim and disperse easily across oceanic currents. As such, although some degree of dispersion across currents is possible (Ward *et al.*, 2021), water masses act as powerful barriers for these poorly mobile organisms, creating biogeographical niche effects [when several taxa respond similarly to common environmental conditions (Lima-Mendez *et al.*, 2015)], and effectively restricting community types to their cores. Heterogeneous dispersal inside community types, whereby successive samples in a given water mass have a high degree of compositional variability, was nevertheless observed (e.g. subtropical-associated community on day 1, Figure 4), and could be linked with sampling events near to water mass transitions. These areas indeed tend to present communities composed of taxa from both water masses (Bagnaro *et al.*, 2020).

Even when the distribution of the water masses was less clear during the second voyage on sampling day 23 February 2017 (Supplementary Figure S2), biological communities from our eDNA signal still separated according to water masses, with a clear break between the mixed water type and the front and sub-Antarctic waters, and a weaker break between the neritic and mixed water types. The appearance of mixed neritic and subtropical waters during summer in the area has been previously described (Hopkins *et al.*, 2010; Jones *et al.*, 2013), and linked with the occurrence of eddies, or the residual effects of weather conditions (e.g. winds, rain, riverine discharged) on surface water characteristics. Community composition between front and sub-Antarctic communities did not; however, seem to differ for day two, with no indicator species identified for the sub-Antarctic water mass. Microbial community differences across the front and sub-Antarctic water have been reported to be transient elsewhere (Allen *et al.*, 2020; Bagnaro *et al.*, 2020), with the two community types being often closely related—possibly as a consequence of the sub-Antarctic origin of the frontal waters (Sutton, 2003).

The main community gradients were, however, also coinciding with strong nitrate gradients [passing from the low concentrations typical of the near-shore environment to the relatively higher concentrations of the sub-Antarctic waters (Jones *et al.*, 2013)]. Nutrient gradients are strong planktonic community determinants in the global oceans—directly controlling microbial traits and functions—and are known to drive community differentiation over space (Song *et al.*, 2022). Recently, research on temperature control of the global biogeographic differentiation of algal microbiomes also found that a major breakpoint existed in the oceans around sea surface temperatures of 14°C [separating cold and warm upper ocean systems, see (Martin *et al.*, 2021)]. This temperature threshold was crossed by the Munida transect during our sampling, and we could thus be observing the larger impact of these gradient (nitrate, temperature) superimposed on the more local impact of water masses niche effect.

The ability of eDNA to capture taxonomic diversity a fine spatial scales is being increasingly recognized [e.g. (Closek *et al.*, 2019; Jo *et al.*, 2019; Jeunen *et al.*, 2020; West *et al.*, 2020; Gold *et al.*, 2021)], and gives weight to the interpretation of the spatial distribution of the organisms in this study. The fact that most of the recorded species did match their known distribution [for example, we confirm the previously reported *Ascidia* tunicate and *Nyctiphanes* krill presence as indicative of neritic waters (Jillett, 1976; Murdoch, 1989)] further strengthens the position of eDNA sampling as a spatially explicit species monitoring approach. In that scope, several plankton of interest could be detected, with potential value for the monitoring of toxic algal bloom [e.g. *Pseudochattonella* spp. (Mardones *et al.*, 2021); *Pseudo-nitzschia* spp. (Trainer *et al.*, 2012)], or for investigations on biogeochemical cycles [e.g. carbon and sulphur cycles, through occurrence of *Phaeocystis* spp. (Verity *et al.*, 2007)].

As previous studies demonstrated, multi-trophic sampling—making use of several assays—as the potential to reveal trophic links (Zhang *et al.*, 2020; Blackman *et al.*, 2022). We detected species of krill, e.g. *Euphausia* spp.—a important source of food for humpback whales [*Megaptera novaeangliae* (Findlay *et al.*, 2017)]—and *Nyctiphanes* spp., a known source of food for whales and muttonbirds [*Puffinus tenuirostris* (Cruz *et al.*, 2001)]. The co-occurrence of diatoms or other motile prey with copepods (e.g. *Oithona* spp.) was also commonly reported, which is consistent with known predator–prey interactions (Djeghri *et al.*, 2018). The sampling intensity of this study does not allow for the description of the dynamic (spatial or temporal) of these trophic interactions, but does strongly suggest that this type of investigation would be possible at the scale of oceanic transects, provided more repeated sampling.

More intensive sampling would also be required for the spatially explicit characterization of fish communities (and other large mobile metazoans) along the Munida transect. Although marked community structure was not expected for multicellular metazoans—due to their ability to swim across water masses—other eDNA studies have successfully reported the spatial structure of fish communities in coastal areas (Closek *et al.*, 2019; West *et al.*, 2020; Gelis *et al.*, 2021; Gold *et al.*, 2021; García-Machado *et al.*, 2022). These studies, however, relied on much larger sample sizes (i.e. from 130 to 284 samples), or were located much closer to shore, where eDNA retention might be higher (Gelis *et al.*, 2021; Gold *et al.*, 2021). Metazoan homogeneity has also been reported, e.g. across the southern coast of South Africa, where metazoan communities were not significantly different, but bacterial communities differed across the southern coast (Holman *et al.*, 2021). Samples in our study were further limited to 10 L of water at each station across all replicates, which likely did not contain sufficient eDNA of all animal taxa in the surrounding water mass, particularly if these organisms are mobile, transient, and in low densities. Increasing water volume generally

increases the number of species detected (Hunter *et al.*, 2019), but—for practical reasons—this needed to be weighed against the number of replicates taken. Furthermore, eDNA dilutes and degrades in marine water quickly (Jeunen *et al.*, 2018; Murakami *et al.*, 2019). Infrequent and inconsistent sampling may thus miss pelagic species that only occasionally transverse the Otago coast. For example, rare sightings of cetaceans have been reported off the coast of Otago, and although no sightings occurred during the sampling period (Hawke, 1989), their presence could be potentially detected in future water samples, in the context of longer-term monitoring programs. Seasonality also plays a large role in the pelagic community structure across the Munida transect [mostly characterized for planktonic bacteria, protists, and single-cell eukaryotic communities (Baltar *et al.*, 2016; Morales *et al.*, 2018; Allen *et al.*, 2020)], as water masses are highly variable throughout the year (Jones *et al.*, 2013; Morales *et al.*, 2018; Berry *et al.*, 2019). A more complete picture of metazoan community composition of the Munida transect, making use of eDNA, would likely need to include repetitive, seasonal sampling, including night-time sampling.

The presence of a number of fish species was nevertheless detected (e.g. slender tuna, *Allothunnus* spp., barracouta, *Thyrsites* spp., and bream, *Brama* spp.), which is promising in the context of future fish monitoring in the area. Increased sample size—similar to the sample sizes used in the studies cited above—would, however, be needed to propose a clearer view of fish community differentiation in the area. The position of the Munida Observational Time Series (MOTS) as the longest on-going oceanic transect in the Southern Hemisphere provides a remarkable opportunity to achieve such sampling effort.

Community differences between surface and depth

As expected, at-depth community composition differed from surface water community composition. Known mesopelagic fish species could be detected with eDNA signal, e.g. Snaggletooth (*Rhadinesthes* spp.) (Flynn and Pogonoski, 2012), lanternfish (*Lampanyctodes* spp.), and smoothtongue (*Leuroglossus* spp.). Surface-level eDNA detection for the last two species could indicate vertical migration (Easson *et al.*, 2020; Allan *et al.*, 2021), or faecal traces from predators samples (Isaacs *et al.*, 1974; Allan *et al.*, 2021). Typically, the vertical migration patterns have been documented, and these species have been found in the diet of predatory seals off the coast of Otago (Harcourt *et al.*, 2011; Robertson *et al.*, 1978).

Many planktonic organisms were detected in both surface and mesopelagic waters, and were thus a probable constituent of marine snow, or other forms of transfer of DNA from the surface to the deep (Allan *et al.*, 2021). Sinking particles are among the main contributors to downward carbon fluxes in the oceans, and thus to the strength of the biological carbon pump (Duret *et al.*, 2020). The presence of a large proportion of diatoms and other chlorophytes at-depth illustrates the carbon pump activity in the sampled area. Further sampling may help identify the main organisms driving this carbon sink processes, and subsequently improve the parameterization of carbon export models that integrate sinking particles (Durkin *et al.*, 2021).

Mesopelagic communities were also more variable than surface water samples (Jillett, 1969; Clayton *et al.*, 2017). The paucity of total eDNA from deep water could indicate limited biomass in the mesopelagic environment, yielding stochastic taxonomic results. This could also have been driven by slightly different sampling locations on either day (Figure 1), or by differences in sinking particles (Mestre *et al.*, 2018), as the compositional diversity of these particles can be high (Durkin *et al.*, 2021). Finally, we suspect false positives in at least three depth samples, despite clean negatives, from triplefin (*Forsterygion* spp.), slender tuna (*Allothunnus* spp.), and barracouta (*Thyrites* spp.) due to these only having one DNA sequence each in at-depth replicates for day one. We cannot rule out, however, their presence in surface waters. Their detection in the mesopelagic zone might thus be the consequence of eDNA transfer to the deep [e.g. predators eating and defecating prey, or DNA from dead organisms, in our eDNA samples (Miller, 2015; Guilfoyle and Schultz, 2017; Mestre *et al.*, 2018)]. These current limitations are likely to be mitigated with increased sampling effort, and highlight the need for repeated and more intense sampling across seasons, especially for understudied areas such as the deep seas.

Taxonomic resolution, database considerations, and missed taxa

Overall, OTU-level diversity was a better descriptor of each water mass than genus-level diversity. In some cases, OTUs may have differentiated species within the same genus, providing higher taxonomic resolution for taxa such as single-cell eukaryotes that may not have been sequenced previously. However, caution is warranted for interpreting these results as PCR error may artificially inflate OTUs (Patin *et al.*, 2013; Kelly *et al.*, 2019). Genus-level patterns, although less robust, were still evident [as reported elsewhere (Agogué *et al.*, 2011; Martiny *et al.*, 2015)]. This could suggest that members of the same genus might share general ecological strategies (Martiny *et al.*, 2015; Yeh *et al.*, 2019) leading to potential niche effects (Lima-Mendez *et al.*, 2015).

Like all eDNA metabarcoding studies, we rely on taxonomic databases for OTU identification. The paucity of reference sequences (e.g. 16S rRNA), especially for the understudied southern ocean, likely failed to identify some taxonomic diversity simply because it is unknown (Costello, 2015) or undocumented (Weigand *et al.*, 2019). Genetic sequences for every taxa for a particular assay may not have been sequenced, deposited, or well-curated, and assays may have different databases of sequences (Weigand *et al.*, 2019; Schenekar *et al.*, 2020). For example, blue cod (*Parapercis colias*) is a fish common to local waters, yet there was no 16S sequence available for this species in GenBank as of 18 January 2021. Instead, the similar yellow cod, *P. allporti*, was detected, but its distribution is in Australia, not the southern east coast of New Zealand (May and Maxwell, 1980). To help mitigate these issues, genus-level identification was used rather than species-level, but until these gaps in taxonomic databases are filled, we recommend eDNA tools be used in conjunction with traditional sampling methods (e.g. trawls, plankton nets, baited underwater video) to fully characterize community composition especially in understudied areas (Stat *et al.*, 2019).

Conclusions

eDNA signals were able to identify different biological communities within different water masses across the Munida transect, and between surface and mesopelagic waters at our

deep station. The Southland Front and sub-Antarctic water masses shared similar metazoan biological communities that could only be clearly distinguished at OTU level, highlighting the value of increased taxonomic resolution. Achieving satisfactory taxonomic resolution with eDNA is still challenging, given the lack of reference sequences for some taxonomic groups and geographical locations. However, the ability to sample in the same water drop the spatial coincidence of multiple trophic levels (from unicellular eukaryotes to large mobile metazoans, but also bacterioplanktons, if 16S assays are used), has tremendous potential to facilitate the study of the factors governing oceanic communities as whole ecosystems (biotic and abiotic), and the ongoing efforts at describing the architecture of the planktonic interactome (Lima-Mendez *et al.*, 2015; Bjorbækmo *et al.*, 2020). This is particularly important as models integrating multiple trophic levels tend to yield better results in the context of ecosystem dynamics (Priyadarshi *et al.*, 2022), and biogeochemical cycles (Hood *et al.*, 2006; Trull *et al.*, 2019) in our changing oceans.

The present study relied on limited sampling effort but laid the foundation for how eDNA might be used across long temporal datasets for the monitoring of pelagic biodiversity. Repeated sampling along the MOTS could quickly lead to sampling intensities matching previous studies that characterized fish communities with eDNA at small spatial scales. The unique position of the MOTS—crossing three water masses and a front off the Otago coastline—represents an unparalleled opportunity to enhance our understanding of the Southern Ocean.

Acknowledgements

Thank you very much to members of the Knapp laboratory, Gemmell laboratory, and the University of Otago Marine Science and Chemistry departments who facilitated sampling, seasickness recovery, and manuscript review. We thank Kim Currie for providing the MOTS platform, which allowed the samples to be collected. We thank the iwi whose land and water (moana) resources we sailed on and used, especially Te Rūnanga o Ōtākou and Te Rūnanga o Ngāi Tahu.

Supplementary Material

Supplementary material is available at the *ICESJMS* online version of the manuscript.

Conflict of Interest Statement

The authors declare that they have no conflict of interests.

Funding

This research was supported by the Sustainable Seas grant C01 × 1515 "Quantifying marine biodiversity using e-DNA" awarded to M.K. and N.J.G. and institutional funding from the University of Otago awarded to M.K. and N.J.G. C.I.M.A. was supported by the University of Otago Doctoral Scholarship.

Data Availability Statement

Native species are taonga (treasured) to Māori (the indigenous peoples of Aotearoa New Zealand). For Māori, the genetic material from taonga species has value, and Māori have kaitiakitanga (guardianship) over these species and their genetic data. Genomics Aotearoa, Aotearoa New Zealand's collaborative research platform for genomics and bioinformatics, is leading the co-development of inclusive approaches to data sovereignty for genetic and genomic data, including, but not limited to data storage, access, and ownership. These data will be made available on the recommendation of the iwi (tribes) and hapū (subtribes) that are the kaitiaki (guardians) of the Munida transect (Te Rūnanga o Ngāi Tahu, Te Rūnanga o Ōtākou). Code has been made available at https://github.com/Clare-eDNA/Munida. Data are made available through the Aotearoa Genomic Data Repository https://repo.data.nesi.org.nz/discovery/TAONGA-AGDR00030/ with Ben Te Aika as a current katiaki.

Ethics approval and consent to participate

Not applicable. Samples were water samples, which required no ethics approval.

Author Contributions

C.I.M.A. analysed the data—data curation and formal analysis, writing—original draft and review and editing along with visualization, and facilitated iwi consultation. G.J.J. performed the fieldwork and laboratory work (methodology). H.C. formed the databases and advised on analyses and software and provided supervision. H.R.T. advised on writing—review and editing and supervised. A.B. advised on data analysis, writing—review and editing, and software. C.H. supervised C.I.M.A. and A.B. K.C. provided field access, advised on analyses, and provided writing—review and editing. F.B., M.S., and M.B. helped with writing—review and editing. L.U. advised on indicator species analysis. M.K. and N.J.G. secured funding.

References

Adams, C. I. M., Hoekstra, L. A., Muell, M. R., and Janzen, F. J. 2019. A brief review of non-Avian reptile environmental DNA (eDNA), with a case study of painted turtle (*Chrysemys picta*) eDNA under field conditions. Diversity, 11: 50.

Afzali, S. F., Bourdages, H., Laporte, M., Mérot, C., Normandeau, E., Audet, C., and 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.

Agogué, H., Lamy, D., Neal, P. R., Sogin, M. L., and Herndl, G. J. 2011. Water mass-specificity of bacterial communities in the North Atlantic revealed by massively parallel sequencing. Molecular Ecology, 20: 258–274.

Allan, E. A., DiBenedetto, M. H., Lavery, A. C., Govindarajan, A. F., and Zhang, W. G. 2021. Modeling characterization of the vertical and temporal variability of environmental DNA in the mesopelagic ocean. Scientific Reports, 11: 21273.

Allan, E. A., Zhang, W. G., Lavery, A. C., and Govindarajan, A. F. 2021. Environmental DNA shedding and decay rates from diverse animal forms and thermal regimes. Environmental DNA, 3: 492–514.

Allen, R. 2019. Marine microbial community dynamics and responses to ocean acidification. PhD Thesis, University of Otago, Dunedin. https://ourarchive.otago.ac.nz/handle/10523/9591 (last accessed 10 February 2023).

Allen, R., Summerfield, T. C., Currie, K., Dillingham, P. W., and Hoffmann, L. J. 2020. Distinct processes structure bacterioplankton and

protist communities across an oceanic front. Aquatic Microbial Ecology, 85: 19–34.
Ansotegui, A., Sarobe, A., Trigueros, J. M., Urrutxurtu, I., and Orive, E. 2003. Size distribution of algal pigments and phytoplankton assemblages in a coastal—Estuarine Environment: contribution of small eukaryotic algae. Journal of Plankton Research, 25: 341–355.
Backe-Hansen, P., and Throndsen, J. 2002. Pico- and nanoplankton from the inner Oslofjord, eastern Norway, including description of two new species of Luffisphaera (incerta sedis). Sarsia, 87: 55–64.
Bagnaro, A. 2021. EcotoneFinder: Characterising and Locating Ecotones and Communities. https://CRAN.R-project.org/package=EcotoneFinder (last accessed 16 February 2021).
Bagnaro, A., Baltar, F., Brownstein, G., Lee, W. G., Morales, S. E., Pritchard, D. W., and Hepburn, C. D. 2020. Reducing the arbitrary: fuzzy detection of microbial ecotones and ecosystems – focus on the pelagic environment. Environmental Microbiome, 15: 1–14.
Bálint, M., Pfenninger, M., Grossart, H.-P., Taberlet, P., Vellend, M., Leibold, M. A., Englund, G., *et al.* 2018. Environmental DNA time series in ecology. Trends in Ecology and Evolution, 33: 945–957.
Balkis, N. 2003. Seasonal variations in the phytoplankton and nutrient dynamics in the neritic water of Büyükçekmece Bay, Sea of Marmara. Journal of Plankton Research, 25: 703–717.
Baltar, F., Currie, K., Stuck, E., Roosa, S., and Morales, S. E. 2016. Oceanic fronts: transition zones for bacterioplankton community composition. Environmental Microbiology Reports, 8: 132–138.
Baltar, F., Stuck, E., Morales, S., and Currie, K. 2015. Bacterioplankton carbon cycling along the Subtropical Frontal Zone off New Zealand. Progress in Oceanography, 135: 168–175.
Bartle, J. A. 1976. Euphausiids of cook strait: a transitional fauna? New Zealand Journal of Marine and Freshwater Research, 10: 559–576.
Bates, N. R., Astor, Y. M., Church, M. J., Currie, K., Dore, J. E., González-Dávila, M., Lorenzoni, L., *et al.* 2014. A time-series view of changing surface ocean chemistry due to ocean uptake of anthropogenic CO_2 and ocean acidification. Oceanography, 27: 126–141.
Berry, T. E., Osterrieder, S. K., Murray, D. C., Coghlan, M. L., Richardson, A. J., Grealy, A. K., Stat, M., *et al.* 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.
Berry, T. E., Saunders, B. J., Coghlan, M. L., Stat, M., Jarman, S., Richardson, A. J., Davies, C. H., *et al.* 2019. Marine environmental DNA biomonitoring reveals seasonal patterns in biodiversity and identifies ecosystem responses to anomalous climatic events. PLoS Genetics, 15: e1007943.
Bjorbækmo, M. F. M., Evenstad, A., Røsæg, L. L., Krabberød, A. K., and Logares, R. 2020. The planktonic protist interactome: where do we stand after a century of research? The ISME Journal, 14: 544–559.
Blackman, R. C., Ho, H-C., Walser, J-C., and Altermatt, F. 2022. Spatiotemporal patterns of multi-trophic biodiversity and food-web characteristics uncovered across a river catchment using environmental DNA. Communications Biology, 5: 259.
Bokulich, N. A., Kaehler, B. D., Rideout, J. R., Dillon, M., Bolyen, E., Knight, R., Huttley, G. A., *et al.* 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome, 6: 90.
Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., *et al.* 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology, 37: 852–857.
Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P., and Coissac, E. 2016. obitools: a unix-inspired software package for DNA metabarcoding. Molecular Ecology Resources, 16: 176–182.
Bozec, Y-M., Kulbicki, M., Laloë, F., Mou-Tham, G., and Gascuel, D. 2011. Factors affecting the detection distances of reef fish: implications for visual counts. Marine Biology, 158: 969–981.
Brewin, B. 1951. Seasonal changes in micro-plankton in the Otago Harbour during the years 1944 and 1945. Transactions and Proceedings of the Royal Society of New Zealand, 79: 614–627.
Buxton, C. D., and Smale, M. 1989. Abundance and distribution patterns of three temperate reef fishes (Teleostei: Sparidae) in exploited and unexploited areas off the southern Cape coast. Journal of Applied Ecology, 26: 441–451.
Cáceres, M. D., and Legendre, P. 2009. Associations between species and groups of sites: indices and statistical inference. Ecology, 90: 3566–3574.
Cáceres, M., Jansen, F., and Dell, N. 2020. *Package "indicspecies"* (1.7.9). http://sites.google.com/site/miqueldecaceres/ (last accessed 6 March 2022).
Chang, F. H. 1996. A review of knowledge of a group of closely related, economically important toxic gymnodinium/gyrodinium (Dinophyceae) species in New Zealand. Journal of the Royal Society of New Zealand, 26: 381–394.
Chen, I-C., Hill, J. K., Ohlemüller, R., Roy, D. B., and Thomas, C. D. 2011. Rapid range shifts of species associated with high levels of climate warming. Science, 333: 1024 LP–1026.
Clayton, S., Lin, Y-C., Follows, M. J., and Worden, A. Z. 2017. Co-existence of distinct ostreococcus ecotypes at an oceanic front. Limnology and Oceanography, 62: 75–88.
Closek, C. J., Santora, J. A., Starks, H. A., Schroeder, I. D., Andruszkiewicz, E. A., Sakuma, K. M., Bograd, S. J., *et al.* 2019. Marine vertebrate biodiversity and distribution within the Central California current using environmental DNA (eDNA) metabarcoding and ecosystem surveys. Frontiers in Marine Science, 6: 732–732.
Cohen, N. R., McIlvin, M. R., Moran, D. M., Held, N. A., Saunders, J. K., Hawco, N. J., Brosnahan, M., *et al.* 2021. Dinoflagellates alter their carbon and nutrient metabolic strategies across environmental gradients in the central Pacific Ocean. Nature Microbiology, 6: 173–186 .
Costello, M. J. 2015. Biodiversity: the known, unknown, and rates of extinction. Current Biology, 25: R368–R371.
Croot, P. L., and Hunter, K. A. 1998. Trace metal distributions across the continental shelf near Otago Peninsula, New Zealand. Marine Chemistry, 62: 185–201.
Cruz, J. B., Lalas, C., Jillett, J. B., Kitson, J. C., Lyver, P. O., Imber, M., Newman, J. E., *et al.* 2001. Prey spectrum of breeding sooty shearwaters (*Puffinus griseus*) in New Zealand. New Zealand Journal of Marine and Freshwater Research, 35: 817–829.
Currie, K. I., and Hunter, K. A. 1999. Seasonal variation of surface water CO_2 partial pressure in the Southland Current, east of New Zealand. Marine and Freshwater Research, 50: 375–382.
Currie, K. I., Reid, M. R., and Hunter, K. A. 2011. Interannual variability of carbon dioxide drawdown by subAntarctic surface water near New Zealand. Biogeochemistry, 104: 23–34.
Djeghri, N., Atkinson, A., Fileman, E. S., Harmer, R. A., Widdicombe, C. E., McEvoy, A. J., Cornwell, L., *et al.* 2018. High prey-predator size ratios and unselective feeding in copepods: a seasonal comparison of five species with contrasting feeding modes. Progress in Oceanography, 165: 63–74.
Djurhuus, A., Closek, C. J., Kelly, R. P., Pitz, K. J., Michisaki, R. P., Starks, H. A., Walz, K. R., *et al.* 2020. Environmental DNA reveals seasonal shifts and potential interactions in a marine community. Nature Communications, 11: 254–254.
Donelson, J. M., Sunday, J. M., Figueira, W. F., Gaitán-Espitia, J. D., Hobday, A. J., Johnson, C. R., Leis, J. M., *et al.* 2019. Understanding interactions between plasticity, adaptation and range shifts in response to marine environmental change. Philosophical Transactions of the Royal Society B: Biological Sciences, 374: 20180186.
Duret, M. T., Lampitt, R. S., and Lam, P. 2020. Eukaryotic influence on the oceanic biological carbon pump in the Scotia Sea as revealed by 18S rRNA gene sequencing of suspended and sinking particles. Limnology and Oceanography, 65: S49–S70.

Durkin, C. A., Buesseler, K. O., Cetinić, I., Estapa, M. L., Kelly, R. P., and Omand, M. 2021. A visual tour of carbon export by sinking particles. Global Biogeochemical Cycles, 35: e2021GB006985.

Easson, C. G., Boswell, K. M., Tucker, N., Warren, J. D., and Lopez, J. V. 2020. Combined eDNA and acoustic analysis reflects diel vertical migration of mixed consortia in the Gulf of Mexico. Frontiers in Marine Science, 7: 552.

Edgar, G. J., Barrett, N. S., and Morton, A. J. 2004. Biases associated with the use of underwater visual census techniques to quantify the density and size-structure of fish populations. Journal of Experimental Marine Biology and Ecology, 308: 269–290.

Edgar, R. C. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics, 26: 2460–2461.

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

Eikrem, W., Edvardsen, B., and Throndsen, J. 2009. Research note: renaming Verrucophora farcimen Eikrem, Edvardsen et Throndsen. Phycological Research, 57: 170–170.

Eikrem, W., and Throndsen, J. 1990. The ultrastructure of *Bathycoccus gen.* nov. and *B. prasinos* sp. nov., a non-motile picoplanktonic alga (Chlorophyta, Prasinophyceae) from the Mediterranean and Atlantic. Phycologia, 29: 344–350.

Evans, N. T., Shirey, P. D., Wieringa, J. G., Mahon, A. R., and Lamberti, G. A. 2017. Comparative cost and effort of fish distribution detection via environmental DNA analysis and electrofishing. Fisheries, 42: 90–99.

Fediajevaite, J., Priestley, V., Arnold, R., and Savolainen, V. 2021. Meta-analysis shows that environmental DNA outperforms traditional surveys, but warrants better reporting standards. Ecology and Evolution, 11: 4803–4815.

Findlay, K. P., Seakamela, S. M., Meÿer, M. A., Kirkman, S. P., Barendse, J., Cade, D. E., Hurwitz, D., *et al.* 2017. Humpback whale "super-groups"—A novel low-latitude feeding behaviour of Southern Hemisphere humpback whales (*Megaptera novaeangliae*) in the Benguela Upwelling System. PLoS ONE, 12: e0172002.

Flynn, A., and Pogonoski, J. J. 2012. Guide to Mesopelagic Fishes of the Southern Tasman Sea. CSIRO Marine and Atmospheric Research, Hobart. http://stors.tas.gov.au/989883.

García-Machado, E., Laporte, M., Normandeau, E., Hernández, C., Côté, G., Paradis, Y., Mingelbier, M., *et al.* 2022. Fish community shifts along a strong fluvial environmental gradient revealed by eDNA metabarcoding. Environmental DNA, 4: 117–134.

Gelis, E. R. E., Kamal, M. M., Subhan, B., Bachtiar, I., Sani, L. M. I., and Madduppa, H. 2021. Environmental biomonitoring of reef fish community structure with eDNA metabarcoding in the Coral Triangle. Environmental Biology of Fishes, 104: 887–903.

Gilbey, J., Carvalho, G., Castilho, R., Coscia, I., Coulson, M. W., Dahle, G., Derycke, S., *et al.* 2021. Life in a drop: sampling environmental DNA for marine fishery management and ecosystem monitoring. Marine Policy, 124: 104331.

Gold, Z., Sprague, J., Kushner, D. J., Marin, E. Z., and Barber, P. H. 2021. EDNA metabarcoding as a biomonitoring tool for marine protected areas. PLoS One, 16: e0238557.

Goldberg, C. S., Turner, C. R., Deiner, K., Klymus, K. E., Thomsen, P. F., Murphy, M. A., Spear, S. F., *et al.* 2016. Critical considerations for the application of environmental DNA methods to detect aquatic species. Methods in Ecology and Evolution, 7: 1299–1307.

Guilfoyle, M. P., and Schultz, M. T. 2017. The contribution of double-crested cormorants (*Phalacrocorax auritus*) to silver carp (*Hypophthalmichthys molitrix*) DNA loads in the Chicago Area Waterway System. Journal of Great Lakes Research, 43: 1181–1185.

Guillard, R., Keller, M., O'Kelly, C., and Floyd, G. 2004. *Pycnococcus provasolii gen. *et sp. nov., a coccoid prasinoxanthin-containing phytoplankter from the Western North Atlantic and Gulf of Mexico. Journal of Phycology, 27: 39–47.

Harcourt, R. G., Schulman, A. M., Davis, L. S., and Trillmich, F. 2011. Summer foraging by lactating female New Zealand fur seals (*Arctocephalus forsteri*) off Otago Peninsula, New Zealand. Canadian Journal of Zoology, 73: 678–690.

Harmelin-Vivien, M. L., and Francour, P. 1992. Trawling or visual censuses? Methodological bias in the assessment of fish populations in seagrass beds. Marine Ecology, 13: 41–51.

Hawke, D. J. 1989. Dusky dolphins *Lagenorhynchus obscurus* on the continental shelf near Otago peninsula, south-east New Zealand. New Zealand Natural Sciences, 16: 113–116.

Hoe Chang, F., Williams, M. J. M., Schwarz, J. N., Hall, J. A., Maas, E. W., and Stewart, R. 2013. Spatial variation of phytoplankton assemblages and biomass in the New Zealand sector of the Southern Ocean during the late austral summer 2008. Polar Biology, 36: 391–408.

Holman, L. E., de Bruyn, M., Creer, S., Carvalho, G., Robidart, J., and Rius, M. 2021. Animals, protists and bacteria share marine biogeographic patterns. Nature Ecology & Evolution, 5: 738–746.

Hood, R. R., Laws, E. A., Armstrong, R. A., Bates, N. R., Brown, C. W., Carlson, C. A., Chai, F., *et al.* 2006. Pelagic functional group modeling: progress, challenges and prospects. Deep Sea Research Part II: Topical Studies in Oceanography, 53: 459–512.

Hopkins, J., Shaw, A. G. P., and Challenor, P. 2010. The Southland Front, New Zealand: variability and ENSO correlations. Continental Shelf Research, 30: 1535–1548.

Hunter, M. E., Ferrante, J. A., Meigs-Friend, G., and Ulmer, A. 2019. Improving eDNA yield and inhibitor reduction through increased water volumes and multi-filter isolation techniques. Scientific Reports, 9: 5259–5259.

Isaacs, J. D., Tont, S. A., and Wick, G. L. 1974. Deep scattering layers: vertical migration as a tactic for finding food. Deep Sea Research, 21: 651–656.

Jerde, C. L. 2021. Can we manage fisheries with the inherent uncertainty from eDNA? Journal of Fish Biology, 98: 341–353.

Jeunen, G-J., Knapp, M., Spencer, H. G., Lamare, M. D., Taylor, H. R., Stat, M., Bunce, M., *et al.* 2018. Environmental DNA (eDNA) metabarcoding reveals strong discrimination among diverse marine habitats connected by water movement. Molecular Ecology Resources, 19: 426–438,

Jeunen, G-J., Knapp, M., Spencer, H. G., Taylor, H. R., Lamare, M. D., Stat, M., Bunce, M., *et al.* 2019. Species-level biodiversity assessment using marine environmental DNA metabarcoding requires protocol optimization and standardization. Ecology and Evolution, 9: 1323–1335.

Jeunen, G-J., Lamare, M. D., Knapp, M., Spencer, H. G., Taylor, H. R., Stat, M., Bunce, M., *et al.* 2020. Water stratification in the marine biome restricts vertical environmental DNA (eDNA) signal dispersal. Environmental DNA, 2: 99–111.

Jillett, J. B. 1969. Seasonal hydrology of waters off the Otago peninsula, south-eastern New Zealand. New Zealand Journal of Marine and Freshwater Research, 3: 349–375.

Jillett, J. B. 1976. Zooplankton associations off Otago Peninsula, south-eastern New Zealand, related to different water masses. New Zealand Journal of Marine and Freshwater Research, 10: 543–557.

Jo, H., Kim, D-K., Park, K., and Kwak, I-S. 2019. Discrimination of spatial distribution of aquatic organisms in a coastal ecosystem using eDNA. Applied Sciences, 9: Article 17.

Jones, K. N., Currie, K. I., McGraw, C. M., and Hunter, K. A. 2013. The effect of coastal processes on phytoplankton biomass and primary production within the near-shore Subtropical Frontal Zone. Estuarine, Coastal and Shelf Science, 124: 44–55.

Kavehei, A., Gore, D. B., Chariton, A. A., and Hose, G. C. 2021. Impact assessment of ephemeral discharge of contamination downstream of two legacy base metal mines using environmental DNA. Journal of Hazardous Materials, 419: 126483.

Kelly, R. P., Shelton, A. O., and Gallego, R. 2019. Understanding PCR processes to draw meaningful conclusions from environmental DNA studies. Scientific Reports, 9: 12133.

Kooistra, W. H. C. F., Sarno, D., Balzano, S., Gu, H., Andersen, R. A., and Zingone, A. 2008. Global diversity and biogeography of Skeletonema species (Bacillariophyta). Protist, 159: 177–193.

Langlois, T. J., Newman, S. J., Cappo, M., Harvey, E. S., Rome, B. M., Skepper, C. L., and Wakefield, C. B. 2015. Length selectivity of commercial fish traps assessed from in situ comparisons with stereo-video: is there evidence of sampling bias? Fisheries Research, 161: 145–155.

Larson, W. A., Barry, P., Dokai, W., Maselko, J., Olson, J., and Baetscher, D. 2022. Leveraging eDNA metabarcoding to characterize nearshore fish communities in Southeast Alaska: do habitat and tide matter? Environmental DNA, 4: 868–880.

Leray, M., Ho, S-L., Lin, I-J., and Machida, R. J. 2018. MIDORI server: a webserver for taxonomic assignment of unknown metazoan mitochondrial-encoded sequences using a curated database. Bioinformatics, 34: 3753–3754.

Leray, M., Yang, J. Y., Meyer, C. P., Mills, S. C., Agudelo, N., Ranwez, V., Boehm, J. T., *et al.* 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: 34–34.

Li, W. K. W., Andersen, R. A., Gifford, D. J., Incze, L. S., Martin, J. L., Pilskaln, C. H., Rooney-Varga, J. N., *et al.* 2011. Planktonic microbes in the Gulf of Maine area. PLoS One, 6: e20981.

Lima-Mendez, G., Faust, K., Henry, N., Decelle, J., Colin, S., Carcillo, F., Chaffron, S., *et al.* 2015. Determinants of community structure in the global plankton interactome. Science, 348: 1262073.

Lindfield, S. J., Harvey, E. S., McIlwain, J. L., and Halford, A. R. 2014. Silent fish surveys: bubble-free diving highlights inaccuracies associated with SCUBA-based surveys in heavily fished areas. Methods in Ecology and Evolution, 5: 1061–1069.

Machida, R. J., Leray, M., Ho, S-L., and Knowlton, N. 2017. Metazoan mitochondrial gene sequence reference datasets for taxonomic assignment of environmental samples. Scientific Data, 4: 170027.

Marchant, H. J., Buck, K. R., Garrison, D. L., and Thomsen, H. A. 1989. Mantoniella in antarctic waters, including the description of *M. antarctica *sp. nov. Journal of Phycology, 25: 167–174.

Mardones, J. I., Paredes, J., Godoy, M., Suarez, R., Norambuena, L., Vargas, V., Fuenzalida, G. *et al.* 2021. Disentangling the environmental processes responsible for the world's largest farmed fish-killing harmful algal bloom: Chile, 2016. Science of The Total Environment, 766: 144383.

Marshall, N. T., and Stepien, C. A. 2019. Invasion genetics from eDNA and thousands of larvae: a targeted metabarcoding assay that distinguishes species and population variation of zebra and quagga mussels. Ecology and Evolution, 9: 3515–3538.

Martin, K., Schmidt, K., Toseland, A., Boulton, C. A., Barry, K., Beszteri, B., Brussaard, C. P. D., *et al.* 2021. The biogeographic differentiation of algal microbiomes in the upper ocean from pole to pole. Nature Communications, 12: 5483.

Martiny, J. B. H., Jones, S. E., Lennon, J. T., and Martiny, A. C. 2015. Microbiomes in light of traits: a phylogenetic perspective. Science, 350: aac9323.

Mason, D. H., Dysthe, J. C., Franklin, T. W., Williams, C. L., Young, M. K., McKelvey, K. S., and Schwartz, M. K. 2021. Certain detection of uncertain taxa: EDNA detection of a cryptic mountain sucker (*Pantosteus jordani*) in the Upper Missouri River, USA. Environmental DNA, 3: 449–457,

May, J. L., and Maxwell, J. G. H. 1980. Field Guide to Trawl Fish From the Temperate Waters of Australia, 8, CSIRO Division of Fisheries Research, Hobart, 201pp. https://www.frdc.com.au/sites/default/files/products/1981-013-DLD.pdf.

McClanahan, T. R., and Mangi, S. C. 2004. Gear-based management of a tropical artisanal fishery based on species selectivity and capture size. Fisheries Management and Ecology, 11: 51–60.

McClatchie, S., Jaquiery, P., Kawachi, R., and Pilditch, C. 1991. Grazing rates of *Nyctiphanes australis* (Euphausiacea) in the laboratory and Otago Harbour, New Zealand, measured using three independent methods. Continental Shelf Research, 11: 1–22.

Mestre, M., Ruiz-González, C., Logares, R., Duarte, C. M., Gasol, J. M., and Sala, M. M. 2018. Sinking particles promote vertical connectivity in the ocean microbiome. Proceedings of the National Academy of Sciences, 115: E6799–E6807.

Miller, E. J. 2015. Ecology of Hector's dolphin (Cephalorhynchus hectori): quantifying diet and investigating habitat selection at Banks Peninsula, PhD Thesis, University of Otago, Duendin, Otago, New Zealand. https://ourarchive.otago.ac.nz/handle/10523/5490.

Morales, S. E., Meyer, M., Currie, K., and Baltar, F. 2018. Are oceanic fronts ecotones? Seasonal changes along the subtropical front show fronts as bacterioplankton transition zones but not diversity hotspots. Environmental Microbiology Reports, 10: 184–189.

Murakami, H., Yoon, S., Kasai, A., Minamoto, T., Yamamoto, S., Sakata, M. K., Horiuchi, T., *et al.* 2019. Dispersion and degradation of environmental DNA from caged fish in a marine environment. Fisheries Science, 85: 327–337.

Murdoch, R. C. 1989. The effects of a headland eddy on surface macrozooplankton assemblages North of Otago Peninsula, New Zealand. Estuarine, Coastal and Shelf Science, 29: 361–383.

Murray, D. C., Coghlan, M. L., and Bunce, M. 2015. From benchtop to desktop: important considerations when designing amplicon sequencing workflows. PLoS One, 10: e0124671.

Naustvoll, L-J. 2010. Alien Invasive Species Profile (factsheet)—NOBANIS. NOBANIS, Europe. www.nobanis.org.

Nester, G. M., Brauwer, M. D., Koziol, A., West, K. M., DiBattista, J. D., White, N. E., Power, M., *et al.* 2020. Development and evaluation of fish eDNA metabarcoding assays facilitate the detection of cryptic seahorse taxa (family: Syngnathidae). Environmental DNA, 2: 614–626.

Not, F., Latasa, M., Marie, D., Cariou, T., Vaulot, D., and Simon, N. 2004. A single species, *Micromonas pusilla* (Prasinophyceae), dominates the eukaryotic picoplankton in the western English Channel. Applied and Environmental Microbiology, 70: 4064–4072.

O'Donnell, J. L., Kelly, R. P., Shelton, A. O., Samhouri, J. F., Lowell, N. C., and Williams, G. D. 2017. Spatial distribution of environmental DNA in a nearshore marine habitat. PeerJ, 5, e3044.

Overland, J., Dunlea, E., Box, J. E., Corell, R., Forsius, M., Kattsov, V., Olsen, M. S., *et al.* 2019. The urgency of Arctic change. Polar Science, 21: 6–13.

Paavo, B. L., Ham, D., Görlitz, S., and Probert, P. K. 2012. How does tidal submersion time affect macroinvertebrate community patterns on a temperate sheltered sandflat? Marine and Freshwater Research, 63: 68.

Palma, S., and Silva, N. 2004. Distribution of siphonophores, chaetognaths, euphausiids and oceanographic conditions in the fjords and channels of southern Chile. Deep Sea Research Part II: Topical Studies in Oceanography, 51: 513–535.

Parsons, K. M., Everett, M., Dahlheim, M., and Park, L. 2018. Water, water everywhere: environmental DNA can unlock population structure in elusive marine species. Royal Society Open Science, 5: 180537–180537.

Patin, N. V., Kunin, V., Lidström, U., and Ashby, M. N. 2013. Effects of OTU clustering and PCR artifacts on microbial diversity estimates. Microbial Ecology, 65: 709–719.

Pecl, G. T., Araújo, M. B., Bell, J. D., Blanchard, J., Bonebrake, T. C., Chen, I-C., Clark, T. D., *et al.* 2017. Biodiversity redistribution under climate change: impacts on ecosystems and human well-being. Science, 355. https://doi.org/10.1126/science.aai9214.

Perry, A. L., Low, P. J., Ellis, J. R., and Reynolds, J. D. 2005. Ecology: climate change and distribution shifts in marine fishes. Science, 308: 1912–1915.

Port, J. A., O'Donnell, J. L., Romero-Maraccini, O. C., Leary, P. R., Litvin, S. Y., Nickols, K. J., Yamahara, K. M., *et al.* 2016. Assessing

vertebrate biodiversity in a kelp forest ecosystem using environmental DNA. Molecular Ecology, 25: 527–541.
Priyadarshi, A., Chandra, R., Kishi, M. J., Smith, S. L., and Yamazaki, H. 2022. Understanding plankton ecosystem dynamics under realistic micro-scale variability requires modeling at least three trophic levels. Ecological Modelling, 467: 109936.
R Core Team. 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/ (last accessed 10 February 2023).
Renshaw, M. A., Olds, B. P., Jerde, C. L., Mcveigh, M. M., and Lodge, D. M. 2015. The room temperature preservation of filtered environmental DNA samples and assimilation into a phenol–chloroform–isoamyl alcohol DNA extraction. Molecular Ecology Resources, 15: 168–176.
Rhodes, L. L., Peake, B. M., MacKenzie, A. L., and Marwick, S. 1995. Coccolithophores *Gephyrocapsa oceanica* and *Emiliania huxleyi* (Prymnesiophyceae = Haptophyceae) in New Zealand's coastal waters: characteristics of blooms and growth in laboratory culture. New Zealand Journal of Marine and Freshwater Research, 29: 345–357.
Richardson, R. T., Sponsler, D. B., McMinn-Sauder, H., and Johnson, R. M. 2020. MetaCurator: a hidden Markov model-based toolkit for extracting and curating sequences from taxonomically-informative genetic markers. Methods in Ecology and Evolution, 11: 181–186.
Rigual-Hernández, A. S., Trull, T. W., Flores, J. A., Nodder, S. D., Eriksen, R., Davies, D. M., Hallegraeff, G. M., *et al.* 2020. Full annual monitoring of Subantarctic *Emiliania huxleyi* populations reveals highly calcified morphotypes in high-CO_2 winter conditions. Scientific Reports, 10: 2594.
Robertson, A., Dixon, P., and Daniel, P. A. 1988. Zooplankton dynamics in mangrove and other nearshore habitats in tropical Australia. Marine Ecology Progress Series, 43: 139–150.
Robertson, D. A., Roberts, P. E., and Wilson, J. B. 1978. Mesopelagic faunal transition across the Subtropical Convergence east of New Zealand. New Zealand Journal of Marine and Freshwater Research, 12: 295–312.
Rockwell, N. C., Duanmu, D., Martin, S. S., Bachy, C., Price, D. C., Bhattacharya, D., Worden, A. Z., *et al.* 2014. Eukaryotic algal phytochromes span the visible spectrum. Proceedings of the National Academy of Sciences, 111: 3871–3876.
Schapira, M., Vincent, D., Gentilhomme, V., and Seuront, L. 2008. Temporal patterns of phytoplankton assemblages, size spectra and diversity during the wane of a *Phaeocystis globosa* spring bloom in hydrologically contrasted coastal waters. Journal of the Marine Biological Association of the United Kingdom, 88: 649–662.
Schenekar, T., Schletterer, M., Lecaudey, L. A., and Weiss, S. J. 2020. Reference databases, primer choice, and assay sensitivity for environmental metabarcoding: lessons learnt from a re-evaluation of an eDNA fish assessment in the Volga headwaters. River Research and Applications, 36: 1004–1013.
Sickel, W., Ankenbrand, M. J., Grimmer, G., Holzschuh, A., Härtel, S., Lanzen, J., Steffan-Dewenter, I., *et al.* 2015. Increased efficiency in identifying mixed pollen samples by meta-barcoding with a dual-indexing approach. BMC Ecology, 15: 20.
Small, H. J., Shields, J. D., Reece, K. S., Bateman, K., and Stentiford, G. D. (2012). Morphological and molecular characterization of *Hematodinium perezi* (Dinophyceae: Syndiniales), a dinoflagellate parasite of the Harbour Crab, *Liocarcinus depurator*. Journal of Eukaryotic Microbiology, 59: 54–66.
Song, W., Liu, J., Qin, W., Huang, J., Yu, X., Xu, M., Stahl, D., *et al.* 2022. Functional traits resolve mechanisms governing the assembly and distribution of nitrogen-cycling microbial communities in the global ocean. MBio, 13: e0383221.
Stat, M., John, J., DiBattista, J. D., Newman, S. J., Bunce, M., and Harvey, E. S. 2019. Combined use of eDNA metabarcoding and video surveillance for the assessment of fish biodiversity. Conservation Biology, 33: 196–205.
Stevens, C. L., O'Callaghan, J. M., Chiswell, S. M., and Hadfield, M. G. 2019. Physical oceanography of New Zealand/Aotearoa shelf seas—a review. New Zealand Journal of Marine and Freshwater Research, 55: 6–45.
Stewart-Sinclair, P. J., Last, K. S., Payne, B. L., and Wilding, T. A. 2020. A global assessment of the vulnerability of shellfish aquaculture to climate change and ocean acidification. Ecology and Evolution, 10: 3518–3534.
Stoeck, T., Frühe, L., Forster, D., Cordier, T., Martins, C. I. M., and Pawlowski, J. 2018. Environmental DNA metabarcoding of benthic bacterial communities indicates the benthic footprint of salmon aquaculture. Marine Pollution Bulletin, 127: 139–149.
Stoeckle, B. C., Kuehn, R., and Geist, J. 2016. Environmental DNA as a monitoring tool for the endangered freshwater pearl mussel (*Margaritifera margaritifera* L.): a substitute for classical monitoring approaches? Aquatic Conservation: Marine and Freshwater Ecosystems, 26: 1120–1129.
Suarez-Menendez, M., Planes, S., Garcia-Vazquez, E., and Ardura, A. 2020. Early alert of biological risk in a coastal lagoon through eDNA metabarcoding. Frontiers in Ecology and Evolution, 8: 9. https://doi.org/10.3389/fevo.2020.00009.
Sutton, P. J. H. 2003. The Southland Current: a subAntarctic current. New Zealand Journal of Marine and Freshwater Research, 37: 645–652.
Teng, S. T., Tan, S. N., Lim, H. C., Dao, V. H., Bates, S. S., and Leaw, C. P. 2016. High diversity of *Pseudo-nitzschia* along the northern coast of Sarawak (Malaysian Borneo), with descriptions of *P. bipertita* sp. nov. and *P. limii* sp. nov. (Bacillariophyceae). Journal of Phycology, 52: 973–989.
Thomsen, H. A., and Buck, K. R. 1998. Nanoflagellates of the central California waters: taxonomy, biogeography and abundance of primitive, green flagellates (Pedinophyceae, Prasinophyceae). Deep Sea Research Part II: Topical Studies in Oceanography, 45: 1687–1707.
Thomsen, P. F., and Willerslev, E. 2015. Environmental DNA - an emerging tool in conservation for monitoring past and present biodiversity. Biological Conservation, 183: 4–18.
Trainer, V. L., Bates, S. S., Lundholm, N., Thessen, A. E., Cochlan, W. P., Adams, N. G., and Trick, C. G. 2012. *Pseudo-nitzschia* physiological ecology, phylogeny, toxicity, monitoring and impacts on ecosystem health. Harmful Algae, 14: 271–300.
Trull, T., Jansen, P., Schulz, E., Weeding, B., Davies, D., and Bray, S. 2019. Autonomous multi-trophic observations of productivity and export at the australian Southern Ocean Time Series (SOTS) reveal sequential mechanisms of physical–biological coupling. Frontiers in Marine Science, 6: 525.
Verity, P. G., Brussaard, C. P., Nejstgaard, J. C., van Leeuwe, M. A., Lancelot, C., and Medlin, L. K. 2007. Current understanding of phaeocystis ecology and biogeochemistry, and perspectives for future research. Biogeochemistry, 83: 311–330.
von Szalay, P. G., and Somerton, D. A. 2005. The effect of net spread on the capture efficiency of a demersal survey trawl used in the eastern Bering Sea. Fisheries Research, 74: 86–95.
Wakelin, S., Cave, V., Dignam, B., D'Ath, C., Tourna, M., Condron, L., Zhou, J., *et al.* 2016. Analysis of soil eDNA functional genes: potential to increase profitability and sustainability of pastoral agriculture. New Zealand Journal of Agricultural Research, 59: 333–350.
Ward, B. A., Cael, B. B., Collins, S., and Young, C. R. 2021. Selective constraints on global plankton dispersal. Proceedings of the National Academy of Sciences of the United States of America, 118: e2007388118.
Ward-Paige, C., Flemming, J. M., and Lotze, H. K. 2010. Overestimating fish counts by non-instantaneous visual censuses: consequences for population and community descriptions. PLoS One, 5: e11722.
Weigand, H., Beermann, A. J., Čiampor, F., Costa, F. O., Csabai, Z., Duarte, S., Geiger, M. F., *et al.* 2019. DNA barcode reference libraries

for the monitoring of aquatic biota in Europe: gap-analysis and recommendations for future work. Science of The Total Environment, 678: 499–524.

West, K. M., Stat, M., Harvey, E. S., Skepper, C. L., DiBattista, J. D., Richards, Z. T., Travers, M. J., *et al.* 2020. eDNA metabarcoding survey reveals fine-scale coral reef community variation across a remote, tropical island ecosystem. Molecular Ecology, 29: 1069–1086.

Yates, M. C., Fraser, D. J., and Derry, A. M. 2019. Meta-analysis supports further refinement of eDNA for monitoring aquatic species-specific abundance in nature. Environmental DNA, 1: 5–13.

Yeh, C-F., Soininen, J., Teittinen, A., and Wang, J. 2019. Elevational patterns and hierarchical determinants of biodiversity across microbial taxonomic scales. Molecular Ecology, 28: 86–99.

Yoo, Y. D., Seong, K. A., Jeong, H. J., Yih, W., Rho, J-R., Nam, S. W., and Kim, H. S. 2017. Mixotrophy in the marine red-tide cryptophyte teleaulax amphioxeia and ingestion and grazing impact of cryptophytes on natural populations of bacteria in Korean coastal waters. Harmful Algae, 68: 105–117.

Zaiko, A., Martinez, J. L., Ardura, A., Clusa, L., Borrell, Y. J., Samuiloviene, A., Roca, A., *et al.* 2015. Detecting nuisance species using NGST: methodology shortcomings and possible application in ballast water monitoring. Marine Environmental Research, 112: 64–72.

Zaiko, A., Wood, S. A., Pochon, X., Biessy, L., Laroche, O., Croot, P., and Garcia-Vazquez, E. 2020. Elucidating biodiversity shifts in ballast water tanks during a cross-latitudinal transfer: complementary insights from molecular analyses. Environmental Science and Technology, 54: 8443–8454.

Zhang, J., Kobert, K., Flouri, T., and Stamatakis, A. 2014. PEAR: a fast and accurate Illumina Paired-end reAd mergeR. Bioinformatics, 30: 614–620.

Zhang, Y., Pavlovska, M., Stoica, E., Prekrasna, I., Yang, J., Slobodnik, J., Zhang, X., *et al.* 2020. Holistic pelagic biodiversity monitoring of the Black Sea via eDNA metabarcoding approach: from bacteria to marine mammals. Environment International, 135: 105307.

Handling Editor: Kristi Miller-Saunders