Ecological divergence of a mesocosm in an eastern boundary upwelling system assessed with multi-marker environmental DNA metabarcoding

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Abstract

Eastern boundary upwelling systems (EBUS) contribute a disproportionate fraction of the global fish catch relative to their size and are especially susceptible to global environmental change. Here we present the evolution of communities over 50 days in an *in situ* mesocosm 6 km offshore of Callao, Peru and in the nearby unenclosed coastal Pacific Ocean.

30   The communities were monitored using multi-marker environmental DNA (eDNA) metabarcoding and flow cytometry. DNA extracted from weekly water samples were subjected to amplicon sequencing for four genetic loci: 1) the V1-V2 region of the 16S rRNA gene, for photosynthetic eukaryotes (via their chloroplasts) and bacteria; 2) the V9 region of the 18S rRNA gene for exploration of eukaryotes but targeting phytoplankton; 3) cytochrome oxidase I (COI), for exploration of eukaryotic taxa but targeting invertebrates, and 4) the 12S rRNA gene, targeting vertebrates.

35   The multi-marker approach showed a divergence of communities (from microbes to fish) between the mesocosm and the unenclosed ocean. Together with the environmental information, the genetic data furthered our mechanistic understanding of the processes that are shaping EBUS communities in a changing ocean. The unenclosed ocean experienced significant variability over the course of the 50-day experiment with temporal shifts in community composition but remained dominated by organisms that are characteristic of high nutrient, upwelling conditions (e.g.

40   diatoms, copepods, anchovies). A large directional change was found in the mesocosm community. The mesocosm community that developed was characteristic of upwelling regions when upwelling relaxes and waters stratify (e.g. dinoflagellates, nanoflagellates). The selection of dinoflagellates under the salinity-driven experimentally stratified conditions in the mesocosm, as well as the warm conditions brought about by the coastal El Niño, may be an indication of how EBUS will respond under the global environmental changes (i.e., increases in surface temperature and

45   freshwater input, leading to increased stratification) forecast by the IPCC.

1 Introduction

Eastern boundary upwelling systems (EBUS) are exceptionally productive marine ecosystems: they account for 5% of total global primary production (Carr, 2001) and 20% of marine fish production (Chavez and Messié, 2009) while occupying less than 1% of the area of the ocean. Strong physical forcing drives productivity in these ecosystems;

50   upwelling-favourable winds bring macro- and micronutrients from depth to the surface. Under favourable temperature and light conditions phytoplankton bloom (Messié and Chavez, 2015), leading to increases in biomass in higher trophic levels (Chavez and Messié, 2009; Ayón et al., 2008). However, these systems are variable both physically and ecologically, making it difficult to develop mechanistic understanding of the links between ecological, biogeochemical, and physical processes. Given that these systems may be disproportionately affected by climate

55   change (Gruber, 2011) it is key that we develop predictive understanding of how these systems will change over time. Here we present results from a perturbation experiment geared at targeting this problem.

Observational studies provide insights into processes regulating biological production and community structure in upwelling systems, but because of the complex interplay of multiple factors, it is difficult to assess the relative

60   contributions of drivers causing change. The EBUS literature has extensive analyses of correlative relationships in the

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pursuit of causative understanding (e.g. Carr and Kearns, 2003; Messié and Chavez, 2015; Patti et al., 2008). By isolating natural communities in enclosures or mesocosms, one can physically perturb the system in a controlled manner (Stewart et al., 2013; Riebesell et al., 2008; Riemann et al., 2000; Sandaa et al., 2009), providing a method for studying mechanisms driving responses of these systems to perturbations. However, contained mesocosms remove horizontal mixing processes, can modify vertical mixing, and remove top predators (fish and mammals), and thus are not exact analogues of natural marine ecosystems.

Variations in zooplankton, phytoplankton, and/or bacteria have been monitored in mesocosm experiments using a variety of sampling techniques, including nets of various mesh size, direct counts of bacteria from water samples, flow cytometry for enumeration of small phytoplankton and bacteria, or algal pigment analysis (e.g. Hitchcock et al., 2016; Suffrian et al., 2008). Environmental DNA (eDNA) metabarcoding is a complementary, rapidly evolving biomonitoring technique that can survey these communities by examining both extracellular and intracellular DNA present in environmental samples (Taberlet et al., 2012). Here we define eDNA as any DNA captured by filtering seawater through a low porosity filter (Chavez et al. 2021) and as such it includes intact microbial cells and other small live organisms as well as material shed or produced by larger plants and animals that has not yet degraded. By targeting and amplifying a highly variable region of the genome across numerous taxonomic groups, eDNA metabarcoding allows for the simultaneous detection and identification of a diversity of taxa (Valentini et al., 2016) and has been used in a variety of aquatic settings and across a wide range of organisms, recovering greater alpha diversity than visual counts or morphological identification (Djurhuus et al., 2018; Boussarie et al., 2018). While eDNA metabarcoding has been used in mesocosms to demonstrate its effectiveness by detecting and identifying known species assemblages (Kelly et al., 2014; Evans et al., 2016), its utility to detect change across multiple trophic levels has not been demonstrated in perturbation mesocosm experiments, nor have multiple eDNA markers been used simultaneously to monitor community dynamics. By providing information about broad taxonomic groups, eDNA metabarcoding provides a holistic, community-level view of ecological changes occurring within mesocosms.

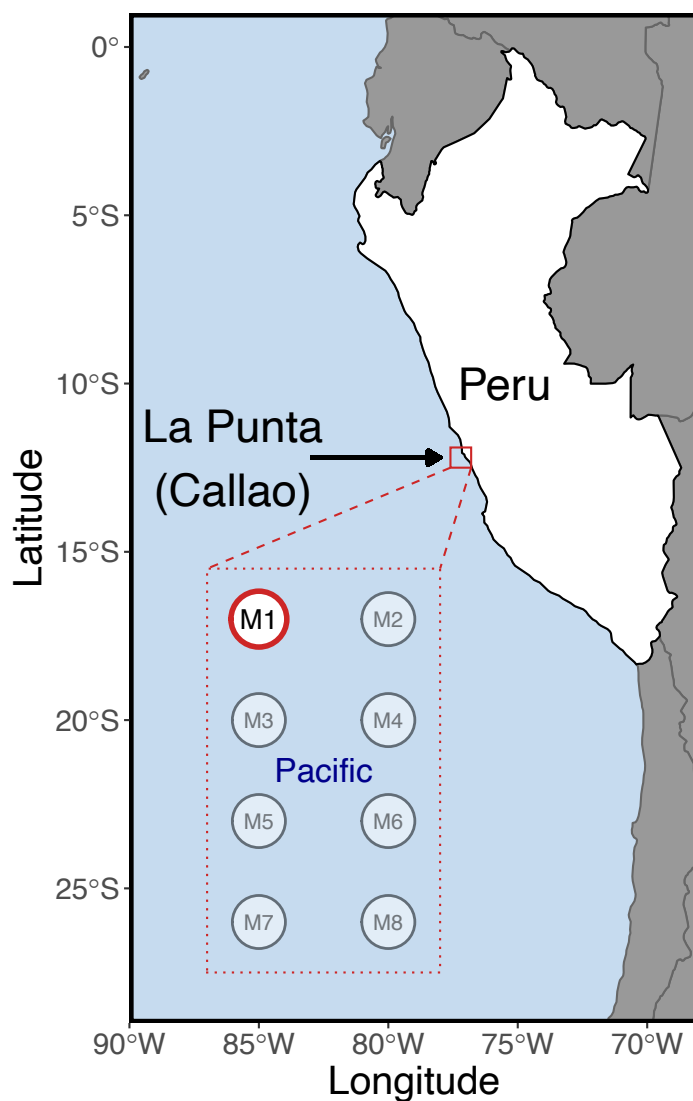
Here, we present results from an *in situ* mesocosm experiment that took place in austral summer 2017 in the coastal Peruvian upwelling system near Callao (Bach et al., 2020). The broad goals of the experiment (detailed in Bach et al. 2020) were to study how marine populations and biogeochemical properties change during an upwelling event, nutrient-rich water collected in the regional oxygen minimum zone (OMZ) was added to the mesocosms. The mesocosms were later modified by the injection of a salt brine solution to maintain the vertical density gradient and prevent full water column mixing. This resulted in heavily stratified mesocosms. A third, unintended perturbation occurred when seabirds began to hover over and perch on the mesocosms during the last month, further modifying biogeochemical and ecological conditions. Alongside the core physicochemical measurements to characterize environmental conditions (Bach et al., 2020), there were multiple ancillary experiments. Our team collected samples for flow cytometry and eDNA from surface waters of the nearby unenclosed Pacific Ocean and from a mesocosm over the 50-day experiment period. Four genetic loci – the V1-V2 region of the 16S rRNA gene (Sudek et al., 2015; Giovannoni et al., 1990), the V9 region of the 18S rRNA gene (Amaral-Zettler et al., 2009; Stoeck et al., 2010; Amaral-

Zettler et al., 2018), mitochondrial cytochrome oxidase I (COI) (Leray et al., 2013; Folmer et al., 1994), and the
110 mitochondrial 12S rRNA gene (Miya et al., 2015) were evaluated, capturing a diversity of bacterial, phytoplankton,
zooplankton, and vertebrate populations, respectively.

Using this multi-marker approach, we detected the ecological divergence of the mesocosm relative to the nearby highly
variable and dynamic unenclosed ocean. The mesocosm communities evolved to be dominated by taxa typical of
115 stratified conditions, whereas the unenclosed ocean retained a community shaped by high nutrients and intermittent
upwelling. The impact of resting seabirds was detected via a "orni-eutrophication" driven phytoplankton bloom (Bach
et al., 2020) and the appearance of DNA of fish, seabirds, and bacteria typical of host-association of animal
microbiomes (Saccharibacteria) (Jaffe et al., 2021). This study reveals a clear community shift driven by an
experimental manipulation that simulated upwelling and subsequent stratification, as well as the impact of external
120 inputs to the experimental system, and demonstrates that eDNA metabarcoding is a powerful tool for detecting
community-level changes over time.

2 Materials and methods

2.1 Mesocosm deployment and manipulations

On February 22, 2017, eight "Kiel Off-Shore Mesocosms for Ocean Simulations" (Riebesell et al., 2013) were
125 deployed just north of Isla San Lorenzo, 6 km off the Peruvian coastline (Fig. 1). Each mesocosm consisted of a
cylindrical 18.7 m long polyurethane bag (2 m diameter, 54.4 ±1.3 m^3 volume) suspended in an 8 m tall flotation
frame (Bach et al., 2020). After allowing water exchange for three days through nets (mesh size 3 mm) at the top and
bottom of each mesocosm, the water mass inside each of the mesocosms was isolated from the surrounding water by
attaching a conical sediment trap to the lower end of the bags and pulling the upper ends of the bags ~1.5 m above the
130 surface. The enclosing of the mesocosms marked the start (day 0) of the 50-day experiment. Sampling of chl-a and
physicochemical and biogeochemical conditions was performed on all 8 mesocosms as well as in the nearby coastal
ocean within a few meters from the mesocosms. The eDNA and flow cytometry samples were collected from all
mesocosms, but due to limited resources, the samples that were analysed were only from mesocosm M1 and the nearby
unenclosed coastal Pacific Ocean, where samples were taken within a few meters of the mesocosm frame. These two
135 collections and their analysis are referred to as mesocosm and Pacific hereafter.

140   **Figure 1. The KOSMOS 2017 study site. The eight mesocosms (M1-M8) were deployed 6 km offshore of La Punta (Callao), just north of Isla San Lorenzo (12.0555°S; 77.2348°W). All mesocosms were sampled for chl-a, various biogeochemical variables, and multiple physicochemical parameters (Bach et al., 2020). Mesocosm M1 (highlighted in red) was also sampled for DNA and flow cytometry, as was the nearby unenclosed Pacific Ocean. Please note that the square marking the study site is not true to scale.**

Over the course of the experiment, three primary intentional manipulations took place in all 8 mesocosms: OMZ water addition, salt additions to control stratification, and additions of organisms. On days 11 and 12 of the experiment, a total of ~20 m^3 of OMZ water collected at a depth of 30 m was exchanged with the water enclosed in M1; this water had been collected on day 5 from the OMZ located at station 1 (12.028323°S; 77.223603°W) of the IMARPE (Instituto

150 del Mar del Perú) time-series transect (Graco et al., 2017) using deep water collectors (Taucher et al., 2017). The OMZ water was injected to a depth of 14-17 m and was designed to simulate upwelling of water from the OMZ. In order to keep the mesocosm stratified and thus preserve the low O$_2$ bottom layer from the injected OMZ water, a NaCl brine solution was injected evenly into the bottom layers of the mesocosm on days 13 (69 L at a depth range of 10 – 17 m) and 33 (46 L at a depth range of 12.5 – 17 m) of the experiment. Some of the research questions from this campaign

155 concerned the responses of endemic organisms to the experimental conditions in the mesocosms, and therefore two endemic organisms, larvae of the Peruvian Scallop (*Argopecten purpuratus*) and eggs of the Fine Flounder (*Paralichthys adspersus*) were added to all mesocosms. On day 14, Peruvian Scallop larvae was added in concentrations of ~10,000 individuals m^{-3}, and on day 31 Fine Flounder eggs were added in concentrations of ~90 individuals m^{-3}. However, few scallop larvae and no fish larvae were detected during subsequent sampling via vertical

160 tows of an Apstein net of mesh size 100 μm (unpublished data), and Fine Flounder DNA was only detected in the two samples directly following the addition, indicating that they either degraded or sank out of the surface layer.

2.2 Sample collection

Salinity, temperature, and chl-a fluorescence were measured with vertical casts of a CTD60M sensor system (Sea & Sun Technologies) in all mesocosms and the Pacific. Samples for inorganic nutrients were collected using a 5 L

165 "integrating water sampler" (IWS) (Hydro-Bios Kiel) that evenly collected water for two separate depth ranges, the surface and bottom waters. However, the inorganic nutrient, chl-a, temperature, and salinity results presented in the first column of Figure 2 were calculated by averaging the IWS-collected data over the two depth ranges. After transport back to an on-shore laboratory, nutrient samples were filtered (0.45 μm filter, Sterivex, Merck) and analysed using an autosampler (XY2 autosampler, SEAL Analytical) and a continuous flow analyser (QuAAtro AutoAnalyzer, SEAL

170 Analytical) connected to a fluorescence detector (FP-2020, JASCO). Silicic acid (Si(OH)$_4$) was analysed colorimetrically following the procedures by Mullin and Riley, (1955). Nitrate (NO$_3^-$) and nitrite (NO$_2^-$) were quantified through the formation of a pink azo dye as established by Morris and Riley (1963). Note that other measurements were made throughout the experiment from all mesocosms as reported elsewhere (Bach et al., 2020). CTD casts and IWS water collections occurred every other day (except for days 1-4 and 12-18, when they were taken

175 daily),

DNA samples that were analysed were taken in M1 and the Pacific on days 1, 8, 15, 24, 32, 36, 42, and 48. A single DNA sample was analysed for each day. Flow cytometry samples were collected roughly every other day. Water for eDNA and flow cytometry measurements was collected using the IWS to evenly sample the upper portion of the water

180 column; the depths sampled were 0 – 5 m on days 1 and 2, 0 – 10 m from day 3 to 28, and 0 – 12.5 m from day 29 to 50. These sampling depths sampled the top layer of the mesocosm, which was almost entirely above the depths at

which the salt brine solution was injected. After transport back to shore, eDNA samples were collected by filtering 250 mL of water onto a 47 mm diameter 0.22 μm pore size polyvinylidene difluoride membrane filter (Millipore, USA) using a vacuum pump. All filters were flash frozen in liquid nitrogen and stored at -80°C before being shipped on dry ice to California, USA for analysis.

Field controls consisting of 250 mL of filtered reverse osmosis (RO) and MilliQ water were collected on Day 3 of the experiment in order to characterize any contaminating taxa in these systems and later steps. No DNA was detected in these controls using NanoDrop 1000 spectrophotometer (ThermoFisher Scientific, Waltham, MA) measurements; environmental samples ranged from 19.9 – 139.3 ng/μL. However, COI, 18S, and 16S rRNA PCR amplification yielded slight amplification and PCR products were sequenced (Table S1). After normalization steps, the dominant reads in the bacterial portion of the 16S rRNA sequences consisted of Betaproteobacteria (36.2%), that were not prominent in field samples (0.27% mean proportion of reads in field samples). The greatest proportion of plastidial reads in the field blanks were multiple diatom ASVs (66.7% of reads), with the same ASVs composing 42.8% of reads in the field samples. In the COI reads, most ASVs found in the field blanks were also not prominent in the field samples, with the exception of the calanoid copepod *Paracalanus* (12.4% of reads in field blanks, 13.3% of reads in field samples). In the 18S reads, the top ASVs in the field blanks were the copepods *Hemicyclops thalassius* (30.0% of reads in field blanks, 3.9% of reads in environmental samples) and *Paracalanus* (11.7% of reads in field blanks, 14.6% of reads in environmental samples). While this indicates that there may have been a low level of field-based cross-contamination in our environmental samples, which is found in most eDNA studies (and is a near inevitability when sampling in a remote location without a dedicated molecular laboratory), these reads were not removed from our analyses.

2.3 Flow cytometry

Triplicate samples of 1 mL volume each were taken and preserved with glutaraldehyde (EM grade, final concentration of 0.25%). The samples were then incubated for 20 minutes in the dark and subsequently treated the same way as the eDNA samples. Photoautotrophs (photosynthetic eukaryotes, *Synechococcus*, and *Prochlorococcus* were it present) and heterotrophic bacteria were enumerated on a BD Influx cell sorter (BD Biosciences, USA) equipped with a 488-nm argon laser (200 mW) and a 70 μm nozzle running with 0.2 μm pre-filtered 1xPBS (10xPBS, pH 7.4, Life Technologies). Prior to the running of each sample, fluorescent polystyrene beads (0.75 μm yellow green beads, Polysciences, Inc) were added for reference. For calculation of the total volume analysed, the samples were weighed before and after each run. For counts of photoautotrophs, the system was triggered on forward angle light scatter (FALS), and red chlorophyll autofluorescence (692/40 nm band-pass filter) as well as orange phycoerythrin autofluorescence (572/27 nm band-pass filter) versus FALS and were recorded over 8 min. running at ~25 μL min^{-1}. To count heterotrophic bacteria, the samples were stained with SYBR Green I (10,000x SYBR Green I, Thermo Fisher; final concentration of 0.5x with 15-minute incubation time in the dark) and was triggered on green fluorescence (520/35 nm band-pass filter). The samples were run for 1 min. at ~25 μL min^{-1}. FCS-files were processed in WinList

220   3D 9.0.1 (Verity Software House, Topsham ME, USA). Among other bacterial populations, a unique population of presumed bacteria appeared in mesocosm and coastal samples after day 24 of the study and was gated in accordance with the representative cytogram (Fig. S1).

2.4 DNA extraction

DNA from filters was extracted using the DNeasy® Blood and Tissue kit (Qiagen, Germantown, MD) following
225   standard protocol with some modifications that included an overnight incubation and increasing the amount of lysis buffer to completely submerge the filter during lysis (protocols.io: dx.doi.org/10.17504/protocols.io.xjufknw). DNA extraction concentrations were quantified using NanoDrop 1000 spectrophotometer (ThermoFisher Scientific, Waltham, MA) measurements.

230   **2.5 DNA amplification and sequencing**

2.5.1 Cytochrome oxidase I (COI), 18S rRNA, and 12S rRNA

PCR reactions for COI and the 12S rRNA gene were run with Fluidigm two-step amplification protocol for each sample (COI protocols.io: dx.doi.org/10.17504/protocols.io.mwnc7de, 12S protocols.io: dx.doi.org/10.17504/protocols.io.bcppivmn), while PCR reactions for the 18S rRNA gene were run using 12-basepair
235   Golay barcoded reverse primers (protocols.io: dx.doi.org/10.17504/protocols.io.n2vdge6). For COI, the primary PCR primers used are from Leray et al., 2013 and are as follows: Fluidigm CS1+**mlCOIinfF** (forward): ACACTGACGACATGGTTCTACA   **GGWACWGGWTGAACWGTWTAYCCYCC**   and   Fluidigm CS2+**HCO2198** (reverse): TACGGTAGCAGAGACTTGGTCT **TAAACTTCAGGGTGACCAAAAAATCA.** For 18S, primary PCR primers used are from Amaral-Zettler et al., 2009 and are as follows: Euk1391F (forward):
240   AATGATACGGCGACCACCGAGATCTACAC TATCGCCGTT CG **GTACACACCGCCCGTC** and EukBr (reverse):   CAAGCAGAAGACGGCATACGAGAT   XXXXXXXXXXXX   AGTCAGTCAG   CA **TGATCCTTCTGCAGGTTCACCTAC** (where XXXXXXXXXXXX is unique 12-bp barcode location, all primers listed in 5' to 3' direction). For 12S, primary PCR primers used are from Miya et al., 2015 and are as follows: Fluidigm CS1 + **12S MiFish_U** (forward): ACACTGACGACATGGTTCTACA**GTCGGTAAAACTCGTGCCAGC** and
245   Fluidigm CS2 + 1**2S MiFish_U** (reverse):
  TACGGTAGCAGAGACTTGGTCT**CATAGTGGGGTATCTAATCCCAGTTTG**.

For each of the three markers, primary PCR amplifications were carried out in triplicate 25 μL reactions using 1 μL DNA extract, 12.5 μL AmpliTaq Gold Fast PCR master mix (Applied Biosystems), 1 μL each of forward and reverse
250   primers (5 μM), and 9.5 μL molecular-biology grade water. PCR reactions were run in 96-well plates with a NTC run in triplicate for each plate. Primary COI cycling parameters were 95 °C for 10 min followed by 16 cycles of 94 °C for 10 seconds, 62 °C for 30 seconds, 68 °C for 60 seconds, next followed by 25 cycles of 94 °C for 10 seconds, 46 °C for 30 seconds, 68 °C for 60 seconds, and a final step of 72 °C for 10 min. Primary 18S rRNA cycling parameters were 95°C for 10 min followed by 35 cycles of 94 °C for 45 seconds, 57 °C for 30 seconds, 68 °C for 90 seconds, and

255 a final elongation step of 72 °C for 10 min. Primary 12S rRNA cycling parameters were 95 °C for 15 min followed by 13 cycles of 94 °C for 30 seconds, 69.5 °C for 30 seconds (changes -1.5°C per cycle), 72 °C for 90 seconds, next followed by 25 cycles of 94 °C for 30 seconds, 50 °C for 30 seconds, 72 °C for 45 seconds, and a final step of 72 °C for 10 min.

260 Following PCR, the pooled PCR products for each genetic marker were run through an agarose gel to confirm the presence of target bands and inspected for degree of amplification, as well as absence of any non-specific amplification. PCR products were purified and size selected using the Agencourt AMPure XP bead system (Beckman Coulter, USA). A second agarose gel was run to confirm primer removal and retention of target amplicons after purification.

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Library preparation and sequencing was conducted at the Research Technology Support Facility (RTSF) Genomics Core at Michigan State University (MSU) as was secondary amplification for COI and 12S. PCR products were run through Invitrogen SequalPrep Normalization Plate (ThermoFisher Scientific) using the manufacturer's protocol to create pooled libraries. The pooled product was loaded on a standard MiSeq v2 flow cell and sequenced in a 2x250bp

270 (COI, 12S rRNA) or 2x150 bp (18S rRNA) paired end format using a v2 500-cycle MiSeq reagent cartridge. The MiSeq run was performed with a 10% PhiX spike. Custom sequencing primers were added to appropriate wells of the reagent cartridge. Base calling was done by Illumina Real Time Analysis (RTA) v1.18.54 and output of RTA was demultiplexed and converted to FastQ format with Illumina Bcl2fastq v2.18.0.

2.5.2 16S rRNA

275 Prior to amplification, DNA was diluted to 5 ng µL^{-1} with TE pH 8. The V1-V2 16S rRNA gene region was amplified as previously described (Sudek et al., 2015) with 5 µL of 10× buffer, 1 U of HiFi-Taq, 1.6 mM MgSO4, 5 ng of template DNA, and 200 nM of 27F (AGRGTTYGATYMTGGCTCAG, Daims et al., 1999) and 338RPL primer (GCWGCCWCCCGTAGGWGT, Morris et al., 2002). PCR cycling parameters were 95 °C for 2 min, 30 cycles of 94 °C for 15 seconds, 55 °C for 30 seconds and 68 °C for 1 min, followed by a final elongation at 68 °C for 7 min.

280 Purification, barcoding, library preparation, and sequencing were performed at University of Arizona with MiSeq 2x300 bp reads.

2.6 Bioinformatics

2.6.1 Cytochrome oxidase I (COI), 18S rRNA, and 12S rRNA

The resulting Illumina sequence data was analysed through a custom shell script adapted from the banzai pipeline

285 (https://github.com/MBARI-BOG/BOG-Banzai-Dada2-Pipeline, O'donnell et al., 2016). Complete script and parameters are included in the Supplement. Within the script, primer sequences were first removed from fastq files through the program Atropos (Didion et al., 2017). Fastq files were then fed into the DADA2 program (Callahan et al., 2016). DADA2 models error on a per-Illumina run basis, controlling for read quality and picking amplicon sequence variant (ASV) sequences that represent biological variability rather than sequencing error (Callahan et al.,

290 2016). Within DADA2, reads were trimmed to remove low-quality regions and filtered by quality score, sequencing errors were modelled and removed, and reads were then merged and chimeric sequences removed. Taxonomy was assigned to the resulting ASV sequences through blastn searches to NCBI GenBank's non-redundant nucleotide database (nt) (Camacho et al., 2009; Agarwala et al., 2018). Blast results were filtered using MEGAN6's lowest common ancestor (LCA) algorithm (Huson et al., 2016). Only hits with ≥80% sequence identity, ≥100 bitscore and

295 whose bitscores were within the top 2% of the highest bitscore value for each ASV were considered by MEGAN6. The MEGAN6 parameter LCA percent was from 0.80 to 0.85, depending on the marker, allowing for up to 15 – 20% of top hits to be off target and still have the majority taxonomy assigned. This parameter value was chosen to allow for minor numbers of incorrectly annotated GenBank entries – effectively allowing for ASVs which had many high-quality hits to a taxa to still be assigned to that taxa even if there existed a high-bitscore hit to another GenBank

300 sequence annotated to an unrelated taxa. Despite the issue that this introduces of potentially assigning species-level identification to an ASV with multiple strong matches, we decided that this compromise was necessary to allow species identification with an imperfectly curated reference database. We decided this was more advantageous than the disadvantage caused by ignoring small numbers of true closely related sequences. Furthermore, post-MEGAN6 filtering was performed to ensure only contigs with a hit of ≥97% sequence identity and ≥200 bitscore were annotated

305 to the species level. Only contigs with a hit of ≥95% sequence identity and ≥150 bitscore were annotated to the genus level. Annotations were elevated to the next highest taxonomic level for contigs that failed those conditions.

2.6.2 16S rRNA

Demultiplexed reads were imported into QIIME2 (Bolyen et al., 2019) and cutadapt (Martin, 2011) trim-paired was used to trim primers. Trimmed reads were denoised with DADA2 (Callahan et al., 2016) denoise-paired command,

310 with a truncation of the forward and reverse reads to 250 and 225 bp, respectively. Resulting ASVs were classified in QIIME2 with the feature-classifier classify-consensus-blast (Camacho et al., 2009) command with a percent identity of 0.95, maximum number of accepted hits of 5 and consensus of 0.7 against the 99% representative sequences of SILVA 132 (Quast et al., 2013) and both 18S and 16S rRNA gene references. A bacterial ASV table was then generated by removing mitochondrial and plastidial, sequences. Plastidial sequences were then selected based on the

315 SILVA classification and reclassified with the Phytoref database (Decelle et al., 2015) using the same settings as before except with a percent-identity of 0.90. The five most abundant cyanobacterial ASVs were further analysed by manual blast against NCBI-nt excluding environmental sequences.

2.7 Quality Control and Decontamination

Following the bioinformatic pipeline, the COI, 18S rRNA, and 12S rRNA sequencing results were passed through

320 custom R ver. 3.6.0 (R Core Team, 2019) decontamination scripts. For each plate, we first removed all singleton ASVs. Next, for each ASV that was detected in at least one of the PCR blanks on the plate, we determined the maximum number of reads of that ASV in any of the individual PCR blanks and subtracted this value from the reads of the ASV in each of the environmental samples. This was done to address cross-contamination from various sources, such as tag-jumping (Schnell et al., 2015). As mentioned previously in Section 2.2, no decontamination steps were

taken using the field blank samples. Finally, we removed all reads assigned to common terrestrial contaminants: orders Rodentia and Lagomorpha, families Hominidae, Bovidae, Felidae, and Canidae, and genera *Gallus* and *Meleagris*. These decontamination steps were not run for 16S rRNA sequences because the PCR blanks were not sequenced.

2.8 Statistical analyses

Beta diversity analyses were run separately on the five datasets (COI, 18S rRNA, 12S rRNA, 16S rRNA bacterial sequences, and 16S rRNA plastidial sequences) using the QIIME2 (Bolyen et al., 2019) DEICODE plugin. The beta diversity analyses for 12S rRNA are included in the Supplement (Fig. S2) since vertebrates were excluded from the mesocosm (water filtered through a 3 mm mesh), and vertebrate eDNA that was detected was: 1) found during our first sampling and then decayed and disappeared at a rate consistent with experimental results (Sassoubre et al., 2016); 2) the result of the intentional addition of fish eggs, and 3) the unintentional addition from seabird faeces. Through matrix completion and robust Aitchison PCA (RPCA), DEICODE (Martino et al., 2019) is particularly well-suited to handle the sparseness inherent to sequencing data. A PERMANOVA test was also run in QIIME2 on the Aitchison distance matrix produced by DEICODE to determine the significance of the variance between M1 samples following OMZ water addition and the other samples (Pacific and first two M1 samples). Based on the results of the RPCA and PERMANOVA, which showed a clear differentiation between the M1 samples following OMZ water addition (day 15 and on) and Pacific surface water samples on PC1 for the four primary datasets analyzed for beta diversity (all but 12S rRNA), the loading scores on PC1 were used to identify ASVs that were driving the divergence between M1 and the Pacific. The PC results presented are constrained to ASVs that were consistently present throughout the experiment as determined by ASVs that composed at least 0.1% of the reads in 25% of the samples. Heatmaps of relative abundances were constructed for all ASVs in all samples (Fig. S3); criteria for inclusion of ASVs in these heatmaps was a relative abundance within the top 10 most abundant ASVs in any sample within a respective dataset and greater than 1% of the reads in any sample.

3 Results

3.1 Physicochemical conditions, inorganic nutrients, and chlorophyll-a

The mesocosm experiment occurred during an unusual warming event described as a coastal El Niño (Garreaud, 2018; Bach et al., 2020). Sea surface temperature (SST) was 1 to 1.5° C warmer than average (Herring et al., 2019) at the start of the experiment. SST fluctuated over the experiment (Fig. 2a) but returned to average values about 5 weeks into the experiment. Temperature in the mesocosms tracked that of the surrounding water (Fig. 2a). Salinity in the mesocosms began with levels similar to the open ocean, and was modified via NaCl brine additions to depths below 10 m on day 13 and again on day 33 (Fig 2b).

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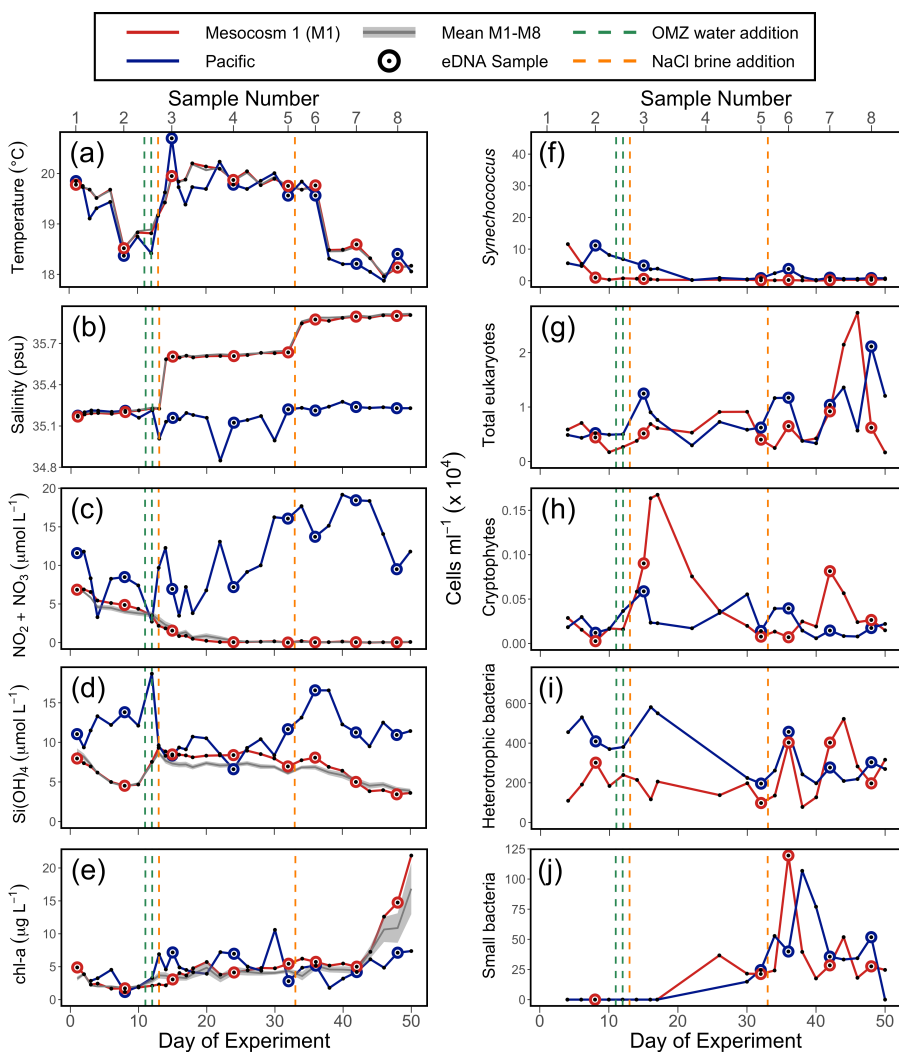
Figure 2. Physicochemical conditions, inorganic nutrients, and chlorophyll-a (chl-a) in the mesocosms and unenclosed Pacific surface waters (a-e), with values averaged over the 0-17 m depth range. Flow cytometry results (f-j) from samples collected in the surface waters; these depths were 0 – 5 m on days 1 and 2, 0 – 10 m from day 3 to 28, and 0 – 12.5 m from

day 29 to 50. For all panels, each individual sample is indicated. For (a), (b), (c), (d), and (e), the solid grey line represents the mean for all eight mesocosms; the shaded grey line is the mean ± SE for all eight mesocosms. The green lines indicate exchange of ~20 m³ of OMZ water with water in the mesocosm (total volume ~ 50 m³) on days 11 and 12. The orange lines indicate NaCl brine additions on days 13 (69 L at a depth range of 10 – 17 m) and 33 (46 L at a depth range of 12.5 – 17 m). (a) Temperature. (b) Salinity. (c) NO_2^-+NO_3^-. (d) $Si(OH)_4$. (e) Chlorophyll-a (chl-a). (f) *Synechococcus*. (g) Photosynthetic eukaryotes. (h) Cryptophytes. (i) Heterotrophic (non-pigmented) bacteria. (j) Small bacteria (smaller than standard non-pigmented bacteria in the system).

NO_2^- + NO_3^- (NO_x^-) concentration in M1 was initially 6.9 µmol L^{-1} and declined steadily over time (Fig. 2c). M1 received addition of OMZ water with NO_x^- concentration of 0.3 µmol L^{-1} on days 11 and 12, and reached the detection threshold of 0.2 µmol L^{-1} around day 20 (Fig. 2c). Conversely, NO_x^- concentrations in the Pacific at the study site were considerably higher and more variable, ranging between 2.7 – 19.2 µmol L^{-1} and reaching particularly high values during the second half of the experiment (Fig. 2c). The concentration of $Si(OH)_4$ in M1 was initially 8.0 µmol L^{-1} and declined to a value of 4.7 µmol L^{-1} on day 10, prior to addition of OMZ water with $Si(OH)_4$ concentration of 17.4 µmol L^{-1}. The addition of OMZ water caused $Si(OH)_4$ in M1 to increase to 9.3 µmol L^{-1} on day 13, after which its concentration declined for the rest of the experiment, reaching a value of 3.6 µmol L^{-1} on day 50 (Fig. 2d). In the Pacific, $Si(OH)_4$ concentrations fluctuated, ranging from a minimum of 6.6 µmol L^{-1} on day 24 to a maximum of 18.7 µmol L^{-1} on day 12 (Fig. 2d).

Chlorophyll-a (chl-a) concentration in M1 was initially 4.9 µg L^{-1} and declined until reaching a minimum of 1.7 µg L^{-1} on day 6. Following OMZ water addition, chl-a gradually increased until approximately day 40, after which it increased rapidly. This rapid increase in chl-a in the final ~10 days of the experiment, which was seen across all eight mesocosms (Fig. 2e), has been attributed to "orni-eutrophication" by defecating sea birds (Inca Terns, *Larosterna inca*), who found the mesocosms to be suitable resting places (Bach et al., 2020). Chl-a in the Pacific was more variable and generally comparable to the range of chl-a concentrations found in the mesocosms until day 40.

The observed values of temperature, salinity, NO_x^-, $Si(OH)_4$, and chl-a in M1 were very similar to the values found in all eight mesocosms (Fig. 2, with more detail in Bach et al. 2020), indicating that M1 was representative of the overall abiotic and hence biotic conditions found within the mesocosms.

3.2 eDNA overview statistics

Across the five datasets, the unenclosed Pacific samples exhibited higher alpha diversity relative to mesocosm at nearly all levels of taxonomy from phyla to ASVs. Summary statistics are given in Table 1. A total of 1,219,350 COI, 1,759,671 18S rRNA, 217,398 12S rRNA, 241,783 16S rRNA plastidial, and 3,125,836 16S rRNA bacterial paired end reads passed filtering and decontamination steps. These amplicons resulted in a total of 2,434 COI ASVs, 3,296 18S rRNA ASVs, 470 12S rRNA ASVs, 258 16S rRNA chloroplast ASVs, and 5,786 16S rRNA bacterial ASVs (Table 1). These resulting ASVs form the basis of the statistical analysis presented below.

Table 1. DNA sequencing statistics. The 16 total samples were divided between 8 M1 (mesocosm) and 8 Pacific samples, with "Avg. reads" and "Stdev" reflecting the average and standard deviation (respectively) of the number of reads per sample. All other values represent the total sum across all samples. Across all five datasets, the Pacific samples consistently show a higher level of alpha diversity at all levels of taxonomy.

	Cytochrome Oxidase I		18S rRNA		12S rRNA		16S rRNA Plastidial		16S rRNA Bacterial	
	M1	Pacific	M1	Pacific	M1	Pacific	M1	Pacific	M1	Pacific
Avg. Reads	78,140	74,279	125,809	94,150	13,236	13,939	9,131	21,092	203,083	187,646
(Stdev)	22,681	6,018	26,365	19,034	9,080	6,052	6,511	10,738	20,875	36,331
ASVs	1,720	1,890	2,105	2,825	360	265	158	203	3,223	4,139
Phyla	25	28	29	39	2	4	5	7	24	38
Classes	54	59	71	84	5	8	13	13	43	72
Orders	104	117	143	176	18	24	15	16	130	166
Families	141	168	193	229	25	32	14	15	183	242
Genera	45	57	214	242	24	27	2	4	348	414
Species	37	48	197	234	21	21	2	5	10	8

3.3 Results of Principal Component Analysis

3.3.1 RPCA and PERMANOVA

The first principal component of COI, 18S rRNA, V1- V2 16S rRNA plastidial ASVs, and V1-V2 16S rRNA bacterial ASVs revealed a swift divergence between the Pacific and mesocosm communities (Fig. 3). In the COI, 18S rRNA, and 16S rRNA plastidial sequences (Fig. 3a, 3b, and Fig. 3c), this divergence is first seen on day 15, the first eDNA sample taken after the OMZ water and salt additions. In the 16S rRNA bacterial sequences (Fig. 3d), this divergence is already seen on day 8, prior to additions. A PERMANOVA test revealed a significant difference between M1 samples following additions and the other samples, confirming the significance of the observed divergence (COI: p = 0.001, F-statistic = 11.78; 18S rRNA: p = 0.001, F-statistic = 11.76;16S rRNA chloroplast: p = 0.002, F-statistic = 7.20; 16S rRNA bacterial: p = 0.001, F-statistic = 10.11). Following this initial divergence, the M1 samples continue to separate from the Pacific samples. The Pacific samples show a slight temporal trend on PC2 across all four datasets. As shown by the proportion of variance explained by each principal component, the degree of divergence between M1 and the Pacific is greater than the degree of change experienced by the Pacific.

420
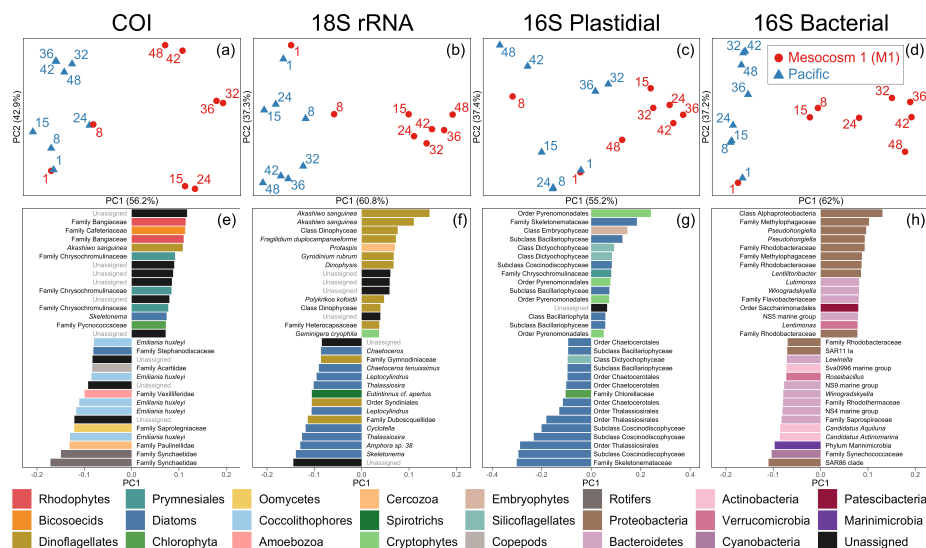
Figure 3. Beta diversity analyses using DEICODE robust Aitchison PCA for (a) COI, (b) 18S rRNA, (c) V1-V2 16S rRNA plastidial ASVs, and (d) V1-V2 16S rRNA bacterial ASVs, including photosynthetic taxa. Data points represent individual samples; the number next to each data point indicates the day collected. ASV loadings on PC1 from the DEICODE robust Aitchison PCA for (e) COI, (f) 18S rRNA, (g) V1-V2 16S rRNA plastidial ASVs, and (h) V1-V2 16S rRNA bacterial ASVs. As

425 **seen in (a), (b), (c), and (d) and confirmed by a PERMANOVA test, PC1 separates the Pacific samples and pre-OMZ water addition M1 samples from the post-OMZ water addition M1 samples (days 15+), and thus positive loading scores are associated with the mesocosm, whereas negative loading scores are associated with the Pacific samples. Text adjacent to loading scores represents the lowest taxonomy to which that ASV was annotated. The taxonomy assigned to 16S rRNA ASVs corresponds to that of the SILVA taxonomy.**

430

3.3.2 Mesocosm-associated ASVs and taxonomy revealed by PC1 loading scores

The taxa that had the highest positive PC1 loading scores, and thus were associated with M1, included various photo-, mixo-, and heterotrophic taxa. For the COI data, this included the dinoflagellate *Akashiwo sanguinea*. Unassigned ASVs made up six of the other mesocosm-associated ASVs for COI, while other mesocosm-associated ASVs included

435 two assigned to the red algae family Bangiaceae, one to the heterotrophic nanoflagellate family Cafeteriaceae, three to the haptophyte family Chrysochromulinaceae, one to the diatom *Skeletonema*, and one to the prasinophyte family Pycnococcaceae (Class V prasinophytes, Bachy et al., 2022).

The mesocosm-associated 18S rRNA ASVs were dominated by dinoflagellates, which were 9 of the top 15 positive

440 loading ASVs. The six other ASVs in the top 15 mesocosm-associated ASVs included one ASV assigned to the cercozoan genus *Protaspis*, another ASV assigned to the cryptophyte species *Geminigera cryophila*, and four unassigned ASVs.

In the V1-V2 16S rRNA plastidial sequences, mesocosm-associated ASVs included four ASVs assigned to the cryptophyte order Pyrenomonadales, six diatom ASVs, an ASV assigned to the class Embryophyceae (land plants), two ASVs assigned to the silicoflagellates (class Dictyophyceae), an ASV assigned to the family Chrysochromulinaceae, and an unassigned ASV. In the V1-V2 16S rRNA bacterial sequences, nine ASVs were assigned to the phylum Proteobacteria, four to the phylum Bacteroidetes, and one each to the phyla Patescibacteria and Verrucomicrobia.

3.3.3 Pacific ASVs and taxonomy revealed by PC1 loading scores

Pacific COI ASVs included three mesozooplankton ASVs, two assigned to the rotifer family Synchaetidae and one assigned to the calanoid copepod family Acartiidae. In the phytoplankton portion of the COI Pacific-associated ASVs, the coccolithophore *Emiliania huxleyi* figured prominently, comprising five of the top 15 Pacific-associated ASVs. The diatom family Stephanodiscaceae was also determined to be associated with the Pacific. Of the COI ASVs associated with the Pacific that weren't mesozooplankton or phytoplankton, one ASV belonged to the amoeboid family Paulinellidae (phylum Cercozoa), one was assigned to the amoebozoan family Vexilliferidae, one was assigned to the oomycete family Saprolegniaceae, and three ASVs were unassigned.

Pacific 18S rRNA ASVs were dominated by diatom ASVs, which made up 9 of the top 15 Pacific-associated ASVs. All of these diatom ASVs were annotated to the genus- or species-level, and included the genera *Skeletonema*, *Amphora*, *Thalassiosira*, *Cyclotella*, *Leptocylindrus*, and *Chaetoceros*. Three of the top 15 Pacific ASVs were dinoflagellate ASVs, and two of which were annotated to the order Syndiniales, with one of these ASVs annotated to the family Duboscquellidae within the Syndiniales; the other dinoflagellate ASV was annotated to the family Gymnodiniaceae. One other ASV was assigned to the tintinnid ciliate species *Eutintinnus* cf. *apertus*, and two other ASVs were unassigned.

The Pacific V1-V2 16S rRNA plastidial ASVs were dominated by diatoms, which represented 13 of the top 15 Pacific-associated ASVs. Of the two other Pacific-associated chloroplast-derived ASVs, one was assigned to the chlorophyte family Chlorellaceae and the other to the class Dictyophyceae (silicoflagellates). Among the 16S rRNA bacterial ASVs, the top 15 contained six assigned to the phylum Bacteroidetes, three each assigned to the phyla Proteobacteria and Actinobacteria and one each assigned to the phyla Marinimicrobia, Verrucomicrobia and Cyanobacteria. Surprisingly, the cyanobacterial ASV is identical to a *Cyanobium* sp. strain, Suigetsu-CR5, isolated from a Japanese saline lake (Ohki et al., 2012), while further analysis of other, less abundant cyanobacterial ASVs identified more typical marine *Synechococcus* strains (see Supplement).

3.4 Vertebrate detection

12S rRNA detected a diversity of vertebrate taxa in the Pacific, dominated by anchovy (genus *Engraulis*) (Fig. 4a), and with fluctuating numbers of reads. In contrast, the mesocosm showed an initial high number of reads, similarly dominated by anchovy, followed by a drastic drop in reads in the second and third samples (taken on days 8 and 15),

which had only 10 and 37 vertebrate reads, respectively (Fig. 4b). The number of vertebrate reads in the mesocosm
then increased on day 24 due to reads assigned to the family Sciaenidae (drums and croakers), and subsequently an
increase in anchovy reads was also observed. In Fig. 4c, reads assigned to the genus *Paralichthys* appear on days 32
and 36 following the addition of eggs of Fine Flounder *Paralichthys adspersus* on day 31, but are absent in all other
samples. Other taxa of note that were detected by 12S rRNA but are not pictured in the above plots because they did
not meet the abundance threshold to be included were Inca tern *Larosterna inca* (6 reads each in the mesocosm on
days 42 and 48) and brown pelican *Pelecanus occidentalis* (159 reads in the mesocosm on day 36); the detection of
these species is indicated by the corresponding symbols above the bars in Fig. 4b.
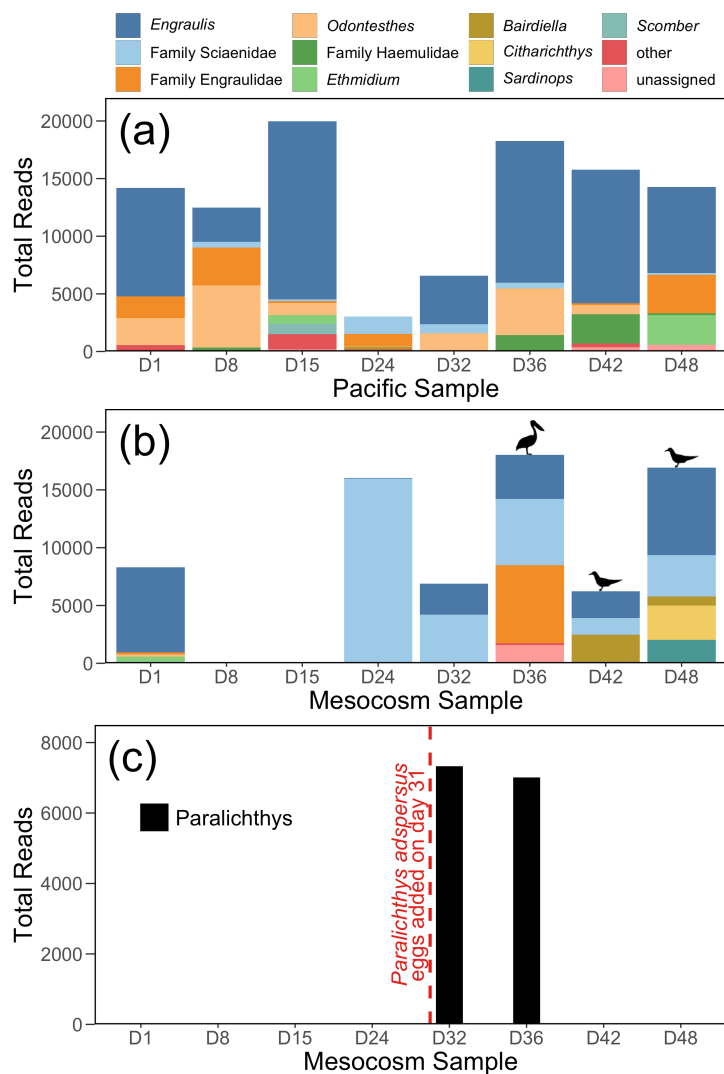
Figure 4. 12S rRNA results, showing top 10 genera, (or families, for ASVs that were not annotated to the genus level) for (a) the Pacific and (b) the mesocosm samples, by total number of reads. Detections of *Larosterna inca* and *Pelecanus occidentalis* are indicated by the symbols in (b). All reads of *Paralichthys* have been removed from (a) and (b). (c) shows the total number of reads of *Paralichthys*, the genus of the fish eggs (*Paralichthys adspersus*) that were added to the mesocosm on day 31. Because there is no available reference sequence for *Paralichthys adspersus*, genus-level annotations are used to assess the detections of this species using eDNA.

490

4 Discussion

495 The Peruvian EBUS is known for its dynamic nature (Penven et al., 2005; Huyer et al., 1991; Echevin et al., 2004), reflected by the physicochemical (warming shift, NO_x^- fluctuations) and ecological (Fig. 3) changes observed in the unenclosed Pacific ocean over our 50-day sampling period. The variability observed in the Pacific during the sampling period is contrasted by the large shift from these upwelling conditions in the mesocosm, which was isolated from the surrounding Pacific and stratified by NaCl brine injections. This resulted in reduced horizontal and vertical mixing,

500 leading to depletion of nutrients (Fig. 2); NO_x^- (Fig. 2c) in particular exhibited a quick and continuous decline, reaching the threshold of detection by day 20. This is in stark contrast to the concentration of NO_x^- in the Pacific, which varied from 2.7 – 19.2 µmol L^{-1}. The observed high concentrations and high day-to-day variability of nitrogen in the natural Pacific samples was indicative of intermittent upwelling, whereby nutrients in the surface waters are replenished from below (Chavez and Messie, 2009; Graco et al., 2017). $Si(OH)_4$ (Fig. 2d) is also lower in the mesocosm and declined

505 in concentration throughout (except for when OMZ water, rich in $Si(OH)_4$, was injected), but never reached levels below 3.4 uM.

The differences in physical and biogeochemical conditions between the Pacific and the eight mesocosms were pronounced. These physical and chemical differences are reflected in the eDNA metabarcoding comparisons of

510 diversity and taxonomic composition of the biological communities in the unenclosed Pacific Ocean and one of the mesocosms (M1). The communities responded in a somewhat predictable manner, with those in the Pacific being characteristic of higher nutrient, upwelling conditions and those in the mesocosms being characteristic of lower nutrient, stratified conditions.

4.1 Phytoplankton communities

515 Phytoplankton are strongly linked to changes in abiotic conditions in upwelling systems, and the connection between environmental conditions and phytoplankton was reflected in the differing communities of the mesocosm and Pacific. Upwelling conditions, as were observed in the Pacific during the experiment, are associated with increased nutrient availability and turbulence (Chavez and Messié, 2009), favouring fast growing non-motile phytoplankton such as diatoms (Margalef, 1978), and newly discovered prasinophyte species (see e.g., Worden et al., 2004; Simmons et al.,

520 2016). Conversely, relaxing upwelling conditions and subsequent stratification (as were observed in the mesocosm) result in reduced mixing, and exhaustion of nutrients (nitrate) in the surface layer. These conditions favour motile organisms like dinoflagellates who are able to vertically migrate to the deeper nutrient-rich layer at night and back to the surface during the day without the hindrance of turbulence (Margalef, 1978; Smayda, 2010; Smayda and Trainer, 2010).

525

The stratified mesocosm was dominated by the mixotrophic dinoflagellate *Akashiwo sanguinea*, a signature bloom species in EBUS (Trainer et al., 2010; Bach et al., 2020). *A. sanguinea* ASVs were found to be strongly mesocosm-associated in both the COI and 18S rRNA datasets (Fig. 4e and 4f), and in the 18S rRNA sequences in particular, *A. sanguinea* was dominant in the mesocosm in the later stages of the experiment, comprising over 50% of the reads in

530 some samples (Fig. S3). *A. sanguinea* reads were a minor proportion of the reads in the COI dataset and absent from the 16S rRNA plastid sequences; the disparity in the detection of *A. sanguinea* by our different PCR primers can be explained by inefficient dinoflagellate amplification by COI primers (Lin et al., 2009) and the loss of most chloroplast genes from dinoflagellates (Koumandou et al., 2004), causing them not to be detected in our 16S rRNA plastidial sequences (Needham and Fuhrman, 2016). While the correlation between cell biomass and amplicon sequence

535 abundance is complicated both by technical biases in metabarcoding and variation in gene copy numbers (Martin et al., 2022), the high biomass of certain taxa as inferred using read numbers is corroborated by other sampling techniques used to sample community composition. In this experiment, *A. sanguinea* was also identified as the dominant dinoflagellate by imaging flow cytometry and microscopy in mesocosm M1, and in seven of eight mesocosms overall (Bach et al., 2020). Bach et al. (2020) report the common diurnal migration pattern of dinoflagellates, at the surface

540 during the day, migrating to the nutricline at night to take up nutrients. Mixotrophy and vertical migration allow *A. sanguinea* to exploit nutrients found below the nutrient-depleted surface layer, especially when there is a shallow thermocline (Kudela et al., 2010), as was the case in the mesocosm in this experiment (Fig. 3a in Bach et al., 2020). Blooms of *A. sanguinea* are regularly observed in EBUS (Du et al., 2011; Kudela et al., 2008; Kahru et al., 2004; Dugdale et al., 1977) under shallow thermocline, stratified conditions, following upwelling relaxation. *A. sanguinea*

545 is typically not found in EBUS phytoplankton communities outside these specific conditions (Kolody et al., 2019; Limardo et al., 2017). Hence, the mesocosm experiment provided the right environmental conditions to initiate a dinoflagellate bloom from the propagules inside the mesocosm, which were either already present in the enclosed water or introduced from the OMZ water additions.

550 In addition to *A sanguinea*, many other dinoflagellate ASVs were found to be mesocosm-associated. These dinoflagellate ASVs included the mixotrophic dinoflagellate *Fragilidium duplocampanaeforme* and its prey, the genus *Dinophysis* (Park and Kim, 2010). A heterotrophic dinoflagellate known to feed on bloom-forming dinoflagellates, *Polykrikos kofoidii* (Matsuyama et al., 1999; Tillmann, 2004), was also detected to be mesocosm-associated. Dinoflagellates comprised about two-thirds of the top organisms in our 18S rRNA PC loadings analysis (Fig. 3f)

555 indicating a strong selection of these organisms under stratified conditions and interesting predator-prey relationships. Other mesocosm-associated ASVs included ones annotated to the family Chrysochromulinaceae in the COI and V1-V2 16S rRNA plastidial datasets, which also likely reflect stratification, as blooms of *Chrysochromulina* have previously been reported in strongly stratified, stable conditions (Edvarsen and Paasche, 1998). Multiple ASVs assigned to the cryptophyte order Pyrenomonadales were found to be mesocosm-associated in the 18S rRNA and V1-

560 V2 16S rRNA plastidial datasets; this could be connected to their green light harvesting phycobiliproteins, which appear to facilitate their growth in low-light conditions (Spear-Bernstein and Miller, 1989), as were found in the mesocosms (Fig. 3c in Bach et al., 2020). However, some Pyrenomondales are predatory mixotrophs, thus changes in relative abundance could also connect to changes in the bacterial community that provisioned prey resources.

565 In contrast, the phytoplankton community of the coastal Pacific ocean was dominated by diatoms, typical of communities during higher nutrient upwelling conditions (Pennington et al., 2006; Chavez et al., 2017; Choi et al.,

2020) as well as the cosmopolitan coccolithophore *Emiliania huxleyi*. As seen by the high levels of NO_x^- in the Pacific (Fig. 2c), upwelling conditions were maintained during the period of study, favouring *r*-selected species like diatoms and maintaining an early succession community (Margalef, 1978; Tyrrell and Merico, 2004). The presence of coccolithophores is indicative of the mixing of some open ocean waters that had come closer to shore due to the coastal

575 El Niño. Although Class II prasinophytes (Mamiellophyceae) were detected in the 16S amplicon data (Fig. S3), they were present at lower relative abundances than the above groups. This is different from observations of prasinophyte importance in more mesotrophic water columns in EBUS off California, USA (Limardo et al., 2017; Kolody et al., 2019; Choi et al., 2020) and off the coast of Chile (De la Iglesia et al., 2020). In the mesocosm, the low levels of nutrients in the mixed layer resulted in lower diatom growth rates and they sank due to increased stratification (Smayda

580 and Trainer, 2010). Some diatom ASVs remained prevalent in the mesocosm, particularly ASVs assigned to the family Skeletonemataceae (Fig. S3), perhaps as seed populations ready to exploit a change in the environment. In the latter part of the experiment they may also have been seeded by the seabird faeces, given the increased presence of birds in the local area. Diatoms are ubiquitous in anchovy stomachs (Espinoza and Bertrand, 2008) and anchovies are favoured by seabirds (Duffy, 1983), and both anchovy and seabird eDNA appeared later in the mesocosm.

585

There were also three dinoflagellate ASVs common in the Pacific. While one of these was assigned to the family Gymnodiniaceae, a broad family of free-living dinoflagellates, the other two were both assigned to the Syndiniales, a parasitic order (Guillou et al., 2008). Among these two ASVs, one was annotated to the family Duboscquellidae, with some species within this family known to parasitize the tintinnid ciliate *Eutintinnus* (Coats, 1988). The ciliate

590 *Eutintinnus* has also been documented to form symbiotic relationships with multiple diatom species (Gómez, 2007, 2020; Vincent et al., 2018). Interestingly, an ASV assigned to the genus *Eutintinnus* was also found in the Pacific, suggesting that both the parasite-host relationship and the symbiotic relationship of this ciliate was found in the ocean surrounding the mesocosms.

595 An unusual and abundant ASV identical to *Cyanobium* sp. Suigetsu-CR5 (Ohki et al., 2012) was found in the coastal Pacific samples. The primers used to amplify 16S rRNA did not provide full-length 16S rRNA gene sequences to confirm that this ASV was the same organism as that isolated from a Japanese lake. However, *Cyanobium* sp. Suigetsu-CR5–like sequences have been reported from the open ocean Northeastern Pacific (Sudek et al., 2015). Taken together, these results suggest that a marine *Cyanobium* sp. Suigetsu-CR5–like organism exists and given the right

600 environmental conditions can become a dominant cyanobacterium.

4.2 Zooplankton communities

The mesozooplankton communities of the mesocosm and Pacific as detected via COI were differentiated primarily by Pacific-associated ASVs assigned to the rotifer family Synchaetidae and the calanoid copepod family Acartiidae (Fig. 3e); these results are in agreement with vertical tows of an Apstein net of mesh size 100 μm during the experiment

605 (unpublished data). Rotifers were detected initially in similar abundance in both the mesocosm and Pacific in the COI data, but declined in abundance in the mesocosm while remaining more abundant, albeit highly variable, in the Pacific

(Fig. S3b). Conversely, ASVs assigned to the copepod family Acartiidae were more abundant in the Pacific than the mesocosm from the start of the experiment in both the net tow and COI data (unpublished data, Fig. S3b), indicating that fewer individuals of this family were trapped within the mesocosm when it was closed, owing to the patchy distribution of zooplankton (Wiebe and Holland, 1968). The most dominant zooplankton taxa found were the calanoid copepod *Paracalanus* (detected by COI and 18S) and the cyclopoid copepod *Hemicyclops* (detected only by 18S); however, these taxa were found in similar abundances in both the mesocosm and Pacific samples throughout the experiment by both eDNA metabarcoding and net tow data and thus did not differentiate the two sampling sites (Fig. S3b and S3c). Copepods have lifespans that are greater than the duration of the experiment (Ianora, 1998), so a significant response of the copepod communities was not captured within the mesocosm relative to the Pacific. Longer term experiments will be required to study responses of animals with generation times of months or greater.

The nanozooplankton community of the mesocosm was separated from that of the Pacific by the higher relative abundance of the family Cafeteriaceae, the higher relative abundance of the cercozoan genus *Protaspis*, and the relative lower abundance of the families Paulinellidae and Vexilliferidae. The family Cafeteriaceae is a made up of heterotrophic nanoflagellates, many of which are filter-feeding bacterivores (Schoenle et al., 2020). Their increased abundance in the mesocosm may reflect their tendency to associate with detritus (Patterson et al., 1993) or adhere to structure (Baker et al., 2018; Boenigk and Arndt, 2000), as was available in the mesocosm. The amoebozoan family Vexilliferidae is ubiquitous in both marine and estuarine environments (Page, 1983), while the amoeboid family Paulinellidae (phylum Cercozoa) contains phototrophic and heterotrophic species which inhabit freshwater, brackish, and marine environments (Kim and Park, 2016).

4.3 Heterotrophic and photosynthetic bacterial community

As seen in the beta diversity analyses in Fig. 3, the bacterial community of the mesocosm diverged from that of the Pacific faster than the communities detected by COI, 18S rRNA, and 16S plastidial sequences, separating on PC1 by day 8 rather than day 15. This may be at least partially explained by the fast growth rate of prokaryotic cells (Zubkov, 2014). The ASVs that were Pacific-associated were representative of typical coastal, phytoplankton rich communities (Needham and Fuhrman, 2016; Buchan et al., 2014). These ASVs included an ASV assigned to the 16S rRNA clade SAR86, one of the most abundant constituents of microbial communities in the surface ocean (Dupont et al., 2012), and an ASV assigned to the family Synechococcaceae, a ubiquitous family of cyanobacteria that is most abundant in nutrient-rich surface waters (Partensky et al., 1999), as was found in the Pacific during the course of the experiment (Fig. 2). Mesocosm-associated ASVs included the methylotrophic family Methylophagaceae (Neufeld et al., 2007), *Pseudohongiella*, a genus recently isolated from the northwest Pacific (Xu et al., 2016; Park et al., 2014), multiple ASVs of the family Rhodobacteraceae, and an ASV assigned to the NS5 marine group. Rhodobacteraceae have previously been documented to increase in response to a bloom of *A. sanguinea* (Yang et al., 2012), as was documented within the mesocosm, and have also been documented to increase in blooms of the harmful dinoflagellate *Alexandrium* (Hattenrath-Lehmann and Gobler, 2017). Similarly, the NS5 marine group has previously been documented to have

increased in abundance with blooms of both *A. sanguinea* (Yang et al., 2015) and *Alexandrium* (Hattenrath-Lehmann and Gobler, 2017).

645 One of the most unusual taxa that was detected was from the order Saccharimonadales within the superphylum *Cand.* Patescibacteria (Parks et al., 2018), which was first detected in M1 on day 15 and in the Pacific on day 24, and subsequently increased in abundance in both M1 and the Pacific. The rise corresponded with an increase in small bacteria and heterotrophic bacteria overall in flow cytometry samples. While this group (Saccharibacteria) has been detected in seawater previously (Hugenholtz et al., 2001) and is commonly found across many different environments

650 from soil to the human gut and oral microbiomes (Kuehbacher et al., 2008; Marcy et al., 2007; Ferrari et al., 2005), some representatives of this group have an unusual symbiotic lifestyle. They were recently isolated from the human oral microbiome where they were found to be epibionts of Actinobacteria (He et al., 2015). Recent genomic evidence reveals that Saccharibacteria have small genomes (< 1 Mb) with genomic contents consistent with a symbiotic lifestyle (Lemos et al., 2019), and microscopy and filter size-fractionation have found them to be very small cells (or "ultra-

655 small bacteria"). To account for their rapid rise in prevalence, it is possible that these Saccharibacteria colonized the mesocosms via a biofilm (outside and inside the experiment itself) or were introduced via seabird faeces, as Saccharibacteria have previously been detected in the avian microbiome (Hird et al., 2015). The timing of the increase is consistent with the appearance of the seabirds on the mesocosms. Since the seabirds were foraging in the vicinity of the mesocosms it is possible that they may have contributed to the increase in the Pacific samples as well. In any

660 case, this observation warrants further study to understand the lifestyle and genomic potential of this enigmatic group.

4.4 Insights into vertebrates

The 12S rRNA metabarcoding data detected distinctly different communities in the Pacific and in the mesocosm. Bony fishes typical of the coastal Peruvian upwelling system, dominated by the Peruvian anchoveta (*Engraulis ringens*) were found in the Pacific samples. In the mesocosm, the species assemblage reflected three experimental

665 perturbations: 1) the exclusion of vertebrates via a 3 mm mesh, 2) the addition of eggs of *Paralichthys adspersus*, and 3) the impact of resting seabirds. During the deployment of the mesocosm, the initial water was filtered through a net of mesh size 3mm, and collection of the OMZ water that was later added was conducted through a net of mesh size 10mm. The fine mesh sizes effectively excluded most, if not all, living vertebrate stages from the enclosure but not their eDNA. The initial vertebrate eDNA in the mesocosm decayed rapidly as evidenced by the extremely low read

670 counts on days 8 and 15 (10 and 37 vertebrate reads, respectively). The virtual disappearance of vertebrate eDNA a week after the start of the experiment is in line with estimates of bony fish eDNA decay rates (Sassoubre et al., 2016), which show an exponential decline in eDNA concentrations with time. Quantification of eDNA decay rates of northern anchovy (*Engraulis mordax*), which is congeneric with the Peruvian anchoveta *E. ringens*, showed that *E. mordax* DNA concentration reached the threshold of detection by a qPCR assay within three days of the fish being removed

675 from the enclosure (Sassoubre et al., 2016). There may have been some eDNA introduced by the addition of the OMZ water; however, the ten-day period between when this water was collected on day 5 and when the first eDNA sampling

occurred following OMZ water addition (day 15) coupled with the decay rate of eDNA makes the detection of this eDNA unlikely.

680 The addition of eggs of the fish *Paralicthys adspersus* ("Fine Flounder") to the mesocosm on day 31 led to a notable spike in *Paralichthys* reads on days 32 and 36; *Paralichthys* was not detected in any other samples in the mesocosm or Pacific. Because *P. adspersus* does not have a reference sequence available in GenBank (release 238.0) for the region targeted by the MiFish primers, no species-level detections were possible, and thus we assessed the detection of this species by eDNA through annotations to the genus *Paralichthys*. The strong signal of *Paralichthys* in the 12S

685 rRNA results is particularly notable because the introduction of fish eggs was not detected via net sampling (Bach et al., 2020). The fish eggs apparently did not develop and decomposed or sank out of the mesocosm fairly quickly. Based on their size, the estimated sinking rate for *P. adspersus* eggs, which average a diameter of 0.66-0.80 mm (Silva and Oliva, 2010), is about 5 mm s^{-1} (Robertson, 1981). Additionally, for any eggs that hatched, larval settling rates for two other species of *Paralichthys* is estimated to be about 10 mm s^{-1} (Hare et al., 2006). With the very stable, stratified

690 waters of the mesocosm, the lack of turbulence likely would have caused any eggs or larvae to quickly settle out of the water column, and coupled with eDNA decay rates, it is not surprising that this signal was no longer detected in the eDNA sample taken on day 42.

The final and most pronounced influence on the vertebrate composition within the mesocosm was the impact of
695 seabirds, which occasionally rested on the mesocosms until day 36, but increased abruptly in abundance thereafter (Bach et al., 2020). DNA from both seabirds and their prey was detected in the second half of the experiment. Inca tern (*Larosterna inca*), the most common bird observed on the mesocosms, was detected in the last two mesocosm samples (days 42 and 48), while brown pelican (*Pelecanus occidentalis*) was detected in the mesocosm on day 36 (Fig. 4b). While bony fish are the intended target of the MiFish primers, these primers are also capable of detecting

700 other vertebrates due to sequence similarity in the targeted region of 12S rRNA gene, albeit with weaker amplification (Miya et al., 2015; Monuki et al., 2021). As such, while we detected seabirds using our primers, they are likely not well represented in our data; primers that specifically target birds should improve assessments of their eDNA (Ushio et al., 2018). Inca terns are known to feed primarily on *E. ringens*, and the defecation by these seabirds into the mesocosm is inferred by the increase in *Engraulis* DNA in the last four samples. Additionally, many reads assigned

705 to the family Sciaenidae (drums and croakers) were detected in the mesocosm, but because of the lack of reference sequences for most of the species in this family that are found in the coastal waters of Peru, it is impossible to determine the identity of these species. However, members of the family Sciaenidae have been documented to be important prey species for seabirds in other regions (Lamb et al., 2017). In addition, the increase in small bacteria (Fig. 2j) in both the mesocosm and the Pacific may be a result of the seabirds defecating in and around the mesocosm.

710 **4.5 Strengths and limitations of eDNA metabarcoding in mesocosm experiments**

Through the use of eDNA metabarcoding targeting multiple genetic loci, we were able to examine community-level changes, detecting 12,244 ASVs assigned to 85 unique phyla; amongst these ASVs, the taxonomy of 816 ASVs could

be resolved to the genus level. Sampling methods commonly used to assess communities often inherently size-select (vertical net tows, Skjoldal et al., 2013), are hindered by both the labour-intensive nature of morphological assessments and the inherent variation in expertise amongst taxonomists (Harvey et al., 2017), and are limited in the taxonomic resolution they provide (e.g. flow cytometry). eDNA metabarcoding provides a method that overcomes some of these limitations: by sampling genetic material in seawater, there is higher sensitivity for detection, no minimum size threshold (as there is in light microscopy or net tows), and metabarcoding of genetic material provides objective taxonomic assignments at higher resolution than morphological identification (Berry et al., 2015), depending on the variable region sequenced and its efficacy for resolving different taxonomic groups (Wear et al., 2018; Parada et al., 2016).

eDNA metabarcoding also comes with a number of challenges and limitations, particularly the issue of incomplete reference databases. For example, amongst the 88 COI ASVs that met the criteria for inclusion in the analysis of PC1 loading scores (Fig. 3e-h), only 17 had a match greater than 95% to a reference sequence. Most of the COI ASVs identified as driving differences between the mesocosm and Pacific samples and visualized in Fig. 3e had percent matches to reference sequences of 80-90% and thus were either annotated to higher orders of taxonomy or left unassigned; the only exceptions to this are the ASVs assigned to *Akashiwo sanguinea*, *Emiliania huxleyi*, *Skeletonema*, and Class V prasinophytes. A similar issue occurred with the 12S rRNA data – because of the lack of reference sequences for Peruvian fishes, most ASVs could only be annotated to the genus or family level. While reference databases are constantly improving thanks to large-scale sequencing efforts (e.g., Rimet et al., 2016; Gold et al., 2021), the lack of reference sequences will continue to hinder metabarcoding efforts, especially for uncharismatic and cryptic taxa.

4.6 Summary and conclusion

In this study multiple marker eDNA metabarcodes were used to follow the evolution of diverse communities in an *in situ* mesocosm and the nearby unenclosed Pacific Ocean over 50 days. A quick divergence between mesocosm and open ocean communities was observed at multiple trophic levels. The mesocosm community evolved quickly into one that is common in stable, stratified conditions (Smayda and Trainer, 2010; Margalef, 1978). Spilling et al., (2018) have noted that in the Baltic, dinoflagellates respond to increases in stratification driven by either changes in salinity or temperature. The Pacific community retained the character of communities found under nutrient-replete, upwelling conditions. Upwelling conditions are associated with increased nutrient availability and turbulence (Chavez and Messié, 2009), favouring fast-growing, non-motile phytoplankton such as diatoms (Margalef, 1978). Conversely, weak upwelling and increased stratification result in reduced mixing and removal of nutrients from the shallow mixed layer (Smayda and Trainer, 2010), favouring motile organisms like dinoflagellates who are able to migrate to the nutricline at night without the hindrance of turbulence (Smayda, 2010; Margalef, 1978). The bacterial communities reflected the changes in phytoplankton composition and likely the influence of seabirds. Many of the abundant bacterial taxa in the mesocosm are reported to increase in response to blooms of dinoflagellates, while typical open-ocean cyanobacteria (Synechococcaceae) and heterotrophic bacteria (e.g., SAR86) were more abundant in the Pacific.

The increase in resting seabirds towards the end of the experiment led to unusual bacterial groups that have been reported to be present in the guts of animals (namely the Saccharibacteria). Primary and secondary consumers in upwelling ecosystems, such as zooplankton and fish, have slower responses to physicochemical conditions so their responses were incompletely resolved in the 50-day experiment.

The method of eDNA metabarcoding is rapidly evolving, facing a series of challenges and opportunities. For example, incomplete reference database issues hindered the confidence of our taxonomic assignments. This was particularly notable off the coast of Peru, where few species have been well-characterized genetically. Nonetheless, the multiple-marker eDNA metabarcoding results presented here show how single samples can yield results that are comparable and complementary to a multitude of traditional and other emerging methods to survey marine biodiversity across the tree of life. The application of multiple genetic markers provided insight into how multiple trophic levels interact under changing physical and biological (seabirds) conditions, revealing coupled changes in bacterial (16S), phytoplankton (18S), zooplankton (COI), and vertebrate (12S) communities. They also revealed evidence of potential predator-prey and parasite-host relationships, whose complexity could be explored further in interaction networks.

The effects of perturbations, either purposeful (additions of OMZ water, brine solution, and fish eggs) or unintended (seabird droppings), on marine communities were clearly resolved with our methods. The perturbations provided new insights into ecosystem processes that are difficult to study otherwise. Mesocosm experiments are challenging because of the difficulties of reproducing physical conditions in contained systems. However, fundamental ecosystem processes in the ocean (i.e. stratification) can be well studied with mesocosms as described here. The selection of dinoflagellates under the combined effect of the salinity-driven stratified conditions in the mesocosm and the warm conditions brought about by the coastal El Niño (which affected both the mesocosm and Pacific) may be an indication of how EBUS could respond to the global environmental changes (in both salinity and temperature) forecast for the future (IPCC, 2014). In support of this, evidence from the fossil record indicates that during the Paleocene thermal maximum, when temperatures where warmer than present, there was a global response of surface-dwelling coastal dinoflagellate communities (Crouch et al., 2001). Mesocosm experiments, like the one studied here, provide a valuable complement to traditional observational studies of upwelling systems. Insights from the genetic methods applied here will guide us towards a more mechanistic understanding of the processes that are shaping EBUS communities in a changing ocean.

Author contributions

MM, DMN, SS, NKT, KP, AZW, and FPC wrote the paper. MM and DMN conducted the data analysis. GMC led the collection and preservation of eDNA samples with assistance from DMN and CP. CP led the collection and preservation of flow cytometry samples with assistance from GMC and DMN. BG analysed the flow cytometry data. EE analysed the nutrients. UR and AL designed the overall mesocosm experiment. FPC and AZW designed the sampling/analyses of samples described in the paper and secured funding to carry out the work.

Deleted: and stratified conditions in the mesocosm

Deleted: will

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Deleted: (i.e. continued global warming)

795 **Competing interests**

The authors declare that they have no conflict of interest.

Special issue statement

This article is part of the special issue "Ecological and biogeochemical functioning of the coastal upwelling system
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Code and data availability

All code and data required to reproduce the analytical results, figures, and tables for this study are available on GitHub
810 at https://github.com/MBARI-BOG/KOSMOS_eDNA_paper (last access: 26 October 2022;
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Ecological divergence of a mesocosm in an eastern boundary upwelling system assessed with multi-marker environmental DNA metabarcoding

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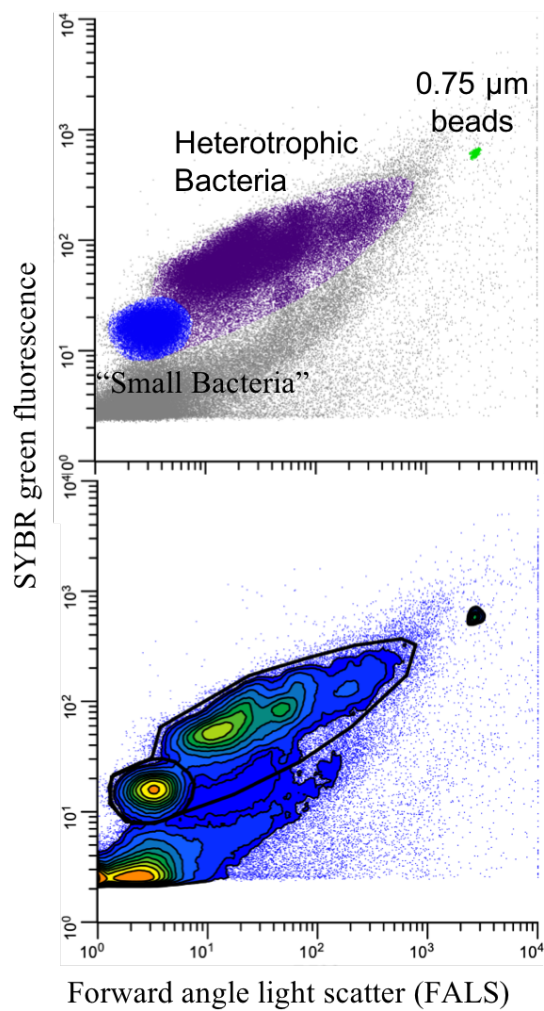
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Figure S1: Representative flow cytogram from Mesocosm 1, day 36 indicating the population of "small bacteria" that became highly prevalent later in the experiment in both Mesocosm 1 and Pacific (Figure 2j). As shown, gating of this population was based on lower forward angle light scatter and SYBR green fluorescence than the larger population of heterotrophic bacteria.

12S
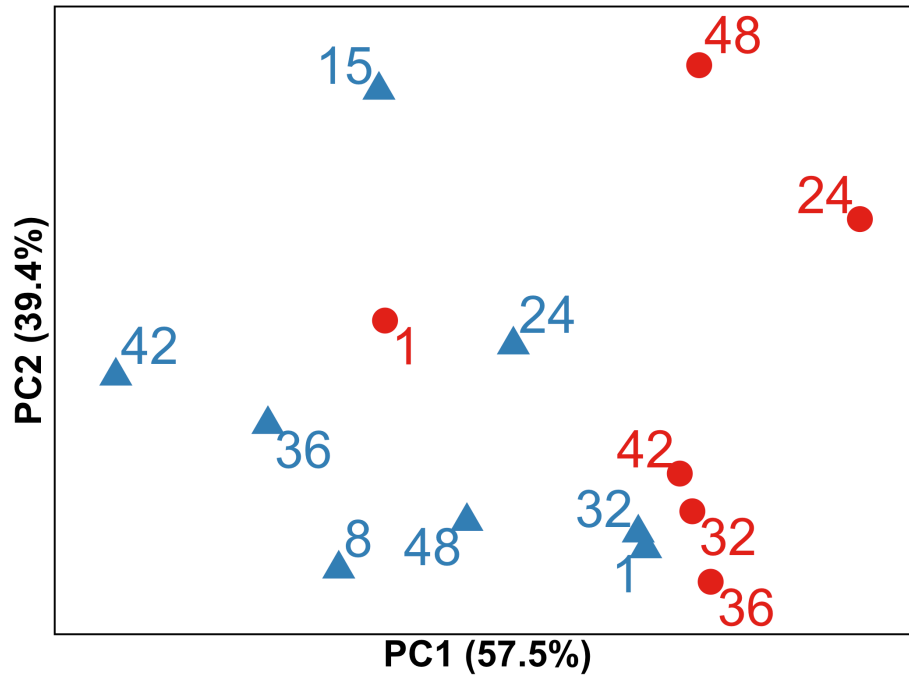
Figure S2. Beta diversity analyses using DEICODE robust Aitchison PCA for 12S rRNA ASVs, subset for only chordate sequences. M1 samples are shown as red circles and Pacific samples are shown as blue triangles. M1 days 8 and 15 were excluded from the analysis because of their low read counts.

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Figure S3. Relative abundance (percent of reads per sample) of ASVs that were among the top 10 most abundant ASVs in any individual sample and that were ever more than 1% of the reads in any sample for (a) 12S rRNA, (b) COI, (c) 18S rRNA, (d) 16S rRNA plastidial ASVs, and (e) 16S rRNA bacterial ASVs. Here, the QIIME 2 classify-consensus-blast plugin using the SILVA 132 database and the 7-level majority taxonomy was used for 18S rRNA sequence classification. The

1290 taxonomy assigned to 16S rRNA ASVs corresponds to that of the SILVA taxonomy.

Supplementary Results 1. Taxon Distributions in M1 and Pacific (Heatmap)

The COI, 18S rRNA and 16S rRNA communities showed differentiation between M1 and the Pacific and over time; for 12S rRNA, because of the deliberate exclusion of nekton from M1, this differentiation is also present but is due to differences in initial conditions rather than change due to experimental manipulation. In the 12S rRNA dataset, the exclusion of vertebrates in M1 is evidenced by lack of data for days 8 and 15, due to the decay of the vertebrate DNA initially trapped in the mesocosm. Throughout the experiment, the Pacific 12S rRNA community is dominated by Peruvian anchoveta, *Engraulis ringens*, which also comes to dominate M1 in the latter half of the experiment due to the impact of resting seabirds defecating into the mesocosm (Fig. S3a). For COI, the dominant zooplankton sequences were the calanoid copepod *Paracalanus* (abundant in both M1 and the Pacific), the family Acartiidae (abundant only in the Pacific), and rotifers of the family Synchaetidae (abundant in both M1 and the Pacific at the start of the experiment, abundant only the Pacific from day 15 on) (Fig. S3b). The phytoplankton community as detected by COI was dominated by the haptophyte *Emiliania huxleyi* (abundant in both M1 and the Pacific initially, only abundant in the Pacific in subsequent samples) and diatoms (more abundant in the Pacific, with the exception of *Skeletonema*, which was more abundant in M1) (Fig. S3b). Additionally, the heterotrophic protist family Caferiaceae dominated for much of the latter dates in M1. In the 18S rRNA dataset, the primary taxa differentiating M1 and the Pacific is the mixotrophic dinoflagellate *Akashiwo sanguinea*, which dominated 18S rRNA reads in M1 beginning on day 24. The phytoplankton community as detected via chloroplast sequences were most dominated by diatoms, in particular those of the groups Skeletonemataceae (especially M1), Thalassiosirales (Pacific), and cryptophytes (mostly M1) (Fig. S3d). The bacterial communities were relatively more "even" and dominated by fewer taxa, but among those most relatively abundant in both M1 and Pacific were Alphaproteobacteria (SAR11 and Rhodobacterales), Bacteroidetes, and Saccharimonadales (Fig. S3e).

Six cyanobacterial ASVs were represented with 500+ reads in at least one sample. Five ASVs had at most one difference over the 275 nucleotides of the V1-V2 16S rRNA amplicon to a cultured representative of *Synechococcus* clade I, II, IV (Rocap et al., 2002) and WPC2 (Choi and Noh, 2009), and *Prochlorococcus* LLIV (Rocap et al., 2002). Surprisingly, the last ASV is identical over its entire length to the V1-V2 region of Cyanobium sp. Suigetsu-CR5, isolated from a Japanese saline lake (Ohki et al., 2012). The same ASV has two mismatches to a *Synechococcus* clade III representative, which is a marine strain and thus the more likely relative of this ASV, but we do not have the additional phylogenetic information (i.e. full length 16S sequence) to resolve this. ASVs most similar to Cyanobium sp. Suigetsu-CR5 have been identified in the Northeastern Pacific before (Sudek et al., 2015). The relative abundances of all six ASVs decreased dramatically over the course of the experiment, both in Pacific and mesocosm. This is in agreement with our flow cytometry data (Fig. 2f) which for *Synechococcus* shows a precipitous decline in the mesocosm from ~340,000 cells ml^{-1} on day 2 to less than 10,000 cells ml^{-1} from day 8 onward. The Pacific control sample shows the same trend though less drastic, starting from only 123,000 cells ml^{-1} on day 2 and dropping to 13,000 cells ml^{-1} or less from day 22 onward (Fig. 2f).

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