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Characterizing the plankton communities of Monterey Bay using eDNA metabarcoding

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ABSTRACT

Environmental DNA (eDNA) is a promising new tool for monitoring marine ecosystem and will be an integral part of a marine biodiversity observation network. In particular, monitoring plankton communities will be of increasing importance, as they form the basis of marine food webs and dominate the biomass of these systems. This study examines eDNA metabarcoding data using the 18S rRNA and cytochrome oxidase I (COI) genes to examine plankton communities in Monterey Bay National Marine Sanctuary (MBNMS) along a latitudinal transect at depths ranging from 0 to 200 m. Plankton communities detected with eDNA were analyzed to determine their correlation with depth, distance from shore, and relation to a dynamic oceanographic front. Communities varied most significantly with depth across all samples, while distance from shore had a smaller but still significant effect. This study identifies drivers of differences in plankton communities as detected by eDNA, helping to inform targeted eDNA sampling to capture the highest amount of biodiversity.

INTRODUCTION

As our oceans continue to undergo unprecedented changes (e.g. Sorte et al. 2010; Doney et al. 2011), monitoring our marine ecosystems has become increasingly critical in improving our understanding of these systems to inform management, conservation, and policy measures. To this end, many integrating sampling efforts, such as the Marine Biodiversity Observation Network (MBON), have begun coordinated and systematic efforts to survey and record the distributions of marine species (Muller-Karger et al. 2014). However, many traditional survey methods for marine organisms are costly as well as time and labor intensive, often limiting the scope of our assessments of marine populations (Murphy & Jenkins, 2010). To address these limitations and needs, genomic methods have recently emerged as alternative ways of assessing marine population health (Bourlat et al. 2013). One of these techniques, environmental DNA, or eDNA, shows promise as a cost-effective and non-invasive biomonitoring tool for marine organisms (Thomsen & Willerslev, 2015).

eDNA is any genetic material that has been shed by organisms into the environment, in forms such as fecal matter, metabolic wastes, skin cells, or gametes (Taberlet et al. 2012). By isolating, amplifying, and sequencing this genetic material, organisms can be detected without ever being directly observed. While early eDNA studies were often focused on a single species in aquatic environments (Ficetola et al. 2008), a technique known as eDNA metabarcoding has emerged as the primary method for characterizing ecosystem-level biodiversity (Ji et al. 2013). eDNA metabarcoding involves the identification of many different taxa simultaneously by targeting a highly variable region of the genome, flanked by regions on either side conserved across the taxa of interest (Valentini et al. 2015). Thus, by comparing amplicons to a DNA reference library, a variety of taxa can be identified from an environmental sample at far less cost and with higher taxonomic coverage (Deiner et al. 2017) than many traditional methods.

While fish are the group currently most studied using eDNA (Hansen et al. 2018), less attention has been paid to lower trophic level organisms, namely zooplankton and phytoplankton. Phytoplankton are responsible for roughly half of all global primary

production (Field et al. 1998), while both zooplankton and phytoplankton play important roles in biogeochemical cycling, have important ecosystem functions, and form the basis of marine food webs (Reynolds 2006, Richardson 2008). Environmental DNA has great potential for monitoring these plankton communities, as a recent comparison of eDNA with morphological identification of plankton revealed that eDNA recovered a considerably higher diversity of species (Djurhuus et al. 2018).

In this study, we characterize the plankton communities of Monterey Bay National Marine Sanctuary (MBNMS) through eDNA metabarcoding, using the Cytochrome Oxidase I (COI) and 18S rRNA metabarcoding markers to recover a wide variety of phytoplankton and metazoan taxa. This study aims to 1) describe the phytoplankton and zooplankton communities of MBNMS, 2) determine the physical drivers of observed differences in plankton communities, and 3) examine the relationship between plankton communities and an evolving oceanographic front. By describing these plankton communities and the factors that determine the distribution and abundance of their associated eDNA, we identify how sampling efforts can be targeted and improved to better characterize marine communities using eDNA metabarcoding.

MATERIALS AND METHODS

FIELD SAMPLING

Sampling was carried out aboard the R/V Western Flyer during the spring CANON (Coordinated, Agile, and Novel Observing Network) Cruise from June 6, 2018 to June 10, 2018. Samples were collected along a latitudinal line offshore of Davenport, CA (36.98 N, -122.29 W to -122.77 W), and were focused on characterizing plankton populations across a developing front created by an upwelling plume. Samples (N = 50) were taken at depths ranging from the surface to 200 m, but were focused at 30 m (N = 20) and 200 m (N = 15). Sampling occurred at five different stations centered around an oceanographic front detected during the cruise. Two of these stations (MWT123, MWT124) were inshore of the front, two (time-series or TS and MWT126) were in the front detected during the cruise, and one (Hot Spot or HS) was offshore of the front. The TS station was established during the cruise at the location where an oceanographic front

was detected. The Hot Spot (HS) station was similarly established during the cruise at a location offshore of the front where there was an increased acoustic signal. All MWT (midwater trawl) stations were not part of the adaptive sampling of the cruise, but rather were established previously.

Seawater samples for environmental DNA (eDNA) were collected using Niskin bottles on a CTD rosette. At each sampling point, a single 1-liter water sample was filtered onto a 0.22 μm polyvinylidene difluoride (PVDF) membrane filter (Millipore, USA). All filters were flash frozen in liquid nitrogen and preserved at -80°C until further analysis. Additionally, a wave glider was used to collect SST data along the transect during the cruise.

DNA EXTRACTION AND AMPLIFICATION

DNA was extracted from filters using the DNeasy Blood and Tissue Kit following the protocol found in Walz et al. 2019. Following DNA extraction, samples were metabarcoded for the 18S rRNA (Amaral-Zettler et al. 2009) and COI (Leray et al. 2013) genes. PCR reactions for each primer set were carried out in triplicate in a 25 μL reaction volume, using 1 μL of DNA, 12.5 μL Amplitag Gold Fast PCR master mix (Applied Biosystems, USA), 1 μL of a 5 μM solution of the forward and reverse primers, and 9.5 μL nuclease-free water. Three PCR blanks (no-template controls) were also used to check for contamination during the PCR process. Thermal cycling parameters for the PCR reactions can be found in Closek et al. 2018. Following the PCR reaction, technical replicates were pooled and visualizing on an agarose gel. Successful amplification was confirmed by the presence of the target band and the absence of non-specific amplification, as well as the absence of bands in the no-template controls (NTCs). PCR products were purified and size selected using the Agencourt AMPure XP bead system (Beckman Coulter, USA). A second agarose gel was run following the bead cleaning step to confirm the retention of the target band and confirm removal of primer-dimer. Purified product was quantified using a Quant-It Picogreen dsDNA Assay (Life Technologies) on an fmax Molecular Devices Fluorometer with SoftMaxPro v1.3.1.

DNA SEQUENCING

Equimolar pools were constructed and quantified to confirm pool concentration prior to library preparation. One library was constructed from the pooled product for each genetic locus using the KAPA HyperPrep and Library Quantification kits following manufacturer's protocol. Libraries were loaded on a standard MiSeq v2 flow cell and one sequencing run per genetic locus was performed in a 2x250bp paired end format using a v2 500-cycle MiSeq reagent cartridge. The MiSeq run for each genetic locus was performed with A 10% PhiX174 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 the output of RTA was demultiplexed and converted to FastQ format with Illumina Bcl2fastq v2.18.0.

BIOINFORMATICS

Sequences were run through a modified version of the banzai pipeline, a custom shell script that uses different programs to process raw DNA sequences from an Illumina sequencing run (O'Donnell et al. 2016). PEAR was used to assemble and filter paired-end reads (Zhang et al. 2013). Homopolymers were removed with grep and awk commands. Samples were concatenated and tags were removed. Primers were removed with cutadapt (Martin 2011) and singletons were removed. Operational Taxonomic Units (OTUs) were clustered with SWARM (Mahé et al. 2014) and Chimeras removed with VSEARCH v1.8.0. Taxonomic annotations for the OTUs for both markers were performed with the GenBank nr BLASTN database downloaded from NCBI. Annotations with >80% identities were retained. These annotations were then interpreted through MEGAN6, which only considered hits that had a bitscore of greater than 100 and were within the top 2% highest scoring hits per contig. The most recent common ancestors of these hits were subsequently determined.

DECONTAMINATION OF SEQUENCING DATA

After running sequences through the banzai pipeline, samples were decontaminated using a custom Python script. This script took the maximum number of reads for each OTU found in any of the three PCR blanks and subtracted them from each sample. This is a conservative way of dealing with index-jumping, as it is more likely that the indices in PCR blanks would bind to DNA on a plate than the indices in environmental samples, due to the lack of DNA to bind to in PCR blanks.

DATA PREPARATION

The decontaminated OTU table was randomly subsampled to an even depth of 16,762 reads for the 18S rRNA marker and 14,487 reads for the COI marker, the minimum number of reads found in a sample. Rarefying accounts for the fact that sequencing depth is not uniform, and that raw read counts are not necessarily biologically informative (McMurdie & Holmes, 2014). Following rarefaction of read counts, all unwanted taxa were removed: unassigned reads, terrestrial contamination (human, pig, and cow DNA), and known incorrect taxonomic assignments (insects, arachnids). Finally, all OTUs were merged together on the basis of unique taxonomic assignment. By doing so, we ignored different OTUs for the same species in order to remove the influence of intraspecific genetic diversity.

To examine our sampling depth as well as patterns in alpha diversity, we also merged the un-rarefied OTU table based on unique taxonomic assignments. As rarefying data to an even sampling depth removes rare OTUs from samples, this was necessary to look at the presence/absence of species. Species accumulation curves were plotted using the un-rarefied data; all other statistical analyses were carried out on the rarefied data.

STATISTICAL ANALYSES

To examine what factors carried the most weight in determining clustering between different samples, a Permutational Analysis of Variance (PERMANOVA) was carried out. This test apportioned variance between four different variables: 1) depth, 2)

time of day, 3) sampling station, and 4) day of the cruise. Nonmetric multidimensional scaling (NMDS) was performed using the Bray-Curtis dissimilarity indices on the 50 samples to look into clustering between samples.

To determine what taxa were influential in determining the clustering of samples, we re-merged the rarefied data by family and carried out a canonical correspondence analysis (CCA), constrained by depth. We then filtered out only those families that had at least 1,000 reads across all samples and a CCA score of at least 0.7 to ensure that we only examined the most influential families. All statistical analyses were carried out in R (RC Team, 2013), using the packages phyloseq (McMurdie & Holmes, 2013) and vegan (Oskanen et al. 2007).

To analyze community change with the movement of an oceanographic front, we calculated the stepwise Bray-Curtis index for samples taken at the front (time series) station at 30 m depth and plotted these values against the temperature measured with the CTD rosette at this depth.

RESULTS

OBSERVED RICHNESS

Sequencing resulted in 1,587,913 reads for the COI gene and 3,793,119 reads for the 18S gene. From these reads, DNA metabarcoding detected 560 unique taxonomic assignments/annotations using the COI marker, corresponding to 31 phyla, 63 classes, 147 orders, 325 families, 128 genera, and 115 species. For the 18S marker, 639 unique taxonomic assignments/annotations were detected, corresponding to 37 phyla, 89 classes, 201 orders, 305 families, 266 genera, and 182 species.

DETERMINANTS OF DIFFERENCES IN COMMUNITY

For both markers, residuals (noise/stochasticity) are the biggest contributors to differences, followed by depth and sampling station. Depth was the most significant driver in community differences for both markers ($p = 0.001$). Sampling station had a significant effect for 18S ($p = 0.01$) but was barely not significant for COI ($p = 0.06$).

Time of day and day of the cruise were both insignificant; however, it should be noted that sampling was not even across days of the cruise and time of day, and as such small sample sizes are considerable contributors to the lack of significance for each variable.

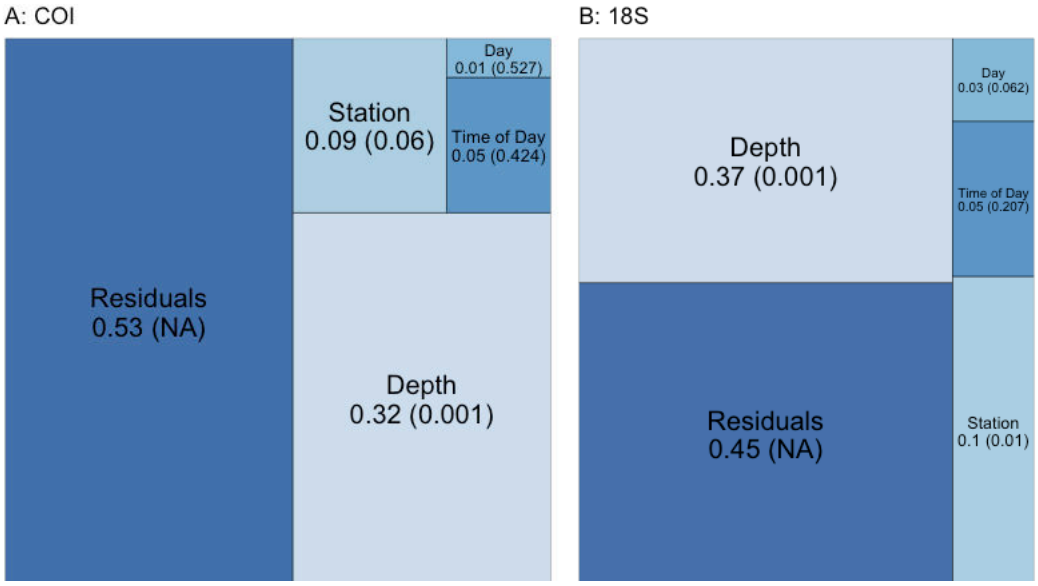

Figure 1. Results of PERMANOVA to apportion differences in samples among different variables. The first number is the amount of variation that the variable explains, and the number in parentheses is the p-value.

EFFECT OF DEPTH

As seen in Fig. 1, depth was the strongest factor in determining the community composition of our samples for both the COI and 18S genes. When plotted in an NMDS plot according to Bray-Curtis distances and colored by depth, a strong depth signature is seen, with the samples being farther apart as the depth difference increases (Fig. 2). Species accumulation curves are shown in Fig. 3, with the 200 m samples showing a higher number of unique taxonomic annotations as well as a steeper slope than the 30 m samples. Curves for both depths begin to flatten out as more samples are added, but none of the curves plateau.

CCA analysis of the different taxa associated by depth across the two markers reveals 19 taxa significantly associated with shallow (30 m) samples (7 detected with 18S, 12 with COI) and 13 taxa significantly associated with deep (200 m) samples (7

detected with 18S, 6 with COI) (Fig. 4). Of the 32 taxa that have a strong depth association, 22 are annotated to the family level; the rest are annotated to a higher taxonomic level (order through phylum). As such, there is some overlap between the taxa, but we will treat them all as unique. Of the 19 shallow-associated taxa, 9 are diatoms, 3 are copepods, two are pelagic crustaceans (families Euphausiidae and Sergestidae), one (Babesiidae) is a family of apicomplexan parasites, one (Nitrosopumilaceae) is a family of marine archaea, one (Flavobacteriaceae) is a family of bacteria, one is a family of brown algae (Chordariaceae), and one is a pipeline classification error (family Planorbidae, a family of air-breathing freshwater snails). Of the 13 deep-associated taxa, four are diatoms (families Bacillariaceae, Thalassionemataceae, and Gomphonemataceae, as well as the reads assigned only to the phylum Bacillariophyta), four are copepods, one is a phylum of green algae (Chlorophyta), one is a phylum of picophytoplankton (Bolidophyceae), one is a family of siphonophores (Forskaliidae), one is the class Siphonophorae (siphonophores; includes Forskaliidae) and one is the class Appendicularia (the larvaceans).

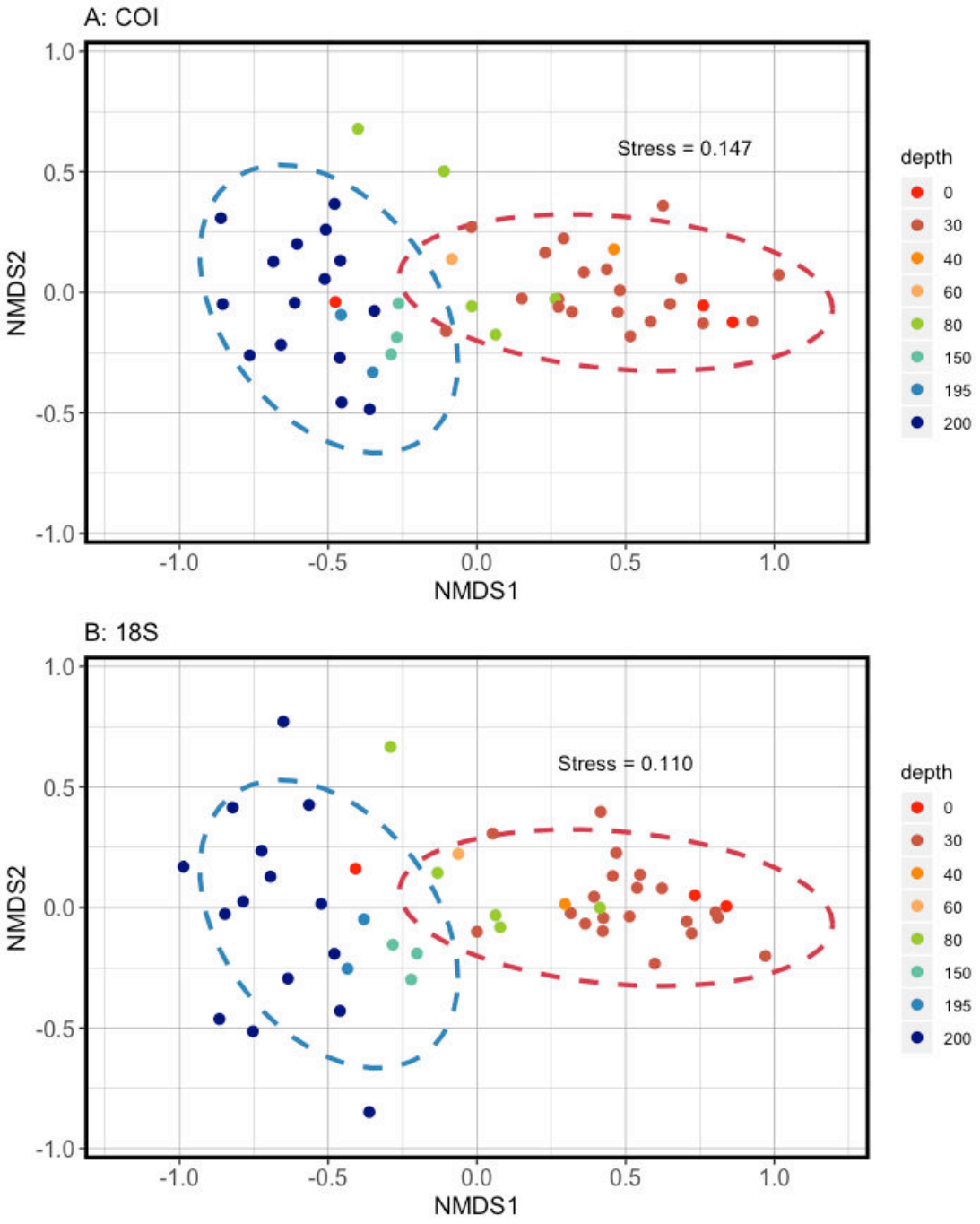

Figure 2. NMDS plots using Bray-Curtis dissimilarity for COI (A) and 18S (B). Ellipses are 95% confidence intervals for two distinct clusters in the ordination.

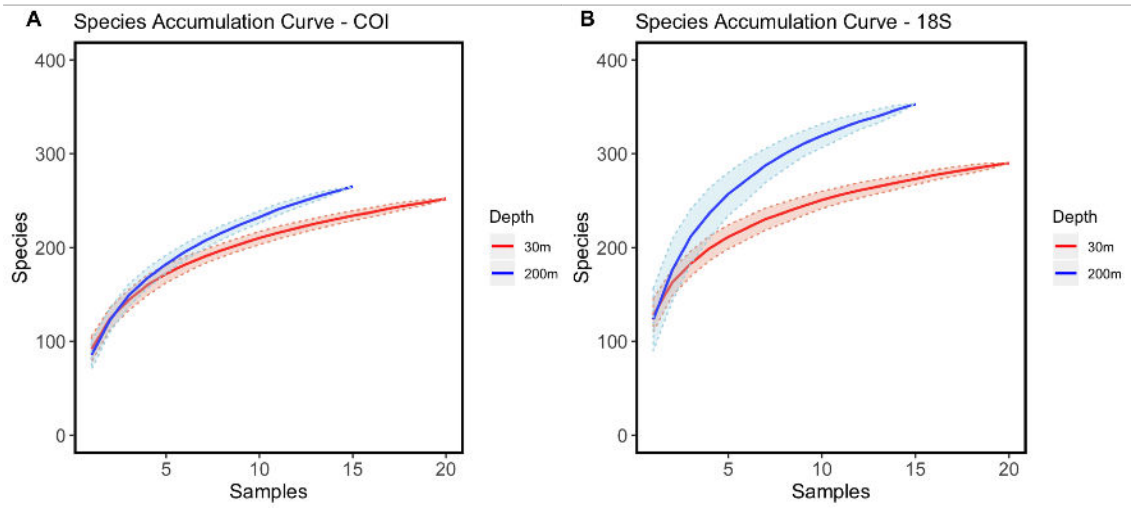

Figure 3. Species (unique taxonomic assignment) accumulation curves for COI (A) and 18S (B). 30 m samples are shown in red, and 200 m samples are shown in blue.

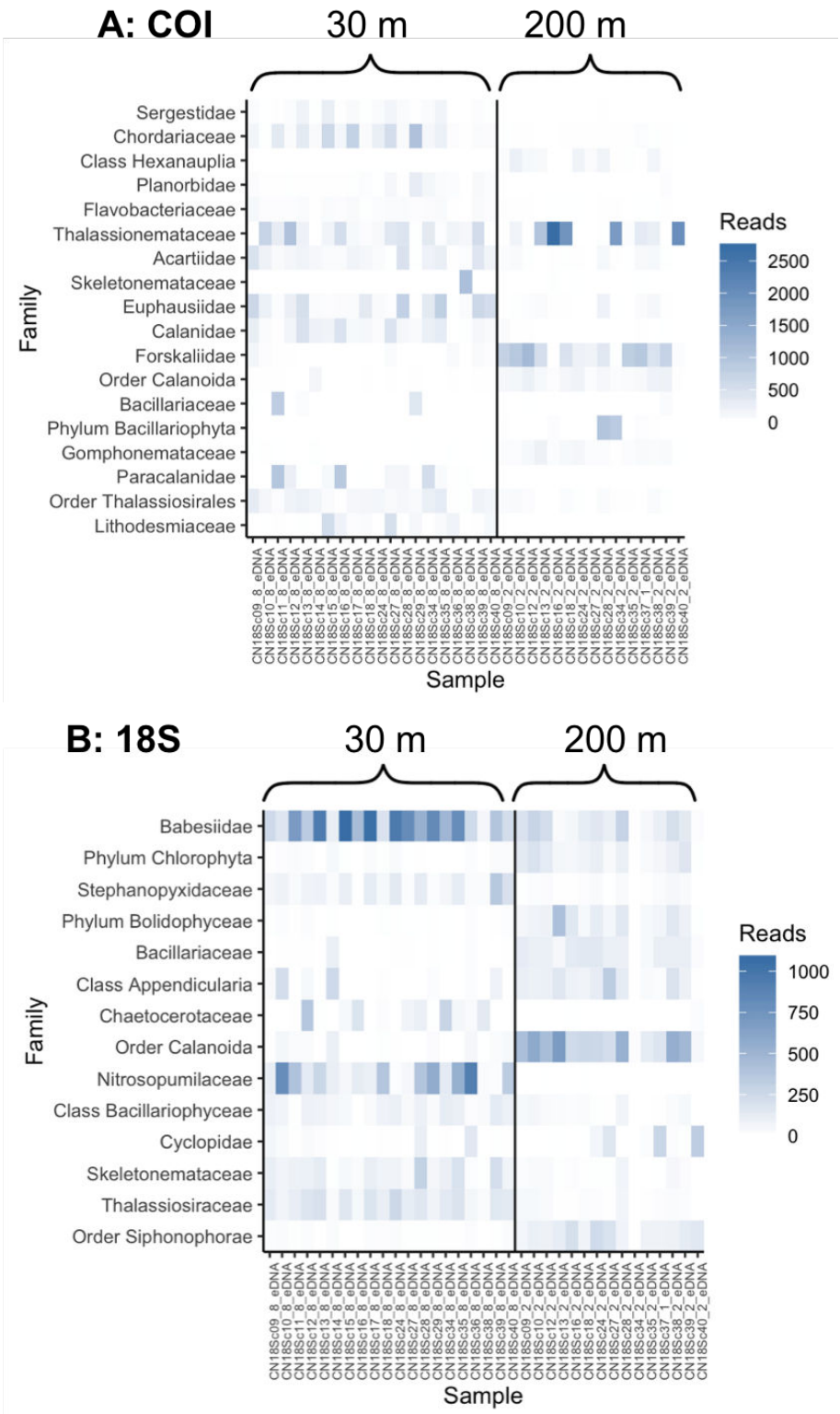

Figure 4. Most influential taxa in differentiating 30 m and 200 m samples, merged by family. If taxonomy was only annotated to a higher rank, that is listed instead of the family.

EFFECT OF SAMPLING STATION

As seen in Fig. 1, sampling station was the second greatest determinant of plankton community following depth, explaining about 10% of the variance for both 18S and COI, with its effect for the community detected with the 18S marker being significant ($p = 0.01$) and the community detected with the COI marker being barely insignificant ($p = 0.06$). These differences are shown in Fig. 5, where inshore and offshore samples taken at 30 m cluster separately, with samples taken in the front clustering in between the two groups. The clustering is tighter for 18S than for COI, aligning with the differing levels of significance for the two markers according to the PERMANOVA.

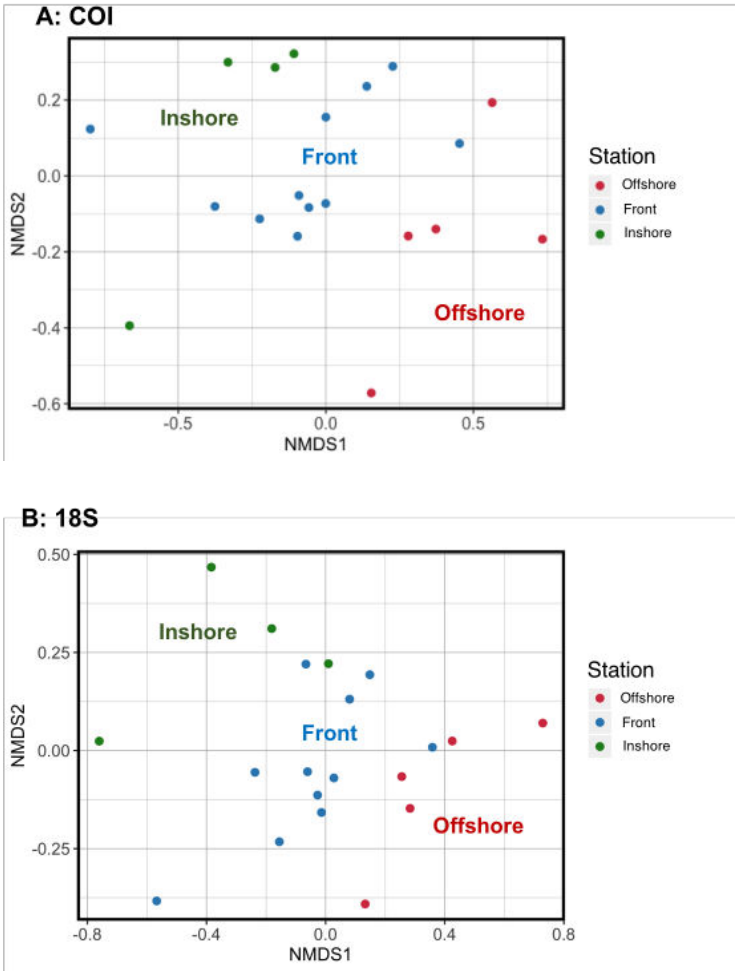

Figure 5. NMDS plot for all samples taken at 30m throughout the research cruise (N = 20) for the COI (A) and 18S (B) genes. The five different sampling stations are grouped into offshore (hot spot, or HS), inshore (MWT123 and MWT124), and front (time series and MWT 126) for ease of visualization.

As seen in Fig. 6, the slopes of the species accumulation curves for the inshore, front, and offshore stations are considerably different, as are the sample sizes. These curves reveal a pattern of alpha diversity where the number of taxa detected decreases as you move farther inshore.

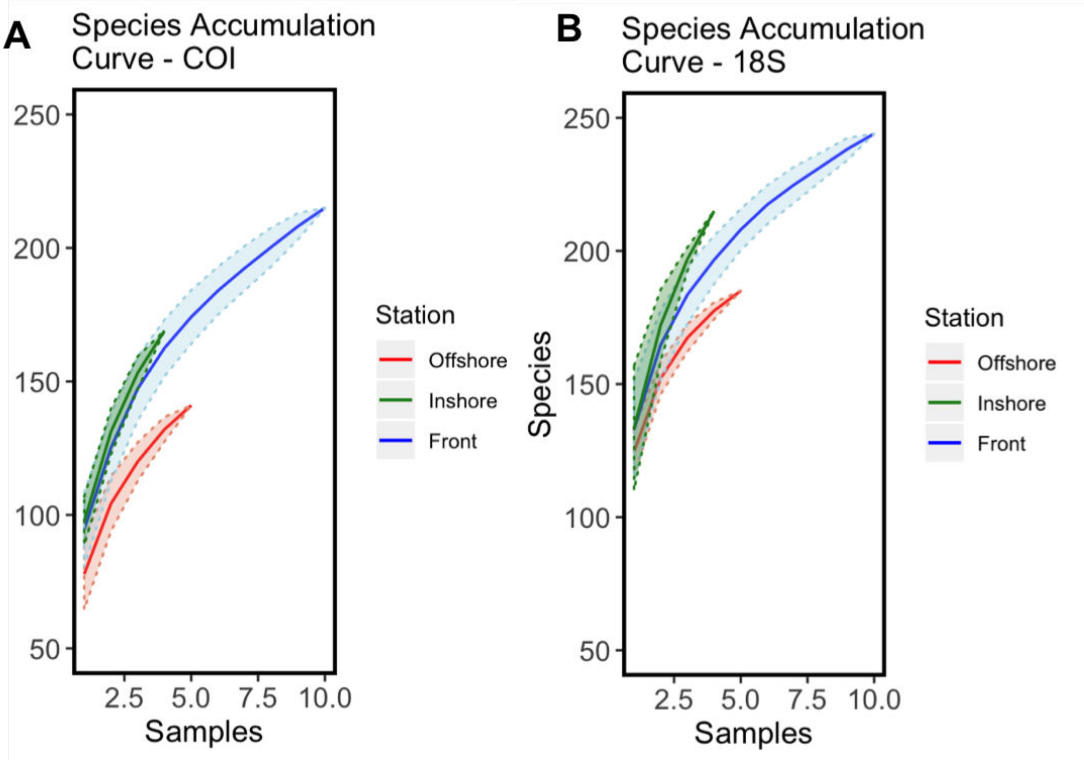

Figure 6. Species (unique taxonomic assignment) accumulation curves for COI (A) and 18S (B) at 30 m, grouped by distance from shore.

COMMUNITIES IN RELATION TO A FRONT

Community change at the front station, which was established during the cruise in the front and sampled 11 times over five days, was measured using stepwise Bray-Curtis dissimilarity values. This index incorporates the abundance of different taxa, measured in number of reads (as opposed to the Jaccard index, which only measures presence or absence), and by measuring the stepwise Bray-Curtis values (one sample compared to the previous sample), we can detect the degree of community change. At the same time, the movement of the front was tracked a waveglider collecting SST data, which created the profiles shown in Fig. 7. By plotting temperature along with these Bray-Curtis values

(Fig. 8), we can compare the amount of community change to the rate and amount of temperature change, which is used as a proxy for changing water mass with the movement of the front. The highest stepwise Bray-Curtis values for both 18S and COI are seen at the fourth time point in the cruise, which corresponds with the greatest rate of temperature change. However, it is difficult to separate the effect of changing water mass from the stochasticity in sampling, PCR, or sequencing.

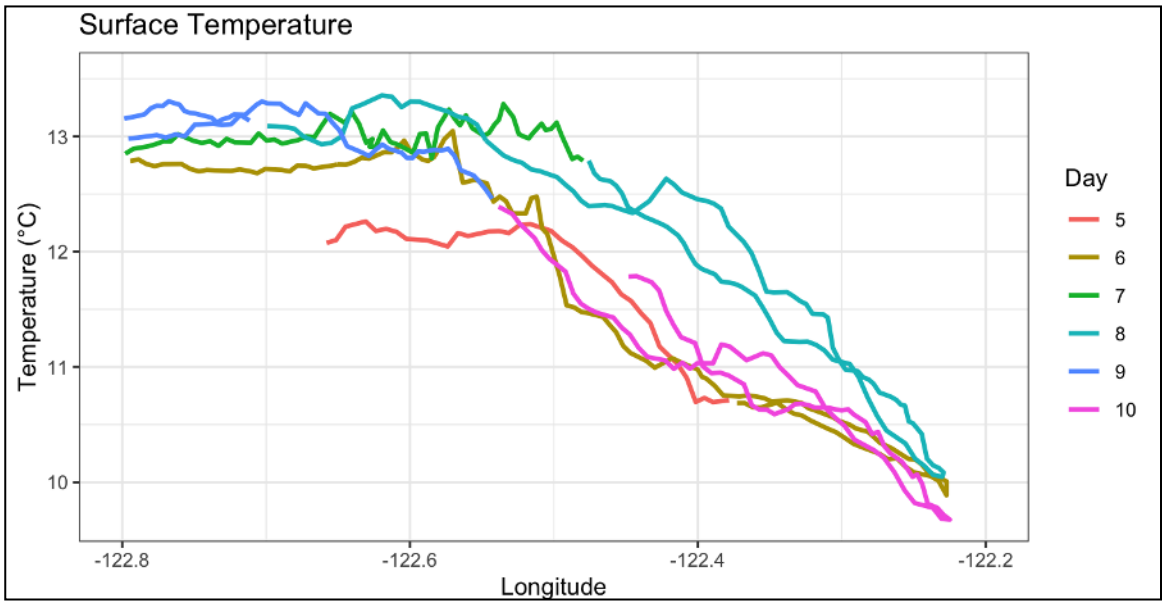

Figure 7. Sea surface temperature as measured by a waveglider during the sampling cruise. There is a clear signature of a warmer, offshore water mass meeting cooler, upwelled water and creating a front. Additionally, the different days show differences in the position of the front, revealing the dynamics nature of the front.

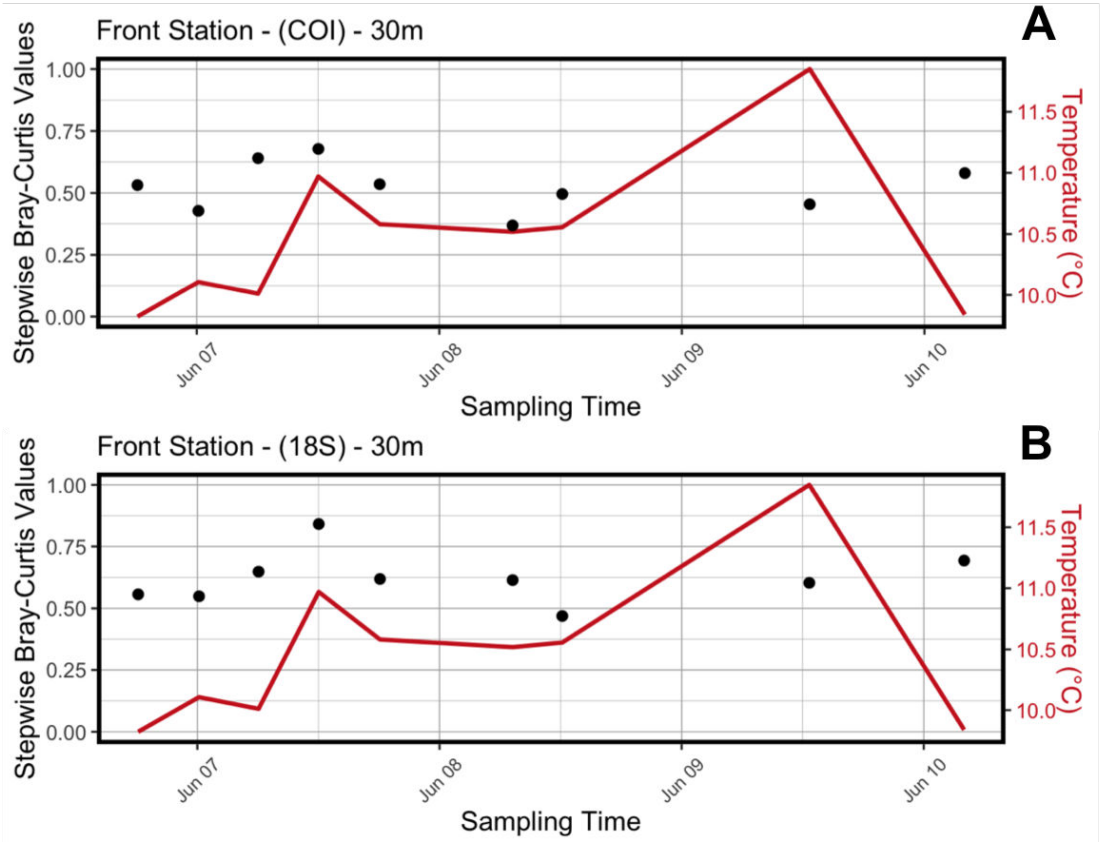

Figure 8. Stepwise Bray-Curtis dissimilarity indices plotted for COI (A) and 18S (B) at the front (time series) station, along with the temperature of the water sampled. These Bray-Curtis values measure the dissimilarity in the community between one sample and the one taken immediately prior, and thus are an index of community change, with higher values representing greater change in the community.

DISCUSSION

DRIVERS OF COMMUNITY DIFFERENCES

As most eDNA studies have focused on a particular, highly localized system such as a kelp forest (e.g. Port et al. 2016) or only at one depth (e.g. Djurhuus et al. 2018), this expansive dataset allowed us to examine gradients of a number of factors and how these affect eDNA signatures.

The fact that eDNA detected the greatest changes in plankton communities with depth shore aligns with previous research using traditional survey methods (Banse 1964). While a handful of eDNA studies have sampled the deep ocean immediately above the

seafloor (e.g. Thomsen et al. 2016), to our knowledge this is the first study looking at midwater eDNA. Although vertical differences in plankton communities have long been known from traditional survey methods (Russell 1927), this distinct depth signature has not yet been detected via eDNA.

The detection and significant association of some photosynthetic organisms, such as diatoms, at a depth of 200 m, has multiple potential explanations. This may be a result of the diatoms detected vertically migrating (Pearre 2003) and their DNA being present at depth because of their presence in the layer. Additionally, as many diatoms are larger than the 0.22 μm membrane size on our filter (Round et al. 1990), it is possible that whole diatoms were captured. Another possible explanation, which differs in that the diatoms are not actually present at depth, is the sinking of genetic material from the surface layers and being detected at depth. eDNA has been well documented to sink in aquatic systems (Turner et al. 2014, Turner et al. 2015), and the sinking of particles containing genetic material has been well documented in the ocean as well (Shanks & Trent 1980). As such, their detection may not be actually be indicative of their presence at depth, but rather the sinking of their genetic material, but this requires further research.

The differences in communities with distance from shore are likely explained by two factors: 1) the topography of Monterey Bay and 2) the relation of the sampling locations to an upwelling plume. A previous study of phytoplankton in Monterey Bay showed the effect of sea floor topography on the pelagic plankton community (Ryan et al. 2005), which similarly would have had an effect on our sampling transect, as the line off of Davenport starts on the shelf and moves off the shelf break. As Point Año Nuevo is known to be an upwelling center for Monterey Bay (Rosenfeld et al. 1994), our sampling transect off of Davenport also started closer to the upwelling plume and moved offshore, which would have also had a significant effect on the plankton communities.

COMMUNITIES IN RELATION TO AN OCEANOGRAPHIC FRONT

One of the primary goals of the adaptive sampling used by the 2018 spring CANON cruise was to detect and monitor the evolution of a front, collecting eDNA data to see how the community changed over time. By establishing a stationary time series

station during the cruise at the location that the front was at the start of the cruise, we were not able to track the front directly, but instead tracked a location where the front was repeatedly moving back and forth, as seen in Fig. 7. Fig. 8 reveals a slight correlation between Bray-Curtis dissimilarity values and changes in temperature, but it is difficult to discern these differences from stochasticity arising from sampling, PCR, or sequencing due to the lack of biological replicates. Without biological replicates to establish a baseline level of stochasticity (between replicates), it is difficult to parse apart actual ecological insights from noise.

CONCLUSIONS/RECOMMENDATIONS

Plankton community signatures as detected by eDNA varied significantly with both depth and distance from shore, matching observations made with traditional survey methods. Depth in particular is of great interest for deeper analysis and future research, as it is unknown how eDNA varies with depth and how this matches observations from other sampling methods such as video transects or acoustic data.

eDNA has great potential for providing a cheap, quick, and non-invasive method for observing our oceans and detecting patterns in marine biodiversity. By determining what aspects of where a sample was taken are the biggest contributors to the eDNA recovered, we can target our sampling efforts to capture the greatest amount of biodiversity. Thus, by identifying physical drivers of communities detected by eDNA, we can improve the utilization of eDNA metabarcoding in observing marine ecosystems. However, while eDNA metabarcoding holds great promise for characterizing ecological communities, there is still a great amount of uncertainty surrounding how well eDNA reflects the organisms of the sampled water mass. Processes like eDNA production, degradation, and transport are poorly understood and vary by organism and system studied (Hansen et al. 2018). Thus, a better understanding of these processes is vital in improving confidence in the ecological significance of eDNA results and will improve the applicability of eDNA for conservation and management.

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