Monterey Bay Aquarium Research Institute

Using eDNA to Observe How Marine Life Responds to Changing Ocean Conditions in Monterey Bay, California

Emma Rosso, Masters of Conservation Medicine Candidate, Tufts University Cummings School of Veterinary Medicine
Mentors: Francisco Chavez, Kathleen Pitz
Summer 2021

Keywords: community diversity, environmental DNA (eDNA), metabarcoding, spatial/ temporal distribution

ABSTRACT:

[will write after all other sections are completed]

…

INTRODUCTION:

As the world's oceans face threats associated with global climate change, such as ocean acidification and rising sea temperatures, there is a growing need to monitor how marine life is changing to adapt [citation]. This can be difficult when marine communities are already poorly understood, especially when they are microscopic and elusive to traditional monitoring methods [citation]. Environmental DNA (eDNA), which is the DNA that is shed by an organism into its environment [citation, USGS], can be a useful tool for keeping track of the organisms that have been "swimming through" an area. More specifically, eDNA can be used to better understand community diversity and abundance in a marine ecosystem [citation], by a process known as metabarcoding, or multi-sequence genetic analysis [citation].

Among the most recent applications of eDNA metabarcoding is determining how a marine community can change with spatial, temporal, and environmental changes (Djurhuus et al., 2020). Different types of organisms can be detected with eDNA, however certain genetic markers are more optimal than others in doing so, depending on the taxa of interest. The 18S ribosomal RNA (rRNA) marker is commonly used to detect planktonic eukaryotes, mainly protozoa like diatoms and dinoflagellates, which are common in most marine ecosystems [citation]. Because of plankton's high sensitivity to their environment, their community structure can change dramatically with minute changes [citation], which makes them valuable in helping predict large-scale changes in the future due to climate change.

Time-series eDNA metabarcoding data was collected in Monterey Bay, California, and analyzed to determine whether there appeared to be a relationship between change in marine community structure with that of time, location, and sea surface temperature. Monterey Bay is the study site of interest because of its unique upwelling system (Chavez & Messié, 2009) and regularly recorded seasonal and interannual changes in sea surface temperature, as can be seen in Figure 1D, taken from Chavez et al. (2017). The Monterey Bay Aquarium Research Institute (MBARI) has three main sampling stations, one directly on the coast (C1), and two moorings further out into the bay (M1 and M2) (Figure 1D), which adds a dimension of spatial location in the eDNA metabarcoding data.

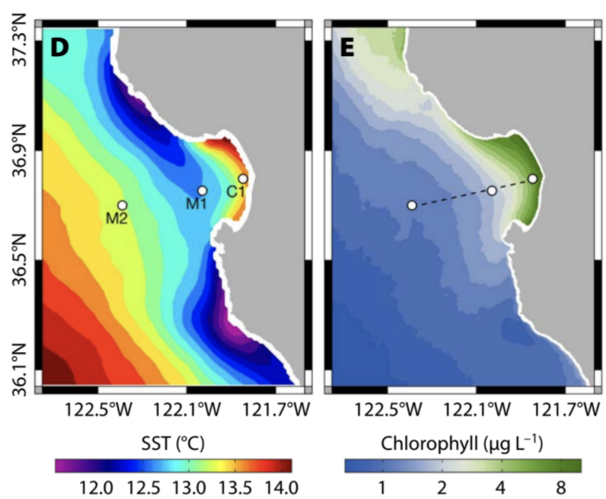


Figure 1. (D) Average springtime SST. (E) Surface chlorophyll for Monterey Bay and the central California Current System with locations of time-series stations C1, M1, and M2, as well as the underway ship transect (dashed line).

METHODS:

SAMPLE COLLECTION:

[prior to internship, investigate how methods were done]

(Time Series: 2008-2017) ...

DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING:

[prior to internship, investigate how methods were done]

...

DATA PREPARATION AND BIOINFORMATICS:

The raw 18S eDNA sequencing data was processed in Qiime2 2021.4, a pipeline program that turns DNA sequences into readable data frames that can be used for bioinformatics and other analyses [citation]. Through Qiime2, a DEICODE Aitchison Distance was run to convert the data frames into non-zero matrices of principal components. Principal components, or PCs are highly-variable, independent variables [better word?] that are given a score to be compared with other PCs. Here, the two primary principal components are the variability between seasons of the data points, which is classified as PC1, and the variability between years, classified as PC2.

STATISTICAL ANALYSIS:

In RStudio Version 1.4.1103, seasonality (PC1) and interannuality (PC2) were compared to one another via a principal component analysis (PCA) according to differences in location (sampling station) and time (month and year). This was done to show how much planktonic community diversity was changing in response to these different spatiotemporal factors. Also, each individual PC score was plotted over time, to show when variability in community diversity was at its highest and lowest, within and between years. Correlation tests and plots were also run to make comparisons between each of the three sampling stations (C1 vs. M1, C1 vs. M2, etc.) according to either their PC1 score or their PC2 score. For each station-to-station correlation, a Pearson's correlation coefficient was calculated to assess its strength.

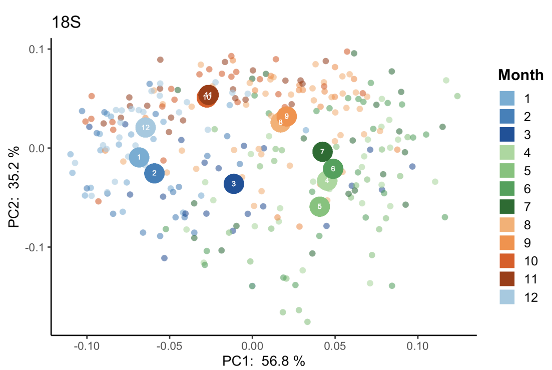
[how to mention/cite tidyverse packages?]

To assess for changes in community diversity in response to sea surface temperature (SST), data from [Diego Sancho - citation] of SSTs converted to PC1 scores were plotted against this study's PC2 scores in a correlation plot. This was done to determine if the interannual variability in planktonic community structure is associated with seasonal changes in SST in Monterey Bay. The PC1 and PC2 scores represented all of those collected at the three stations, with SST data modified to only reflect data collected between 2008 and 2017.

RESULTS:

PRINCIPAL COMPONENT ANALYSIS (PCA):

In the robust PCA plot for PC1 vs. PC2 by month, there was high variance in community diversity in both seasonality and interannuality, however there was more spread in seasonality. Also, January (Month 1) has a low PC1 score, with each progressive month increasing in PC1 score until June (Month 6), where it decreases again until January, showing a cyclical change in diversity with a change in the time of year (Figure 2a). In the RPCA by year, on the other hand, variance was high in interannuality (PC2) and low for seasonality (PC1), and there appears to be an overall increase in PC2 score each year (Figure 2b).

(a)


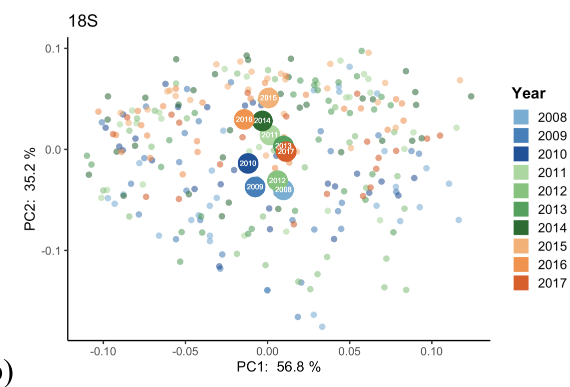
(b)


Figure 2. Mean RPCA Scores for 18S eDNA marker, plotted as PC1 vs. PC2, colored by (a) Month and (b) Year.

In the RPCA plot for PC1 vs. PC2 by sampling station, there is moderate variance for seasonality (PC1), but little to no variance for interannuality (PC2). Also, points for Mooring 1 are mostly clustered in the positive PC1 range, while points for Mooring 2 are mostly clustered in the negative PC1 range, and points for C1 are more generally clustered (Figure 3).

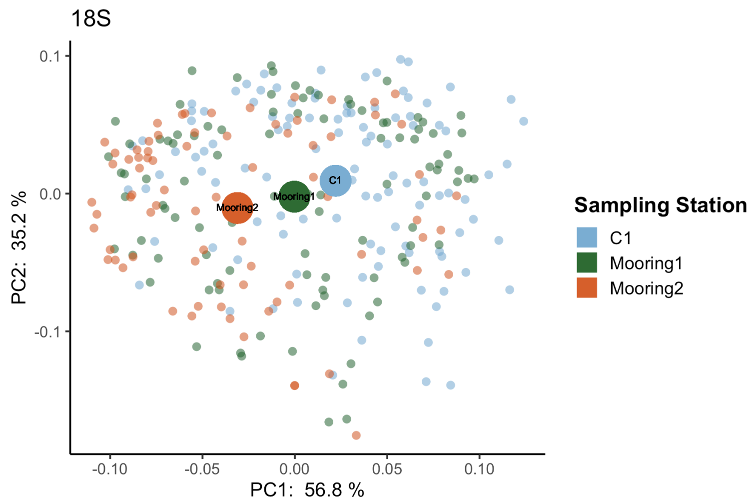
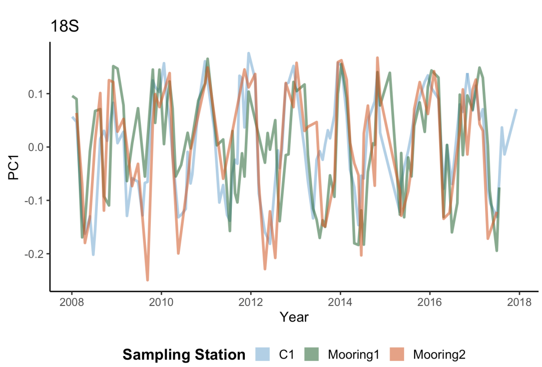


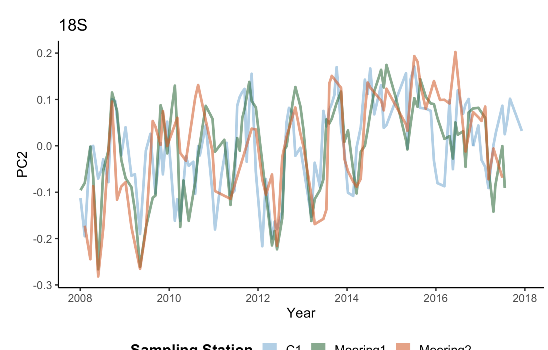
Figure 3. Mean RPCA Scores for 18S eDNA marker, plotted as PC1 vs. PC2, colored by Sampling Station.

CHANGE IN PC SCORES OVER TIME:

Plotting PC1 scores over time, seasonal shifts in community diversity of plankton can be seen at all three stations, with the variance being its highest during the winter and lowest during the summer (Figure 4a). For PC2 scores over time, shifts in diversity are cyclical overall, however, since around 2015, the variance has remained at higher levels, and only began decreasing again in 2017 or so [associated with ENSO?]. For both Figure 4a and 4b, the three sampling stations all exhibit similar trends in planktonic diversity variance over time.



(a)



(b)

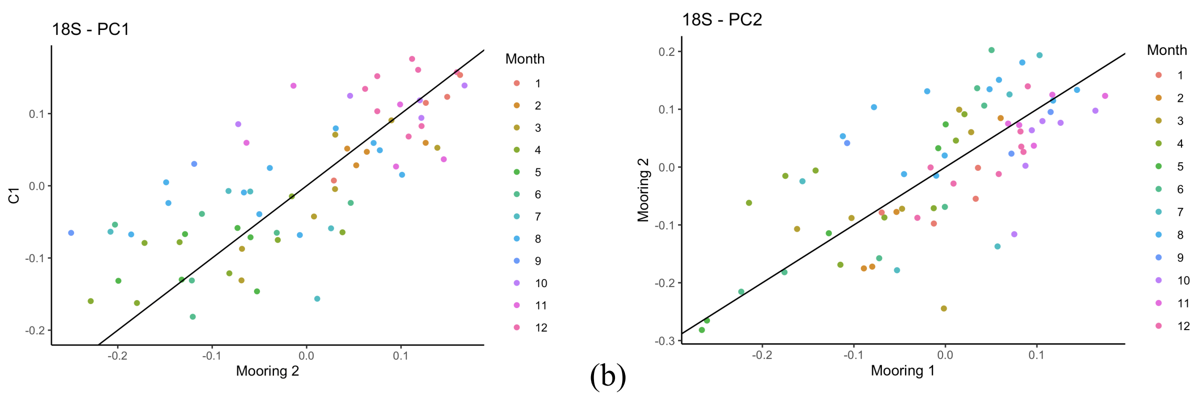
Figure 4. (a) Change in PC1 over time (Year) at 18S eDNA marker, colored by station. (b) Change in PC2 over time (Year) at 18S eDNA marker, colored by station. In (b), PC2 for Mooring 1 represents -PC2.

STATION VS. STATION CORRELATION:

According to Table 1, each Pearson's coefficient indicates moderate to high correlation, which means each station's PC1 and PC2 scores are moderately to highly correlated with those of the other stations'. The highest correlation for PC1 was between Mooring 1 and C1 (r = 0.75), which means that the season-to-season difference in diversity was highest between the coast and one of the moorings further out in the bay (Figure 5a). One the other hand, the highest correlation for PC2 was between Mooring 1 and Mooring 2 (r = 0.69), meaning the year-to-year difference was highest between the two moorings (Figure 5b).

PC SCORE	STATION VS. STATION	r
PC1	M1 vs. M2	0.5733858
PC1	M1 vs. C1	0.6104453
PC1	M2 vs. C1	0.7450341
PC2	M1 vs. M2	0.6927605
PC2	M1 vs. C1	0.5807497
PC2	M2 vs. C1	0.5319611

Table 1. Pearson's coefficients (r) for all PC1 and PC2 comparisons between the three stations.



(a) (b)

Figure 5. (a) Correlation plot for PC1 scores at C1 vs. M2, with Pearson's correlation coefficient of r_A=0.7450341. (b) Correlation plot for PC2 scores at M2 and M1, with Pearson's correlation coefficient of r_B=0.6927605.

SEA SURFACE TEMPERATURE VS PC SCORE CORRELATION PLOTS:

[calculate p-value to confirm significance]

There was a low, yet still significant, correlation between interannual variability (PC2) in planktonic community diversity and seasonal (PC1) sea surface temperatures at all three stations (r = 0.34). There does not appear to be a noticeable month-to-month trend in this correlation (Figure 6).

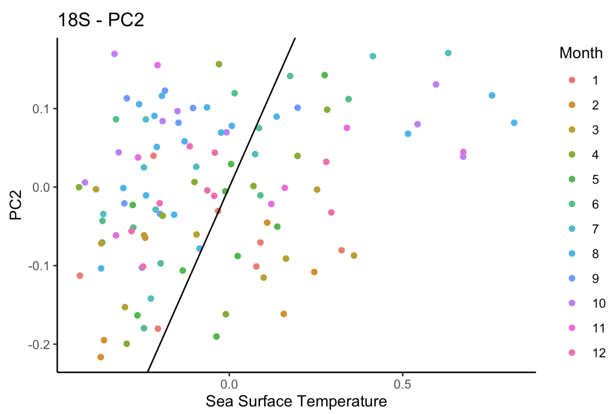


Figure 6. Correlation plot of PC2 score vs. sea surface temperature with Pearson's coefficients r_B=0.335851). SST is a PC1 score, representing its seasonal variance.

DISCUSSION:

DIVERSITY IN RELATION TO TIME, LOCATION, AND TEMPERATURE:

Temporal trends in planktonic community diversity in Monterey Bay, California, occurred seasonally and inter-annually, however, seasonal changes were generally more cyclical and consistent from year to year, while inter-annual changes followed a more irregular pattern, possibly in response to the El Niño-Southern Oscillation (ENSO) or perhaps even factors associated global climate change [citation]. Spatial trends appeared to have seasonal variance, gradually moving from nearshore to offshore, with the greatest correlation in change being between the C1 station right on the coast and Mooring 2, which is farthest away from it. In terms of interannual variability, the change in proportional plankton abundance was more similar between the two moorings, further out into the bay.

Data plotting year-to-year community structure change against seasonal changes in sea surface temperature (SST) suggest that a relationship between the two phenomena exists, however, it is not as strong as with spatiotemporal factors. That being said, perhaps SST affects planktonic communities in Monterey Bay in ways other than the one assessed for this study. Perhaps the interannual changes in SST have more of an impact, especially with the concern that ocean temperatures are rising worldwide due to climate change.

COMPARISON WITH OTHER DATA:

[will look further into this]

CONCLUSIONS/RECOMMENDATIONS:

- Include Taxonomic Data
- Investigate Further: eDNA and pCO_2, Temperature, Salinity
- Upwelling, Ocean Acidification

ACKNOWLEDGEMENTS:

I would like to thank my mentor, Francisco Chavez, for his support and guidance throughout the course of this internship. I would also like to thank Katie Pitz and Jacoby Baker for the R scripts I used for my analysis, as well as for their troubleshooting support. I would also like to thank Reiko Michisaki, Nathan Truelove, and Monique Messié for all of their feedback and advice. Finally, I would like to thank George Matsumoto, Megan Bassett, and Lyndsey Claassen for coordinating the MBARI Summer Internship Program. Funding for the program includes the Dean and Helen Witter Family Fund, the Rentschler Family Fund in memory of former MBARI board member Frank Roberts (1920-2019), and by the David and Lucile Packard Foundation.

References:

Chavez, F. P., & Messié, M. (2009). A comparison of eastern boundary upwelling ecosystems. *Progress in Oceanography*, *83*(1-4), 80-96.

Chavez, F., Pennington, J., Michisaki, R., Blum, M., Chavez, G., Friederich, J., . . . Messié, M. (2017). Climate Variability and Change: Response of a Coastal Ocean Ecosystem. *Oceanography, 30*(4), 128-145. Retrieved July 9, 2021, from http://www.jstor.org/stable/26367631

Djurhuus, A., Closek, C. J., Kelly, R. P., Pitz, K. J., Michisaki, R. P., Starks, H. A., ... & Breitbart, M. (2020). Environmental DNA reveals seasonal shifts and potential interactions in a marine community. *Nature communications*, *11*(1), 1-9.

Gallego, R., Jacobs-Palmer, E., Cribari, K., & Kelly, R. P. (2020). Environmental DNA metabarcoding reveals winners and losers of global change in coastal waters. *Proceedings of the Royal Society B*, *287*(1940), 20202424.

Tsuji, S., Takahara, T., Doi, H., Shibata, N., & Yamanaka, H. (2019). The detection of aquatic macroorganisms using environmental DNA analysis—A review of methods for collection, extraction, and detection. *Environmental DNA*, *1*(2), 99-108.

USGS eDNA (cite):

https://www.usgs.gov/special-topic/water-science-school/science/environmental-dna-edna?qt-science_center_objects=0#qt-science_center_objects

[citation for Qiime2 and RStudio]

[citation for Diego Sancho's SST data]