Monterey Bay Aquarium Research Institute

Exploring drivers of Northern Anchovy abundance in Monterey Bay using an environmental DNA time series

Emma Keller, M.S., University of California, Santa Cruz

Mentors: Dr. Lis Henderson, Dr. Francisco Chavez

Summer 2022

Keywords: eDNA, Northern anchovy, time series

ABSTRACT:

Efforts to predict the abundance of the Northern anchovy population in the California Current Ecosystem have been pursued for decades due to their ecological and commercial importance. Previous studies have explored dietary preferences, climate influence, and top-down mechanisms such as predation. Environmental DNA (eDNA) is a new tool in the oceanographic field being used for assessing biodiversity, relative abundance, and inter-species interactions. We examined factors that contribute to anchovy abundance using a 12-year time series of eDNA data in Monterey Bay. We found little linear correlation with diet, but a significant negative correlation with the North Pacific Gyre Oscillation Index. In addition, we explored how the eDNA data varies in different locations in Monterey Bay and found spatial and temporal patterns for both anchovies and sardines that are potentially reflective of the species habitat range, migratory patterns, and spawning activity.

INTRODUCTION:

The Northern anchovy, *Engraulis mordax*, serves as a critical energy conduit between upwelling-driven productivity and upper trophic level organisms in the California Current Ecosystem (CCE). Despite only encompassing less than 1% of the global ocean, primary productivity in eastern boundary upwelling currents such as the CCE is responsible for nearly 17% of the total global harvest of fish (Pauly & Christensen, 1995). Anchovies in Monterey Bay attract a cascade of biological activity; including whales, seabirds, and other fishes that come to feed off them (Santora et al., 2020; Sydeman et al., 2020). Northern anchovy and Pacific sardine (*Sardinops sagax*) populations flourish in eastern boundary current upwelling systems globally, and one typically exists in abundance without the other, referred to as a "regime." These regimes were previously thought to be alternated by changes in temperature, where anchovies favored cooler temperatures, and sardines warmer temperatures, changes ignited by basin scale processes such as the Pacific Decadal Oscillation and the El Niño Southern Oscillation (Chavez et al., 2003). However, the current anchovy regime we are seeing now in Monterey Bay is being accompanied by a warm sea surface temperature anomaly (Chavez et al., 2017; Thompson et al., 2019). This prompts us to investigate other ocean conditions that may be affecting anchovy recruitment, physiology, and behavior.

Forage fish such as the Northern anchovy are highly sensitive to changes in their surrounding environment, especially when switching from yolk sac feeding to first feeding (Portner & Peck, 2010; Lasker et al., 1970). Prey availability, species composition, and extended periods of mixing that displace prey are all factors that can contribute to anchovy population abundance (Lasker et al., 1970; Wroblewski & Richman, 1987). Prey density is critical for first-feeding anchovies that need to consistently feed to reach adult stages. As our climate continues to change, and as fisheries start to incorporate ecosystem-based management practices, it is becoming increasingly important to identify the reasons behind large shifts in species composition, particularly for forage fish which are an essential food source for other organisms (Field et al., 2006).

Environmental DNA (eDNA) is emerging as an effective tool to identify ocean community composition and inter-species interactions by applying established genomic principles to seawater samples (Djurhuus et al., 2020). Organisms leave behind microscopic tissue, cells, and other debris in the ocean that are then sequenced by collecting seawater samples. This provides a less invasive, less costly, and less labor-intensive method of species identification in comparison to traditional methods. Species that would not normally be detected due to their elusive nature or rarity are captured, which has the potential to provide a more comprehensive and accurate consensus of the population (Truelove et al., 2022). The relationship between eDNA data and raw abundance of a species is developing, as different methods are being explored and DNA shedding rates of different organisms are being taken into consideration (Ficetola et al., 2014; Sassoubre et al., 2016). There is some promising evidence that data reflects abundance that is estimated by traditional methods, but the current most reliable way of interpreting the data is that eDNA shows relative presence of a species (Hänfling et al., 2016; Langlois et al., 2020).

In this study, we aimed to examine how a 12-year time-series of eDNA samples collected in Monterey Bay can provide insight into what factors drive Northern anchovy abundance. Many studies have investigated anchovy and sardine diet preferences through laboratory experiments and in situ observations but analyzing diet from an eDNA time series perspective is relatively novel (Schmitt, 1986; Theilacker, 1987). Climate indices such as the North Pacific Gyre Oscillation (NPGO) were used for further insight into conditions that would affect the Northern anchovy.

MATERIALS AND METHODS:

The Monterey Bay Aquarium Research Institute (MBARI) time series started in 1989, originally collecting CTD (conductivity, temperature, depth) measurements in addition to biological and chemical data. Beginning in 2008, eDNA samples have been collected on time-series cruises, at stations C1, M1, and M2. Pictured below, stations C1, M1, and M2 represent coastal, mid-bay, and outer bay moorings (Figure 1).

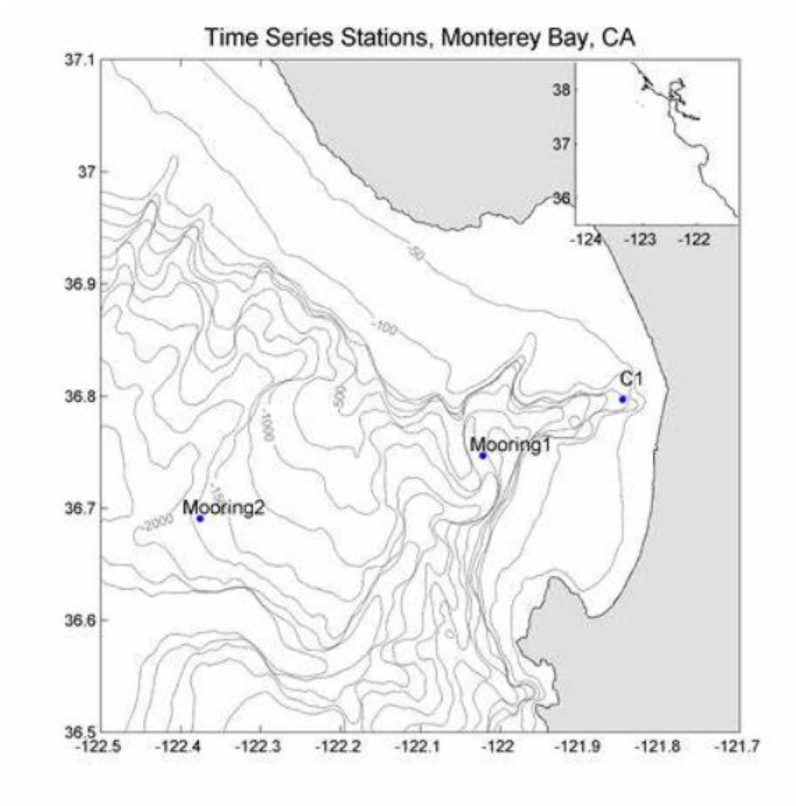

Figure 1 - Location of sample sites C1, M1 (Mooring 1), and M2 (Mooring 2) in Monterey Bay. Source: https://www3.mbari.org/bog/roadmap/major_stations.htm

The markers 12S, 18S, and COI were used to target three distinct groups of organisms: bony fishes, phytoplankton, and zooplankton. Amplicon sequence variant (ASV) reads for each species were adjusted from the raw amount to percent of total reads for each species. Species of interest from the 12S, 18S and COI markers were extracted and plotted to view any potential inter-species interaction in the programming language R. The NPGO Index was taken from ERDDAP, NOAA's database that provides publicly available oceanographic data. The ASV reads for anchovies and sardines were separated by station in Monterey Bay to view any notable spatial or temporal patterns.

RESULTS:

COMMUNITY SHIFTS IN SITES C1, M1, AND M2:

The top 10 species for the 12S marker of bony fishes (Figure 2) is an excellent example of the potential that eDNA has for monitoring biodiversity and species of concern on a fisheries level. We can see that the Northern anchovy dominates much of this time series, particularly from 2017-2019. The top 10 species for phytoplankton is more diverse, and species shifts are less obvious (Figure 3). *Akashiwo sanguinea*, a species that is proven to be a nutritious prey preference for first-feeding anchovies, appears to be more present later in the time series, in addition to *Dinophyceae*. *Pseudocalanus*, a small copepod that is a preferred prey for Pacific Sardine, appears to be relatively more present in the sardine regime of 2008-2014 in the zooplankton plot (Figure 4). From 2014 onward, *Synchaetidae*, a family of rotifers, and *Emiliana huxleyi,* a species of coccolithophore, are more present.

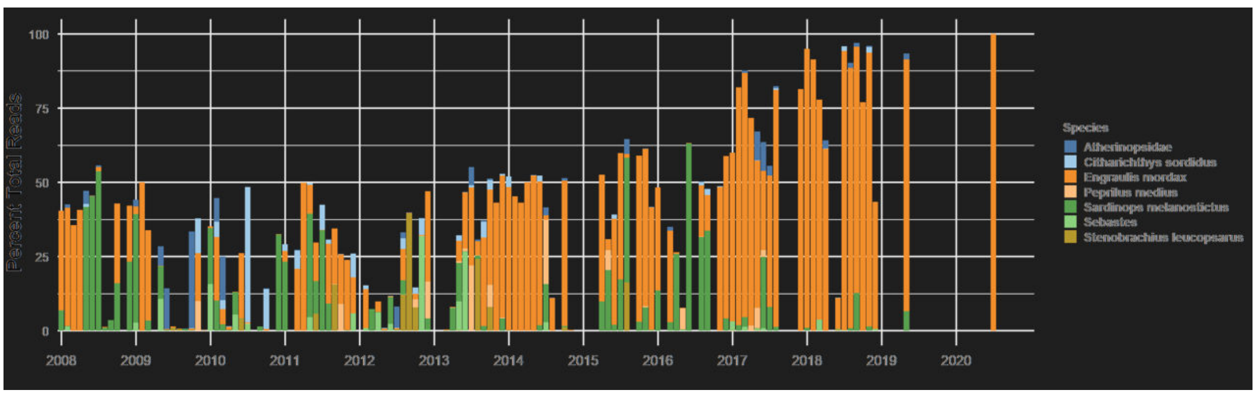

Figure 2 – Stacked bar plot of top 10 species in 12S (bony fish) series from 2008-2020.

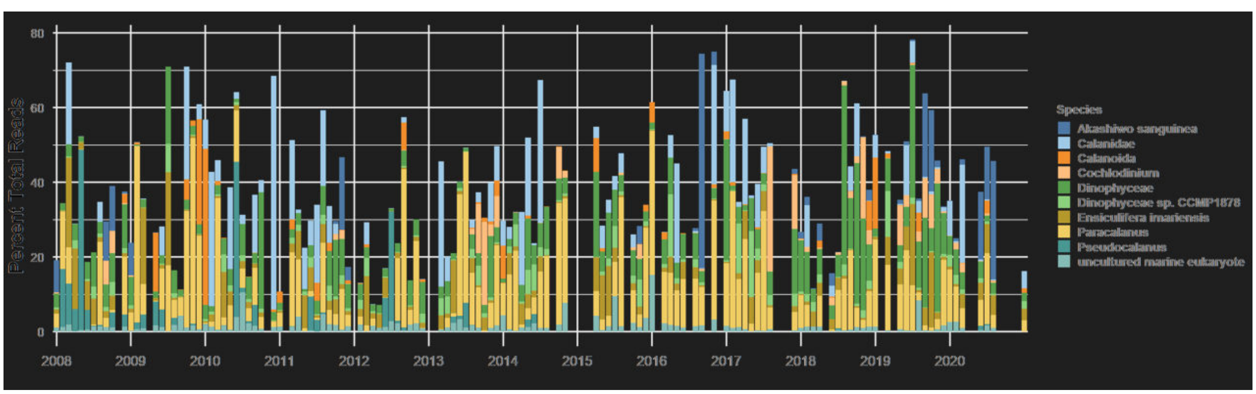

Figure 3 – Stacked bar plot of top 10 species in 18S (phytoplankton) series from 2008-2020.

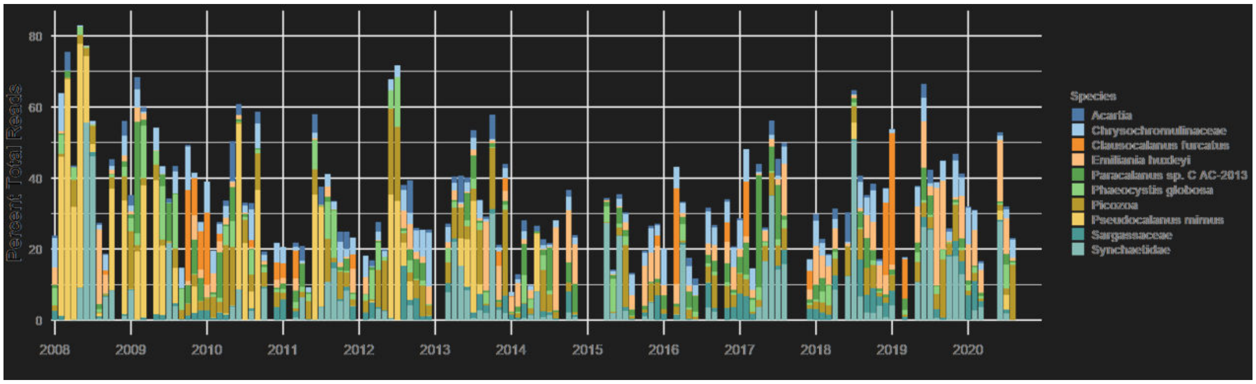

Figure 4 – Stacked bar plot of top 10 species in COI (zooplankton) series from 2008-2020.

DIETARY PREFERENCES

The ASV reads for Northern anchovy and Pacific sardine from all sample sites confirm a regime shift from sardine to anchovy around 2014 that is also evident in the top 10 12S species bar plot (Figure 5, Figure 2). Around this same time, a similar shift from cold to warm SST temperatures occurred, often referred to as the "warm blob," counterintuitive to the previous notion that anchovies prefer colder waters (Leising et al., 2015). An analysis was done on dietary preferences of anchovies and sardines to extract species of interest from the 18S and COI markers. Evidence that prey density and species composition is critical for fish in their first-feeding stages is robust, and that successful recruitment in the following years is a result of this (Cushing, 1990). Anchovies are not able to withstand starvation periods beyond 1.5 days, and certain species have proven to be more nutritious than others, such as *Akashiwo sanguinea* (Lasker et al., 1970). Similar laboratory studies concluded that first-feeding anchovies prefer dinoflagellates over diatoms, and that diatoms were spit out from the anchovies as most species are too large and spiny (Lasker, 1981; Lasker and Zweifel, 1978). Therefore, I extracted dinoflagellates and diatoms from the time series by the class *Dinophyceae* and phylum *Bacillariophyta*. When plotted, dinoflagellate reads show a marked increase relative to diatoms around the same time that anchovies increase (Figure 6). This is also consistent with ocean observations that we are in "the age of dinoflagellates" (Fischer et al., 2020). However, Pearson's correlation coefficient of 0.33 between anchovies and dinoflagellates doesn't constitute a significant linear relationship (Figure 7). *Emiliana*, a coccolithophore which also appeared to increase relative to anchovies, had a Spearman correlation coefficient of R = 0.39 (Figure 8).

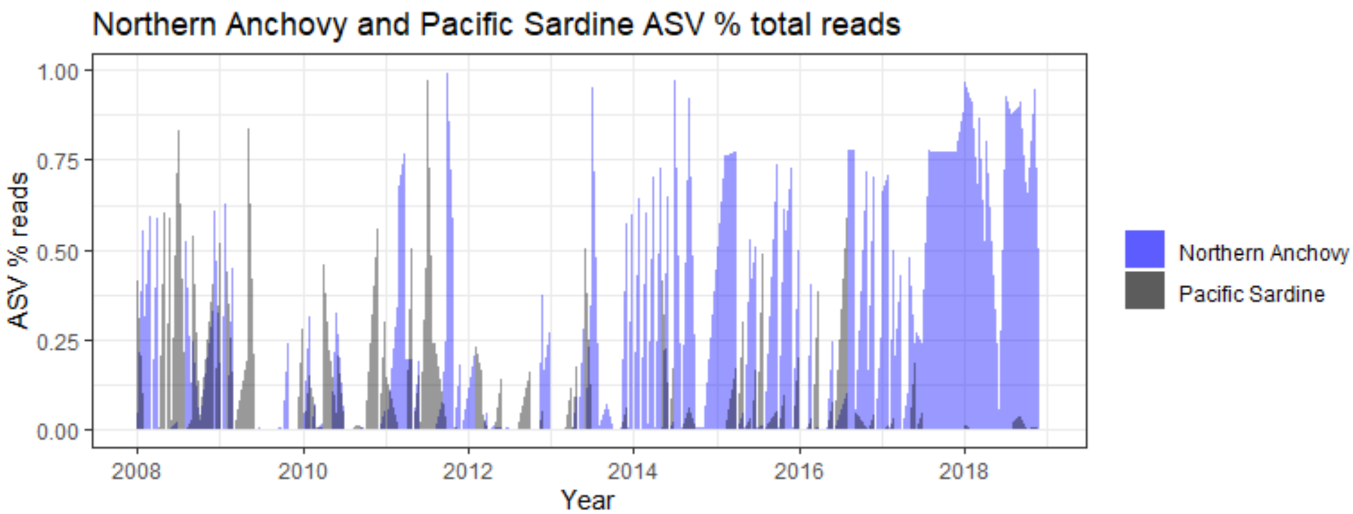

Figure 5 – Shaded ribbon plot of Northern Anchovy and Pacific Sardine amplicon sequence variant reads as a function of percent of the total reads, from 2008-2020.

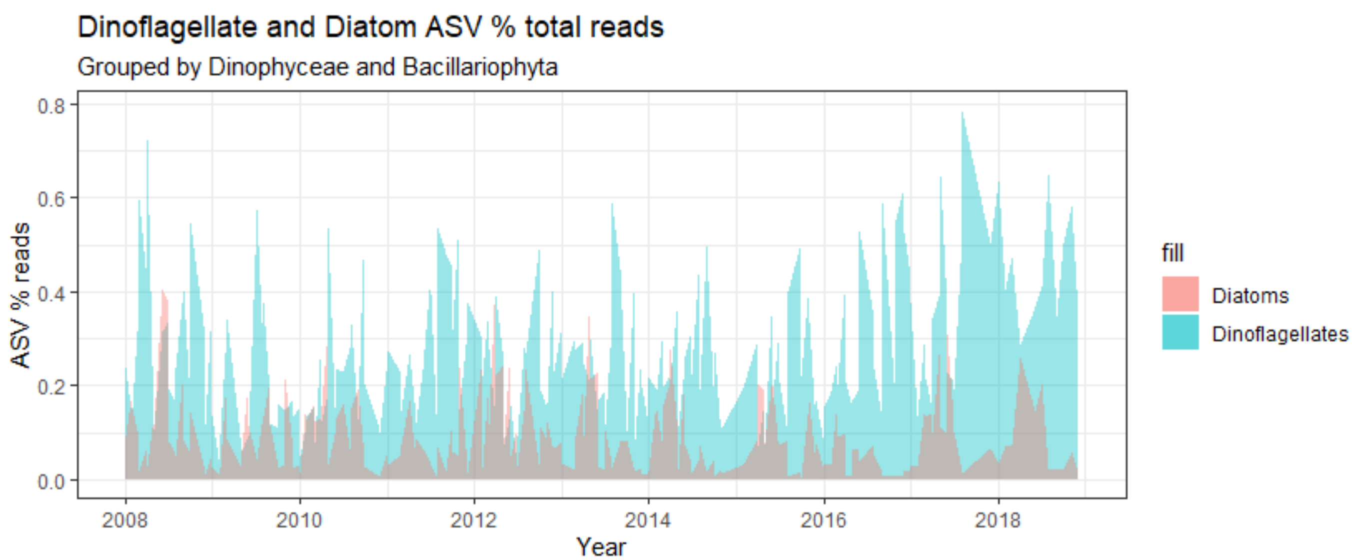

Figure 6 – Shaded ribbon plot of Dinoflagellates and Diatoms amplicon sequence variant reads as a function of percent of the total reads, from 2008-2020. Dinoflagellates were grouped by the class Dinophyceae, and Diatoms grouped by the phylum Bacillariophyta.

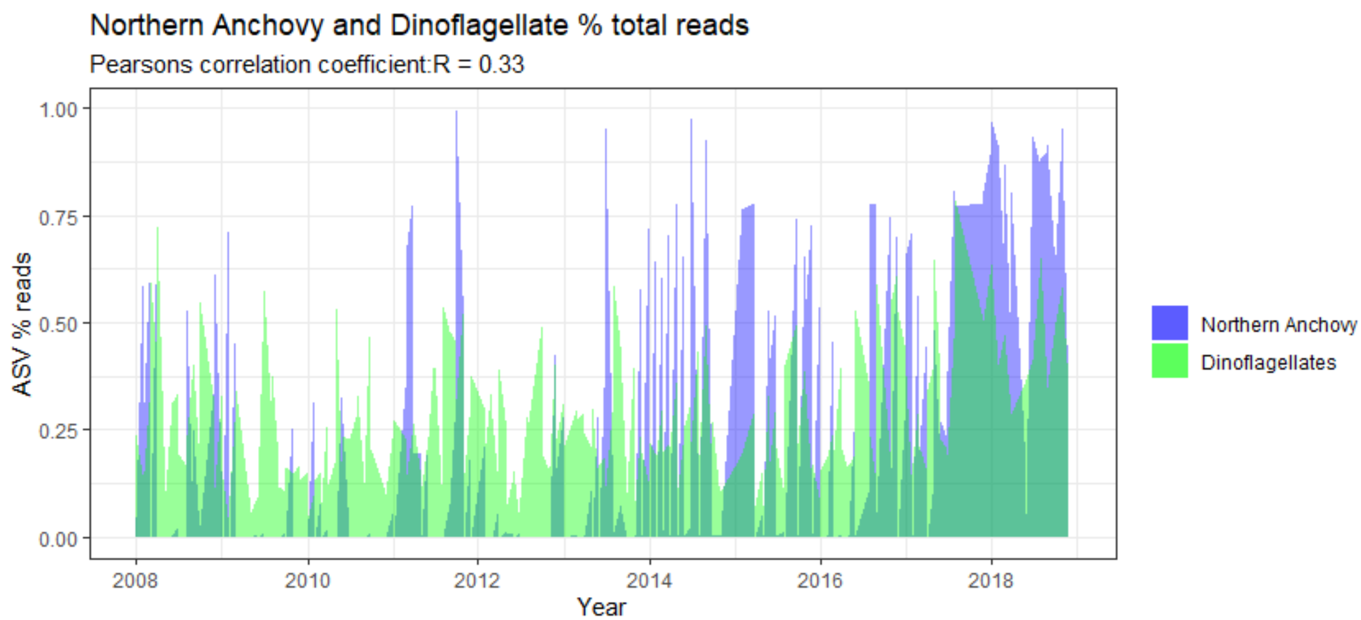

Figure 7 - Shaded ribbon plot of Northern Anchovy and Dinoflagellates amplicon sequence variant reads as a function of percent of the total reads, from 2008-2020. Pearson's correlation coefficient was calculated to assess any linear relationship between the two to find dietary preferences of Northern Anchovy (R = 0.33).

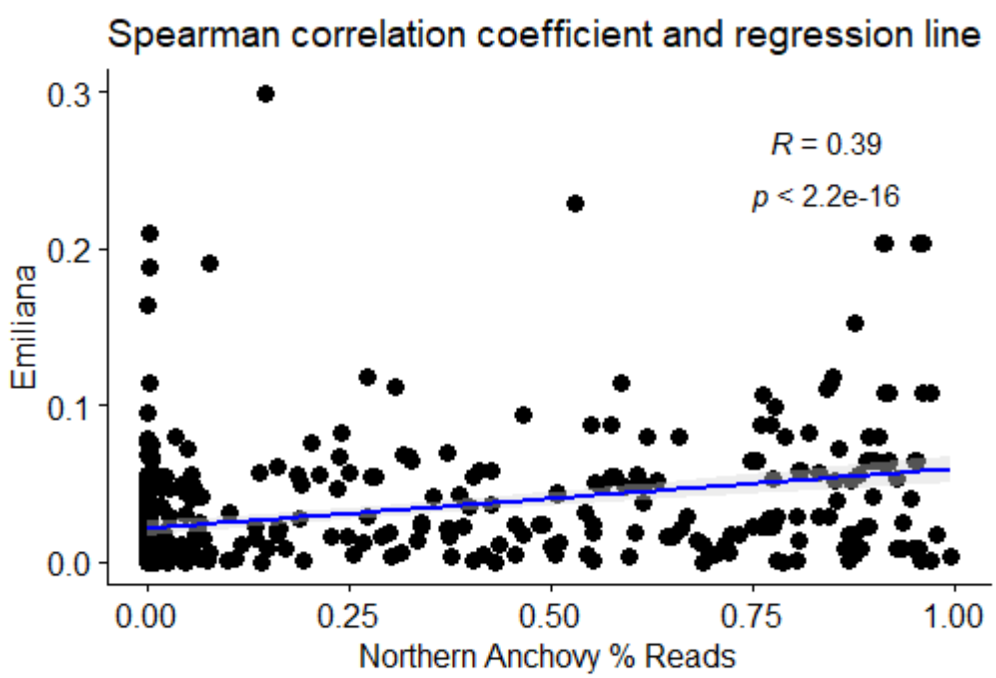

Figure 8 – Spearman correlation coefficient and regression line plotted for Northern Anchovy and genus Emiliana, a coccolithophore R = 0.39.

A Principal Component Analysis (PCA) was plotted for all species of interest and some climate indices to determine how much variability they were causing, in addition to viewing their relationship between each other (Figure 9). Only about 23% of the variability in the data set is explained, but there is lots of information on how these variables relate to one another. Again, we see anchovies and dinoflagellates close together, in addition to diatoms and the upwelling index grouped close together. The NPGO index is directly opposite to the Northern Anchovy, indicating a high negative correlation which was explored later.

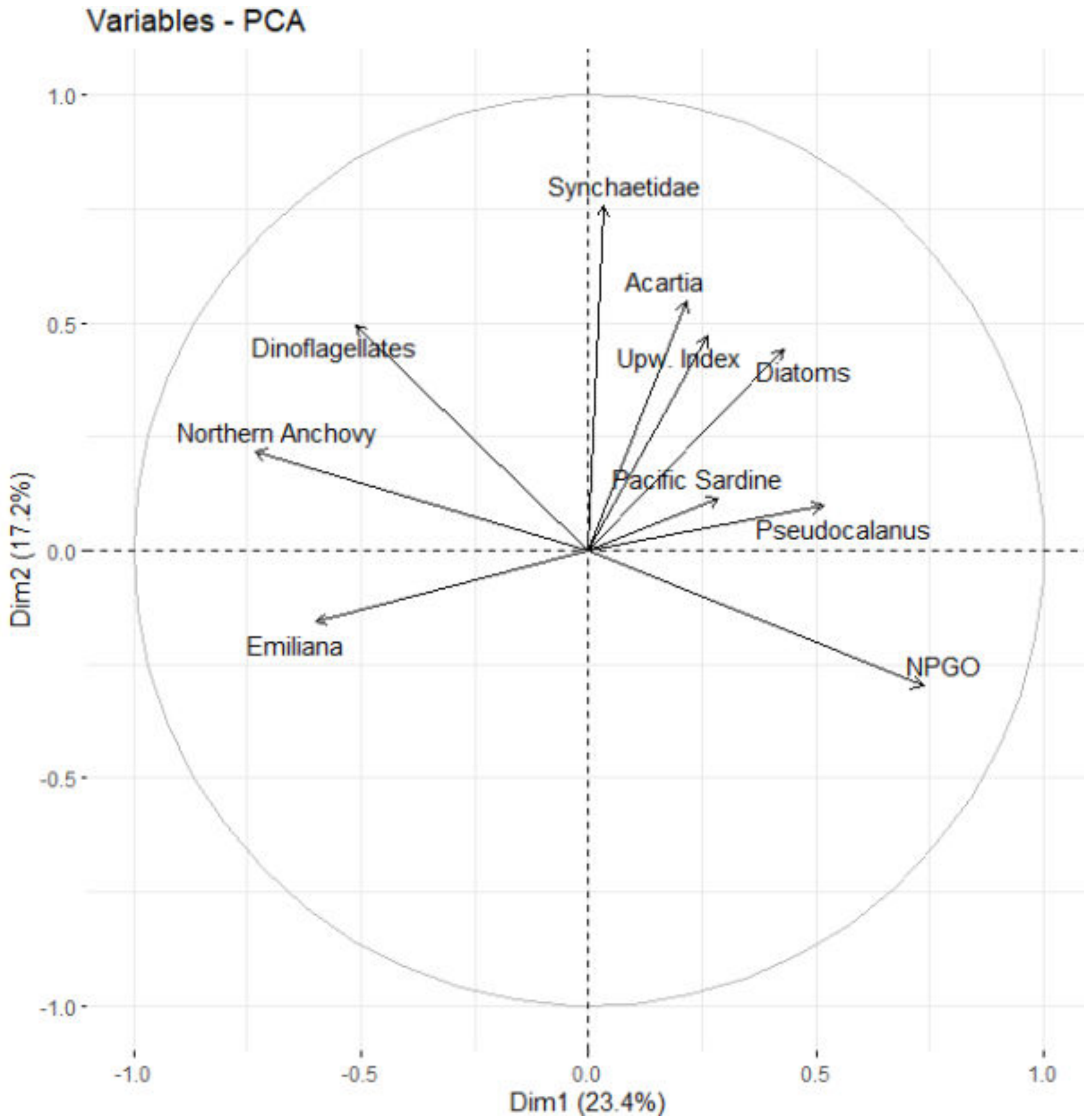

Figure 9 – Principal component analysis of all species that were extracted from 12S, 18S, and COI time series.

CLIMATE INFLUENCE:

The NPGO and anchovy amplicon sequence variant reads are negatively correlated, with Pearson's correlation coefficient R = -0.53 (Figure 10). The NPGO is a basin scale climate influence, the second dominant mode of sea surface height variability that is highly correlated with previously unexplained fluctuations in nitrate, salinity, and chlorophyll (di Lorenzo et al., 2008). This "negative" phase of the NPGO is associated with lower nitrate, lower salinity, lower chlorophyll, and less coastal upwelling.

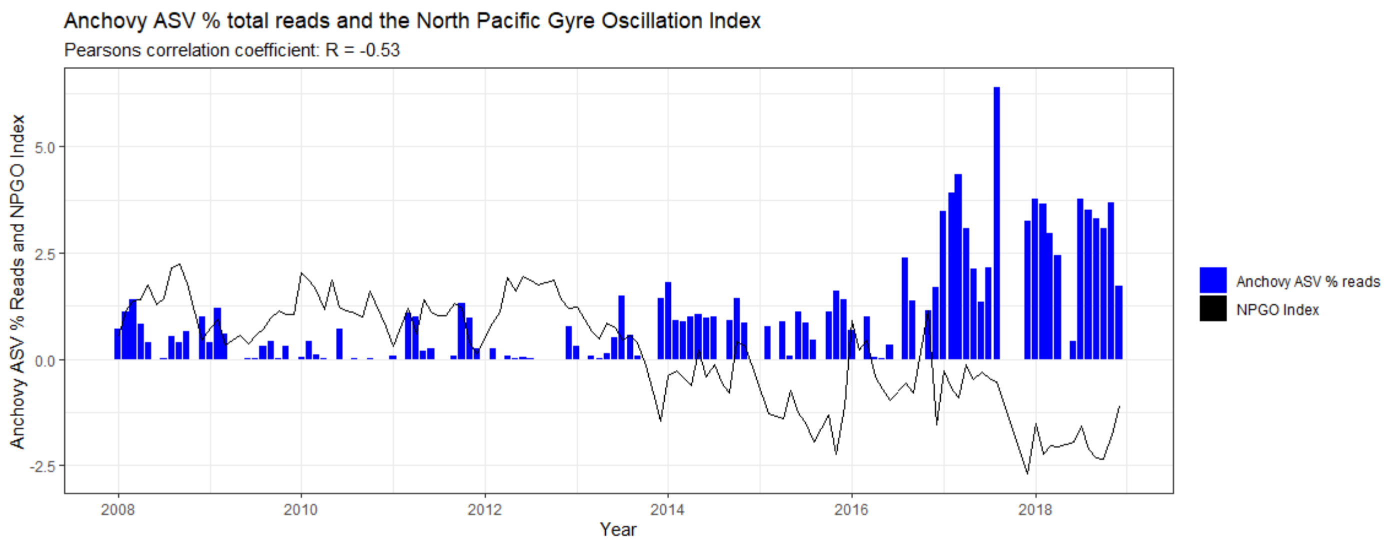

Figure 10 – Pearson's correlation coefficient between Northern Anchovy amplicon sequence variant reads and the North Pacific Gyre Oscillation Index (R = -0.53).

NEARSHORE VS. OFFSHORE HABITATS

The Northern Anchovy and Pacific Sardine are known to inhabit slightly differing regions of the California Current, where anchovies are generally found more nearshore, and sardines are found in offshore waters (Emmett et al., 2005; Sydeman et al., 2020). The definition of nearshore vs. offshore can differ among disciplines, but generally shoreward of ~500m bottom depth distinguishes the open ocean pelagic environment from nearshore habitats and demersal fish. Monterey Bay is unique in that it contains nearshore, offshore, and "deep sea" habitats in the Monterey Canyon. Stations C1, M1, and M2 present three largely differing habitats for marine fish, at bottom depths approximately ~200m, ~1000m, and ~2000m. When plotting the amplicon sequence variant reads for anchovy and sardine by station, it appears that sardines are more present in M1 and M2 compared to C1 (Figure 12). In contrast, there appears to be a mix of anchovy presence at all 3 sites (Figure 11).

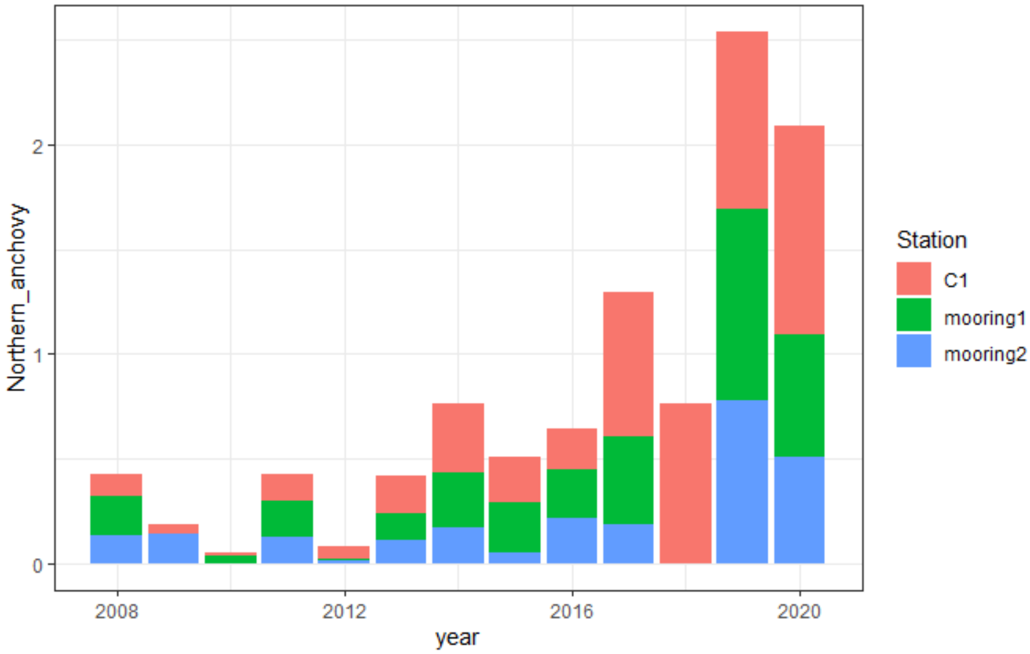

Figure 11 – Northern Anchovy amplicon sequence variant reads for 2008-2020 separated by station.

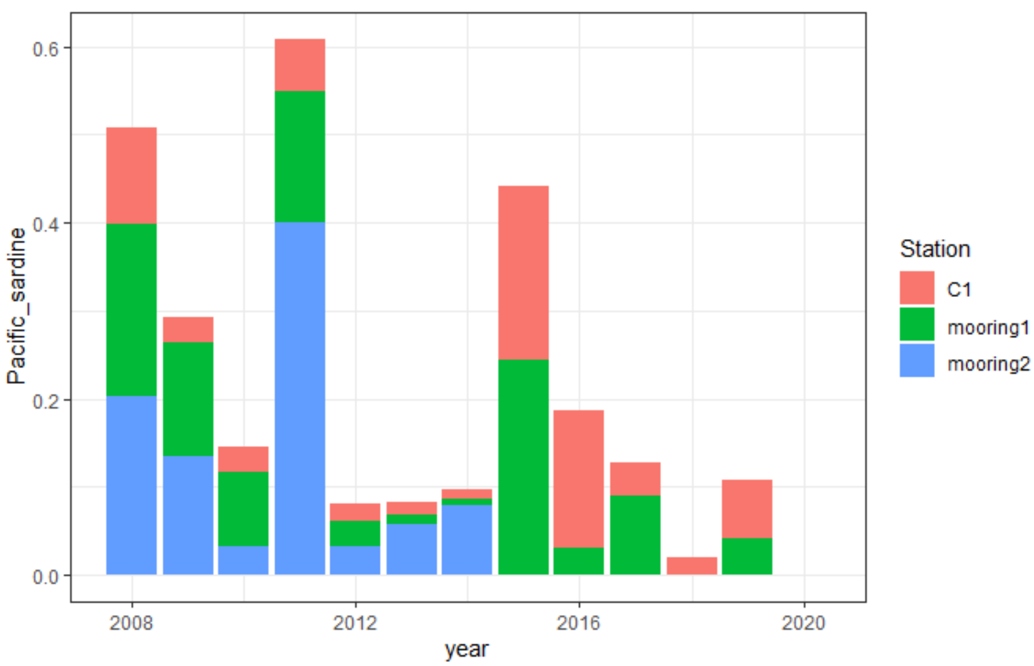

Figure 12 – Pacific Sardine amplicon sequence variant reads for 2008-2020 separated by station.

CLIMATOLOGICAL MEAN AND SEASONAL TRENDS

When plotting the mean of all anchovy and sardine reads by month from 2008-2020, there appears to be some notable patterns. Sardines are known to be migratory fish that favor warmer waters, some migrating as far as the coast of Canada from Southern California in their adult stages (Demer et al., 2012). The low abundance of sardines from October-December could be reflective of migration to warmer waters in winter or other behavioral patterns (Figure 13). In addition, we can also see the distribution of sardines at sites C1, M1, and M2 in the climatological mean, where sardines appear to be more present in offshore areas (M1 & M2).

For the climatological mean of anchovies, we see a trend in low anchovy presence from April-June (Figure 14). Anchovies can spawn throughout the year when food availability is high, but mostly spawn in the springtime. Since the eDNA water samples are taken within ~10 meters of the surface, we can speculate as to whether anchovies are going elsewhere in the bay to spawn, or perhaps deeper in the water column where their presence may be less detected. In addition, the anchovies collected by MBARI for biological data by hook and line methods were less catchable during these months.

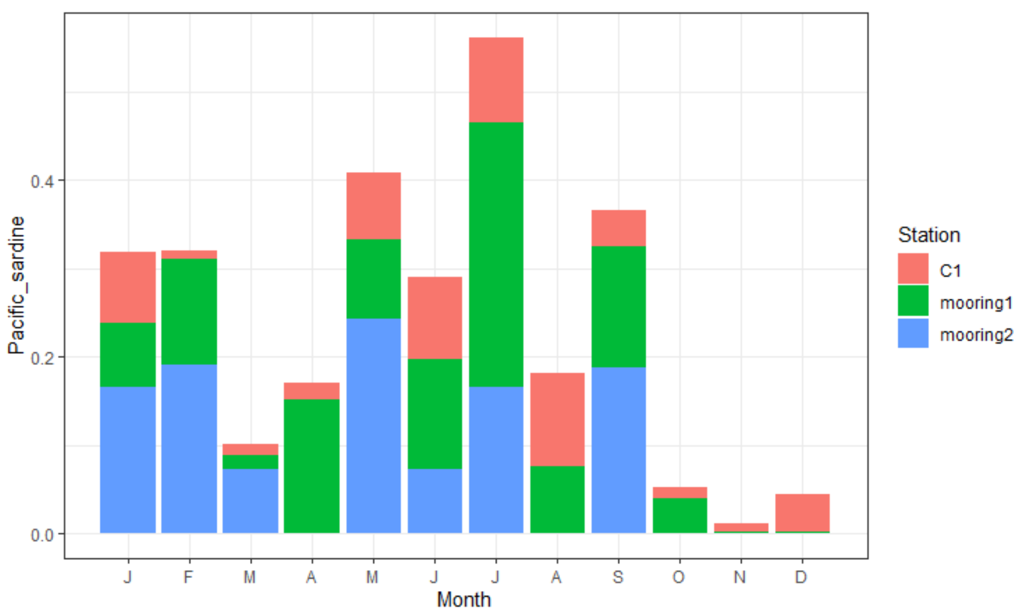

Figure 13- Mean percent total reads of Pacific Sardine by station and month.

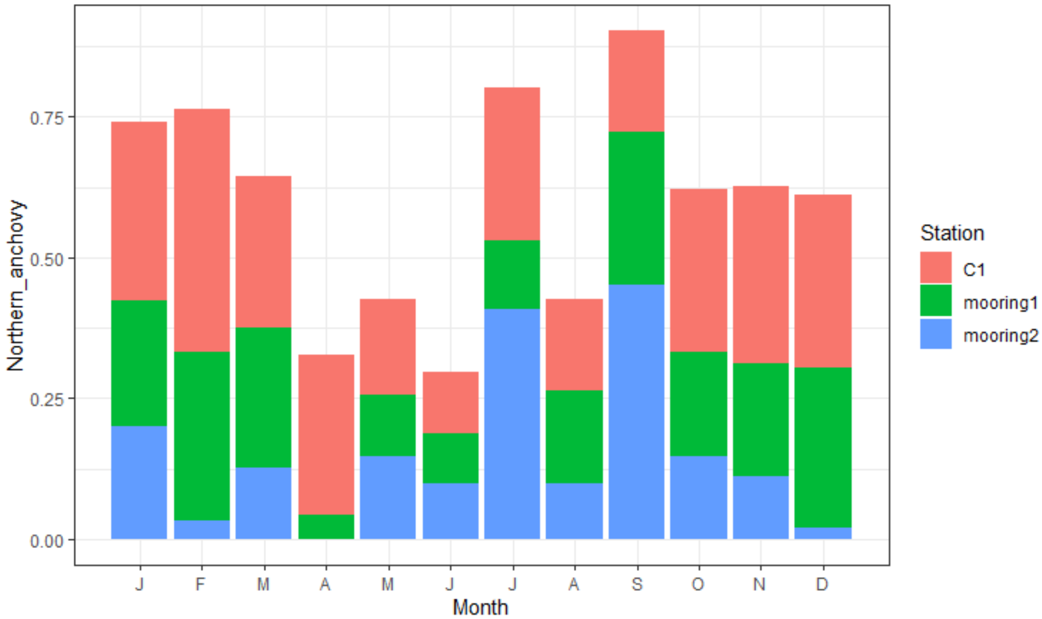

Figure 14- Mean percent total reads of Northern Anchovy by station and month.

CONCLUSIONS/RECOMMENDATIONS:

Forage fish are highly dynamic, short-lived fish, therefore finding answers to their abundance is difficult and likely due to combination of many factors that may change throughout time. The global fluctuations of anchovies and sardines in eastern boundary current upwelling systems remains a puzzle to be solved by both oceanographers and fisheries experts. A more thorough analysis of diet (lag-correlations, eDNA of anchovy stomach tissue samples) can provide further insight into this. A significant correlation was found between anchovies and the NPGO index. This relationship should be explored further in addition to other climate conditions, on a basin, regional, and local scale. Stratification in the water column caused by warm SST conditions may be beneficial to anchovies in that prey density is increased, that could be examined with local upwelling indices. Warm SSTs are also conducive to algal blooms, red tides, and production of dinoflagellates that are preferred prey of first-feeding anchovies, and an analysis of algal bloom occurrence in Monterey Bay along with anchovy abundance could investigate this.

We successfully explored new applications for eDNA, including examining fish diet, habitat range, and potential behavioral patterns such as spawning and migration. eDNA holds promising potential for capturing spatial and temporal variability of fish. Future studies could "test" the eDNA data by examining other well-established patterns of fish abundance over time and space to see if the eDNA data supports this. The 12S time series contains many different fish species and inter-species interactions with anchovies could be examined as well. Other considerations include exploring how different amplicon sequence variant reads may be biased to capture some organisms more than others, and how these reads change throughout time.

ACKNOWLEDGEMENTS:

I would like to thank my mentors, Lis Henderson and Francisco Chavez, for their support and guidance in my project. Thank you to everyone in Chavez Lab who quickly familiarized me with eDNA. Many thanks to George Matsumoto, Megan Basset, and Lyndsey Claassen for taking the time to thoughtfully put together this internship. The MBARI internship is made possible through the Dean and Helen Witter Family Fund, the Rentschler Family Fund, the David and Lucile Packard Foundation, and the Maxwell/Hanrahan Foundation. Lastly, thank you to all my peers in this program for the support and good times shared.

References

Chavez, F., Min, M., Pitz, K., Truelove, N., Baker, J., LaScala-Grunewald, D., Blum, M., Walz, K., Nye, C., Djurhuus, A., Miller, R., Goodwin, K., Muller-Karger, F., Ruhl, H., & Scholin, C. (2021). Observing Life in the Sea Using Environmental DNA. *Oceanography*, *34*(2), 102–119. https://doi.org/10.5670/oceanog.2021.218

Chavez, F. P., Ryan, J., Lluch-Cota, S. E., & ÑIquen C., M. (2003). From Anchovies to Sardines and Back: Multidecadal Change in the Pacific Ocean. *Science*, *299*(5604), 217–221. https://doi.org/10.1126/science.1075880

Cushing, D. (1990). Plankton Production and Year-class Strength in Fish Populations: An Update of the Match/Mismatch Hypothesis. *Advances in Marine Biology*, 249–293. https://doi.org/10.1016/s0065-2881(08)60202-3

Demer, David & Zwolinski, Juan & Byers, Kyle & Cutter, George & Renfree, Josiah & Sessions, Thomas & Macewicz, Beverly. (2012). Prediction and confirmation of seasonal migration of Pacific sardine (Sardinops sagax) in the California Current Ecosystem. *Fishery Bulletin.* 110. 52-70.

di Lorenzo, E., Schneider, N., Cobb, K. M., Franks, P. J. S., Chhak, K., Miller, A. J., McWilliams, J. C., Bograd, S. J., Arango, H., Curchitser, E., Powell, T. M., & Rivière, P. (2008). North Pacific Gyre Oscillation links ocean climate and ecosystem change. *Geophysical Research Letters*, *35*(8). https://doi.org/10.1029/2007gl032838

Djurhuus, A., Closek, C. J., Kelly, R. P., Pitz, K. J., Michisaki, R. P., Starks, H. A., Walz, K. R., Andruszkiewicz, E. A., Olesin, E., Hubbard, K., Montes, E., Otis, D., Muller-Karger, F. E., Chavez, F. P., Boehm, A. B., & Breitbart, M. (2020). Environmental DNA reveals seasonal shifts and potential interactions in a marine community. *Nature Communications*, *11*(1). https://doi.org/10.1038/s41467-019-14105-1

Emmett, Robert & Brodeur, Richard & Miller, Todd & Pool, Suzan & Krutzikowsky, Gregory & Bentley, Paul & Mccrae, Jean. (2005). Pacific sardine (Sardinops sagax) abundance, distribution, and ecological relationships in the Pacific Northwest. *California Cooperative Oceanic Fisheries Investigations Reports*. 46.

Ficetola, G. F., Pansu, J., Bonin, A., Coissac, E., Giguet-Covex, C., de Barba, M., Gielly, L., Lopes, C. M., Boyer, F., Pompanon, F., Rayé, G., & Taberlet, P. (2014). Replication levels, false presences and the estimation of the presence/absence from eDNA metabarcoding data. *Molecular Ecology Resources*, *15*(3), 543–556. https://doi.org/10.1111/1755-0998.12338

Field, J., Francis, R., & Aydin, K. (2006). Top-down modeling and bottom-up dynamics: Linking a fisheries-based ecosystem model with climate hypotheses in the Northern California Current. *Progress in Oceanography*, *68*(2–4), 238–270. https://doi.org/10.1016/j.pocean.2006.02.010

Fischer, A. D., Hayashi, K., McGaraghan, A., & Kudela, R. M. (2020). Return of the "age of dinoflagellates" in Monterey Bay: Drivers of dinoflagellate dominance examined using automated imaging flow cytometry and long-term time series analysis. *Limnology and Oceanography*, *65*(9), 2125–2141. https://doi.org/10.1002/lno.11443

Hänfling, B., Lawson Handley, L., Read, D. S., Hahn, C., Li, J., Nichols, P., Blackman, R. C., Oliver, A., & Winfield, I. J. (2016). Environmental DNA metabarcoding of lake fish communities reflects long-term data from established survey methods. *Molecular Ecology*, *25*(13), 3101–3119. https://doi.org/10.1111/mec.13660

Langlois, V. S., Allison, M. J., Bergman, L. C., To, T. A., & Helbing, C. C. (2020). The need for robust qPCR-based eDNA detection assays in environmental monitoring and species inventories. *Environmental DNA*, *3*(3), 519–527. https://doi.org/10.1002/edn3.164

Lasker, R., Feder, H. M., Theilacker, G. H., & May, R. C. (1970). Feeding, growth, and survival of Engraulis mordax larvae reared in the laboratory. *Marine Biology*, *5*(4), 345–353. https://doi.org/10.1007/bf00346901

Lasker, R. (1981). The role of a stable ocean in larval fish survival and subsequent recruitment. *Marine fish larvae: morphology, ecology and relation to fisheries*, *1*, 80-89.

Lasker, R., & Zweifel, J. R. (1978). Growth and survival of first-feeding northern anchovy larvae (Engraulis mordax) in patches containing different proportions of large and small prey. *Spatial pattern in plankton communities* (pp. 329-354).

Leising, A., Schroeder, I., Bograd, S., Abell, J., Durazo, R., Gaxiola-Castro, G., Bjorkstedt, E., Field, J., Sakuma, K., Robertson, R., Goericke, R., Peterson, W., Brodeur, R., Barceló, C., Auth, T., Daly, E., Suryan, R., Gladics, A., Porquez, J., McClatchie, S., Weber, E., Watson, W., Santora, J., Sydeman, W., Melin, S., Chavez, F., Golightly, R., Schneider, S., Fisher, J., Morgan, C., Bradley, R., & Warybok, P. (2015). State of the California Current 2014-15: Impacts of the Warm-Water "Blob". *Scripps Institution of Oceanography*.

Pauly, D., & Christensen, V. (1995). Primary production required to sustain global fisheries. *Nature*, *374*(6519), 255–257. https://doi.org/10.1038/374255a0

Pörtner, H. O., & Peck, M. A. (2010). Climate change effects on fishes and fisheries: towards a cause-and-effect understanding. *Journal of Fish Biology*, *77*(8), 1745–1779. https://doi.org/10.1111/j.1095-8649.2010.02783.x

Santora, J. A., Mantua, N. J., Schroeder, I. D., Field, J. C., Hazen, E. L., Bograd, S. J., Sydeman, W. J., Wells, B. K., Calambokidis, J., Saez, L., Lawson, D., & Forney, K. A. (2020). Habitat compression and ecosystem shifts as potential links between marine heatwave and record whale entanglements. *Nature Communications*, *11*(1). https://doi.org/10.1038/s41467-019-14215-w

Sassoubre, L. M., Yamahara, K. M., Gardner, L. D., Block, B. A., & Boehm, A. B. (2016). Quantification of Environmental DNA (eDNA) Shedding and Decay

Rates for Three Marine Fish. *Environmental Science & Technology*, *50*(19), 10456–10464. https://doi.org/10.1021/acs.est.6b03114

Schmitt, P. D. (1986). Prey size selectivity and feeding rate of larvae of the northern anchovy, Engraulis mordax Girard. *CalCOFI Rep*, *27*, 153-161.

Sydeman, W. J., Dedman, S., García-Reyes, M., Thompson, S. A., Thayer, J. A., Bakun, A., & MacCall, A. D. (2020). Sixty-five years of northern anchovy population studies in the southern California Current: a review and suggestion for sensible management. *ICES Journal of Marine Science*, *77*(2), 486–499. https://doi.org/10.1093/icesjms/fsaa004

Theilacker, G. H. (1987). Feeding ecology and growth energetics of larval northern anchovy, Engraulis mordax. *Fishery Bulletin*, *85*(2), 213.

Thompson, Andrew & Schroeder, Isaac & Bograd, Steven & Hazen, Elliott & Jacox, Michael & Leising, Andrew & Wells, Brian & Largier, John & Fisher, Jennifer & Jacobson, Kym & Zeman, Samantha & Bjorkstedt, Eric & Robertson, Roxanne & Kahru, Mati & Goericke, Ralf & Peabody, Clare & Baumgartner, Timothy & Lavaniegos, Bertha & Miranda, Luis & Melin, Sharon. (2019). State of the California Current 2018-19: A novel anchovy regime and a new marine heatwave? https://par.nsf.gov/servlets/purl/10156903

Truelove, N. K., Patin, N. V., Min, M., Pitz, K. J., Preston, C. M., Yamahara, K. M., Zhang, Y., Raanan, B. Y., Kieft, B., Hobson, B., Thompson, L. R., Goodwin, K. D., & Chavez, F. (2022). Expanding the temporal and spatial scales of environmental DNA research with autonomous sampling. *Environmental DNA*. https://doi.org/10.1002/edn3.299

Wroblewski, J., & Richman, J. G. (1987). The non-linear response of plankton to wind mixing events — implications for survival of larval northern anchovy. *Journal of Plankton Research*, *9*(1), 103–123. https://doi.org/10.1093/plankt/9.1.103