Enhancing PISCO Kelp Forest Surveys Through Environmental DNA (eDNA) Metabarcoding

Haylee A. Bregoff ¹², Jacoby Baker ³ & Dr. Francisco Chavez ³

¹ Moss Landing Marine Laboratories (MLML)

² CSU Monterey Bay (CSUMB)

³ Monterey Bay Aquarium Research Institute (MBARI)

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ABSTRACT

Technological advancements in the field of environmental DNA (eDNA) have shown great potential to improve contemporary environmental management practices, established by traditional monitoring and survey methods. Kelp forests are one of the most highly productive marine systems but have reached historical population lows. Giant kelp (Macrocystis pyrifera), is a foundation species that provides a wide range of ecosystem services. Monitoring and restoration efforts are necessary along the central California coastline as kelp coverage has drastically declined with low rates of recovery observed. Wide-scale kelp surveys are highly intensive and expensive. Here, we investigate the application of eDNA metabarcoding as a tool to enhance kelp forest monitoring and restoration in the Monterey Bay National Marine Sanctuary. Using the COI primer for eDNA metabarcoding gave us a snapshot of the diverse array of organisms present. The COI gene region is one of the most widely available and used sequence regions in public reference libraries, making it ideal for community analyses. The Partnership for Interdisciplinary Studies of Coastal Oceans (PISCO) has monitored kelp forests from southern California to southern Oregon since 1999. In 2020, PISCO divers collected water samples from two paired sites of reference and state marine reserves, in the Big Sur marine protected areas. We found that eDNA detected a greater number of taxa in comparison to the PISCO visual surveys and utilizing both methods allowed us to identify all trophic levels in complex marine food webs.

INTRODUCTION

Environmental DNA metabarcoding, or eDNA, is a promising new molecular method to detect bulk species from the environment using materials like soil, sediment, and water (Taberlet *et al.* 2012). eDNA metabarcoding capture free-floating short fragments of DNA shed by organisms swimming in the water column (Taberlet *et al.* 2012; Deiner *et al.* 2017). eDNA has the capacity to capture taxa from microbes to large marine mammals, eDNA can also be a strong addition to biomonitoring and ecosystem-based monitoring (Stat *et al.* 2017). Although molecular ecology ais a relatively new field, researchers have proven the efficacy of eDNA in a variety of marine ecosystems (Ruppert *et al.* 2019). As a result, eDNA is now increasingly used to observe community structure in marine systems; becoming increasingly common over the past 10 years (Díaz-Ferguson *et al.* 2014).

eDNA metabarcoding offers many advantages in marine systems. Firstly, eDNA can be used to be sampled passively or sample processing to be fully automated (Chavez *et al.* 2021). The molecular methods of biomonitoring are relatively cost-effective compared to the expensive and intensive costs of traditional visual scuba-based surveys (Gold *et al.* 2021); only one liter of sea

water is needed for eDNA sampling. Secondly, the community composition is not limited by taxonomic identification or survey designs. DNA barcoding does not require visual taxonomic identification skills, which means that a broad range of marine taxa can be identified from a single sample (Rossouw *et al.* 2024). By using eDNA, one can sample areas that have less access, low visibility, and eliminating potential risks for diver safety at sites where multiple scuba-based visual surveys are needed. Therefore, eDNA greatly enhances traditional monitoring techniques in highly complex and biodiverse systems that may be less than ideal to sample frequently.

However, eDNA is not yet a standalone tool for ecosystem-based monitoring. Although the technology is promising, there are a lot of unknowns like methodological biases from primer selection and variation in detecting eDNA signatures (Ruppert et al. 2019). As of now, using universal primers with eDNA is only able to give us a snapshot of taxa present in the community from when the water samples were collected (Taberlet et al. 2012). Research has shown that some universal primers show bias to certain phyla due to the targeted genetic region having lower rates of assignment leading to primer bias (Abellan-Schneyder et al. 2021). Furthermore, there needs to be an expansion of sequence reference data base availability to give us insight into lesser studied or rare organism. Key questions still remain regarding the spatial and temporal variability of eDNA signatures in marine environments; critical information for standardizing effective eDNA biomonitoring efforts. Previous research has shown that eDNA signatures tend to degrade within hours (Kelly & Palmer 2018; Thomsen et al. 2012), in marine environments, with laboratory studies observed degradation rates around 3 to 5 days (Ely et al. 2021). Thus, in highly heterogenous environments, a rapid rate of eDNA turnover can be expected. In highly biodiverse habitats, like kelp forests, we would expect eDNA to capture varying levels of eDNA signatures dependent on the collection depth and proximity to shore.

Applying eDNA metabarcoding for kelp forest monitoring is not a novel endeavor. Past research has supported that eDNA is sensitive enough to detect differences in temporal and horizontal variability in kelp forest ecosystems. Multiple studies that describe the variation in marine eDNA signatures across depth (Lamy et al. 2021). Port et al. (2016) distinguished distinct vertebrate communities within a kelp forest ecosystem in Monterey Bay in his pioneering work from 2015. Additionally, Monuki et al. (2021) showed that eDNA was sensitive enough to distinguish depth partitioning within kelp forest sites and captured significantly different community assemblages from nearshore to offshore zones on a finer scale. On a wider scale, Chavez et al. (2021), has been able to show the utility of eDNA through observing the community of Monterey Bay through time-series cruises. These findings demonstrate that molecular methodologies complement traditional monitoring techniques; posing several advantages, including the enhancement of our understanding complex food webs - like kelp forests, improving the efficiency of surveying commercially important fish and invertebrates with reduced costs and higher resolution, increasing the detection capability for invasive, protected, or pathogenic species, advancing quantitative molecular methods for estimating age and population sizes, and enabling large-scale ocean observation at a global level (Chavez et al. 2021). By applying biomonitoring protocols to kelp forest monitoring, one has the potential to gain greater insight into complex interactions and infer management decisions.

Giant kelp (*Macrocystis pyrifera*) is a foundational species that provides nutrients, shelter, and refugia to a plethora of fish and invertebrate species (Dayton 1972; Steneck et al. 2002). Giant kelp forests are iconic and important marine habitats along the coast of central California (Steneck *et al.* 2002); they are one of the most abundant marine habitats in the California Current System (CCS) (Graham *et al.* 2007). However, giant kelp populations in central California are vulnerable to warming waters, intense marine heat waves like the 2013 "the blob", and loss of important

grazer predators - including sunflower sea stars (Smith *et al.* 2021). There is a need for research to understand how trophic interactions in such a biodiverse and complex community changes with varying environmental stressors. Understanding how trophic interactions respond to high stress events will allow us to identify how to improve the recovery of local kelp forests along Big Sur, and apply these concepts to other regions in central California. The Monterey Bay National Marine Sanctuary uses PISCO data to publish annual reports, but these reports can be enhanced through the addition of eDNA to visual surveys to mitigate the loss of kelp density.

In this study, I propose to enhance traditional kelp forest monitoring through the addition of eDNA metabarcoding. This paper analyzes data collected from Big Sur in 2020. The visual survey and water sampling occurred within the Monterey Bay National Marine Sanctuary at Northern sites in Point Sur State Marine Reserve and Southern sites in Point Buchon State Marine Reserve. We sought out to answer two major questions: (1) Can eDNA detect differences within and between kelp forests in Big Sur? (2) Does eDNA enhance traditional visual surveys in kelp forest ecosystems? As scuba-surveys are dependent upon diver training and visibility we expect that visual surveys will have less species observed. However, eDNA will detect a diverse array of taxon despite low visibility months. We expect that visual surveys will have less taxa observed at all sites. The visual survey data will have less differences distinguished between the North and South regions. Visual surveys are predicted to provide fine-scale shifts in abundance for the closed-list of taxa observed, which provides alpha diversity estimates. In addition, eDNA will detect a greater number of taxa, a greater number of phyla, lesser observed species like small plankton, cryptic, and rare. Moreover, eDNA will do a better job at distinguishing within and between sites by providing a finer resolution to beta diversity estimates. By pairing eDNA with traditional monitoring methods, we can provide robust estimates of the total biodiversity in complex and biodiverse ecosystems that can be difficult to sample.

MATERIALS AND METHODS

STUDY SYSTEM

The Monterey Bay National Marine Sanctuary (MBNMS) was designated in 1992 and protects over 250 miles of Central California Coastline (Greene *et al.* 2002). In 2007, 29 Marine Protected Areas (MPA) were instituted in Central CA, protecting over 85 square miles (Greene *et al.* 2002). This study was performed in MPAs in Big Sur (Figure 1A & 1B). In 2020, PISCO sampled two State Marine Reserves (SMR). The northern sites are located in Point Sur SMR, while the southern sites are in Point Buchon SMR. In Point Sur SMR, the MPA site is False Sur, paired with the reference site South Wreck Downcoast. In Point Buchon SMR, the MPA site is Point Buchon, paired with the reference site Green Peak. Reference refers to any site found outside of an MPA. The northern SMRs are located around 85 miles North of the southern SMRs (Figure 1A & 1B; Figure 2). The northern site pairs, Point Sur SMR, are about six and a half miles from each site. The southern site pairs, Point Buchon SMR, are located approximately five and a half miles from each site (Figure 2).



Figure 1A and 1B. Maps of Point Sur and Point Buchon State Marine Reserve. These maps were produced by the California Department of Fish and Wildlife (CDFW). The red area is State Marine Reserve (SMR) and the blue area is State Marine Conservation Area (SMCA). State Marine Reserves allow for no take, no recreational fishing, and no commercial fishing. State Marine Conservation Areas allow for some recreational and commercial fishing with restrictions in place.

PISCO DIVER SURVEYS

The Partnership for Interdisciplinary Studies of Coastal Oceans (PISCO) uses scuba surveys to quantify the relative abundance and density of macroalgae, invertebrates, and fish to characterize kelp forest ecosystems along the central California and the West Coast of the United States (Menge *et al.* 2019). PISCO has surveyed over 300 sites annually, anytime from June to September, since 1999 (Menge *et al.* 2019). PISCO divers employ three different subtidal survey methods: fish transects, swaths, and uniform point contact (UPC.) This study will only focus on the invertebrate and algal communities.

The sampling period began on 08/11/2020, at the most southern site, Green Peak and ended on 10/19/2020, at the most northern site, False Sur. Two 30m transects were laid across three stratified depths of 5 m, 12.5 m, and 20 m. Six transects were run at each site. On each sampling date, benthic sampling was performed through invertebrate and algal swaths and UPC. A "swath" surveys one meter on either side of the benthic transect. Uniform point contact (UPC) data is collected every meter along the 30m transect. Divers preformed subtidal benthic swaths to estimate the density of kelp and targeted macroinvertebrates and UPC to estimate the substrate type and the percent cover of understory invertebrates and algae.



Figure 2. Big Sur MPA and reference sampling sites created in R. Northern sites are found in the Point Sur State Marine Reserve. Southern sites are found in the Point Buchon State Marine Reserve.

eDNA SAMPLING PROTOCOLS

PISCO surveyors collected three liters of sea water were collected at each sampling point. Water samples were filtered on site after the subtidal sampling was complete. Water samples for eDNA

extracts were collected along the 5 m and 20 m transects, a total of four sampling points at each site. Along the 5 m transect, the shallow transects, the bottom collection depth was at 5 m while the surface collection depth was 0m. Along the 20 m transect, the deep transect, the bottom collection depth was at 20 m while the surface collection depth was 0m. Each sampling point was processed in triplicate, a full liter of water was filtered for each eDNA sample. Twelve samples were processed from each site.

The first site sampled was South Wreck Downcoast; all four transects and water samples were collected on 10/18/2020. False Sur had all four transects and water samples collected on 10/19/2020. Due to the natural topography of the seafloor and landscape at False Sur, the shallow and deep transects are located farther than other sites. Green Peak had all four transects and water samples collected on 10/29/2020. Point Buchon was the only site that had samples taken over two separate days. The first two shallow transects, and water samples were collected on 10/26/2020, the two deep transects and water samples completed on 10/30/2020.

DNA EXTRACTION & SEQUENCING

All samples were processed at MBARI in 2023. Environmental DNA (eDNA) was extracted using the Qiagen DNeasy 96-well Blood and Tissue Kit. A blank extraction of sterile Milli-Q water was used as a negative control for each plate. PCR was used to amplify target amplicons: cytochrome c oxidase subunit I (*COI*) (Table 1). Each round of PCR included a PCR blank, an additional negative control of sterile Milli-Q water. The Agencourt AMPure XP bead system was used to purify PCR products. Gel electrophoresis was run to verify that only target amplicons were captured. The PCR product was then shipped to Michigan State University's Genomics Core for the remaining library preparation steps of pooling, index PCR, and sequencing on an Illumina MiSeq.

Table 1. An overview of the genes targeted, primers, primer nucleotide sequences, expected sequence fragment length (in base pairs), and thermocycler conditions for PCR.

GeneTaxaPrimersPrimer SequencesPCR ConditionsReference
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COI	Invertebrat es	mlCO1int F HCO2198 ~313(bp)	5'~GGWACWGG WTGAACWGTW TAYCCYCC~3' 5'~TAAACTTCA GGGTGACCAA AAAATCA~3'	 95 °C for 10 minutes 16 cycles of: 94 °C for 10 seconds 62 °C for 30 seconds (this changes -1°C for each subsequent cycle) 68 °C for 60 seconds Then 25 cycles of: 94 °C for 10 seconds 46 °C for 30 seconds 68 °C for 60 seconds Final elongation step of 72 °C for 10 minutes Hold at 4 °C 	Leray <i>et al.</i> 2013 Folmer <i>et</i> <i>al.</i> 1994
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BIOINFORMATICS

All analyses were performed in R Studio (Version 2024.04.2+764). The *COI* raw nucleotide sequence data was transformed by using Atropos (Didion *et al. In review*) to remove primer sequences. The DADA2 Banzai pipeline was used to trim regions of poor sequence quality, infer true sample composition, and merge paired forward and reverse reads (Callahan *et al.* 2016). Once the data had been cleaned and merged, taxa were assigned through NCBI GenBank database with the blastn algorithm (NCBI Resource Coordinators, 2018). Taxa identifications were followed by MEGAN6's lowest common ancestor algorithm (Huson *et al.* 2016). Taxize was used to verify ASV's taxonomic identification (Chamberlain & Szocs 2013) through the WoRMS database. The phyloseq package (McMurdie & Holmes 2013) was used to remove non-target taxa from the dataset, including terrestrial contaminants such as bacteria, fungi, and insects.

RESULTS

PISCO VISUAL SURVEYS

The most abundant phylum observed at all sites was Echinodermata with the most abundant species being the purple sea urchin (*Strongylocentrotus purpuratus*). The diversity between sites did not vary by much as PISCO divers used a closed-species list to identify invertebrate species of interest. General trends observed include MPA sites having a greater abundance of red algae compared to paired reference sites (Figure 3).

At the northern MPA site False Sur, 3004 organisms were counted, and 63.81% of those taxa were purple urchin (Figure 3). Bat stars (*Patiria miniata*) were the second most abundant species observed (n=256), and the third most abundant species were the white spotted rose anemone (*Urticina eques*) (n=163) and Monterey Stalked tunicate (*Styela montereyensis*)(n=148). At the northern reference site of South Wreck, a total of 3146 organisms were counted, including 60.68% of purple urchin (Figure 3). Bat stars were the second most abundant taxa observed (n=396), followed by the red urchin (n=276), and lastly the white spotted rose anemone (n=204). The southern MPA had the lowest number of organisms observed at 1608 total, with 61.69% of purple urchins (Figure 3). Bat stars were the second most abundant organism observed (n=63). Lastly, at the southern reference site at Green Peak, 3087 with 68.03% of taxa observed being purple urchin (Figure 3). The next abundant species observed were bat stars (n=280), red urchins (*Mesocentrotus franciscanus*)(n=172), and giant rock scallops (n=157).



Figure 3. The total number of individuals observed was grouped by phylum for the comparison to eDNA estimates. Sites are arranged from north to south. PISCO visual observations were collected by species or identification to the lowest taxonomic classification. The proportion of the community was calculated into percent count. The most abundant species observed were sea stars and sea urchins.

SUMMARY STATISTICS: COI GENE SEQUENCES

The total number of assigned amplicon sequence variants (ASVs) produced were 3,948,620 from the *COI* gene. The northern sites, False Sur and South Wreck Downcoast, assigned 893,207 and 797536 ASVs respectively. The southern sites, Point Buchon and Green Peak, assigned 78,4177 and 856,863 ASVs. The site with the most ASVs identified was Point Buchon (n=2781) then Green Peak (n = 2310), then False Sur (n = 2259), with South Wreck having the least amount of ASVs observed (n= 1707). The average number of reads per sample was about 147, ranging anywhere from 10 to 89,799 reads. The number of ASVs assigned per sample ranged from 1 to about 800. After running computing the diversity with Shannon's diversity indices, Pileou's evenness was calculated to ensure that reads were distributed evenly across ASV assignment.

COMMUNITY DYNAMICS FOR COI GENE: ALPHA DIVERSITY

Each site had a unique top five most abundant taxa observed from the *COI* primer. The main groups observed were red algae, coccolithophores, and copepods. Rotifer abundances increased southward (Figure 4). Rotifer and sponge abundances increased in the southern region. MPA sites appear to have higher abundances of red algae compared to reference sites. Offshore transects had a higher abundance of plankton and smaller phyla compared to nearshore transects. False Sur top species observed *Mazzaella flaccida, Emiliania huxleyi, Rhodymenia pacifica, Chondracanthus exasperatus, Chone magna.* South Wreck top species observed *Emiliania huxleyi, Chone magna, Tetraclita rubescens, Paracalanus sp. C AC-2013, Mazzaella flaccida.* The top species at Point Buchon were *Calliarthron tuberculosum, Mazzaella flaccida, Emiliana huxleyi, Tetraclita rubescens, Paracalanus species C AC-2013.* Lastly, the Green Peak sites top species observed were *Clausocalanus parapergens,Emiliania huxleyi, Chone magna, Clausocalanus furcatus, Nereocystis luekanta.*





COMUNITY DYNAMICS FOR COI GENE: BETA DIVERSITY

There are significant differences observed between sites when using eDNA. Using *COI* allows for communities to be distinguished not only by region, but both between sites and within sites. Figure 5 shows that data points group closely by region with some subset grouping between reference and reserve and circle and triangle respectively. The major difference is between MPAs

and references. Figure 6 shows the same pattern, but is subset by site and transect depth, which is nearshore (5 m depth) or offshore (20 m depth). Rather than seeing the sites group closely together like as to be expected, there is grouping by MPA protection and transect depth.



Figure 5. A robust principal component analysis (RPCA) comparing the regional differences in community composition and diversity. The northern region is found within Big Sur's Point Sur State Marine Reserve (Figure 1A.) and the southern region is found within Big Sur's Point Buchon State Marine Reserve (Figure 1B.).



Figure 6. A robust principal components analysis (RPCA) comparing the similarity and dissimilarity between sites and transects. The RPCA was calculated in DADA2 with QIIME2. Sites are grouped with nearshore (5 m depth transects) and offshore (20 m depth transects). Shapes of the datapoints indicate a collection level of bottom or surface and circle or triangles, respectively. The top loading scores for the False Sur and Point Buchon nearshore transects were taxa from the phyla Rhodophyta, which are red algae.



COMPARING SURVEY METHODS

Figure 7. The average number of species observed using each survey method. Environmental DNA had a greater number of species observed compared to PISCO visual surveys.

Overall, there was a total of 241 species observed when both methods were combined. When using eDNA, there was a total of 205 unique species observed with PISCO capturing 36 species. All the phyla observed by PISCO were observed using eDNA. The eDNA community captured smaller organisms, whereas PISCO captured larger macroinvertebrates. PISCO allows us to quantitatively track the populations through time by incorporating the historical data. The nine species shared in both detection methods were giant kelp (*Macrocystis pyrifera*), bull kelp (*Nereocystis luetkeana*), leather stars (*Dermasterias imbricata*), bat stars (*Patiria miniata*), gumboot chitons (*Cryptochiton stelleri*), cryptic kelp crab (*Pugettia richii*), northern kelp crab (*Pugettia producta*), purple urchin (*Strongylocentrotus purpuratus*), and sandcastle tube worms (*Phragmatopoma californica*).

DISCUSSION

This study determined if eDNA can detect differences within and between kelp forests in Big Sur sites. To understand how eDNA taxa detected differed from the PISCO visual survey results, we explored the most abundant taxa observed in each method. eDNA was shown to have the taxonomic resolution to detect differences not only between region but between sites and within sites and transects. With additional time, a PERMANOVA would have been run to determine if communities were significant different between methods. Overall, eDNA detected a greater number of phyla, species, and trophic levels compared to visual surveys alone (Figure 3. and 4.). Using the *COI* universal primer, we captured a diverse array of taxa. This provides a compositional snapshot of the kelp forest community, which provides greater estimates of the true diversity of kelp forests. However, eDNA detected a greater number of plankton and smaller organisms, PISCO detected a greater number of large important kelp-dwelling invertebrates. PISCO does a great job of identifying and quantifying important kelp dwelling macroinvertebrate species.

Both eDNA and visual surveys captured similar trends like MPAs harboring higher abundance and diversity of red algae compared to the paired reference sites. The visual surveys show high abundance of purple urchins and bat stars. Following the 2013 "the blob", urchin populations began to rise in central California, which lowered the diversity in kelp forest herbivores (Smith *et al.* 2021; Galloway *et al.* 2023). Purple and red urchin abundance increased through time with low persistent abundance in other grazers (Pearse 2006; Rodgers-Bennet *et al.* 2024). The MPA sites appear to do a better job of protecting the intertidal and nearshore communities compared to the reference sites. Both MPA sites appear to have greater abundances of more phyla observed. Previous studies have shown that MPA status increases biodiversity of kelp forests (Caselle *et al.* 2015).

Using eDNA metabarcoding of the COI region has many implications for kelp forest monitoring. The primer detected a high number of invertebrate and algal species common in kelp forests. The molecular method identified the plankton community like rotifers, coccolithophores, copepods, diatoms, and dinoflagellates. These zooplankton and phytoplankton are important food sources for filter feeders and lower trophic levels in kelp forest ecosystems. However, eDNA is not a standalone tool. Pairing visual surveys and environmental DNA metabarcoding is highly complementary as visual surveys allow us to quantify a targeted group of organisms while eDNA gives us a snapshot of organisms present in the community. Utilizing eDNA supplements the groups that visual surveys can miss in low visibility or if they have less taxonomic identification skills. Even though eDNA and visual surveys variably captured different groups in kelp forests, methods are highly complementary to one another. Collecting one liter of sea water can enhance the detection of rare, cryptic, or smaller organisms that are generally missed with traditional monitoring techniques. Additionally, PISCO's closed species list weakens the methodology's ability to discriminate differences between sites. Including eDNA for PISCO type visual surveys will increase the taxonomic resolution and distinguish differences in community composition between sites.

It is important to address the limitations of eDNA to make progress and enhance kelp forest monitoring. As previously discussed, eDNA is not yet able to quantify the abundance of organisms observed using universal primers like *COI*. Certain groups can have lower alignment rates due to primer biases (Pinto & Raskin 2012). For example, Echinodermata has been shown to have lower assignment when *COI* is used (Ward *et al.* 2008). Researchers in Japan produced the 16SOph1 primer to detect the class *Ophiuroidea*, which are brittle and basket stars (Okanishi *et al.* 2023). The answer to these primer bias solutions is using taxon-specific primers to directly select groups that one is interested in detecting for and even quantifying through qPCR. Another major limitation is the varying rate of DNA shed and degradation in the water column. Environmental DNA is a relatively new technology, and it is important to identify these contemporary limitations to establish more succinct methodological design. As we can identify major restriction for eDNA, visual surveys are limited by diver's air supply, taxonomic identification skills, and the visibility of the water column. Furthermore, divers need consistent training over time to ensure that visual

surveys are accurate and consistent. High-frequency diving can be energetically intensive and expensive to access these isolated kelp forests. Big Sur was a great study system for the pairing of these methods because the diving conditions are cold water with low visibility. As each method has major limitations, combining them is highly complementary and gives us deeper insight into trophic interactions in highly biodiverse marine ecosystems like giant kelp forests.

The objective of this study is to enhance traditional monitoring techniques to better infer management and restoration in the Monterey Bay National Marine Sanctuary. The Monterey Peninsula has experienced drastic declines in kelp coverage so there is an urgent need for proper management. Recently, Dr. Tom Bell with Kelpwatch, host to the world's largest and public repository of satellite derived kelp canopy coverage, published a paper (Bell *et al.* 2024) that used satellite imagery to map kelp coverage's fluctuation through time stating "Understanding differences in environmental conditions and trophic interactions around the Monterey Peninsula and nearby locations that have exhibited high kelp canopy recovery may shed light on important drivers that are best assessed by instrumented moorings and diver-based survey methods". By exploring how community composition changes through time and in the face of variable environmental stress, we understand why some sites are recovering faster than other sites. By assessing environmental variation and shifts in community dynamics over time, we can model how kelp forests will respond to major environmental stressors like heatwaves or storm surges.

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