**SUPPLEMENT 1**

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**Introduction**

This supplemental material provides additional details on the methods, results, and discussion to support the main findings and conclusion of the manuscript.

The CalCOFI program (<https://calcofi.com/>) serves to provide fisheries-independent ecosystem assessments of fish assemblages in the Southern California Current and has provided decades of data on ichthyoplankton assemblages (*1*). The current CalCOFI surveys sample four times per year from the U.S. Mexican Border to Monterey Bay (*1*). We used this rich sample archive to interrogate ichthyoplankton assemblages from 1996-2019 (See Supplemental Methods). Importantly, the occurrence of a marine heatwave (MHW) within the study region and sampling period provided an additional opportunity to investigate the utility of having a non-destructive means of interrogating the valuable CalCOFI sample archive.

**Methods**

*Study Design*

To evaluate the efficacy of metabarcoding methods used to analyze ethanol preserved samples and investigate potential changes in the ichthyoplankton assemblages over decadal scales, we identified ichthyoplankton by metabarcoding and microscopy in ethanol-preserved samples collected over two decades (1996,1998-2019; Figure S5) of spring CalCOFI cruises (*2*–*5*). We note that samples collected in 1997 were stored in <50% ethanol and were discarded due to failed preservation.

Samples were collected in late March or early April of each year (calcofi\_metadata\_analysis\_20210907.csv). Here we focus on spring samples because the majority of species in the California Current spawn in spring and historically the annual California Current Ecosystem Report has relied on the spring data (*6*, *7*). This decision is supported by recent work using ichthyoplankton data across the full set of yearly CalCOFI cruises which found little evidence for phenological trends (*6*, *7*), thus aiding the ability to look at impacts from the marine heatwave.

Samples were collected from four well-separated stations (up to 370 km apart) from distinct vicinities of the California Current with differing water properties (*8*) (*2*–*5*) (Figure S1). The northernmost station was located offshore of Point Conception, CA within the California Current (34.14833˚N -121.1567˚W). The second station was located off San Nicholas Island, CA (33.32333 ˚N, -119.6667˚W) that experiences high variation in annual temperature depending on the respective strengths of the California Current and Southern California Counter Current (*8*). The third station was a southern coastal inshore site off San Diego, CA (32.84667˚N, -117.5383˚W) characterized by relatively warmer waters from the California Counter Current with seasonal (spring) upwelling of cool, nutrient-rich water (*8*). The fourth site was a southern offshore site (31.85000˚N, -119.5683˚W) characterized by sub-tropical oceanic waters (Figure 4).

At each station, oblique bongo net tows were conducted from 210 m to the surface using standard CalCOFI methods (*9*–*12*). Each side of the bongo net had a 0.71 m-diameter mouth opening and a net size of 0.505 mm mesh. Cod end contents of both bongo nets were preserved at sea. The starboard side was preserved in sodium borate-buffered 2% formaldehyde and the port side was preserved in Tris-buffered 95% ethanol. Ethanol was replaced after 24 hours to account for dilution from tissue water loss. Microscopy was conducted to identify species abundance from formaldehyde-preserved samples following standardized CalCOFI techniques (*8*) while metabarcoding was conducted on the ethanol in which port side samples were stored; consequently, we expected the contents of the paired samples to differ slightly as a function of sampling stochasticity.

*Metabarcoding Collection Isolation, Amplification, and Sequencing*

Prior to filtration, the ethanol-preserved samples were inverted three times and let rest for 30 minutes to resuspend and homogenize samples in the preservative. Filtration of ethanol from the port-side bongo samples was conducted in a pre-PCR clean room at the NOAA Southwest Fisheries Science Center within a biological safety cabinet in July 2019. The pre-PCR room had no previous post-PCR work conducted within and all surfaces and equipment were sterilized frequently with 10% bleach and 70% ethanol. The pre-PCR clean room was at ambient pressure and reasonable precautions to limit contamination were conducted including only wearing clean clothes that have not been exposed to labs with PCR product, no food brought into the lab, and gloves were exchanged regularly.

Ethanol preservative was filtered using a vacuum filtration manifold with Nalgene Analytical Test Filter Funnels (Thermofisher Scientific, Waltham, MA, USA) with the manufacturer’s 0.45 µm filters replaced with 0.2 µm Durapore PVDF filters (Sigma Aldrich, St. Louis, MO, USA) using sterile forceps. Up to 125 mL of ethanol was then transferred from the preserved jars into the filter funnels using a 10 mL pipette, carefully avoiding any sample contents and thus preserving CalCOFI specimens for future research and analysis. Sample jars were refilled using freshly prepared tris-buffered ethanol before being returned to the collection archive. We included two negative controls to test for lab contamination by filtering 125 mL of molecular grade water. Filters were stored at -20˚C before DNA extraction.

Filters were extracted using the standard Qiagen DNAeasy Kit (Qiagen Inc., Valencia, CA, USA) in a pre-PCR molecular lab. Extracted DNA was amplified using the MiFish Universal Teleost primer sets to capture fish diversity (*13*)*.*

Here, we highlight our decision to utilize the MiFish Universal Teleost *12S* primers. First, these primers have been rigorously validated for fish barcoding (*14*–*20*) and shown to provide accurate taxonomic assignments for a broad range of fishes (*15*). We recognize that there are limitations for this, and indeed all, metabarcoding primer sets (*21*) which are forced to balance specificity [how well target species can be taxonomically resolved] against breadth [range of species across the tree of life that can be amplified] (*22*). Even a “gold standard” like the *16S* rRNA gene marker for prokaryotic sequences struggles with taxonomic assignment accuracy (*23*), especially with short-read sequences. Although taxonomic resolution limitations and compromises remain for the *12S* target (*15*, *24*), the taxonomic resolution has been improved and best practices for taxonomic classification have been identified through the development of a nearly comprehensive California Current Large Marine Ecosystem *12S* reference database along with a full factorial cross-validation analysis of bioinformatic approaches (*15*).

Second, there are no widely used or benchmarked *CO1* metabarcoding primer sets for fish applications although *CO1* barcoding is a common barcoding target. This is because a) the conserved nature of the locus across the tree of life which results in amplification of a broad array of taxa (*25*, *26*), and b) the mismatch in high throughput sequencing platform length (max is paired-end 300 bp) and rate of *CO1* evolution/accumulation of sequence differences between species (*19*, *27*). In fact, these shortcomings were the original motivation for researchers to develop alternative fish metabarcoding loci targeting *12S* loci for fishes (*20*). Together, the research community has largely converged on the MiFish Universal Teleost *12S* primer set as standard practice for fish metabarcoding given its balance of high specificity and breadth (*20*). Thus we feel confident that the MiFish Universal Teleost *12S* primer set was an appropriate choice for metabarcoding here.

Each metabarcoding extraction was subsampled for three PCR reactions using the MiFish *12S* primer set. PCR amplification for the MiFish primer set was conducted following the thermocycler profile of Curd *et al.* (*16*). MiFish PCR reactions had 25 μL reaction volume containing 12.5 μL QIAGEN Multiplex Taq PCR 2x Master Mix (Qiagen Inc., Valencia, CA, USA), 6.5 µL of molecular grade water, 2.5 µL of each primer (2 µmol/L), and 1 μL DNA extraction. MiFish PCR thermocycling employed a touchdown profile with an initial denaturation at 95°C for 15 min to activate the DNA polymerase, followed by 13 cycles of a 30s denaturation at 94°C, a 30s annealing that started at 69.5°C and then decreased by 1.5°C for each subsequent cycle (last cycle was 50°C), finishing with a 1 min extension at 72°C. This initial touchdown profile was followed by 35 additional cycles using identical parameters except a constant annealing temperature of 50°C and ending with a final extension at 72°C for 10 min.

Two non-native non-marine vertebrates, American alligator (*Alligator mississippiensis*) and dromedary camel (*Camelus dromedarius*), were purchased at a local market and used as positive controls. For all positive controls, tissues were extracted using the Qiagen Blood and Tissue kit following the manufacturer’s instructions. All PCR products were visualized via electrophoresis on 2% agarose gels to ensure amplification success and correct product size. Only filters from four jars failed to amplify, and upon further inspection within the archived notes, all these samples had known preservation issues (e.g., preservative dried out, observed mold, etc.). All other DNA extractions successfully amplified.

We prepared libraries following the methods of Curd *et al.* using a two-step PCR amplification method with one final pool per primer set. Previous work indicated that two-step PCR amplification can reduce amplification biases (*28*, *29*) perhaps introduced by the inclusion of various indices during one-step PCR procedures. Variations in the relative amplification efficiency of each PCR is a concern here given the desire to study an array of targets in an oceanic region over space and time. Overall, there are review papers available that outline the advantages and disadvantages for one-step and two-step PCR protocols (*30*).

Prior to the second indexing PCR reaction, PCR samples from the first reaction were cleaned using the Serapure magnetic bead protocol. We quantified bead-cleaned samples with the Quant-iT™ broad range dsDNA Assay Kit (Thermofisher Scientific, Waltham, MA, USA) on a Victor3 plate reader (Perkin Elmer Waltham, MA, USA). We indexed the sample libraries using unique combinations of the Nextera Index A, B, C, and D Kit (Illumina, San Diego, CA, USA) and KAPA HiFi HotStart Ready Mix (Kapa Biosystems, Sigma Aldrich, St. Louis, MO, USA). Indexing was performed with a second PCR using a 25 μL reaction mixture containing 12.5 μL of Kapa HiFi Hotstart Ready mix, 1.25 μL of index primers, 10 ng of template DNA to ensure equal copy number, and the remaining volume was filled using molecular grade water depending on cleaned PCR product concentration. Index thermocycling parameters were: denaturation at 95˚C for 5 min, 5 cycles of denaturation at 98˚C for 20 sec, annealing at 56˚C for 30 sec, extension at 72˚C for 3 min, followed by a final extension at 72˚C for 5 min. To confirm successful PCR and correct product size, we electrophoresed PCR products on 2% agarose gels. We then bead cleaned and quantified DNA concentration, as described above so that we could pool samples so as to have equal copy number for each unique library. Pooled libraries were sequenced on an Illumina NextSeq PE 2x150 at UCLA Technology Center for Genomics and Bioinformatics.

*Bioinformatics*

The resulting metabarcoding data were processed using the *Anacapa Toolkit* to conduct quality control, amplicon sequence variant (ASV) parsing, and taxonomic assignment using user-generated custom reference databases. We processed sequences using default parameters except using a Q score cutoff of 30 and assigned taxonomy using *CRUX*-generated metabarcode specific reference databases (*15*). The MiFish sequencing data was assigned taxonomy using the California fish specific reference database and a bootstrap confidence cutoff score of 60 following Gold *et al .*(*15*).

The two resulting raw ASV community tables were decontaminated following Kelly et al. (*31*). First, only merged paired reads that occurred at least twice (e.g., no singletons) were retained. Second, we estimated index hopping between samples by calculating the proportion of sequences within the positive control samples and then subtracting reads from each sample by the sample read depth multiplied by the proportion of reads observed in the positive controls. Third, we discarded technical replicates with fewer than 30,000 reads. Fourth, we calculated Bray-Curtis dissimilarities between technical PCR replicates and fit a skewed beta distribution (a= 0.6, b= 9.5). We then removed all replicates with greater than 95% probability of belonging to the beta distribution. Resulting tables were then combined into a final ASV community table in *R.*

*Microscopy Identification of Ichthyoplankton*

Plankton samples were processed at the NOAA Southwest Fisheries Science Center ichthyoplankton laboratory. From each plankton sample, fish larvae were sorted and identified through microscopy to the lowest practical taxon (*10*, *12*). Most taxa were identified to species although some were only characterized to genus or family level (See larval\_counts\_20210305.csv). The number of larvae per species per jar, total abundance of filtered ichthyoplankton, and proportion of jar sorted were recorded.

*Estimating Abundance*

We estimated the abundance of ichthyoplankton in each jar using a novel joint Bayesian hierarchical model described in detail in Supplement 2.

*Environmental Covariates*

We specifically examined the relationship of ichthyoplankton communities to sea surface temperatures (SST). Two month prior mean SSTs were obtained using the *rerddapXtracto* package (*32*) in *R* to collect PathFinder Ver 5.3 monthly remotely sensed composites. To calculate two-month prior means we first obtained monthly composites from April 1995 to April 2019 for each station. We then averaged across monthly composite sea surface temperatures ignoring any missing values. Prior two month sea surface temperatures were chosen given the average age of spring larvae (*33*) (Figure 4).

We then investigated the relationship of ichthyoplankton assemblages against sea surface temperature as a proxy for a multitude of environmental shifts associated with the MHW. In addition, we specifically characterized fish that are uniquely present or absent before (1996-2013) and after the 2014-16 Marine Heatwave (2014-2019). Analyses were repeated using the mean average water column temperature (MWCT) obtained from nearly simultaneously conducted CTD rosette deployments (*9*). The mean water column temperature was averaged across 10 to 100m depth, where the majority of ichthyoplankton reside, following Thompson et al. (*9*).

*Data Analysis*

After model estimation, we calculated mean abundance estimates (larvae counts per standardized volume towed) per species per site per year. To explore species-specific sea surface temperature (SST) relationships, we fit a Bayesian generalized linear model using log (abundance) as the response variable and SST (˚C) as a continuous predictor variable. Models were implemented for each species using Stan as implemented in R (*34*). We then summarized the affinity between each species and SST by calculating a T-statistic based on each species' estimated coefficients (mean slope/standard deviation). We further plotted the estimated slope for each “species grouping” by habitat associations derived from previous CalCOFI research (habitat\_association\_to\_check\_art.csv)(*35*). We summed total log(abundance) per habitat association per site per year and fit a Bayesian generalized linear model using log (total abundance) as the response variable and SST (˚C) as a continuous predictor variable.

We repeated the above analyses using a Bayesian binomial model using presence as the response variable and SST (˚C) as a continuous predictor variable across the data set to explore occurrence relationships with temperature and identify warm- and cool- associated taxa. We set a threshold of presence/absence based on the model using a threshold of < 0.01 larvae per standardized volume to be considered absent within a site.

We further explored species occurrence and abundance relationships with SST by fitting the above Bayesian generalized linear models with site as a random effect. In addition, we repeated all of the above analyses using MWCT instead of SST.

To explore how fish assemblages change over time we plotted a heatmap of observed abundance summed across sites each year. Chronological clustering was conducted across years using Bray Curtis dissimilarities of abundances using a K of 8 using the package *rioja* in R (*36*) and a dendrogram of years was constructed using the *ggdenro* package (*37*). Similarly, hierarchical clustering was conducted across species using Bray Curtis dissimilarities of abundances using a K of 6. To further explore fish assemblage changes NMDS Ordination of Bray-Curtis dissimilarities were calculated from estimated abundances of each year summed across sites as implemented by the *metaMDS* function from *vegan* in R (*38*). The above analyses were also conducted with site separated as well as each site on it’s own. To investigate the relative effect of year, SST, and site to the explained variance in fish assembalges across the ata set, we ran a PERMANOVA on Bray-Curtis dissimilarities using the following model: ~ Year + SST + Site.

We visualized anchovy and sardine abundance over time by calculating the median log (abundance) of each species per station per year. We then plotted the log (median) abundance of each of the four sites while error bars represent the 95% confidence intervals observed for a given species at a given site in that year.

To explore co-occurrence patterns across species, we fit a generalized linear latent variable model (GLLVM) following the methods of Niku et al. (*39*), specifically conducting model fitting to determine the best distribution fit as well as number of latent variables to use. The highest performing GLLVM employed 3 latent variables and applied a negative binomial distribution with variational approximation (*40*) on the joint model predicted larvae count data. We then plotted the correlation matrix of the linear predictors across species with and without incorporating SST in the GLLVM to identify co-occurring species and the effect of SST on co-occurrence patterns.

To evaluate the effect of the marine heatwave (MHW) on CCLME fishes we compared estimated species abundances before the MHW (1996-2013), to both during and after the MHW (2014-2019), at each site respectively. We first calculated the mean abundance for each species at each site for each model run. We then subtracted the means for each model run to evaluate changes in MHW abundance per species per site per model run. We then calculated a 95% CI of change in MHW abundance per species to identify which species were significantly different before vs. during and after the MHW at each site.

All data and code to conduct analyses and generate all figures are available on GitHub (https://github.com/zjgold/CalCOFI\_eDNA) and associated Google Drive link (https://drive.google.com/drive/folders/12cU9mY\_CWoro-x6Hgh\_pgv\_66zZEzm1h?usp=sharing) [will be replaced with a Dryad repository upon acceptance].

**Results**

*Fish Assemblage Structure*

We observed substantial changes in fish assemblage structure across sites, time, and temperature sampled (NMDS stress =0.03) (Figure S11-26). Site explained the greatest observed variance (12%) which is unsurprising given the intentionally chosen distinct biogeographic characteristics of each site (PERMANOVA p <0.05). However, despite the > 370km distance between sites, we captured significant synchronous changes in fish assemblage dynamics in response to year (2.4%) and temperature (4.6%) (PERMANOVA p <0.05). In particular, we observed strong clustering of the post MHW period from 2017-2019, the 2005 El Niño and the 1998 El Niño along with southern mesopelagic species. Both 2014 and 2016 were distinct from other years and associated with a suite of mesopelagic species, although the MHW itself was not strongly clustered largely due to the differential onset and characterization of the warming event within the region (*3*).

We also found strong positive and negative co-occurrence patterns through GLLVM analyses across species when controlling for temperature. Specifically, we observed strong negative co-occurrence patterns between fisheries targets like benthic fisheries targets (e.g. *Citharichtys* sp. sanddabs) and mesopelagic fishes (S27). We also observed strong positive co-occurrence patterns between a suite of benthic species as well as strong positive co-occurrence patterns between a suite of mesopelagic fishes. These results suggest that when controlling for temperature, we observe strong benthic versus pelagic tradeoffs as observed previously (*6*, *41*, *42*).

When focusing on co-occurrence patterns without controlling for temperature, we observed strong negative co-occurrence patterns with fisheries targets (North Pacific Hake *Merluccius productus*) and mesopelagic fishes (S28). Here, temperature explained 19% of the variability among species co-occurrence and was particularly important in driving negative co-occurrence patterns between North Pacific Hake and mesopelagic species. These results suggest that temperature may mediate tradeoffs between fisheries versus southern mesopelagic fish assemblages. Further work exploring the underlying mechanisms of these negative co-occurrence patterns is warranted.

*Analysis Using Mean Water Column Temperature*

We repeated the analyses presented in the main manuscript with mean water column temperature (MWCT) as opposed to two-month SST (Figures 1-3). Two-month averaged SST and instantaneous MWCT were only 29% correlated (linear regression, p<0.01). This finding is perhaps unsurprising given the substantial difference in spatial and temporal integration time of these temperature measurements. Despite these apparent differences, we found nearly identical results in the direction and significance of species–temperature associations as well as temperature-driven variation in fish assemblage dynamics (Figures S6-S13). These results suggest that species-temperature associations and our conclusions are largely robust to temperature metrics.

*Metabarcoding Signal Appears Stable in the Ethanol-Preserved Samples*

For each site-species combination, if metabarcoding signals appear auto-correlated in time -- that is, if one year's metabarcoding signal is correlated with the previous year's signal -- then we require a time-series model that incorporates such autocorrelation into the error structure. If, by contrast, years appear independent of one another, we can treat model variation as time-independent and therefore treat each data point as being independent. We observe no such correlation (mean = -0.014, standard deviation = 0.35) and so we treat all observations as independent of one another.

In further investigating the question of whether these samples can be considered time-independent, we considered whether or not older samples might have less metabarcoding signal due to sample degradation. If the metabarcoding signal were degrading overtime in the preserved samples, we would expect several parameters to change as a function of sample age: (1) a decrease in precision with which we observe amplicon abundance, (2) a decrease in richness of species detected, and (3) a decrease in the confidence in posterior estimates of larval abundances from our joint Bayesian model. We test for these effects in turn.

First, among triplicate PCR reactions, we might expect degraded DNA to behave more stochastically than non-degraded DNA, such that technical replicates would yield increasingly divergent amplicon abundances with greater degradation. Here, we measure the precision of our estimates with the coefficient of variation (CV) of species-specific amplicons across three technical replicates. An increase in CV with the age of the sample would signal degradation, but we saw no such trend (Figure S29). Second, rare amplicons often make up a large fraction of metabarcoding datasets, and because of their rarity, these often show up stochastically across replicates or sequenced samples. If older DNA samples were degraded, we would expect fewer of these rare species, and by extension, fewer species overall. We saw no such effect (linear regression p> 0.5; linear mixed effect model failed convergence). Third, we might expect -- if DNA were degrading -- that such degradation would impair our ability to estimate the larval abundance of each species in older samples. Again, we saw no evidence of this effect (Figure S30-31).

*Overlap in Species Detections*

The maximum observed morphological counts in which metabarcoding failed to detect a given taxa was 9 (mean = 1.61). Across a total of 4,704 possible detections, 70.2% were non-detections by both methods, 11.2% were detections by both methods, 16.4% were detections only made by metabarcoding, and 2.1% were detections only made my microscopy (Figure S4).

*Stochasticity in Metabarcoding Data*

We conducted a deep dive into the origin and source of variation in amplicon sequence data. These analyses identified stochastic dropouts in whereby a taxon is amplified in one PCR reaction but not in a replicate PCR reaction as a main driver of variation in this data set. This is a well-known phenomenon general to PCR with rare templates (*43*–*45*) and we document such behavior in this manuscript (Figures S2-S4). For example, for *Symbolophorus californiensis* we observed an instance of 3,897 reads, 165 reads, and 0 reads across three technical PCR replicates with sample read depths of 132,731, 196,260, 55,400 from the same DNA extraction. These stochastic dropouts are easily visualized along the X axis in Figure S2. We note that the highest observed species-specific amplicon sample read proportion associated with a stochastic dropout was 2.9% (3,897 /132,731) with the vast majority of such dropouts occurring below 0.03% read proportion within a technical replicate. These results suggest that stochasticity is largely driven by the abundance of DNA molecules within a sample rather than a specific feature associated with a particular primer set, especially given that dozens of other metabarcoding studies have identified similar patterns (*44*–*46*).

This phenomenon of stochastic dropouts adds noise to the observations and limits the accuracy with which we might predict amplicon abundances (particularly rare ones). This is best visualized by the noise near the origin at Figures S3 & S4. To address this, we developed a comprehensive joint Bayesian model that incorporates stochasticity in observed amplicon read counts through a multinomial subsampling process (See supplement 2 for full model description). Thus, we explicitly account for stochasticity in the model through sampling distributions and using the resulting parameters to estimate the uncertainty around our given estimated larvae counts. Ultimately, such noise in the dataset does not fundamentally change the interpretation of our observations or of our model but serves to limit our confidence in the abundance of rare targets, a persistent problem in community ecology (*47*).

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Map

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**Figure S1. Site Map**

Ichthyoplankton samples were collected from four stations with distinct biogeographic characteristics.

Chart, scatter chart

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**Figure S2. Observed Sequence Reads vs. Observed Morphological Counts**

Observed sequencing reads and morphological counts do not follow a clear linear relationship. The one-to-one line is plotted in red and Pearson correlation coefficient is 0.56. This non-linearity is unsurprising given that observed reads are a function of both DNA concentrations (here assumed proportional to morphological counts) as well as species-specific amplification efficiencies (here are unknown) (See Supplement 2). Thus without accounting for species-specific amplification efficiencies within the compositional amplicon data set, we do not expect to observe a clear positive relationship. This apparent lack of relationship depicted here motivated the creation of the mechanistic joint Bayesian model. We also note the occurrence of stochastic dropouts (technical replicates with zero reads) can be observed along the X-axis. We also note that variance is highest at low observed morphological counts.

Chart, scatter chart

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**Figure S3. Predicted Counts vs. Observed Morphological Counts**

Predicted counts are generated from the joint Bayesian model. The one-to-one line is plotted in red and Pearson correlation coefficient is 0.81. Variance in predicted counts markedly decreases with higher observed morphological counts. We note variance in predicted counts is substantially less than that between observed reads and morphological counts Figure S3.

Chart, scatter chart

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**Figure S4. Predicted Sequence Reads vs. Observed Sequence Reads**

Predicted sequence reads are generated from the joint Bayesian model. The one-to-one line is plotted in red and Pearson correlation coefficient is 0.95. In general, predicted sequence reads track observed sequence reads, and show substantially less variance than observed sequence reads in Figure S3. However, unexpected zeros across multiple technical PCR replicates (stochastic dropouts), deviate notably from expected, low variance results (points along Y-axis). We note that all observed stochastic dropouts occur in less than 2.9% of sample read proportions and less than 9 morphological counts for a given sample. Such dropouts are likely a function of subsampling rare DNA molecules associated with molecular biology processing (See Supplement 2).

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**Figure S5. Co-detection of Taxa By Metabarcoding and Microscopy**

Of the 56 taxa used for modeling efforts (Supplemental Methods), both metabarcoding and microscopy detected 46 taxa, with nine detected only be metabarcoding and one detected only by microscopy.

Graphical user interface, application, PowerPoint

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**Figure S6. Temperature Associations in Fish Species**

Changes in species occurrence patterns in response to MWCT, with Southern Mesopelagic species increasing in prevalence with elevated temperature (A). T statistic (slope coefficient/ standard error) from generalized binomial mixed model was calculated for each species across all sites. Only species with significantly different slopes (95% CI greater or less than zero) are plotted. Importantly, metabarcoding identified cold associated variants of the Northern Lanternfish (*Stennobrachius leucopsarus*) which cannot be morphologically identified (B) as well as warm-associated species like the Mexican Lampfish (*Triphoturus mexicanus*) (C).

Chart

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**Figure S7. Southern Oceanic Species Drive Fish Community Shifts**

We capture changes in species biomass in response to MWCT, with Southern Mesopelagic species increasing in abundance with elevated temperature (A). T statistic (slope coefficient/standard error) from generalized linear models were calculated for each species across all sites. Only species with significantly different slopes (95% CI greater or less than zero) are plotted. Southern mesopelagic fishes were associated with increased temperature as indicated by the boxplots of all species-specific slopes from generalized linear models (B) and by the aggregated abundance relationship (C). In contrast, benthic species, as well as Northern Hake and Pacific Sardine abundances, were correlated with cooler temperatures.

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**Figure S8. Significant Species Occurrence and SST Correlations at each Site**

Occurrence of the Mexican lampfish (*Triphoturus mexicanus*)was positively correlated with increased SST at the three northernmost sites. Generalized binomial mixed model of occurrence versus SST was calculated for each species at each site. Only species with significantly different slopes (95% CI greater or less than zero) are plotted. Colors correspond to T statistic (slope coefficient/ standard error). Colors of species names correspond to habitat associations described in Figure 2.A picture containing chart

Description automatically generated

**Figure S9. Significant Species Occurrence and MWCT Correlations at each Site**

Occurrence of the Mexican lampfish (*Triphoturus mexicanus*)was positively correlated with increased MWCTat the three northernmost sites. Generalized binomial mixed model of occurrence versus MWCTwas calculated for each species at each site. Only species with significantly different slopes (95% CI greater or less than zero) are plotted. Colors correspond to T\_statistic (slope coefficient/ standard error). Colors of species names correspond to habitat associations described in Figure 2.

**Chart

Description automatically generated Figure S10. Significant Species Abundance and SST Correlations at each Site**

The bbundance of the Mexican lampfish (*Triphoturus mexicanus*)was positively associated with increased SST at all sites. Likewise, the abundance of suite of mesopelagic species including *Vinciguerria* sp., *Symbolophorus californiensis,* and *Stomias atriventer* among others increased with warmer SST. Generalized linear mixed model of log (abundance) versus SST was calculated for each species at each site. Only species with significantly different slopes (95% CI greater or less than zero) are plotted. Colors correspond to T statistic (slope coefficient/ standard error). Colors of species names correspond to habitat associations described in Figure 2.Chart, bar chart

Description automatically generated

**Figure S11. Significant Species Abundance and MWCT Correlations at each Site**

The abundance of the Mexican lampfish (*Triphoturus mexicanus*)was positively associated with increased MWCTat all sites. Likewise, the abundance of suite of mesopelagic species including *Vinciguerria* sp., *Symbolophorus californiensis,* and *Stomias atriventer* among others increased with warmer SST. Generalized linear mixed model of log (abundance) versus MWCTwas calculated for each species at each site. Only species with significantly different slopes (95% CI greater or less than zero) are plotted. Colors correspond to T statistic (slope coefficient/ standard error). Colors of species names correspond to habitat associations described in Figure 2.

Chart

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**Figure S12.** **Bar Plot** **of Significant Species Abundance and SST Correlations Across All Sites**

The abundance of southern mesopelagic species increased with warmer temperatures while fisheries targets like Pacific Sardine (*Sardinops sagax*) and North Pacific Hake (*Merluccius productus*) decreased. Generalized linear mixed model of log (abundance) versus SST was calculated for each species across all sites. Only species with significantly different slopes (95% CI greater or less than zero) are plotted. Colors correspond to T statistic (slope coefficient/ standard error).

**Chart, bar chart

Description automatically generated**

**Figure S13.** **Bar Plot** **of Significant Species Abundance and MWCT Correlations Across All Sites**

The abundance of southern mesopelagic species increased with warmer temperatures while fisheries targets like Pacific Sardine (*Sardinops sagax*) and North Pacific Hake (*Merluccius productus*) decreased. Generalized linear mixed model of log (abundance) versus SST was calculated for each species across all sites. Only species with significantly different slopes (95% CI greater or less than zero) are plotted. Colors correspond to T statistic (slope coefficient/ standard error).

Chart, scatter chart

Description automatically generated

**Figure S14. Increased Abundance of Southern Mesopelagic Species with Higher SST**

Species specific regressions of log (Abundance) vs. SST (˚C). Three of the four southern mesopelagic species had significant positive associations between abundance and SST. Although not significant across all sites, the abundance of *Ceratoscopelus townsendi* significantly increased with higher SST at the San Diego Offshore site (Figure S7).

Diagram

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**Figure S15. Increased Abundance of Southern and Central Mesopelagic Species with Higher SST**

Abundances of each habitat association were summed at each site. Habitat association regressions were fit using sum total log (Abundance) vs. SST (˚C).

Chart, box and whisker chart

Description automatically generated

**Figure S16. Heat Map of Abundances Over Time**

Northern Anchovy (*Engraulis* mordax) and rockfishes *Sebastes* sp. dominated predicted counts. Estimated abundance of each year, averaged across sites, plotted over time. Years are color coded by chronological clustering. Species are grouped by hierarchical clustering. Lighter colors indicate higher abundance, white is a lack of detection. Species are color coded by habitat association matching Figure 1.

Diagram

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**Figure S17. NMDS Ordination of Species and Years**

Fish assemblages changed across time with southern mesopelagics clustering with the 1998 and 2005 El Niños as well as 2017-2019 after the peak of the marine heat wave. NMDS Ordination of Bray-Curtis dissimilarities calculated from summed abundance of each year averaged across sites. Marine heatwave patterns are obscured by differential onset and receding of the warming event across sites. Years are color coded by chronological clustering (k =8). Species are color coded by habitat association matching Figure 2.

Graphical user interface

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**Figure S18. NMDS Ordination of Species and Samples**

Fish assemblages were strongly structured by sites, particularly the station just offshore of San Nicholas Island which had the highest abundance of *Sebastes* sp. NMDS Ordination of Bray-Curtis dissimilarities calculated from summed abundance of each year averaged across sites. Marine heatwave patterns are obscured by differential onset and receding of the warming event across sites. Samples are color coded by station. Species are color coded by habitat association matching Figure 2.Chart

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**Figure S19. Heat Map of San Diego Offshore Abundances Over Time**

Estimated abundance of each year plotted over time. Years are color coded by chronological clustering. Species are grouped by hierarchical clustering. Lighter colors indicate higher abundance, white is a lack of detection. Species are color coded by habitat association matching Figure 1.

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**Figure S20. NMDS Ordination of San Diego Offshore Species and Years**

NMDS Ordination of Bray-Curtis dissimilarities calculated from abundance of each year. Years are color coded by chronological clustering (k =8). Species are color coded by habitat association matching Figure 2.A picture containing chart

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**Figure S21. Heat Map of San Diego Inshore Abundances Over Time**

Estimated abundance of each year plotted over time. Years are color coded by chronological clustering. Species are grouped by hierarchical clustering. Lighter colors indicate higher abundance, white is a lack of detection. Species are color coded by habitat association matching Figure 1.

**Chart

Description automatically generatedFigure S22. NMDS Ordination of San Diego Inshore Species and Years**

NMDS Ordination of Bray-Curtis dissimilarities calculated from abundance of each year. Years are color coded by chronological clustering (k =8). Species are color coded by habitat association matching Figure 2.A picture containing chart

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**Figure S23. Heat Map of Pt. Conception Abundances Over Time**

Estimated abundance of each year plotted over time. Years are color coded by chronological clustering. Species are grouped by hierarchical clustering. Lighter colors indicate higher abundance, white is a lack of detection. Species are color coded by habitat association matching Figure 1.

Diagram

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**Figure S24. NMDS Ordination of Pt. Conception Species and Years**

NMDS Ordination of Bray-Curtis dissimilarities calculated from abundance of each year. Years are color coded by chronological clustering (k =8). Species are color coded by habitat association matching Figure 2.

**Figure S25. Heat Map of San Nicholas Island Abundances Over Time**

Estimated abundance of each year plotted over time. Years are color coded by chronological clustering. Species are grouped by hierarchical clustering. Lighter colors indicate higher abundance, white is a lack of detection. Species are color coded by habitat association matching Figure 1.

Diagram

Description automatically generated

**Figure S26. NMDS Ordination of San Nicholas Island Species and Years**

NMDS Ordination of Bray-Curtis dissimilarities calculated from abundance of each year. Years are color coded by chronological clustering (k =8). Species are color coded by habitat association matching Figure 2.

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Description automatically generated

**Figure S27. Co-occurrence Patterns of Species Controlling for SST**

Benthic fisheries targets like sanddabs were negatively correlated with mesopelagic species when controlling for SST. These results capture a strong benthic vs. pelagic community dynamics when controlling for SST. We plot the correlation matrix of the generalized linear latent variable model predictors across species. Species in blue correlate are positively correlated with each other while species in red are negatively correlated. Strength of association is visualized by size of square. Species are color coded by habitat association matching Figure 2.

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**Figure S28. Co-occurrence Patterns of Species**

Fisheries targets like North Pacific Hake were negatively correlated with mesopelagic species. We plot the correlation matrix of the generalized linear latent variable model predictors across species. Species in blue correlate are positively correlated with each other while species in red are negatively correlated. Strength of association is visualized by size of square. Species are color coded by habitat association matching Figure 2.

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**Figure S29. Stable Precision of Amplicon Abundance Over Time**

Here we measure the coefficient of variation (CV) of species-specific amplicons across three technical replicates. An increase in CV with the age of the sample would signal degradation; we see no such trend.

Chart

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**Figure S30. Stable Precision of Abundance Estimates Over Time**

Coefficient of variation of model estimates of abundance over time. We observe no evidence of change in precision over time.