Received: 28 July 2022 | Revised: 10 February 2023 | Accepted: 17 February 2023

DOI: 10.1002/edn3.400

Environmental DNA
Open Access
Dedicated to the study and use of environmental DNA for basic and applied sciences

WILEY

SPECIAL ISSUE ARTICLE

Archived DNA reveals marine heatwave-associated shifts in fish assemblages

Zachary Gold[1,2,3,4,5] | Ryan P. Kelly[6] | Andrew Olaf Shelton[2] | Andrew R. Thompson[3] | Kelly D. Goodwin[3,7] | Ramón Gallego[2] | Kim M. Parsons[2] | Luke R. Thompson[7,8] | Dovi Kacev[9] | Paul H. Barber[4]

[1]Cooperative Institute for Climate, Ocean, & Ecosystem Studies, University of Washington, Seattle, Washington, USA

[2]Northwest Fisheries Science Center, NMFS/NOAA, Seattle, Washington, USA

[3]Southwest Fisheries Science Center, NMFS/NOAA, La Jolla, California, USA

[4]Department of Ecology and Evolutionary Biology, UCLA, Los Angeles, California, USA

[5]NOAA Pacific Marine Environmental Laboratory, Seattle, Washington, USA

[6]School of Marine and Environmental Affairs, University of Washington, Seattle, Washington, USA

[7]Ocean Chemistry and Ecosystems Division, Atlantic Oceanographic and Meteorological Laboratory, Miami, Florida, USA

[8]Northern Gulf Institute, Mississippi State University, Mississippi State, Mississippi, USA

[9]Scripps Institution of Oceanography, UCSD, La Jolla, California, USA

Correspondence
Zachary Gold, Cooperative Institute for Climate, Ocean, & Ecosystem Studies, University of Washington, Seattle, WA, USA.
Email: zachary.gold@noaa.gov

Funding information
Howard Hughes Medical Institute; NSF GRFP & GRIP, Grant/Award Number: 2015204395; UCLA Department of Ecology and Evolutionary Biology; UCLA La Kretz Center for Conservation Genomics

Abstract

Marine heatwaves can drive large-scale shifts in marine ecosystems, but studying their impacts on whole species assemblages is difficult. Analysis combining microscopic observations with environmental DNA (eDNA) metabarcoding of the ethanol preservative of an ichthyoplankton biorepository spanning a 23 years time series captures major and sometimes unexpected changes to fish assemblages in the California Current Large Marine Ecosystem during and after the 2014–2016 Pacific Marine Heatwave. Joint modeling efforts reveal patterns of tropicalization with increases in southern, mesopelagic species and associated declines in commercially important temperate fish species (e.g., North Pacific Hake [*Merluccius productus*] and Pacific Sardine [*Sardinops sagax*]). Data show shifts in fisheries assemblages (e.g., Northern Anchovy, *Engraulis mordax*) even after the return to average water temperatures, corroborating ecosystem impacts found through multiple traditional surveys of this study area. Our innovative approach of metabarcoding preservative eDNA coupled with quantitative modeling leverages the taxonomic breadth and resolution of DNA sequences combined with microscopy-derived ichthyoplankton identification to yield higher-resolution, species-specific quantitative abundance estimates. This work opens the door to economically reconstruct the historical dynamics of assemblages from modern and archived samples worldwide.

KEYWORDS
amplicon sequencing, CalCOFI, California Current Ecosystem, eDNA, ichthyoplankton, joint model, marine heatwave, quantitative metabarcoding

[Correction added on 8 May 2023, after first online publication: the article title has been revised in this version.]

1 | INTRODUCTION

Climate-induced marine heatwaves are increasing in frequency and severity with far-reaching consequences in marine ecosystems (Oliver et al., 2018), ranging from severe organismal stress to cascading ecosystem effects (Frölicher & Laufkötter, 2018). Notable recent examples include repeated bleaching events across the Great Barrier Reef (2016, 2017, 2020) (Hughes et al., 2018), near-total deforestation in Northern California, USA, kelp forests (2016–2019) (Rogers-Bennett & Catton, 2019), and rapid collapse of Bering Sea snow crab (Szuwalski et al., 2021). These marine heatwaves precipitated drastic, unprecedented changes in dominant foundational species across hundreds of thousands of square kilometers of shallow, coastal ecosystems and also drove concurrent range shifts of marine species in response to these warming events (Thompson et al., 2022; Walker et al., 2020).

The impacts of large environmentally driven disturbances on coastal marine ecosystems have been ecologically and economically significant (Cheung & Frölicher, 2020; Nielsen et al., 2021; Pinsky et al., 2020). For example, the dramatic collapse of Pacific Sardine (*Sardinops sagax*) in the 1940s disrupted marine food webs, causing broad-scale, negative socio-economic impacts across the Northeast Pacific (Becker et al., 2019; Chavez et al., 2003; Checkley et al., 2017). The California Cooperative Oceanic Fisheries Investigations (CalCOFI) was formed in 1949 to better understand the processes driving these complex marine ecosystem dynamics and to avert similar fisheries collapses within the California Current Large Marine Ecosystem (CCLME). CalCOFI has continuously conducted systematic fisheries-independent surveys of the southern CCLME from 1951 until the present (Gallo et al., 2019; Lindegren et al., 2013; McClatchie, 2016) with a focus on monitoring larval fish assemblages, as larval fish dynamics are a key predictor of ecosystem health and function (Gallo et al., 2019; Nielsen et al., 2021; Smith & Moser, 2003).

Larval fish abundances help to characterize the state of marine ecosystems as they track spawning-stock biomass (Hsieh et al., 2006). Over 70 years, CalCOFI has documented decadal and annual changes in fish assemblages in response to environmental conditions, identifying major shifts in response to Pacific Decadal Oscillations and El Niño Southern Oscillations (Gallo et al., 2019; Moser et al., 1987, 2001; Thompson et al., 2012). These decadal and annual changes in ichthyoplankton dynamics are superimposed over strong biogeographic assemblages associated with distinct water masses typical for the Southern California Bight (Moser et al., 2001). For example, ichthyoplankton assemblages differ among three characteristic water types: the relatively cold and fresh California Current, the warmer and saltier California Counter Current and Central Pacific water mass, and deeper water upwelled across the continental shelf (Asch, 2015; Lindegren et al., 2013; Smith & Moser, 2003; Snyder et al., 2003). Importantly, periods of elevated temperatures are historically associated with higher abundances of southern, mesopelagic species and Pacific Sardine. In contrast, colder periods have been associated with higher abundances of northern, mesopelagic species and Northern Anchovy (*Engraulis mordax*; Chavez et al., 2003; Thompson et al., 2022).

Such historical insights into forage-fish community dynamics across decadal climatic regime shifts help form the basis for understanding the effects of climate change on the CCLME (Asch, 2015; Checkley et al., 2017; Lindegren et al., 2013). However, there is concern that the rate and scope of recent changes may drive ecosystem impacts not predicted by past patterns, motivating a fresh look at the biological history available in biorepositories. However, manual identification of larvae is labor-intensive, and taxonomic resolution is often limited by a lack of discernible morphological characteristics (Thompson, Chen, et al., 2017). For example, the larvae of only three of the 66 species of rockfishes in the genus *Sebastes* that occur in the California Current can be identified morphologically, thus requiring genetic-based identification (Thompson, Chen, et al., 2017).

Decades of research within the study region (Moser et al., 1987, 1993, 2001; Smith & Moser, 2003; Thompson et al., 2022) indicate the majority of species spawn in spring and the resulting ichthyoplankton closely track adult biomass (Hsieh et al., 2005). Thus, spring ichthyoplankton assemblages have been used as an important indicator of underlying changes in the local fish assemblages (McClatchie, 2016). Here, we leverage historical CalCOFI samples to reconstruct spring ichthyoplankton assemblages over a 23-year time series, using a novel "environmental DNA" (eDNA) approach. Specifically, we pair MiFish Universal Teleost 12S rRNA gene amplicon sequence data (Miya et al., 2015) acquired from ichthyoplankton sample preservative with morphological count observations in a joint Bayesian model to estimate species-specific larval abundance. These data span the 2014–2016 Pacific Marine Heatwave, the warmest 3-year period in the North Pacific in over 100 years of recorded history driven by a massive influx of warm, saline water from the central Pacific (Jacox et al., 2018), provided an opportunity to investigate the effects of marine heatwaves on ichthyoplankton assemblages in the southern CCLME.

2 | MATERIALS AND METHODS

2.1 | Study design

To investigate decadal changes in southern California Current ichthyoplankton assemblages, we identified fish larvae from four CalCOFI stations sampled during spring months over 2 decades (1996, 1998–2019). Archived spring ichthyoplankton samples were collected across four biogeographically dissimilar stations (up to 370 km apart) with variable water properties (McClatchie, 2016; Nielsen et al., 2021; Thompson et al., 2019). 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) which experiences high variation in annual temperature depending on the respective strengths of the California Current and Southern California Counter Current. The third station was a

southern coastal inshore station 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. The fourth station was a southern offshore station (31.85000° N, −119.5683° W) characterized by sub-tropical oceanic waters (Figure 1).

At each station, oblique bongo net tows were conducted from 210 m depth to the surface using standard CalCOFI methods (Kramer et al., 1972; McClatchie, 2014; Thompson et al., 2012; Thompson, McClatchie, et al., 2017). 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 samples were archived in the Pelagic Invertebrate Collection at Scripps Institution of Oceanography stored at room temperature and out of direct sunlight.

To characterize species abundance, a team of expert ichthyologists conducted microscopy on the 84 collection lots (21 sampling years, four stations) of formaldehyde-preserved bulk zooplankton including fish larvae (>505 µm). Identification of ichthyoplankton followed standardized CalCOFI techniques in which all larvae are sorted from the other invertebrate zooplankton assemblages within each cod end and then subsequently identified to the lowest possible taxonomic rank (McClatchie, 2016). We note that eggs of a few species are typically sorted from each sample but were not included in our analyses (Section 4). Six of the 84 collection lots were preserved in multiple jars due to the large volume of zooplankton, resulting in 90 total jars (Appendix S1).

To better resolve larval identifications, we conducted DNA metabarcoding on DNA isolated by filtering the ethanol in which paired port-side samples were preserved, thereby maintaining the integrity of the historical larvae and egg samples. Given that liquid preservative was used as the target substrate (as opposed to water, soil, or air) and the mass of larvae and eggs in the jar were not directly disturbed, we refer to this process as eDNA metabarcoding herein. Up to 125 mL of ethanol preservative (mean = 121.7 mL, $n = 90$; only 6 of these jars had <125 mL with min. = 34 mL) was pipetted off archived samples and vacuum filtered onto 0.2 µm PVDF filters.

DNA was extracted from filters using a modified Qiagen DNeasy Blood and Tissue kit (Curd et al., 2019) and amplified using the MiFish Universal Teleost (Miya et al., 2015) PCR primer set targeting the 12S rRNA mitochondrial gene region (Table S1). eDNA from each extraction was amplified in triplicate with each technical PCR replicate sequenced separately to capture stochastic variation within the amplification process (Appendix S1). This resulted in a final data set of 84 collection lots across four stations spanning 23 years that were morphologically identified as well as 90 unique DNA extractions and 244 unique PCR technical replicates that were sequenced. A total of 26 technical PCR replicates with either low sequencing depth ($n < 30{,}000$) or high dissimilarity (Bray Curtis dissimilarity >0.7) were removed from the final dataset. See Appendix S1 for full description.

2.2 | Estimating larval species abundance

We used a novel Bayesian hierarchical model based on the quantitative metabarcoding framework described in Shelton et al. (2022) to jointly estimate the abundance of ichthyoplankton in each jar and the taxon-specific amplification efficiencies, given the two complementary datasets (ichthyoplankton estimated by visual counts and sequence-read counts from the metabarcoding observations). We expect morphological and molecular analyses to be independent,

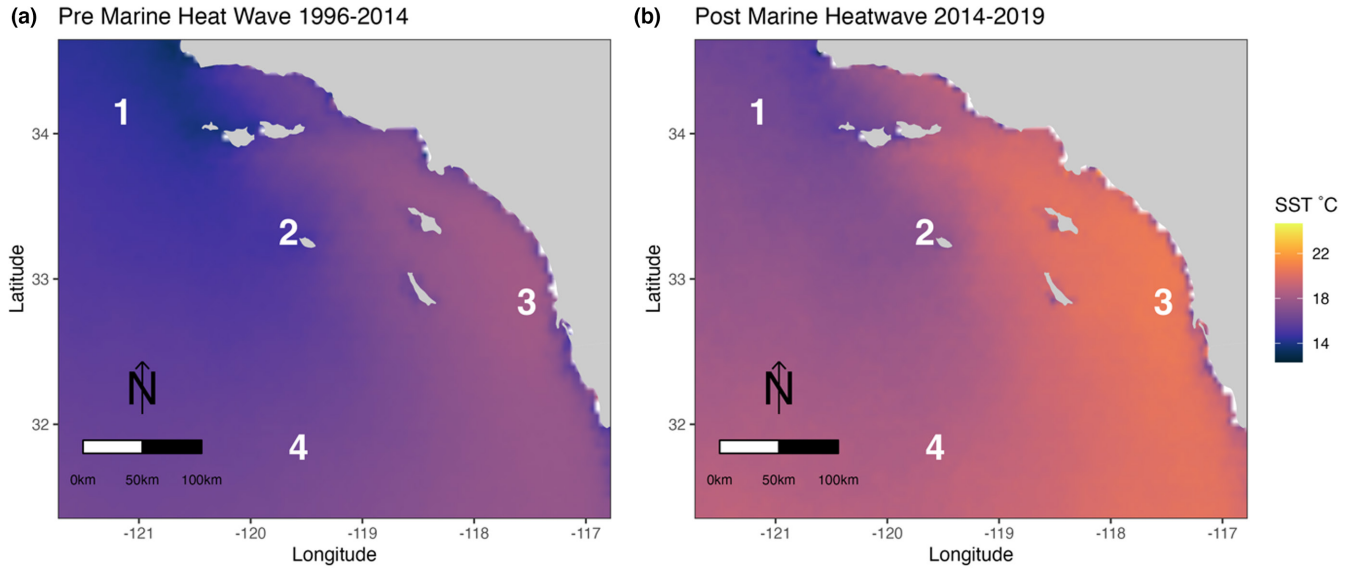

FIGURE 1 Sea surface temperature (SST) pre and post marine heat wave. To visualize the dramatic shift in ecological conditions, we plotted average daily SSTs from (a) pre (April 1, 1996–April 1, 2014) and (b) post (April 1, 2014–April 1, 2019) marine heatwave. Average SST was dramatically elevated during and after the marine heatwave. The four sites sampled are plotted in white: (1) Point Conception, (2) San Nicholas Island, (3) San Diego Inshore, and (4) San Diego Offshore.

imperfect reflections of a common biological community because port- and starboard-side samples are not precisely identical (Section 4).

Briefly, we model the observed number of sequence reads, for any species i, as a nonlinear function of the species-specific fraction of DNA in the template (McLaren et al., 2019; Silverman et al., 2021). We use i to represent species, but it can be generalized to represent ASVs or other molecular targets. Our framework is built upon the premise that the amplicons produced during a PCR reaction are dictated by the amplicon efficiency parameter a_i, which is characteristic of the interaction between the particular PCR reaction and each species being amplified. Thus, for any species i and in the absence of other species in the reaction, the number of amplicons should be directly related to the efficiency of amplification and the starting concentration of DNA template such that

$$A_i = c_i(a_i + 1)^{N_{PCR}} \quad (1)$$

where A_i is amplicon abundance, c_i is the true number of DNA copies in the reaction attributable to species i, a_i is the species-specific amplification efficiency (bounded on (0,1)), and N_{PCR} is the number of PCR cycles used in the reaction (Lalam, 2006). The multispecies nature of a metabarcoding run complicates this single-species picture insofar as a limited read-depth means only a small subset of the total molecules will be read by the sequencing instrument. This creates competition among template molecules in the mix, resulting in a compositional dataset. Here we account for the compositional nature of metabarcoding via a model term shared among species in a sample that estimates the fraction of amplicons sequenced (see Appendix S1 for full description of the model and assumptions).

The parameters c_i and a_i (Equation 1) are not identifiable using the observed metabarcoding data alone. To address this, Shelton et al. (2022) highlighted four general strategies to help estimate solutions to the above equation through the inclusion of additional independently derived sources of information. The first two strategies involve estimating amplification efficiencies alongside metabarcoding data, thereby providing estimates of two parameters (A_i and a_i) and allowing for estimation of input DNA concentrations, c_i. These amplification efficiencies can be derived either by the sequencing of (1) mock communities of known DNA template composition or (2) samples of unknown composition across a range of PCR cycles. The third strategy employs unique molecular identifiers to identify source molecules prior to amplification, functionally bypassing the need for the above equation. These three strategies have each been successfully demonstrated (Hoshino et al., 2021; Hoshino & Inagaki, 2017; Shelton et al., 2022; Silverman et al., 2021).

However, to date, no work has demonstrated the fourth strategy described in Shelton et al. (2022), which employs the use of a second independent set of observations of the same community to constrain the total possible parameter space of starting DNA concentrations and amplification efficiency values (c_i and a_i respectively). Here, we demonstrate this fourth strategy by linking sequencing data to the morphological ichthyoplankton counts from paired samples to constrain the species-specific starting concentrations of DNA in the ethanol jars. Essentially, we have imperfect information about the abundances of different species from two different sources (morphological counts and metabarcoding sequence-reads), and each of these constrains our understanding of the other: the count information helps to estimate the amplification efficiencies in light of the observed read-abundance, and the read-abundances suggest places where morphological counts under- or over-estimated the abundance of particular species in the jar (Appendix S1). One benefit of this strategy is that traditional observations are used to constrain eDNA measurements, providing a mechanistic framework to incorporate molecular and traditional surveys that align with current fisheries management indicators.

We highlight that the above modeling framework allows us to take advantage of the strengths of metabarcoding over visual assessment of samples, namely higher sensitivity and taxonomic resolution (Gold, Wall, et al., 2022; Thompson et al., 2016), as well as the strengths of morphological counts, namely quantitative estimates of abundance (larvae counts per standardized volume towed). The resulting joint model leverages the taxonomic breadth and resolution of amplicon sequencing (Gold et al., 2021; Miya et al., 2020), combining these with the power of morphological counts to yield species-specific quantitative abundance estimates. The joint model provides abundance estimates for a broader diversity of species than observed by morphological counts alone.

2.3 | Data analysis

We first compared the results of metabarcoding and morphological surveys. We focus these comparisons on the 59 species that were either observed in >9 technical PCR replicates within the metabarcoding data or enumerated >25 total times within the morphological data to ensure sufficient representation to achieve model convergence. We then focus our analysis on the results of the joint model, using the mean posterior estimates for abundance estimates per species per station per year as our response variable in the following analyses.

A suite of environmental variables – not just sea surface temperature (SST) – changed dramatically during the marine heat wave event. Upwelling strength and location, dissolved oxygen, salinity, current strength, and other environmental covariates also shifted during the climate-change influenced marine heatwave (Gentemann et al., 2017; Morgan et al., 2019; Ren & Rudnick, 2021; Schroeder et al., 2019). For simplicity, we use SST as a proxy for the onset and continuation of this suite of changes, documenting the resulting shift in community assemblage without attempting to identify any singular mechanistic driver responsible for this shift. To visualize the dramatic shift in ecological conditions, we plotted average SSTs from pre (April 1, 1995–April 1, 2014) and post (April 1, 2014–April 1, 2019) marine heatwave using the *rerddapXtracto* package (Mendelssohn, 2020) in R to collect daily PathFinder Ver 5.3 remotely sensed composites (Figure 1).

To evaluate the effect of the marine heatwave on CCLME fishes, we compared estimated species abundances for periods before (1996–2013), during (2014–2016), and after (2017–2019) the marine heatwave. We compare ichthyoplankton assemblages before the marine heatwave to assemblages during and after the marine heatwave because altered ocean conditions persisted well beyond 2019 (Ren & Rudnick, 2021). We first calculated the mean abundance for each species at each station for each model run. We then subtracted the pre-marine heatwave species-site abundance means from the post-marine heatwave species-site abundance means for each model run to evaluate changes in marine heatwave abundance per species per station per model run. We then calculated a 95% CI of change in marine heatwave abundance per species to identify which species were significantly different before versus during and after the marine heatwave at each station. We further plotted the change in marine heatwave abundance for each "species grouping" by habitat associations derived from previous CalCOFI research (see Appendix S1; Hsieh et al., 2005). We further visualized anchovy and sardine – key taxa of management interest – abundance over time by calculating the mean log-abundance of each species per station per year. We plotted the mean log-abundance of each of the four stations with error bars represent the 95% credible intervals (CI) observed for a given species at a given station in that year (Figure 5).

3 | RESULTS

eDNA metabarcoding of ethanol preservative with MiFish 12S (Miya et al., 2015) generated a total of 59.9 million sequence reads across 84 collection lots representing 90 unique DNA extractions and 244 unique PCR technical replicates. All sequence data were processed using the *Anacapa Toolkit* (Curd et al., 2019). After quality control, amplicon sequence-variant (ASV) dereplication, and decontamination processes (Curd et al., 2019; Gallego et al., 2020; Gold et al., 2021), we retained a total of 54.5 million reads (technical replicate range: 36,050–1.2 million reads) (Appendix S1). From these data, we classified 130 unique taxa including 103 species-level assignments (79%), 15 genus-level assignments (12%), 11 family-level assignments (8.5%), and one class-level assignment. Independent microscopy-count data from paired, matching formalin-preserved samples classified a total of 92 unique taxa including 76 species-level assignments (83%) and 16 genus-level assignments (17%) from of 9610 larvae sorted across 84 collection lots. See Dryad repository for full ASV table and complimentary detailed summaries.

Molecular taxonomic assignments identified two distinct lineages of the Northern Lanternfish (*Stennobrachius leucopsarus*) that are indistinguishable by morphological examination. The two lineages were identified by ASV clusters that differed by a single conserved base pair (99.5% sequence similarity). The two Northern Lanternfish lineages were repeatedly detected across samples and in high sequence read counts (variant 1: 6,145,100 total reads, 236 detections across technical replicates; variant 2: 259,989 total reads, 97 detections across technical replicates). These two lineages exhibited dramatically different ecological patterns across the samples (Figure 2 and Figure S1), and were therefore treated separately.

The joint Bayesian model focused on the 59 taxa that had sufficient representation across datasets to achieve model convergence (i.e. observed in >9 technical PCR replicates and >25 total larvae counted across the dataset), representing 99.0% of all larvae identified and reads sequenced. Of these 59 species, 48 were identified via microscopy and eDNA metabarcoding. eDNA metabarcoding alone detected 11 additional taxa including six additional species (*Benthalbella dentata*, *Ceratoscopelus townsendi*, *Diaphus theta*, *Microstomus pacificus*, *Parophrys vetulus*, *Peprilus simillimus*), one additional family (Opisthoproctidae), one additional variant of *Stennobrachius leucopsarus*, and three distinct higher-level taxonomic groups that likely represent distinct taxa lacking reference barcodes (Bathylagidae and *Stenobrachius* sp.).

Detection levels varied by species and survey methods (Figure 2). The majority of species ($n = 58$) were detected more often with DNA metabarcoding, while only one species (*Argyropelecus sladeni*) was more frequently detected via morphological identification. The 10 most abundant taxa by sequence reads and larval counts were identical across both methods. Non-detection of species across PCR replicates was only observed for samples where microscopy counted 9 larvae or fewer (Figure S2).

3.1 | Quantitative abundance estimates

The joint Bayesian model had successful convergence yielding station-, species-, and year-specific larval abundances for 59 fish species spanning a 23-year period (Figure 3). We observed a poor correlation between uncorrected amplicon abundances from eDNA metabarcoding and larval counts from visual morphological analysis (Figure 3a). In contrast, model estimates of larval counts and sequence reads (posterior means shown) show a close relationship to observed data (Figure 3b,c). Our model was able to link larval abundance and sequence counts well, particularly for larvae with abundant amplicon sequences and morphological counts.

3.2 | Displacement of target fish species and tropicalization of fish assemblages associated with the marine heatwave

Ichthyoplankton assemblages shifted over time throughout the study region (Figure 4, Figures S3 and S4), but transformed during the 2014–2016 marine heatwave. Specifically, southern, mesopelagic species increased in abundance while several temperate species of ecological and economic importance declined; these changes were synchronous despite sampling stations being geographically distant and environmentally unique. For example, the mesopelagic Mexican Lampfish (*Triphoturus mexicanus*) was at peak abundance during the marine heatwave and extended its typical range both poleward and into coastal shelf waters by hundreds of kilometers

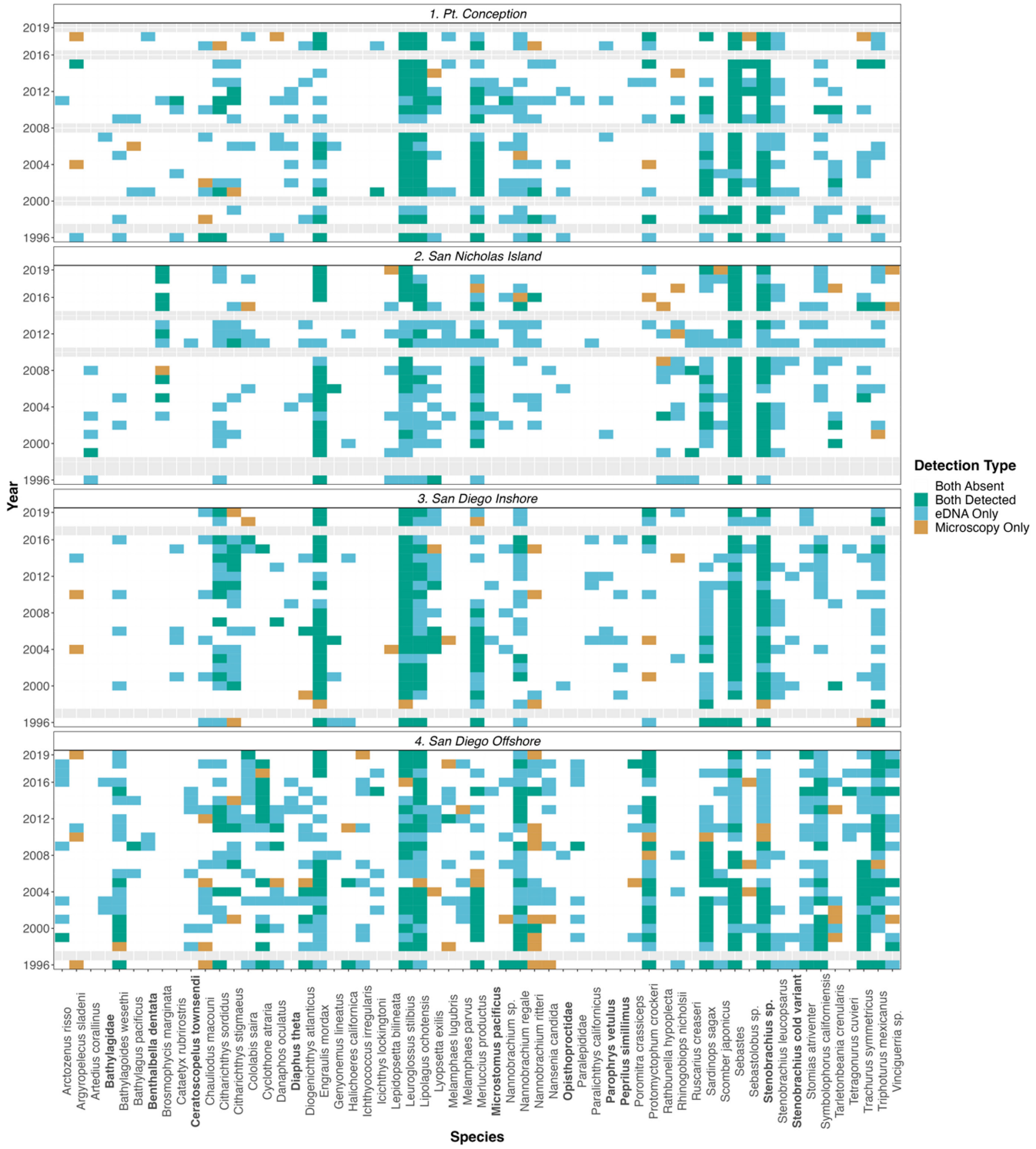

FIGURE 2 Co-detection of ichthyoplankton by metabarcoding and morphological methods. Detection plot of 59 species detected via microscopy ($n = 48$) and metabarcoding ($n = 59$). Metabarcoding detected all species observed in morphological counts and 11 additional species (in bold) that were not. Gray lines indicate missing data.

(Figure 4). In contrast, the abundances of northern, mesopelagic species and fisheries targets such as Pacific Sardine (*S. sagax*) and North Pacific Hake (*Merluccius productus*) were significantly lower after the onset of the marine heatwave and tended not to co-occur with warm associated southern, mesopelagic taxa (Figure 4).

3.3 | Abundance changes in forage fishes

Results show dramatic changes in anchovy and sardine abundance (larvae counts per standardized volume towed) across the 23-year time series (Figure 5 and Figure S5). Anchovy abundance was

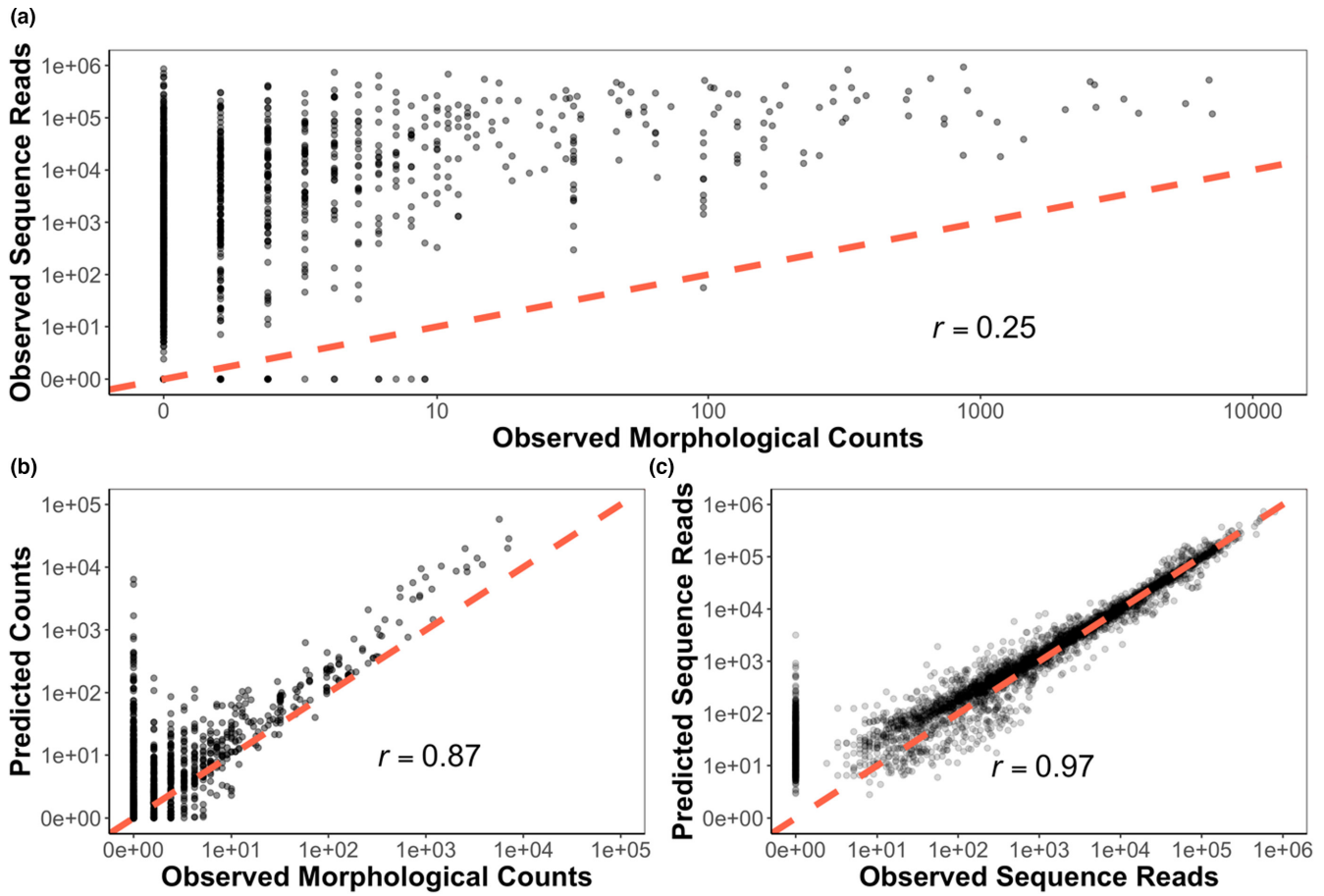

FIGURE 3 Bayesian joint model improves quantitative abundance estimates. Plot of observed (uncorrected) eDNA metabarcoding derived sequencing reads against observed morphological counts (a). Observed-predicted plot for larval counts (b) and sequence reads (c) from our joint model results. The one-to-one line is plotted in red and Pearson correlation coefficients (*r*) are reported.

~5-times higher during and after the marine heatwave (max: 3548, mean ± SD: 397 ± 834) than prior (mean ± SD: 62 ± 192). This increase was particularly dramatic given the low abundances immediately preceding the marine heatwave (mean ± SD: 1 ± 1.4). In contrast, on average, sardine abundances remained low before (mean ± SD: 31 ± 65) as well as during and after the marine heatwave (mean ± SD: 8 ± 19). However, there were regional variations in this pattern with relatively high sardine abundances at the San Diego Offshore station from 2005 to 2008 (mean ± SD: 119 ± 72) and an increase in Sardine abundance in nearshore coastal waters at the San Nicholas station after the marine heatwave (mean ± SD: 50 ± 6).

4 | DISCUSSION

The novel combination of morphological data and eDNA metabarcoding of ethanol preserved larvae from an ichthyoplankton biorepository in a joint modeling framework (Shelton et al., 2022) revealed marked shifts in California Current Large Marine Ecosystem ichthyoplankton communities across a 23-year time series. Across geographically distant and environmentally distinct sampling sites, ichthyoplankton communities show remarkably similar patterns of tropicalization during the 2014–2016 marine heatwave. Results demonstrate the power of eDNA metabarcoding from ethanol preserved bulk samples to unlock important insights into community dynamics over time, creating novel research opportunities from preserved sample collections (Gallo et al., 2019).

The observed ichthyoplankton assemblage shifts during the marine heatwave are consistent with recent studies reporting the tropicalization of terrestrial and marine ecosystems in response to climate change (Chaudhary et al., 2021; Vergés et al., 2016). These deviations can induce novel species interactions, catalyzing changes in ecosystem function (Frölicher & Laufkötter, 2018; Morgan et al., 2019). The high abundances of both Northern Anchovy and southern mesopelagic species seen in our dataset during and after the 2014–2016 marine heatwave is unique in the previous >70-year CalCOFI dataset (Moser et al., 1987; Smith & Moser, 2003). This result, corroborated by Thompson et al. (2022), suggests that climate-associated biological shifts in the CCLME are likely to be without modern analog (Nielsen et al., 2021; Sydeman et al., 2020; Thompson et al., 2019, 2022).

Our model estimates of ichthyoplankton assemblages are consistent with studies (Sydeman et al., 2020; Thompson et al., 2022) that documented a decline in both sardine and anchovy abundance

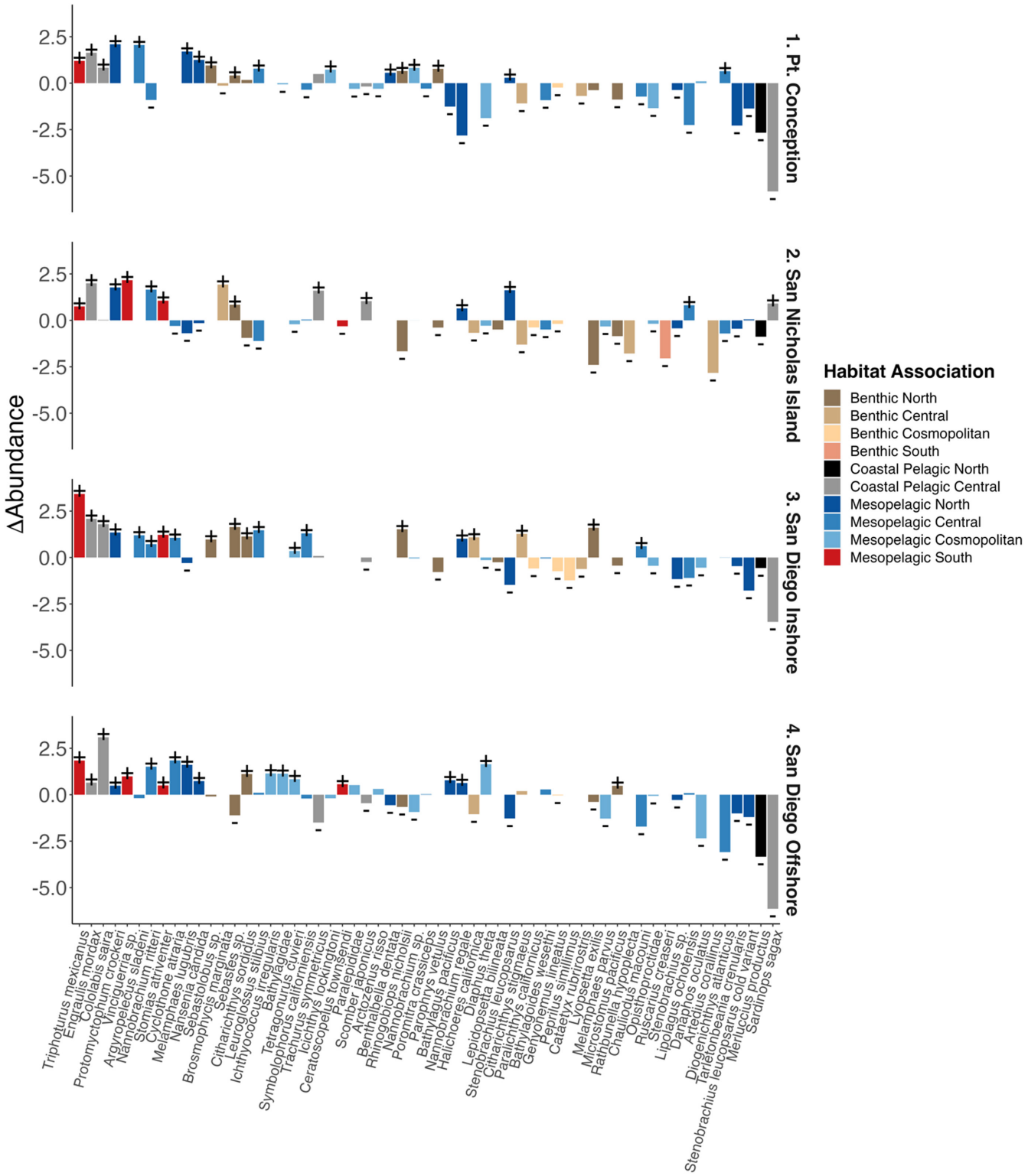

FIGURE 4 Novel marine heatwave assemblages. Shifts in species modeled abundances with the onset of the marine heatwave (1996–2013 vs. 2014–2019). Synchronous increases in southern mesopelagic species and Northern Anchovy (*Engraulis mordax*) were observed across all stations. Stations are in rows, species in columns, and the joint model estimated change in abundance between the two ecological phases is shown as the response variable. Fisheries targets including Pacific Sardine (*Sardinops sagax*) and North Pacific Hake (*Merluccius productus*), as well as many other benthic and coastal species, had concurrent negative associations. Significant differences during and after the marine heatwave are marked with + or −.

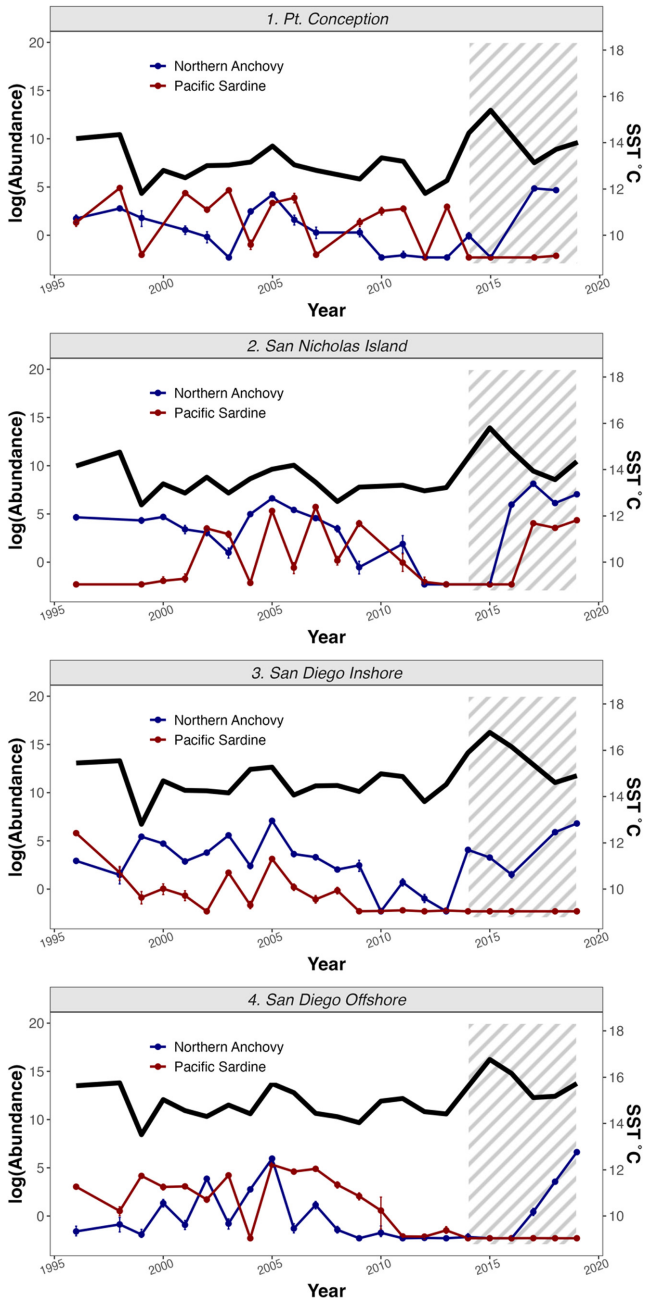

FIGURE 5 Synchronous increase in anchovy abundance during and after marine heatwave. Model posterior estimates for larval fish abundances (larvae counts per standardized volume towed) over time at each of the four sampled stations. Joint modeling of metabarcoding and morphological counts reconstructed increases in Northern Anchovy (*Engraulis mordax*) during the recent Pacific Marine Heatwave and low spawning of Pacific Sardine (*Sardinops sagax*) over the past decade (points are means and error bars are 95% credible intervals; the shaded region is during and after the marine heatwave). Northern Anchovy abundances are in blue while Pacific Sardine abundances are in red. Sea surface temperature is plotted above the Northern Anchovy and Pacific Sardine abundances for reference.

beginning in 2005 followed by a dramatic increase in anchovy abundance in the wake of the marine heatwave (Figure 5). This rise in anchovy and continued low abundances of sardine during the marine heatwave is an ecological surprise. Correlative analyses between basin-scale environmental indices such as the Pacific Decadal Oscillation indicate that, for the latter half of the 20th century, anchovy thrived under cooler conditions and sardine under warmer conditions (Chavez et al., 2003). However, our results corroborate recent observations of anchovy-dominated forage-fish assemblages arising in response to marine heatwave-associated ocean warming conditions (Checkley et al., 2017; McClatchie, 2012; Nielsen et al., 2021; Santora et al., 2020; Thompson et al., 2019). Specifically, increases in Northern Anchovy and southern mesopelagic fishes were found to be associated with decreases in Pacific Sardine and North Pacific Hake in the Southern CCLME (Piatt et al., 2020; Robinson et al., 2018), suggesting fundamentally changed ecosystems and fisheries relative to the recent past (Thompson et al., 2022).

The unexpected response of ichthyoplankton communities to the 2014–2016 Pacific Marine Heatwave suggests that the mechanisms that govern the population dynamics of forage fish species are not a mere function of temperature, but governed by more complex factors including trophic ecology as well as spatial and temporal variation of distinct, favorable oceanic conditions (Checkley et al., 2017; Schroeder et al., 2019; Thompson et al., 2022). Thus, improvement of our mechanistic understanding of drivers of forage fish dynamics is needed to inform ecological predictions in the face of extreme ocean events such as marine heatwaves, which are likely to increase in frequency and duration under climate change (Deutsch et al., 2015; Frölicher et al., 2018; Howard et al., 2020; Oliver et al., 2021). The quantitative metabarcoding approaches described here provide an important avenue to characterize larval prey field and gut contents, allowing for the characterization of a critical, yet poorly understood driver of recruitment volatility in coastal pelagic and other fishes (Barbato et al., 2019; Erdozain et al., 2019; Garcia-Vazquez et al., 2021; James et al., 2022; Kelly et al., 2019; Mariac et al., 2018; Nielsen et al., 2021; Pitz et al., 2020; Sydeman et al., 2020).

4.1 | Quantitative metabarcoding framework

Across-species variation in amplification efficiencies resulted in relatively poor correlation between raw sequence abundance and morphological ichthyoplankton counts (Figure 3). However, after leveraging relative abundances provided by morphological counts to estimate differences in PCR amplification efficiency, Pearson correlations between observed and predicted counts approached 1. This result delivers strong empirical support for the mechanistic metabarcoding framework proposed by Shelton et al. (2022), and greatly improves the quantitative reach of DNA metabarcoding.

This joint modeling framework provides a valuable foundation for the development of metabarcoding based quantitative abundance estimates that could ease the burden of morphological work. Although we informed our model from abundance estimates from morphological larval identification, as noted by Shelton et al. (2022), this joint modeling approach can utilize any estimate of abundance,

including qPCR or dPCR derived estimates of absolute DNA concentration. Therefore, the combination of multiple quantitative single-species molecular assays, rather than morphological counts, could provide the information needed to for our joint model, providing a purely molecular approach to estimating abundance from metabarcoding data (McLaren et al., 2019, 2022; Pont et al., 2023). Such molecular based approaches are needed as few biological monitoring programs have access to the expert taxonomists needed to painstakingly identify the >9000 individual ichthyoplankton collected within our study (Gallo et al., 2019).

An important avenue of future research efforts is to unite the multiple amplification efficiency estimation strategies outlined in Shelton et al. (2022) to derive quantitative estimates of taxa detected through eDNA metabarcoding. Here, our model solves for amplification efficiencies by constraining the underlying starting DNA concentrations with observed morphological counts, allowing for a model estimate of amplification efficiencies and underlying starting DNA concentrations for all taxa observed through metabarcoding data. In contrast, previous work has utilized mock communities or variable PCR cycling efforts to estimate amplification efficiencies directly (McLaren et al., 2019; Silverman et al., 2021). Future efforts should leverage a fully integrated model that derives independent information for all three parameters (observed reads, amplification efficiencies, and underlying DNA concentrations) to derive accurate quantitative estimates of molecularly observed biological communities.

Despite its utility, our joint modeling efforts also has limitations. First, we assume exponential PCR across all 49 cycles, which was likely violated given theoretical and analytical work that suggests a decline in the efficiency of PCR reactions over time (Boggy & Woolf, 2010; Chatterjee et al., 2012; Kubista et al., 2006). Fortunately, such PCR saturation would not affect the interpretation of our results because bias would be applied uniformly as a scalar across all estimated amplification efficiencies. Future efforts could address this issue by simply incorporating a decay coefficient in the underlying PCR equation (Boggy & Woolf, 2010; Chatterjee et al., 2012; Kubista et al., 2006).

Another limitation of our modeling framework is the assumption that morphological counts directly correlated with the starting DNA concentrations in each ethanol preserved sample. This assumption is likely not completely accurate because: (1) our work did not incorporate morphological counts of egg abundance from the jars, (2) metabarcoding and morphological taxonomic identification are imperfect, leading to mismatches between survey approaches, and (3) we made the explicit assumption that all larvae share an identical DNA shedding rate into the ethanol preservative, ignoring any allometric or physiological effects (Appendix S1). These limitations impact our ability to both directly compare and jointly model genetic and morphological methods (Figure 2) (Kelly et al., 2017).

Due to these limitations, care should be taken before extending our model framework to eDNA obtained directly from environmental sources and more complex metabarcoding applications as our model cannot account for the many processes impacting captured DNA prior to the PCR reaction. For example, ethanol-preserved larvae in a bulk collection have experienced the same conditions in a constant volume of ethanol. As such, these samples may be more amenable to estimating abundance accurately because only a few processes are likely to affect the relationship between captured DNA and the number of larvae in a jar. In contrast, eDNA derived from environmental samples are impacted many processes beyond species specific allometry and morphology, including life stage, behavior, temperature, state, origin, and fate and transport of the collected DNA, among others (Andruszkiewicz Allan et al., 2021; Barnes & Turner, 2016; Harrison et al., 2019; Jo et al., 2019; Sassoubre et al., 2016; Shelton et al., 2022; Thalinger et al., 2021; Yates et al., 2021). Understanding the impact of these processes on metabarcoding results will require a fully integrated modeling approach accounting for all potential mechanisms impacting metabarcoding read abundances. Such an approach would necessarily rely on a unified theory of eDNA metabarcoding pulling together our collective mechanistic understanding of shedding, degradation, and fate and transport processes (Harrison et al., 2019) alongside the subsampling and PCR amplification processes (Diana et al., 2022; Gold, Shelton, et al., 2022; Shelton et al., 2022) to ultimately derive accurate quantification and detection estimates.

To this end, recent work has begun to characterize the effects of additional mechanistic processes prior to amplification, particularly the effect of subsampling processes on observed read abundances and non-detections (Egozcue et al., 2020; Gold, Shelton, et al., 2022). Utilizing the dataset described in this manuscript in conjunction with mock communities, Gold, Shelton, et al. (2022) demonstrate that rates of non-detection are a function of amplification efficiencies and underlying DNA concentration. These results highlight the future importance of not only modeling the deterministic PCR process as we have done here, but also modeling the stochastic subsampling process prior to amplification as both processes together drive observed biological signal (abundance of observed reads) and noise (the patterns of non-detections across technical replicates) in metabarcoding data sets (Gold, Shelton, et al., 2022; Gold, Wall, et al., 2022).

Despite the current limitations of our modeling approach, results demonstrate the clear value of the Shelton et al. (2022) framework for deriving abundance estimates from metabarcoding data (Figure 3). This approach is particularly well-suited to controlled systems such as the CalCOFI ethanol-preserved samples, microbiomes, and bulk metabarcoding approaches (Carew et al., 2018; McLaren et al., 2019). Addressing the above limitations by advancing mechanistic modeling efforts will further enhance the accuracy of quantitative estimates derived from metabarcoding data in the future, allowing for applications to aquatic eDNA.

4.2 | Novel insights from legacy collections

Ultimately, our novel approach generating metabarcoding sequence data from ethanol preservative combined with joint Bayesian

hierarchical modeling provides valuable quantitative estimates by non-destructively sampling legacy collections via metabarcoding. At the same time this novel approach provides a mechanistic framework for determining absolute abundance estimates from compositional amplicon sequencing data (Gloor et al., 2017; McLaren et al., 2019; Silverman et al., 2021). Importantly, the integration of metabarcoding approaches allowed the differentiation of 11 additional variants and species that were not morphologically identifiable in ichthyoplankton (Thompson, Chen, et al., 2017). For example, metabarcoding identified unique variants of the Northern Lampfish (*Stennobrachius leucopsarus*) that are morphologically indistinguishable and combined as a complex exhibited little change before and after the marine heatwave. However, these two ASVs had markedly different responses to the marine heatwave with one variant largely disappearing after the marine heatwave onset (Figure 4), illustrating the power of molecular methods reveal ecological dynamics otherwise hidden by shared larval morphology.

Beyond improved taxonomic resolution, the key advantage of our framework is the ability to derive quantitative estimates from metabarcoding data, albeit with the many caveats described above. Determining abundance from eDNA metabarcoding has been challenging, with mixed results (Fonseca, 2018; Lacoursière-Roussel, Côté, et al., 2016; Yates et al., 2019). Unlocking such quantitative metabarcoding approaches expands the potential for linking ecological assemblages to environmental processes beyond presence-absence (Lacoursière-Roussel, Côté, et al., 2016; Lacoursière-Roussel, Rosabal, & Bernatchez, 2016; Stoeckle et al., 2021; Yates et al., 2019). Such quantitative approaches may prove critical in modeling and predicting future ecosystem change, particularly difficult to study ecosystems like coastal pelagic fishes, although directly linking assemblage dynamic responses to climate-driven forces remains inherently challenging. While the CalCOFI samples are specific to ichthyoplankton from the CCLME, bulk collection of community samples is commonly used to survey plankton, insects, pollen, gut contents, and microbiomes, among many other targets (Deiner et al., 2017). As such, the methodology we present here is broadly applicable to such bulk collections to efficiently understand modern and historical changes in ecological communities.

AUTHOR CONTRIBUTIONS

Conceptualization: ZG, DK, KDG, ART, LRT, PHB. Performed Research: ZG, RPK, AOS, RG, DK, ART, PHB, KMP. Funding Acquisition: ZG, PHB, ART, KDG, DK. Data Curation: ZG, RPK, AOS, RG. Formal Analysis: ZG, RPK, AOS, RG, ART. Writing – Original Draft Preparation: ZG, RPK, AOS, ART, KDG, RG, KMP, LRT, DK, PHB.

ACKNOWLEDGEMENTS

We acknowledge NSF GRFP and GRIP [DEG No. 2015204395], UCLA La Kretz Center for Conservation Genomics, a Howard Hughes Medical Institute Professor award to PHB, and the UCLA Department of Ecology and Evolutionary Biology for funding this research.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to report.

DATA AVAILABILITY STATEMENT

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Appendix S1. All data and code to conduct analyses and generate all figures are available on GitHub (https://doi.org/10.5281/zenodo.7872130) and associated Dryad Data Repository (https://doi.org/10.5068/D1267D) and NCBI SRA (BioProject: PRJNA966243).

[Correction added on 8 May 2023, after first online publication: the Data Availability Statement section has been updated in this version.]

ORCID

Zachary Gold https://orcid.org/0000-0003-0490-7630
Ryan P. Kelly https://orcid.org/0000-0001-5037-2441
Paul H. Barber https://orcid.org/0000-0002-1486-8404

REFERENCES

Andruszkiewicz Allan, E., Zhang, W. G., C Lavery, A., & F Govindarajan, A. (2021). Environmental DNA shedding and decay rates from diverse animal forms and thermal regimes. *Environmental DNA*, *3*(2), 492–514. https://doi.org/10.1002/edn3.141

Asch, R. G. (2015). Climate change and decadal shifts in the phenology of larval fishes in the California current ecosystem. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(30), E4065–E4074. https://doi.org/10.1073/pnas.1421946112

Barbato, M., Kovacs, T., Coleman, M. A., Broadhurst, M. K., & de Bruyn, M. (2019). Metabarcoding for stomach-content analyses of pygmy devil ray (*Mobula kuhlii* cf. eregoodootenkee): Comparing tissue and ethanol preservative-derived DNA. *Ecology and Evolution*, *9*(5), 2678–2687. https://doi.org/10.1002/ece3.4934

Barnes, M. A., & Turner, C. R. (2016). The ecology of environmental DNA and implications for conservation genetics. *Conservation Genetics*, *17*(1), 1–17. https://doi.org/10.1007/s10592-015-0775-4

Becker, E. A., Forney, K. A., Redfern, J. V., Barlow, J., Jacox, M. G., Roberts, J. J., & Palacios, D. M. (2019). Predicting cetacean abundance and distribution in a changing climate. *Diversity and Distributions*, *25*(4), 626–643. https://doi.org/10.1111/ddi.12867

Boggy, G. J., & Woolf, P. J. (2010). A mechanistic model of PCR for accurate quantification of quantitative PCR data. *PLoS One*, *5*(8), e12355.

Carew, M. E., Coleman, R. A., & Hoffmann, A. A. (2018). Can non-destructive DNA extraction of bulk invertebrate samples be used for metabarcoding? *PeerJ*, *2018*(6), e4980. https://doi.org/10.7717/peerj.4980

Chatterjee, N., Banerjee, T., & Datta, S. (2012). *Accurate estimation of nucleic acids by amplification efficiency dependent PCR*.

Chaudhary, C., Richardson, A. J., Schoeman, D. S., & Costello, M. J. (2021). Global warming is causing a more pronounced dip in marine species richness around the equator. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(15), e2015094118. https://doi.org/10.1073/pnas.2015094118

Chavez, F. P., Ryan, J., Lluch-Cota, S. E., & Ñiquen, C. M. (2003). Climate: From anchovies to sardines and back: Multidecadal change in the Pacific Ocean. *Science*, *299*(5604), 217–221. https://doi.org/10.1126/science.1075880

Checkley, D. M., Asch, R. G., & Rykaczewski, R. R. (2017). Climate, anchovy, and sardine. *Annual Review of Marine Science*, *9*(1), 469–493. https://doi.org/10.1146/annurev-marine-122414-033819

Cheung, W. W. L., & Frölicher, T. L. (2020). Marine heatwaves exacerbate climate change impacts for fisheries in the Northeast Pacific. *Scientific Reports*, *10*(1), 1–10. https://doi.org/10.1038/s41598-020-63650-z

Curd, E. E., Gold, Z., Kandlikar, G. S., Gomer, J., Ogden, M., O'Connell, T., Pipes, L., Schweizer, T. M., Rabichow, L., Lin, M., Shi, B., Barber, P. H., Kraft, N., Wayne, R., & Meyer, R. S. (2019). Anacapa toolkit: An environmental DNA toolkit for processing multilocus metabarcode datasets. *Methods in Ecology and Evolution*, *10*(9), 1469–1475. https://doi.org/10.1111/2041-210X.13214

Deiner, K., Bik, H. M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., Creer, S., Bista, I., Lodge, D. M., de Vere, N., Pfrender, M. E., & Bernatchez, L. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular Ecology*, *26*(21), 5872–5895. https://doi.org/10.1111/mec.14350

Deutsch, C., Ferrel, A., Seibel, B., Pörtner, H. O., & Huey, R. B. (2015). Climate change tightens a metabolic constraint on marine habitats. *Science*, *348*(6239), 1132–1135. https://doi.org/10.1126/science.aaa1605

Diana, A., Matechou, E., Griffin, J., Yu, D., Luo, M., Tosa, M., Bush, A., & Griffiths, R. (2022). *eDNAPlus: A unifying modelling framework for DNA-based biodiversity monitoring*. ArXiv Preprint ArXiv:2211.12213.

Egozcue, J. J., Graffelman, J., Ortego, M. I., & Pawlowsky-Glahn, V. (2020). Some thoughts on counts in sequencing studies. *NAR Genomics and Bioinformatics*, *2*(4), 1–10.

Erdozain, M., Thompson, D. G., Porter, T. M., Kidd, K. A., Kreutzweiser, D. P., Sibley, P. K., Swystun, T., Chartrand, D., & Hajibabaei, M. (2019). Metabarcoding of storage ethanol vs. conventional morphometric identification in relation to the use of stream macroinvertebrates as ecological indicators in forest management. *Ecological Indicators*, *101*, 173–184. https://doi.org/10.1016/j.ecolind.2019.01.014

Fonseca, V. G. (2018). Pitfalls in relative abundance estimation using edna metabarcoding. *Molecular Ecology Resources*, *18*(5), 923–926. https://doi.org/10.1111/1755-0998.12902

Frölicher, T. L., Fischer, E. M., & Gruber, N. (2018). Marine heatwaves under global warming. *Nature*, *560*(7718), 360–364. https://doi.org/10.1038/s41586-018-0383-9

Frölicher, T. L., & Laufkötter, C. (2018). Emerging risks from marine heat waves. *Nature Communications*, *9*(1), 650. https://doi.org/10.1038/s41467-018-03163-6

Gallego, R., Jacobs-Palmer, E., Cribari, K., & Kelly, R. P. (2020). Environmental DNA metabarcoding reveals winners and losers of global change in coastal waters: EDNA and climate change. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1940), 20202424. https://doi.org/10.1098/rspb.2020.2424rspb

Gallo, N. D., Drenkard, E., Thompson, A. R., Weber, E. D., Wilson-Vandenberg, D., McClatchie, S., Koslow, J. A., & Semmens, B. X. (2019). Bridging from monitoring to solutions-based thinking: Lessons from CalCOFI for understanding and adapting to marine climate change impacts. *Frontiers in Marine Science*, *6*, 695. https://doi.org/10.3389/fmars.2019.00695

Garcia-Vazquez, E., Georges, O., Fernandez, S., & Ardura, A. (2021). eDNA metabarcoding of small plankton samples to detect fish larvae and their preys from Atlantic and Pacific waters. *Scientific Reports*, *11*(1), 1–13. https://doi.org/10.1038/s41598-021-86731-z

Gentemann, C. L., Fewings, M. R., & García-Reyes, M. (2017). Satellite sea surface temperatures along the west coast of the United States during the 2014–2016 Northeast Pacific marine heat wave. *Geophysical Research Letters*, *44*(1), 312–319. https://doi.org/10.1002/2016GL071039

Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V., & Egozcue, J. J. (2017). Microbiome datasets are compositional: And this is not optional. *Frontiers in Microbiology*, *8*, 2224. https://doi.org/10.3389/fmicb.2017.02224

Gold, Z., Curd, E. E., Goodwin, K. D., Choi, E. S., Frable, B. W., Thompson, A. R., Walker, H. J., Burton, R. S., Kacev, D., Martz, L. D., & Barber, P. H. (2021). Improving metabarcoding taxonomic assignment: A case study of fishes in a large marine ecosystem. *Molecular Ecology Resources*, *21*(7), 2546–2564. https://doi.org/10.1111/1755-0998.13450

Gold, Z., Shelton, A. O., Casendino, H. R., Duprey, J., Gallego, R., van Cise, A., Fisher, M., Jensen, A. J., D'Agnese, E., & Allan, E. A. (2022). Distinguishing signal from noise: Understanding patterns of non-detections to inform accurate quantitative metabarcoding. *BioRxiv*, 2022–09.

Gold, Z., Wall, A. R., Schweizer, T. M., Pentcheff, N. D., Curd, E. E., Barber, P. H., Meyer, R. S., Wayne, R., Stolzenbach, K., Prickett, K., Luedy, J., & Wetzer, R. (2022). A manager's guide to using eDNA metabarcoding in marine ecosystems. *PeerJ*, *10*, e14071. https://doi.org/10.7717/PEERJ.14071

Harrison, J. B., Sunday, J. M., & Rogers, S. M. (2019). Predicting the fate of eDNA in the environment and implications for studying biodiversity. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1915), 20191409. https://doi.org/10.1098/rspb.2019.1409

Hoshino, T., & Inagaki, F. (2017). Application of stochastic labeling with random-sequence barcodes for simultaneous quantification and sequencing of environmental 16S rRNA genes. *PLoS One*, *12*(1), e0169431.

Hoshino, T., Nakao, R., & Minamoto, T. (2021). Simultaneous absolute quantification and sequencing of fish environmental DNA in a mesocosm by quantitative sequencing technique. *Scientific Reports*, *11*(1), 1–9.

Howard, E. M., Penn, J. L., Frenzel, H., Seibel, B. A., Bianchi, D., Renault, L., Kessouri, F., Sutula, M. A., McWilliams, J. C., & Deutsch, C. (2020). Climate-driven aerobic habitat loss in the California current system. *Science Advances*, *6*(20), eaay3188.

Hsieh, C. H., Reiss, C., Watson, W., Allen, M. J., Hunter, J. R., Lea, R. N., Rosenblatt, R. H., Smith, P. E., & Sugihara, G. (2005). A comparison of long-term trends and variability in populations of larvae of exploited and unexploited fishes in the Southern California region: A community approach. *Progress in Oceanography*, *67*(1–2), 160–185. https://doi.org/10.1016/j.pocean.2005.05.002

Hsieh, C. H., Reiss, C. S., Hunter, J. R., Beddington, J. R., May, R. M., & Sugihara, G. (2006). Fishing elevates variability in the abundance of exploited species. *Nature*, *443*(7113), 859–862. https://doi.org/10.1038/nature05232

Hughes, T. P., Kerry, J. T., Baird, A. H., Connolly, S. R., Dietzel, A., Eakin, C. M., Heron, S. F., Hoey, A. S., Hoogenboom, M. O., Liu, G., McWilliam, M. J., Pears, R. J., Pratchett, M. S., Skirving, W. J., Stella, J. S., & Torda, G. (2018). Global warming transforms coral reef assemblages. *Nature*, *556*(7702), 492–496. https://doi.org/10.1038/s41586-018-0041-2

Jacox, M. G., Alexander, M. A., Mantua, N. J., Scott, J. D., Hervieux, G., Webb, R. S., & Werner, F. E. (2018). 6. Forcing of multiyear extreme ocean temperatures that impacted California current living marine resources in 2016. *Bulletin of the American Meteorological Society*, *99*(1), S27–S33. https://doi.org/10.1175/BAMS-D-17-0119.1

James, C. C., Barton, A. D., Allen, L. Z., Lampe, R. H., Rabines, A., Schulberg, A., Zheng, H., Goericke, R., Goodwin, K. D., & Allen, A. E. (2022). Influence of nutrient supply on plankton microbiome biodiversity and distribution in a coastal upwelling region. *Nature Communications*, *13*(1), 1–13.

Jo, T., Murakami, H., Yamamoto, S., Masuda, R., & Minamoto, T. (2019). Effect of water temperature and fish biomass on environmental DNA shedding, degradation, and size distribution. *Ecology and Evolution*, *9*(3), 1135–1146. https://doi.org/10.1002/ece3.4802

Kelly, R. P., Closek, C. J., O'Donnell, J. L., Kralj, J. E., Shelton, A. O., & Samhouri, J. F. (2017). Genetic and manual survey methods yield different and complementary views of an ecosystem. *Frontiers in Marine Science*, *3*, 283. https://doi.org/10.3389/FMARS.2016.00283

Kelly, R. P., Shelton, A. O., & Gallego, R. (2019). Understanding PCR processes to draw meaningful conclusions from environmental DNA studies. *Scientific Reports*, *9*(1), 1–14. https://doi.org/10.1038/s41598-019-48546-x

Kramer, D., Kalin, M. J., Stevens, E. G., Thrailkill, J. R., & Zweifel, J. R. (1972). *Collecting and processing data on fish eggs and larvae in the California Current*. NOAA Tech. Rep. NMFS Circ. (Vol. 370). US Department of Commerce, National Oceanic and Atmospheric Administration ….

Kubista, M., Andrade, J. M., Bengtsson, M., Forootan, A., Jonák, J., Lind, K., Sindelka, R., Sjöback, R., Sjögreen, B., & Strömbom, L. (2006). The real-time polymerase chain reaction. *Molecular Aspects of Medicine*, *27*(2–3), 95–125.

Lacoursière-Roussel, A., Côté, G., Leclerc, V., & Bernatchez, L. (2016). Quantifying relative fish abundance with eDNA: A promising tool for fisheries management. *Journal of Applied Ecology*, *53*(4), 1148–1157. https://doi.org/10.1111/1365-2664.12598

Lacoursière-Roussel, A., Rosabal, M., & Bernatchez, L. (2016). Estimating fish abundance and biomass from eDNA concentrations: Variability among capture methods and environmental conditions. *Molecular Ecology Resources*, *16*(6), 1401–1414. https://doi.org/10.1111/1755-0998.12522

Lalam, N. (2006). Estimation of the reaction efficiency in polymerase chain reaction. *Journal of Theoretical Biology*, *242*(4), 947–953.

Lindegren, M., Checkley, D. M., Rouyer, T., MacCall, A. D., & Stenseth, N. C. (2013). Climate, fishing, and fluctuations of sardine and anchovy in the California current. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(33), 13672–13677. https://doi.org/10.1073/pnas.1305733110

Mariac, C., Vigouroux, Y., Duponchelle, F., García-Dávila, C., Nunez, J., Desmarais, E., & Renno, J. F. (2018). Metabarcoding by capture using a single COI probe (MCSP) to identify and quantify fish species in ichthyoplankton swarms. *PLoS One*, *13*(9), e0202976. https://doi.org/10.1371/journal.pone.0202976

McClatchie, S. (2012). Sardine biomass is poorly correlated with the Pacific decadal oscillation off California. *Geophysical Research Letters*, *39*(13). https://doi.org/10.1029/2012GL052140

McClatchie, S. (2014). Regional fisheries oceanography of the California current system: The CalCOFI program. In *Regional fisheries oceanography of the California Current System: The CalCOFI program*. Springer. https://doi.org/10.1007/978-94-007-7223-6

McClatchie, S. (2016). *Regional fisheries oceanography of the California Current System*. Springer.

McLaren, M. R., Nearing, J. T., Willis, A. D., Lloyd, K. G., & Callahan, B. J. (2022). Implications of taxonomic bias for microbial differential-abundance analysis. *Biorxiv.Org*. https://www.biorxiv.org/content/10.1101/2022.08.19.504330

McLaren, M. R., Willis, A. D., & Callahan, B. J. (2019). Consistent and correctable bias in metagenomic sequencing experiments. *eLife*, *8*, e46923. https://doi.org/10.7554/eLife.46923

Mendelssohn, R. (2020). *rerddapXtracto: Extracts environmental data from "ERDDAP" web services*. R package version 1.0.0.

Miya, M., Gotoh, R. O., & Sado, T. (2020). MiFish metabarcoding: A high-throughput approach for simultaneous detection of multiple fish species from environmental DNA and other samples. *Fisheries Science*, *86*(6), 939–970. https://doi.org/10.1007/s12562-020-01461-x

Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J. Y., Sato, K., Minamoto, T., Yamamoto, S., Yamanaka, H., Araki, H., Kondoh, M., & Iwasaki, W. (2015). MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: Detection of more than 230 subtropical marine species. *Royal Society Open Science*, *2*(7), 150088. https://doi.org/10.1098/rsos.150088

Morgan, C. A., Beckman, B. R., Weitkamp, L. A., & Fresh, K. L. (2019). Recent ecosystem disturbance in the northern California current. *Fisheries*, *44*(10), 465–474. https://doi.org/10.1002/fsh.10273

Moser, H. G., Charter, R., Smith, P., Ambrose, D., Watson, W., Charter, S., & Sandknop, E. (2001). Distributional atlas of fish larvae and eggs in the Southern California bight region: 1951–1998. *California Cooperative Oceanic Fisheries Investigations Atlas*, *34*, 1–166.

Moser, H. G., Charter, R. L., Smith, P. E., Ambrose, D. A., Charter, S. R., Meyer, C. A., Sandknop, E. M., & Watson, W. (1993). Distributional atlas of fish larvae and eggs in the California current region: Taxa with 1000 or more total larvae, 1951 through 1984. In *CalCOFI Atlas No. 31* (Vol. *53*). Marine Life Research Program, Scripps Institution of Oceanography.

Moser, H. G., Smith, P. E., & Eber, L. E. (1987). Larval fish assemblages in the California current region, 1954–1960, a period of dynamic environmental change. *CalCOFI Report*, *28*(28), 97–127.

Nielsen, J. M., Rogers, L. A., Brodeur, R. D., Thompson, A. R., Auth, T. D., Deary, A. L., Duffy-Anderson, J. T., Galbraith, M., Koslow, J. A., & Perry, R. I. (2021). Responses of ichthyoplankton assemblages to the recent marine heatwave and previous climate fluctuations in several Northeast Pacific marine ecosystems. *Global Change Biology*, *27*(3), 506–520. https://doi.org/10.1111/gcb.15415

Oliver, E. C. J., Benthuysen, J. A., Darmaraki, S., Donat, M. G., Hobday, A. J., Holbrook, N. J., Schlegel, R. W., & Sen Gupta, A. (2021). Marine heatwaves. *Annual Review of Marine Science*, *13*, 313–342. https://doi.org/10.1146/annurev-marine-032720-095144

Oliver, E. C. J., Perkins-Kirkpatrick, S. E., Holbrook, N. J., & Bindoff, N. L. (2018). 9. Anthropogenic and natural influences on record 2016 marine heat waves. *Bulletin of the American Meteorological Society*, *99*(1), S44–S48. https://doi.org/10.1175/BAMS-D-17-0093.1

Piatt, J. F., Parrish, J. K., Renner, H. M., Schoen, S. K., Jones, T. T., Arimitsu, M. L., Kuletz, K. J., Bodenstein, B., García-Reyes, M., Duerr, R. S., Corcoran, R. M., Kaler, R. S. A., McChesney, G. J., Golightly, R. T., Coletti, H. A., Suryan, R. M., Burgess, H. K., Lindsey, J., Lindquist, K., … Sydeman, W. J. (2020). Extreme mortality and reproductive failure of common murres resulting from the Northeast Pacific marine heatwave of 2014-2016. *PLoS One*, *15*(1), e0226087. https://doi.org/10.1371/journal.pone.0226087

Pinsky, M. L., Selden, R. L., & Kitchel, Z. J. (2020). Climate-driven shifts in marine species ranges: Scaling from organisms to communities. *Annual Review of Marine Science*, *12*, 153–179. https://doi.org/10.1146/annurev-marine-010419-010916

Pitz, K. J., Guo, J., Johnson, S. B., Campbell, T. L., Zhang, H., Vrijenhoek, R. C., Chavez, F. P., & Geller, J. (2020). Zooplankton biogeographic boundaries in the California Current System as determined from metabarcoding. *PLoS One*, *15*(6), e0235159. https://doi.org/10.1371/journal.pone.0235159

Pont, D., Meulenbroek, P., Bammer, V., Dejean, T., Erős, T., Jean, P., Lenhardt, M., Nagel, C., Pekarik, L., & Schabuss, M. (2023). Quantitative monitoring of diverse fish communities on a large scale combining eDNA metabarcoding and qPCR. *Molecular Ecology Resources*, *23*(2), 396–409.

Ren, A. S., & Rudnick, D. L. (2021). Temperature and salinity extremes from 2014–2019 in the California Current System and its source waters. *Communications Earth & Environment*, *2*(1), 1–9.

Robinson, H., Thayer, J., Sydeman, W. J., & Weise, M. (2018). Changes in California Sea lion diet during a period of substantial climate variability. *Marine Biology*, *165*(10), 1–12. https://doi.org/10.1007/s00227-018-3424-x

Rogers-Bennett, L., & Catton, C. A. (2019). Marine heat wave and multiple stressors tip bull kelp forest to sea urchin barrens. *Scientific Reports*, *9*(1), 1–9. https://doi.org/10.1038/s41598-019-51114-y

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), 1–12. 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 and Technology*, *50*(19), 10456–10464. https://doi.org/10.1021/acs.est.6b03114

Schroeder, I. D., Santora, J. A., Bograd, S. J., Hazen, E. L., Sakuma, K. M., Moore, A. M., Edwards, C. A., Wells, B. K., & Field, J. C. (2019). Source water variability as a driver of rockfish recruitment in the California current ecosystem: Implications for climate change and fisheries management. *Canadian Journal of Fisheries and Aquatic Sciences*, *76*(6), 950–960.

Shelton, A. O., Gold, Z. J., Jensen, A. J., D'Agnese, E., Andruszkiewicz Allan, E., van Cise, A., Gallego, R., Ramón-Laca, A., Garber-Yonts, M., Parsons, K., & Kelly, R. P. (2022). Toward quantitative metabarcoding. *Ecology*, *104*, e3906.

Silverman, J. D., Bloom, R. J., Jiang, S., Durand, H. K., Dallow, E., Mukherjee, S., & David, L. A. (2021). Measuring and mitigating PCR bias in microbiota datasets. *PLoS Computational Biology*, *17*(7), e1009113. https://doi.org/10.1371/journal.pcbi.1009113

Smith, P. E., & Moser, H. G. (2003). Long-term trends and variability in the larvae of Pacific Sardine and associated fish species of the California current region. *Deep-Sea Research Part II: Topical Studies in Oceanography*, *50*(14–16), 2519–2536. https://doi.org/10.1016/S0967-0645(03)00133-4

Snyder, M. A., Sloan, L. C., Diffenbaugh, N. S., & Bell, J. L. (2003). Future climate change and upwelling in the California current. *Geophysical Research Letters*, *30*(15), 1823. https://doi.org/10.1029/2003GL017647

Stoeckle, M. Y., Adolf, J., Charlop-Powers, Z., Dunton, K. J., Hinks, G., & Vanmorter, S. M. (2021). Trawl and eDNA assessment of marine fish diversity, seasonality, and relative abundance in coastal New Jersey, USA. *ICES Journal of Marine Science*, *78*(1), 293–304. https://doi.org/10.1093/icesjms/fsaa225

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

Szuwalski, C., Cheng, W., Foy, R., Hermann, A. J., Hollowed, A., Holsman, K., Lee, J., Stockhausen, W., & Zheng, J. (2021). Climate change and the future productivity and distribution of crab in the Bering Sea. *ICES Journal of Marine Science*, *78*(2), 502–515.

Thalinger, B., Rieder, A., Teuffenbach, A., Pütz, Y., Schwerte, T., Wanzenböck, J., & Traugott, M. (2021). The effect of activity, energy use, and species identity on environmental DNA shedding of freshwater fish. *Frontiers in Ecology and Evolution*, *9*, 73. https://doi.org/10.3389/fevo.2021.623718

Thompson, A. R., Ben-Aderet, N. J., Bowlin, N. M., Kacev, D., Swalethorp, R., & Watson, W. (2022). Putting the Pacific marine heatwave into perspective: The response of larval fish off southern California to unprecedented warming in 2014–2016 relative to the previous 65 years. *Global Change Biology*, *28*(5), 1766–1785. https://doi.org/10.1111/gcb.16010

Thompson, A. R., Chen, D. C., Guo, L. W., Hyde, J. R., & Watson, W. (2017). Larval abundances of rockfishes that were historically targeted by fishing increased over 16 years in association with a large marine protected area. *Royal Society Open Science*, *4*(9), 170639. https://doi.org/10.1098/rsos.170639

Thompson, A. R., Harvey, C. J., Sydeman, W. J., Barceló, C., Bograd, S. J., Brodeur, R. D., Fiechter, J., Field, J. C., Garfield, N., Good, T. P., Hazen, E. L., Hunsicker, M. E., Jacobson, K., Jacox, M. G., Leising, A., Lindsay, J., Melin, S. R., Santora, J. A., Schroeder, I. D., … Williams, G. D. (2019). Indicators of pelagic forage community shifts in the California current large marine ecosystem, 1998–2016. *Ecological Indicators*, *105*, 215–228. https://doi.org/10.1016/j.ecolind.2019.05.057

Thompson, A. R., Hyde, J. R., Watson, W., Chen, D. C., & Guo, L. W. (2016). Rockfish assemblage structure and spawning locations in southern California identified through larval sampling. *Marine Ecology Progress Series*, *547*, 177–192. https://doi.org/10.3354/meps11633

Thompson, A. R., McClatchie, S., Weber, E. D., Watson, W., & Lennert-Cody, C. E. (2017). Correcting for bias in calcofi ichthyoplankton abundance estimates associated with the 1977 transition from ring to bongo net sampling. *California Cooperative Oceanic Fisheries Investigations Reports*, *58*, 1–11.

Thompson, A. R., Watson, W., McClatchie, S., & Weber, E. D. (2012). Multi-scale sampling to evaluate assemblage dynamics in an oceanic marine reserve. *PLoS One*, *7*(3), e33131. https://doi.org/10.1371/journal.pone.0033131

Vergés, A., Doropoulos, C., Malcolm, H. A., Skye, M., Garcia-Pizá, M., Marzinelli, E. M., Campbell, A. H., Ballesteros, E., Hoey, A. S., Vila-Concejo, A., Bozec, Y. M., & Steinberg, P. D. (2016). Long-term empirical evidence of ocean warming leading to tropicalization of fish communities, increased herbivory, and loss of kelp. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(48), 13791–13796. https://doi.org/10.1073/pnas.1610725113

Walker, H. J., Hastings, P. A., Hyde, J. R., Lea, R. N., Snodgrass, O. E., & Bellquist, L. F. (2020). Unusual occurrences of fishes in the Southern California Current System during the warm water period of 2014–2018. *Estuarine, Coastal and Shelf Science*, *236*, 106634. https://doi.org/10.1016/j.ecss.2020.106634

Yates, M. C., Fraser, D. J., & Derry, A. M. (2019). Meta-analysis supports further refinement of eDNA for monitoring aquatic species-specific abundance in nature. *Environmental DNA*, *1*(1), 5–13. https://doi.org/10.1002/edn3.7

Yates, M. C., Glaser, D. M., Post, J. R., Cristescu, M. E., Fraser, D. J., & Derry, A. M. (2021). The relationship between eDNA particle concentration and organism abundance in nature is strengthened by allometric scaling. *Molecular Ecology*, *30*(13), 3068–3082.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Gold, Z., Kelly, R. P., Shelton, A. O., Thompson, A. R., Goodwin, K. D., Gallego, R., Parsons, K. M., Thompson, L. R., Kacev, D., & Barber, P. H. (2023). Archived DNA reveals marine heatwave-associated shifts in fish assemblages. *Environmental DNA*, *00*, 1–14. https://doi.org/10.1002/edn3.400