Message in a Bottle: Archived DNA Reveals Marine Heatwave-Associated Shifts in Fish Assemblages

Zachary Gold1,2, Ryan P. Kelly3, Andrew Olaf Shelton2, Andrew Thompson4, Kelly D. Goodwin5, Ramon Gallego2, Kim Parsons2, Luke R. Thompson5,6, Dovi Kacev7, Paul H. Barber8

1 Cooperative Institute for Climate, Ocean, & Ecosystem Studies, UW, Seattle, WA

2 Northwest Fisheries Science Center, NMFS/NOAA, Seattle, WA

3 School of Marine and Environmental Affairs, UW, Seattle, WA

4 Southwest Fisheries Science Center, NMFS/NOAA, La Jolla, CA

5 Ocean Chemistry and Ecosystems Division, Atlantic Oceanographic and Meteorological Laboratory, Miami, FL

6 Northern Gulf Institute, Mississippi State University, Mississippi State, MS

7 Scripps Institution of Oceanography, UCSD, La Jolla

8 Department of Ecology and Evolutionary Biology, UCLA, Los Angeles, CA

**Abstract**

Marine heatwaves can drive large-scale shifts in marine ecosystems, but their impacts can be difficult to study from a species assemblage perspective. Here, we combine DNA sequences derived from the ethanol of a 23-year longitudinal sample collection with microscopy-derived ichthyoplankton identification to demonstrate tropicalization of fish assemblages in the California Current Large Marine Ecosystem during and after the 2014-16 unprecedently large Pacific marine heatwave. Our quantitative model estimated assemblage composition and abundance to reveal increases in southern, mesopelagic species associated with declines in important temperate fisheries targets (e.g. North Pacific Hake (*Merluccius productus*) and Pacific sardine (*Sardinops sagax*)). We observed novel assemblages of southern mesopelagic fishes and temperate species (e.g. Northern Anchovy, *Engraulis mordax*) even after the return to average water temperatures. Overall, the integrated dataset captured temporal assemblage dynamics of 56 fishes including important temperate marine indicator species that lack morphologically distinguishing characteristics. The technique of jointly modeling morphological and molecular data from ethanol-derived samples opens the door to reconstructing the historical dynamics of assemblages from modern and archived samples worldwide.

**Introduction**

Climate-induced marine heatwaves (MHWs) are increasing in frequency and severity with far-reaching consequences in marine ecosystems (*1*) ranging from severe organismal stress to cascading ecosystem effects (*2*). Notable recent examples include repeated bleaching events across the Great Barrier Reef (2016, 2017, 2020)(*3*) and near-total kelp deforestation in Northern California, USA (2016-19)(*4*). These marine heatwaves precipitated drastic, unprecedented changes in dominant foundational species across hundreds of thousands of square kilometers of shallow, coastal ecosystems.

The impacts of such large environmentally driven disturbances on coastal marine ecosystems have been ecologically and economically significant (*5*–*7*). In the 1940s, the dramatic collapse of Pacific Sardine (*Sardinops sagax*) disrupted marine foodwebs, causing broad-scale, negative socio-economic impacts across the Northeast Pacific (*8*–*10*). 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), the California Cooperative Oceanic Fisheries Investigations (CalCOFI) was formed in 1949. CalCOFI has continuously conducted systematic fisheries-independent surveys of the southern CCLME from 1951 until present (*11*, *12*), focusing on monitoring larval fish assemblages, as larval fish dynamics are a key predictor of ecosystem health and function (*5*, *12*, *13*).

Larval fish abundances help to characterize the state of marine ecosystems as they track spawning-stock biomass (*14*). Over 70 years of CalCOFI research 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 (*12*, *15*–*17*). These decadal and annual changes in ichthyoplankton dynamics are superimposed over the strong biogeographic assemblage associations with distinct water mass characteristics within the Southern California Bight (*17*). Importantly, ichthyoplankton assemblages differ between the colder and fresher California Current; warmer and saltier California Counter Current and Central Pacific water mass; as well as upwelling conditions across the continental shelf (*11*, *13*, *18*, *19*). Importantly, periods of elevated temperatures were historically associated with higher abundances of southern mesopelagic species and Pacific Sardine while colder periods were associated with higher abundances of northern mesopelagics and Northern Anchovy (*Engraulis mordax*) (*10*, *20*). These critical insights into forage fish community dynamics across decadal climatic regime shifts are vital to understanding the effects of climate change on the CCLME (*9*, *11*, *19*).

Despite the value of previous CalCOFI ichthyoplankton efforts, such traditional manual identification of larvae is labor-intensive and taxonomic resolution is often limited by a lack of discernible morphological characteristics (*21*). Here, we reconstruct ichthyoplankton assemblages over 23 years, applying genetic metabarcoding (amplicon sequencing) to the ethanol surrounding preserved CalCOFI plankton samples. We pair these data with morphological count observations in a joint Bayesian model to estimate species-specific larval abundance.

We model taxon sequence-read counts from metabarcoding as an outcome of the PCR process, wherein each taxon has a different amplification efficiency for the primer set used (see Methods). We link the sequencing data to morphological ichthyoplankton counts to constrain the species-specific starting concentrations of DNA in the ethanol jars. The resulting integrated model leverages the taxonomic breadth and resolution (*22*, *23*) of amplicon sequencing, combining these with the power of morphological counts to yield species-specific quantitative abundance estimates. By jointly interpreting these datasets, we can track changes in abundance for a broad diversity of larval fish species, yielding a much higher-resolution picture of these assemblages.

The resulting estimates capture major and sometimes highly unexpected changes to the fish assemblages during and after the 2014–2016 Pacific Marine Heatwave (MHW), the warmest 3-year period in the North Pacific over 100 years of recorded history (*24*). We directly examine the responses of ichthyoplankton assemblages to sea surface temperature (SST) as an abiotic proxy for the MHW as a whole, recognizing that a suite of environmental variables including upwelling strength and location, dissolved oxygen, and salinity, among other environmental covariates, changed dramatically during the climate-driven MHW (*25*–*28*). Complementary analyses using mean water-column temperature – rather than SST – yielded similar results (See Supplement).

**Methods**

*Study Design*

To investigate decadal changes in the ichthyoplankton assemblages in the California Current vicinity, we identified ichthyoplankton from four distinct stations in spring. Archived spring ichthyoplankton samples were collected across four biogeographically distinct stations with variable water properties (*29*) over 2 decades (1996,1998-2019; Figure S1; See Supplemental Methods)(*5*, *30*). Decades of research indicate the majority of species spawn in spring within the study region (*16*, *17*, *20*, *31*) and closely track adult biomass (*32*). Hence we expect the spring ichthyoplankton to reflect underlying changes in the local fish assemblages.

At each station, oblique bongo net tows were conducted from 210 m to the surface using standard CalCOFI methods (*15*, *33*–*35*). 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. Microscopy was conducted to identify species abundance from formaldehyde-preserved samples following standardized CalCOFI techniques (*29*). DNA metabarcoding targeting the *12S* rRNA mitochondrial gene region using the MiFish Universal Teleost (*36*) PCR primer set was conducted on the ethanol in which port side samples were preserved. See supplemental methods for full description.

*Estimating Abundance*

We estimated the abundance of ichthyoplankton in each jar using a novel joint Bayesian hierarchical model described in detail in Supplement 2. In brief, we estimate that, for any species *i*, the number of sequenced amplicons is proportional to the fraction of DNA available from that species in the PCR template. The amplicons produced during a PCR reaction are governed by the amplicon efficiency parameter , which is characteristic of the interaction between the particular primer set and each species being amplified. Thus, for any species *i*, the number of amplicons should be directly related to the efficiency of amplification and the starting concentration of DNA template such that

(1)

where is amplicon abundance, is the true number of DNA copies in the reaction attributable to species *i*, is the species-specific amplification efficiency (bounded on (0,1)), and is the number of PCR cycles used in the reaction.

If we could perfectly observe amplicons, the above equation would be all that we needed to relate observed amplicons to the true number of template DNA copies . Unfortunately, PCR and sequencing technology does not allow for such direct observation for two main reasons. First, the actual observed amplicon read counts are driven primarily by the variable values among species and (*37*–*39*). Second, DNA sequencing machines do not read all of the copies from a PCR reaction (*40*, *41*). This subsampling of amplicons changes what in eq. 1 appears to be a single-species process – each species being amplified independently – into a multi-species process; the number of amplicons observed for species *i* will depend upon both the amplicons produced for species *i* = 1 and the amplicons produced for species *i* = 2, 3, ..., *I* in the same reaction. Observations of amplicons are thus compositional data and need to be analyzed as such (*42*).

To retain the data-generating structure from eq.1 as much as possible, we develop a model for a single sample with many species. As above, if we let *i* index species with *i* = 1, 2, ..., *I*, then we can write a deterministic equation for the number of amplicons observed in log-space as

(2)

where the only new term is η, representing the proportion of reads observed from a given sampling run. Note that in this formulation η is a single value shared across all species and serves to scale the number of amplicons observed. Additionally, we can rewrite the number of DNA copies in terms of proportional number of counts, log() = log() − log() . Note that the second term in this equation is a sum of the counts across all species, and so is a single shared value for all species. As such it can be integrated into the value η that scales the overall abundance for each species *i*,

(3)

This equation is appealing because it provides a process-oriented description of the biology of interest (the βs), a term for how PCR modifies our amplicon sequence count observation of the true abundance ( , and a term for how subsampling of DNA reads in the sequencer will adjust the number of amplicons observed (log(η)).

However, in the absence of additional information, it is not possible to estimate the β and *a* parameters from metabarcoding data alone. Including morphological count data enables us to estimate the confounded parameters from the metabarcoding. For each sampled station, we have two independent sets of observed data: 1) counts of larval/juvenile fishes for each taxon from the formaldehyde jars and 2) counts of amplicons for each taxon from ethanol jars. These observed data arise from a common (but unobserved) biomass for each species at each station-year combination, and thus we can model them jointly.

Importantly in the model implementation, microscopy counts were modeled as Poisson-distributed given their relatively small absolute values and low variance (*33*) whereas amplicon sequence data were modeled using a Negative Binomial distribution given their relatively high absolute values and high variability among replicates (Figure S2-S4). These statistical distributions are commonly used in models of amplicon and count data, respectively (*35*–*37*). A full description of model implementation and assumptions are provided in Supplement 2.

*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 (*46*). 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 (See supplemental methods)(*32*). 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 (See supplemental results).

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 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 and Discussion**

Metabarcoding with MiFish *12S* (*47*) generated a total of 59.9 million sequence reads across 84 stations representing 90 unique DNA extractions and 262 unique PCR technical replicates. All sequence data were processed using the *Anacapa Toolkit* (*48*). After quality control, sequence-variant (ASV) dereplication, and decontamination processes (*22*, *48*, *49*), we retained a total of 54.5 million reads (See Supplemental Methods). From these data, we classified 130 unique taxa including 103 species-level assignments, 15 genus-level assignments, 11 family-level assignments, and 1 class-level assignment. We identified two distinct morphologically indistinguishable lineages of the Northern Lanternfish (*Stennobrachius leucopsarus*). The two lanternfish lineages exhibited dramatically different ecological patterns across the samples and were therefore treated separately.

Independent microscopy-count data from paired, matching formalin-preserved samples consisted of 9,610 larvae sorted across 84 jars. From these data, we classified a total of 92 unique taxa including 76 species-level assignments and 16 genus-level assignments.

For our integrated Bayesian model we focused on the 56 species which had sufficient representation across the metabarcoding data set to achieve model convergence (observed in >10 technical PCR replicates) and thus provided reliable quantitative estimates (Figures S3-S5). Model fits yielded site-, species-, and year-specific larval abundances for 56 fish species spanning a 23-year period.

***Displacement of Target Fish Species and Tropicalization of Fish Assemblages Associated with the Marine Heatwave***

We observed a transformation of marine ichthyoplankton assemblages during the 2014–2016 MHW where southern mesopelagic species increased while several temperate species of critical ecological and economic importance declined. Such synchronous changes in the marine ichthyoplankton assemblages occurred during the MHW despite the hundreds of kilometers between stations and unique biogeographic characteristics associated with each sampled geographic location (See supplemental results). For example, the mesopelagic Mexican Lampfish (*Triphoturus mexicanus*) was at peak observed abundance during the MHW, and extended its typical range both poleward and into coastal shelf waters (Figures 1a & c, S6-10).

These shifts occurred throughout the ichthyoplankton assemblage. On the one hand, subtropical mesopelagic species uniformly increased in association with elevated sea-surface temperatures. On the other hand, many coastal species tended to decrease (Figure 2, S6-26). In particular, the abundances of northern mesopelagic species and fisheries targets such as Pacific Sardine (*Sardinops sagax*) and North Pacific Hake (*Merluccius productus*) were significantly correlated with colder sea surface temperatures and displayed negative co-occurrence patterns with warm associated southern mesopelagic taxa (Figure 3, S27-28). These results suggest multiple coastal pelagic fisheries targets may continue to be scarce as environmental conditions that are similar to the 2014-2016 MHW become more common (*5*, *30*, *50*), and suggest future ecological disruptions with potentially major socio-economic consequences may arise under continued unabated climate change (*2*).

Graphical user interface, application

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**Figure 1. Temperature Associations in Fish Species**

Changes in species *occurrence* patterns associated with SST, with southern mesopelagic species increasing in prevalence with elevated temperature (A). T statistic (slope coefficient/ standard error) from a generalized binomial mixed model was calculated for each species across all sites. Only 23 out of 56 total species with significant slopes (95% CI that exclude zero) are plotted. Importantly, metabarcoding identified cold-associated variants of the Northern Lanternfish (*Stennobrachius leucopsarus*) that cannot be morphologically identified (B) as well as common warm-associated species such as the Mexican Lampfish (*Triphoturus mexicanus*) (C).

**Chart

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**Figure 2. Southern Oceanic Species Drive Fish Community Shifts**

Changes in species *biomass* in response to temperature, with Southern Mesopelagic species increasing in abundance with elevated temperature (A). As in Fig 1, significant T statistics from generalized linear models are plotted. Southern mesopelagic fishes were associated with increased temperature as indicated by summarizing species-specific slopes from generalized linear models (B) and by the aggregated abundance relationship (C; each point is the mean posterior estimate of abundance for an individual southern mesopelagic species in a single year). In contrast, abundances of benthic species and coastal pelagic species such as Northern Hake and Pacific Sardine abundances, were associated with cooler temperatures.

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**Figure 3. Novel Marine Heatwave Assemblages**

Shifts in species abundances before the MHW (1996-2013) compared to during and after the MHW (2014-2019). Synchronous increases in southern mesopelagics and Northern Anchovy were observed across all sites. Here, sites are shown in rows, species in columns, and the change in abundance between the two ecological phases shown as the response variable. Fisheries targets including Pacific Sardine and North Pacific Hake, as well as many other benthic and coastal species, had concurrent negative associations. Significant differences during and after the MHW are marked with + or -.

Recent studies demonstrate the tropicalization of terrestrial and marine ecosystems in response to climate change (*40*, *41*). These shifts can induce novel species interactions, catalyzing changes in ecosystem function (*2*, *25*). For example, we observe the combination of high abundances of both anchovy and southern mesopelagics (*5*, *30*) – a pattern otherwise undocumented in the previous >70-year CalCOFI dataset (*16*, *20*). Although the ecological implications of these novel assemblages are, by definition, unpredictable, our results suggest that if future assemblages resemble those seen in the MHW, increases in Northern Anchovy and southern mesopelagic fishes are likely to be associated with decreases in Pacific Sardine and North Pacific Hake in the Southern CCLME (*53*, *54*); these shifts have fundamentally changed ecosystems and fisheries relative to the recent past (*20*).

***Biomass Changes in Forage Fishes***

Both because of their own commercial value and because they are prey for other high-value fishery species, sardine and anchovy fluctuations have been a major focus of fisheries research since the 1950s (*9*). Our model estimates are consistent with other studies (*20*, *55*) that documented a decline in both sardines and anchovy beginning in 2005. This was followed by high abundances of anchovy, but continued low abundances of sardine in the wake of the MHW, 2015-2019 (Figure 4). Although anchovy larvae abundance was low in spring during the 2014-2016 MHW, anchovy recruitment was high in summer 2015 (*56*). Anchovy mature in approximately one to two years(*55*, *57*) and thus the 2015 class likely began spawning in mid-2016 (*20*), leading to high anchovy spawning stock biomass and larval abundances by 2016 and lasting into 2019 and 2020 (*58*).

Graphical user interface, application

Description automatically generated

**Figure 4. Synchronous Increase in Anchovy Abundance During and After Marine Heatwave**

Posterior estimates for larval fish abundances (counts/10m2) over time at each of the four sampled sites. Joint modeling of metabarcoding and morphological counts reconstructed increases in Northern Anchovy (*Engraulis mordax*) [blue] during the recent Pacific Marine Heatwave and low spawning of Pacific Sardine (*Sardinops* sagax) [red] over the past decade (points are means and error bars are 95% credible intervals; shaded region is during and after the MHW). SST is plotted above the Northern Anchovy and Pacific Sardine abundances, for reference.

The rise in anchovy and continued low abundances of sardine during the MHW 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 (*10*). However, our findings and others (*5*, *9*, *30*, *59*) suggest that the mechanisms that govern the population dynamics of these species are not a mere function of temperature, but that more complex factors drive recruitment dynamics of these species (*20*). For example, despite largely synchronous responses of fish assemblages to the marine heatwave, sardine declines were not consistent across the CCLME, with refugia of localized abundance in nearshore waters potentially driven by distinct, favorable conditions (*9*, *20*, *55*).

Further improving our mechanistic understanding of drivers of fish assemblage dynamics will better inform ecological predictions in the face of climate change (*60*). As we demonstrate, a combination of metabarcoding and visual surveys can characterize species across trophic levels (*61*) and this has the potential to reveal ecological mechanisms. Here, we used metabarcoding to accurately characterize the composition of larval fishes in CalCOFI plankton samples. Future efforts could focus on documenting the phytoplankton and zooplankton assemblages that comprise both larval prey and predators. Several major hypotheses seeking to explain recruitment variability are underpinned by the capacity of young larvae to consume appropriate prey that facilitates faster growth (*62*). Unfortunately, accurately characterizing the larval prey field has traditionally been difficult as prey are generally too small to be accurately sampled by nets (*63*). Metabarcoding of water samples from the same locations where larvae are collected, however, can characterize the larval prey field at an unprecedentedly high level of detail. In addition, metabarcoding of the stomachs of larval fishes can then identify actual prey items that were consumed by larvae. Evaluating the larval prey field and gut contents through metabarcoding will help us to finally understand the drivers of recruitment volatility in coastal pelagic and other fishes (*5*, *55*, *64*–*68*).

The described unexpected rise in anchovy during the 2014-16 MHW resonated throughout the CCLME (*50*). For example, California sea lion pups grew at anomalously high rates after their mothers consumed copious anchovy forage and produced ample milk (*53*). High rates of almost exclusively anchovy consumption also seemingly induced thiamine deficiency in adult salmon resulting in poor condition of recruits (*69*). Birds capable of feeding on anchovy thrived (*30*) while those unable to consume anchovy perished (*54*). Given that conditions comparable to the 2014–2016 MHW are predicted to be more common in the CCLME in the future (*1*), our results suggest that continued biological responses to both anchovy-dominated forage-fish assemblages and MHW-associated ocean warming conditions are likely to be without modern analog (*20*).

***Novel Insights from Legacy Collections***

Molecular tools differentiated variants and species that were not morphologically identifiable in the ichthyoplankton (*21*).For example, metabarcoding identified unique cold-associated variants of the Northern Lampfish (*Stennobrachius leucopsarus*) that are morphologically indistinguishable and combined as a complex are only weakly associated with cooler temperatures. By illuminating such unseen variation, molecular methods reveal ecological dynamics otherwise hidden by shared larval morphology.

Ultimately, our approach to studying historical fluctuations in ichthyoplankton assemblages reveals climate-associated biological changes in the CCLME, and suggests ways in which these changes could alter the function and socio-economic benefits derived from marine ecosystems. Importantly, this novel approach provides quantitative estimates by non-destructively sampling legacy collections via metabarcoding, and at the same time provides a mechanistic framework for determining absolute abundance estimates from compositional amplicon sequencing data (*37*, *42*, *70*). Unlocking such quantitative metabarcoding approaches expands the potential for linking ecological assemblages to environmental processes beyond just presence-absence analyses (*71*–*73*). Such quantitative approaches may prove critical in modeling and predicting future ecosystem change, 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 (*74*). As such, here we provide broadly applicable methodology with which to efficiently understand modern and historical changes in ecological communities.

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