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

2   Assemblages

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22   Summary: Novel quantitative abundance estimates from archived DNA reveals marine

23   heatwave-associated shifts in fish assemblages.

Abstract

Marine heatwaves can drive large-scale shifts in marine ecosystems, but studying their impacts from a species assemblage perspective can be difficult. Here, we leverage the taxonomic breadth and resolution of DNA sequences derived from the ethanol of a 23-year longitudinal sample collection, combining these with microscopy-derived ichthyoplankton identification to yield higher-resolution species-specific quantitative abundance estimates of fish assemblages in the California Current Large Marine Ecosystem during and after the 2014-16 Pacific marine heatwave. This integrated dataset reveals patterns of tropicalization with increases in southern, mesopelagic species associated and 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. Our novel modeling approach 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.

47   The impacts of such large environmentally driven disturbances on coastal marine

48   ecosystems have been ecologically and economically significant (*5–7*). In the 1940s, the

49   dramatic collapse of Pacific Sardine (*Sardinops sagax*) disrupted marine foodwebs, causing

50   broad-scale, negative socio-economic impacts across the Northeast Pacific (*8–10*). To better

51   understand the processes driving these complex marine ecosystem dynamics and to avert similar

52   fisheries collapses within the California Current Large Marine Ecosystem (CCLME), the

53   California Cooperative Oceanic Fisheries Investigations (CalCOFI) was formed in 1949.

54   CalCOFI has continuously conducted systematic fisheries-independent surveys of the southern

55   CCLME from 1951 until present (*11*, *12*), focusing on monitoring larval fish assemblages, as

56   larval fish dynamics are a key predictor of ecosystem health and function (*5*, *12*, *13*).

57   Larval fish abundances help to characterize the state of marine ecosystems as they track

58   spawning-stock biomass (*14*). Over 70 years of CalCOFI research has documented decadal and

59   annual changes in fish assemblages in response to environmental conditions, identifying major

60   shifts in response to Pacific Decadal Oscillations and El Niño Southern Oscillations (*12*, *15–17*).

61   These decadal and annual changes in ichthyoplankton dynamics are superimposed over the

62   strong biogeographic assemblage associations with distinct water mass characteristics within the

63   Southern California Bight (*17*). Ichthyoplankton assemblages differ among the colder and fresher

64   California Current, warmer and saltier California Counter Current and Central Pacific water

65   mass, and in upwelling conditions across the continental shelf (*11*, *13*, *18*, *19*). Importantly,

66   periods of elevated temperatures were historically associated with higher abundances of

67   southern, mesopelagic species and Pacific Sardine while colder periods were associated with

68   higher abundances of northern, mesopelagics and Northern Anchovy (*Engraulis mordax*) (*10*,

69 *20*). These insights into forage fish community dynamics across decadal climatic regime shifts

70 are vital to understanding the effects of climate change on the CCLME (*9*, *11*, *19*).

71 Despite the value of previous CalCOFI ichthyoplankton efforts, such traditional manual

72 identification of larvae is labor-intensive, and taxonomic resolution is often limited by a lack of

73 discernible morphological characteristics (*21*). Here, we reconstruct ichthyoplankton

74 assemblages over 23 years, applying genetic metabarcoding (amplicon sequencing) to the

75 ethanol surrounding preserved CalCOFI plankton samples. We pair these data with

76 morphological count observations in a joint Bayesian model to estimate species-specific larval

77 abundance.

78 We model taxon sequence-read counts from metabarcoding as an outcome of the PCR

79 process, wherein each taxon has a different amplification efficiency for the primer set used (see

80 Materials and Methods). We link the sequencing data to morphological ichthyoplankton counts

81 to constrain the species-specific starting concentrations of DNA in the ethanol jars. The resulting

82 integrated model leverages the taxonomic breadth and resolution (*22*, *23*) of amplicon

83 sequencing, combining these with the power of morphological counts to yield species-specific

84 quantitative abundance estimates. By jointly interpreting these datasets, we can track changes in

85 abundance for a broad diversity of larval fish species, yielding a much higher-resolution picture

86 of these assemblages.

87 The resulting estimates capture major and sometimes highly unexpected changes to the

88 fish assemblages during and after the 2014–2016 Pacific Marine Heatwave (MHW), the warmest

89 3-year period in the North Pacific over 100 years of recorded history (*24*). We directly examine

90 the responses of ichthyoplankton assemblages to sea surface temperature (SST) as an abiotic

91 proxy for the MHW as a whole, recognizing that a suite of environmental variables including

92   upwelling strength and location, dissolved oxygen, and salinity, among other environmental

93   covariates, changed dramatically during the climate change influenced MHW (*25–28*).

94   Complementary analyses using mean water-column temperature – rather than SST – yielded

95   similar results (See Supplement S1).

96

97   **Results**

98   Metabarcoding with MiFish *12S* (*29*) generated a total of 59.9 million sequence reads

99   across 84 jars representing 90 unique DNA extractions and 262 unique PCR technical replicates.

100   All sequence data were processed using the *Anacapa Toolkit* (*30*). After quality control,

101   sequence-variant (ASV) dereplication, and decontamination processes (*22*, *30*, *31*), we retained a

102   total of 54.5 million reads (See Supplement 1 Methods). From these data, we classified 130

103   unique taxa including 103 species-level assignments (79%), 15 genus-level assignments (12%),

104   11 family-level assignments (8.5%), and 1 class-level assignment. We identified two distinct

105   morphologically indistinguishable lineages of the Northern Lanternfish (*Stennobrachius*

106   *leucopsarus*). The two lanternfish lineages exhibited dramatically different ecological patterns

107   across the samples and were therefore treated separately.

108   Independent microscopy-count data from paired, matching formalin-preserved samples

109   consisted of 9,610 larvae sorted across 84 jars. From these data, we classified a total of 92 unique

110   taxa including 76 species-level assignments (83%) and 16 genus-level assignments (17%).

111   For our integrated Bayesian model, we focused on the 56 species that had sufficient

112   representation across the metabarcoding data set to achieve model convergence (observed in >10

113   technical PCR replicates) and thus provided reliable quantitative estimates (Figures S3-S5).

114 Model fits yielded station-, species-, and year-specific larval abundances for 56 fish species

115 spanning a 23-year period.

116 *Displacement of Target Fish Species and Tropicalization of Fish Assemblages Associated with*

117 *the Marine Heatwave*

118   We observed a transformation of marine ichthyoplankton assemblages during the 2014–

119 2016 MHW where southern, mesopelagic species increased while several temperate species of

120 critical ecological and economic importance declined. Such synchronous changes in the marine

121 ichthyoplankton assemblages occurred during the MHW despite the hundreds of kilometers

122 between stations and unique biogeographic characteristics associated with each sampled

123 geographic location (See Supplement 1 results). For example, the mesopelagic Mexican

124 Lampfish (*Triphoturus mexicanus*) was at peak abundance during the MHW, and extended its

125 typical range both poleward and into coastal shelf waters (Figures 1a & c, S6-10).

126   Shifts were observed throughout the ichthyoplankton assemblages of the study region

127 (Figures 2-3). On the one hand, subtropical, mesopelagic species uniformly increased in

128 association with elevated sea-surface temperatures. On the other hand, many coastal species

129 typically seen in the region tended to decrease (Figure 2, S6-26). In particular, the abundances of

130 northern, mesopelagic species and fisheries targets such as Pacific Sardine (*Sardinops sagax*)

131 and North Pacific Hake (*Merluccius productus*) were significantly correlated with colder sea

132 surface temperatures and displayed negative co-occurrence patterns with warm associated

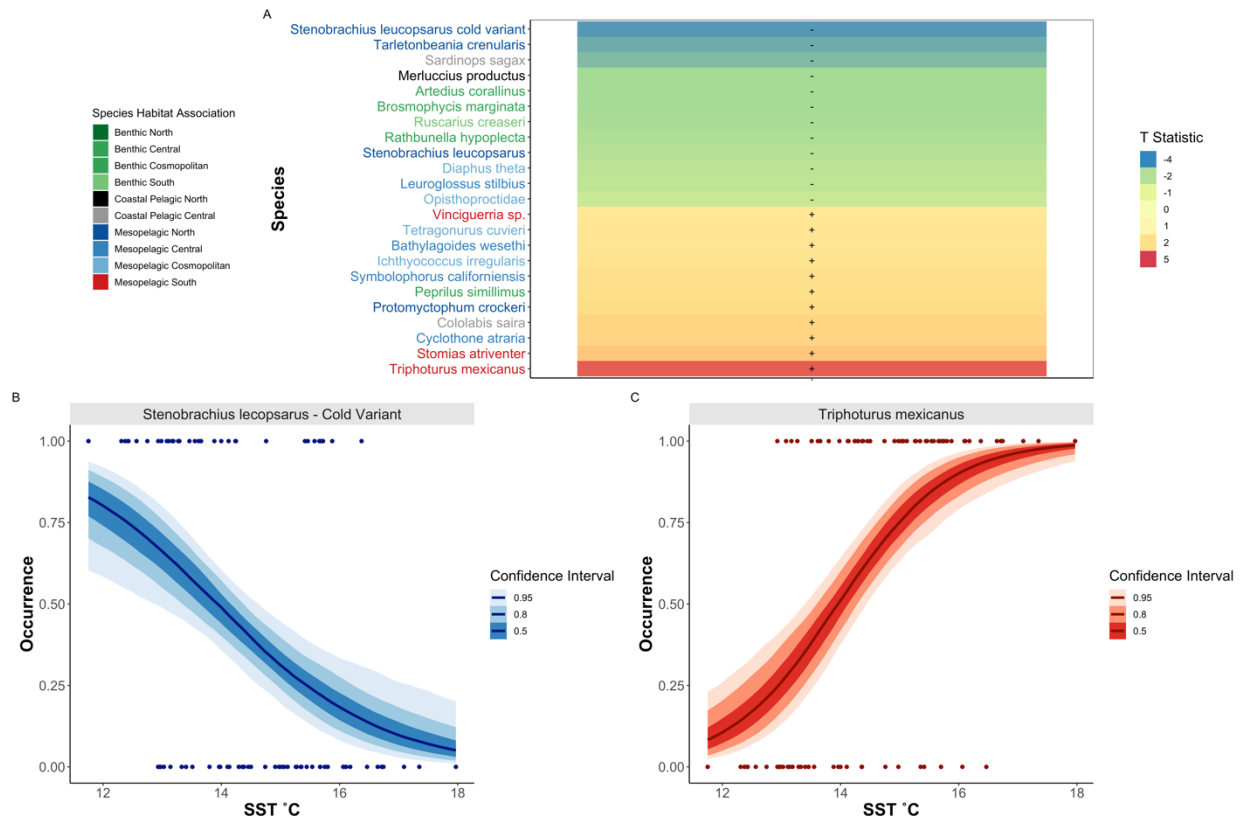133 southern, mesopelagic taxa (Figure 3, S27-28).

134

Figure 1. Temperature-Occurrence 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 stations. 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).
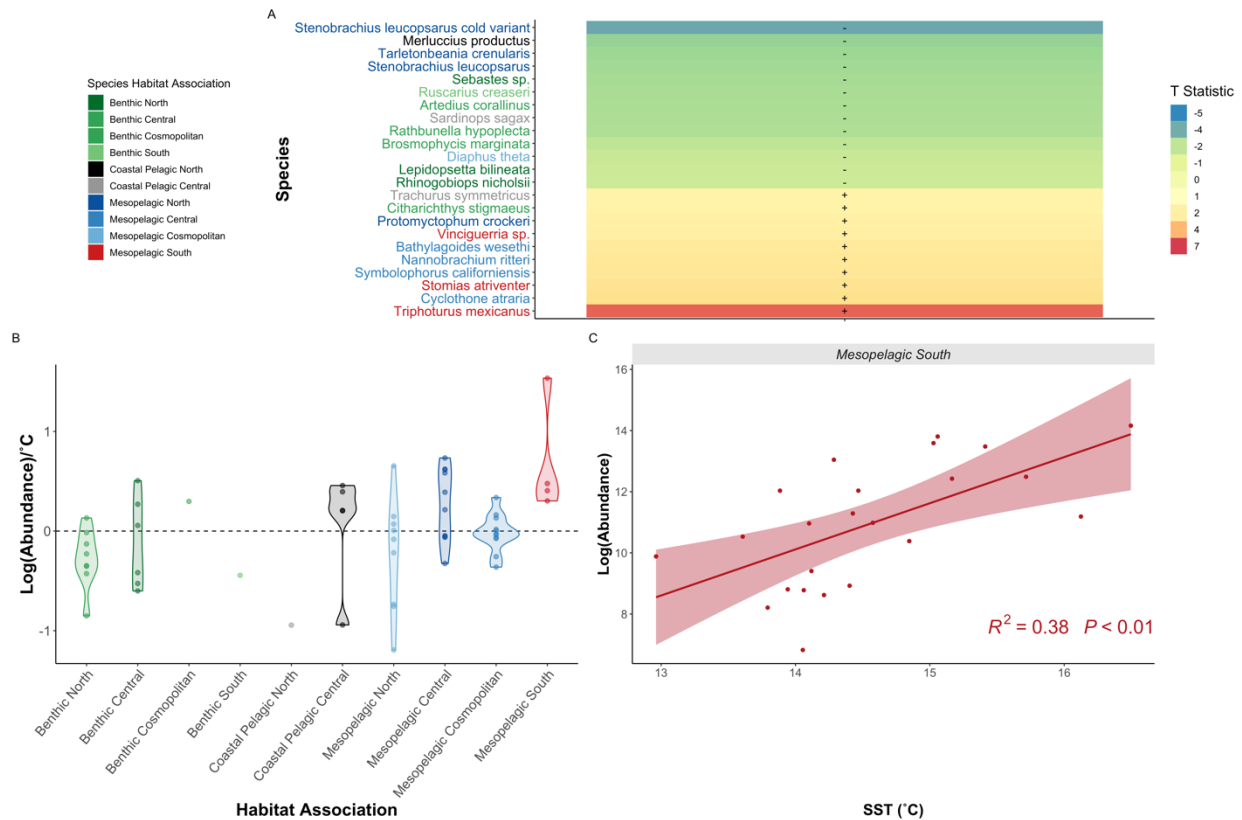
Figure 2. Increased Abundance of Southern Oceanic Species Drive Fish Community Shifts

Changes in species abundance in response to SST ˚C, with southern, mesopelagic species increasing in abundance with elevated sea surface 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 all 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.

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 stations. Here, stations are shown in rows, species in

166   columns, and the change in abundance between the two ecological phases shown as the

167   response variable. Fisheries targets including Pacific Sardine and North Pacific Hake, as

168   well as many other benthic and coastal species, had concurrent negative associations.

169   Significant differences during and after the MHW are marked with + or -.

170   *Biomass Changes in Forage Fishes*

171   Both because of their own commercial value and because they are prey for other high-

172   value fishery species, sardine and anchovy fluctuations have been a major focus of fisheries

173   research since the 1950s (*9*). Our model estimates are consistent with other studies (*20*, *32*) that

174   documented a decline in both sardines and anchovy beginning in 2005. This period of time was

175   followed by high abundances of anchovy, but continued low abundances of sardine in the wake

176   of the MHW, 2015-2019 (Figure 4). Although anchovy larvae abundance was low in spring

177   during the 2014-2016 MHW, anchovy recruitment was high in summer 2015 (*33*). Anchovy

178   mature in approximately one to two years (*32*, *34*) and thus the 2015 class likely began spawning

179   in mid-2016 (*20*), leading to high anchovy spawning stock biomass and larval abundances by
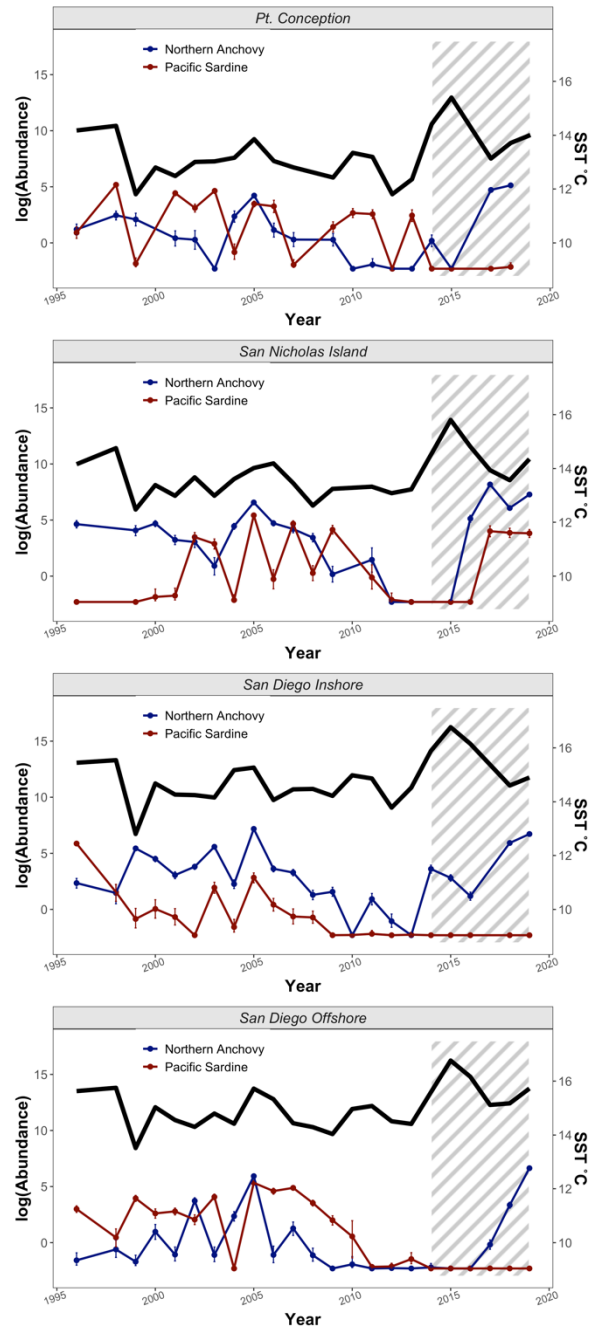
180   2016 and lasting into 2019 and 2020 (*35*).

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

Posterior estimates for larval fish abundances (counts/10m^2) over time at each of the four sampled stations. Joint modeling of metabarcoding and morphological counts reconstructed increases in Northern Anchovy (*Engraulis mordax*) [blue] during the recent

11

187   Pacific Marine Heatwave and low spawning of Pacific Sardine (*Sardinops* sagax) [red]

188   over the past decade (points are means and error bars are 95% credible intervals; shaded

189   region is during and after the MHW). SST is plotted above the Northern Anchovy and

190   Pacific Sardine abundances, for reference.

191

192   **Discussion**

193   *Displacement of Target Fish Species and Tropicalization of Fish Assemblages Associated with*

194   *the Marine Heatwave*

195   Recent studies demonstrate the tropicalization of terrestrial and marine ecosystems in

196   response to climate change (*40*, *41*). These shifts can induce novel species interactions,

197   catalyzing changes in ecosystem function (*2*, *25*). For example, we observe the combination of

198   high abundances of both Northern Anchovy and southern, mesopelagics (*5*, *38*) – a pattern

199   otherwise undocumented in the previous >70-year CalCOFI dataset (*16*, *20*). Furthermore, our

200   results suggest multiple coastal pelagic fisheries targets may continue to be scarce as

201   environmental conditions that are similar to the 2014-2016 MHW become more common (*5*, *38*,

202   *39*). Although the ecological implications of these novel assemblages are, by definition,

203   unpredictable, our results suggest that if future assemblages resemble those seen in the MHW,

204   increases in Northern Anchovy and southern, mesopelagic fishes are likely to be associated with

205   decreases in Pacific Sardine and North Pacific Hake in the Southern CCLME (*40*, *41*); these

206   shifts have fundamentally changed ecosystems and fisheries relative to the recent past (*20*).

207

208   *Biomass Changes in Forage Fishes*

209   The rise in anchovy and continued low abundances of sardine during the MHW is an

210   ecological surprise. Correlative analyses between basin-scale environmental indices such as the

211   Pacific Decadal Oscillation indicate that, for the latter half of the 20th century, anchovy thrived

212   under cooler conditions and sardine under warmer conditions (*10*). However, our findings and

213   others (*5*, *9*, *38*, *42*) suggest that the mechanisms that govern the population dynamics of these

214   species are not a mere function of temperature, but that more complex factors drive recruitment

215   dynamics of these species (*20*). For example, despite largely synchronous responses of fish

216   assemblages to the marine heatwave, sardine declines were not consistent across the CCLME,

217   with refugia of localized abundance in nearshore waters potentially driven by distinct, favorable

218   conditions (*9*, *20*, *32*).

219   Further improving our mechanistic understanding of drivers of fish assemblage dynamics

220   will better inform ecological predictions in the face of extreme ocean events such as MHWs

221   which are likely to increase in frequency and duration under climate change (*43*). As we

222   demonstrate, a combination of metabarcoding and visual surveys can characterize species across

223   trophic levels (*44*) and this has the potential to reveal ecological mechanisms. Here, we used

224   metabarcoding to accurately characterize the composition of larval fishes in CalCOFI plankton

225   samples. Future efforts could focus on documenting the phytoplankton and zooplankton

226   assemblages that comprise both larval prey and predators. Several major hypotheses seeking to

227   explain recruitment variability are underpinned by the capacity of young larvae to consume

228   appropriate prey that facilitates faster growth (*45*). Unfortunately, accurately characterizing the

229   larval prey field has traditionally been difficult as prey are generally too small to be accurately

230   sampled by nets (*46*). Metabarcoding of water samples from the same locations where larvae are

231   collected, however, can characterize the larval prey field at an unprecedentedly high level of

232   detail. In addition, metabarcoding of the stomachs of larval fishes can then identify actual prey

233   items that were consumed by larvae. Evaluating the larval prey field and gut contents through

234   metabarcoding will help us to finally understand the drivers of recruitment volatility in coastal

235   pelagic and other fishes (*5, 32, 47–51*).

236   The described unexpected rise in anchovy during the 2014-16 MHW resonated

237   throughout the CCLME (*39*). For example, California sea lion pups grew at anomalously high

238   rates after their mothers consumed copious anchovy forage and produced ample milk (*40*). High

239   rates of almost exclusively anchovy consumption also seemingly induced thiamine deficiency in

240   adult salmon resulting in poor condition of recruits (*52*). Birds capable of feeding on anchovy

241   thrived (*38*) while those unable to consume anchovy perished (*41*). Given that conditions

242   comparable to the 2014–2016 MHW are predicted to be more common in the CCLME in the

243   future (*1*), our results suggest that continued biological responses to both anchovy-dominated

244   forage-fish assemblages and MHW-associated ocean warming conditions are likely to be without

245   modern analog (*20*).

246   *Novel Insights from Legacy Collections*

247   Molecular tools differentiated variants and species that were not morphologically identifiable in

248   the ichthyoplankton (*21*). For example, metabarcoding identified unique cold-associated variants

249   of the Northern Lampfish (*Stennobrachius leucopsarus*) that are morphologically

250   indistinguishable and combined as a complex are only weakly associated with cooler

251   temperatures. By illuminating such unseen variation, molecular methods reveal ecological

252   dynamics otherwise hidden by shared larval morphology.

253   Ultimately, our approach to studying historical fluctuations in ichthyoplankton

254   assemblages reveals climate-associated biological changes in the CCLME, and suggests ways in

255 which these changes could alter the function and socio-economic benefits derived from marine

256 ecosystems. Importantly, this novel approach provides quantitative estimates by non-

257 destructively sampling legacy collections via metabarcoding, and at the same time provides a

258 mechanistic framework for determining absolute abundance estimates from compositional

259 amplicon sequencing data (*53–56*). Here we relied on morphological counts to ground

260 compositional metabarcoding data, but our framework suggests that any estimate of abundance

261 (e.g. qPCR) or amplification efficiency (e.g. derived from mock communities) can achieve

262 similar results (See Supplement 2) (*55, 56*). Unlocking such quantitative metabarcoding

263 approaches expands the potential for linking ecological assemblages to environmental processes

264 beyond just presence-absence analyses (*57–59*). Such quantitative approaches may prove critical

265 in modeling and predicting future ecosystem change, although directly linking assemblage

266 dynamic responses to climate-driven forces remains inherently challenging. While the CalCOFI

267 samples are specific to ichthyoplankton from the CCLME, bulk collection of community

268 samples is commonly used to survey plankton, insects, pollen, gut contents, and microbiomes,

269 among many other targets (*60*). As such, here we provide broadly applicable methodology with

270 which to efficiently understand modern and historical changes in ecological communities.

271

272 **Materials and Methods**

273 *Study Design*

274 To investigate decadal changes in the ichthyoplankton assemblages in the California Current

275 vicinity, we identified ichthyoplankton from four distinct stations during spring months.

276 Archived spring ichthyoplankton samples were collected across four biogeographically

277 dissimilar stations with variable water properties (*61*) over 2 decades (1996,1998-2019; Figure

278   S1; See Supplement 1 methods)(*5, 38*). Decades of research within the study region (*16, 17, 20,*

279   *62*) indicate the majority of species spawn in spring and closely track adult biomass (*63*). Hence,

280   we expect the spring ichthyoplankton to reflect underlying changes in the local fish assemblages.

281   At each station, oblique bongo net tows were conducted from 210 m depth to the surface

282   using standard CalCOFI methods (*15, 64–66*). Cod end contents of both bongo nets were

283   preserved at sea. The starboard side was preserved in sodium borate-buffered 2% formaldehyde

284   and the port side was preserved in Tris-buffered 95% ethanol. Microscopy was conducted to

285   identify species abundance from formaldehyde-preserved samples following standardized

286   CalCOFI techniques (*61*). DNA metabarcoding was conducted on the ethanol in which port side

287   samples were preserved using the MiFish Universal Teleost (*67*) PCR primer set targeting the

288   *12S* rRNA mitochondrial gene region. See Supplement 1 methods for full description.

289   *Estimating Abundance*

290   We estimated the abundance of ichthyoplankton in each jar using a novel joint Bayesian

291   hierarchical model described in detail in Supplement 2. In brief, we estimate that the number of

292   sequenced amplicons, for any species *i,* is proportional to the species-specific fraction of DNA in

293   the PCR template (*53, 55, 56*). The amplicons produced during a PCR reaction are dictated by

294   the amplicon efficiency parameter a_i, which is characteristic of the interaction between the

295   particular primer set and each species being amplified. Thus, for any species *i*, the number of

296   amplicons should be directly related to the efficiency of amplification and the starting

297   concentration of DNA template such that

298 $$A_i = c_i(a_i + 1)^{N_{PCR}} \tag{1}$$

299   where A_i is amplicon abundance, c_i is the true number of DNA copies in the reaction attributable

300   to species i, a_i is the species-specific amplification efficiency (bounded on (0,1)), and N_{PCR} is

301   the number of PCR cycles used in the reaction (*68*).

302   However, not all molecules of DNA are transferred through each molecular step (*69, 70*),

303   changing what in eq. 1 appears to be a single-species process – each species being amplified

304   independently – into a multi-species process; the number of amplicons observed for species i will

305   depend upon both the amplicons produced for species $i = 1$ and the amplicons produced for

306   species $i = 2, 3, ..., I$ in the same reaction. Observations of amplicons are thus compositional

307   data, meaning they are the proportions of the sample amplicon reads and convey relative

308   quantitative information of the observed species and therefore need to be analyzed as such (*54*).

309   To retain the data-generating structure from eq.1 as much as possible, we develop a

310   model for a single sample with many species. As above, if we let I index species with $I = 1, 2, ...,$

311   I, then we can write a deterministic equation for the number of amplicons observed in log-space

312   as

$$\log (A_i) = \log(c_i) + N_{PCR} \log(a_i + 1) + \log(\eta) \qquad (2)$$

314   where the only new term is η, representing the proportion of reads observed from a given

315   sampling run (See supplement 2 for details). Note that in this formulation η is a single value

316   shared across all species and serves to scale the number of amplicons observed. Additionally, we

317   can rewrite the number of DNA copies in terms of proportional number of counts, $\log(\beta_i) =$

318     $\log(c_i) - \log(\sum_i c_i)$. Note that the second term in this equation is a sum of the counts across all

319   species, and so is a single shared value for all species. As such it can be integrated into the value

320   η that scales the overall abundance for each species i,

$$\log (A_i) = \log(\beta_i) + N_{PCR} \log(a_i + 1) + \log(\eta) \qquad (3)$$

322   This equation is appealing because it provides a process-oriented description of the

323   biology of interest (the βs), a term for how PCR modifies our amplicon sequence count

324   observation of the true abundance ($N_{PCR} \log(a_i + 1)$), and a term for how subsampling of DNA

325   reads will adjust the number of amplicons observed ($\log(\eta)$).

326   However, in the absence of additional information, it is not possible to estimate the β and

327   *a* parameters from metabarcoding data alone. Including morphological count data enables us to

328   estimate the confounded parameters from metabarcoding data. For each sampled station, we have

329   two independent sets of observed data: 1) counts of larval/juvenile fishes for each taxon from the

330   formaldehyde jars and 2) counts of amplicons for each taxon from ethanol jars. These observed

331   data arise from a common (but unobserved) biomass for each species at each station-year

332   combination, and thus we can model them jointly (*71*).

333   Microscopy counts were modeled as Poisson-distributed given their relatively small

334   absolute values and low variance (*64*), and amplicon sequence data were modeled using a

335   Negative Binomial distribution given their relatively high absolute values and high variability

336   among replicates (Figure S2-S4). These statistical distributions are commonly used in models of

337   amplicon and count data, respectively (*35–37*). A full description of model implementation and

338   assumptions are provided in Supplement 2.

339   *Data Analysis*

340   After model estimation, we calculated mean abundance estimates (larvae counts per

341   standardized volume towed) per species per station per year. To explore species-specific sea

342   surface temperature (SST) relationships, we fit a Bayesian generalized linear model using log

343   (abundance) as the response variable and SST (˚C) as a continuous predictor variable. Models

344   were implemented for each species using Stan as implemented in R (*75, 76*). We then

345　summarized the affinity between each species and SST by calculating a T-statistic based on each

346　species' estimated coefficients (mean slope/standard deviation). We further plotted the estimated

347　slope for each "species grouping" by habitat associations derived from previous CalCOFI

348　research (See Supplement 1 methods)(*63*). We summed total log (abundance) per habitat

349　association per station per year and fit a Bayesian generalized linear model using log (total

350　abundance) as the response variable and SST (˚C) as a continuous predictor variable.

351　　　We repeated the above analyses using a Bayesian binomial model using presence as the

352　response variable and SST (˚C) as a continuous predictor variable across the data set to explore

353　occurrence relationships with temperature and identify warm- and cool- associated taxa. We set a

354　threshold of presence/absence based on the model using a threshold of < 0.01 larvae per

355　standardized volume to be considered absent within a station.

356　　　We further explored species occurrence and abundance relationships with SST by fitting

357　the above Bayesian generalized linear models with station as a random effect (See Supplement 1

358　results).

359　　　We visualized anchovy and sardine abundance over time by calculating the mean log

360　(abundance) of each species per station per year. We then plotted the mean log (abundance) of

361　each of the four stations while error bars represent the 95% confidence intervals observed for a

362　given species at a given station in that year.

363　　　To evaluate the effect of the marine heatwave (MHW) on CCLME fishes we compared

364　estimated species abundances before the MHW (1996-2013), to both during and after the MHW

365　(2014-2019), at each station respectively. We first calculated the mean abundance for each

366　species at each station for each model run. We then subtracted the means for each model run to

367　evaluate changes in MHW abundance per species per station per model run. We then calculated a

368   95% CI of change in MHW abundance per species to identify which species were significantly

369   different before vs. during and after the MHW at each station.

370   All data and code to conduct analyses and generate all figures are available on GitHub

371   (https://github.com/zjgold/CalCOFI_eDNA) and associated Google Drive link

372   (https://drive.google.com/drive/folders/12cU9mY_CWoro-

373   x6Hgh_pgv_66zZEzm1h?usp=sharing) [will be replaced with a Dryad repository upon

374   acceptance].

375

381   *Author Contributions*

382   • Conceptualization: ZG, DK, KDG, ART, LRT, PHB

383   • Performed Research: ZG, RPK, AOS, RG, DK, ART, PHB, KMP

384   • Funding Acquisition: ZG, PHB, ART, KDG, DK

385   • Data Curation: ZG, RPK, AOS, RG

386   • Formal Analysis: ZG, RPK, AOS, RG, ART

387   • Writing – Original Draft Preparation: ZG, RPK, AOS, ART, KDG, RG, KMP, LRT, DK,

388   PHB

389   *Competing Interests*

390   The authors declare no competing interests.

Data and Materials Availability

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. 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].

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