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Large interannual variation in spawning in San Diego marine protected areas captured by molecular identification of fish eggs

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ABSTRACT: Long-term monitoring of marine ecosystems is critical to assessing how global processes such as natural environmental variation and climate change affect marine populations. Ichthyoplankton surveys provide one approach to such monitoring. We conducted weekly fish egg collections off the Scripps Institution of Oceanography (SIO) Pier (La Jolla, CA, USA) for 3 yr (2014 to 2017) and added a second sampling site near the La Jolla kelp forest for 1 yr (2017). Fish eggs were identified using DNA barcoding and data were compared to previous work from SIO Pier surveys from 2012 to 2014. We documented large interannual variability in fish egg abundance associated with climatic fluctuations, including an El Niño event captured during our sampling years. Overall egg abundance was reduced by >50% during periods of anomalously warm water in 2014 to 2016. Fish egg abundance rebounded in 2017 and was accompanied by a phenological shift of peak spawning activity. We found that interannual fish egg abundance may be linked with upwelling regimes and winter temperatures. Across the period of joint sampling, we found no distinct differences in community composition between the SIO Pier (soft bottom) and kelp forest habitat we sampled (2 km distant). Long-term monitoring of fish spawning can contribute to our understanding of how natural environmental variation, such as El Niño events, affects fish reproductive activity. This understanding may extend to trends in marine resource availability associated with climate and aid in evaluating the efficacy of existing management efforts.

KEY WORDS: Fish spawning · DNA barcoding · Ichthyoplankton · Long-term monitoring

INTRODUCTION

Management of marine resources can be informed by both fisheries-dependent and fisheries-independent data. Fisheries-independent data that includes species not directly targeted by fisheries can be useful to management efforts by providing a broader view of ecosystem status and may increase the ability to detect ecosystem changes that are not immediately affected by fisheries activity (Anderson et al. 2008). Ichthyoplankton surveys have long played a key role in providing fisheries-independent data for ecosystem monitoring and fisheries management. Fish egg and larval surveys can be a useful tool in assessing fish faunal diversity and the spatial and temporal distribution of spawning activity (Ahlstrom 1968, Ahern et al. 2018). By providing data on early life stages, egg and larval surveys are important complements to traditional diver surveys and trawls that are limited to adult and juvenile fish (Ahlstrom 1968, Harada et al. 2015). For example, ichthyoplankton surveys have been used to document the spawning grounds of many commercially important fish species such as the northern anchovy in the Gulf of California, and cod and plaice in the North Sea (Green-Ruiz & Hinojosa-Corona 1997, Fox et al. 2000).

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However, because the eggs of many fish species are morphologically indistinguishable, it has been difficult, until recently, to accurately determine which species were spawning, with the exception of a few morphologically distinct species (Ahlstrom 1968). New molecular approaches based on DNA barcoding have made possible the accurate identification of fish eggs and larvae (e.g. Hyde et al. 2005, Gleason & Burton 2012, Harada et al. 2015). DNA barcoding uses species-specific differences in DNA sequences to identify individual eggs by matching their sequences to a database of sequences obtained from identified adult specimens. If the available database is complete, PCR amplification and sequencing permits identification of each egg in a collection, including cryptic taxa that may go unobserved in other types of habitat monitoring.

This study documents spawning activity of fish populations in the marine protected areas (MPAs) adjacent to the Scripps Institution of Oceanography (SIO, La Jolla, CA, USA), which include the San Diego-Scripps Coastal State Marine Conservation Area (SMCA) and Matlahuayl State Marine Reserve (SMR). The San Diego-Scripps Coastal SMCA prohibits the take of marine resources except for coastal pelagic species by hook and line and Matlahuayl SMR prohibits the take of all marine resources. Fishes present in the study area are well documented in the literature and physically in the Scripps Marine Vertebrates collection (Craig et al. 2004, Hastings et al. 2014); however, there is less information about species-specific spawning patterns and how they might change with annual environmental variation. Finding and identifying fish eggs in the plankton demonstrates recent local spawning activity since most fish eggs in the southern California Current Ecosystem hatch in 2 to 4 d (Zwiefel & Lasker 1976). We built upon the study of Harada et al. (2015), using DNA barcoding to identify fish eggs in the plankton off the SIO Pier. Through continued monitoring, we aim to document any changes in spawning activity that might be associated with changes in oceanographic conditions, such as ocean temperature increases associated with the 'Warm Blob' event in 2014 and the 2015–2016 El Niño event (Bond et al. 2015, Jacox et al. 2016). We documented large interannual variation in spawning activity across sampling years 2012 to 2017. Beginning in 2017, we further expanded our survey area to include sampling from the nearby kelp forest habitat adjacent to the Matlahuayl SMR. By sampling both kelp forest and sandy beach (SIO Pier) habitat with weekly collections over a 7 mo period, we can begin to assess if there are habitat-specific patterns of spawning across the species found in the La Jolla MPAs.

MATERIALS AND METHODS

Sampling locations and techniques

Sampling sites were located in or immediately outside 2 of La Jolla's MPAs, the San Diego-Scripps Coastal SMCA and the Matlahuayl SMR (coordinates in Table S1 in the Supplement at www.int-res.com/articles/suppl/m604p199_supp.pdf). The San Diego-Scripps Coastal SMCA is dominated by soft bottom sandy habitat while the Matlahuayl SMR contains soft bottom, rocky bottom, and kelp forest habitat. Surface transport models of this area were constructed in Harada et al. (2015) and demonstrated that eggs had a high probability of being spawned almost completely within these MPA boundaries.

Weekly plankton samples were collected from the end of SIO Pier (32° 52′ 2″ N, 117° 15′ 26″ W) from August 2014 to August 2017, continuing previous work started in August 2012 (Harada et al. 2015). As a shore station in the Southern California Ocean Observing System, a variety of environmental data from the Scripps Pier are publically available (see http://www.sccoos.org). Samples were collected by lowering a 505 µm mesh 1 m diameter plankton net until the net reached the seafloor around midday each sampling day. This was repeated 3 more times for a total of 4 pulls, sampling approximately 16 m^3 of water (based on average water depth of about 5 m). In addition, weekly plankton samples were collected from kelp forest habitat adjacent to the Matlahuayl reserve (32° 51′ 15″ N, 117° 16′ 52″ W) from February 2017 to August 2017. This site is located approximately 2 km from the SIO Pier. Samples were collected by pulling a 333 µm mesh 0.5 m diameter plankton net behind a small boat at 0.5 knots for 5 min. The net was weighted for a sampling depth of about 1 m, sampling approximately 60 m^3 of water. Although we used different mesh sizes at different sampling sites, both mesh sizes (505 and 303 µm) were smaller than the fish eggs we sampled, which ranged from 0.7 to 1.2 mm (A. E. Harada unpubl. data). Moreover, fish eggs that were found only in the kelp samples using the smaller mesh size were much larger than even our larger mesh size (ranging from 0.6 to 2.1 mm) (Moser et al. 1983). The collected plankton samples from both sites were manually sorted using a dissecting microscope, and fish eggs were individually

counted and removed. Northern anchovy *Engraulis mordax* and Pacific sardine eggs *Sardinops sagax*, both morphologically distinct, were counted and removed from the sample. The remaining eggs were stored in 95% ethanol at 4°C at least 12 h prior to further processing. If a collection contained over 500 fish eggs, a subset of approximately 400 eggs was selected for DNA barcoding for species identification. Samples were processed within 2 wk of collection.

Processing eggs, PCR, and sequencing

After storage in ethanol, individual fish eggs were rinsed with deionized water and placed in 15 µl of buffer (2/3 Qiagen AE buffer, 1/3 water). Eggs were then physically crushed with a clean pipette tip to release the DNA. No further DNA extraction or purification was needed. Samples were stored at −20°C prior to PCR. To amplify DNA, universal fish cytochrome *c* oxidase subunit I (COI) primers were used (Ward et al. 2005): COI VF1 forward primer (5′-TTC TCA ACC AAC CAC AAA GAC ATT GG-3′) and COI VR1 reverse primer (5′-TAG ACT TCT GGG TGG CCA AAG AAT CA-3′). These primers produced an amplicon of 710 bp. PCR was performed using 25 µl reaction volume, with 12.5 µl of GoTaq Green Master Mix (Promega), 5 pmol of each primer, and 1 µl of DNA extract. Thermal cycling was initiated at 95°C for 2 min followed by 35 cycles of 95°C for 30 s, 50°C for 45 s, and 72°C for 1 min, followed by 72°C for 5 min. After PCR, samples were run on a 1.5% agarose gel and visualized with GelRed (Biotium) or SybrSafe (Invitrogen) to detect presence of amplified DNA. About 64% of samples were successfully amplified using COI primers. Samples that failed to amplify with COI were amplified using the mitochondrial 16S ribosomal rRNA gene, using forward primer 16Sar (5′-CGC CTG TTA TCA AAA ACA T-3′) and reverse primer 16Sbr (5′-CCG GTC TGA ACT CAG ATC ACG T-3′) for a 570 bp amplicon (Palumbi 1996). Of the samples that failed to amplify with COI primers, about 53% were successfully amplified using 16S primers. Overall, about 14% of the fish eggs could not be amplified with either 16S or COI primer sets. Samples with either the COI or 16S product were purified using Sephadex G-50 fine spin columns (GE Healthcare) and sequenced using Sanger sequencing (commercial sequencing service). Samples were sequenced in one direction using the forward primer of either the COI or 16S primer for their respective amplicons. Sequences were identified using BLAST searches of the NCBI database, which contains COI and 16S rRNA barcodes from over 500 species of California marine fishes, most of which are vouchered in the SIO Marine Vertebrate Collection, allowing for nearly complete coverage of species in California marine waters (Hastings & Burton 2008). The top BLAST hit with 95% sequence similarity or greater was used for species identification. In some cases, there were multiple species that had equal scores and were identified to only to the genus level. For example, there were 2 sanddab species (*Citharichthys sordidus* and *C. xanthostigma*) observed in our collections that have 99% sequence similarity and in many cases were unable to be distinguished based on our sequence data, therefore these species were grouped in our analysis.

Data analysis

The total number of eggs collected each day was recorded along with species identifications from DNA barcoding each egg for each collection for each site. Sea surface temperature (SST) was measured and recorded at 2 m depth approximately every 6 min from the SIO Pier. Data were accessed through the Southern California Coastal Ocean Observing System and used for analysis of fish egg collections with respect to variation in ocean temperature (www.sccoos.org). To test the correlation between annual winter temperatures and annual spring–summer fish egg abundance, winter temperature was averaged for each year (December to February) and regressed against the average number of fish eggs per collection during spring and summer (March to August) for that year.

We estimated upwelling using daily upwelling indices calculated at 33° N, 119° W from all collection years (2012 to 2017) in m^3 s^{-1} by Pacific Fisheries Environmental Laboratories (www.pfeg.noaa.gov). To test the correlation between annual spring upwelling and annual spring–summer fish egg abundance, the cumulative upwelling (sum of daily upwelling indices) over spring (March to May) was regressed against the average number of fish eggs per collection during spring and summer for that year. Additionally, cumulative monthly upwelling was regressed against the average fish eggs per collection for each month.

Non-metric multidimensional scaling was used to visualize community matrix data using Bray-Curtis

dissimilarity matrices, using the 'vegan' package in R. Counts for each species were normalized to the number of fish eggs collected for that sampling day in order to reduce the weight of highly abundant species. Non-metric multidimensional scaling was produced in R to visually compare differences between our 2 sampling sites. Collections from both sites that were made within 24 h were paired and counts for each species were normalized to m^3 of water sampled. We fit linear mixed effects models for species with the largest difference in percentage between sites to test if there were significant differences in abundance at each site or an interaction between site and spawning period (indicated by month). For each year, the date of the highest species richness per collection was recorded. We tested for phenological changes in spawning across years; in order to ensure accuracy, only species for which we found 50 or more eggs were included in this analysis.

RESULTS

Abundance of fish eggs

We observed fish spawning patterns in 2 different habitats over time and compared these data to spawning data previously obtained from the SIO Pier (Harada et al. 2015). We found extensive interannual variability in fish spawning. During the years 2015 and 2016, we observed a 53 % decline in the average number of fish eggs per collection during the summer (June through August) compared to data from the previous 2 yr (Fig. 1). Although we did observe a seasonal increase in the number of fish eggs in the summer (compared to winter) across all years (consistent with Harada et al. 2015), there were fewer fish eggs per collection from 2015 and 2016 than from 2013 and 2014. This pattern was not due to any particular species or dominant group of species. Species-specific abundance through time for the 5 most com-

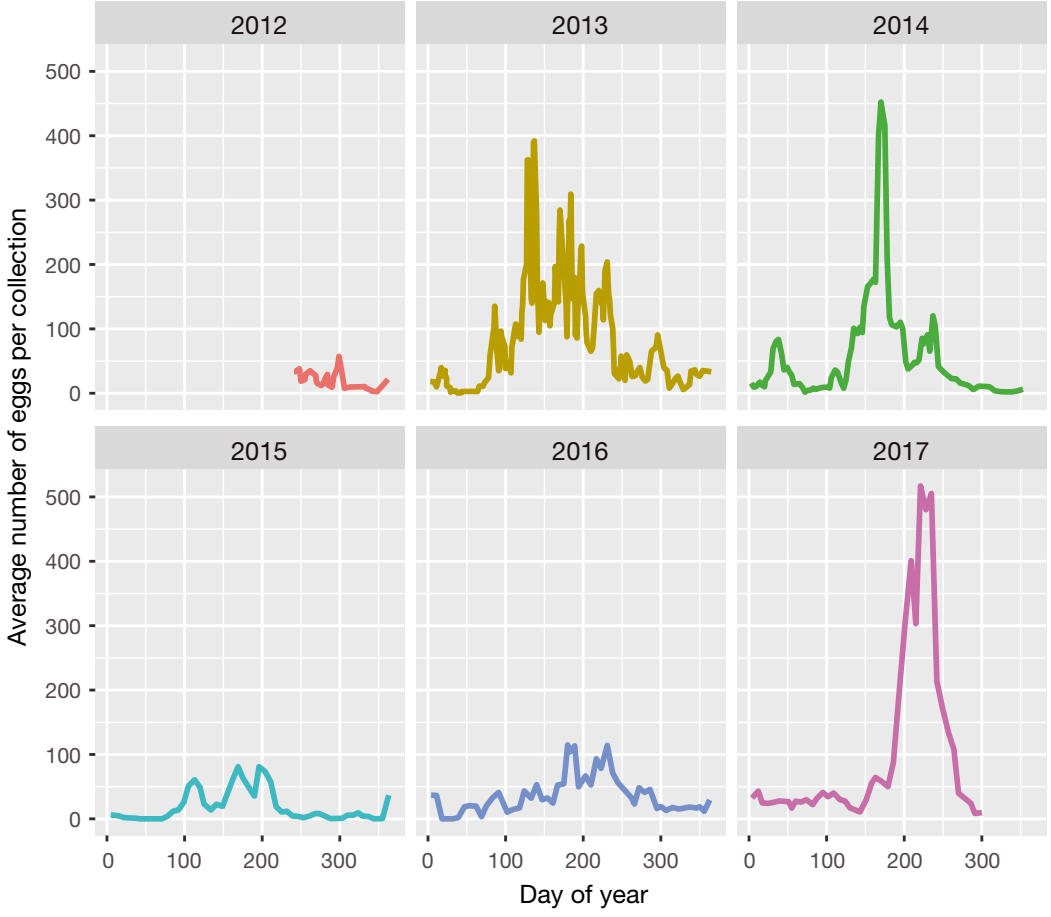

Fig. 1. Average number of fish eggs collected in each weekly collection at the San Diego Scripps Coastal Reserve from 2012 to 2017. Average depicted is a moving average of eggs collected over a 3 wk period overlapping by 1 wk. The years 2012 through 2014 were taken with permission from Harada et al. (2015)

mon species in our samples and one sport fish, the California Corbina *Menticirrhus undulatus*, are shown in Fig. 2. Although represented by relatively few eggs in our collections, the sharp peaks in spawning in *M. undulatus* showed very little annual variation, while *Engraulis mordax* eggs largely disappeared in 2015 and 2016. *Citharichthys stigmaeus*, while notably reduced in 2015, showed the broadest spawning season among all species and remained a dominant component of the ichthyoplankton throughout the sampling period.

Interestingly, during the summer of 2017 we observed a recovery of fish eggs numbers similar to numbers observed in 2013 and 2014. However, peak spawning season during 2017 appears to have shifted to later in the year, from May/June in 2013 to 2016 to July/August in 2017 (Fig. 1). To assess how spawning seasonality compared across years, we recorded the number of species found per collection (see Fig. S1 in the Supplement). The highest number of species recorded per sample occurred approximately 1 mo later in 2017 compared with earlier years in which spawning was recorded (2013 and 2014; Table S2); this parallels the overall phenological change in peak egg abundance. For 4 species, Pacific sardine *Sardinops sagax*, queenfish *Seriphus politus*, Pacific chub mackerel *Scomber japonicus*, and jack mackerel *Trachurus symmetricus*, we found that seasonal spawning started approximately 1 mo later in 2017 than in previous years.

Community composition

Overall, in collections from the SIO Pier from September 2014 to August 2017, we collected 6939 fish eggs; of those, 4150 eggs were identified as 37 different fish species. During the collection period of the kelp forest from February 2017 to August 2017, we collected 11 163 fish eggs and identified 5546 as 35 species. There were 7 species found in our previous study (Harada et al. 2015) that we did not find in the current study: ocean white fish *Caulolatilus princeps*, California lizardfish *Synodus lucioceps*, California opaleye *Girella nigricans*, Pacific pompano *Peprilus simillimus*, mussel blenny *Hypsoblennius jenkinsi*, giant sea bass *Stereolepis gigas*, and Pacific barracuda *Sphyraena argentea.* We documented 5 species that had not previously been recorded to spawn in our study area by Harada et al. (2015): the yellowtail

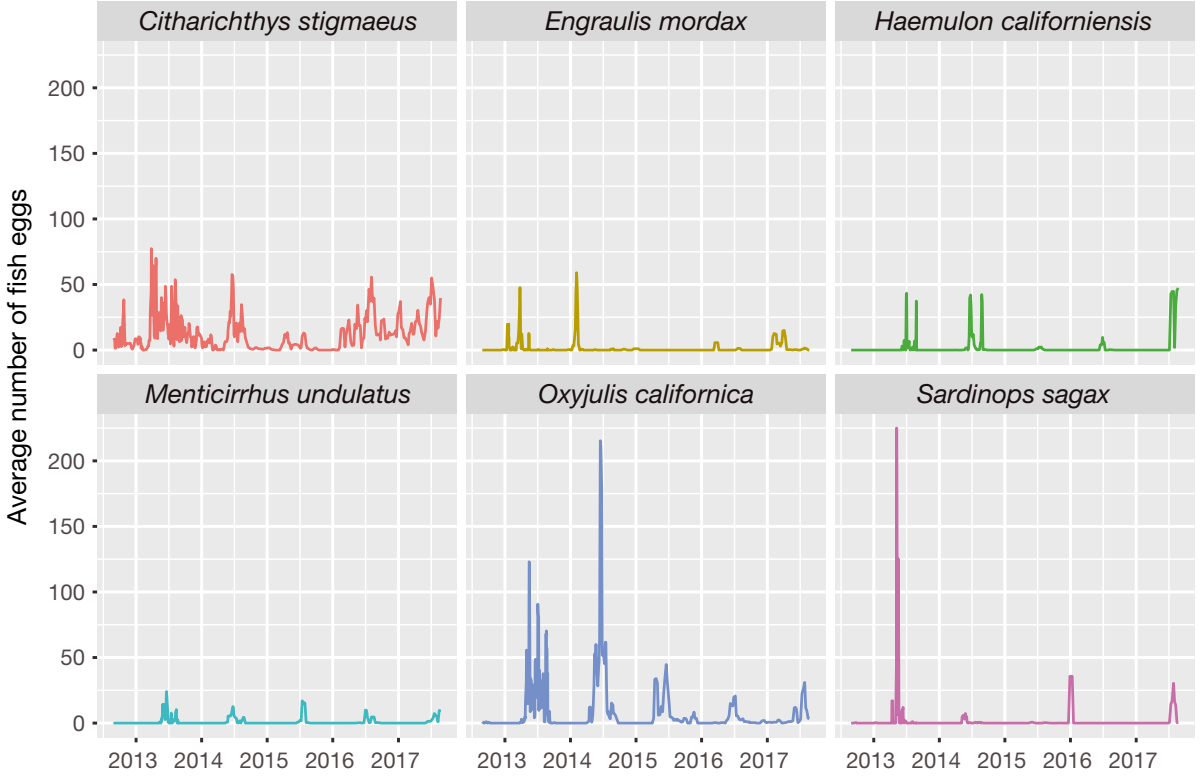

Fig. 2. Average number of fish eggs of 6 species collected from 2012 to 2017. The 5 most common species are shown with one additional species, *Menticirrhus undulatus.* Average is a moving average of eggs collected over a 3 wk period overlapping by 1 wk

jack *Seriola lalandi*, flat-head grey mullet *Mugil cephalus*, blackbelly eelpout *Lycodes pacificus*, basketweave cusk-eel *Ophidion scrippsae*, and California scorpion fish *Scorpaena guttata*. However, all of these newly documented species contributed <0.2% of all fish eggs that were identified; these rare species in egg collections contributed little to overall community composition. Multivariate analysis of community composition showed that there were no distinct changes in community composition over time, but there were distinct differences in the seasonal spawning community between fall–winter and spring–summer months (Fig. 3). Note that warm years did not cluster together; rather all years overlapped in multidimensional space.

We sampled the kelp forest habitat from February 2017 to August 2017 and were able to compare community composition between kelp and Pier habitats (Table 1). Nine species in the kelp forest plankton samples were not found at the SIO Pier in that time period: hornyhead turbot *Pleuronichthys verticalis*, red-eye round herring *Etrumeus acuminatus*, opaleye *G. nigricans*, diamond turbot *Hypsosetta guttulata*, C-O sole *Pleuronichthys coenosus*, white seabass *Atractoscion nobilis*, bigmouth flounder *Hippoglossina stomata*, Pacific barracuda *S. argentea*, and giant sea bass *S. gigas*. With the exception of 2 species (*H. stomata* and *E. acuminatus*), all other species had been observed in previous collections from the SIO Pier. Five species were found in SIO Pier samples but were absent from kelp forest plankton samples: zebra-perch sea chub *Hermosilla azurea*, spotted sand bass *Paralabrax maculatofasciatus*, Pacific pompano *P. simillimus*, spotted cusk-eel *Chilara taylori* and the California lizardfish *S. lucioceps*. Some of the presence/absences likely reflect the limited sampling period for the kelp site. For example, our Pier data show that most of our Pacific pompano eggs were collected between November to February, with a smaller number in the spring, and the November to February period was not covered in our kelp collections. Despite the discrepancies in species presence or absence between sites, these differences accounted for 0.2% or fewer of the total eggs sampled and therefore did not appear to contribute substantially to overall differences in community composition. A global analysis of community composition between sites using non-metric multidimensional scaling did not find evidence for distinct differences in community composition between sites (Fig. 4). If communities differed between sites we would expect to see greater clustering of samples between sites; however, we see extensive overlap (Fig. 4). Moreover, non-metric multidimensional scaling plots between sites separated by month show extensive overlap between months, indicating there were similar spawning communities at both sites in a given month (Fig. S2). Species with the largest difference in percentage between our sampling sites were the northern anchovy *E. mordax*, speckled sanddab *C. stigmaeus*, Pacific sardine *S. sagax*, and California salema *Haemulon californiensis* (Table 1). However, linear mixed effects models fit for each of these species found non-significant differences between the numbers of eggs m^{-3} between sites (Fig. S3). Furthermore, these models found no evidence for temporal habitat differences, as there were non-significant interactions between site and month for each of these species. Though we found no differences in community composition between sites

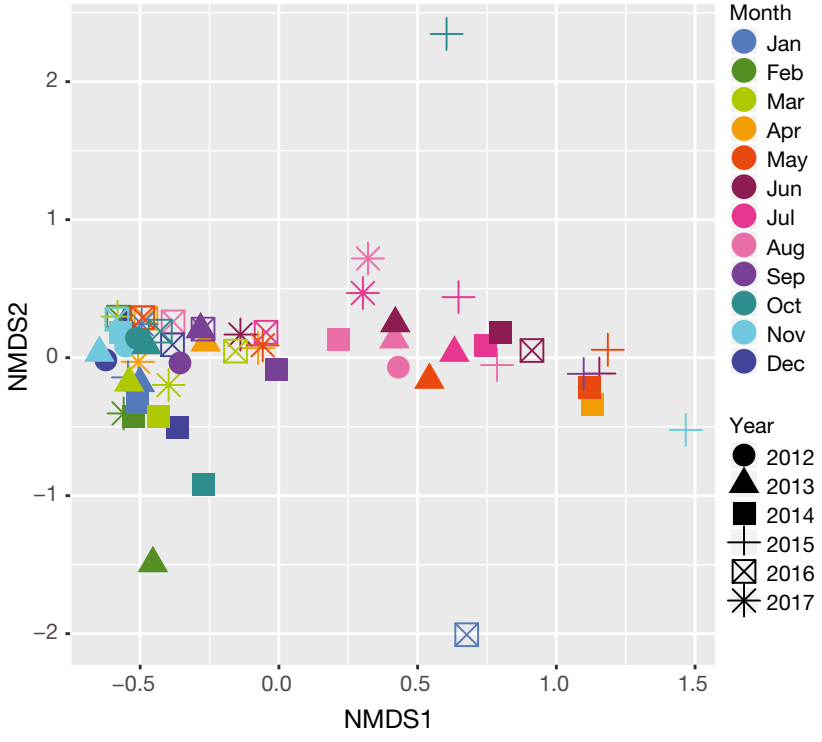

Fig. 3. Non-metric multidimensional scaling plot of the number of fish eggs for each species per month normalized to number of eggs identified for each month from 2012 to 2017 based on Bray-Curtis dissimilarity. Stress value of 0.10 indicates the plot gives an adequate representation of the data

Table 1. Species collected from weekly samples at 2 sites: Matlahuayl State Marine Reserve (kelp site; n = 25 collections) and San Diego Scripps Coastal State Marine Conservation Area (Pier site; n = 30 collections) between February and August 2017. Both number of eggs collected from individual species and percent of total eggs collected is shown for each site, listed in order of percent difference between sites. NA: species not found. Note: *Haemulon californiensis* was previously *Xenistius californiensis*, and *Halichoeres californica* was previously *Halichoeres semicinctus*

Species	No. of eggs identified		% of total		No. of collections		% Diff. between sites
	Kelp site	Pier site	Kelp site	Pier site	Kelp site	Pier site	
Engraulis mordax	1946	106	35.09	5.38	13	12	29.71
Citharichthys stigmaeus	582	619	10.49	31.39	23	28	20.90
Sardinops sagax	891	97	16.07	4.92	15	6	11.15
Haemulon californiensis	307	271	5.54	13.74	7	4	8.21
Citharichthys xanthostigma/sordidus	47	116	0.85	5.88	16	8	5.03
Roncador stearnsii	24	80	0.43	4.06	6	10	3.62
Seriphus politus	14	62	0.25	3.14	7	10	2.89
Oxyjulis californica	599	162	10.80	8.22	20	18	2.59
Menticirrhus undulatus	87	57	1.57	2.89	6	8	1.32
Halichoeres californica	652	254	11.76	12.88	12	11	1.12
Genyonemus lineatus	2	22	0.04	1.12	3	5	1.08
Scomber japonicus	107	19	1.93	0.96	9	7	0.97
Paralichthys californicus	98	17	1.77	0.86	15	11	0.90
Umbrina roncador	16	21	0.29	1.06	4	3	0.78
Paralabrax clathratus	35	4	0.63	0.20	10	4	0.43
Ophidion scrippsae	19	1	0.34	0.05	3	1	0.29
Xystreurys liolepis	1	6	0.02	0.30	2	2	0.29
Symphurus atricaudus	22	3	0.40	0.15	6	2	0.24
Anisotremus davidsonii	27	6	0.49	0.30	7	2	0.18
Paralabrax nebulifer	5	5	0.09	0.25	5	4	0.16
Semicossyphus pulcher	15	8	0.27	0.41	6	5	0.14
Cynoscion parvipinnis	11	2	0.20	0.10	3	1	0.10
Seriola lalandi	11	2	0.20	0.10	4	1	0.10
Mugil cephalus	1	1	0.02	0.05	2	1	0.03
Trachurus symmetricus	7	2	0.13	0.10	4	2	0.02
Cheilotrema saturnum	2	1	0.04	0.05	3	1	0.01
Hermosilla azurea	0	17	NA	0.86	0	3	NA
Paralabrax maculatofasciatus	0	4	NA	0.20	0	3	NA
Peprilus simillimus	0	3	NA	0.15	0	1	NA
Chilara taylori	0	2	NA	0.10	0	2	NA
Synodus lucioceps	0	2	NA	0.10	0	1	NA
Pleuronichthys verticalis	4	0	0.07	NA	3	0	NA
Etrumeus acuminatus	3	0	0.05	NA	3	0	NA
Girella nigricans	3	0	0.05	NA	2	0	NA
Hypsopsetta guttulata	2	0	0.04	NA	3	0	NA
Pleuronichthys coenosus	2	0	0.04	NA	3	0	NA
Atractoscion nobilis	1	0	0.02	NA	2	0	NA
Hippoglossina stomata	1	0	0.02	NA	2	0	NA
Sphyraena argentea	1	0	0.02	NA	2	0	NA
Stereolepis gigas	1	0	0.02	NA	2	0	NA

generalized through time, it is noteworthy that on a given sampling day there could be large differences in the percentage of species collected at each site. For example, on 5 July 2017, *C. stigmaeus* comprised 9 and 63% of the collection at the kelp and Pier sites respectively, and *Oxyjulis californica* comprised 36 and 6% of collections at the kelp and Pier sites respectively, with 442 and 74 total eggs collected at each site respectively.

Environmental effects on spawning

SST data collected from the SIO Pier show variability in temperatures across sampling years (Fig. 5). During winters 2014–2015 and 2015–2016, we observed warmer temperatures than previous years (Table 2). Additionally, these 2 yr showed the highest annual average temperatures. The data suggest that when winter temperatures were warmest, the

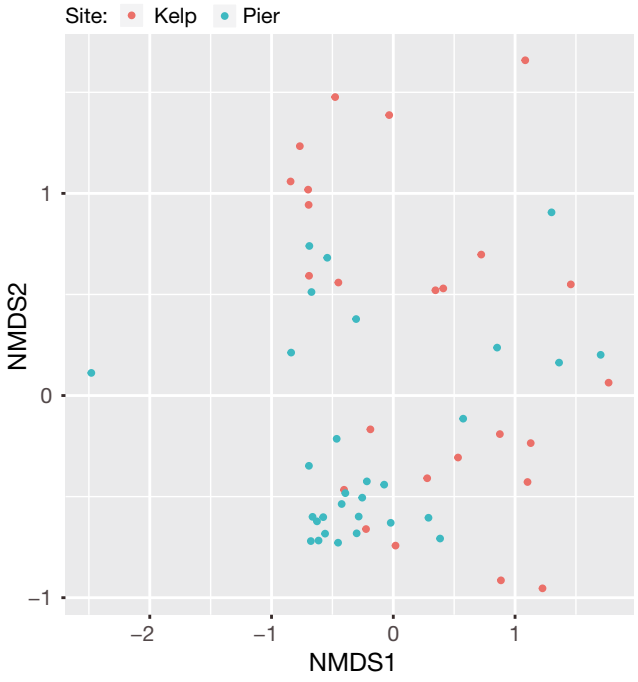

Fig. 4. Non-metric multidimensional scaling plot of number of fish eggs for each species normalized to number of eggs identified per collection between 2 sampling sites from February 2017 to August 2017. Based on Bray-Curtis dissimilarity. Stress value 0.166 indicates plot gives an adequate representation of data

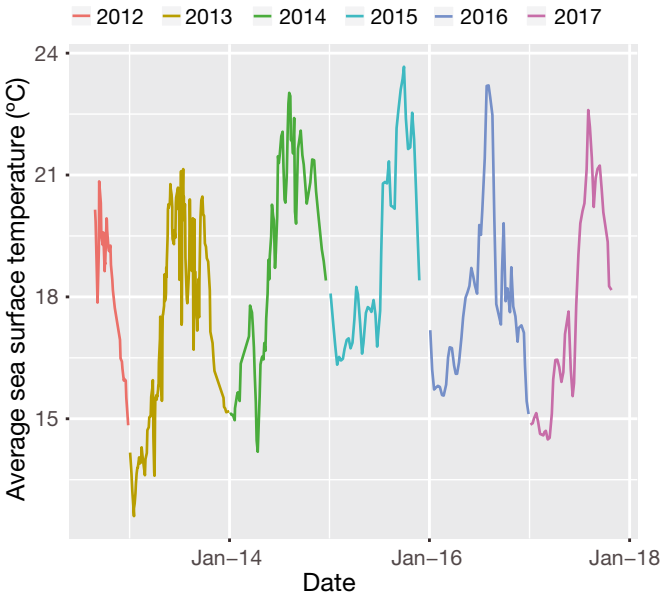

Fig. 5. Average sea surface temperature at the Scripps Institution of Oceanography Pier from 2012 to 2017. Average depicted is a moving average of temperatures recorded on egg collection days over a 3 wk period overlapping by 1 wk

Table 2. Annual and winter (December to February) average (±SE) sea surface temperatures at the Scripps Institution of Oceanography Pier. Data were collected approximately every 6 min at 2 m depth

Year	Annual average (°C)	Winter	Winter average (°C)
2012	17.50 ± 0.01	2012–2013	14.37 ± 0.01
2013	17.25 ± 0.01	2013–2014	15.47 ± 0.00
2014	19.57 ± 0.01	2014–2015	17.10 ± 0.01
2015	19.26 ± 0.01	2015–2016	16.06 ± 0.01
2016	18.25 ± 0.01	2016–2017	15.03 ± 0.00
2017	18.37 ± 0.01		

following spring and summer fish spawning was depressed. In order to examine the relationship between winter temperatures and spring and summer spawning, we plotted average number of spring and summer (March to August) fish eggs collected from the Pier for each year against the average winter temperature (December to February). We found a significant negative correlation between winter temperatures and spring and summer fish egg abundance ($R^2 = 0.83$, $p < 0.05$; Fig. 6). In contrast, there was no relationship between annual average summer temperature (June to August) and the annual spring–summer fish egg abundance ($R^2 = 0.38$, $p > 0.05$; Fig. S4). In general, higher winter temperatures corresponded with lower cumulative spring upwelling for that year, though we found only a marginally significant relationship between the 2 variables ($R^2 = 0.67$, $p = 0.06$).

There was a significant positive relationship between spring (March to May) upwelling measured by the sum of daily upwelling indices (cumulative upwelling index, CUI) and the average spring and summer fish egg abundance for each year ($R^2 = 0.75$, $p < 0.05$; Fig. 7). We also found a significant positive correlation between the CUI for each month and the logarithmic mean fish egg abundance by month grouped across all species and all years ($R^2 = 0.33$, $p < 0.01$; Fig. 8). This result is consistent with our previous study (Harada et al. 2015), which recorded abundant fish eggs when temperatures were highest, coinciding with seasonal spawning, which peaks in the summer.

DISCUSSION

In this study, we collected and identified fish eggs spawned in or near La Jolla's MPAs to examine temporal changes in abundance and community composition of spawning fishes as represented by their eggs collected from the plankton. Although ship-board

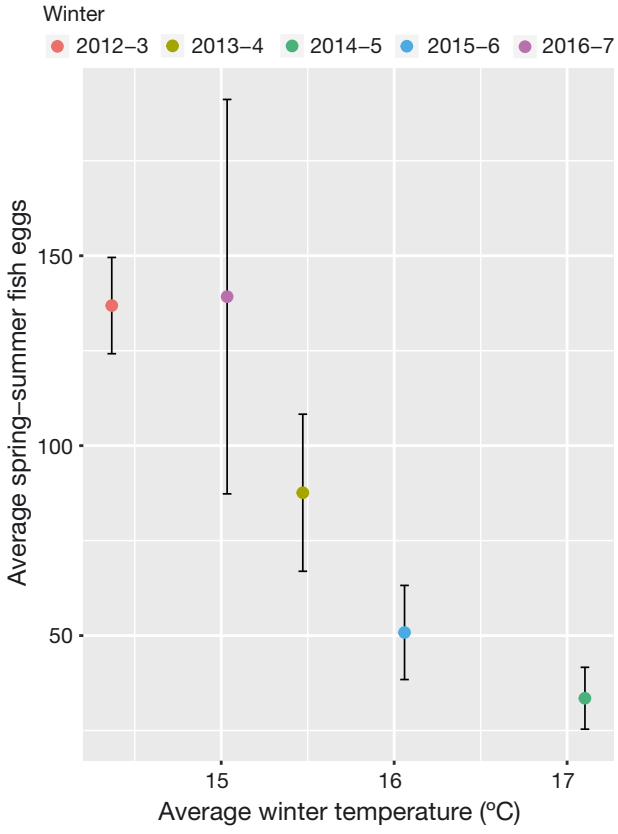

Fig. 6. Spring and summer (March to August) average (±SE) fish egg abundance plotted against previous average winter temperature (December to February) for each year. Fish eggs were collected from the Scripps Institution of Oceanography Pier. There is a significant negative correlation between winter temperatures and spring–summer spawning ($R^2 = 0.83$, $p < 0.05$)

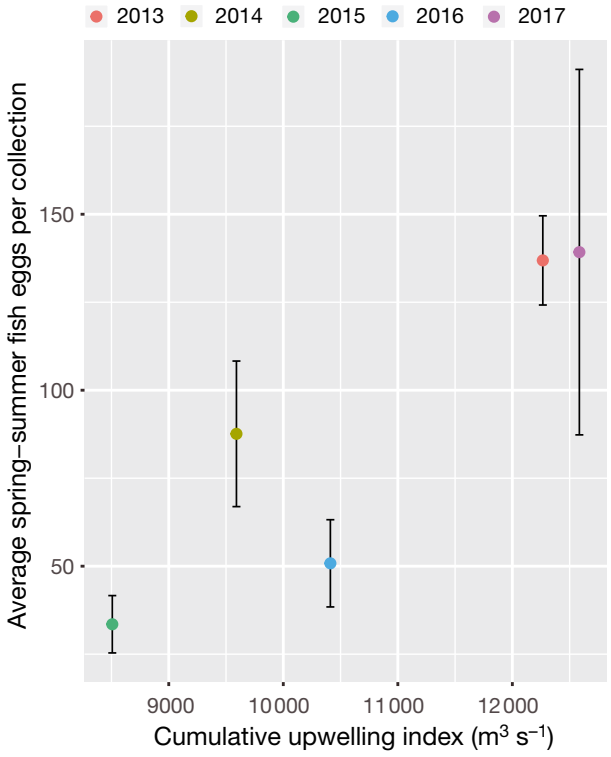

Fig. 7. Average (±SE) spring and summer (March to August) fish egg abundance per collection plotted against spring cumulative upwelling index (sum of daily upwelling indices over spring). Fish eggs were collected from the Scripps Institution of Oceanography Pier. Significant positive relationship between spring upwelling and spring–summer fish egg abundance ($R^2 = 0.75$, $p < 0.05$)

sampling of ichthyoplankton has a long history off the California coast (e.g. California Cooperative Oceanic Fisheries Investigations, CalCOFI, see http://calcofi.org/about-calcofi.html), sustained shore-based monitoring has been limited. Our 5 yr of weekly near-shore monitoring including taxonomic resolution to species via DNA barcoding provides new insights into the spawning activity of coastal marine fish communities.

The single most striking observation of this study was the massive decline in spawning during 2 anomalously warm years. We documented a decline of over 50% in the average number of fish eggs per collection in the summer months of 2015 and 2016 compared to previous spawning data from 2013 and 2014. The depressed spawning activity observed in 2015 and 2016 could be the result of changes in upwelling regimes and resulting changes in bottom-up processes impacting ecosystem productivity. In 2014, an anomalously warm water region termed the 'Warm Blob' formed in the Gulf of Alaska and sub-

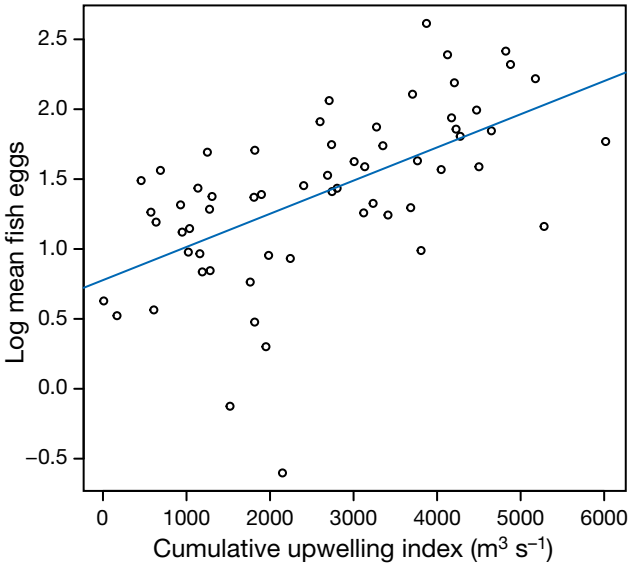

Fig. 8. Cumulative upwelling indices (sum of daily upwelling index) over a 1 mo period vs. log-transformed average number of fish eggs collected during the same month from the Scripps Institution of Oceanography Pier from August 2012 to October of 2017 ($R^2 = 0.33$, $p < 0.01$)

sequently extended down the eastern Pacific coastline, accounting for 1 to 5°C higher than average SSTs that continued to persist until May 2015 (Bond et al. 2015, Zaba & Rudnick 2016). The following year experienced above-average SSTs characteristic of El Niño events in the California current (Jacox et al. 2016). We observed a 1 to 2°C increase in annual average temperature during these years (2014 to 2016) compared to previous years (2013 and 2014) and the following year (2017; Table 2). These positive temperature anomalies increased vertical stratification and deepened the thermocline and nutricline, which can limit fluxes of cold, nutrient-rich deep water to the surface and decrease phytoplankton biomass (Kahru & Mitchell 2000, Jacox et al. 2016, Zaba & Rudnick 2016). Previous time-series data from the Southern California Bight found that there was in fact an inverse relationship between SIO Pier temperatures and primary production in the region (Smith & Eppley 1982). Decreases in primary production would presumably have negative consequences in terms of food availability for higher trophic levels, including fishes. Decreased food availability or food quality can negatively impact growth rates, survivorship, and reproduction, and could potentially decrease spawning activity during the following spawning season (Ruttenberg et al. 2005). This time period was marked by mass strandings of tuna crabs and starvation of sea lion pups that could indicate the far-reaching effects of decreased primary productivity (Zaba & Rudnick 2016). Because sea lion pups largely feed on fish, starvation of pups could indicate decreased fish biomass (McClatchie et al. 2016). Though we cannot definitively confirm that these temperature anomalies resulted in changes in primary productivity that significantly affected spawning activity, it is likely that fish populations experienced effects similar to other organisms.

Decreased fish spawning could also be directly related to physiological effects of increased temperature during 2014 and 2015. Changes in temperature can alter reproductive endocrine homeostasis, gametogenesis, and rates of gonadal development (Genner et al. 2010, Pankhurst & Munday 2011). Inhibition of reproduction at higher temperatures has been shown in a range of species, though temperature thresholds vary across these species (Taranger & Hansen 1993, Pankhurst & Van Der Kraak 2000, Ruttenburg et al. 2005). The species of fish in our study are temperate species that likely have a wide range of thermal tolerances and have varying geographic distributions (Hastings et al. 2014). Species-specific analysis of the 5 most common species in our collections showed that they did not respond to temperature increases in the same way (Fig. 2). It would be unlikely for increased temperature during warm years to affect all species uniformly; however there could be a range of responses, including altered spawning season, depressed spawning, or reproductive failure (Munday et al. 2008).

A third potential explanation for decreased fish egg abundance during 2015 and 2016 could be an offshore or northward shift in spawning location. Such a shift in spawning could result in a decline of eggs captured at our sampling site. This result would indicate modification of spawning behavior in response to environmental change, which is consistent with observed changes in marine ectotherm distributions in response to temperature and dissolved oxygen concentrations resulting from climate change (Stramma et al. 2012, Deutsch et al. 2015).

During 2017, we observed peak spawning and highest species richness approximately 1 mo later than in previous years. This pattern of species richness was driven by delayed spawning in a relatively small number of fish species (4); for most species, total spawning season remained unchanged, although the height of spawning was shifted to later in the year. Surprisingly, relatively few studies have investigated how environmental variability can influence phenology in marine organisms (Genner et al. 2010). Warmer temperatures are associated with delayed spawning in flounder *Platichthys flesus* and earlier spawning in capelin *Mallotus villosus* and Pacific herring *Clupea harengus pallasi* (Ware & Tanasichuk 1989, Carscadden et al. 1997, Sims et al. 2004). In contrast, our data show that there was no apparent change of seasonal spawning during warm years; however, peak spawning was shifted 1 mo later during a cooler year (2017) that followed successive warm years. Although based on only a single El Niño event, our results suggest such climate fluctuations may alter the phenology of fish spawning in following years. Indeed, the phenology of fish larvae in the California Current Ecosystem exhibits interannual variation associated with the El Niño Southern Oscillation (Asch 2015), and these climate fluctuations can impact pelagic fish populations such as the northern anchovy *Engraulis mordax* and Pacific sardine *Sardinops sagax* (Lindegren & Checkley 2013, Checkley et al. 2017). If changes in spawning phenology in response to climate fluctuations are asynchronous with larval food resources, there can be negative consequences for survivorship and recruitment (Cushing 1990). These results highlight the importance of understanding the phenology of mar-

ine organisms in order to predict marine population dynamics and manage populations.

We found a significant positive relationship between CUI and average fish eggs by month. This was likely driven by seasonal upwelling in the California Current, occurring during the spring and summer and coinciding with peak spawning activity (Robinette et al. 2007). We also found a significant positive relationship between annual spring upwelling and annual spring–summer fish egg abundance for each year, though additional sampling years are needed to adequately test this relationship. Consistent with our study, others have found that annual spring upwelling coincided with maximum spawning of the northern anchovy, and more persistent spring upwelling led to increased larval abundance of *Citharichthys stigmaeus* in central California (Lasker & Smith 1974, Robinette et al. 2007). We found a significant negative correlation between annual winter temperatures and annual spring and summer spawning for each year. In contrast, we found no relationship between annual summer temperatures and spring and summer spawning. Temperature can exert large physiological effects on fish reproduction such as alteration of endocrine homeostasis, vitellogenesis, and oocyte development (Pankhurst & Munday 2011). In some fish genera such as *Citharichthys*, a flatfish genus commonly found in our sampling area, vitellogenesis begins as early as February, in which case winter temperatures could impact physiological mechanisms of fish reproduction and lead to variability in fish egg abundance across years (Rackowski & Pikitch 1989). Alternatively, higher winter temperatures could be indicative of changes in other oceanographic variables that could have an indirect effect on spawning activity the following spawning season. For example, *Citharichthys* spawning is triggered by a sudden decline in bottom water temperature associated with seasonal upwelling (Rackowski & Pikitch 1989). Therefore, interannual variability in upwelling could alter fish reproductive activity (Robinette et al. 2007). If the patterns we observed here are maintained across years, winter temperatures could be used to predict spring and summer spawning for at least some species and have important applications in fisheries management. Again, additional years of data are needed to determine the strength and consistency of this relationship.

In this study, we documented large interannual variability in fish egg abundance that was associated with large climatic fluctuations, including an El Niño event captured during our sampling years. Decreased abundance of fish eggs during anomalously warmer years was likely due to changes in primary productivity, physiological effects of increased temperature on fish species, behavioral avoidance, or a combination of these mechanisms. Furthermore, we documented a phenological delay of peak fish egg abundance of approximately 1 mo in the most recent cooler year. Lastly, we found that annual fish egg abundance was negatively correlated with winter temperatures and positively correlated with annual spring upwelling. These results underscore the importance of understanding how natural environmental variation affects marine fish populations, and these data will help us understand how temperature increases associated with climate change may impact future populations and communities. Temperature-mediated effects will likely depend on a variety of factors including physiological tolerances, behavioral response, dispersal capability, and capacity for adaption (Pankhurst & Munday 2011). By providing fisheries-independent data, ichthyoplankton surveys resolved to species by DNA barcoding can play an important role in fisheries management by providing information regarding the spatial and temporal distribution of spawning activity as well as whole ecosystem responses to environmental variability (Ahlstrom 1968, Smith & Eppley 1982, Moser et al. 2001). These data offer insights into the spawning activity of coastal inshore fish communities that complement other ichthyoplankton surveys conducted further offshore.

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