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Authors

Duke, EM
Harada, AE
Burton, RS

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

Elena Maria Duke*, Alice E. Harada, Ronald S. Burton

Marine Biology Research Division

Scripps Institution of Oceanography

University of California, San Diego

La Jolla, CA 92093-0202

1*Corresponding author: emduke@ucsd.edu

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 Pier (La Jolla, CA, USA) for three years (2014-2017) and added a second sampling site near the La Jolla kelp forest for one year (2017). Fish eggs were identified using DNA barcoding and data were compared to previous work from Pier surveys from 2012-2014. 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 2014-2016. Fish egg abundance rebounded in 2017 and was accompanied by a phenological shift of peak spawning activity. We found 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 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 affect 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.

Keywords: 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 that are 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 (Alstrom & Moser 1976, 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 (Alstrom & Moser 1976, 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).

However, because the eggs of many fish species are morphologically indistinguishable, it had been difficult until recently to accurately determine which species were spawning, with the exception of a few morphologically distinct species (Alstrom & Moser 1976). 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 adjacent to Scripps Institution of Oceanography (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 Marine Conservation Area prohibits the take of marine resources except 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 2-4 days (Zwiefel & Lasker 1976). We build upon the study of Harada et al. (2015) using DNA barcoding to identify fish eggs in the plankton off the Scripps 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 document large interannual variation in spawning activity across sampling years 2012-2017. Beginning in 2017, we further expand 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 seven-month period, we can begin to assess if there are habitat-specific patterns of spawning across the species found in the La Jolla MPAs.

Methods

Sampling locations and techniques

Sampling sites were located in or immediately outside two of La Jolla's marine protected areas, the San Diego Scripps Coastal SMCA and the Matlahuayl SMR bounded by coordinates in (Table S1). 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 eggs had a high probability of being spawned within or almost completely within these MPA boundaries.

Weekly plankton samples were collected from the end of Scripps Pier (32°52′2″N, -117°15′26″W) from August 2014 to August 2017, continuing previous work started in August of 2012 (Harada et al. 2015). Samples were collected by lowering a 505 micron-mesh one-meter diameter plankton net until the net reached the seafloor around midday each sampling day. This was repeated three more times for a total of four pulls, sampling approximately 16 cubic meters 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 (32º 51′15″ N, -117º 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 micron mesh one half-meter diameter plankton net behind a small boat at 0.5 knots for 5 minutes. The net was weighted for a sampling depth of about 1 m, sampling approximately 60 cubic meters of water. Although we used different mesh sizes at different sampling sites, both mesh sizes (0.5 mm, and 0.3 mm) were smaller than the fish eggs we sample, which range from 0.7 mm- 1.2 mm (A. Harada, unpubl. data). Moreover, fish eggs that were found only in the kelp samples using the smaller mesh size have much larger egg sizes than even our larger mesh size ranging from (0.6 mm-2.1 mm) (Budd 1940, 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. The

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 hours prior to further processing. If a collection contained over five hundred fish eggs, a subset of approximately four hundred eggs were selected for DNA barcoding for species identification. Samples were processed as soon as possible, never exceeding two weeks before processing

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 squished 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 polymerase-chain reaction (PCR). To amplify DNA, universal fish cytochrome *c* oxidase subunit I (COI) primers were used (Ward et al. 2005): COI VF1 forward primer (5′-TTCTCAACCAACCACAAAGACATTGG-3’) and COI VR1 reverse (5′-TAGACTTCTGGGTGGCCAAAGAATCA-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 sixty four percent 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’ CGCCTGTTATCAAAAACAT-3’) and reverse primer 16Sbr (5’-CCGGTCTGAACTCAGATCACGT-3’) for a 570 bp amplicon (Palumbi 1996). Of the samples

that failed to amplify with COI primers, about fifty three percent were successfully amplified using 16S primers. Overall, about fourteen percent 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 NCBI database, which contains COI and 16S rRNA barcodes from over 500 species of California marine fishes, most of which are vouchered in the Scripps Institution of Oceanography 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 two species (*Citharichthys sordidus* and *Citharichthys 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 was measured and recorded at 2 m depth approximately every 6 minutes from the Scripps 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 (http://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-February) and regressed against the average number of fish eggs per collection during spring and summer (March-August) for that year.

We estimated upwelling using daily upwelling indices calculated at 33°N, 119°W from all collection years (2012-2017) in cubic meters per second 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-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 two sampling sites. Collections from both sites that were made within twenty-four hours were paired and counts for each species were normalized to cubic meters 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

189*Abundance of fish eggs*

190 We observed fish spawning patterns in two different habitats over time and compared
191these data to spawning data previously obtained from the SIO Pier (Harada et al. 2015). We
192found extensive interannual variability in fish spawning. During the years 2015 and 2016 we
193observed a 53% decline in the average number of fish eggs per collection during the summer
194(June through August) compared to data from the previous two years (Fig. 1). Although we did
195observe a seasonal increase in the number of fish eggs in the summer (compared to winter)
196across all years (consistent with Harada et al. 2015), there were fewer fish eggs per collection
197from 2015 & 2016 than from 2013 & 2014. This pattern was not due to any particular species or
198dominant group of species. Species-specific abundance through time for the five most common
199species in our samples and one sport fish, the California Corbina (*Menticirrhus undulatus*) are
200shown in Figure 2. Although represented by relatively few eggs in our collections, the sharp
201peaks in spawning in *M.undulatus* showed very little annual variation while *Engraulis mordax*
202largely disappeared for 2015-2016. *Citharichthys stigmaeus* while notably reduced in 2015
203showed the broadest spawning season among all species and remained a dominant component of
204the icthyoplankton throughout the sampling period.

205 Interestingly, during the summer of 2017 we observed a recovery of fish eggs numbers
206similar to numbers observed in 2013 and 2014. However, peak-spawning season during 2017
207appears to have shifted to later in the year, from May/June in 2013-2016 to July/August in 2017
208(Fig. 1). To assess how spawning seasonality compared across years, we recorded the number of
209species found per collection (Figure S1). The highest number of species recorded per sample
210occurred approximately one month later in 2017 compared with earlier years in which spawning
211was recorded (2013-2014; Table S1); this parallels the overall phenological change in peak egg

abundance. For four 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 one month later in 2017 than in previous years.

Community composition

Overall, in collections from the Scripps Pier from September of 2014 to August of 2017, we collected 6,939 fish eggs and of those 4,150 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 5,546 as 35 species. There were seven 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 Lizard Fish (*Synodus lucioceps*), California Opal Eye *(Girella nigricans*, Pacific Pompano (*Peprilus simillimus),* Mussel Blenny (*Hypsoblennius jenkinsi)*, Giant Sea Bass (*Stereolepis gigas*), Pacific Barracuda *(Sphyraena argentea).* We documented five species that had not previously been recorded to spawn in our study area by Harada et al. 2015: the Yellowtail 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 less than 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 shows there were no distinct changes in community composition over time, but distinct differences in seasonal spawning community between fall-winter and spring-summer months (Fig. 3). Note that warm years do not cluster together; rather all years overlap in multidimensional space.

From February 2017 to August 2017 we sampled the kelp forest habitat and were able to compare community composition between kelp and pier habitats (Table 2). Nine species in the kelp forest plankton samples were not found at the Scripps 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 two species (the Bigmouth Flounder, *H. stomata*, and Red-eye Round Herring, *E. acuminatus*), all other species had been observed in previous collections from the Scripps Pier. Five species were found in Pier samples and 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 may 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 of 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. S1). 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 2). However, linear mixed effects models fit for each of these species found non-significant differences between the numbers of eggs per cubic meter between sites (Fig. S2). 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 generalized through time, it is noteworthy that on a given sampling day there could be large differences in percentage of species collected at each site. For example on July 5th 2017 *C. stigmaeus* comprised 9% and 63% of the collection at kelp and pier sites respectively, *Oxyjulis californica* comprised 36% and 6% of collections at kelp and pier respectively, with total eggs collected at each site, 442 and 74 eggs respectively.

Environmental effects on spawning

Sea surface temperature data collected from the Scripps 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 3). Additionally, these two years show the highest annual average temperatures. The data suggest that when winter temperatures were warmest the 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-August) fish eggs collected from the Pier for each year against the average winter temperature (December-February). We found a significant negative correlation between winter temperatures and spring and summer fish egg abundance ($R^2 = 0.83$, p

281< 0.05; Fig. 6). In contrast, there was no relationship between annual average summer
282temperature (June-August) and the annual spring-summer fish egg abundance ($R^2 = 0.38$, $p >$
2830.05; Fig. S3). In general, higher winter temperatures corresponded with lower cumulative spring
284upwelling for that year, though we found only a marginally significant relationship between the
285two variables ($R^2 = 0.67$, $p = 0.06$).

286 There was a significant positive relationship between spring (March-May) upwelling
287measured by the sum of daily upwelling indices (cumulative upwelling index) and the average
288spring and summer fish egg abundance for each year ($R^2 = 0.75$, $p < 0.05$; Fig. 7). We also found
289a significant positive correlation between the cumulative upwelling index (CUI) for each month
290and the logarithmic mean fish egg abundance by month grouped across all species and all years
291($R^2 = 0.33$, $p < 0.01$; Fig. 8). This result is consistent with our previous study (Harada et al.
2922015), we observed abundant fish eggs when temperatures were highest, coinciding with
293seasonal spawning which peaks in the summer.

294**Discussion**

295 In this study we collected and identified fish eggs spawned in or near La Jolla's marine
296protected areas to examine temporal changes in abundance and community composition of
297spawning fishes as represented by their eggs collected from the plankton. Although ship-board
298sampling of icthyoplankton has a long history off the California coast (e.g, California
299Cooperative Oceanic Fisheries Investigations CalCOFI, see http://calcofi.org/about-calcofi.html),
300sustained shore-based monitoring has been limited. Our five years of weekly near-shore
301monitoring including taxonomic resolution to species via DNA barcoding provides new insights
302into the spawning activity of coastal marine fish communities.

303 The single most striking observation of this study was the massive decline in spawning
304during two anomalously warm years. We documented a decline of over 50% in the average
305number of fish eggs per collection in the summer months of 2015 and 2016 compared to
306previous spawning data from 2013 and 2014. The depressed spawning activity observed in 2015-
3072016 could be the result of changes in upwelling regimes and resulting changes in bottom up
308processes impacting ecosystem productivity. In 2014, an anomalously warm water region
309termed the “Warm Blob” formed in the Gulf of Alaska and subsequently extended down the
310eastern Pacific coastline, accounting for 1-5 ºC higher than average SST that continued to persist
311until May of 2015 (Bond et al. 2015, Kintisch 2015, Zaba & Rudnick 2016). The following year
312experienced above-average SST characteristic of El Niño events in the California current (Jacox
313et al. 2016). We observed a 1-2 ºC increase in annual average temperature during these years
314(2014-2016) compared to previous years (2013-2014) and the following year (2017; Table 3).
315These positive temperature anomalies increased vertical stratification and deepened the
316thermocline and nutricline, which can limit fluxes of cold nutrient-rich deep water to the surface
317and decrease phytoplankton biomass (Kahru & Mitchell 2000, Jacox et al. 2016,, Zaba &
318Rudnick 2016). Previous time series data from the Southern California Bight found that there
319was in fact an inverse relationship between Scripps Pier temperatures and the primary production
320in the region (Smith & Eppley 1982). Decreases in primary production would presumably have
321negative consequences in terms of food availability for higher trophic levels, including fishes.
322Decreased food availability or food quality can negatively impact growth rates, survivorship and
323reproduction, and could potentially decrease spawning activity during the following spawning
324season (Ruttenberg et al. 2005). This time period was marked by mass strandings of tuna crabs
325and 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 will 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 five 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).

343 A third potential explanation for decreased fish egg abundance during 2015-2016 could
344be an offshore or northward shift in spawning location. Such a shift in spawning could result in a
345decline of eggs captured at our sampling site. This result would indicate modification of
346spawning behavior in response to environmental change, which is consistent with observed
347changes marine ectotherm distributions in response to temperature and dissolved oxygen
348concentrations resulting from climate change (Stramma et al. 2012, Deutsch et al. 2017).
349 During 2017, we observed peak spawning and highest species richness approximately
350one month later than in previous years. This pattern of species richness was driven by delayed
351spawning in a relatively small number of fish species (four); for most species, total spawning
352season remained unchanged, although the height of spawning was shifted to later in the year.
353Surprisingly, relatively few studies have investigated how environmental variability can
354influence phenology in marine organisms (Genner et al. 2010). Warmer temperatures are
355associated with delayed spawning in Flounder (*Platichthys flesus*) and earlier spawning in
356Capelin **(***Mallotus villosus*) and Pacific Herring (*Clupea harengus pallasi*) (Ware & Tanasichuk
3571989, Carscadden et al. 1997, Sims et al. 2004). In contrast, our data show that there was no
358apparent change of seasonal spawning during warm years; however, peak spawning was shifted
359one month later during a cooler year (2017) that followed successive warm years. Although
360based on only a single El Niño event, our results suggest such climate fluctuations may alter
361phenology of fish spawning in following years. Indeed the phenology of fish larvae in the
362California Current Ecosystem exhibits interannual variation associated with El Niño Southern
363Oscillation (Asch 2015) and these climate fluctuations can impact pelagic fish populations such
364as the Northern Anchovy (*Engralus mordax*) and Pacific Sardine (*Sardinops sagax*) (Lindegren
365& Checkley 2013, Checkley et al. 2017). If changes in spawning phenology in response to

366climate fluctuations are asynchronous with larval food resources, there can be negative
367consequences for survivorship and recruitment (Cushing 1990). These results highlight the
368importance of understanding phenology of marine organisms in order to predict marine
369population dynamics and manage populations.

370 We found a significant positive relationship between cumulative upwelling index and
371average fish eggs by month. This is likely driven by seasonal upwelling in the California Current,
372occurring during the spring and summer that coincides with peak spawning activity (Robinette et
373al. 2007). We also found a significant positive relationship between annual spring upwelling and
374annual spring-summer fish egg abundance for each year, though additional sampling years are
375needed to adequately test this relationship. Similar to our study, Robinette et al. (2007) found that
376more persistent annual spring upwelling led to increased larval abundance in central California.
377We found a significant negative correlation between annual winter temperatures and annual
378spring and summer spawning for each year. In contrast, we found no relationship between annual
379summer temperatures and spring and summer spawning. Temperature can exert large
380physiological effects on fish reproduction such as alteration of endocrine homeostasis,
381vitellogenesis, and oocyte development (Pankhurst & Munday 2011). In some fish genera such
382as *Citharichthys*, a flatfish genus commonly found in our sampling area, vitellogenesis begins as
383early as February, in which case winter temperatures could impact physiological mechanisms of
384fish reproduction and lead to variability in fish egg abundance across years (Rachowski &
385Pikitch 1989). Alternatively, higher winter temperatures could be indicative of changes in other
386oceanographic variables that could have an indirect effect on spawning activity the following
387spawning season. For example, *Citharichthys* spawning is triggered by sudden decline in bottom
388water temperature associated with seasonal upwelling (Rachowski & Pikitch 1989). Therefore

interannual variability in upwelling could alter fish reproductive activity (Robinette et al. 2007). If the patterns we observe 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 document large interannual variability in fish egg abundance that is 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 one month in the most recent cooler year. Lastly, we found 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 (Alstrom & Moser 1976, Smith & Eppely 1982, Moser et

al 2001). These data offer insights in to the spawning activity of coastal inshore fish communities that complement other icthyoplankton surveys conducted further offshore.

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Table 1. List of species collected from weekly samples from two sites: Kelp and Pier during Feb-Aug 2017. The total number of collections at each site was 25 and 30 collections for Kelp and Pier sites, respectively. 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 represents species that were not found in one of the two sites. Table 1, continued.

Species	Eggs identified: Site~ Kelp	Eggs Identified: Site~Pier	% of total ~Kelp	% of total ~Pier	# of collections ~Kelp	# of collections ~Pier	% difference between sites
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

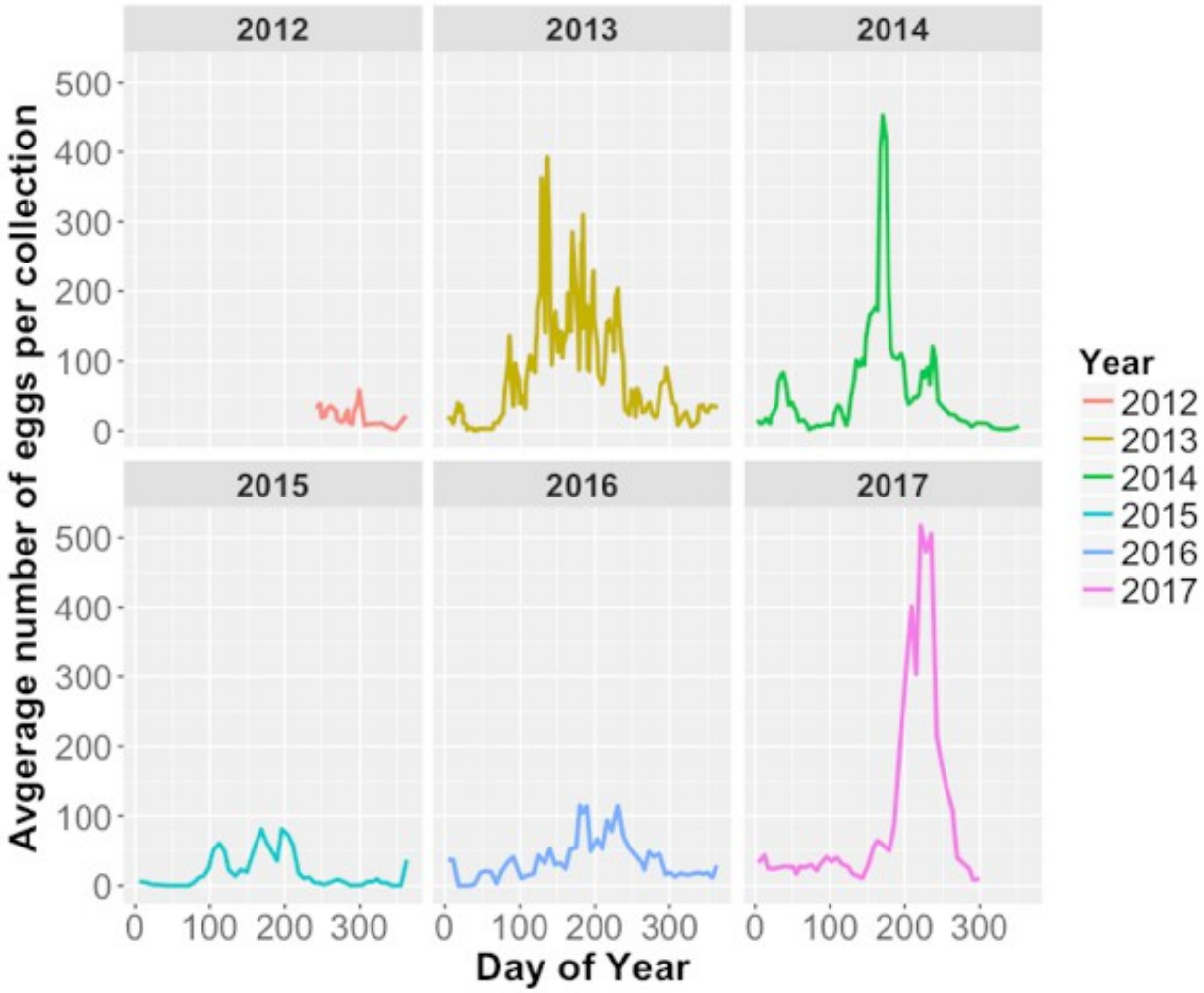
Table 2. List of species collected from weekly samples from two sites: Kelp and Pier during Feb-Aug 2017. The total number of collections at each site was 25 and 30 collections for Kelp and Pier sites, respectively. Both number of eggs collected from individual species and percent of total eggs collected is shown for each site, listed in order of percent difference between sites. NA represents species that were not found in one of the two sites. Note: *Haemulon californiensis* was previously *Xenistius californiensis* and *Halichoeres californica* was previously *Halichoeres semicinctus.*

Species	**Eggs identified: Site~ Kelp**	**Eggs Identified: Site~Pier**	**% of Total ~Kelp**	**% of Total ~Pier**	**# of Collections ~Kelp**	**# of Collections ~Pier**	**% Difference between sites**
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	47	116	0.85	5.88	16	8	5.03

xanthostigma /sordidus							
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

Table 3. Annual average and winter average (December-February) sea surface temperatures are shown with standard error in degrees Celsius. Data were collected approximately every six minutes at 2m depth from the Scripps pier.

Year	Annual average temperature ± SEM (°C)	Winter	Winter average temperature ± SEM (°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		



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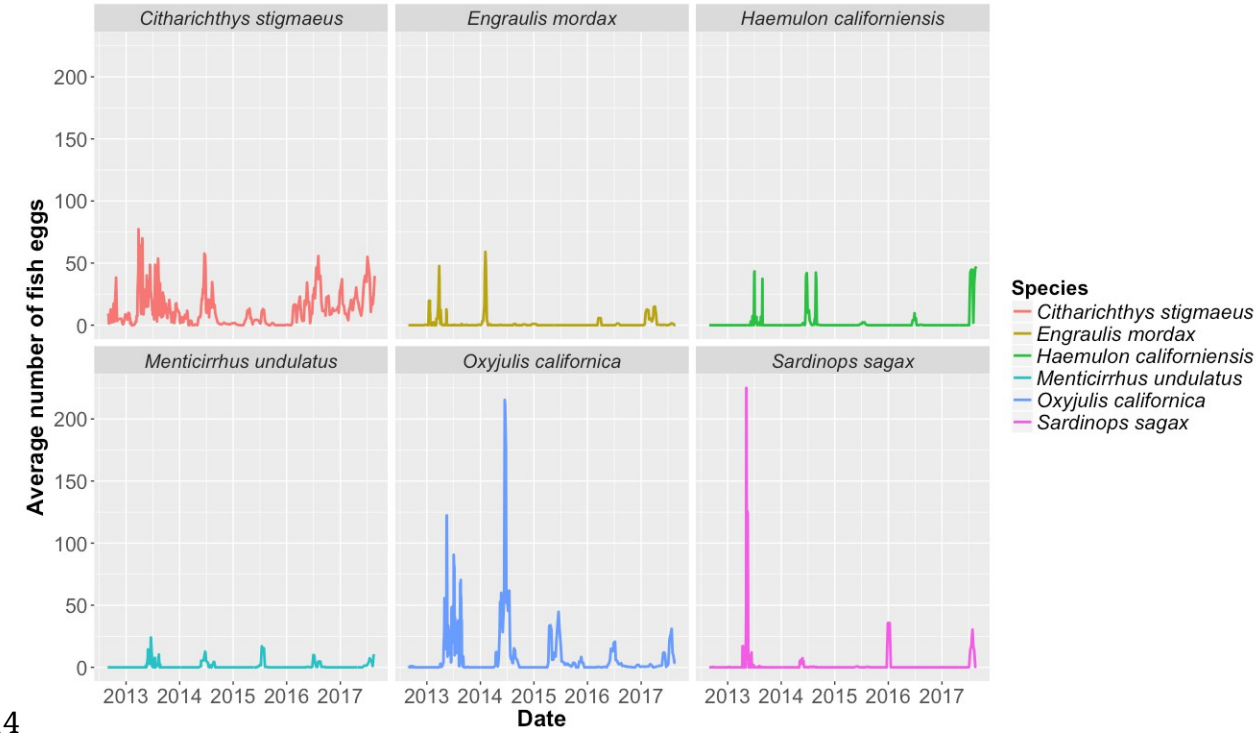

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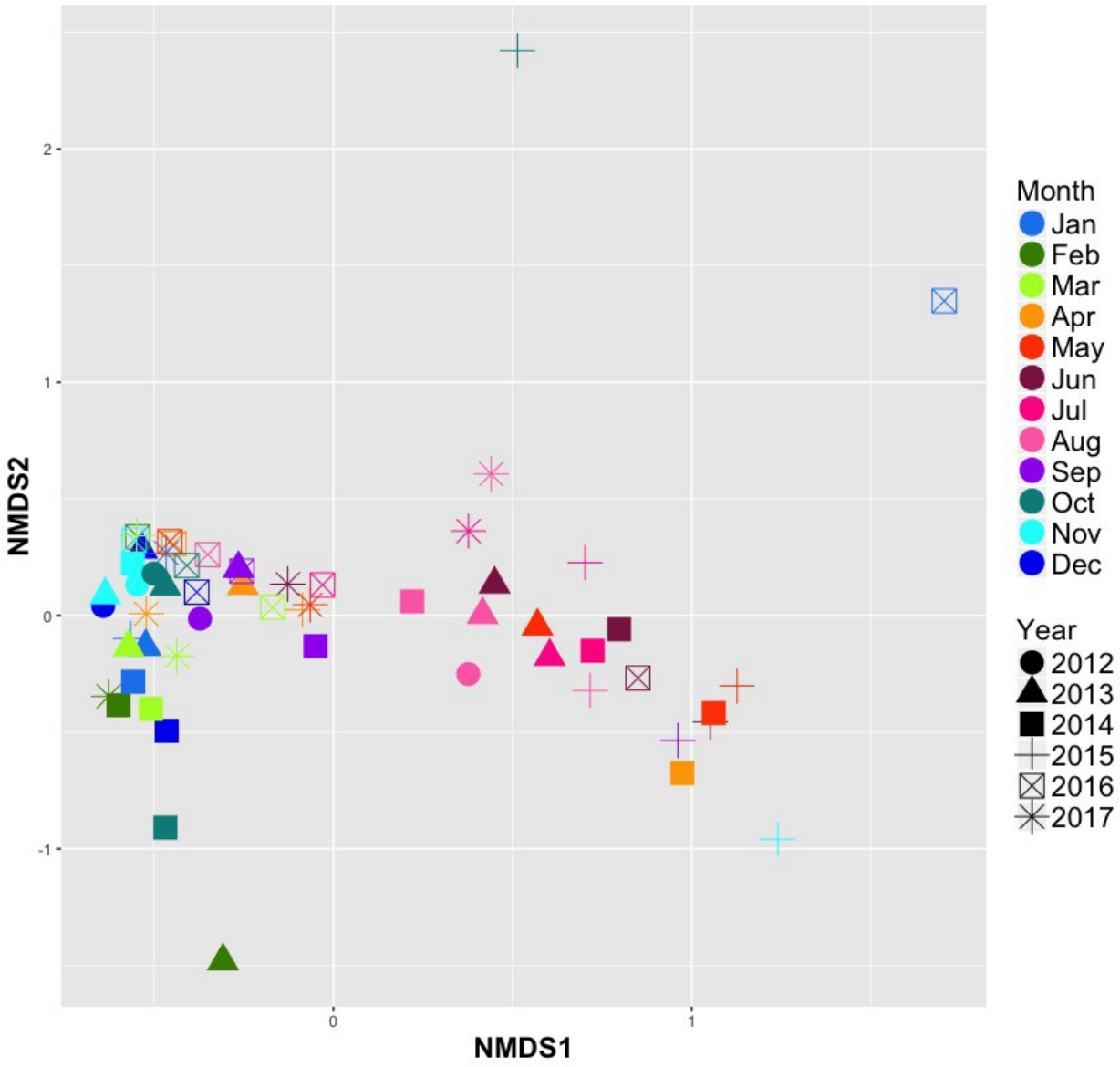

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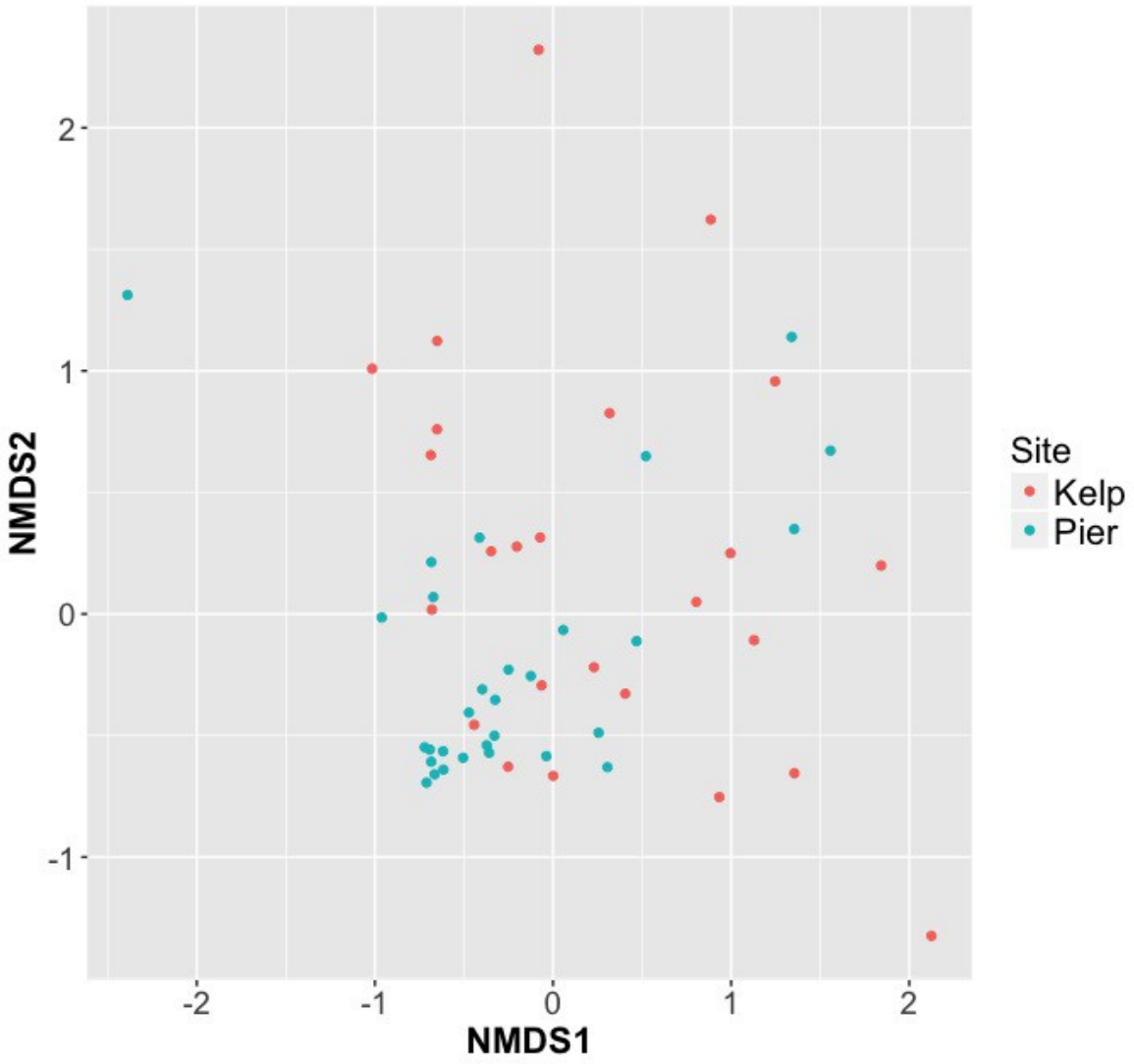

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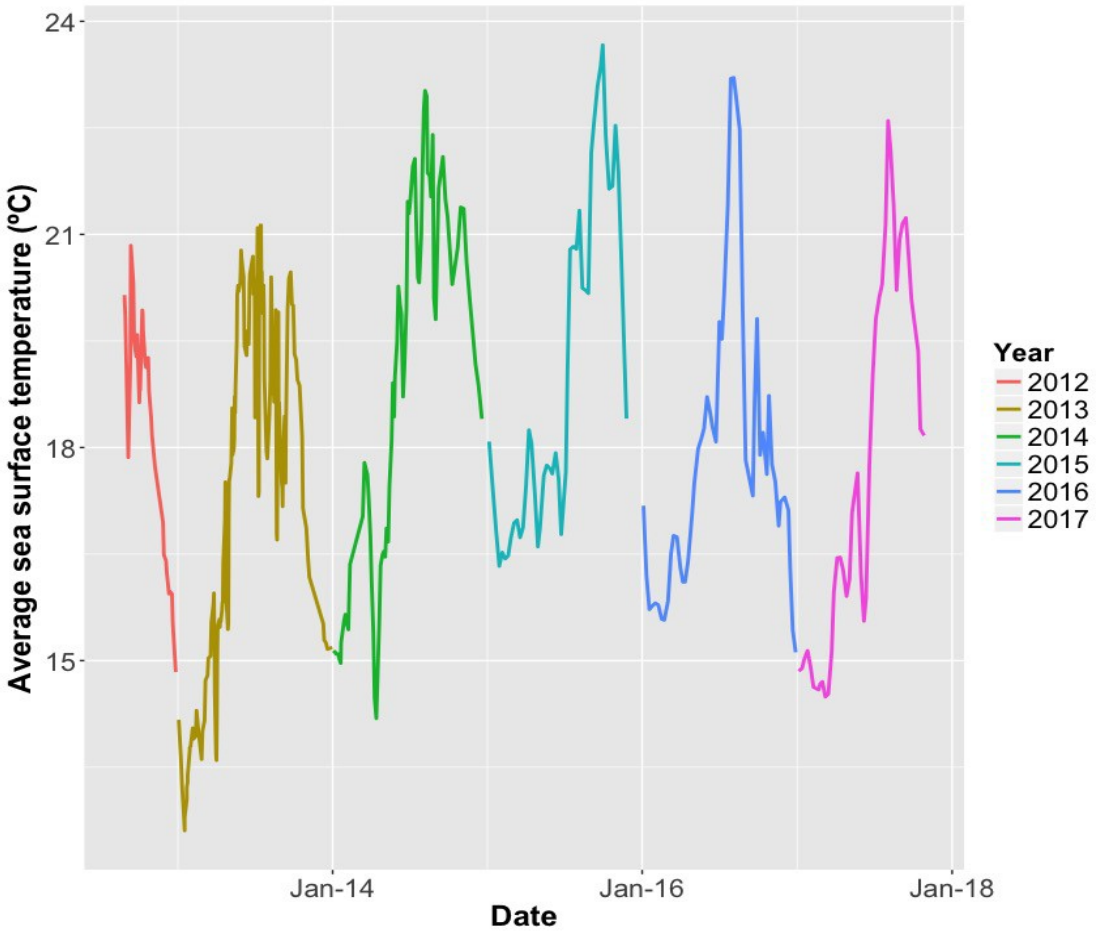

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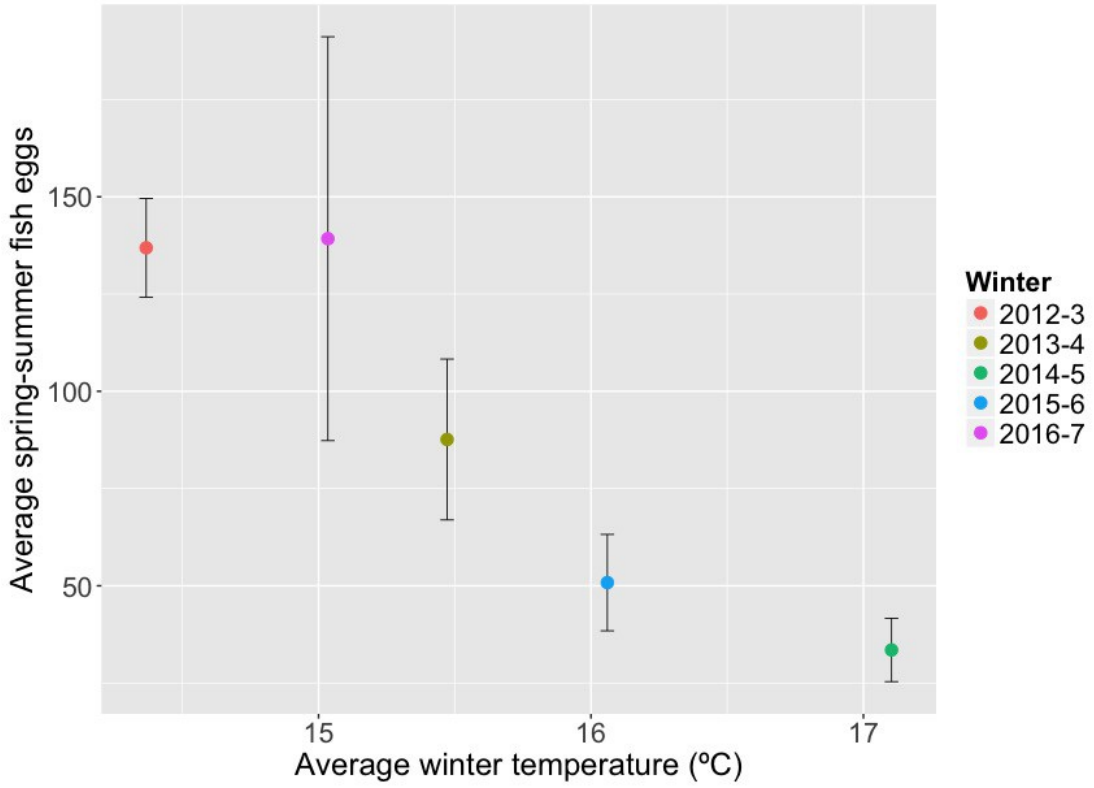

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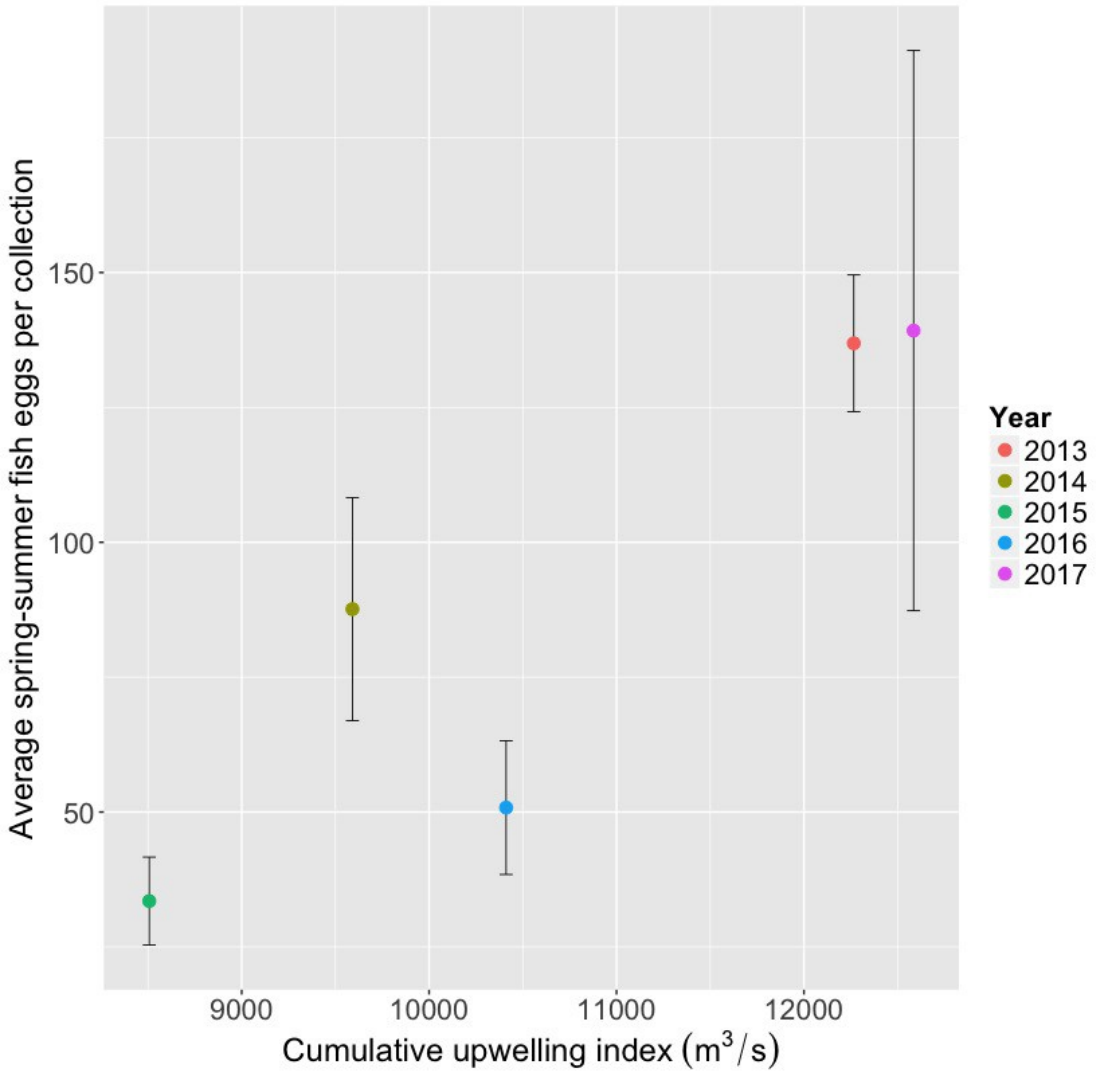

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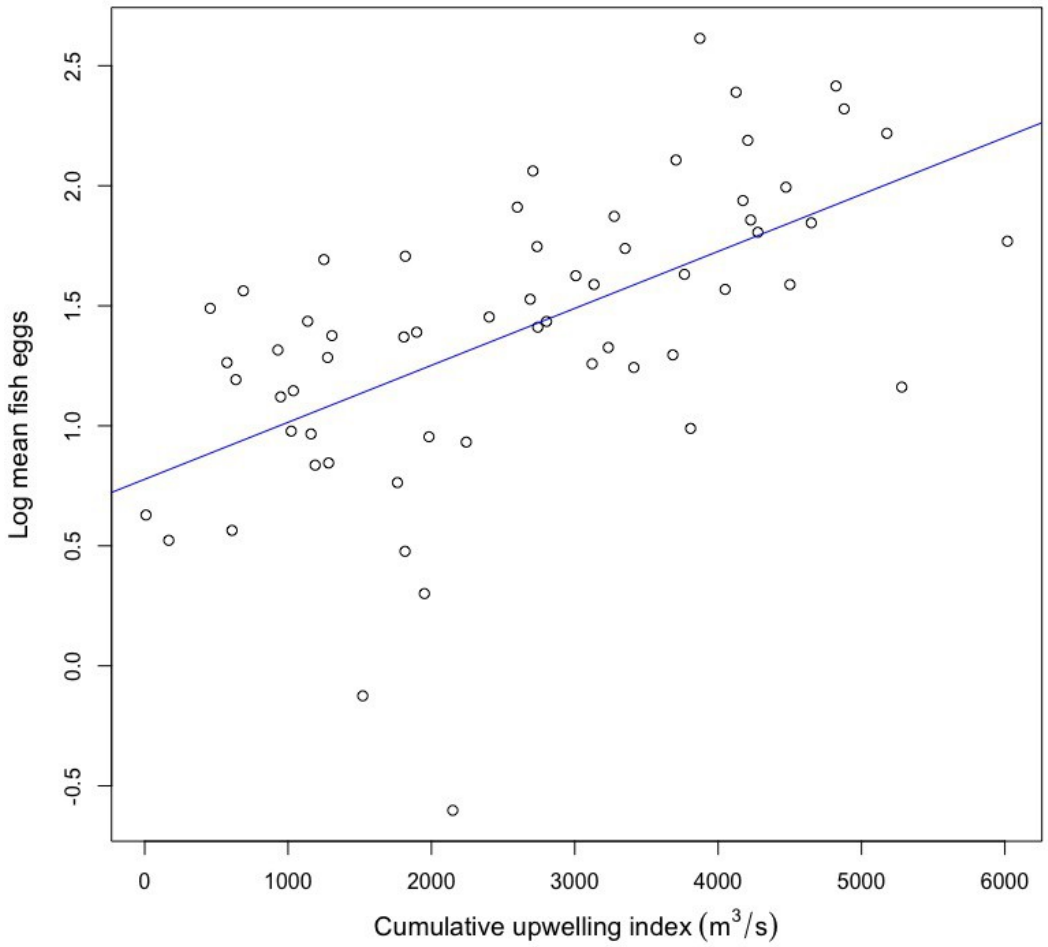

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637Figure 8.
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646 Figure Legends

647Figure 1. Average number of fish eggs collected in each weekly collection in the San Diego-
648Scripps Coastal Reserve from 2012-2017. Average depicted is a moving average of eggs
649collected over a three-week period overlapping by one week. The years 2012 and 2014 were
650taken with permission from Harada et al. (2015).
651
652Figure 2. Average number of fish eggs collected is shown for six species from 2012-2017. Five
653most common species are shown with one additional species *Menticirrhus undulatus.* Average
654depicted is a moving average of eggs collected over a three-week period overlapping by one
655week.
656Figure 3. Non-metric multidimensional scaling plotted from number of fish eggs per month for
657each species normalized to number of eggs identified for each month from 2012-2017 based on
658Bray-Curtis dissimilarity. Stress value of 0.10 indicates the plot gives an adequate representation
659of the data.
660
661Figure 4. Non-metric multidimensional scaling plotted from number of fish eggs for each species
662normalized to number of eggs identified per collection between two sampling sites from
663February 2017 to August 2017. Based on Bray-Curtis dissimilarity. Stress value 0.166 indicates
664plot gives an adequate representation of data.
665
666Figure 5. Average sea surface temperature at the Scripps Pier 2012-2017. Average depicted is a
667moving average of temperatures recorded on egg collection days over a three-week period
668overlapping by one week.
669
670Figure 6. Spring and summer (March-August) average fish egg abundance plotted against
671previous average winter temperature (December -February) for each year. Fish eggs were
672collected from the Scripps Pier. There is a significant negative correlation between winter
673temperatures and spring-summer spawning ($R^2 = 0.83$, $p < 0.05$). Error bars represent SEM.
674
675Figure 7. Average spring and summer (March-August) fish egg abundance per collection plotted
676against spring cumulative upwelling index (sum of daily upwelling indices over spring). Fish
677eggs were collected from the Scripps Pier. Significant positive relationship between spring
678upwelling and spring-summer fish egg abundance ($R^2 = 0.75$, $p < 0.05$). Error bars represent
679SEM.
680
681Figure 8. Cumulative upwelling indices (sum of daily upwelling index) over one month period
682vs. log transformed averaged number of fish eggs collected during the same month from the
683Scripps Pier from August 2012 to October of 2017. ($R^2 = 0.33$, $p < 0.01$).
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688 Supplemental Materials

689Table S1.Two of La Jolla, California's Marine Protected Areas and their boundaries.

MPA	Boundaries of Reserve (Latitude, Longitude)
San Diego- Scripps Coastal State Marine Conservation Area	32° 53.000' N, -117° 15.166' W; 32° 53.000' N, -117° 16.400' W; 32° 51.964' N, -117° 16.400' W; 32° 51.964' N, -117° 15.252' W
Matlahuayl State Marine Reserve	32° 51.964' N, -117° 15.252' W; 32° 51.964' N, -117° 16.400' W; 32° 51.067' N, -117° 16.400' W

690

691

692Table S2. For each collection year from 2013 to 2017 the date in which the highest species
693richness per collection is shown. Two dates are shown for 2015, because there were two
694collections with the highest species richness.

Year	Date	Day of Year	Number of species
2013	19-Jun	170	15
2014	19-Jun	170	18
2015	24-Jun, 23-Jul	175	10
2016	1-Jul	182	14
2017	20-Jul	201	19

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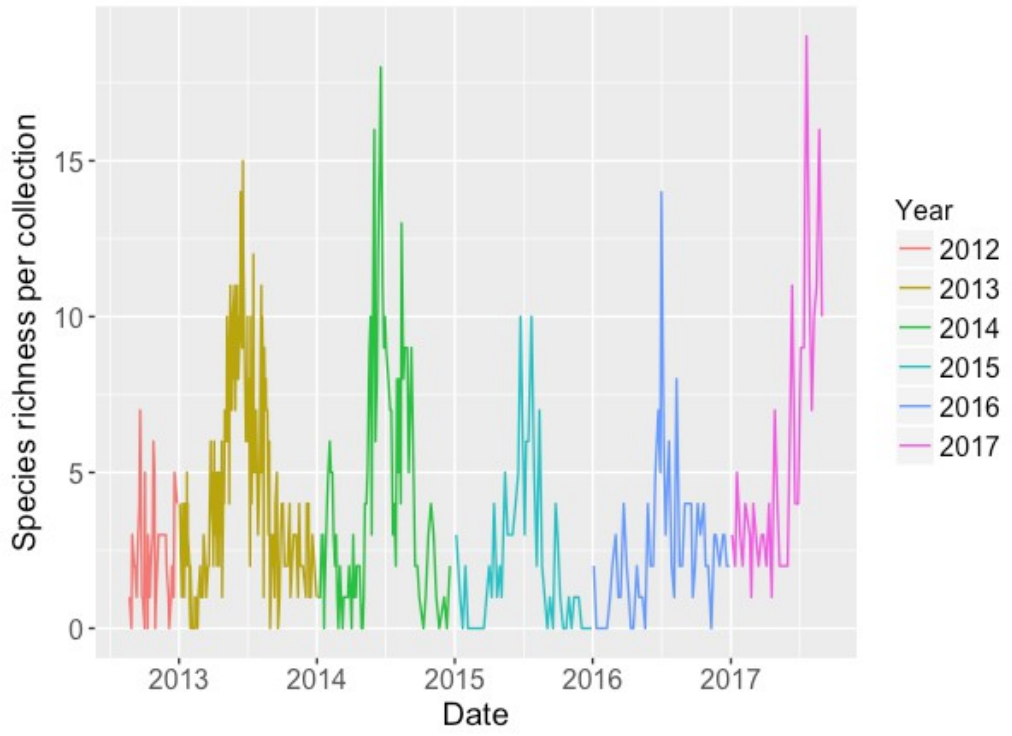
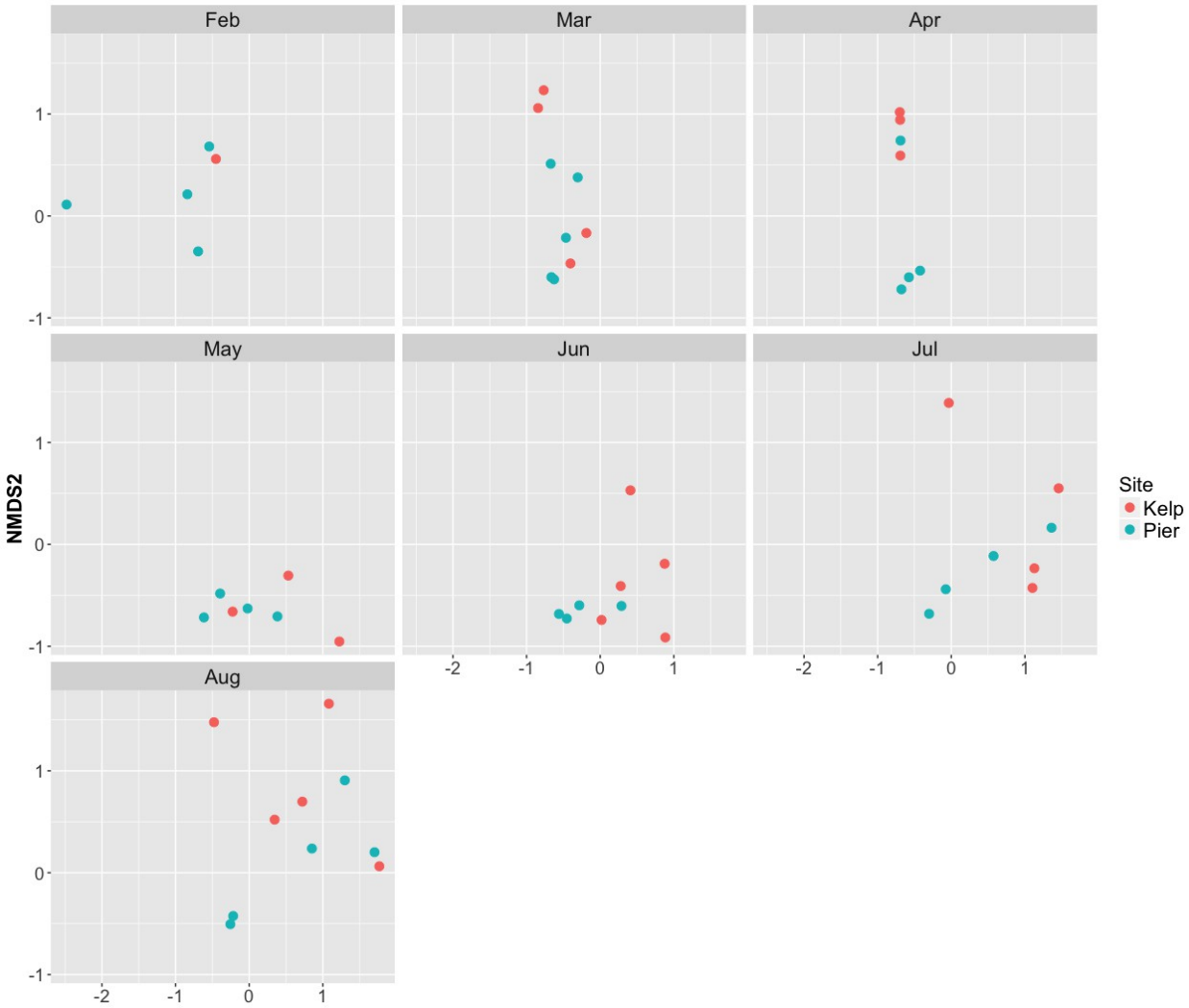


697

698Figure S1. Number species found in each weekly collection of fish eggs in the San Diego-
699Scripps Coastal Reserve from 2012-2017.



700
701Figure S2. Non-metric multidimensional scaling plotted from number of fish eggs for each
702species normalized to number of eggs identified per collection between two sampling sites
703from February 2017 to August 2017 separated by month of collection. Based on Bray-
704Curtis dissimilarity. Stress value 0.166 indicates plot gives an adequate representation of
705data.

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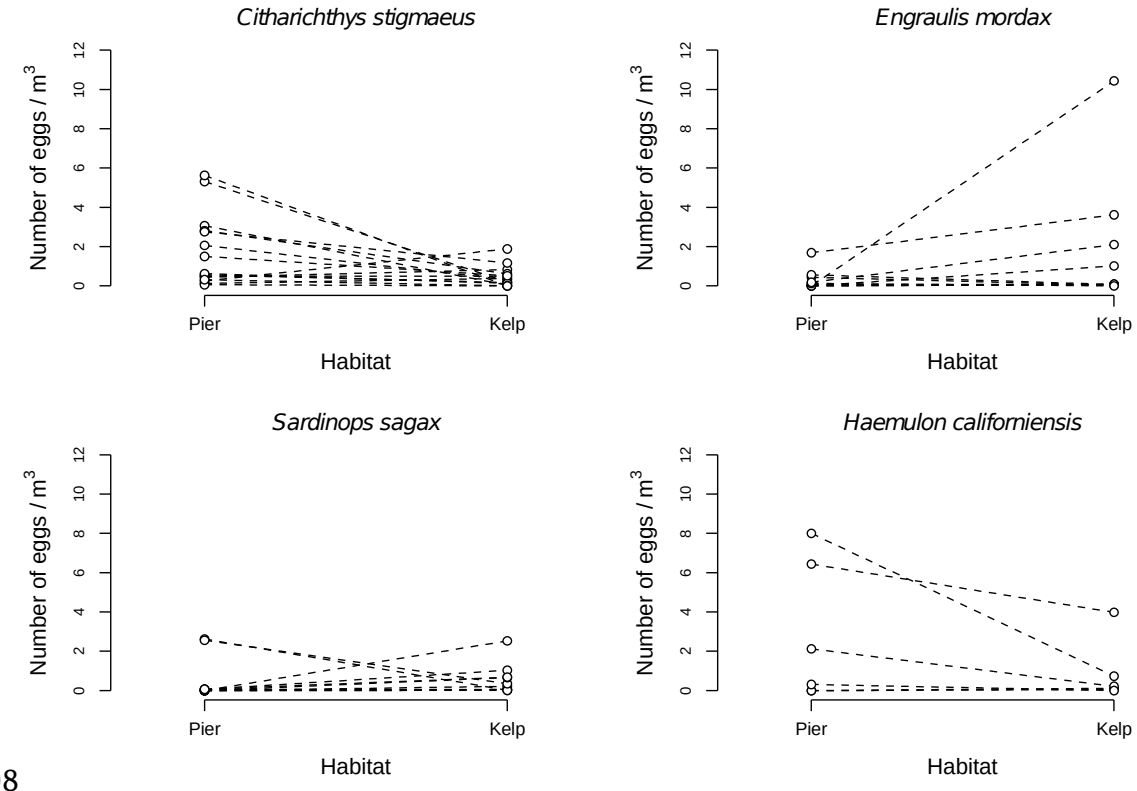

708

709Figure S3. Number of fish eggs per cubic meter between two sites shown for four species.
710Species shown had the largest difference in percentage between two sites overall. Linear mixed
711effects models fit for each showed non-significant differences between sites, and non-significant
712interaction between site and spawning period (indicated by month of collection).
713

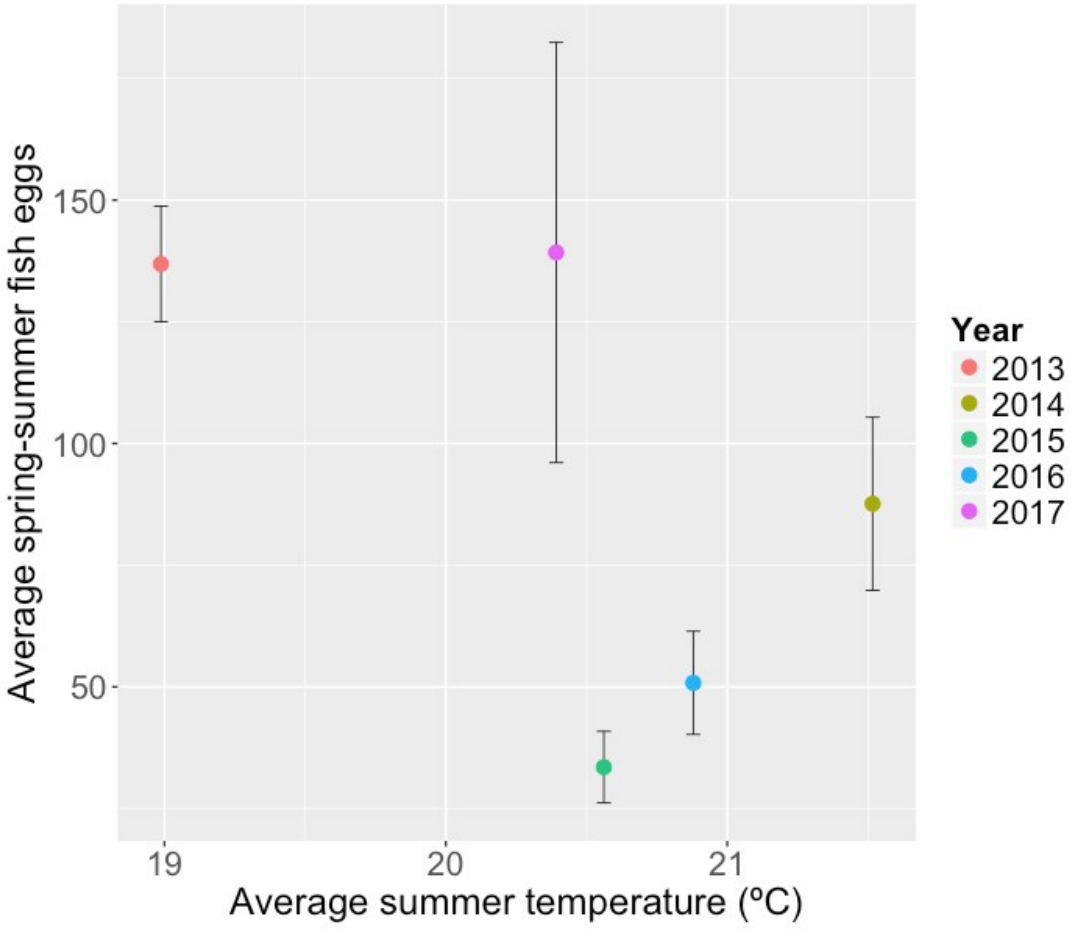

Figure S4. Spring and summer (March-August) average fish egg abundance plotted against average summer temperature (June-August) for each year. Fish eggs were collected from the Scripps Pier. There is no relationship between summer temperatures and spring-summer spawning ($R^2 = 0.380$, $p > 0.05$).