UNIVERSITY OF CALIFORNIA SAN DIEGO

# Monitoring temporal and spatial spawning variability through molecular identification of marine fish eggs

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in

Marine Biology

by

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The Thesis of Laura Emily Furtado is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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ABSTRACT OF THE THESIS

Monitoring temporal and spatial spawning variability through molecular identification of marine fish eggs

by

Laura Emily Furtado

Master of Science in Marine Biology

University of California San Diego, 2019

Professor Ronald Burton, Chair

Monitoring marine protected areas is essential in order to assess if they are effectively conserving marine species and their habitats. Long-term studies allow us to track changes in fish populations in response to environmental variability, and these data can aid in the management of protected areas and fished stocks. This study sampled ichthyoplankton from Scripps Pier for one year and identified them to species using DNA barcoding. These data were compared to

results from Harada *et al*. (2015) and Duke *et al.* (2018), to look for variability in egg abundance and species diversity in response to seasonal temperature changes, including the record-breaking sea surface temperature recorded at Scripps Pier in August 2018. We observed peak egg abundance in June 2018, which was most similar to data from 2013 and 2014, a shift from later spawning seen in July or August during collections from 2015-2017. Overall egg abundance during the spring and summer months of 2018 and sea surface temperature data from the previous winter fits in with the correlation found in Duke, Harada, and Burton (2018). High temperatures recorded in August had no significant effect on number of eggs collected. In January 2019, sampling began at five other California locations, to establish baseline data and monitor sites across latitudes. Initial results have shown that species assemblages north and south of Point Conception, California are different from one another. Evaluating fish spawning in response to environmental variability over time and space will help with the management and conservation of marine resources.

## Introduction

Marine protected areas (MPAs) are a vital resource in conservation, serving as designated areas with goals including the protection of marine ecosystems from human interference (Lubchenco *et al.*, 2003). Some of the benefits of these protections are that they aid in maintaining biodiversity, serve as refuges for the enhancement of fish stocks and resilience against climate change, and can be used as baseline sites to assess human impacts in the oceans (Allison, Lubchenco, and Carr, 1998; Gell and Roberts, 2003; McLeod *et al.*, 2008). Despite knowing that these are the benefits of MPAs, it is difficult to quantify their effectiveness, which, in turn, makes it difficult to justify their existence to stakeholders who would rather make money by fishing there (Lubchenco *et al.*, 2003; Gell and Roberts, 2003).

Extensive monitoring is key to ensuring that MPAs are adequately meeting conservation goals, which requires knowledge of species present in the area and how they are affected by their environment (McLeod *et al.*, 2008). Terrestrial reserves are often simpler to assess because it is easier to define an area of land as a reserve, fence it off, and keep track of the organisms within; marine systems are more complicated because they are more variable in scale, as well as biotic and abiotic processes, and more difficult to monitor (Allison, Lubchenco, and Carr, 1998). Life stages of the same marine species can often be found in completely different habitats, making their protection more difficult. Also, anthropogenic, weather-related, and warm-water events such as the recent Warm Blob or El Niño can cause population ranges to shift temporarily (Leis and Miller, 1976; Perry *et al.*, 2005), while climate change may cause permanent range shifts in marine species, forcing them to move to cooler waters (Perry *et al.*, 2005). Biogeographic barriers, such as steep temperature gradients, land masses, and currents, could impede these necessary range changes (Wares, Gaines, and Cunningham, 2001). Tracking several sites across

latitudes to look at how populations move and are interconnected will give insight into how MPA boundaries will need to move with the organisms over time in order to be most effective.

Long-term observations of changes in species abundance and distribution in conjunction with abiotic factors, such as warming, pollution, and ocean acidification can reveal trends that may help better predict the effects of climate change on fish populations. Previous studies on salmonids and sole have shown negative effects on gamete quality and caused shortened larval development time when exposed to higher temperatures (Taranger and Hansen, 2003; Pankhurst and Munday, 2011; Fincham, Rijnsdorp, and Engelhard, 2013). Changes in migration and spawning phenology during years of abnormal water temperatures have also been well-documented in a variety of species from the northern Atlantic (Carscadden, Nakashima, and Frank, 1997; Genner *et al.*, 2010; Sims *et al.*, 2004) and eastern Pacific (Asch, Cheung, and Reygondeau, 2018). The eastern Pacific has recently experienced several warm-water anomalies, including the "Warm Blob" in 2014 and 2015, El Niño Southern Oscillation, in 2015 and 2016, and record-high sea surface temperatures in San Diego in 2018 (Peterson, Robert, and Bond, 2015; L'Heureux *et al.* 2017; Monroe, 2018). MPAs are excellent candidates for monitoring these changes, as they are relatively free from other external stressors, such as fishing and resource extraction (Gell and Roberts, 2003). Information collected from these controlled sites can then be applied to the management of populations outside reserves.

Monitoring efforts in the two MPAs adjacent to Scripps Institution of Oceanography, the San Diego-Scripps Coastal State Marine Conservation Area (SMCA) and the Matlahuayl State Marine Reserve (SMR), have primarily involved trawling and diver surveys (Craig, Fodrie, and Hastings, 2004; Hastings *et al.*, 2014). These methods are sufficient to assess species diversity, but only for juveniles and adults, not planktonic life stages. These surveys are also more likely to

miss migratory species that are found only during certain times of the year, and cryptic species that are difficult to observe and quantify (Lubchenco *et al.*, 2003). As an added tool, ichthyoplankton surveys have been used to address these concerns along with traditional sampling methods in these two reserves since 2012 (Harada *et al.*, 2015; Duke, Harada, and Burton, 2018). Ichthyoplankton aids in quantifying what species are using existing reserves as spawning grounds or habitat during critical early life stages, and can help determine areas that should continue to be protected.

Ichthyoplankton surveys have been used to estimate the biomass and adult spawning population of a target species, such as the Pacific sardine, *Sardinops sagax* (Ahlstrom and Moser, 1976). Studies like these rely on morphological identification of fish eggs, which is a well-established field (Markle and Frost, 1985). However, some studies have found difficulty in morphological identification, and have instead turned to more reliable molecular methods (Ahern *et al.*, 2018; Hofmann *et al.*, 2017). Molecular identification through amplicon sequencing, known as DNA barcoding, has proved to be both simple and accurate in identifying the species of individual eggs (Harada *et al.*, 2015; Duke, Harada, and Burton, 2018; Ward *et al.*, 2005). Because not all fish species have pelagic eggs and larvae, ichthyoplankton surveys serve as a complement to other survey methods.

This study aims to continue long-term monitoring of the San Diego-Scripps Coastal SMCA and Matlahuayl SMR, assessing species diversity and fish egg abundance, and how those observations relate to sea surface temperature in the area as compared to previously documented interannual variation (Duke, Harada, and Burton, 2018). Additionally, at the start of 2019, monitoring began at five other pier locations in California. These locations are not associated with MPAs, but were chosen due to their association with existing shore stations that have long-

term time series of oceanographic conditions. This expansion is meant to establish a baseline at these sites, which will eventually allow us to look for latitudinal trends and comparisons over several sites at the same time, rather than the single site over a long time. By creating baselines at other locations, we will be able to see the reach of oceanographic trends, and how they may affect populations differently.

## Methods

### *Egg Sampling and Sorting – San Diego*

Sampling for the San Diego location took place at the end of Scripps Pier (32° 52’ 2” N, 117° 15’ 26” W), which has the San Diego-Scripps SMCA to the north and Matlahuayl SMR to the south. The habitat immediately surrounding the pier is predominantly sandy, soft-bottom substrate; however, Matlahuayl SMR includes areas of rocky substrate and kelp forest habitat. Harada *et al.* (2015) previously constructed surface transport models of both MPAs and concluded that eggs collected at the pier had a high likelihood of originating within the MPA boundaries. Therefore, all eggs collected for this study were assumed to have come from within one of these MPAs.

Plankton tows were collected weekly during spring, fall, and winter, and three times per week during the summer (June-September). There is higher variability in number of eggs collected during summer as seen in previous sampling done by Harada *et al.* (2015) and Duke, Harada, and Burton (2018) from August 2012 to December 2017, and so the more frequent summer samples were intended to capture better resolution of that variability.

Samples were taken by lowering a 505-micron mesh, 1-meter diameter conical net to the seafloor (about 5 meters depth) and retrieving it by hand a total of 4 times, for an estimated

volume of 16 cubic meters sampled. The water collected in the cod end was immediately transferred to a 1-liter container and taken back to the lab, where it was filtered through a 300-micron mesh. The concentrated sample in the mesh was then inverted into petri dishes containing a small volume of seawater. Concentrated samples were searched for fish eggs using a dissecting microscope and pipette. Northern anchovy (*Engraulis mordax*) and Pacific sardine (*Sardinops sagax*) eggs are morphologically distinct: anchovy eggs are oblong and sardine eggs are 2mm in diameter with two distinct membranes. These species' eggs were visually identified, separated, and counted independently; all others were approximately 1mm in diameter, spherical, with a single oil droplet, and were subsequently counted together. Eggs were placed in a 1.7 mL tube with 95% ethanol and stored at -20$^{o}$C for at least 12 hours before further processing.

*Egg Sampling and Sorting – Santa Cruz, San Luis Obispo, Santa Barbara, Santa Monica, and Newport*

Samples from other locations were taken off of Newport Beach Pier (33°36'21.7"N 117°55'52.0"W), Santa Monica Pier (34°00'27.0"N 118°29'60.0"W), Stearns Wharf (34°24'29.1"N 119°41'05.9"W), Cal Poly Pier (35°10'12.6"N 120°44'26.4"W), and Santa Cruz Wharf (36°57'26.2"N 122°01'02.2"W). Nets at all locations were also 505-micron mesh, but varied in diameter from 1-meter to ½-meter. Smaller nets were towed proportionally more to match the estimated 16 cubic meters of water sampled from San Diego. These samples were concentrated according to the same protocol, and then the entire plankton sample was placed into 95% ethanol in a 50mL conical tube and shipped to the Burton Lab at Scripps. Once there, fish eggs were removed from the preserved plankton samples, sorted, and placed in 1.7mL tubes with ethanol to await further processing. The rest of the plankton samples were returned to their respective 50mL tubes and stored for potential future projects.

*Egg Processing*

Each egg was removed from the 1.7 mL tube of ethanol and placed into its own 0.2mL PCR strip tube. Excess ethanol was removed from the tube and each egg was washed using 90µL of nuclease-free water. Finally, 15µL of buffer (2/3 Qiagen AE buffer, 1/3 DI water) was added to each tube. Eggs were then crushed using a clean micropipette tip, releasing the genetic material inside. No additional DNA extraction or purification was necessary. DNA samples were stored at -20ºC.

*PCR and Gel Electrophoresis*

Polymerase chain reaction (PCR) was used to amplify two selected mitochondrial amplicons. The PCR master mix included 12.5 microliters of Promega GoTaq Green, 10.5 microliters of nuclease-free water, and 0.5 microliters of each primer (forward and reverse) per sample, plus 1 microliter of DNA extract (from the 15 microliters of crushed egg and AE buffer mixture). The first amplicon targeted was cytochrome oxidase subunit 1 (COI) gene, the widely accepted barcode locus for animals. A set of universal COI primers is used for this amplicon across all fish species: 5' TTCTCAACCAACCACAAAGACATTGG 3' (forward) and 3' ACTTCYGGGTGRCCRAARAATCA 5' (reverse), from Ivanova *et al*. (2006). The reverse was a degenerate primer - Rs and Ys used in the primer sequence indicated A/G and T/C, respectively. This degenerate primer was used to achieve better amplification of a wider variety of species that deviated slightly from the conserved sequence.

PCR reactions were placed in a thermal cycler to undergo amplification, which was initiated at 95ºC for 2 minutes followed by 35 cycles of 95ºC for 30 seconds, 50ºC for 45 seconds, and 72ºC for 1 minute, and finally 72ºC for 5 minutes.

After amplification, the PCR product was run through gel electrophoresis on a 1.5% agarose gel at 95 volts for 35 minutes and visualized using SYBR Safe (Invitrogen). The COI amplicon was 710 bp in length, and so the presence of a band that size on the gel indicated that amplification of COI was successful and could be sequenced. If a band was not present, the DNA sample was run through a new PCR amplification with a 16S primer set from Palumbi *et al.* (1996): 5' CGCCTGTTATCAAAAACAT 3' (forward) and 3' TGCACTAGACTCAAGTCTGGCC 5' (reverse). The PCR for 16S used the same master mix and annealing temperature, but used 30 cycles instead of 35. If a band appeared indicating a 570 bp amplicon, then it would be sequenced.

*Sequencing*

PCR product was cleaned using Sephadex G-50 Fine spin columns (GE), leaving only the amplified DNA solution. 9 microliters of cleaned, amplified DNA plus 1 microliter of the corresponding forward primer were placed in a tube and sent to Retrogen to be sequenced using Sanger sequencing methods.

Hundreds of marine fish species have their COI and 16S gene sequences in GenBank and are easily accessible through NCBI's Basic Local Alignment Search Tool, or BLAST. Many of the fish commonly found in coastal Southern California waters have voucher specimens in the SIO collections, which had their COI and 16S genes sequenced by Hastings and Burton (2008) and submitted to GenBank. All sequences were sent through BLAST, which returns the top species matches for each sequence, plus the percentage of bases from the sample that match the GenBank voucher sequence. A majority of sequences were 99% similar or greater to voucher specimens; however, samples with 95% similarity or greater were acceptable in some cases of lower read quality with no other close matches.

*Environmental Data*

Environmental data is publicly available through automated shore stations associated with the Southern California Coastal Ocean Observing System (www.sccoos.org), for San Diego, Newport, Santa Monica, and Santa Barbara, and the Central and Northern California Ocean Observing System (www.cencoos.org), for San Luis Obispo and Santa Cruz. The Scripps Pier shore station is located two meters below the surface at the end of the pier and takes temperature ($^{o}$C), pressure (dbar), salinity (PSU), and chlorophyll (micrograms/L) measurements every 4 minutes.

## Results

*Scripps Pier*

Weekly samples of ichthyoplankton on the Scripps Pier showed peak spawning activity for 2018 occurred in June (Figure 1), consistent with baseline data set in Harada *et al.* (2015). Duke, Harada, and Burton (2018) had found a shift during 2016 and 2017, in which peak spawning occurred in July and August, as well as a distinct decrease in the number of fish eggs collected during 2015 and 2016, which returned to baseline abundance in 2017. New data collected over the past year revealed that spawning abundance remained at baseline levels through 2018.

The depressions in egg abundance seen in the spring and summer months of 2015 and 2016 were attributed to warmer sea surface temperatures (SST) during the previous winter months of December through February (Duke, Harada, and Burton, 2018). While SST was abnormally high in late July and early August of 2018, SST from the winter of 2017-2018 is more consistent with data from 2012-2014 (Figure 2), as are values for egg abundance (Figure

1). Correlation between SST for December 2017 through February 2018 and egg abundance for March through August 2018 is consistent with the correlation previously calculated by Duke, Harada, and Burton (2018), with an $R^2$ value of 0.79, p=0.01 (Figure 3). There is no significant correlation between concurrent SST and egg abundance during June-August 2018 based on collections made three times per week (Figure 4). However, data from 2018 was consistent with Harada *et al.* (2015), where the most abundant fish eggs occurred during the season in which temperatures are highest, which is attributed to seasonal spawning.

The day with the highest species diversity has gotten later since baseline data was first collected (Table 1). While the day of highest diversity closely matches the time period with the highest SST in 2018 (Figure 2), evidence from previous years cannot support that this is anything more than a coincidence, as SST is usually highest around the month of August each year.

During 2018, a total of 5333 fish eggs were collected in 75 samples, with 4217 of them successfully identified, either morphologically or through molecular methods, representing 37 unique species (Table 2). Three species were collected in 2018 that had not been seen in any prior collections: *Fodiator rostratus*, *Strongylura exilis*, and *Etrumeus acuminatus*. *E. acuminatus* had previously been found in collections from the La Jolla kelp forest, adjacent to Matlahuayl SMR (Duke, Harada, and Burton, 2018), but never before from pier samples. *F. rostratus* was found in two separate collections, while the other two were found in one collection each. These three species accounted for approximately 0.1% of all eggs identified during 2018 and therefore do not contribute significantly to the species diversity. Additionally, *Seriola lalandi* eggs had only been found twice before, one in 2014 and one in 2015, and two *S. lalandi* eggs were found in two separate pier collections in 2018. Ten species previously found from pier collections were not seen in 2018: *Synodus lucioceps* (California lizardfish), *Cheilotrema*

*saturnum* (Black croaker), *Trachurus symmetricus* (Pacific jack mackerel), *Citharichthys gordae* (Mimic sanddab), *Atractoscion nobilis* (White seabass), *Stereolepis gigas* (Giant seabass), *Sphyraena argentea* (Pacific barracuda), *Mugil cephalus* (Flathead gray mullet), *Lycodes pacificus* (Blackbelly eelpout), and *Scorpaena guttata* (California scorpionfish). *C. saturnum*, *T. symmetricus*, and *A. nobilis* combined made up 1.2% and 1.5% of identified eggs in 2013 and 2014, respectively, but otherwise, these ten species have not contributed heavily to species diversity in previous years.

Species vary in the seasonality of spawning behavior (Figure 5), and nine common species were selected to look for phenological changes in spawning. *Citharichthys stigmaeus*, which is known to spawn year-round, is present in most collections. *Engraulis mordax* usually peaks in the first half of the year, but has become more abundant in 2018 and the first three months of 2019. *Menticirrhus undulatus* shifted its peak spawning activity to July during 2015-2017, but appears to have shifted back into June, similar to 2013 and 2014, and increased in abundance during 2018. *Oxyjulis californica* eggs have decreased in abundance since 2013 and 2014 and not recovered, but also returned to peak abundance in June after shifting into July during 2016 and 2017. *Sardinops sagax* does not have a clear pattern, other than only appearing in large numbers during one month of the year, sometimes in the winter and sometimes in the summer. The month of *Xenistius californiensis'* peak egg abundance has remained relatively unchanged, occurring in July, but has also regained overall abundance after a large drop during 2015 and 2016. Duke, Harada, and Burton (2018) did not analyze *Halichoeres semicinctus*, *Roncador stearnsii*, or *Genyonemus lineatus*, but those species have increased in abundance during collections in the last two years. *H. semicinctus* was most abundant in 2017 but 2018 was still significantly higher than 2013-2016. *R. stearnsii* abundance in 2017 was similar to 2013 and

2014, but increased substantially in 2018. *G. lineatus* eggs have not been found nearly as often as the other species, but are present in collections from all other sites starting in 2019, and population comparisons with those sites may be interesting in the future.

*California Shore Stations*

Samples taken at other shore station-associated piers in California (Figure 6) revealed differences in both species composition and ichthyoplankton abundance. In terms of quantity, samples taken from San Luis Obispo most closely reflect those from Scripps Pier, with peaks of well over 100 eggs in a single sample on several occasions. Stearns Wharf in Santa Barbara and Santa Cruz have similar egg abundances to one another, up to almost 50 eggs in a single sample, while Santa Monica and Newport Beach Piers have not amassed more than a few eggs per collection (Figure 7).

Approximately 80% of eggs from all locations were able to be sequenced, which revealed that San Luis Obispo and Santa Cruz share the same four species' eggs in all collections, while samples from Santa Barbara, Newport Beach, and Scripps have more similar species' eggs present with one another (Figure 8). Santa Monica has only collected a small number of eggs (<10), all of which were identified as speckled sanddabs (*Citharichthys stigmaeus*). Due to the lack of data for that location, it was not included in any further analyses.

Three species have been found in all five locations: *Citharichthys stigmaeus*, *Paralichthys californicus*, and *Genyonemus lineatus*. The Pacific sand sole *(Psettichthys melanostictus*), found in both San Luis Obispo and Santa Cruz, has never been observed in San Diego egg surveys since 2012. All other species present in the samples have previously been identified in San Diego at some point from 2012-2019.

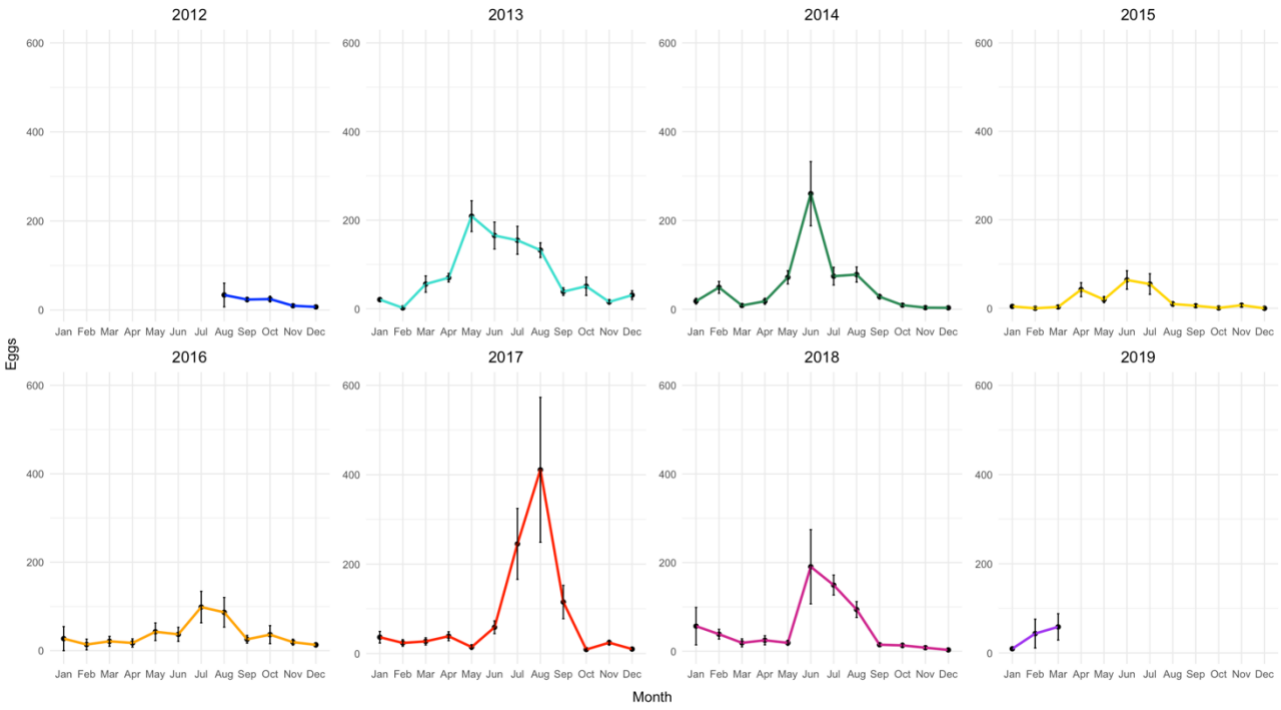


Figure 1: Monthly averages of fish eggs sampled per collection from the Scripps Pier in San Diego, shown with standard error of the means. Data from 2012-2014 from Harada *et al*. (2015), data from 2015-2017 from Duke, Harada, and Burton (2018).

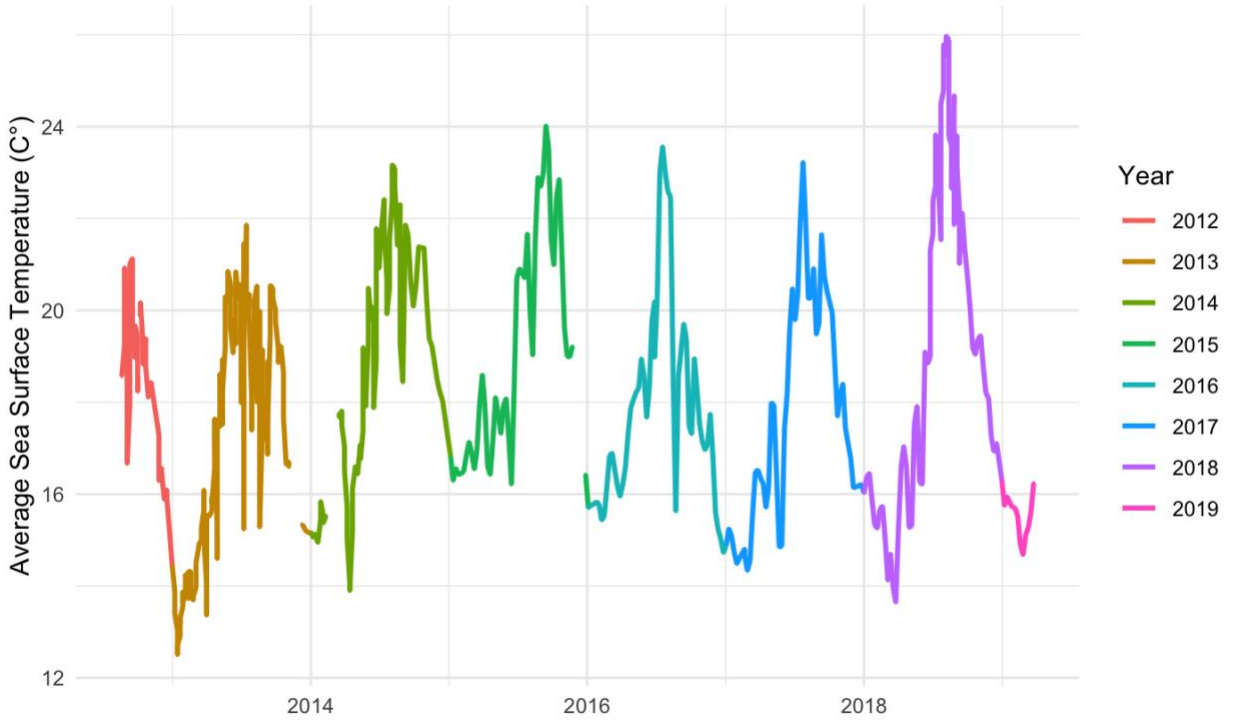


Figure 2: Average sea surface temperature on collection days taken at 2m depth on Scripps Pier, calculated as a moving average over a three-week period with one-week overlap. Data from 2012-2014 from Harada *et al*. (2015), data from 2015-2017 from Duke, Harada, and Burton (2018).

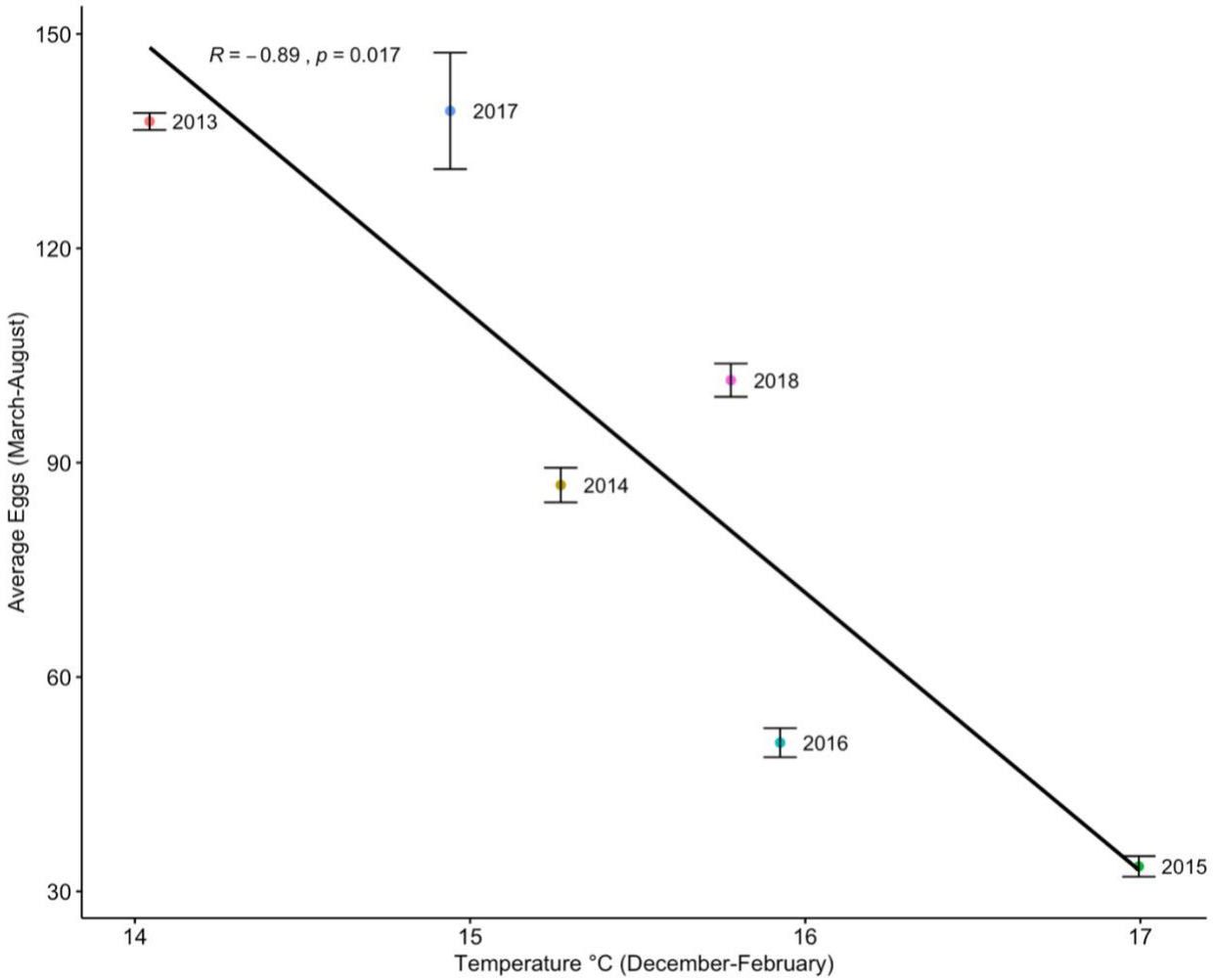


Figure 3: Significant correlation between winter (December-February) sea surface temperature and spring-summer (March-August) egg abundance, with standard error of the means for egg abundance. $R^2$=0.79, p=0.01. Data from 2012-2014 from Harada *et al.* (2015), data from 2015-2017 from Duke, Harada, and Burton (2018).

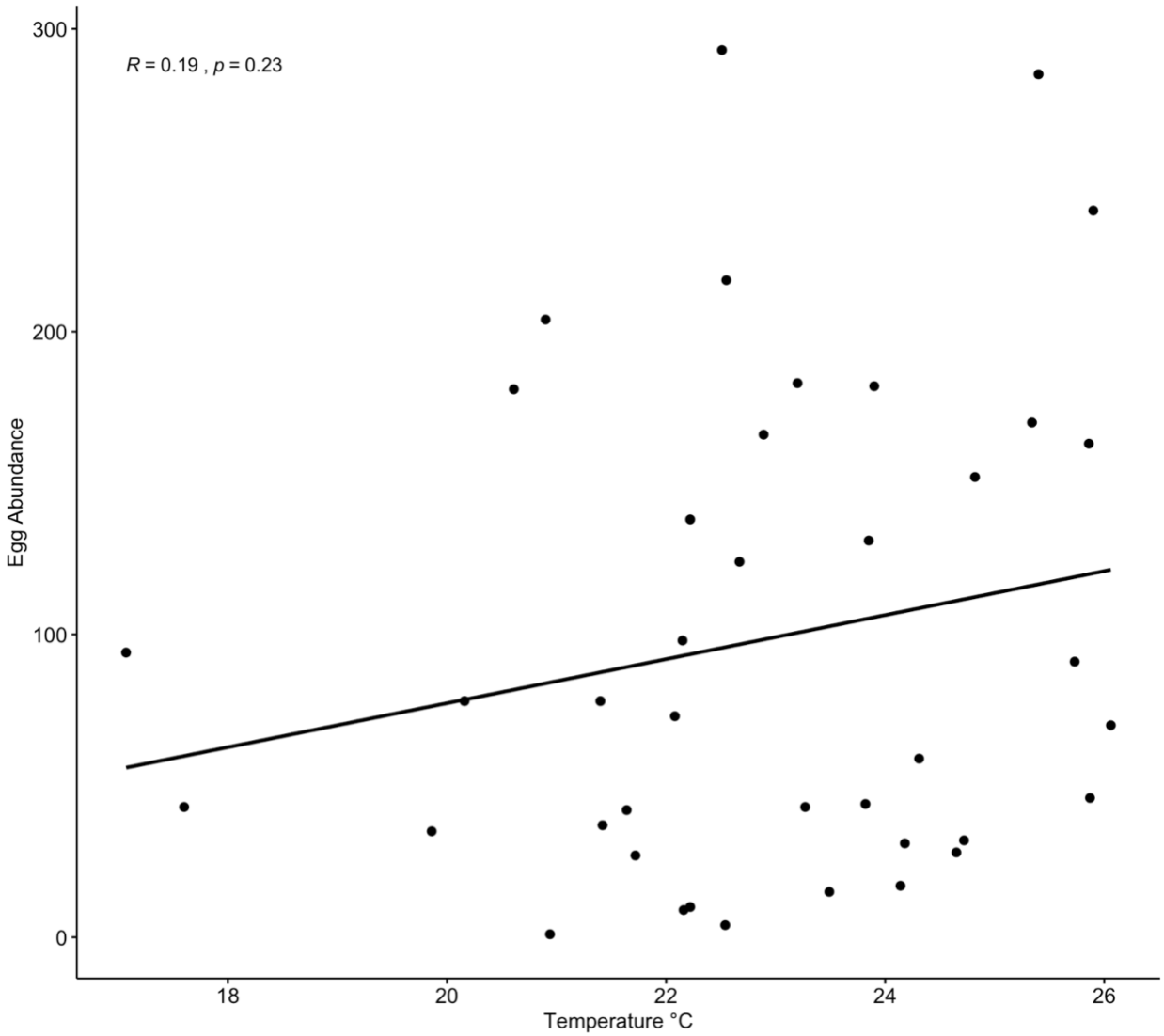


Figure 4: Lack of correlation between number of eggs collected and sea surface temperature on the day of collection from June-September 2018. Collections were made three times per week. $R^2 = 0.036$, p=0.23.


Figure 5: Monthly average spawning activity for nine species of interest, based on collections from Scripps Pier. Data from 2012-2014 from Harada *et al*. (2015), data from 2015-2017 from Duke, Harada, and Burton (2018).

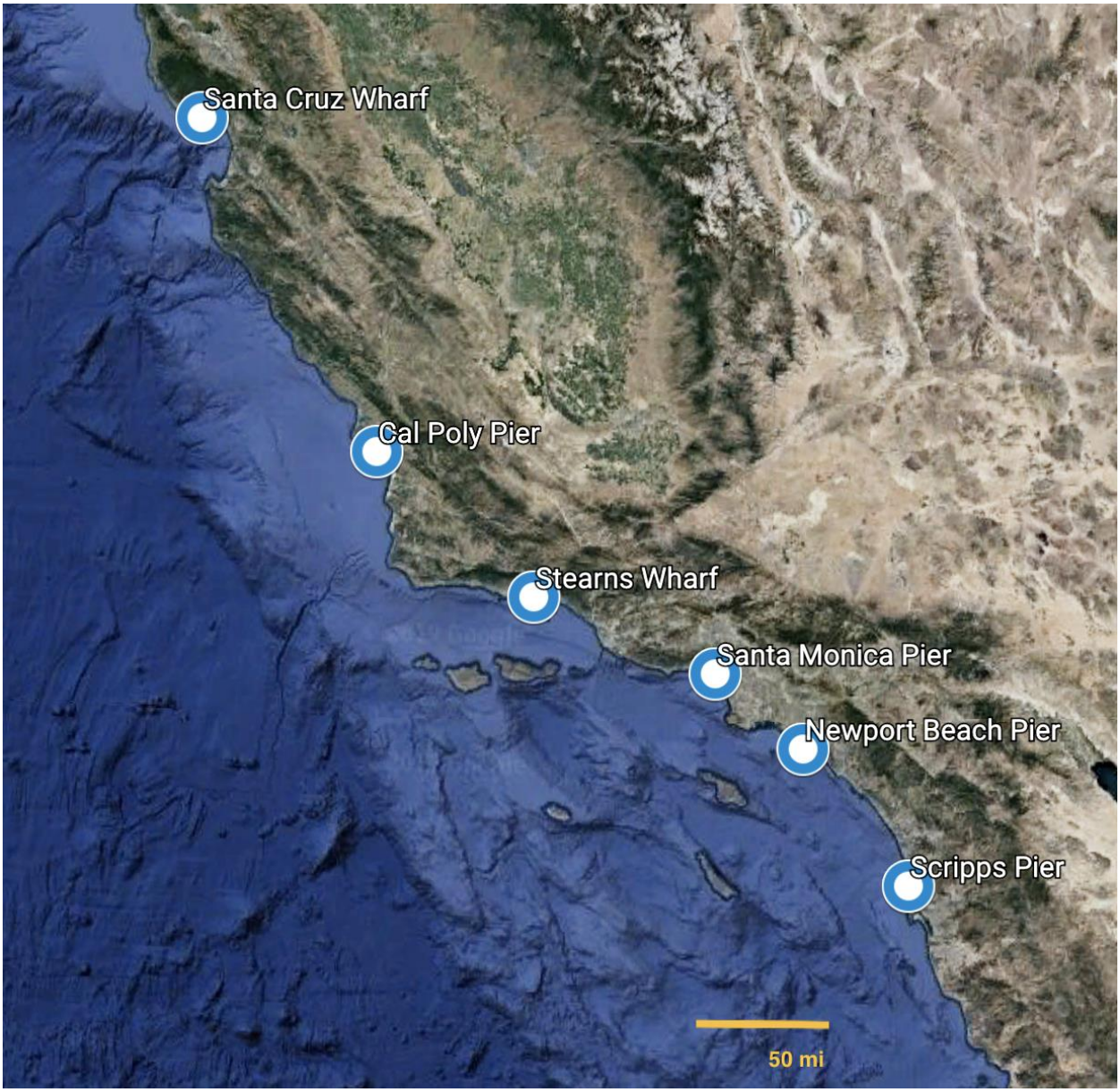

Figure 6: Locations of collection sites.

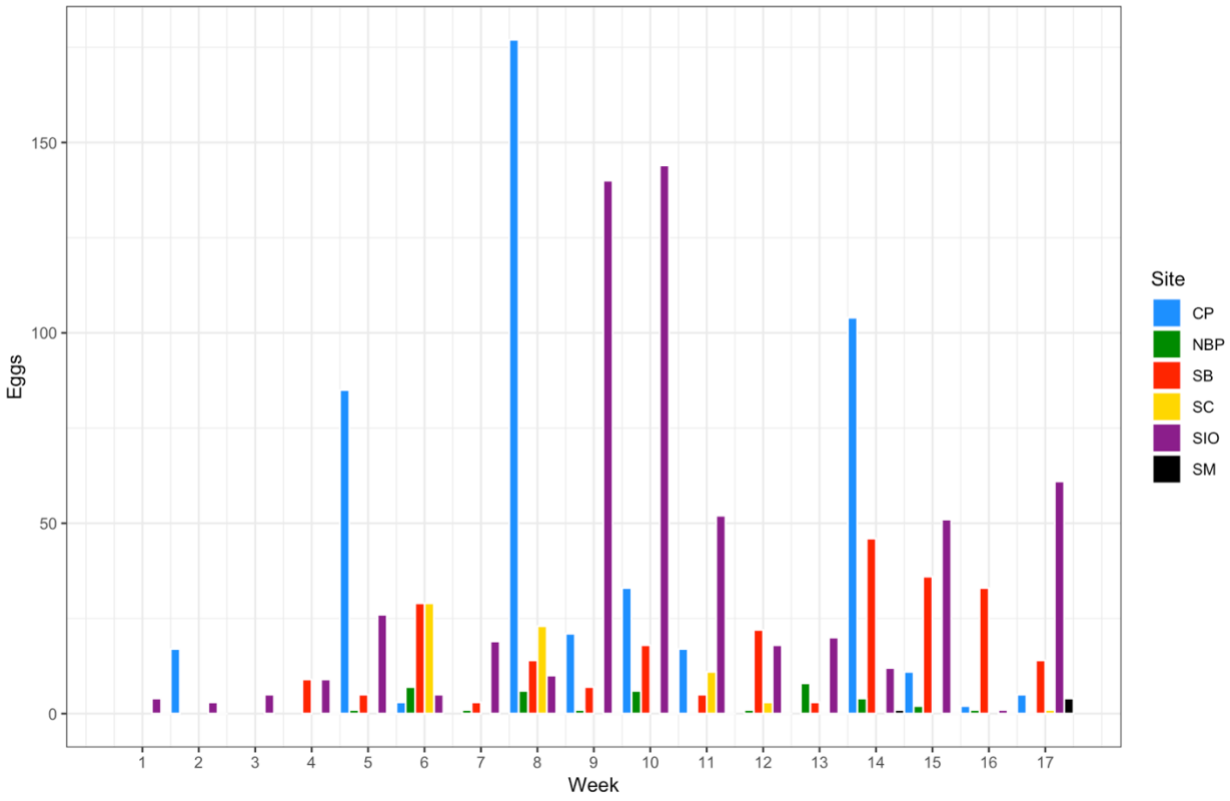


Figure 7: Weekly ichthyoplankton collections taken from six piers in California, from January through April 2019: Santa Cruz (SC), Cal Poly San Luis Obispo (CP), Santa Barbara (SB), Newport Beach (NBP), Santa Monica (SM), and Scripps Institution of Oceanography (SIO). Sites were unable to make collections during some weeks due to rough weather conditions that made collecting unsafe.

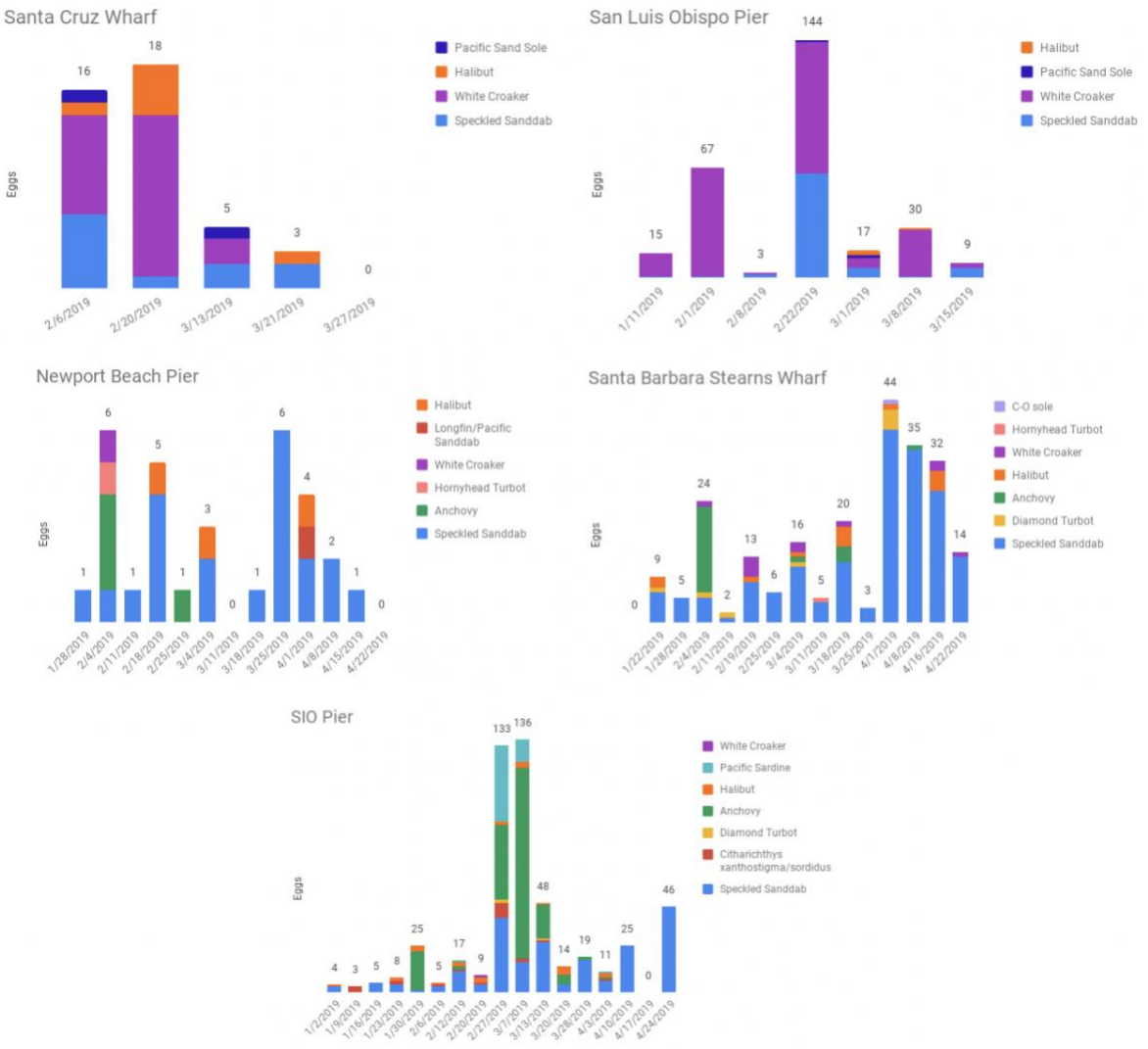


Figure 8: Species diversity in collections from five locations, January-April 2019

Table 1: Date of peak species diversity per year, excluding 2012 and 2019 due to lack of data for the summer months of those years. Data from 2013-2014 from Harada *et al.* (2015), data from 2015-2017 from Duke, Harada, and Burton (2018).

| Year | Date | Day of Year | Species |
|---|---|---|---|
| **2013** | 19-Jun | 170 | 15 |
| **2014** | 19-Jun | 170 | 18 |
| **2015** | 24-Jun, 23-Jul | 175, 204 | 10 |
| **2016** | 1-Jul | 183 | 14 |
| **2017** | 20-Jul | 201 | 19 |
| **2018** | 1-Aug | 213 | 17 |

Table 2: Complete species list of eggs identified from 2018 collections from Scripps Pier. List of all species collected (in order of egg abundance), total number of eggs collected, and number of collections (of 75 total) in which eggs were found.

| Species | Common name | Number of eggs collected | Number of collections |
|---|---|---|---|
| *Citharichthys stigmaeus* | Speckled sanddab | 1485 | 66 |
| *Sardinops sagax* | Pacific sardine | 429 | 22 |
| *Xenistius californiensis* | Californian salema | 406 | 31 |
| *Roncador stearnsii* | Spotfin croaker | 340 | 23 |
| *Halichoeres semicinctus* | Rock wrasse | 316 | 36 |
| *Menticirrhus undulatus* | California corbina | 307 | 27 |
| *Engraulis mordax* | Northern anchovy | 248 | 22 |
| *Oxyjulis californica* | Señorita | 191 | 29 |
| *Anisotremus davidsonii* | Xantic sargo | 59 | 15 |
| *Umbrina roncador* | Yellowfin croaker | 56 | 16 |
| *Paralichthys californicus* | California halibut | 54 | 30 |
| *Seriphus politus* | Queenfish | 48 | 19 |
| *Genyonemus lineatus* | White croaker | 41 | 9 |
| *Citharichthys sordidus* | Pacific sanddab | 34 | 17 |
| *Scomber japonicus* | Chub mackerel | 30 | 8 |
| *Citharichthys xanthostigma* | Longfin sanddab | 28 | 14 |
| *Paralabrax clathratus* | Kelp bass | 25 | 9 |
| *Symphurus atricaudus* | California tonguefish | 22 | 10 |
| *Semicossyphus pulcher* | California sheephead | 19 | 11 |
| *Pleuronichthys coenosus* | C-O sole | 13 | 12 |
| *Hermosilla azurea* | Zebra-perch sea chub | 12 | 9 |
| *Hypsopsetta guttulata* | Diamond turbot | 9 | 7 |
| *Paralabrax maculatofasciatus* | Spotted sand bass | 9 | 6 |

Table 2: Complete species list of eggs identified from 2018 collections from Scripps Pier. List of all species collected (in order of egg abundance), total number of eggs collected, and number of collections (of 75 total) in which eggs were found.

| **Species** | **Common name** | **Number of eggs collected** | **Number of collections** |
|---|---|---|---|
| *Girella nigricans* | Opaleye | 5 | 5 |
| *Paralabrax nebulifer* | Barred sand bass | 5 | 4 |
| *Xystreurys liolepis* | Fantail sole | 4 | 3 |
| *Cynoscion parvipinnis* | Shortfin weakfish | 4 | 2 |
| *Pleuronichthys verticalis* | Hornyhead turbot | 4 | 4 |
| *Etrumeus acuminatus* | Red-eye round herring | 3 | 1 |
| *Hypsoblennius jenkinsi* | Mussel blenny | 2 | 2 |
| *Seriola lalandi* | Yellowtail amberjack | 2 | 2 |
| *Fodiator acutus* | Sharpchin flyingfish | 2 | 2 |
| *Caulolatilus princeps* | Ocean whitefish | 1 | 1 |
| *Peprilus simillimus* | Pacific pompano | 1 | 1 |
| *Chilara taylori* | Spotted cusk-eel | 1 | 1 |
| *Ophidion scrippsae* | Basketweave cusk-eel | 1 | 1 |
| *Strongylura exilis* | Californian needlefish | 1 | 1 |

## Discussion

This study continued long-term monitoring efforts, observing temporal shifts in spawning and community composition of marine fishes in San Diego-Scripps Coastal SMCA and Matlahuayl SMR, and applied the trends and concepts seen over the last six years to set up baseline data at new monitoring sites along the California coast. Samples from 2018 showed a return to values similar to the baseline set in 2013-2014 (Harada *et al.*, 2015), after a period of depressed egg abundance (Duke, Harada, and Burton, 2018). This implied depressed spawning activity and was attributed to the Warm Blob and El Niño warm water events (Peterson and Bond, 2015; L'Heureux *et al.*, 2017). While the summer of 2018 also experienced a warm sea surface temperature event, reaching a record-high (Monroe, 2018), there was no evidence to suggest that this negatively affected the spawning activity. This is consistent with previous data showing no correlation between egg abundance and SST on the date of collection. Duke, Harada, and Burton (2018) found a significant negative correlation between winter SST and abundance of fish eggs collected at Scripps Pier the following summer, and the data from 2018 further supports this correlation. Based on SST from December 2018 to February 2019, and average of 15.85 $^{o}$C, we could tentatively predict that there will be an average of 50-100 eggs per collection during March through August of 2019. Sampling will continue for the foreseeable future and further monitor this relationship.

Effects of temperature on gametogenesis, spawning, migration, and larval development in fishes have been well-documented, although responses vary from species to species. Taranger and Hansen (1993) and Pankhurst and Munday (2011) showed that elevated temperatures can either shift or completely inhibit ovulation in some fish species, depending on the severity and duration of the thermal stress. Sims *et al.* (2004) and Genner *et al.* (2010) both found that

seasonal migrations, gonad maturation, and spawning occurred earlier during cooler years, which may explain the later shifts in peak spawning seen in some species during warmer years in San Diego. Genner *et al*. (2010) also found a significant relationship between SST in November and December, and the timing of spring spawners in April through July of the following year, much like the correlation found in this study. Pauly and Pullin (1987), as well as Pankhurst and Munday (2011), have observed that hatching time and larval development rate are generally faster when exposed to elevated temperatures, because the warmer temperatures decrease incubation time. The effects of this rushed hatching time vary, but may negatively influence individuals if there is a mismatch between timing of hatching and optimal timing for larval survival, such as emerging at night to avoid predation (Pankhurst and Munday, 2011). Elevated temperatures also increase metabolic rates, especially in larval fish, and so thermal stress, coupled with low oxygen or low food availability, would decrease chance of larval fish survival (Pankhurst and Munday, 2011). While all species will react differently to thermal stress, these observations are likely explanations for why 2018 was a relatively "normal" year for fishes spawning in the San Diego-Scripps Coastal SMCA and Matlahuayl SMR, although it is currently unknown if larval survivorship was negatively affected by the record-high SST in August 2018.

Based on data from this study, temperature has no discernible effect of species diversity. Occasionally, there are species found outside of their native range when the water is warmer, such as the Scalloped Hammerhead, *Sphyrna lewini,* and Blue Marlin, *Makaira nigricans,* both of which were spotted off the coast of California between 2013 and 2015 (Cavole *et al*., 2016). In general, though, the same species' eggs are present off the coast of San Diego every year, with little evidence of permanent range changes or new colonizing species thus far (Perry *et al*., 2005). However, Pinsky *et al.* (2019) concluded that marine ectotherms will be more vulnerable

than their terrestrial counterparts to warming and climate change, and are likely to experience more frequent species extirpations. This was attributed in part to the lack of access to thermal refugia in marine systems, as opposed to on land. While the data in this study have not shown evidence of local extinctions, further monitoring and the potential for more record-breaking sea surface temperatures or more frequent El Niño events may reveal long-term shifts (Cai *et al*., 2013).

Monitoring changes in peak spawning activity for specific species, particularly sportfish, coupled with knowledge of their life cycles, can be used to help regulate fishing seasons. For example, if the data showed that the local California corbina (*M. undulatus*) population spawned the most in July for the last three years, fishermen should know that they should target corbina after July to ensure that they are catching mature fish that have had the chance to spawn, thereby maintaining a sustainable fishery. If a warm-water event depresses spawning the previous year, managers of the corbina fishery could place stricter regulations for a year in order to allow the stock population to recover.

Ichthyoplankton surveys will not be a replacement for all monitoring of fish species in a given area. Trawl counts and diver surveys will still be needed, but ichthyoplankton can serve as a tool to supplement these methods. Expanding ichthyoplankton monitoring to other locations in California is necessary to ensure that these trends and methods are applicable outside of San Diego, and other automated shore stations were ideal candidates due to their existing oceanographic monitoring. The initial results taken from piers in Santa Cruz, San Luis Obispo, Santa Barbara, Santa Monica, and Newport Beach have revealed useful information on community composition in those locations, and continued sampling during the summer months will help establish a baseline for trends. These preliminary results suggest that there are

differences in community composition between sites north and south of Point Conception, California. Point Conception is a well-documented biogeographic barrier, especially for planktonic larvae (Doyle, 1985; Burton, 1998; Hohenlohe, 2004). A combination of the California Current, the Southern California Eddy, and upwelling at Point Conception sends plankton kilometers offshore and creates an abrupt temperature gradient, thereby making larval dispersal between the two sites difficult (Wares, Gaines, and Cunningham, 2001; Gaylord and Gaines, 2000). This separation would also contribute to a lack of gene flow between populations on either side, but further data from the ichthyoplankton samples will be needed in order to explore that possibility (Burton, 1998; Hohenlohe, 2004). If ichthyoplankton monitoring proves to be applicable and useful at these other locations, it is my hope that it will become a widespread tool, especially as costs and accessibility of DNA barcoding and next-generation sequencing continue to become more practical.

Temperature is not the only driver of variability in spawning, development, and population health in marine systems. Future research will be able to use these findings to look for similar patterns in factors such as pH, terrestrial runoff, or upwelling. Maintaining long-term time series data of both current reserves and potential areas of importance is vital to understanding how environmental variation and climate change will collectively affect marine organisms. Marine protected areas can serve as "control regions," that provide data on the impacts of anthropogenic influence, while serving as refuges for marine life and their habitats. Likewise, monitoring can be used to identify candidate sites that may become refuges in the future, providing data on populations before and after protections are put in place. Maintaining a network of monitoring stations across different latitudes will help establish how populations move and/or change their spawning habits in response to environmental change, and how MPAs

can move with them. As the climate changes, so must conservation efforts, and monitoring spawning activity in these areas will be essential to their success.

**References**


Ahern, A. L. M., Gómez-Gutiérrez, J., Aburto-Oropeza, O., Saldierna-Martínez, R. J., Johnson, A. F., Harada, A. E., Sánchez-Uvera, A. R., Erisman, B., Arvizú, D. I.C., & Burton, R. S. (2018). DNA sequencing of fish eggs and larvae reveals high species diversity and seasonal changes in spawning activity in the southeastern Gulf of California. *Marine Ecology Progress Series*. https://doi.org/10.3354/meps12446

Ahlstrom, E. H., & Moser, G. (1976). EGGS AND LARVAE OF FISHES AND THEIR ROLE IN SYSTEMATIC INVESTIGATIONS AND IN FISHERIES. *Revue Des Travaux de l'Institut Des Pêches Maritimes*, *40*, 379–398.

Allison, G. W., Lubchenco, J., & Carr, M. H. (2019). Marine Reserves are Necessary but not Sufficient for Marine Conservation. *Ecological Applications*, *8*(1).

Asch, R. G., Cheung, W. W. L., & Reygondeau, G. (2018). Future marine ecosystem drivers, biodiversity, and fisheries maximum catch potential in Pacific Island countries and territories under climate change. *Marine Policy*, *88*, 285–294. https://doi.org/https://doi.org/10.1016/j.marpol.2017.08.015

Burton, R. S. (1998). Intraspecific Phylogeography Across the Point Conception Biogeographic Boundary. *Evolution*, *52*(3), 734–745. https://doi.org/10.2307/2411268

Cai, W., Borlace, S., Lengaigne, M., van Rensch, P., Collins, M., Vecchi, G., Timmermann, A., Santoso, A., McPhaden, M.J., Wu, L., England, M.H., Wang, G., Guilyardi, E., Jin, F.-F. (2014). Increasing frequency of extreme El Niño events due to greenhouse warming. *Nature Climate Change*, *4*, 111. Retrieved from https://doi.org/10.1038/nclimate2100

Carscadden, J., Nakashima, B. S., & Frank, K. T. (1997). Effects of fish length and temperature on the timing of peak spawning in capelin ( Mallotus villosus ). *Canadian Journal of Fisheries and Aquatic Sciences*, *54*, 781–787.

Cavole, L. M., Demko, A. M., Diner, R. E., Giddings, A., Koester, I., Pagniello, C. M. L. S., Paulsen, M.-L., Ramirez-Valdez, A., Schwenck, S.M., Yen, N.K., Zill, M.E., & Franks, P. J. S. (2016). Biological impacts of the 2013–2015 warm-water anomaly in the Northeast Pacific: Winners, losers, and the future. *Oceanography*, *29*(2), 273–285.

Craig, M., Fodrie, F. J., & Hastings, P. (2004). The Nearshore Fish Assemblage of the Scripps Coastal Reserve, San Diego, California. *Coastal Management*, *32*, 341–351. https://doi.org/10.1080/08920750490487188

Doyle, R. F. (1985). *Biogeographical Studies of Rocky Shores Near Point Conception, California*. University of California, Santa Barbara. Retrieved from https://books.google.com/books?id=M8ITAQAAIAAJ

Duke, E. M. (2018). Large inter-annual variability of spawning in San Diego's marine protected areas captured by molecular identification of fish eggs. University of California San Diego.

Duke, E. M., Harada, A. E., & Burton, R. S. (2018). Large interannual variation in spawning in San Diego marine protected areas captured by molecular identification of fish eggs. *Marine Ecology Progress Series*, *604*, 199–210. https://doi.org/10.3354/meps12738

Fincham, J. I., Rijnsdorp, A. D., & Engelhard, G. H. (2013). Shifts in the timing of spawning in sole linked to warming sea temperatures. *Journal of Sea Research*, *75*, 69–76. https://doi.org/10.1016/j.seares.2012.07.004

Gaylord, B., & Gaines, S. D. (2000). Temperature or Transport ? Range Limits in Marine Species Mediated Solely by Flow. *The American Naturalist*, *155*(6), 771–789.

Gell, F. R., & Roberts, C. M. (2003). Benefits beyond boundaries : the fishery effects of marine reserves. *TRENDS in Ecology and Evolution*, *18*(9), 448–455. https://doi.org/10.1016/S0169-5347(03)00189-7

Genner, M. J., Halliday, N. C., Simpson, S. D., Southward, A. J., & Hawkins, S. J. (2010). Temperature-driven phenological changes within a marine larval fish assemblage. *Journal of Plankton Research*, *32*(5), 699–708. https://doi.org/10.1093/plankt/fbp082

Harada, A. E., Lindgren, E. A., Hermsmeier, M. C., Rogowski, P. A., Terrill, E., Burton, R. S., & Bernardi, G. (2015). Monitoring spawning activity in a Southern California marine protected area using molecular identification of fish eggs. *PLoS ONE*, *10*(8), 1–21. https://doi.org/10.1371/journal.pone.0134647

Hastings, P. A., & Burton, R. S. (2008). *Establishing a DNA Sequence Database for the Marine Fish Fauna of California*.

Hastings, P. A., Craig, M. T., Erisman, B. E., & Hyde, J. R. (2014). Fishes of Marine Protected Areas Near La Jolla , California. *Bulletin of the Southern California Academy of Sciences*, *113*(3), 200–231.

Hofmann, T., Knebelsberger, T., Ulleweit, M., Kloppmann, J., & Raupach, M. J. (2017). Egg identification of three economical important fish species using DNA barcoding in comparison to a morphological determination. *Journal of Applied Ichthyology*, *33*, 925–932. https://doi.org/10.1111/jai.13389

Hohenlohe, P. A. (2004). Limits to gene flow in marine animals with planktonic larvae: models of Littorina species around Point Conception , California. *Biological Journal of the Linnean Society*, *82*, 169–187.

Ivanova, N. V., Zemlak, T. S., Hanner, R. H., & Hebert, P. D. N. (2007). Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes*. https://doi.org/10.1111/j.1471-8286.2007.01748.x

L'Heureux, M. L., Takahashi, K., Watkins, A. B., & Wittenberg, A. T. (2017). Observing and predicting the 2015/16 el Niño, (July), 1363–1382. https://doi.org/10.1175/BAMS-D-16-0009.1

Leis, J. M., & Miller, J. M. (1976). Offshore distributional patterns of Hawaiian fish larvae. *Marine Biology*, *36*(4), 359–367. https://doi.org/10.1007/BF00389198

Lubchenco, J., Palumbi, S. R., Gaines, S. D., & Andelman, S. (2003). PLUGGING A HOLE IN THE OCEAN : THE EMERGING SCIENCE OF MARINE RESERVES 1. *Ecological Applications*, *13*(1), 3–7.

Markle, D. F., & Frost, L.-A. (1985). Comparative morphology , seasonality , and a key to planktonic fish eggs from the Nova Scotian shelf. *Canadian Journal of Zoology*, *63*(2), 246–257.

McLeod, E., Salm, R., Green, A., & Almany, J. (2009). Designing marine protected area networks to address the impacts of climate change. *Frontiers in Ecology and the Environment*, *7*(7), 362–370. https://doi.org/10.1890/070211

Monroe, R. (2018). Highest-Ever Seawater Temperature Recorded at Scripps Pier.

Pankhurst, N. W., & Munday, P. L. (2011). Effects of climate change on fish reproduction and early life history stages. *Marine and Freshwater Research*, *62*, 1015–1026.

Pauly, D., & Pullin, R. S. V. (1988). Hatching time in spherical, pelagic, marine fish eggs in response to temperature and egg size. *Environmental Biology of Fishes*, *22*(4), 261–271.

Perry, A. L., Low, P. J., Ellis, J. R., & Reynolds, J. D. (2005). Climate Change and Distribution Shifts in Marine Fishes. *Science*, *308*, 1912–1916.

Peterson, W., Robert, M., & Bond, N. (2015). The warm Blob continues to dominate the ecosystem of the northern California Current. *PICES Press*, *23*(2), 44–47.

Pinsky, M. L., Eikeset, A. M., McCauley, D. J., Payne, J. L., & Sunday, J. M. (2019). Greater vulnerability to warming of marine versus terrestrial ectotherms. *Nature*, *569*(7754), 108–111. https://doi.org/10.1038/s41586-019-1132-4

Psettichthys melanostictus. (n.d.). Retrieved from https://www.fishbase.se/summary/4255

Sims, D. W., Mouth, V. J. W., Genner, M. J., Southward, A. J., & Hawkins, S. J. (2004). Low-temperature-driven early spawning migration of a temperate marine fish. *Journal of Animal Ecology*, *73*, 333–341.

Taranger, G. L., & Hansen, T. (1993). Ovulation and egg survival following exposure of Atlantic salmon , Salmo salar L ., broodstock to different water temperatures. *Aquaculture and Fisheries Management*, *24*, 151–156.

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. N. (2005). DNA barcoding Australia ' s fish species. *Philosophical Transactions of the Royal Society B*, *360*, 1847–1857. https://doi.org/10.1098/rstb.2005.1716

Wares, J. P., Gaines, S. D., & Cunningham, C. W. (2001). A COMPARATIVE STUDY OF ASYMMETRIC MIGRATION EVENTS ACROSS A MARINE BIOGEOGRAPHIC BOUNDARY, *55*(2), 295–306.