UNIVERSITY OF CALIFORNIA SAN DIEGO

Monitoring spatial and temporal variation in the spawning activity of nearshore marine fishes along the California coast via the molecular identification of fish eggs

A thesis submitted in partial satisfaction of the requirements for

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in

Marine Biology

by

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The thesis of Emma Choi is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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ABSTRACT OF THESIS

Monitoring spatial and temporal variation in the spawning activity of nearshore marine fishes along the California coast via the molecular identification of fish eggs

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Many studies have used the spawning activity of fishes to assess the abundance, distribution, and reproductive success of populations. While spawning activity of marine fishes in offshore waters has been studied extensively, little is known about the spawning activity that occurs in near shore communities. This study utilized weekly fish egg sampling in order to construct a seven-year time series of fish spawning near the Scripps Pier in La Jolla, California. Additionally, fish egg sampling was recently introduced to five other sites

along the California coast – Santa Cruz, San Luis Obispo, Santa Barbara, Santa Monica, and Newport Beach. The majority of fish eggs collected have been identified to a species level through the use of DNA barcoding of the COI and 16S genes. Strong seasonal trends have been identified in the spawning seasons of fishes in La Jolla. However, the spawning activity within the summer spawning season, varies greatly among years, both in egg production and species diversity. The unpredictable shifts in spawning activity pose a threat to the stability of their populations and need to be analyzed further to identify factors responsible for these changes. While only one year of data has been collected from the newer sites, the two sites North of Point Conception show greatly reduced diversity in species composition compared to the Southern sites. The extent of spatial variation between sites will become more evident as more data is gathered from continuous sampling.

Introduction

Nearshore ecosystems are highly productive, contributing a great deal to the prosperity of the economy and the environment (Beck et al. 2001; Costanza et al. 1997; Mann 2000; Barth et al. 2007). In particular along the California coast, the diverse and abundant populations of marine fish serve as a valuable resource for both commercial and recreational fisheries (Methot 1983; California Department of Fish and Wildlife 2002). However, the dynamic conditions of the coastal marine environment and fishing pressures can lead to fluctuations in the abundance, diversity, and distributions of these species (Mann 2000). As a result, they need to be monitored across robust spatial and temporal scales in order to implement effective management and conservation strategies. Despite this, only a limited number of studies have been conducted on these scales for fish communities in near-shore environments along the California Coast.

Traditional survey methods include diver surveys and trawls, however, using these methods it is possible to miss cryptic species and early life stages (Brock 1982; Steward and Beukers 2000). Ichthyoplankton surveys, the collection of fish eggs and larvae, can complement the traditional methods by accounting for the species at risk of being overlooked (Jaafar et al. 2012). Through the use of molecular methods, even the morphologically similar ichthyoplankton can be accurately identified to species level (Ward et al. 2009; Gleason and Burton 2012; Harada et al. 2015; Duke et al. 2018). Ichthyoplankton sampling has been successfully employed to classify spawning seasons, estimate the abundance of adult spawning biomass, and assess the species composition of spawning communities (Ahlstrom and Moser, 1976; Hunter and Lo 1993; Duke et al. 2018; Harada et al. 2015). Additionally,

identifying changes in larval fish assemblages has been used to classify environmental changes as ecosystem indicators (Brodeur et al. 2006).

Ichthyoplankton surveys have been successfully employed to monitor the spawning activity of fishes in the California Current. For example, the California Cooperative Oceanic Fisheries Investigations (CalCOFI) survey cruises have produced notable temporally and spatially robust datasets for ichthyoplankton located in offshore communities in the California Current. As a complement to these surveys, Brewer and Smith (1982) deployed cruises for nearshore ichthyoplankton monitoring from 1978-1980, focusing on larvae from Northern anchovy (*Engraulis mordax*) and Pacific sardine (*Sardinops sagax*), and Jahn et al. (1984) gathered coastal ichthyoplankton samples from 1977-1979 documenting shifts in ichthyoplankton as the distance from shore increased. Through these cruises, differences in larval abundance between the nearshore and offshore environments have been observed in multiple species emphasizing the need for monitoring coastal ecosystems.

This study continues the sampling efforts of Harada (2015) and Duke (2018), which were initiated in 2012 at the Scripps Pier (SIO) located in La Jolla, California at the boundary of two Marine Protected Areas (MPAs), the San Diego-Scripps Coastal State Marine Conservation Area (SMCA) and the Matlahuayl State Marine Reserve (SMR). Through these data, extensive interannual variation in egg abundance during the summer spawning season has been established and linked to anomalously warm winter sea surface temperatures (SST). We continued sampling at SIO through 2019 to determine the productivity of the 2018 and 2019 spawning seasons, determine if the correlation between SST and egg abundance is upheld, and assess the relationship between egg abundance and species diversity. Additionally, in 2019, we initiated sampling at five other locations, Santa Cruz (SC), San Luis

Obispo (CP), Santa Barbara (SB), Santa Monica (SM), and Newport Beach (NBP), spanning a gradient of latitudes along the California coast. Using these baseline data, we explore how species diversity changes across latitude and provide preliminary results as to which species are spawning at the study locations.

Methods

Egg Collection and Quantification

Weekly fish egg collections were completed using vertical plankton tows conducted off the ends of Scripps Pier (SIO), Newport Beach Pier (NBP), Santa Monica Pier (SM), Stearns Wharf Pier in Santa Barbara (SB), Avila Pier in San Luis Obispo (CP), and the Santa Cruz Wharf Pier (SC). A plankton net (505µ mesh) was lowered to the seafloor and raised back out of the water, funneling pelagic eggs into the bottle at the cod end as it rises. This process was repeated multiple times to increase the volume of water being sampled, however, due to local logistics, the number of tows and other sampling factors, varies by location. A comparison of sampling sites and methods can be seen in Table 1. After the tows were completed, the net was lowered a final time, but only until the rim touched the surfaced of the water, and then brought up guiding any residual eggs left in the net into the bottle at the cod end. The contents of the cod end were transferred to a 1-Liter container and brought back to the lab where they were immediately poured through a mesh screen (330µm) to concentrate the plankton.

At SIO, the concentrated plankton sample was placed in a petri dish with seawater and put under a microscope. At the other 5 locations, the concentrated plankton sample was stored in 95% ethanol in a 50 mL falcon tube and shipped to SIO where it was poured into a petri dish and put under a microscope. There, the fish eggs were removed and placed in 1.5 mL microtubes with 95% ethanol. The morphologically distinct eggs of the Northern anchovy (*Engraulis mordax*) and the Pacific sardine (*Sardinops sagax*) were quantified and stored separately from the rest of the eggs. The eggs that remained to be identified were stored at -20°C for at least 24 hours until further processing.

DNA Extraction, Amplification, Sequencing, and Identification

The extraction, amplification, sequencing, and identification steps are in accordance with the protocols used by Harada et al. (2015) and Duke et al. (2018). Each egg was placed in an individual well of a 0.2 mL PCR strip tube. The ethanol was removed from each well and each egg was rinsed with 90 µL of nuclease-free water. The water was removed and 15 µL of a 66% AE buffer solution (Qiagen) was added to each well. The samples were then placed in a thermal cycler at 95ºC for 15 minutes and maintained in a 72ºC hold until their removal. A clean pipette tip was used to compress each egg until it popped, expelling the DNA into the AE buffer solution. The DNA was stored at -20ºC until further processing.

The DNA was thawed at room temperature. A 25 µL PCR reaction was prepared for each egg's DNA with 12.5 µL of GoTaq Green Master Mix, 10.5 µL of molecular grade water, 0.5 µL of each primer, and 1 µL of DNA. The first primer pair used was the CO1 universal primers from Ivanova et al. (2006): 5' TTCTCAACCAACCACAAAGACATTGG 3' (forward) and 3' ACTTCYGGGTGRCCRAARAATCA 5' (reverse). Each sample was vortexed to ensure the contents of each well were mixed. The samples were then placed in the thermocycler following the cycler conditions utilized by Harada and Duke. The PCR product of each sample was checked on a 1.5% agarose gel for a band length of 710 base pairs. The samples with the correct band size were purified and sent for Sanger sequencing. The PCR step was repeated for the samples lacking bands using the 16S primer set: 5' CGCCTGTTATCAAAAACAT 3' (forward) and 3' TGCACTAGACTCAAGTCTGGCC 5' (reverse) from Palumbi et al. (1996). The thermocycler conditions remain the same, with the exception of reducing the number of cycles to 30 instead of 35. The PCR products of the 16S

PCR reaction were checked on a 1.5% agarose gel for a 570 base pair band. Samples with the correct sized band were purified and sent for sequencing.

PCR products were purified according to Harada and Duke and sent to Retrogen Inc. (San Diego) for Sanger sequencing in 10 μL reactions, with 9 μL of purified PCR product and 1 μL of either CO1 or 16S forward primer, depending on which primer was used in the corresponding PCR. The sequencing results were run through NCBI's Basic Local Alignment Search Tool, or BLAST, which compares our samples to thousands of sequences available on GenBank. The addition of sequences from Hastings and Burton (2008) greatly contribute to the robustness of the database for CO1 and 16S sequences of marine fish common to southern Californian waters. If our sequences matched a sequence in the database at 95% or higher, it was classified as the species corresponding to that sequence. However, two closely related species, Longfin sanddab (*Citharichthys xanthostigma*) and Pacific Sanddab (*Citharichthys sordidus*), could only be differentiated from each other if the sequences matched at greater than 99%. For these two species, if sequences matched between 95% and 99% they were recorded as ambiguous (one of the two species).

Temperature Data

The data used to calculate the average annual SST (°C) and the average annual winter SST (°C) were obtained from the Southern California Coastal Ocean Observing System (SCCOOS) website. Temperature measurements are recorded approximately every four minutes from a sensor located two meters below the surface. The annual and seasonal averages were calculated from a subset dataset of daily averages and the standard error was

calculated using the formula $SE = \frac{sd}{\sqrt{n}}$ where SE is the standard error, sd is the standard deviation, and n is the number of samples.

Species Diversity Analysis

The temporal and spatial analyses for species diversity were performed on subsets of data from each year/site to mitigate the effects of variable sampling efforts. The minimum number of samples (n) collected in a year at SIO 2013-2019 (temporal analysis) and at a site during 2019 (spatial analysis) was identified. Then, n samples from each of the other years/sites were chosen at random, and the total egg abundance, species richness, and effective number of species (ENS) were calculated and stored in R. This process was repeated 1000 times and the mean, standard deviation, and standard error of the egg abundance, species richness, and ENS were calculated from the 1000 trials. The mean and standard deviation were used to create the plots displayed in the species diversity analysis section of the results.

The egg abundance, species richness, and ENS were calculated in the following ways: total egg abundance = the sum of eggs identified in each sample, species richness = the number of unique species identified, and the effective number of species (ENS) = exp(H) as described by Hill (1973) where H is the Shannon diversity index given by Shannon and Wiener (Shannon and Weaver 1949). The Shannon diversity index was calculated using the vegan package in RStudio (Oksanen et al. 2019) with the formula: $H = -\sum_{i=1}^{S} p_i \ln p_i$ where p_i is the proportional abundance of each species i and S is the number of species so that $\sum_{i=1}^{S} p_i = 1$.

Table 1: 2019 Location Methodology Comparison

Location	SIO	NBP	SM	SB	CP	SC
Sampling Start Date	1-2-2019	1-28-2019	1-2-2019	1-22-2019	1-11-2019	2-6-2019
Sampling End Date	12-26-2019	12-31-2019	12-23-2019	12-30-2019	12-13-2019	12-19-2019
Sampling Effort	65	44	45	49	29	34
Latitude	32° 52’ 2 ” N	33°36'21.7 "N	34°00'27.0 "N	34°24'29.1 "N	35°10'12.6 "N	36°57'26.2 "N
Longitude	117° 15’ 26 ” W	117°55'52.0 "W	118°29'60.0 "W	119°41'05.9 "W	120°44'26.4 "W	122°01'02.2 "W
Net Diameter (m)	1	0.5	0.75	0.5	1	0.75
Number of Tows	4	4	4	16	4	4
Depth (m)	5	7	6	6	9	5
Sample Volume (m^3)	64	30	44	64	112	45
Method	Crane	Hand	Hand	Hand	Crane	Hand

Results

SIO Temporal Monitoring and Analysis

Over the seven-year period, 24,579 eggs have been identified to species level representing 47 different species. Eighteen species were observed every year with Speckled sanddab (*Citharichthys stigmaeus*), Senorita (*Oxyjulis californica*), Pacific sardine (*Sardinops sagax*), Californian salema (*Xenistius californiensis*), and Northern anchovy (*Engraulis mordax*) being the most abundant (Figure 1). While there are fewer species present in the low abundance years (2015, 2016, and 2019), there are no species present in all the high egg abundance years and absent from the low egg abundance years. Further, the addition of 2018 and 2019 sampling at SIO reveals that the egg abundance per sample, throughout the spawning season (roughly May 1st – August 30th) is elevated in 2018 but reduced in 2019 (Figure 2). Although it is worth noting that even within the years that have depressed summer spawning activity, the summer has slightly elevated activity compared to the rest of the year, distinguishing the spawning season.

We wanted to explore the hypothesis proposed by Duke, pertaining to the relationship between the average winter SST and the average spring-summer egg abundance, so we reproduced the analysis adding our two additional data points (2018 and 2019). The weekly SST calculated over a 3-week rolling average is shown in Figure 3A. and the annual and seasonal SST averages are given by Table 2. There is a negative correlation, (σ = -0.89), between the average winter (December – February) SST and the average spring-summer (March – August) egg abundance (Figure 3B). The standard error of the mean spring-summer egg abundance is given by the error bars and the additional 2018 and 2019 points are colored in red. The strength of this relationship has slightly decreased from Duke's initial observation

but remains fairly strong maintaining the idea that temperature may play an important role for determining levels of fish reproduction.

We aimed to resolve whether the reduced egg abundance in the less productive spawning seasons is related to the number of species contributing to the spawning season by looking at the species diversity of the fish eggs we collected during the summer spawning seasons of each year. There is a strong, positive relationship ($\sigma = 0.92$) between the total number of eggs identified during the spawning season and the species richness of the corresponding season (Figure 4A.). However, species richness (defined from our sampling) is expected to increase as the number of eggs from each species (in the wild) increases because it increases the likelihood of the species being captured through our sampling. The Shannon diversity index, which takes into account both richness and eveness, is less susceptible to the same bias. When using Shannon diversity, converted to ENS, to compare the relationship between egg abundance and species diversity (Figure 4B.) the relationship weakens ($\sigma = 0.7$). In particular, the ENS of 2015 and 2019 (low egg abundance years), which both have much lower species richness than the high abundance years, is nearly identical to the ENS of 2014.

Spatial Monitoring and Analysis

During 2019 4,277 eggs were identified, belonging to 32 different species across the six sites with only two, Speckled sanddab (*Citharichthys stigmaeus*) and California halibut (*Paralichthys californicus*), being present at all sites (Figure 5). There are six species, California tonguefish, Queenfish, California corbina, Spotfin croaker, C.O. sole, and Rock wrasse, present at all four southern sites that are absent at the two northern sites and there is one species, Pacific sand sole, that is only present at the two northern sites and absent from

the other four. Interestingly, at SIO, the only location located within an MPA, there are nine species present that are absent from the other five locations.

In addition to the differences in species' distributions of eggs, the introduction of sampling at new locations revealed that the sites vary in egg abundance and apparent spawning seasons (or lack thereof). SC, SM, and NBP lack large peaks in egg abundance, while CP, SB, and SIO all display periods of elevated egg abundance (Figure 6). It is interesting to note that of the three sites with large peaks in egg abundance, the peak in CP is during winter, whereas the peaks at SB and SIO occur during summer months. Further sampling is required to determine if the baseline data shown here are representative of the trends at each site.

Species richness and Shannon diversity were used to compare species diversity across a latitudinal gradient (Figure 7). Latitude and species richness hold a strong, negative relationship (σ = - 0.84) with SIO having the highest species richness (N = 25) by a large margin and CP (N = 4) and SC (N = 4) having the lowest species richness, also by a large margin (Figure 7A). This finding complements the distribution of species' eggs shown by the presence/absence chart (Figure 5), in which there are very few species present at CP and SC. A similar, although weaker, trend (σ = - 0.66) is given by the ENS defined through Shannon diversity (Figure 7B). It is significant that despite the limited number of eggs collected from NBP and SM, there are greater than 10 species identified and regardless of the considerable number of eggs from CP, there are only 4 species identified. The ENS at SB is lower than both CP and SC, however, the three most northern sites are still markedly less diverse than the three southern sites.

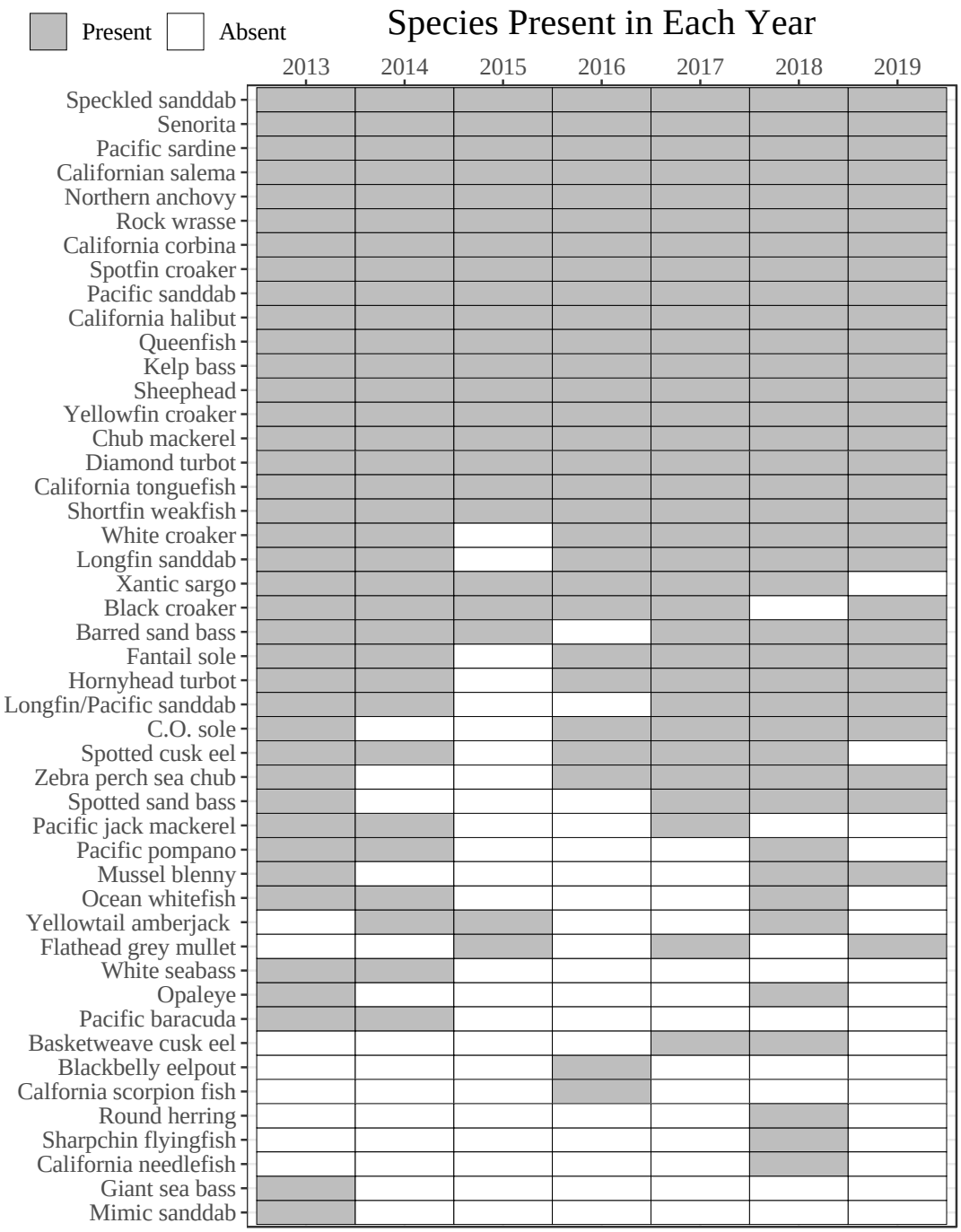

Figure 1: SIO Annual Species Presence. The chart below displays the species present in each year. A gray box indicates the presence of at least one egg from the given species in our samples in the given year, while a white box indicates the absence of eggs from that species. The species are primarily ranked in descending order of the number of years present and within those rankings are in descending order of egg abundance throughout all seven years.

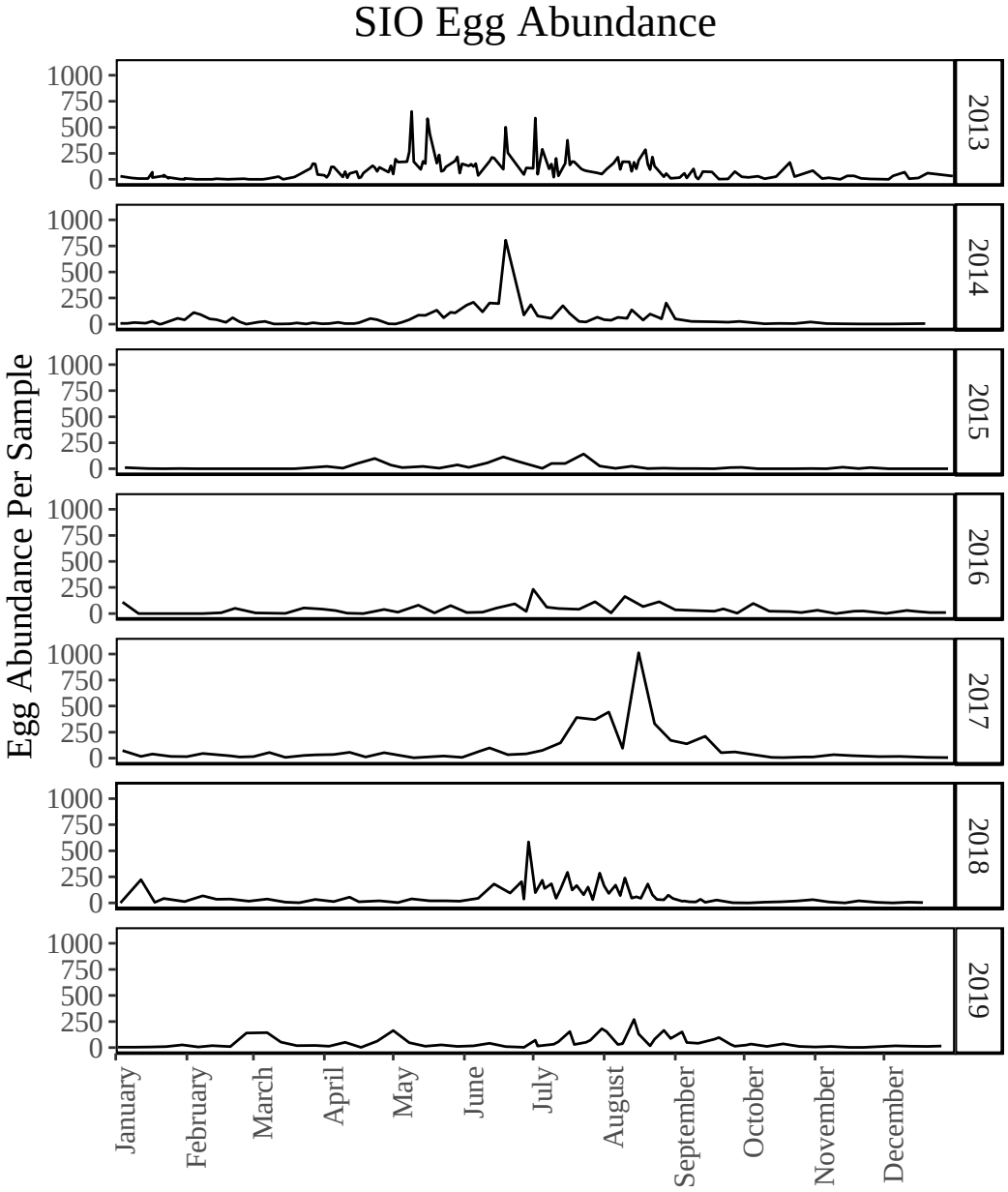

Figure 2: SIO Egg Abundance 2013 – 2019. The raw annual distribution of the number of eggs present in each sample (eggs per ~16m^3 seawater) plotted by the calendar day and paneled out by year.

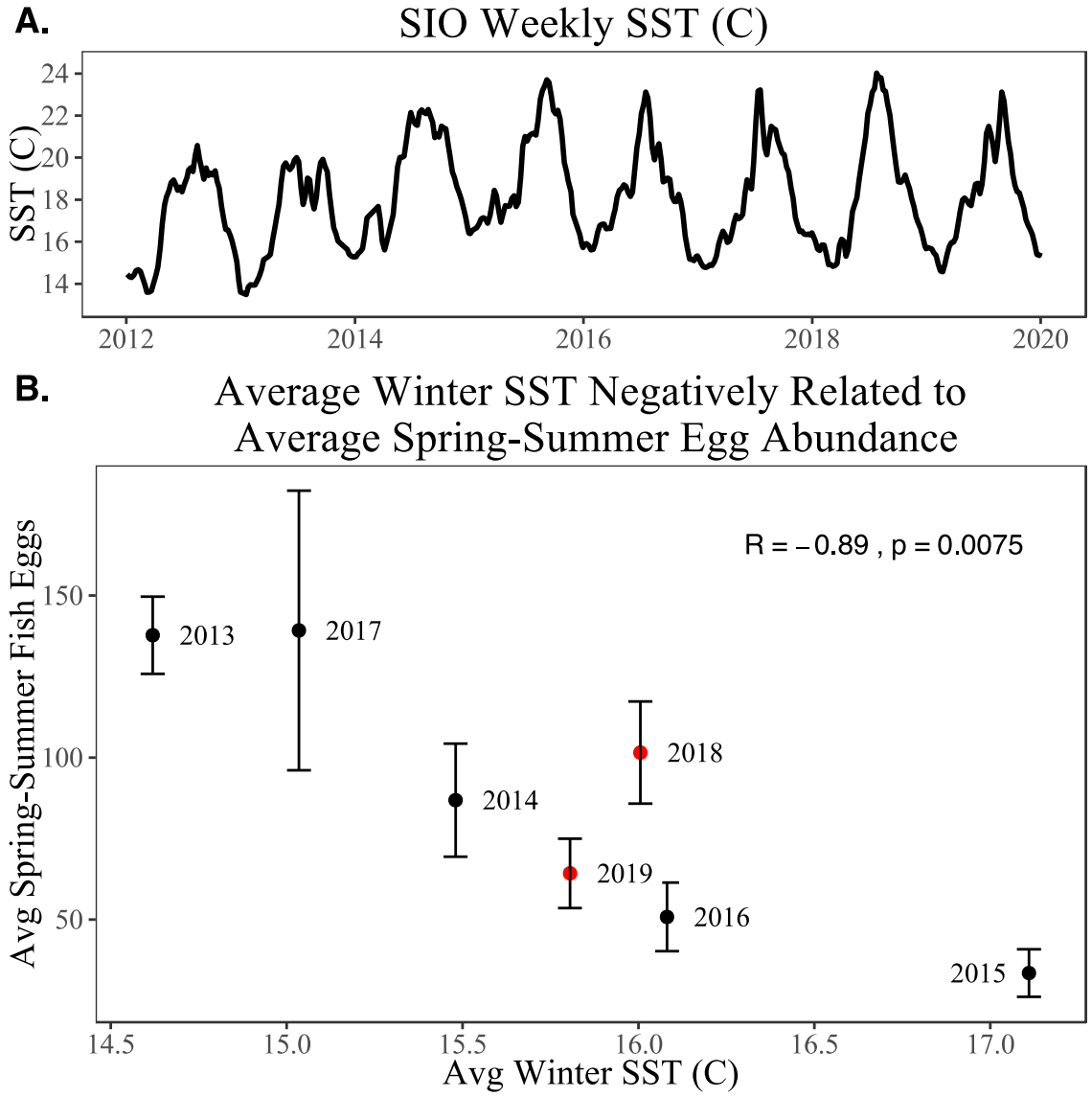

Figure 3: SIO Pier SST. 3A. The weekly average of SST calculated on a three-week rolling average, from the daily averages of SST measurements provided by the SCCOOS sensors attached to the SIO pier at 2m depth. 3B. The correlation (σ = -0.89) between the average winter (December – February) SST, calculated from the daily averages of SST measurements, and the average spring – summer (March – August) fish eggs. The error bars represent the standard error of the annual spring – summer mean in fish egg abundance. The black points (2013 – 2017) are data points originally identified and calculated by Duke (2018) and the red points are the additional 2018 and 2019 data.

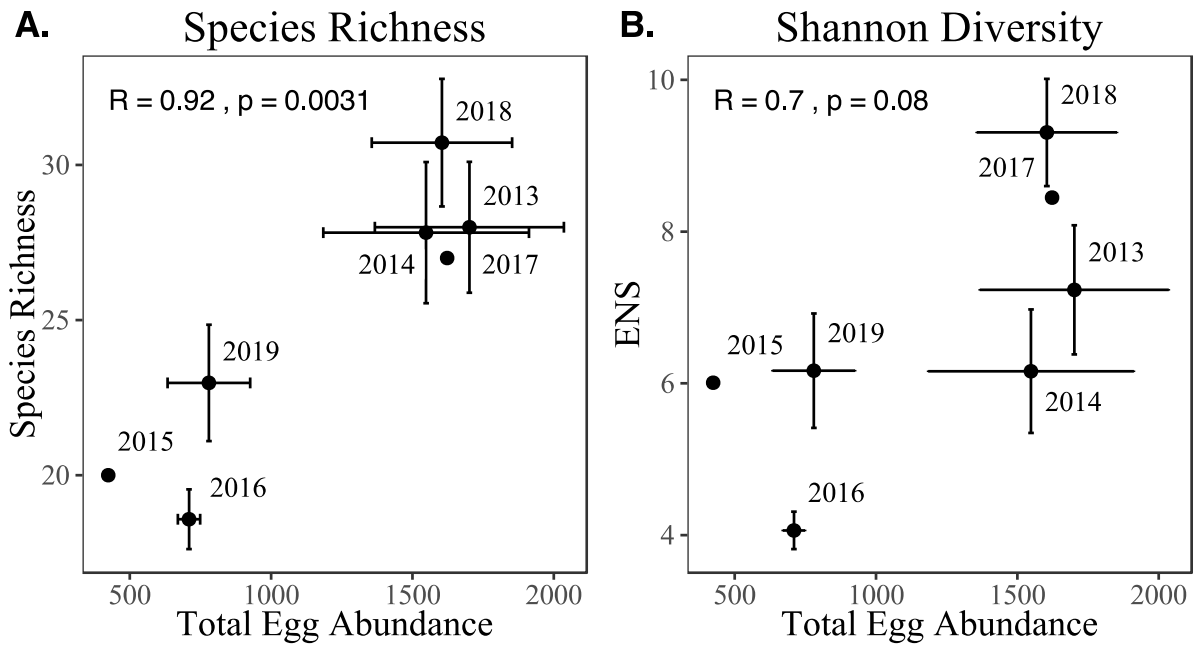

Figure 4: SIO Species Diversity of Spawning Season. 4A. The relationship between the mean total egg abundance and the mean species richness ($\sigma = 0.92$) within the spawning season (May 1 – August 31) of each year. The samples in each year were subset to contain 17 random samples (the minimum sampling effort throughout the spawning season during the seven years) before calculating the total egg abundance and species richness. Figure 4A. contains the relationship between the average total egg abundance and species richness ($\sigma = 0.70$) of the 1000 trials. 4B. The relationship between total egg abundance and Shannon diversity within the spawning season of each year. The total egg abundance and Shannon diversity index were calculated using the same repeated subset method used for A.

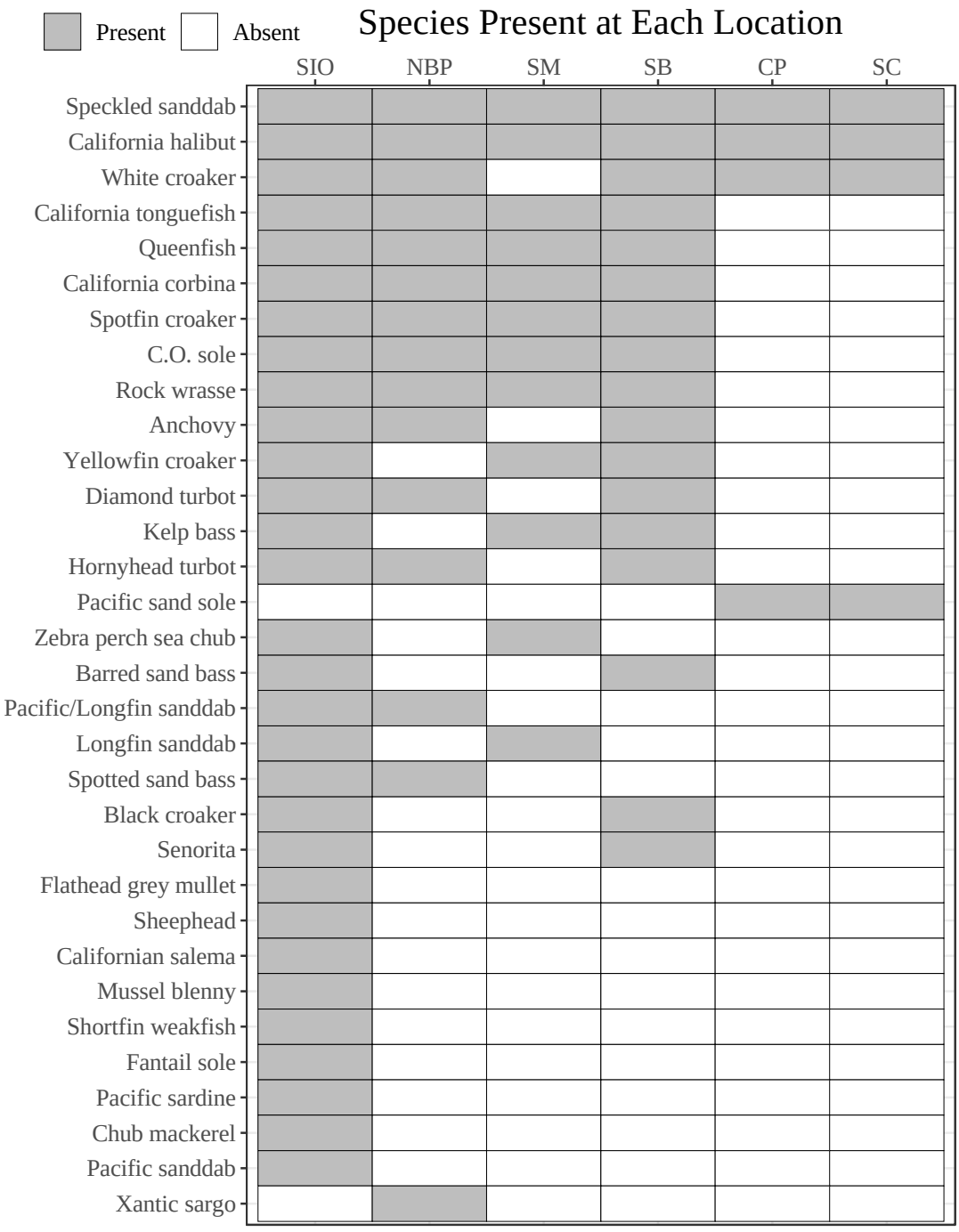

Figure 5: Species Present at Each Location. The figure below shows which species were identified in the samples from each of the locations during 2019. The species are primarily listed in descending order of the number of locations their eggs were identified at. Within that classification species are listed in descending order of egg abundance totaled for all 6 locations.

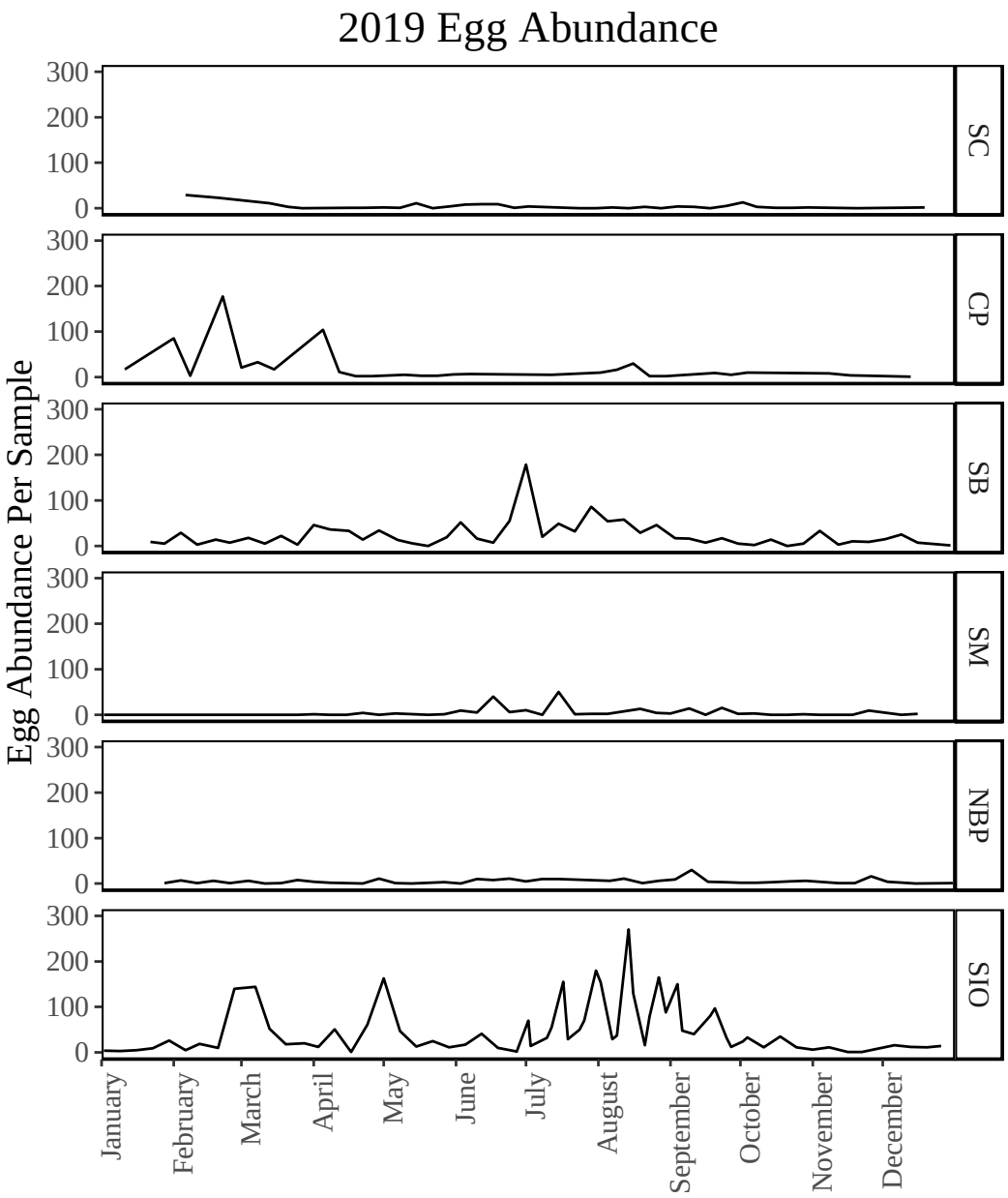

Figure 6: Spatial Variation in Egg Abundance. The number of eggs collected in each sample during 2019, separated by location. The locations are presented in descending latitude. The location labels are as follows - SC = Santa Cruz, CP = Cal Poly San Luis Obispo, SB = Santa Barbara, SM = Santa Monica, NBP = Newport Beach, SIO = Scripps Institution of Oceanography.

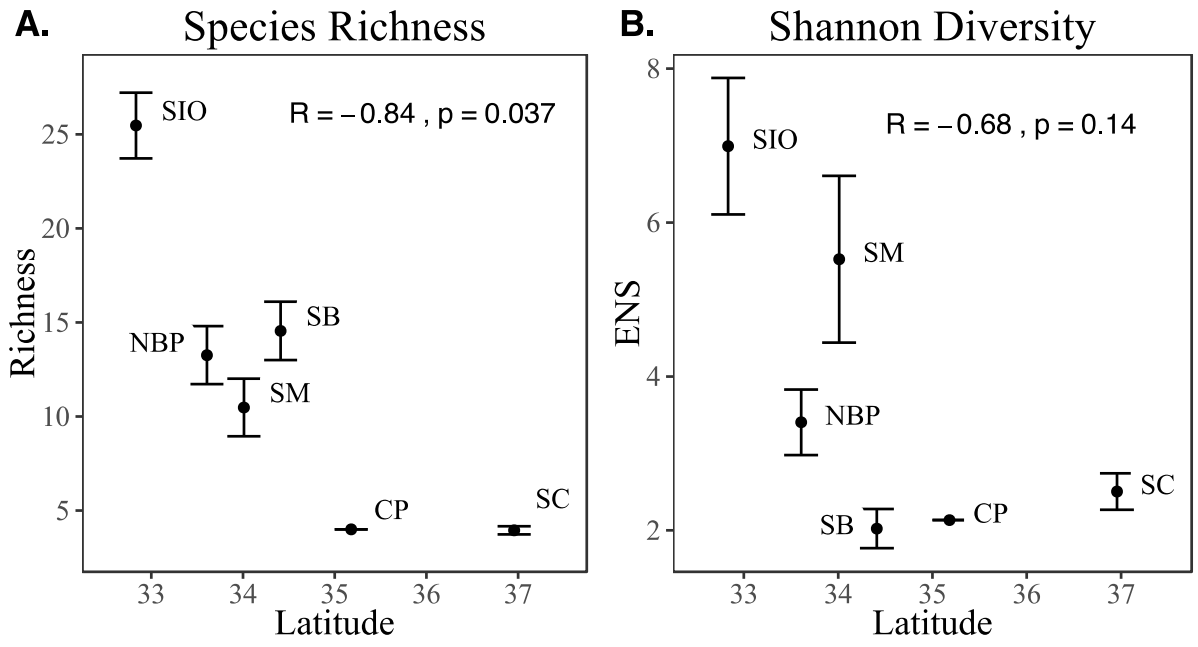

Figure 7: Spatial Variation in Species Diversity. 7A. The relationship between latitude and mean species richness ($\sigma = 0.84$). The latitude refers to the coordinates of each site. The mean species richness was calculated from 1000 trials of determining species richness from 29 (the minimum sampling effort at a given location during 2019) randomly chosen samples at each location. The error bars represent the standard deviation given by the 1000 trials. 7B. The relationship between latitude and ENS ($\sigma = 0.92$), calculated from exp(H) where H is the Shannon diversity. The mean ENS was calculated using the same 1000 trials of 29 random samples used for richness and the error bars represent the standard deviation of those trials.

Table 2: Annual and Seasonal SST averages with standard error.

Year	*Annual Average SST (C)*	*Winter*	*Winter Average SST (C)*
2013	17.23 ± 0.01	2012-2013	14.62 ± 0.12
2014	19.20 ± 0.01	2013-2014	15.48 ± 0.03
2015	19.25 ± 0.01	2014-2015	17.11 ± 0.07
2016	18.15 ± 0.01	2015-2016	16.08 ± 0.07
2017	17.92 ± 0.01	2016-2017	15.04 ± 0.04
2018	18.49 ± 0.01	2017-2018	16.01 ± 0.06
2019	17.92 ± 0.01	2018-2019	15.81 ± 0.09

Discussion

The addition of 2018-2019 data at SIO solidifies the previous notion by Duke et al. (2018) and that there is extensive interannual variation in the egg abundance exhibited during a spawning season at SIO. While years of low egg abundance may be viewed as abnormal, variation in ichthyoplankton abundance is quite common, as it has been observed a number of times in Pacific sardine and Northern anchovy (Ahlstrom 1966; Van der Lingen and Huggett 2003), as well as other larval fish assemblages (Chiu and Hsyu 1994; Smith, and Moser 1983, Duke et al. 2018). The seasonal and annual variation observed has been attributed to a number of variables including salinity, upwelling, anomalous water temperatures, decreased nutrient availability, and global events such as El Nino or La Nina.

The effects of water temperature on the reproductive processes of fish has been extensively studied and anomalous sea surface temperatures have been linked to numerous reproductive difficulties (reviewed in Pankhurst and Munday 2011). Therefore, the relationship between winter SST and egg abundance (Figure 3B.) could indicate that warm winter SST decreases the total egg abundance in the subsequent summer. The depressed egg abundance seen in 2015 and 2016 is associated with the El Nino warm blob event, explored by Duke et al. (2018), however, it cannot explain the reduced egg abundance in 2019 because the warm blob had subsided. SST higher than the physiological limits a species is normally exposed to could lead to reproductive failure or shifts in species' ranges (Munday et al., 2008). Increased SST could also impact the nutrient availability, as water temperatures are often associated with upwelling events characteristic of bringing colder, more nutrient-rich water to the surface (Ulloa et al., 2001). Further, the combination of increased SST and reduced nutrient availability could have significant effects on reproductive activity (Donelson

et al. 2010). Although temperature is likely to be an important factor regulating the reproductive success of fishes, it can have variable effects depending on the species (Pankhurst and Munday 2011). In order to conclusively determine how SST can influence the productivity of a spawning season, more needs to be understood about the species contributing to the spawning season.

The years with reduced egg abundance could be a result of various scenarios: 1) a large reduction in the representation of specific species of fish in our samples, while the other species remained relatively unaffected, 2) all of the species of fish represented in our samples in productive years were equally affected by a factor(s) leading to reduced egg abundance from all species, or 3) multiple species of fish contributed less eggs to the spawning season, than they do during productive years, however some species were more heavily impacted than others. The results given by the temporal species richness analysis (Figure 3A.) indicate that there are, in fact, less species contributing to the total egg abundance of the spawning season during less productive years. However, even an equal reduction in the number of eggs produced by each species, such that the proportion of eggs from each species remained the same, would likely result in decreased representation of rarer species in our samples. The weakened trend between total egg abundance and ENS, given by Shannon diversity (Figure 3B.), suggests that the reduction in total egg abundance is not purely a result of the absence of certain species. The nearly equivalent ENS values of 2015, 2019 (low egg abundance years), and 2014 (high egg abundance year) indicates that regardless of the disparities in species richness, the diversity, defined by both, species richness and eveness, is very similar. The presence/absence chart (Figure 2) shows that of the species only present in four of the seven years, not a single species was absent from 2015, 2016, and 2019 meaning that it is not the

lack of the same, specific species missing from our samples causing the decrease in egg abundance.

Based on these results a few conclusions can be made. The first is that extensive variation in ichthyoplankton abundance could be characteristic of the spawning activity at SIO, rather than an anomaly, although with only seven years of data it may be too soon to make that claim. The second is that there is a fairly strong relationship between winter SST and spring-summer egg abundance. The third is that spawning seasons with fewer eggs also tend to have lower species diversity.

Spatial Monitoring and Analysis

The considerable variation between locations in egg abundance (Figure 6), spawning season (Figure 6), and species diversity (Figure 7) could be attributed to a number of factors including differences in oceanographic variables, geographic barriers, or anthropogenic effects. SST, a well-studied factor influencing fish reproduction (Pankhurst and Munday, 2011), has been shown to be quite variable within the California Current (Blanchette et al., 2007; Mendelssohn et al., 2003) and could therefore, play a role in the spatial variation of spawning activity. Point Conception, a biogeographic barrier located between CP and SB, could contribute to differences between the two northern locations and the four southern locations. The marine protected areas that encompass SIO and are absent from the other five sites could account for the increased egg abundance and species diversity at that location.

The SST gradient along the California coast could influence the differences observed in spawning season and species diversity. For many fish that exhibit seasonal spawning, the phases of the reproductive cycle, such as initiation of gametogenesis or oviposition, have been

linked to the seasonal changes in water temperature (reviewed in Bye, 1984). Autumn decreases in

SST often trigger species that spawn in the autumn or winter to begin vitellogenesis, while spring increases in SST trigger species that spawn in the spring or summer (reviewed in Pankhurst and Munday, 2011). Therefore, the winter peak in egg abundance at CP could be the result of the species contributing to that peak responding to an autumn trigger, while the species contributing to the summer peaks at SB and SIO could be responding to a spring trigger. SST can also dictate species' ranges (Perry et al., 2005), so the cooler water of the northern sites may limit the extent of the southern species or vice versa. Further, warmer or cooler SST may be preferential for spawning activity, so while a species range could include all six sites, spawning may only occur in sites characteristic of that SST (Lluch-Belda et al., 1992). This could explain the lack of Pacific sand sole south of Point Conception, as well as, the lack of California tonguefish (*Symphurus atricaudus*), Queenfish (*Seriphus politus*), California corbina (*Menticirrhus undulatus*), Spotfin croaker (*Rondacor stearnsii*), C.O. sole (*Pleuronichthys coenosus*), and Rock wrasse (*Halichoeres semicinctus*) north of Point Conception. Future work can focus on the relationships between species ranges, spawning locations, and SST to determine if SST does in fact, play a role in dictating the spawning grounds of these species. The influence of SST on the spawning activity occurring at each site will become clearer as the study continues over a longer period of time.

Point Conception, a well-known biogeographic barrier could be responsible for the marked differences in species richness between the two sites north of point conception ($N < 5$) and the four sites south of Point Conception ($N > 10$). Point Conception has been observed to mark both, southern and northern range limits for multiple species with pelagic dispersal

phases (Wares et al., 2001). This could explain why only two of the 32 species, California halibut (*Paralichthys californicus*) and Speckled sanddab (*Citharichthys stigmaeus*), identified in 2019 were found at all six sites. For these two species, it would be interesting to determine whether the eggs collected on either side of the boundary are genetically distinct from one another.

The MPAs surrounding the SIO Pier could be responsible for SIO having the highest egg abundance and the highest species diversity in 2019. While there are a lot of additional factors that could contribute to the observed pattern in abundance and diversity across sites, SIO is the only location within an MPA. A major goal of MPAs are to serve as a refuge for fish to spawn in without the risk of human interference (Lubchenco et al., 2003), so it is interesting, although fairly speculative, to suggest that the spawning success SIO compared to other locations is due to the MPAs. In order to further explore this question, future work could expand the spatial analysis to more locations within MPAs and with more similar habitat and oceanographic conditions.

We demonstrate that fish egg abundance, as well as, species diversity can vary quite extensively both, temporally and spatially, and that the two variables are possibly related to each other. Further, from datasets that span a long period of time and incorporate oceanographic variables, relationships between biological and physical variables, such as SST and egg abundance can be drawn and continuously monitored. Our spatial analysis provides insight into the range of a species spawning grounds and which factors (i.e. SST, point conception, MPAs) may be important for dictating spawning locations for a species. However, in both our SIO temporal analysis and the California coast shore station spatial

analysis, continued sampling is required in order to track changes and identify trends in abundance, diversity, and spawning species' distributions.

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