UNIVERSITY OF CALIFORNIA, SAN DIEGO

Fish Spawning Activities in Southern California Marine Protected Areas as Determined by Molecular Identification of Fish Eggs

A Thesis submitted in partial satisfaction of the requirements for the degree
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by

Elise Anna Lindgren

Committee in charge:

Professor Ronald S. Burton, Chair
Professor Eric Allen, Co-Chair
Professor Kaustuv Roy

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The Thesis of Elise Anna Lindgren is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Co-Chair

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Chair

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ABSTRACT OF THE THESIS

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by

Elise Anna Lindgren

Master of Science in Biology

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Professor Ronald S. Burton, Chair

Professor Eric Allen, Co-Chair

A network of marine protected areas (MPAs) has recently been established off the coast of southern California. In order to monitor species within these reserves, it is

necessary to assess the current state of populations. A baseline for monitoring fish spawning within two of these MPAs was established from 2012 to 2013 using a standardized vertical plankton net tow to collect fish eggs off the pier at Scripps Institution of Oceanography multiple times per week. Molecular methods were used to identify eggs, allowing for more accurate identification than the more commonly used morphological methods. The mitochondrial COI or 16S gene was amplified from extracted DNA, and sequences were compared to DNA barcode databases. A total of 8588 eggs and 38 different species of fish were identified, including commercially and recreationally important species such as *Engraulis mordax* (Northern anchovy), *Sardinops sagax* (Pacific sardine), and *Semicossyphus pulcher* (CA sheephead). Species that are uncommon in this region, including *Cynoscion parvipinnis* (shortfin weakfish) and *Citharichthys gordae* (mimic sanddab), were observed, which may be indicative of ongoing range shifts in breeding habitat. Permutational multivariate analysis of variance (PERMANOVA) was used to test for differences in species assemblages between months. Using a likelihood ratio test, spawning was found to be significantly correlated with sea surface temperature for many species. This survey can allow future studies to monitor changes in spawning activities over time.

Introduction

With increasing frequency over the last several decades, we are seeing significant changes in the abundance and behavior of marine species, and in the nature of entire communities in our oceans (Schiel *et al.* 2004). Rising levels of atmospheric carbon dioxide are contributing to increases in sea surface temperatures, which have risen by 0.11°C per decade since the 1970s (Rhein *et al.* 2013). Studies have shown significant pole-ward range shifts of species as well as earlier breeding and migration associated with this increase in global temperatures (Beaugrand 2004; Parmesan & Yohe 2003; Perry 2005). The greater incidence of invasive species correlated with higher temperatures can displace native species and radically alter habitats and species interactions (Molnar *et al.* 2008; Stachowicz 2002).

Even with these and other studies showing the effects of climate change on the marine world, the response of most species is still unknown (Schiel *et al.* 2004). Without careful monitoring of species and communities, unforeseen changes may be especially destructive due to lack of preparation. Monitoring programs, both temporally and spatially widespread, can provide insight into the vulnerability of communities to climate change. This will allow the development of management plans that incorporate an understanding of the changes that population structures will undergo over time (Perry *et al.* 2010). A crucial step in this is to develop a baseline of the current state of communities to which we can compare future data (Ehler 2003). In this study, we establish a baseline for monitoring fish species offshore of Scripps Institution of

Oceanography, using fish egg studies as indicators of relative population size and spawning activity.

The area under study was established as an academic research area in 1929, prohibiting the take of invertebrates and marine aquatic plants (Sobel & Dahlgren 2004). In accordance with the Marine Life Protection Act (MLPA) passed in 1999, California's marine protected areas (MPAs) were redesigned to better protect the diversity of the oceans. The newly designed MPAs came into effect in southern California in 2012, establishing, among others, the San Diego-Scripps Coastal State Marine Conservation Area (SMCA) and Matlahuayl State Marine Reserve (SMR) which contain the sampling area of this study. The San Diego-Scripps SMCA prohibits the take of living marine resources, with the exception of an allowance for recreational take of coastal pelagic species (such as Pacific sardine, Northern anchovy, and jack mackerel) by hook-and-line. Adjacent is the Matlahuayl SMR which prohibits the take of all living marine resources (California Department of Fish and Wildlife, n.d.).

Marine reserves have been shown to be effective in allowing the recovery of depleted fish populations (Aburto-Oropeza *et al.* 2011; Lester *et al.* 2009; Mosquera *et al.* 2000). No-take reserves provide populations with respite from overfishing, and populations outside the reserve are replenished through spillover of fish and larvae (Halpern & Warner 2003). However, there are a multitude of costs associated with establishing and maintaining MPAs. As a result, MPAs are required to have explicit conservation goals and provide stakeholders with evidence towards the achievement of

these goals (Ehler 2003; Syms & Carr 2001). Careful monitoring is therefore necessary to measure the performance of reserves. Studies of fish populations, for example, can track changes in population abundances and spawning activities in response to fishing pressure, pollution, and climate change.

Many fish population studies have previously been conducted off California (Ahlstrom 1968; Ambrose & Swarbrick 1989; Feder *et al.* 1974; Love & Yoklavich 2002; Schroeder & Love 2002), yet there are a limited number of studies concerning fish populations offshore of Scripps Institution of Oceanography. One of the few studies from this region, in which nearshore fish were surveyed by quarterly diver surveys and trawls, was conducted prior to the establishment of the no-take MPAs in San Diego (Craig *et al.* 2004). Adult and juvenile fish surveys such as this may observe species missed in egg surveys, since fish egg studies are limited to species with pelagic eggs. While adult fish surveys provide insight into the variety of fish that inhabit an area, fish eggs studies establish which species use the MPA and surrounding waters as a breeding ground by observing species that spawn in the region (Planque 2007). In this way, diver surveys and trawls may be complemented by fish egg studies.

Studies of fish eggs and larvae, or ichthyoplankton, are useful indicators of the spawning habits and relative population size of fish species (Ahlstrom 1968; Moser & Watson 2006). Fish eggs have been regularly collected off California since 1949, when the California Cooperative Oceanic Fisheries Investigations (calCOFI) began conducting quarterly cruises to collect plankton from the California Current (California Cooperative

Oceanic Fisheries Investigations, n.d.). These studies have provided useful data about the long-term changes in population size and geographic distribution of fish species. Studies conducted on a smaller time scale, however, can detect fine-scale fluctuations in spawning. More frequent collections also provide an opportunity to observe the presence of species that are uncommon in the region and could easily be missed in quarterly collections.

CalCOFI ichthyoplankton and other studies identify eggs based on morphological characteristics such as egg shape, egg size, and number of oil droplets (Bacheler *et al.* 2010; Duffy-Anderson *et al.* 2006; Matarese *et al.* 2003; Watson *et al.* 1999). Morphological methods can be convenient for identifying species with distinct eggs, and have been successful, for example, in monitoring Pacific sardine and Northern anchovy populations in the California Current (Ahlstrom 1967). However, many species are difficult to distinguish based solely on egg morphology, including many commercially important species (Matarese & Sandknopp 1984; Watson *et al.* 1999). In a study of 288 fish species with pelagic eggs, 70% of eggs were between 0.5 and 1.5 mm in length, and most of these were spherical and containing one oil droplet (Ahlstrom & Moser 1980). This may result in unidentified or misidentified eggs, especially those of species that are closely related.

Due to the limitations of morphological identifications, molecular methods are becoming increasingly more common. Species-specific primers and oligonucleotide probes have been designed in previous studies to allow for efficient identification of fish

eggs and larvae (Gleason & Burton 2012; Hyde *et al.* 2005). These techniques are limited to the identification of a set of predetermined species, however, and species without designated primers or probes will be missed in these analyses. When dealing with a diverse range of species, therefore, DNA barcoding may be more appropriate.

DNA barcoding involves sequencing a gene, most commonly the mitochondrial gene cytochrome c oxidase subunit 1 (COI), and comparing the sequence to a database of identified and vouchered adult specimens (Hebert *et al.* 2003a). This technique is limited only to the availability of sequences in the database, and can allow for the detection of rare species. DNA barcoding can distinguish between closely related species, and has been successfully implemented in the identification of fish (Ardura *et al.* 2010; Hebert *et al.* 2003b; Kawakami *et al.* 2010; Lakra *et al.* 2011; Smith *et al.* 2008; Steinke *et al.* 2009; Ward *et al.* 2005). Mitochondrial genes have been shown to evolve more rapidly than nuclear genes, thus allowing for more interspecies divergence. In addition, the lack of recombination in mitochondrial genes leads to less intraspecies divergence (Saccone *et al.* 1999).

In this study, DNA barcoding was used to identify fish eggs collected off the Scripps Pier, located at the junction of the San Diego-Scripps Coastal SMCA and the Matlahuayl SMR. Eggs were collected multiple times per week in order to observe temporal changes in species abundances and correlations between spawning activities and season, water temperature, and lunar phase. By establishing a baseline of fish spawning and relative population size, future studies can be done to monitor populations and

spawning over time. By observing changes in spawning over many years, studies can distinguish between responses to one year anomalies such as El Niño events and long-term changes brought about through climate change.

Methods and Materials

Sample Collection

Eggs were collected using a vertical plankton net tow from the end of the Scripps Pier (Fig. 1) from two to five times per week, from August 2012 through August 2013. For each collection, a 505-micron mesh, one meter diameter plankton net was lowered to a depth of about 6 meters and raised four times. Fish eggs were counted and sorted using microscopy. *Engraulis mordax* (Northern anchovy) and *Sardinops sagax* (Pacific sardine) eggs were identified at this point based on their distinctive morphology: *E. mordax* eggs are oval, and *S. sagax* eggs are large with a wide perivitelline space. Unidentified eggs were placed into 1.5 µL microcentrifuge tubes with about 500 µL 80% ethanol and stored at 4 °C.

DNA Amplification and Sequencing

Eggs were rinsed with deionized water then individually placed into each well of a 96-well plate. Excess water was removed with a pipette, and 20 µL of Qiagen Elution Buffer was added to each well. Each egg was crushed with clean 200 µL pipette tip, after which the mixture was back-pipetted to insure that no tissue remained in the pipette tip. The crushed eggs were incubated for five minutes in 90 °C using a thermal cycler.

The mitochondrial COI or 16S ribosomal rRNA gene was amplified for each sample using universal primers. The forward primer COI VF1 (5'-TTCTCAACCAACC ACAAAGACATTGG-3') and reverse primer COI VR1 (5'-TAGACTTCTGGGTGGCC AAAGAATCA-3') produced a 740 bp amplicon. The forward primer 16Sar (5'-CGCCT

GTTTATCAAAAACAT-3') and the reverse primer 16Sbr (5'-CCGGTCTGAACTCAG ATCACGT-3') produced an amplicon of 610 bp. After establishing that *Citharichthys stigmaeus* (speckled sanddab) and *Oxyjulis californica* (senorita) were the most abundant species, species-specific primers were designed for both to allow for more efficient identification. The *C. stigmaeus* primer (5'-GCTCCCTCCCTCTTTTCTATTAC-3') and *O. californica* primer (5'-GCCCCTGTTTGTCTGAGCTGTA-3') were used with the COI VR1 reverse primer to yield amplicons of about 430 bp and 205 bp respectively.

The PCR reaction contained 1 µL DNA template, 0.5 µL of each primer (four primers were used for the COI reaction: COI VR1, COI VF1, and both species-specific primers), 12.5 µL of Promega GoTaq Green Master Mix, and 10.5 µL dH_2O for a total reaction volume of 25 µL. A thermal cycler was used with the following parameters for both the COI and 16S reactions: 95 °C for 2 min, 35 cycles of 95 °C for 30 s, 50 °C for 1 min, and 72 °C for 1 min, with a final extension of 72 °C for 7 min. 3 µL of each PCR product was run on a 2% agarose gel along with a 100 bp ladder at 120 Volts for 25 minutes, and then visualized with ethidium bromide. From the gel image, *C. stigmaeus* and *O. californica* species were identified by eye using their distinct fragment lengths (Fig. 2).

DNA from unidentified samples with clear COI or 16S bands (e.g. sample A in Fig. 2) was purified using Sephadex and G50 spin columns, then the DNA concentration of the purified samples was measured using a NanoDrop spectrophotometer. The samples were then sequenced by Retrogen, Inc. using the reverse COI VR1 or 16Sar primers.

Results were analyzed using the Basic Local Alignment Search Tool (BLAST), comparing sequences to those available in GenBank and the Barcode of Life Data System (BOLD) in order to identify the species.

Analysis

The likelihood ratio test was used in JMP Statistical Software 10 to test significance of temperature and lunar phase correlations with egg presence. The analysis compared the likelihood of egg presence for individual species on days with water temperatures exceeding 17°C to days with lower temperatures. Water temperature at a depth of 3 meters from the Scripps Pier was obtained from the Southern California Coastal Ocean Observing System (www.sccoos.org). Lunar phase was compared to the presence or absence of eggs in a collection. Each collection was assigned to one of four lunar phases (new moon, first quarter, full moon, last quarter) based on the phase closest to the date of collection.

Permutational multivariate analysis of variance (PERMANOVA) was used in PRIMER 6 to assess differences in species assemblages between months. This is a nonparametric test that allows significance testing of non-normal data, by using permutations to compute p-values. The analyses were conducted using Bray-Curtis dissimilarities, with log-transformed (x+1) data in order to reduce the weight of highly abundant samples. Post hoc pairwise tests were performed to test differences between months. A nonmetric multidimensional scaling ordination (NMDS) plot was produced in PRIMER to visually compare differences between monthly species assemblages. A full

year was analyzed, from September 2012 to August 2013. August 2012 was not included in this analysis, as it was undersampled when compared to other months.

Results

A total of 14,877 fish eggs were collected from off the Scripps Pier in 159 collections between August 23, 2012 and August 23, 2013. 8588 eggs were identified, representing 38 species of fish (Table 1). Unidentified eggs were a result of amplification failure. The most common species found were *Citharichthys stigmaeus* (speckled sanddab), *Oxyjulis californica* (senorita), and *Sardinops sagax* (Pacific sardine), which together made up 75% of the identified eggs. Overall, 18 different families of fish were represented.

Species assemblages

Table 2 lists the four most abundant species collected in each month. *C. stigmaeus* was most common during the majority of the year, however January and February 2013 were dominated by *Engraulis mordax* (Northern anchovy), May 2013 by *S. sagax,* and July and August 2013 by *O. californica.* Spring and summer months had the highest average number of species per collection (Fig. 3). The highest species count was found in June, with an average of 9.3 species per collection, while February had the lowest with 0.7 species per collection. The most species in a single collection occurred on June 19, 2013, with 15 different species. With 297 eggs identified out a total of 502 eggs, this day had the fourth highest egg abundance. May 9, 2013 had the highest abundance of eggs in a single collection, with 574 eggs identified out 652. This day had 9 different species identified.

The NMDS plot (Fig. 4) suggests a possible difference in species assemblages across months, showing winter and fall months in a cluster and summer months in a second cluster. PERMANOVA showed a significant relationship between month and species assemblage (PERMANOVA, pseudo-F=10.94, P=0.0001) using 9859 permutations. Pairwise tests showed different assemblages between fall/winter months compared to spring/summer months (P<0.001), with the exception of March, which did not show a significant difference from November (P=0.07) or January (P=0.25). Differences in assemblages were not seen within fall/winter months (P>0.01) except February which differed significantly from the rest of the year (P<0.01). This is supported by the NMDS plot which shows most fall and winter months in a tight cluster, indicating little difference between these months.

Species assemblages in June differed significantly from those in July and August (P<0.01). July and August did not show a difference in assemblage (P=0.46). Spring months did not cluster together in the NMDS plot, and both April and May showed significant differences with all other months (P<0.01).

Spawning patterns

Average fish eggs collected throughout the year were calculated using 3-week intervals overlapping by 2 weeks (Fig. 5). Species within the family Paralichthyidae (large-tooth flounders) are shown in figures 5a through 5d: *C. stigmaeus*, *Citharichthys sordidus* (Pacific sanddab), *Paralichthys californicus* (CA halibut), and *Citharichthys xanthostigma* (longfin sanddab). These species spawned throughout most of the year,

with the majority of eggs found during spring or summer. *C. stigmaeus, C xanthostigma,* and *C. sordidus* had a smaller peak of spawning during October. Pleuronectidae (righteye flounders), which includes *Hypsopsetta guttulata* (diamond turbot) seen in figure 5e, similarly showed spawning throughout the year. *H. guttulata* had a main peak of spawning in October.

Sciaenidae (drums or croakers), including *Menticirrhus undulatus* (CA corbina, 5f) and *Roncador stearnsii* (spotfin croaker, 5g), spawned mainly during spring and summer. Labridae (wrasses) are shown in figures 5h through 5j: *O. californica, Halichoeres semicinctus* (rock wrasse), and *Semicossyphus pulcher* (CA sheephead). These species also spawned primarily during spring and summer, but with some spawning occurring during September and October.

Xenistius californiensis (Californian salema, 5k), *Seriphus politus* (queenfish, 5l), *Trachurus symmetricus* (Pacific jack mackerel, 5m), and *S. sagax* (5n) showed similar patterns of spawning, with little to no eggs found in fall and winter, and a strong peak in the spring. *E. mordax* (5o) showed a unique pattern, with two strong peaks of spawning during winter and early spring, and few eggs found during the summer.

Spawning relationship to temperature and lunar phase

A possible correlation between water temperature and the presence of eggs was tested using the likelihood ratio test (Fig. 6). *C. sordidus, P. californicus, M. undulatus, R. stearnsii, O. californica, H. semicinctus, S. pulcher, X. californiensis, S. politus, T.*

symmetricus, and *S. sagax* all showed a significant relationship between spawning and water temperature, with a higher likelihood of eggs present when water temperature exceeded 17 °C. *E. mordax* showed the opposite relationship, with a significantly higher likelihood of finding eggs when the water was below 17 °C. *C. stigmaeus*, *C. xanthostigma*, and *H. guttulata* showed no significant relationship between spawning and water temperature. Overall, there was no significant correlation between lunar phase and spawning (Fig. 7). Most species tested had a slightly higher presence of eggs during the first quarter, but this increased likelihood was only significant for *S. sagax*.

DNA barcoding

Amplification of the COI gene was used most frequently for DNA barcoding, as 16S was unable to distinguish between certain closely related species such as *C. sordidus* and *C. xanthostigma*. The 16S gene, however, amplified more consistently for some species.

Fish egg sizes

Figure 8 shows photographs taken of a subsample of eggs that were subsequently identified by sequencing. From the photographs, egg diameters for 19 different species were determined. The mean diameter was calculated for the 6 species with measurements for 5 or more eggs (Table 3). *S. sagax* had the largest eggs with an average egg size of 1.86 mm. The smallest identified eggs belonged to *C. stigmaeus*, with an average length of 0.66 mm.

Discussion

This study establishes a baseline for the spawning of fish species in the San Diego-Scripps Coastal State Marine Conservation Area and Matlahuayl State Marine Reserve. Results indicate that the region is an important spawning habitat for multiple fishes, including commercially important and threatened species. Species within the families Sciaenidae (drums and croakers) and Paralichthyidae (large-tooth flounders) were most common in the region, with the paralichthyid *C. stigmaeus* most dominant overall.

Species assemblages changed throughout the year, with correlations seen between assemblages and seasons. Fall and winter months had a distinct pattern of species assemblages from summer months, while spring months showed an intermediate assemblage. The clustering of fall and winter months seen in the NMDS plot is supported by the PERMANOVA data, which shows a significant difference in species assemblages between fall/winter and summer, but not within fall/winter. March was similar in composition to November and January, which implies a transition from fall/winter assemblages to spring/summer assemblages during this time. While there is a clustering of summer months seen in the NMDS plot, June showed significant differences in assemblages from July and August. This may be due to the higher number of species found during June, with 27 different species compared to 23 species and 18 species found in July and August, respectively.

February and May showed unique species assemblages from all other months. In May, this is due to the high abundance of *S. sagax*, which greatly outnumbered both *C. stigmaeus* and *O. californica*. *T. symmetricus* eggs, which were rarely seen throughout the rest of the year, were fairly abundant (74 eggs) in May. In addition, May had the highest variety of species, with 29 different species found, while February had the lowest with 3 species. Differences in species assemblages seen between individual months may be due to variations in oceanographic conditions or chance events in the single year of sampling.

There was a significant correlation between water temperate and egg presence, with most species showing a higher likelihood of spawning during warmer temperatures, and *E. mordax* showing the opposite pattern. This is in agreement with the observed patterns of spawning seen in Figure 5, with most species spawning more frequently during spring and summer months and *E. mordax* instead spawning during winter and early spring. Previous studies have established the ideal spawning temperature for *E. mordax* to be within 11.5 to 16.5 °C (Lluch-Belda *et al.* 1991), which agrees with our data, as temperatures exceeding 17 °C had significantly lower egg abundance. Three species of flounders (*C. stigmaeus, C. xanthostigma,* and *H. gutullata*) did not show a significant correlation between spawning and water temperature. This again agrees with Figure 5, as flounders are seen to spawn throughout the year rather than only during the warmer season. Species within the families Sciaenidae, Haemulidae (grunts), Serranidae (sea basses), and Kyphosidae (sea chubs), however, were nearly exclusively found during spring and summer months.

Although it is clear that spawning is related to water temperature, we cannot conclude if this relationship is direct or indirect, and previous studies have shown evidence of both. Temperature has been shown to directly affect the timing of spawning by influencing the onset of gametogenesis in certain fish species (Ware & Tanasichuk 1989). Indirect effects include the influence of temperature on food availability and oxygen levels. It has been hypothesized that spawning is timed so that fish larvae begin feeding during times of high plankton biomass, which is in turn affected by temperature (Cushing 1969). Changes in upwelling related to temperature can therefore advance or delay spawning (Kjesbu 1994). Time of intense upwelling may result in hypoxic events, which also affects fish spawning since high dissolved oxygen is necessary for spawning of certain species (Hunter 1984).

A slight increase in spawning during the first quarter lunar phase was seen for most species, yet this was only significant in *S. sagax*. This result may have been influenced by sampling error, however, as *S. sagax* eggs were initially misidentified due to their unique morphology. This resulted in a misrepresentation of days containing *S. sagax* eggs, which may have exaggerated the presence of eggs during the first quarter. Further sampling is therefore needed to examine the relationship between lunar phase and spawning in this region. Previous studies have shown such relationships, believed to be due to changes in moonlight or tidal height, in several fish species (Debruyn & Meeuwigg 2001; Robertson *et al.* 1999).

Several commercially important or threatened species were observed in this study, including *E. mordax, P. californicus, S. sagax, S. pulcher,* and *M. undulatus*. *S. pulcher* is listed as vulnerable by the IUCN Red List due to a history of overexploitation, decreases in population size, and a small geographic range (Cornish & Dormeier 2006). Other species observed in the region that are important to monitor due to past overfishing or signs of decreasing population size include *Genyonemus lineatus* (white croaker), *Atractoscion nobilis* (white seabass), and *R. stearnsii* (Chao & Espinosa 2010; Miller *et al.* 2011). With a baseline established for these species, further monitoring can track changes in relative population size and spawning activities.

Species considered rare in this region that were observed in our study include *Citharichthys gordae* (mimic sanddab) and *Cynoscion parvipinnis* (shortfin weakfish). *C. gordae* has only been previously observed off the coast of Mexico, and there is little data about its population structure (van der Heiden 2010). Its occurrence in southern California waters may be indicative of a northward range shift, potentially as a response to increasing water temperatures. However, more data would be needed, as only one egg was identified from this species. *C. parvipinnis*, which used to be abundant in southern California, is now mostly found in the Gulf of California (Chao *et al.* 2010; Hubbs 1948). We identified 15 eggs from this species during our survey, collected between May and July 2013. This may again point to a possible range shift, but there is also a possibility of errors in identification during earlier surveys as this species has been previously observed in this region.

The San Diego fish survey by Craig *et al.* (2004) likewise found *C. stigmaeus* to be the most common species overall. Many fish species producing pelagic eggs were observed both in the Craig study as well as ours. A number of species, however, were unique to one or the other study. Species found only in the Craig study include *Parophrys vetulus* (slender sole), *Pleuronichthys decurrens* (curlfin sole), and *Pleuronichthys ritteri* (spotted turbot). This may be due to differences in sampling method or survey area, as their study surveyed a variety of habitats apart from those near the Scripps Pier. Species that were observed only in our study include common species such as *O. californica*, *C. sordidus*, *C. xanthostigma*, and *H. semicinctus*, as well as rare species such as *C. parvipinnis*. Rare species were more likely to be observed in our study due to our more frequent sampling.

By continuing to survey fish eggs in this region, future studies can observe trends in spawning over time and in response to changes in temperature. Distinctions can be made between responses to short-term temperature fluctuations such as those due to El Niño events and longer term changes due to climate change. In the event of algal blooms, the effects of increasing chlorophyll levels on fish populations can be observed. The baseline data produced by this study will be useful for studies of fish assemblages across years, and allow for more efficient monitoring of the region.

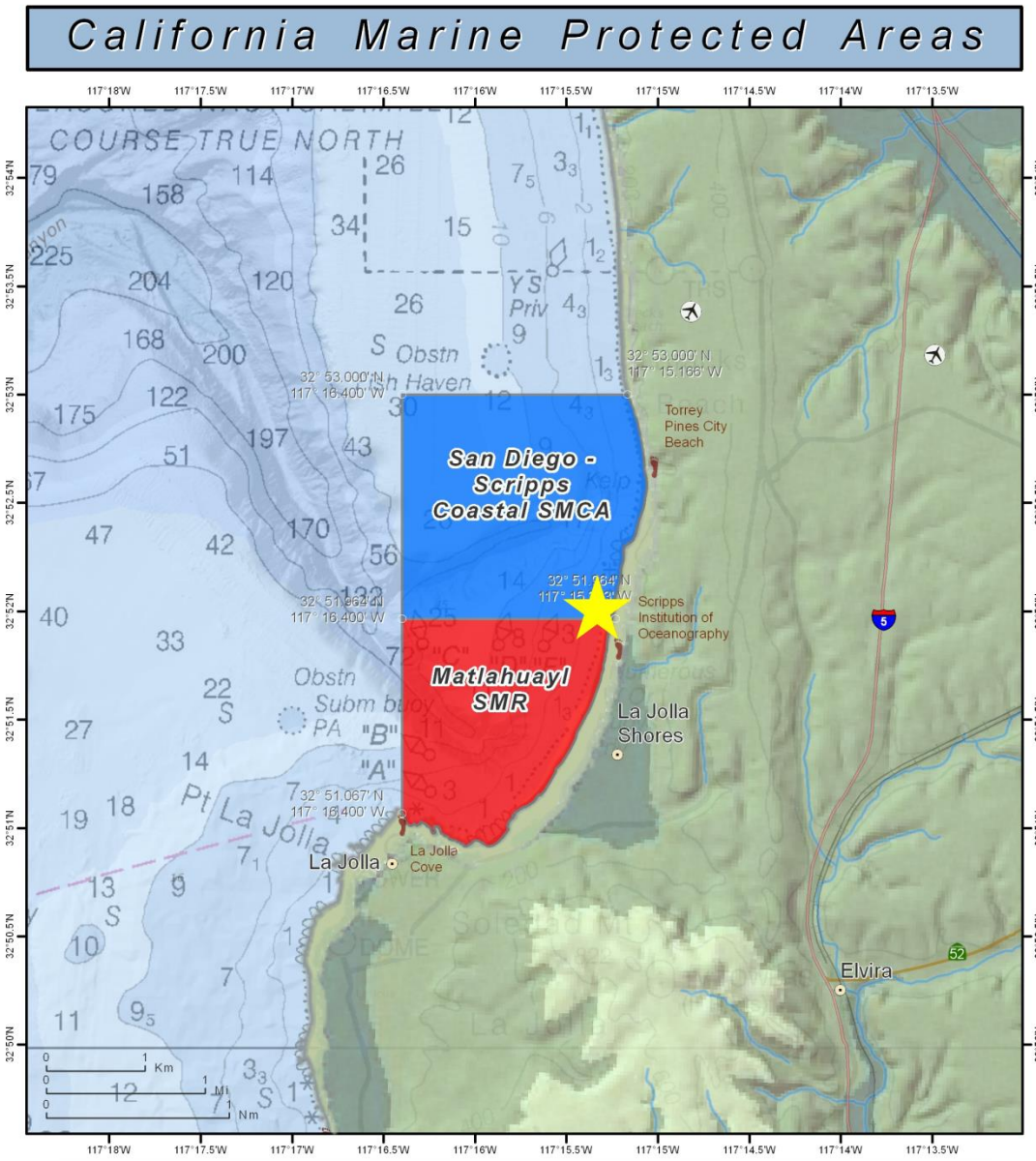


Figure 1. Map of San Diego MPAs. Shows San Diego-Scripps Coastal SMCA and Matlahuayl SMR with star indicating site of collections at the Scripps Pier. California Department of Fish and Wildlife.

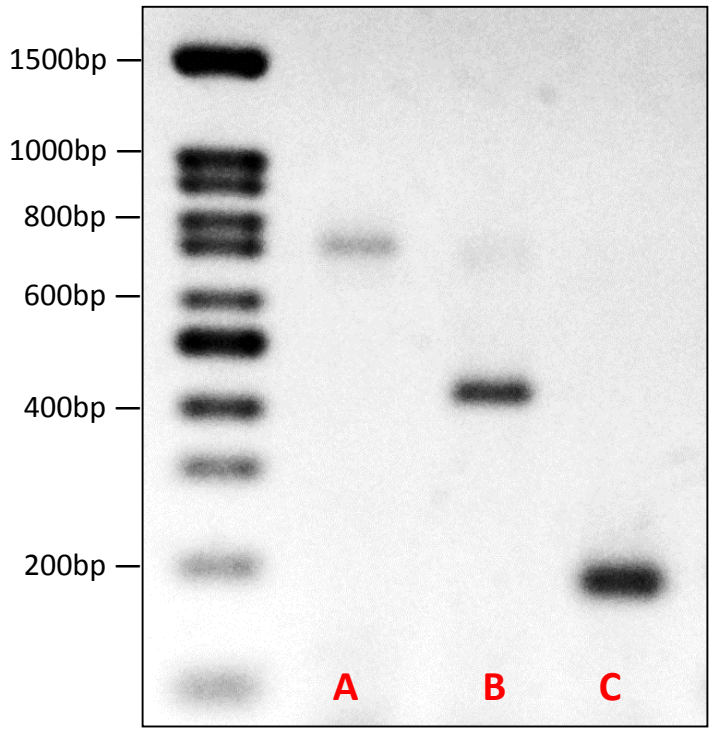


Figure 2. Gel electrophoresis image of fish egg PCR products using species-specific primers. Samples B and C identify *C. stigmaeus* and *O. californica* eggs, respectively. Sample A is the amplified COI gene of a different fish species.

Table 1. Species of fish eggs collected off the Scripps Pier from August 2012 to August 2013, with number of eggs identified for each species and number of collections each species was found in.

Scientific name	Common name	Eggs	Collections
Citharichthys stigmaeus	Speckled sanddab	2929	139
Oxyjulis californica	Senorita	2322	87
Sardinops sagax	Pacific sardine	1199	18
Engraulis mordax	Northern anchovy	413	27
Citharichthys sordidus	Pacific sanddab	278	73
Menticirrhus undulatus	CA corbina	252	34
Xenistius californiensis	Californian salema	219	23
Roncador stearnsii	Spotfin croaker	184	33
Paralichthys californicus	CA halibut	146	50
Halichoeres semicinctus	Rock wrasse	138	35
Seriphus politus	Queenfish	100	19
Trachurus symmetricus	Pacific jack mackerel	84	17
Hypsopsetta guttulata	Diamond turbot	60	37
Citharichthys xanthostigma	Longfin sanddab	44	23
Semicossyphus pulcher	CA sheephead	36	24
Paralabrax clathratus	Kelp bass	27	16
Cheilotrema saturnum	Black croaker	23	14
Genyonemus lineatus	White croaker	20	8
Peprilus simillimus	Pacific pompano	16	8
Scomber japonicus	Chub mackerel	15	7
Cynoscion parvipinnis	Shortfin weakfish	15	9
Chilara taylori	Spotted cusk-eel	9	8
Paralabrax nebulifer	Barred sand bass	9	7
Symphurus atricaudus	CA tonguefish	7	6
Umbrina roncador	Yellowfin croaker	7	4
Xystreurys liolepis	Fantail sole	5	3
Pleuronichthys verticalis	Hornyhead turbot	5	4

Table 1. continued

Scientific name	Common name	Eggs	Collections
Pleuronichthys coenosus	C-O sole	4	4
Caulolatilus princeps	Ocean whitefish	4	3
Girella nigricans	Opaleye	4	3
Atractoscion nobilis	White seabass	4	1
Anisotremus davidsonii	Xantic sargo	3	3
Synodus lucioceps	CA lizardfish	2	1
Citharichthys gordae	Mimic sanddab	1	1
Paralabrax maculatofasciatus	Spotted sand bass	1	1
Hypsoblennius jenkinsi	Mussel blenny	1	1
Hermosilla azurea	Zebra-perch sea chub	1	1
Sphyraena argentea	Pacific barracuda	1	1
	Unidentified	6289	---
	Total	14877 Eggs	159 collections

Table 2. Most abundant species per month. Depicts rank of top 4 most abundant species from August 2012 to August 2013, with N indicating number of collections and n indicating number of eggs identified.

Aug-12 (N=3)		**Sep-12** (N=9)		**Oct-12** (N=13)		**Nov-12** (N=4)	
Species	n	Species	n	Species	n	Species	n
Citharichthys stigmaeus	23	*Citharichthys stigmaeus*	49	*Citharichthys stigmaeus*	187	*Citharichthys stigmaeus*	21
Oxyjulis californica	3	*Halichoeres semicinctus*	4	*Hypsopsetta guttulata*	14	*Citharichthys sordidus*	5
Halichoeres semicinctus	1	*Oxyjulis californica*	3	*Citharichthys sordidus*	13	*Paralichthys californicus*	2
		Hypsopsetta guttulata	3	*Xystreurys liolepis*	5	*Hypsopsetta guttulata*	1
Dec-12 (N=7)		**Jan-13** (N=16)		**Feb-13** (N=9)		**Mar-13** (N=10)	
Species	n	Species	n	Species	n	Species	n
Citharichthys stigmaeus	17	*Engraulis mordax*	125	*Engraulis mordax*	7	*Citharichthys stigmaeus*	293
Citharichthys sordidus	5	*Citharichthys stigmaeus*	111	*Hypsopsetta guttulata*	3	*Engraulis mordax*	193
Paralichthys californicus	4	*Hypsopsetta guttulata*	10	*Citharichthys stigmaeus*	1	*Hypsopsetta guttulata*	10
Pleuronichthys c oenosus;	1	*Peprilus simillimus*	7			*Paralichthys californicus*	7
Engraulis mordax;							
Peprilus simillimus;							
Roncador stearnsii							

Table 2. continued

Apr-13 (N=19)		**May-13** (N=21)		**Jun-13** (N=14)		**Jul-13** (N=19)	
Species	n	Species	n	Species	n	Species	n
Citharichthys stigmaeus	659	*Sardinops sagax*	1102	*Citharichthys stigmaeus*	372	*Oxyjulis californica*	588
Oxyjulis californica	119	*Oxyjulis californica*	819	*Oxyjulis californica*	308	*Citharichthys stigmaeus*	314
Sardinops sagax	51	*Citharichthys stigmaeus*	489	*Menticirrhus undulatus*	128	*Xenistius californiensis*	160
Engraulis mordax	45	*Trachurus symmetricus*	74	*Citharichthys sordidus*	94	*Citharichthys sordidus*	40
Aug-13 (N=15)							
Species	n						
Oxyjulis californica	479						
Citharichthys stigmaeus	384						
Menticirrhus undulatus	51						
Halichoeres semicinctus	42						

Figure 3. Average number of species per collection each month. Depicts mean number of species +/- standard error of the mean.

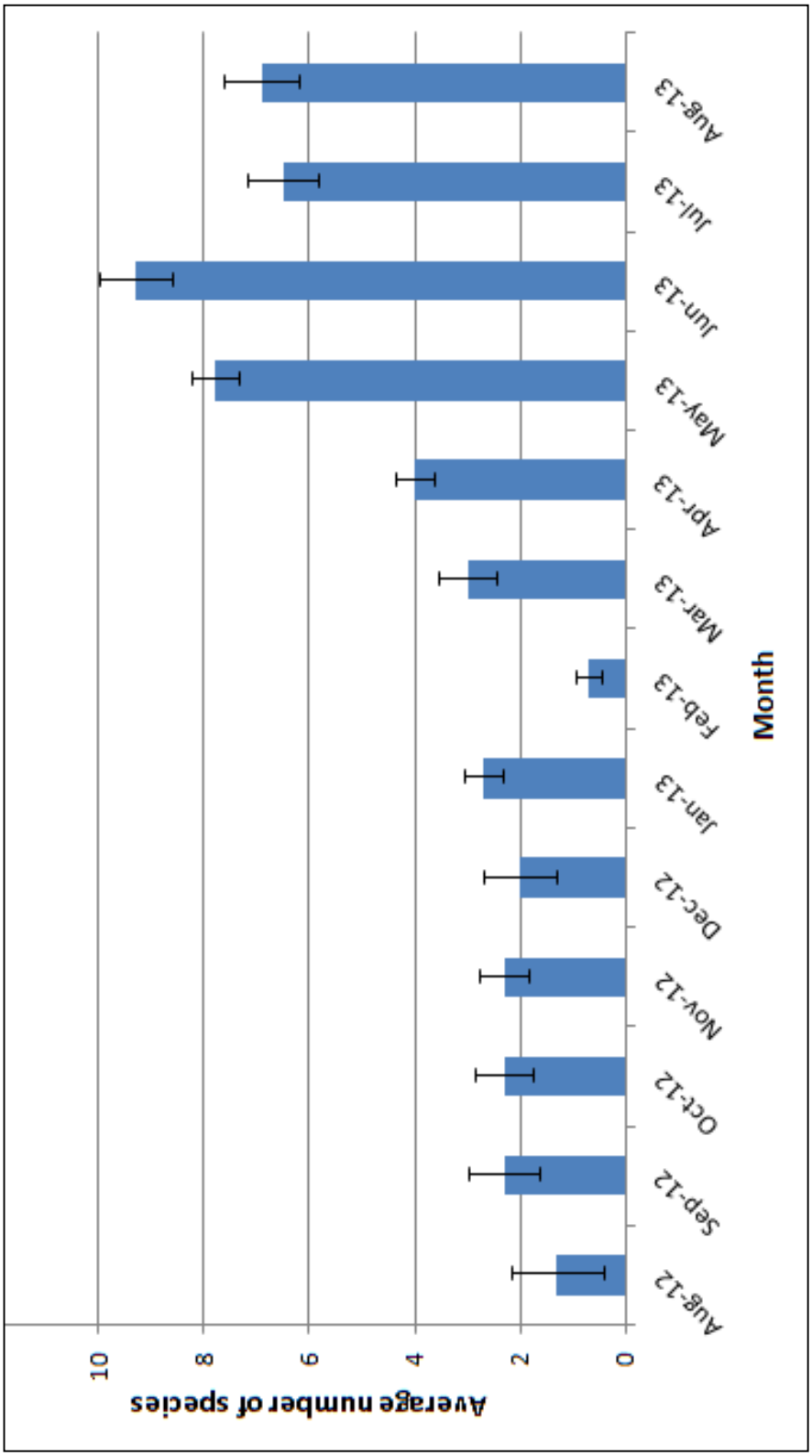

Average number of species
0
2
4
6
8
10
Aug-12
Sep-12
Oct-12
Nov-12
Dec-12
Jan-13
Feb-13
Mar-13
Apr-13
May-13
Jun-13
Jul-13
Aug-13
Month

Figure 4. Nonmetric Multidimensional Scaling ordination (NMDS) plotted from abundances of fish eggs for each species per collection. Based on Bray-Curtis dissimilarities from log-transformed (x+1) data. The stress value indicates that the plot gives an adequate representation of the data.

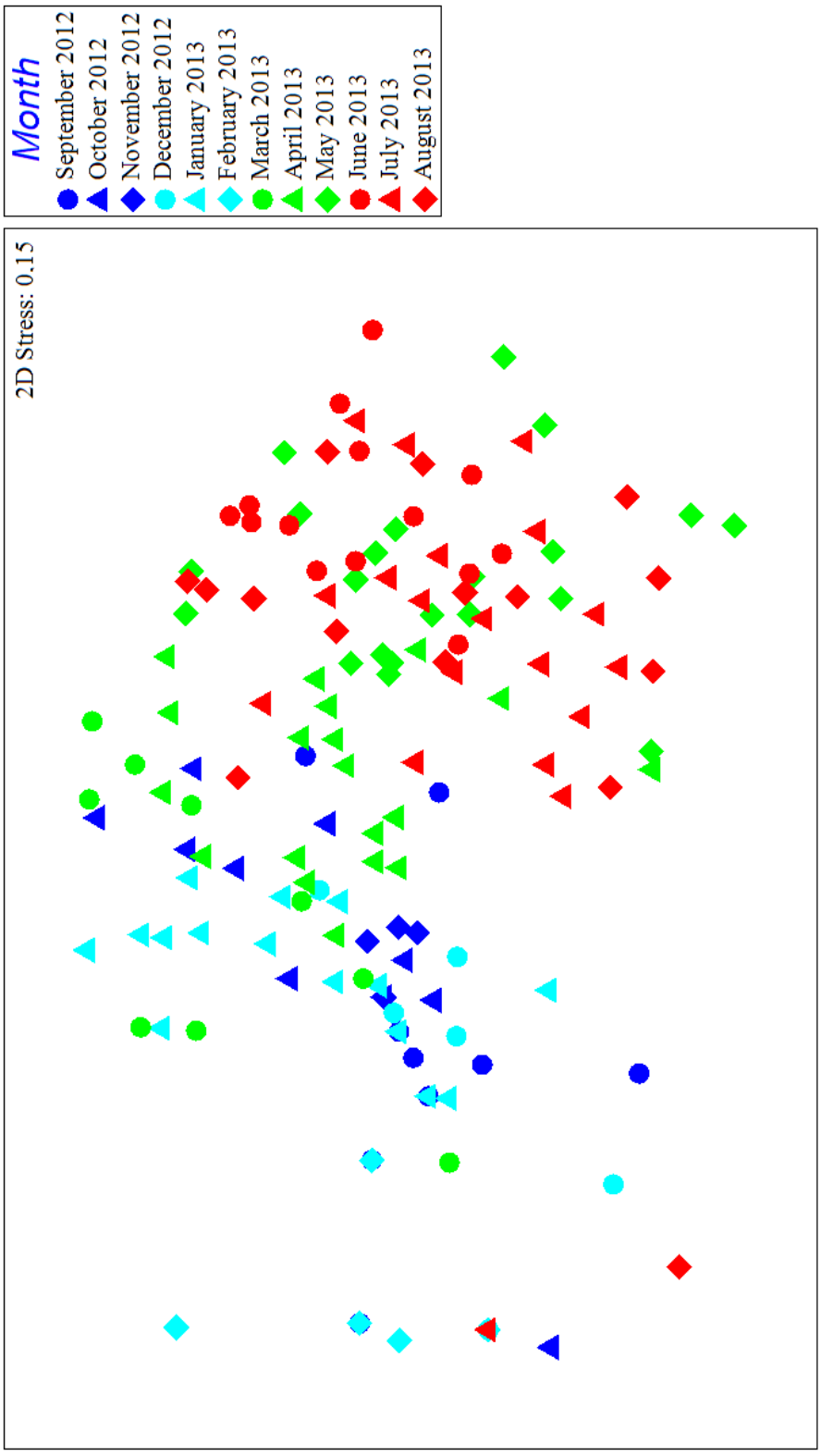
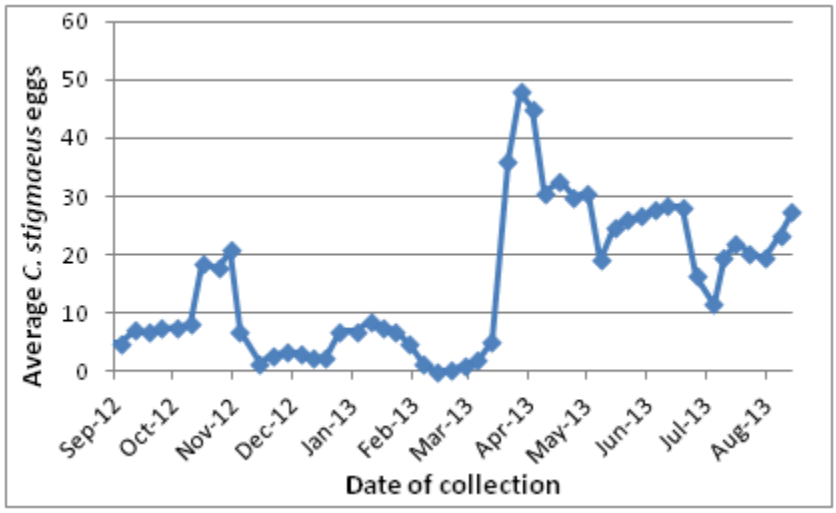
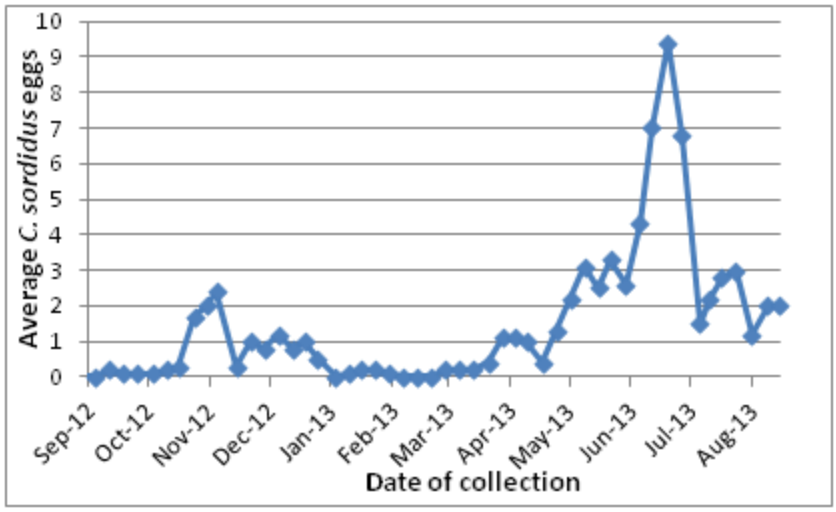

Month
September 2012
October 2012
November 2012
December 2012
January 2013
February 2013
March 2013
April 2013
May 2013
June 2013
July 2013
August 2013
2D Stress: 0.15

Figure 5. Average number of fish eggs per collection from August 2012 to August 2013. Depicts 3-week intervals of collections overlapping by 2 weeks for the 15 most frequent species

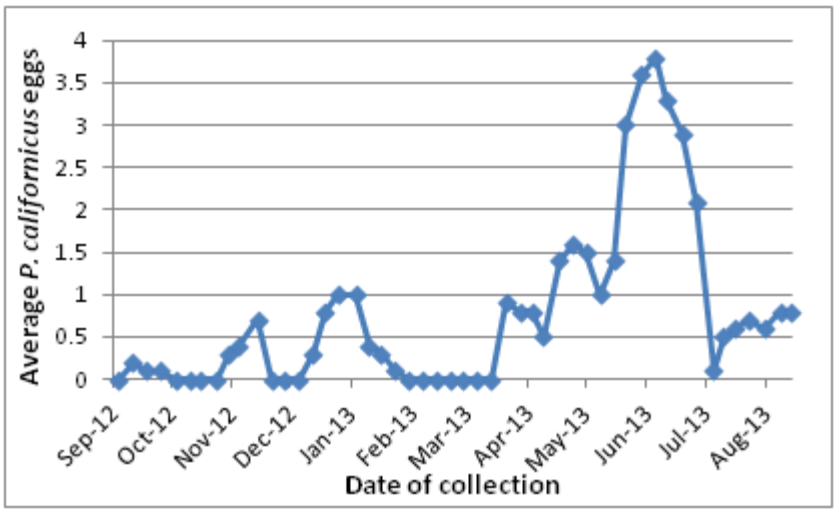
a)


Average C. stigmaeus eggs
60
50
40
30
20
10
0
Sep-12
Oct-12
Nov-12
Dec-12
Jan-13
Feb-13
Mar-13
Apr-13
May-13
Jun-13
Jul-13
Aug-13
Date of collection

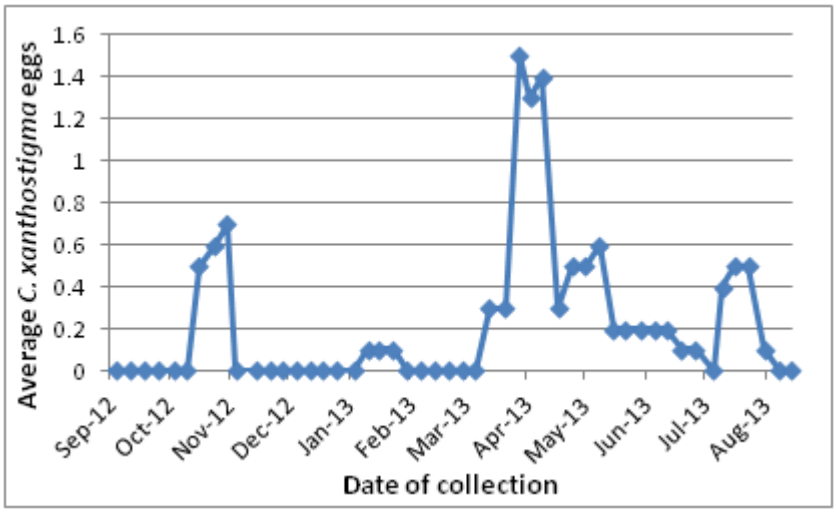
b)


Average C. sordidus eggs
10
9
8
7
6
5
4
3
2
1
0
Sep-12
Oct-12
Nov-12
Dec-12
Jan-13
Feb-13
Mar-13
Apr-13
May-13
Jun-13
Jul-13
Aug-13
Date of collection

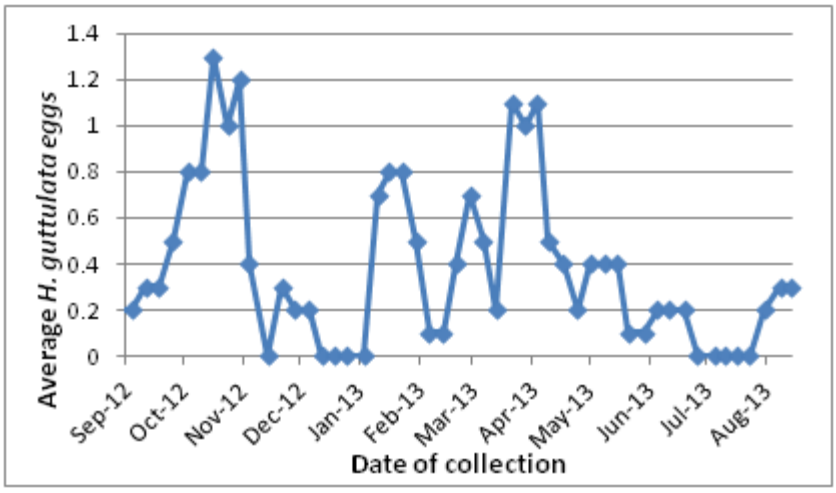
c)


Average P. californicus eggs
4
3.5
3
2.5
2
1.5
1
0.5
0
Sep-12
Oct-12
Nov-12
Dec-12
Jan-13
Feb-13
Mar-13
Apr-13
May-13
Jun-13
Jul-13
Aug-13
Date of collection

d)



e)



f)

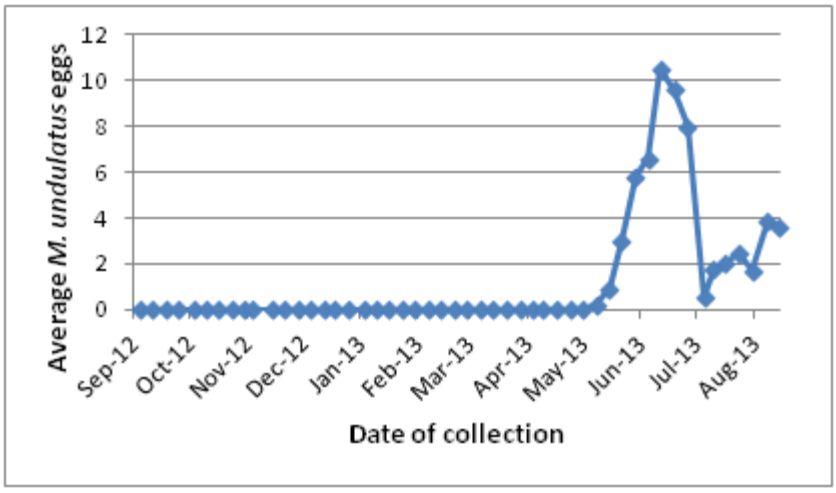
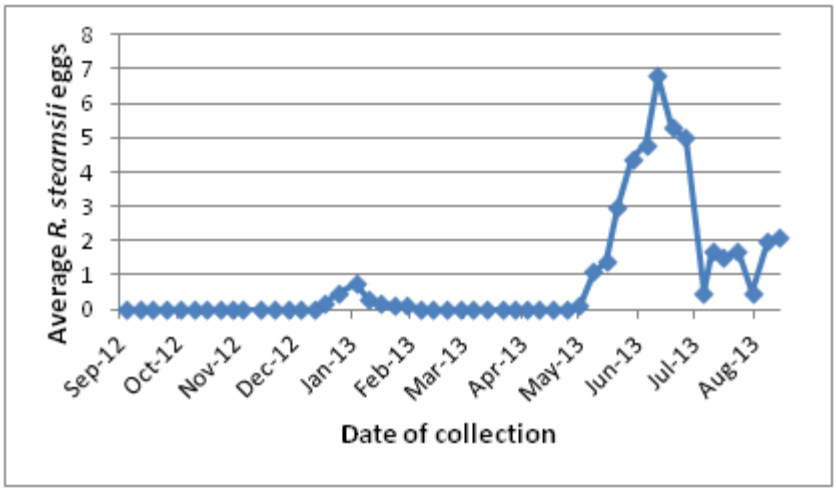
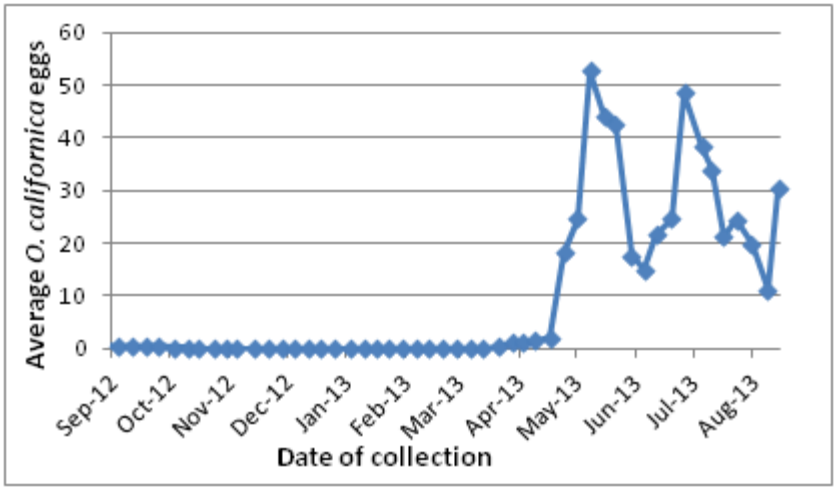


Figure 5. continued

g)



h)



i)

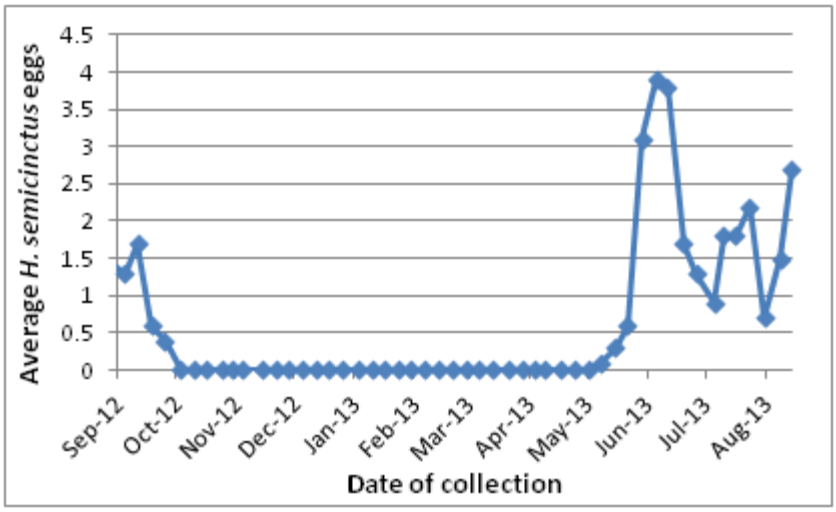
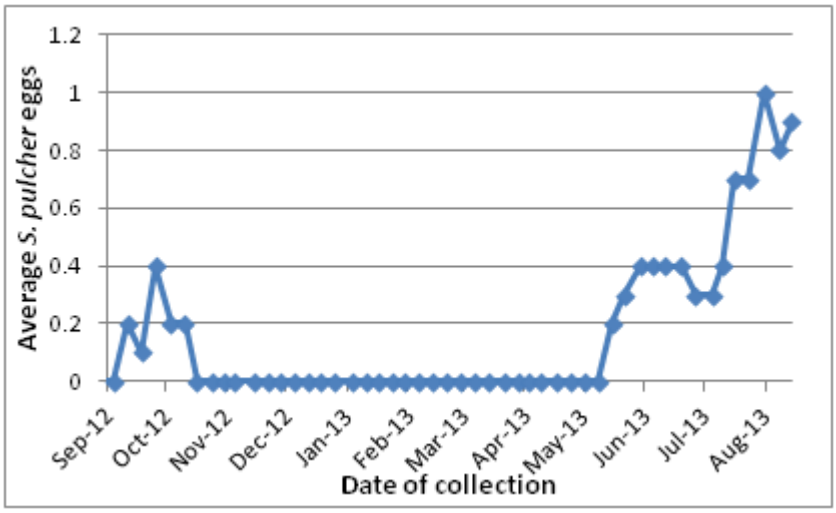
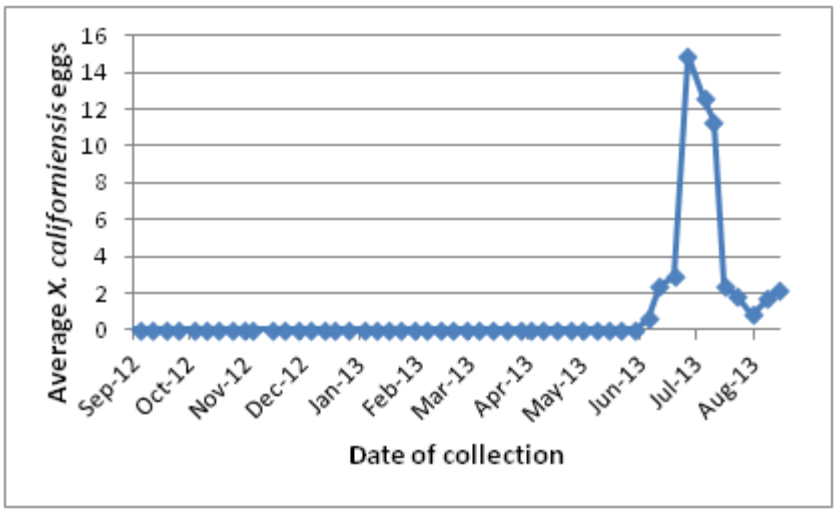


Figure 5. continued

j)



k)



l)

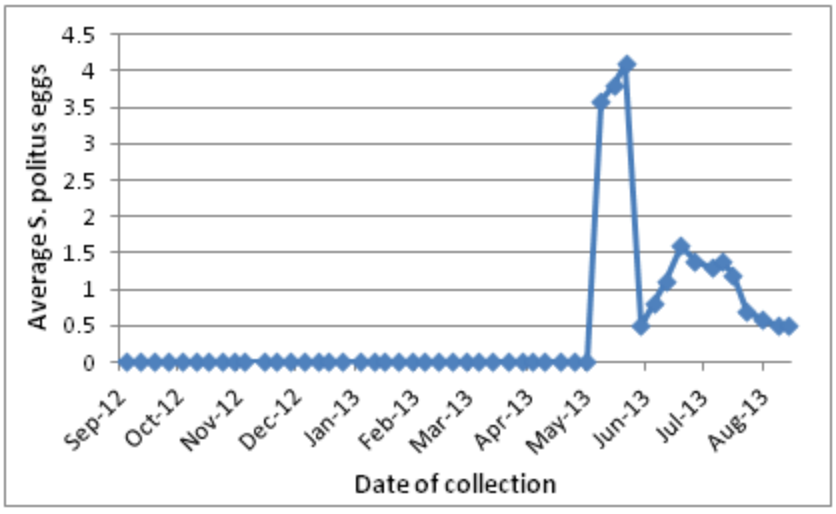
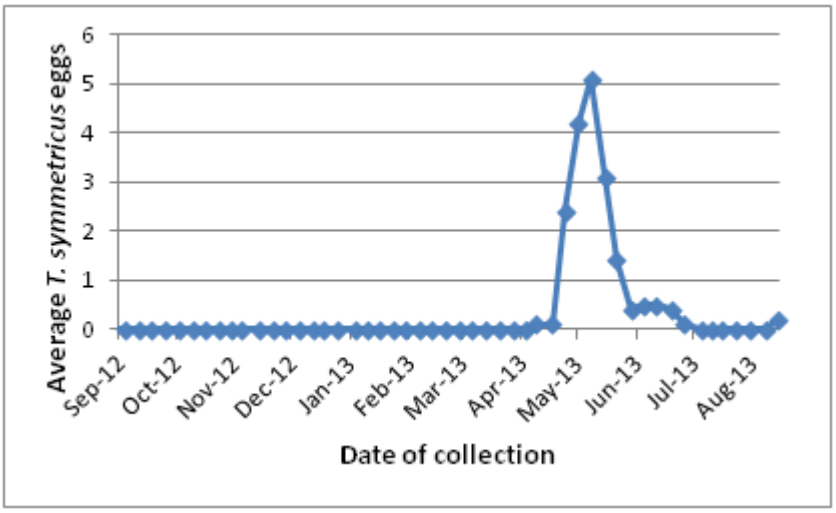
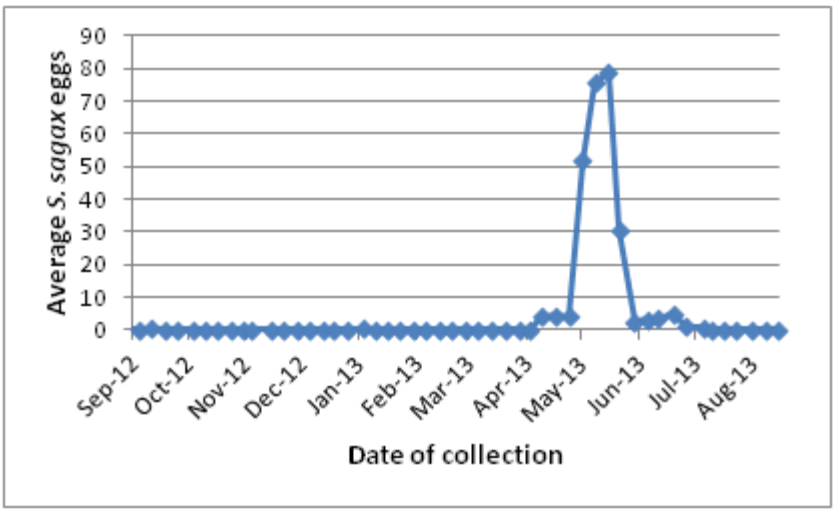


Figure 5. continued

m)



n)



o)

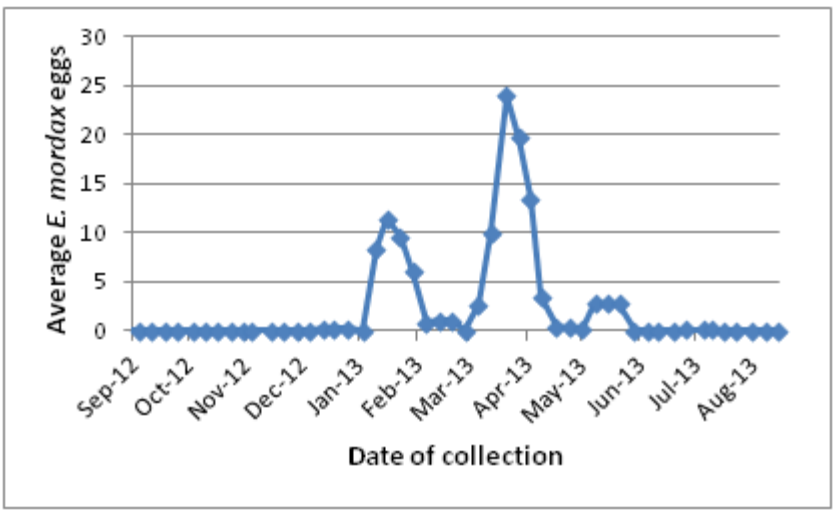
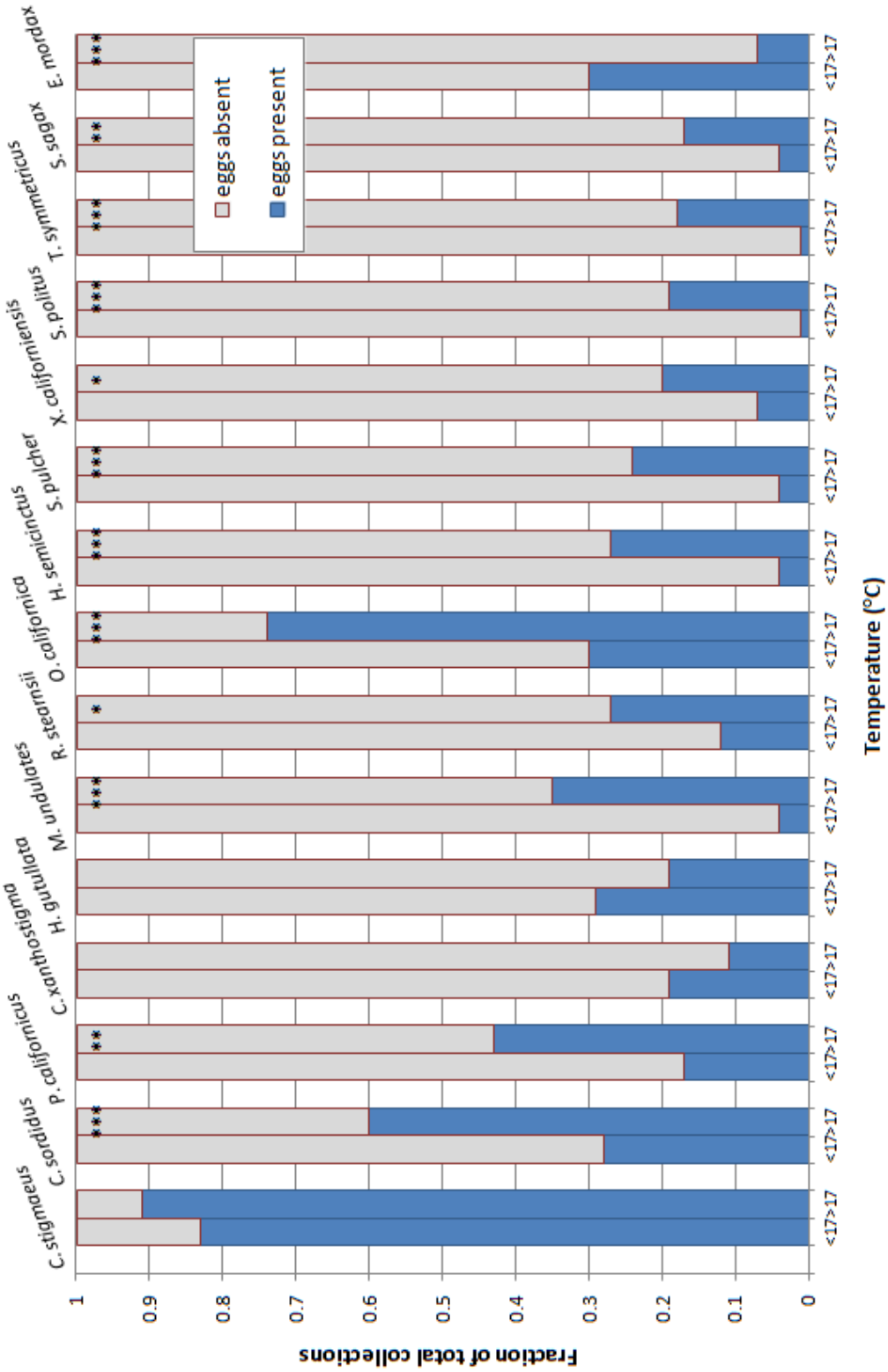


Figure 5. continued

Figure 6. Relationship between spawning and temperature. Depicts fraction of total collections with eggs present and absent, as a function of water temperature. * denotes $P<0.05$; **, $P<0.01$; and ***, $P<0.001$. The likelihood ratio test was used to assess the significance.


C. stigmaeus
C. sordidus
P. californicus
C. xanthostigma
H. gutulata
M. undulates
R. stearnsii
O. californica
H. semicinctus
S. pulcher
X. californiensis
S. politus
T. symmetricus
S. sagax
E. mordax
eggs absent
eggs present
<17
>17
Temperature (°C)
Fraction of total collections
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

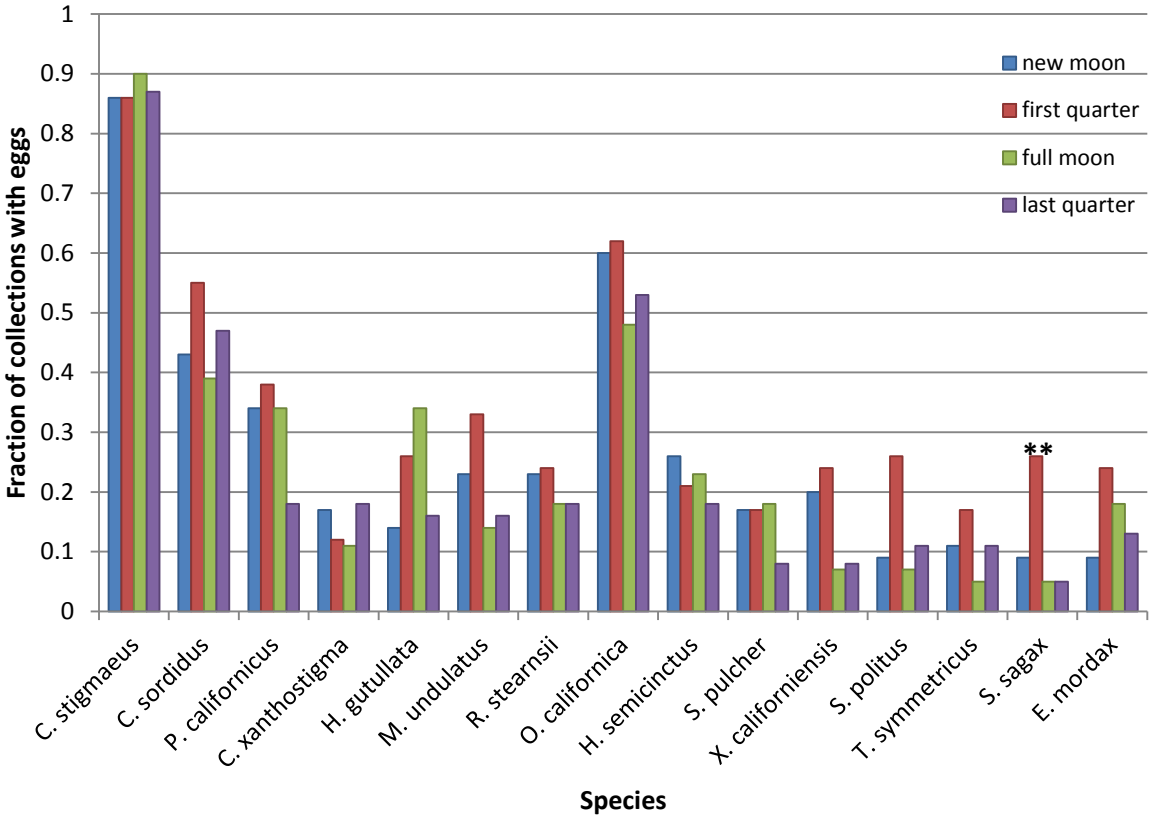


Figure 7. Relationship between spawning and lunar phase. Depicts fraction of total collections with eggs present, as a function of lunar phase. ** denotes P<0.01. The likelihood ratio test was used to assess the significance.

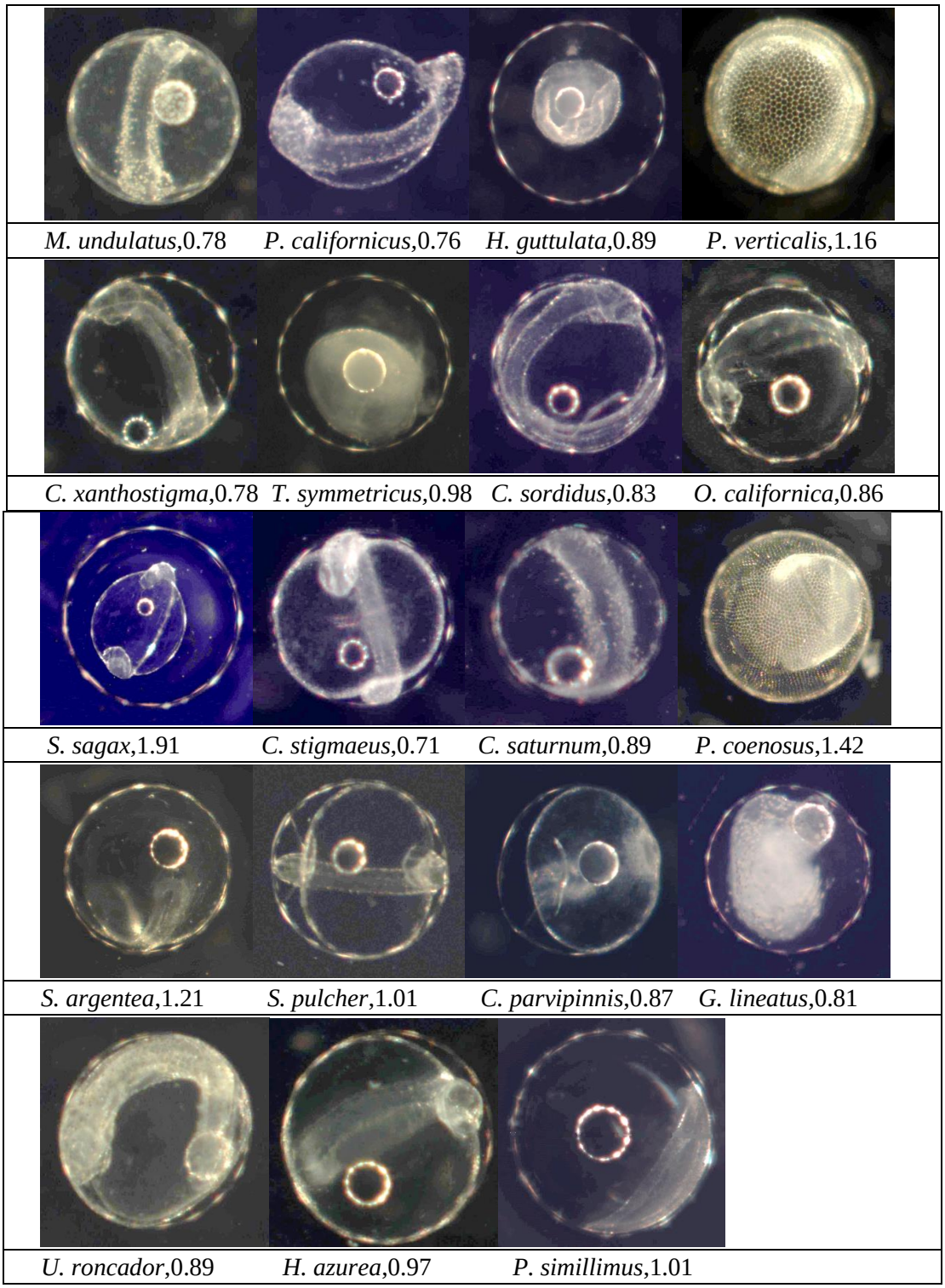


Figure 8. Photographs of fish eggs collected from off the Scripps Pier. Labels denote species name followed by measured diameter of egg in mm.

Table 3. Measured fish egg sizes, giving diameter mean and standard error.

Species	**Mean diameter (mm)**	**SE**
Citharichthys stigmaeus	0.66	0.01
Oxyjulis californica	0.81	0.01
Sardinops sagax	1.86	0.03
Menticirrhus undulatus	0.83	0.01
Trachurus symmetricus	1.04	0.02
Hypsopsetta guttulata	0.85	0.01

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