UNIVERSITY OF CALIFORNIA SAN DIEGO

A Preliminary Comparison of Ichthyoplankton Surveys vs. Environmental DNA Metabarcoding to Survey a Nearshore Marine Fish Community.

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in

Marine Biology

by

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The thesis of Michaela Labare is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

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ABSTRACT OF THE THESIS

A Preliminary Comparison of Ichthyoplankton Surveys vs. Environmental DNA Metabarcoding to Survey a Nearshore Marine Fish Community.

by

Michaela Labare

Master of Science in Marine Biology

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Professor Ronald Burton, Chair

Understanding fish diversity patterns is critical for fisheries management amidst overfishing and climate change. Fish egg surveys have been used to characterize pelagic spawning fish communities, estimate biomass, and track population trends in response to perturbations. Environmental DNA (eDNA) metabarcoding has been implemented to rapidly and non-invasively survey marine ecosystems. To understand the efficacy of eDNA metabarcoding for assessing pelagic spawning fish community composition, concurrent

eDNA metabarcoding and fish egg DNA barcoding off Scripps Institution of Oceanography's Pier (La Jolla, CA) were conducted. Both methods revealed seasonal patterns in agreement with previous fish and fish egg surveys. Species richness was highest in late spring and summer. The presence and spawning of commercially important species and species of conservation concern were detected. Both methods showed overlap for pelagic spawning fishes for broadcast spawners, schooling fish, and locally abundant species. Some actively spawning species were not co-detected with eDNA, likely due to different sampling strategies, taxonomic biases, and abiotic/biotic factors influencing eDNA transport, shedding, and degradation. We identified key advantages and disadvantages of each method. Fish egg barcoding provided information on spawning trends but did not detect taxa with alternate reproduction strategies. Metabarcoding eDNA detected species not found in fish egg sampling, including demersal and viviparous bony fishes, non-spawning adults, Chondrichthyan, and Mammalian species, but missed abundant pelagic fish eggs. This study demonstrates that DNA barcoding of fish eggs and eDNA metabarcoding work best in tandem as each method identified unique fish taxa and provided complementary ecological and biological insight.

INTRODUCTION

Effective and accurate methods for monitoring marine biodiversity are essential in the face of anthropogenic stressors such as climate change, overfishing, and habitat destruction (Sala and Knowlton 2006, Shamshak 2019, FAO 2022). Globally the value of marine and coastal resources/industries is estimated to reach $3 trillion U.S. dollars by 2030, and 3.3 billion people worldwide rely on wild-caught and farmed seafood as a primary source of protein (OECD 2016, FAO 2022). With most U.S. seafood production coming from fully exploited stocks and seafood consumption on the rise, it is imperative to actively assess fish communities to provide the most comprehensive fishery management strategies. Climate change is most notably causing an increase in ocean temperatures leading to species range shifts towards the poles. This can introduce new challenges through invasive species and changes in trophic interactions that can disrupt ecosystems (Auth 2018, Pinksy 2019, Sorte 2010). If we can determine the composition of fish communities and how they change over time, we can better conserve and manage our marine resources and biodiversity. We can also make predictions about responses to climate change for mitigation efforts (Auth 2018, Bakker 2017, Beng 2020, Miya 2021).

The waters surrounding Scripps Institution of Oceanography (SIO, La Jolla, CA) have a long history of fish diversity assessments, making them an ideal location for assessing the detection of a well-documented fish community via eDNA metabarcoding (Craig 2004, Hastings 2014, Duke 2018). The area contains two marine protected areas (MPAs), the Matlahuayl State Marine Reserve (SMR), which is designated as a no-take zone, and the San Diego-Scripps Coastal Marine Conservation Area (SMCA,) which allows recreational take of coastal pelagic species by hook and line (Hastings 2014). These MPAs and the immediate area surrounding them host a diversity of habitats, including pier pilings, eel- and surf-grass stands, sandy beaches,

rocky intertidal, rocky reefs, kelp forests, and submarine canyons (Hastings 2014). Extensive cataloging of the Marine Vertebrate Collection at SIO by Hastings et al. (2014) via trawl, gill net, and diver surveys have identified over 250 different species of fish. Despite comprising only 7 km of coastline, these MPAs serve as a refuge for nearly half of all species seen in California state waters (Allen 2006, Hastings 2014). Regarding economic significance, the MPAs likely serve as spawning grounds for commercially important fish species which can stock areas outside the MPAs (Angulo-Valdés 2010, Harada 2015).

Traditional fish survey methods such as hook and line, seine, trawl, and diver-surveys can be costly, time intensive, weather dependent, and ecologically invasive (Maiello 2022). Additionally, traditional survey methods have biases that can exclude certain species from detection and artificially inflate relative abundance (Cristescu 2018, Maiello 2022). These methods can fail to detect rarer species, species that can evade capture, seasonal species, and those living beyond the survey reaches (He 2022, Suzuki 2022). For example, hook and line surveys are subject to interspecific competition for hooks causing a greater proportion of aggressive species to be recovered (Kuriyama 2019). Likewise, diver-mediated surveying can be biased as cryptic species can be misidentified or species with avoidant behavior are not observed (Pais 2018). There are also limitations to when and where traditional methods can be used; for example, MPAs can have restrictions prohibiting the take of marine organisms, and other environments such as deep-sea habitats are costly to survey and challenging to access (Beng 2020).

Researchers have turned to environmental DNA metabarcoding as a potential solution to overcome some of the issues with traditional surveying. Environmental DNA is derived from the scales, skin cells, fecal matter, saliva, and gametes constantly shed by organisms into the

environment (Taberlet 2012). This material contains small DNA fragments that can be collected from water, air, sediment, and other sources and sequenced via metabarcoding to obtain the species composition (Taberlet 2012). This technology has primarily been used to assess terrestrial and freshwater ecosystems but has begun to be implemented for surveying marine communities (Taberlet 2012, Garlapati 2019, Senapati 2019, Wang 2021). In the last decade, the cost of high-throughput sequencing has declined to the point that eDNA metabarcoding is cheaper than traditional surveying (Cristescu 2018). Additionally, collecting water samples can be faster and simpler, leading to increased use of this technology in the marine environment (Cristescu 2018, Ruppert 2019, Wang, 2021). This technique has also been shown to detect a wider array of taxa compared to other methods and may be sensitive to invasive, cryptic, rare, and endangered species in marine environments (Lehman 2020, Budd 2021, Fediajevaite 2021, Miya 2021, West 2021). Moreover, surveying using environmental DNA (eDNA) has been used to work alongside traditional methods to capture the full range of fish diversity (Thomson 2016, Leduc 2019, Fraija-Fernández 2020, Fediajevaite 2021, Valdivia-Carrillo 2021, He 2022, Keck 2022, Maiello 2022). Environmental DNA, like any method, has biases that can influence the results. Many factors affect the generation, degradation, dispersal, and consequently detection of eDNA. False positives can occur when a species' DNA is detected, but they are not present (Wang 2021). This can happen through contamination during processing or if eDNA is transported into an area (Harrison 2019, Wang 2021). False negatives are when a species present is not detected (Wang 2021). This could be caused by lower eDNA concentration due to degradation or dispersal (Harrison 2019). Organisms have different amplification efficiencies, which can be due to varied primer binding affinity or low initial DNA concentration leading to PCR bias (Kelly 2019). These studies are also limited by their reference database; a species that

lack a reference sequence cannot be detected (Gold and Choi 2020). While eDNA has its own biases that can lead to false positives and negatives, it is a promising tool for monitoring marine ecosystems quickly and cost-effectively (Garlapati 2019, Fediajevaite 2021).

Surveys of adult and juvenile fish supply important insight on species composition, abundance, habitat use, and anatomical data, but they do not capture early life history stages (Harada 2015). Likewise, eDNA metabarcoding can detect DNA from all life history stages but cannot determine the life history stage or morphometrics of the target organism(s). Ichthyoplankton surveys can capture the egg and larval stages of fish populations which can be used as indicators of fish community composition and species-specific spawning trends (Harada 2015, Duke 2018, Choi 2021, Miranda-Chumacero 2020). Morphological ichthyoplankton surveys via microscopy have been regularly conducted off the California coast since 1949 as part of the California Cooperative Oceanic Fisheries Investigations surveys (CalCOFI, calcofi.com). The surveys have been used to estimate adult fish biomass through egg abundance, distribution, and spawning success using fish larvae (MacCall 2016, Sydeman 2020). Results of these surveys have aided in fisheries management, such as tracking the collapse and the recent return of the Northern anchovy (*Engraulis mordax*) (MacCall 2016, Sydeman 2020). Furthermore, they have suggested that the waters surrounding SIO serve as spawning grounds for commercially important fish species, which can 'seed' areas outside the MPAs (Angulo-Valdés 2010, Harada 2015). Earlier work has relied on morphological identification of fish eggs. However, these approaches are limited by the lack of distinct morphological characteristics between species leading to a low accuracy rate for species identification (Ko 2013). To address this, researchers employed DNA barcoding of fish eggs resulting in higher resolution species identification (Harada 2015, Duke 2018, de Lima 2020, Choi 2021).

Given the success of DNA barcoding, the Burton Lab at Scripps Institution of Oceanography has been conducting ongoing weekly plankton tows off Scripps Pier since 2012 to assess the diversity and abundance of pelagic spawning fish (Harada 2015, Duke 2018, Choi 2021). These studies have recorded over 50 species of fish, some of which have not been cataloged as adults by the MVC and capture a different community composition compared to the California current species detected through the CalCOFI surveys (Harada 2015, Duke 2018, Choi 2021). This work has also identified seasonal spawning patterns and interannual variation in spawning associated with winter SST, magnitude of upwelling, and climate oscillations such as the El Nino Southern Oscillation (Harada 2015, Duke 2018, Choi 2021). However, eDNA has the potential to capture the same or greater species composition with equivalent species resolution using only a few liters of water. This study seeks to compare eDNA metabarcoding to fish egg barcoding to determine if eDNA can detect the presence of fishes actively spawning and when they are not spawning. If successful, this could be used to quickly and cost-effectively assess spawning fish populations more frequently and within areas that are difficult to survey. The surveys conducted by the Burton lab provide temporal data on the pelagic spawning fish population off Scripps Pier, which can validate the use of environmental DNA metabarcoding for these purposes. We also seek to understand the information provided through concomitant methods and elucidate biological and ecological patterns in detection. Multiple studies have shown that using eDNA metabarcoding with other methods for assessing fish diversity detects more species than either method alone (Thomson 2016, Leduc 2019, Fraija-Fernández 2020, Fediajevaite 2021, Valdivia-Carrillo 2021, He 2022, Keck 2022, Maiello 2022). Combining eDNA and fish egg barcoding could elucidate patterns in presence and spawning to understand interannual variation in spawning. For example, determining if a species is present but not

spawning or if a species is just not in the study area. Moreover, this data can be used to identify seasonal and interannual patterns in the detection and spawning of protected or commercially relevant species, which can aid in better management of the species through imposing seasonal restrictions and expanding MPAs (de Souza 2016, Lehman 2020, Budd 2021, Troth 2021, Suzuki 2022). The two methods can capture all life history stages and be used to estimate biomass and larval success (MacCall 2016, Beng 2020). Using eDNA can also detect additional taxa that do not spawn in the area or use alternate reproduction strategies such as demersal spawning or viviparous reproduction like many elasmobranchs (Bakker 2017, West 2020, 2021).

METHODS

Study site

To compare eDNA metabarcoding and fish egg DNA barcoding, 17 eDNA samples, and 44 fish egg samples were collected from March to August of 2021 off Ellen Browning Scripps Memorial Pier at the Scripps Institution of Oceanography (SIO) in La Jolla, California, USA (Figure 1). The marine habitat surrounding the pier is primarily sandy bottom and dotted with rocky substrate (Hastings 2014). Metadata including sea surface temperature (SST), salinity, current speed, and chlorophyll level for each sample was retrieved from the Scripps Pier automated shore station dataset (SCCOOS, https://sccoos.org/) and averaged for the day (Table 1).

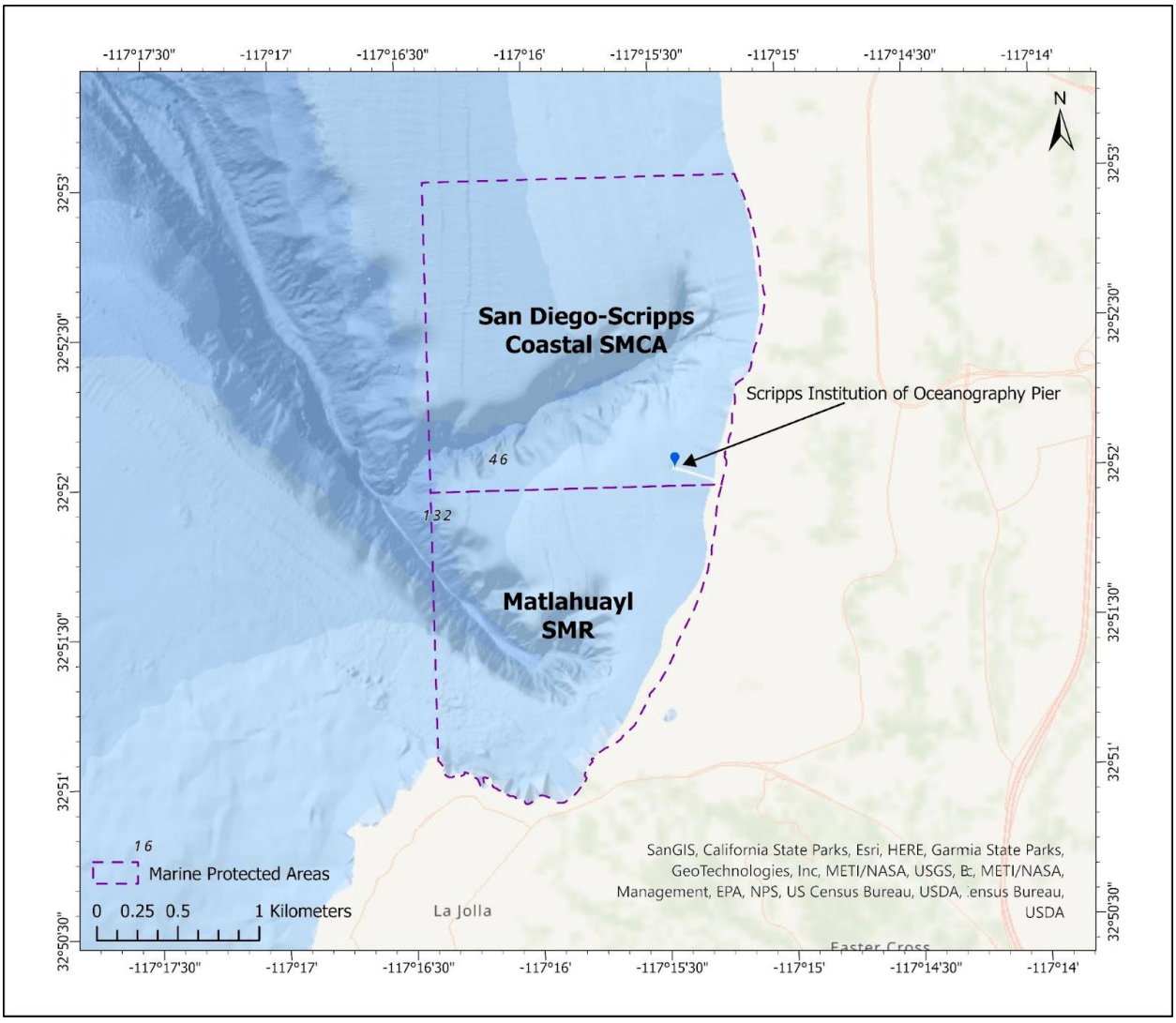

Figure 1. Map of the study site and collection location, Ellen Browning Scripps Memorial Pier at the Scripps Institution of Oceanography in La Jolla, California, USA (SIO). Dashed lines indicate the two surrounding marine protected areas, the Matlahuayl State Marine Reserve (SMR) and the San Diego-Scripps Coastal Marine Conservation Area (SMCA). The map was generated using ArcGIS Pro version 3.0.0 (ESRI).

Table 1. Sample metadata was collected via the Scripps Pier automated shore station dataset (SCCOOS, https://sccoos.org/).

Sample Date	Temperature (Celcius)	Salinity (ppt)	Chlorophyll (µg/L)	Current (mA)
March 4, 2021	14.3	28.1	3	170.3
March 11, 2021	15	33.5	4.5	192.8
April 2, 2021	15.8	33.6	4.4	206.6
April 23, 2021	16.5	33.6	35.2	172.9
April 30, 2021	15.4	33.5	59.1	177
May 7, 2021	18.3	33.6	3.2	192.1
May 28, 2021	19.6	33.1	65.5	163.6
June 2, 2021	19.1	32.3	53.9	159
June 11, 2021	18.9	33.6	2.9	181.9
June 18, 2021	19	33.6	7.7	171
June 24, 2021	19	33.6	18.4	173
July 9, 2021	21.7	33.5	7.2	168.7
July 23, 2021	22	33.3	1.9	171
July 30, 2021	17.8	32.8	6	169
August 5, 2021	15.3	33.1	3.7	180.1
August 13, 2021	17.9	33.1	1.6	172
August 20, 2021	21.2	33.3	3.1	175.4

Fish egg collection and processing

Vertical plankton tows were conducted concurrently with eDNA sampling off Scripps Pier (Figure 1). An additional 37 fish egg samples were collected during the sampling period to assess co-detection between methods by month (Table S1). A plankton net (505 µm) was lowered to the seafloor and back out of the water four times to collect the pelagic fish eggs into a bottle at the cod end. After the final tow, the net was lowered until the rim touched the surface and raised back up to flush any residual eggs in the net into the collection bottle. The sample was then transferred into a 1-liter bottle, immediately transported back to the laboratory (Scripps Institution of Oceanography) and concentrated by pouring the contents through a mesh screen (330 µm). The concentrated plankton sample was placed into 4-6 Petri dishes containing filtered seawater and examined under a microscope at 7.5x magnification. Fish eggs were counted and removed from the sample using a Pasteur pipet and placed in 1.5 mL microfuge tubes with 95%

ethanol. Two species in the survey have morphologically distinct eggs; the Northern anchovy (*Engraulis mordax*) and the Pacific sardine (*Sardinops sagax*), which were quantified and stored in separate 1.5 mL microfuge tubes. All eggs were stored at -20°C for at least 24 hours until further processing. Fish egg DNA extraction, amplification, sequencing, and identification steps were performed in accordance with the protocols used by Harada et al. (2015), Duke et al. (2018), and Choi et al. (2021). Fish eggs were placed individually in 0.2 mL PCR strip tube wells. Ethanol was removed, and each egg was rinsed in 90 µL nuclease-free water to further remove residual ethanol and debris. The water was then removed, and 15 µL of a 66% Buffer AE solution (Qiagen, Hilden, Germany) was added to each tube. The samples were then incubated at 95°C for 15 min in a thermal cycler and maintained at a 72°C hold. A new pipette tip was used to crush each egg, releasing the DNA into the buffer AE solution. The DNA was stored at -20°C until further processing. PCRs were performed on each fish egg DNA sample using the COI (cytochrome oxidase I) universal primers from Ivanova et al. (2007). The PCR reaction contained 1 µL of DNA, 0.5 µL of each of the 10 µM forward (5'-TTCTCAACCAACCACAAAGACATTGG-3') and reverse (5'-ACTTCYGGGTGRCCRAARAATCA-3') primers, 10.5 µL of molecular grade water, and 12.5 µL of GoTaq Green 2X Master Mix (Promega, Madison, WI). Each reaction was vortexed briefly to ensure they were mixed, quickly centrifuged, and then placed in a thermal cycler following the cycling conditions utilized by Harada et al. (2015), Duke et al. (2018), and Choi et al. (2021). The PCR products were then visualized on a 1.5% agarose gel to check for a 710 base pair band. Samples that were successfully amplified were purified and sent for Sanger sequencing. Another PCR was performed to amplify the 16S rDNA gene on samples that were not amplified using COI. The reaction used the same reagents as the COI PCR with the 16S

primer set instead (forward: 5'-CGCCTGTTATCAAAAACAT-3' and reverse: 5'-CCGGTCTGAACTCAGATCACGT-3') from Palumbi et al. (1996). Cycling conditions remained the same, and products were visualized in the same manner to check for a 570 base pair band. Samples that were successfully amplified were purified and sent for Sanger sequencing. Purification was performed according to Harada et al. (2015), Duke et al. (2018), and Choi et al. (2021) and sent to Retrogen, Inc. (San Diego, CA) for Sanger sequencing in 12 µL reactions with 10 µL purified PCR product and 2 µL of either the COI or 16S forward primer (depending on which primer was used in the PCR). Sequences were then trimmed to remove primers and ambiguous bases and assessed for quality. Sequences were then run through BLAST to compare the samples to all sequences in GenBank. If the sequences matched a sequence in the database at 95% similarity or higher, it was classified as the species corresponding to that sequence. Two closely related species, the longfin sanddab (*Citharichthys xanthostigma*) and the Pacific sanddab (*Citharichthys sordidus*) could only be differentiated from one another if the sequences matched at greater than 99% similarity.

An additional analysis was performed using the fish egg survey collection data from 732 collections from August 2012 to May 2022 to assess trends in spawning of the over-exploited Northern anchovy (*Engraulis mordax*).

Environmental DNA collection and processing

Water samples were collected off Scripps Pier via crane, rope, and pulley, with a weighted bucket sterilized with 10% bleach three times and rinsed with Milli-Q ultrapure water three times. The collection bucket and sterile bottles were rinsed with surface ocean water prior to sample collection. Surface water samples (3 L) were obtained and immediately transported to

the lab for further processing in a Polymerase chain reaction-free room. The outside of the bottles was again sterilized with bleach. Two 0.45 µm cellulose filters were used to filter the 3 liters of water with a vacuum pump. Filters were cut in half and stored in separate sterile centrifuge tubes at -80 °C until further processing. One-half of the filter was subjected to DNA extraction in a separate PCR-free sterilized area. The remaining halves were archived at -80 °C. Each sample consisted of one-half of each of the two filters and were cut into smaller pieces and extracted using the Powersoil extraction kit (Qiagen, Hilden, Germany). Extracted DNA concentrations were measured using fluorometry (Qubit HS assay kit, Thermofisher Scientific, Waltham, MA), and samples were normalized to the same concentration. PCRs were performed in triplicate to amplify the fish-specific 196 bp 12S rRNA gene using the 12S MiFish Universal Teleost and Elasmobranch primers (Miya 2015). The 0.2 ml reaction tube contained 1 µL sample DNA, 12.5 µL of GoTaq Green 2X Master Mix (Promega, Madison, WI), 10.5 µL molecular grade water, and 0.5 µL each of the 10 µM 12S MiFish-U forward (5'-GCCGGTAAAACTCGTGCCAGC-3') and reverse primer (5'-CATAGTGGGGTATCTAATCCCAGTTTG-3') which contained Illumina adapter sequences (Illumina, San Diego, CA, Miya 2015). Cycling conditions were an initial denaturation step at 95°C for 3 minutes followed by 30 cycles of denaturation at 95°C for 1 minute, annealing at 54°C for 1 minute, elongation at 72°C for 1 minute, and a final elongation step at 72°C for 7 minutes. PCR products were visualized on a 1.5% agarose gel with SYBR Safe dye (Invitrogen, Waltham, MA).

Triplicate PCRs were pooled and cleaned using SPRI AMPure XP beads (Beckman Coulter). This process was repeated under the same conditions using the 12S MiFish-E primers (forward 5'-GTTGGTAAATCTCGTGCCAGC-3' and reverse 5'-

CATAGTGGGGTATCTAATCCTAGTTTG-3'). A second round of PCR amplification was conducted on the cleaned product to add combinatorial dual indices to each sample using the Nextera XT Index Kit v2 (Illumina, San Diego, CA). The reaction tube contained 1.25 µL of each 10 µM indexing primer (make a table w/index for each sample), 5 µL of the pooled PCR product, 5 µL molecular grade water, and 12.5 µL of GoTaq Green 2X Master Mix (Promega, Madison, WI). Cycling conditions were an initial denaturation step at 95°C for 3 minutes followed by 8 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, elongation at 72°C for 30 seconds, and a final elongation step at 72°C for 5 minutes. The indexed PCR products were visualized on a 1.5% agarose gel with SYBR safe dye (Invitrogen, Waltham, MA) and cleaned using SPRI AMPure XP beads (Beckman Coulter, Indianapolis, IN). Cleaned, indexed products were measured for DNA concentration using fluorometry (Qubit HS assay kit, Thermofisher Scientific, Waltham, MA), and samples were pooled at equimolar concentrations to reach a final concentration of 5 nM. Libraries were then sequenced via 300 bp paired-end sequencing at the IGM Genomics Center, University of California, San Diego, La Jolla, CA, utilizing an Illumina MiSeq.

Bioinformatics

Environmental DNA metabarcoding sequences were processed using the Anacapa Toolkit (version 1) to perform quality control, parse amplicon sequence variants (ASV), and assign taxonomy (Curd 2019). Taxonomic assignment was made using the 12S-fish-specific regional reference database curated for the California Current Large Marine Ecosystem (Gold 2020, 2021). ASVs were then run through NCBI's Basic Local Alignment Search Tool (nucleotide BLAST) to verify taxonomic assignment by comparing the ASV sequence against

those in Genbank to resolve ASVs that were not assigned to a taxon or were resolved to a taxa level above species. If the ASV sequence matched a sequence at 95% or higher, fit the geographic distribution better than the Anacapa results, or was unassigned by Anacapa, the ASV was assigned to that species. For example, an ASV assigned to Embiotocidae was resolved further to the genus *Amphistichus*. Two shark ASVs were assigned to species that did not fit the region, but BLAST matched them to the species known to inhibit the region. There were also three mammalian ASVs not assigned to any taxonomic group which had a >99% identity via BLAST, the common dolphin, harbor seal, and California sea lion. ASVs assigned to Sciaenidae were placed into three groupings (Sciaenidae 1, 2, and 3) as they differed by more than two base pairs. ASVs were then binned by taxonomic assignment and sample.

Data analysis

To compare the results of the eDNA metabarcoding and fish egg DNA barcoding, we looked at total pelagic spawning fish taxa from each method. We classified the ASVs belonging to Actinopterygii based on their spawning strategy to only include broadcast spawners, open water/substratum egg scatterers, and fish which attach their eggs to floating debris such as macroalgae. Additionally, non-pelagic spawning fish taxa detected by eDNA were assessed. Venn diagrams were constructed to visualize the overlapping and unique pelagic fish taxa detected. An Analysis of Variance (ANOVA) was performed to compare species richness between methods using the *vegan* package (version 2.6-2) in *RStudio* (version 2021.09.1-372, R Core Team 2021) (Oksanen 2022). GraphPad Prism (version 8.0.0) was used to assess method detection; the overlap of species using presence-absence data was calculated and performed for the pelagic spawning fish for concurrent sampling. The same assessment was repeated on pelagic

spawning fish from eDNA metabarcoding, and all fish egg barcoding samples taken during the sampling period within a month (additional 27 fish egg samples). A linear regression for species richness was calculated using pelagic spawning fish for concurrent samples (Prism). Relative abundance of eggs and reads for pelagic spawning fish was calculated and assessed (Prism). We compared differences in community composition of pelagic spawning fish from concurrent collections by performing a permutational analysis of variance (PERMANOVA) on Jaccard-binary dissimilarity indices generated using presence/absence data with the *vegan* (version 2.6-2) and *phyloseq* (version 1.38.0) packages (McMurdie and Holmes 2013, Oksanen 2022). A principal components analysis (PCA) was used to visualize differences in sampling method and month via the vegan package (Oksanen 2022). A linear regression was performed to assess spawning trends from 2012-2022 for the Northern anchovy (*Engraulis mordax*). Range, occurrence, and habitat assessments done by Hastings et al. (2014) were used to examine the fish community recovered. Hastings et al. (2014) defined range by determining the range midpoint and binning it into the following categories: Northern (range midpoint North of Point Conception, California), Southern (range midpoint south of Punta Eugenia, Mexico), and Central (range midpoint between Point Conception and Punta Eugenia). If there was no documented collection, we referred to fishbase.org and Allen and Horn (2006) to make the determination following the criteria set by Hastings et al. (2014).

RESULTS

Summary

Our results reveal that while the methods detect distinct species of pelagic spawning fishes, there is substantial overlap in all the pelagic spawning fish species detected between the two methods. Concurrent sampling showed that eDNA often failed to co-detect species which were abundant in the pelagic fish eggs samples. Environmental DNA metabarcoding detected additional taxa with alternate reproductive strategies and non-spawning fishes. Fish egg barcoding showed seasonal spawning patterns corresponding to previous surveys. The two methods together uncovered biological and ecological patterns in spawning and eDNA detection.

Comparison of concurrent eDNA and fish egg samples

In the 17 samples collected from March to August, eDNA detected 42 ASVs from vertebrate taxa representing three classes, 19 orders, 27 families, 36 genera, and 37 species (Table S1). Within the vertebrates, 35 ASVs belonged to the family Actinopterygii, 4 to Elasmobranchii, and 3 to Mammalia (Table S1). There were 28 ASVs belonging to pelagic spawning fishes, with 24 resolved to the species level and 4 to the family level (1 Labridae, 3 Sciaenidae). The pelagic spawning ASVs represented 12 orders, 16 families, 23 genera, and 24 species (Table S1). A total of 655,644 12S reads from the MiSeq run were generated. However, only 7,508 belonged to vertebrate taxa, primarily due to the amplification of non-target bacterial sequences (Table S2). Of the 1811 eggs collected during concurrent sampling, 1,697 were successfully amplified and resolved to the species level (93.7%) using DNA barcoding of either the COI or 16S gene (Table S3). We successfully identified 26 species in the fish egg survey, representing 7 orders, 11 families, and 21 genera (Table S3).

Looking at the pelagic spawning ASVs resolved to the species-level, eDNA captured 61.5% (16/26) of the species detected in the fish egg tows and 70.6% (24/34) of all the pelagic spawning fish species detected by both methods (Figure 2). Environmental DNA failed to detect 10 species seen in the fish egg survey: the Pacific golden-eyed tilefish (*Caulolatilus affinis*), black croaker (*Cheilotrema saturnum*), longfin sanddab (*Citharichthys xanthostigma*), shortfin corvina (*Cynoscion parvipinnis*), rock wrasse (*Halichoeres semicinctus*), spotted sand bass (*Paralabrax maculatofasciatus*), barred sand bass (*Paralabrax nebulifer*), c-o sole (*Pleuronichthys coenosus*), spotfin croaker (*Roncador stearnsii*), and the California sheephead (*Semicossyphus pulcher*). In contrast, eight species were seen only in the eDNA: the striped mullet (*Mugil cephalus*), yellowtail jack (*Seriola lalandi*), sharpchin flyingfish (*Fodiator acutus*), California needlefish (*Strongylura exilis*), California lizardfish (*Synodus lucioceps*), opaleye (*Girella nigricans*), Pacific chub mackerel (*Scomber japonicus*), and the zebraperch (*Kyphosus azureus*) (Figure 2).

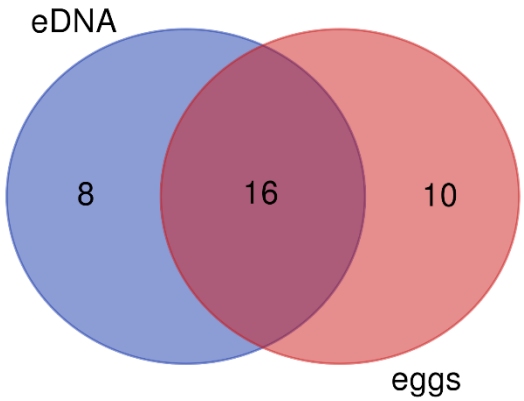

Figure 2. Venn diagram of eDNA and fish egg species detection of pelagic spawning fish from concurrent sampling events (including only taxa resolved to the species level). Environmental DNA captured the bulk of the pelagic spawners detected between both methods (24/34). However, fish egg barcoding captured a greater proportion (26/34) of the total taxa. An additional 4 ASVs detected in the eDNA that were resolved only to the family level (Sciaenidae and Labridae), which likely led to the greater proportion of species detected by fish egg DNA barcoding.

Species richness for eggs and eDNA was calculated for pelagic spawning fishes, including ASVs resolved to higher taxonomic classifications. Fish egg and eDNA richness was positively correlated ($p = 0.0008$, $R^2 = 0.5376$) (Figure 3). Environmental DNA had an average richness of 6.7 (± standard deviation 3.4, range 3-15), and fish eggs had an average richness of 7 (± standard deviation 3.2, range 2-15). Both methods combined had an average richness of 6.9 (± standard deviation 3.3, range 2-15). Richness did not differ significantly by method (ANOVA, $p > 0.05$) (Table S4). June 18th displayed the highest richness for both eDNA and fish eggs (Tables S1 and S2). Average species richness was highest in June for eDNA and August for fish eggs and lowest in March and April for both methods (Table S5). Samples taken in the summer had the highest average species richness for both eDNA and eggs, followed by the spring, then winter (Table S6).

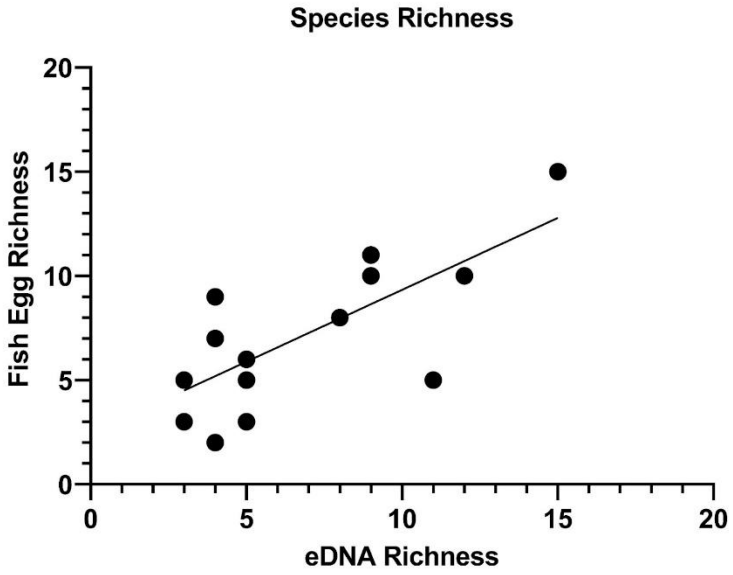

Figure 3. Species richness correlation of pelagic spawning fish from concurrent sampling events (including only taxa resolved to the family level) for eDNA and fish eggs ($p = 0.0008$, $R^2 = 0.5376$).

Speckled sanddab (*Citharichthys stigmaeus*) and Pacific sardine (*Sardinops sagax*) were seen at each sampling event, and the Northern anchovy (*Engraulis mordax*) was detected by both methods more often than all other species for a given sampling event (Figure 4). Twelve species were seen in both the eDNA and egg survey simultaneously in a day, all of which were broadcast spawners belonging to the families: Clupeidae, Haemulidae, Labridae, Paralichthyidae, and Sciaenidae (Figure 4). Seasonal patterns in co-detection were seen in species such as the Northern Anchovy (*Engraulis mordax*), Pacific sardine (*Sardinops sagax*), and sargo (*Anisotremus davidsonii*) (Figure 4).

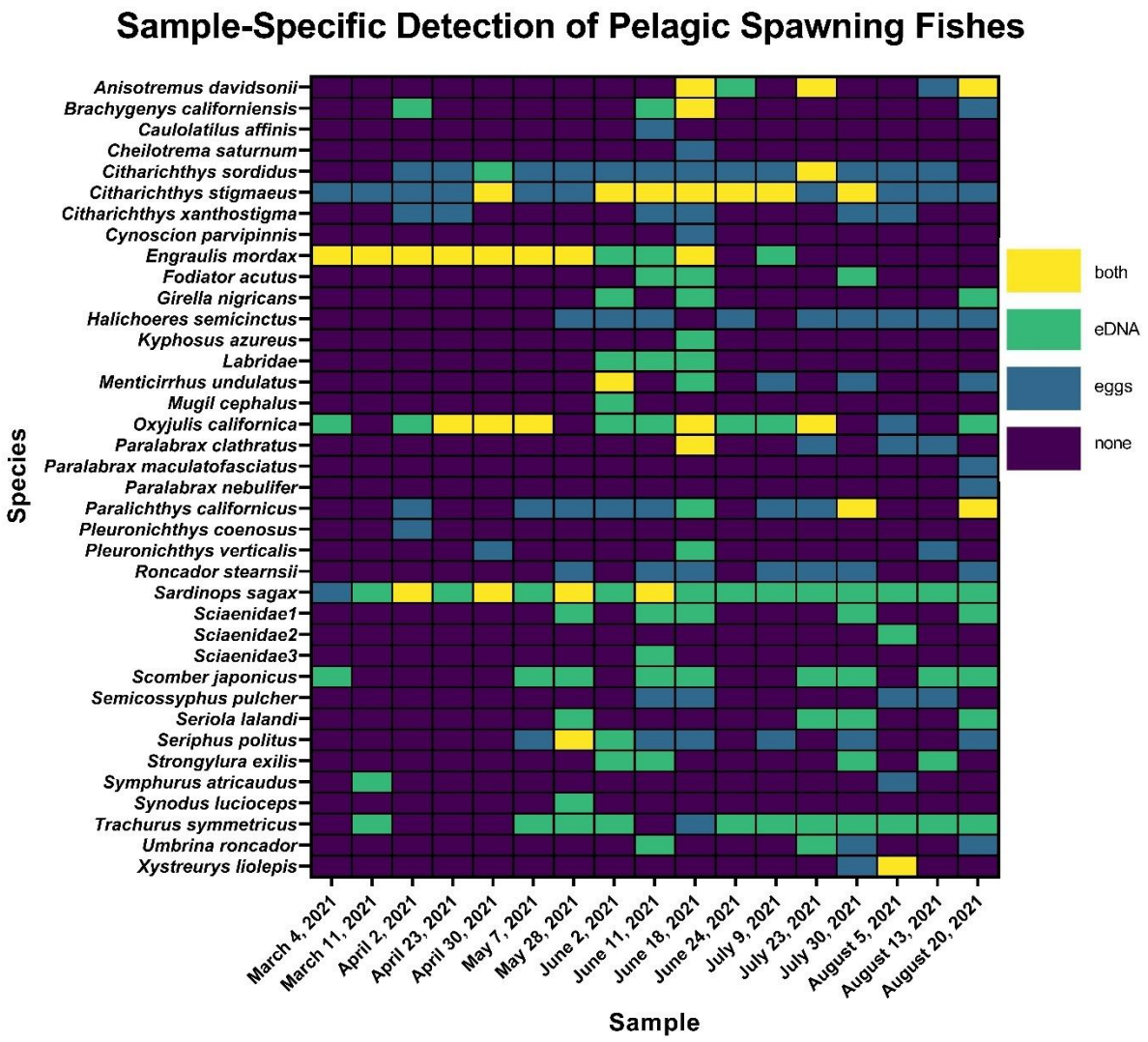

Figure 4. Co-detection of pelagic spawning fish by eDNA and fish eggs, including ASVs resolved to the family level.

We used the metadata collected to assess what variables contributed to the total variation observed among samples (Table S7). Pelagic spawning community composition varied between methods and collection months (Figure 5). Samples were separated along axis 1 by method and along axis 2 by month (Figure 5). Method explained 32% of the total variance, and collection month explained an additional 26% of the total variance (PERMANOVA, $p < 0.001$) (Table S7).

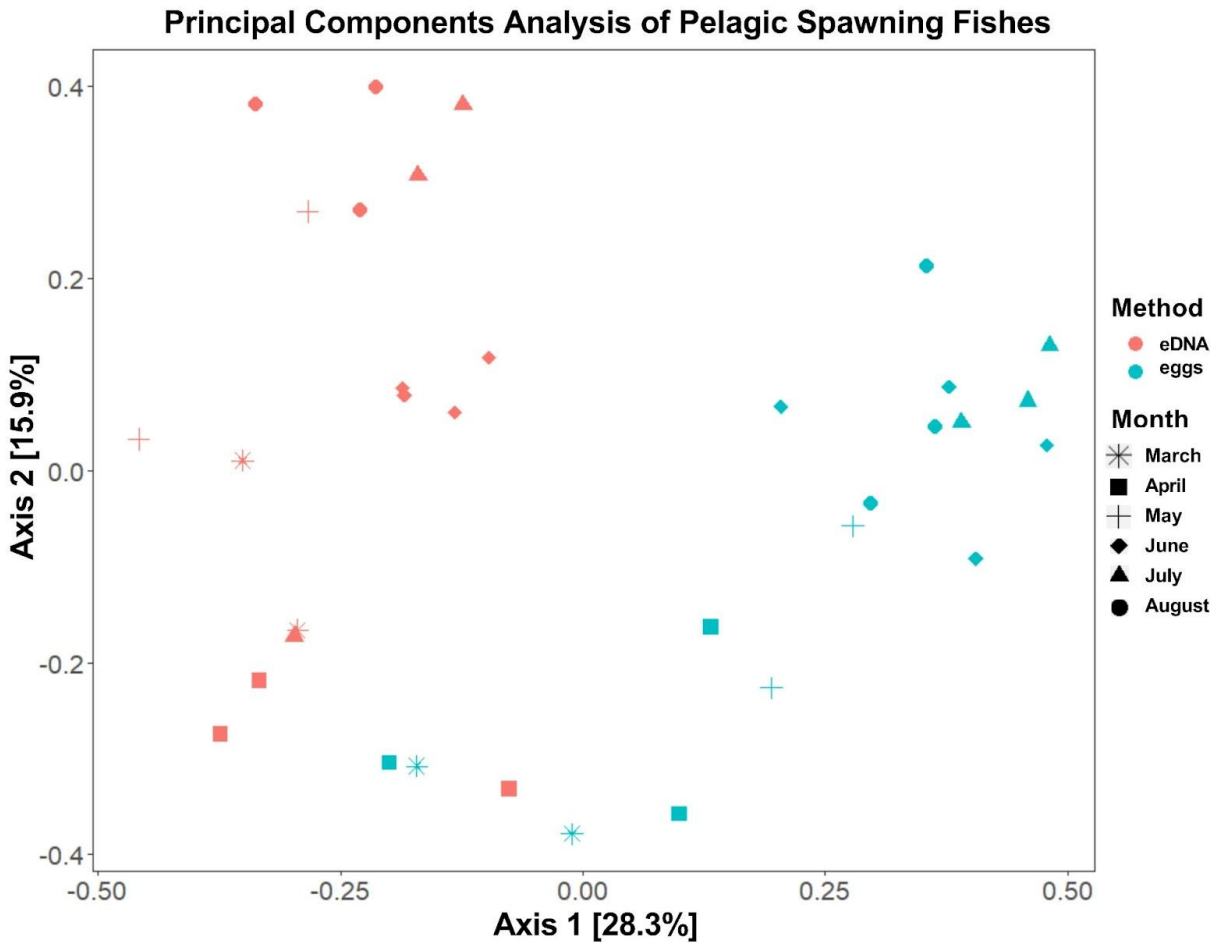

Figure 5. Principal components analysis (PCA) using Jaccard-binary dissimilarities of pelagic spawning fish composition from concurrent sampling. Sample species composition varied by method and collection month. Method explained 32% of the total variance and collection month explained an additional 26% of the total variance (PERMANOVA, $p < 0.001$).

Northern anchovy (*Engraulis mordax*) had the greatest total number of eggs at 762, followed by speckled sanddab (*Citharichthys stigmaeus*) with 671, Pacific sanddab (*Citharichthys sordidus*) with 69, rock wrasse (*Halichoeres semicinctus*) with 45, spotfin croaker (*Roncador stearnsi*i) with 35, señorita (*Oxyjulis californica*) with 28, and longfin sanddab (*Citharichthys xanthostigma*) with 21 (Figure 6 and Table S3). The remaining 19 species each had <20 eggs total, with seven species having only one egg recovered in the samples (Figure 6 and Table S3). There were peaks in egg abundance in March and April primarily due to the

Northern anchovy (*Engraulis mordax*) (Figures 6 and 7). There were also peaks in egg abundance in May, June, July, and August, mainly due to the speckled sanddab (*Citharichthys stigmaeus*), which spawned throughout the entire sampling period (Figures 6 and 7). The Northern anchovy (*Engraulis mordax*) and Pacific sardine (*Sardinops sagax*) spawned from winter to spring and the c-o sole (*Pleuronichthys coenosus*) spawned in the spring (Figure 6). Seasonal trends in spawning are visible from spring to summer for the spotfin croaker (*Roncador stearnsii*), rock wrasse (*Haliochoeres semicinctus*), queenfish (*Serphius politus*), senorita (*Oxyjulis californica*), California corbina (*Menticirrhus undulatus*), sargo (*Anisotremus davidsonii*), longfin sanddab (*Citharichthys xanthostigma*), Pacific sanddab (*Citharichthys sordidus*), and California halibut (*Paralichthys californicus*) (Figure 6). Species seen spawning only in the summer were the yellowfin croaker (*Umbrina roncado*r), jack mackerel (*Trachurus symmetricus*), California tonguefish (*Symphurus atricaudus*), shortfin corvina (*Cynoscion parvipinnis*), salema (*Brachygenys californiensis*), black croaker (*Cheilotrema saturnum*), barred sand bass (*Paralabrax nebulifer*), spotted sand bass (*Paralabrax maculatofasciatus*), kelp bass (*Paralabrax clathratus*), California sheephead (*Semicossyphus pulcher*), fantail sole (Xystreurys liolepis) and the hornyhead turbot (*Pleuronichthys verticalis*) (Figure 6).

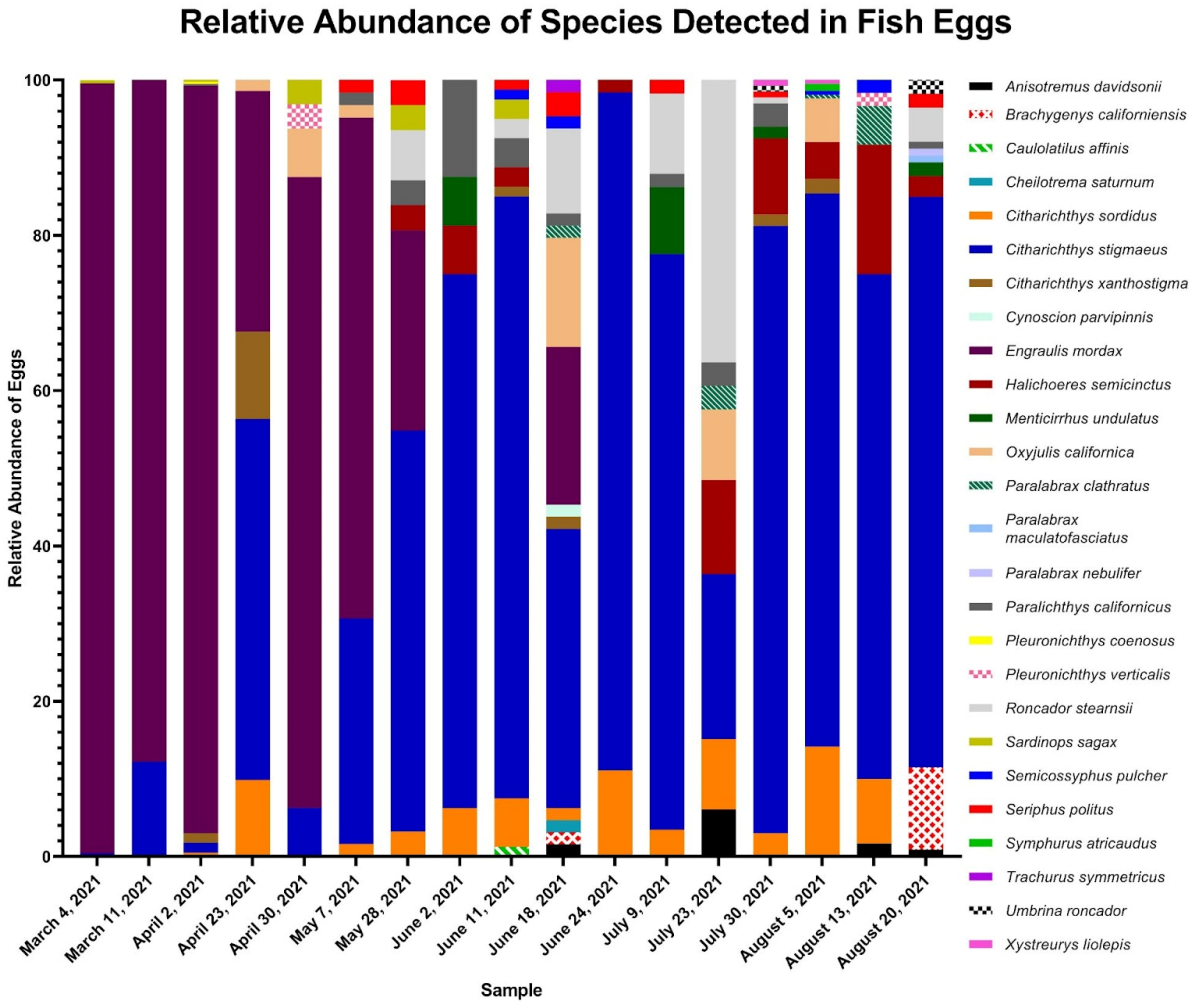

Figure 6. Relative abundance of pelagic spawning fish identified in the fish egg survey calculated using the proportion of eggs collected each day. Seasonal trends in spawning are visible for the most prevalent Northern anchovy (*Engraulis mordax*) and speckled sanddab (*Citharichthys stigmaeus*).

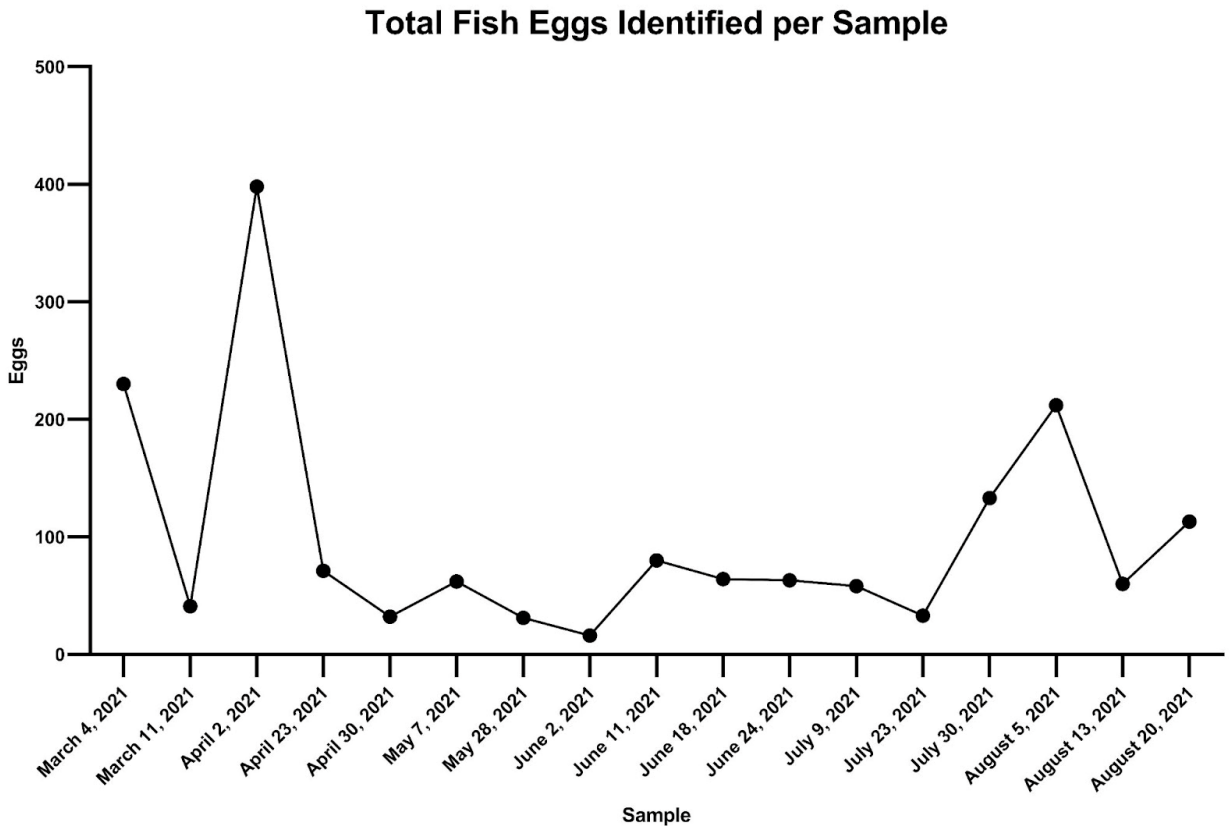

Figure 7. Total identified fish eggs for each sample in which eDNA was collected concurrently. There were 6 peaks in egg abundance primarily due to the spawning of the Northern anchovy (*Engraulis mordax*) and speckled sanddab (*Citharichthys stigmaeus*). April had the highest number of eggs (n = 398).

Seasonal trends in detection via relative abundance of reads are visible for the most prevalent Northern anchovy (*Engraulis mordax)*, Pacific sardine (*Sardinops sagax*), and jack mackerel (*Trachurus symmetricus*) (Table S1). Organisms detected in the eDNA with the greatest relative abundance of reads overall included señorita (*Oxyjulis californica)*, Pacific sardine (*Sardinops sagax*), jack mackerel (*Trachurus symmetricus*), Northern anchovy (*Engraulis mordax*), topsmelt (*Atherinops affinis)*, Pacific chub mackerel (*Scomber japonicus*), bat ray (*Myliobatis californica*), speckled sanddab (*Citharichthys stigmaeus*), sargo (*Anisotremus davidsonii*), yellowfin croaker (*Umbrina roncador*), and an ASV assigned to drums/croakers (Sciaenidae 1) (Table S1). Additional fish taxa detected in the eDNA were the topsmelt

(*Atherinops affinis*), rockpool blenny (*Hypsoblennius gilberti*), and mussel blenny (*Hypsoblennius jenkinsi*), which are demersal spawners. The surfperch (*Amphistichus*), dwarf perch (*Micrometrus minimus*), and the black rockfish (*Sebastes melanops*), which are viviparous species, and the California grunion (*Leuresthes tenuis*), which bury their eggs in the sand, were also all detected (Table S1). There were 4 Elasmobranchii taxa detected, thresher shark (*Alopias vulpinus*), California bat ray (*Myliobatis californica*), spiny dogfish (*Squalus suckleyi*), and round ray (*Urobatis halleri*) (Table S2). Mammalian taxa detected were the harbor seal (*Phoca vitulina*), California sea lion (*Zalophus californianus*), and the common bottlenose dolphin (*Tursiops truncatus*) (Table S1).

Comparison of eDNA to include all fish egg collections

We assessed pelagic spawning fish co-detection within each month using the additional fish egg samples (n = 44) (Table S1). There were 4134 eggs collected, and 3812 were resolved to the species level (92.2%) (Table S3). These eggs represent 10 orders, 14 families, 26 genera, and 32 species. The number of co-detections increased when grouped by month. Sixteen species were seen in both the egg survey and the eDNA, including the speckled sanddab (*Citharichthys stigmaeus*), Pacific sanddab (*Citharichthys sordidus*), señorita (*Oxyjulis californica*), Northern anchovy (*Engraulis mordax*), Pacific sardine (*Sardinops sagax*), salema (*Brachygenys californiensis*), California corbina (*Menticirrhus undulatus*), kelp bass (*Paralabrax clathratus*), Pacific chub mackerel (*Scomber japonicus*), sargo (*Anisotremus davidsonii*), queenfish (*Seriphus politus*), California halibut (*Paralichthys californicus*), jack mackerel (*Trachurus symmetricus*), yellowfin croaker (*Umbrina roncador*), fantail sole (*Xystreurys liolepis*), and the hornyhead turbot (*Pleuronichthys verticalis*) (Figure 8). The month with the most species detected by both

methods was June, with 13 species. The species seen in all five months by both methods were the Northern anchovy (*Engraulis mordax*) and señorita (*Oxyjulis californica*) (Figure 8). Additional species seen the most across months in both methods were the Pacific sardine (*Sardinops sagax*), speckled sanddab (*Citharichthys stigmaeus*), Pacific sanddab (*Citharichthys sordidus*), and sargo (*Anisotremus davidsonii*) (Figure 8). Environmental DNA detected the Pacific sardine (*Sardinops sagax*) more often than the fish egg survey (Figure 8).

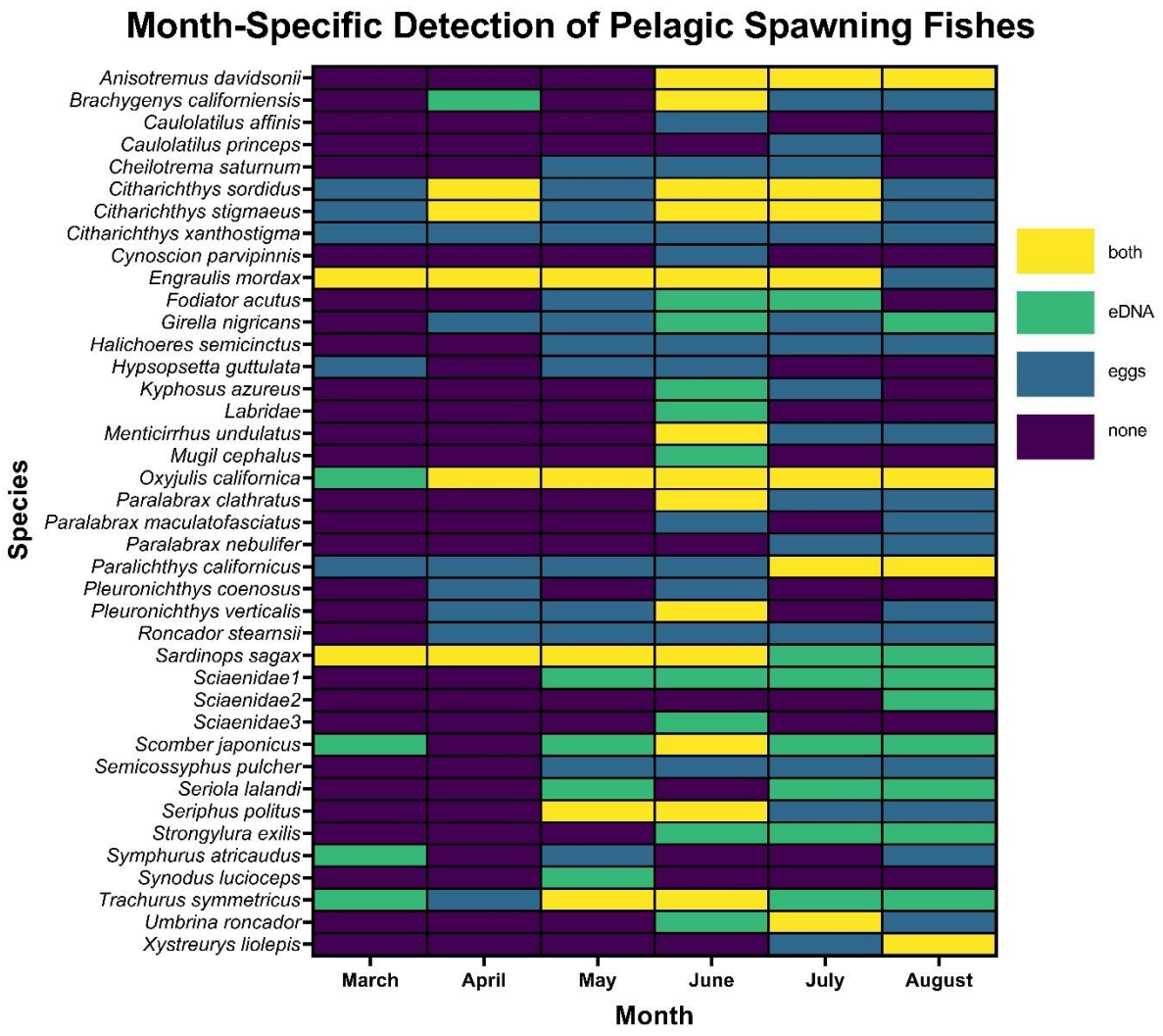

Figure 8. Co-detection of pelagic spawning fish by eDNA and fish eggs, including ASVs resolved to the family level and all fish eggs collected during the entire sampling period (March-August).

Northern Anchovy

We performed an additional analysis using fish egg data from 2012-2022 to assess spawning trends for the Northern anchovy (*Engraulis mordax*) as it was the species detected most frequently and is commercially important. A linear regression was performed using the average number of Northern anchovy eggs per collection each year from 732 fish egg collections (Figure 9). We found that the number of anchovy eggs increased significantly *($p = 0.0046$, R^2 =*

0.6081) (Figure 9). Since the fish egg survey began in 2012 until 2019, the Northern anchovy did not have spawning peaks exceeding 100 eggs on a given day. From 2019 forward, there were multiple days with over 100 eggs each year. Within our own sampling period, there were four collections with >100 eggs, and anchovy eggs were recovered on 29 out of the 44 total collections (Table S3).

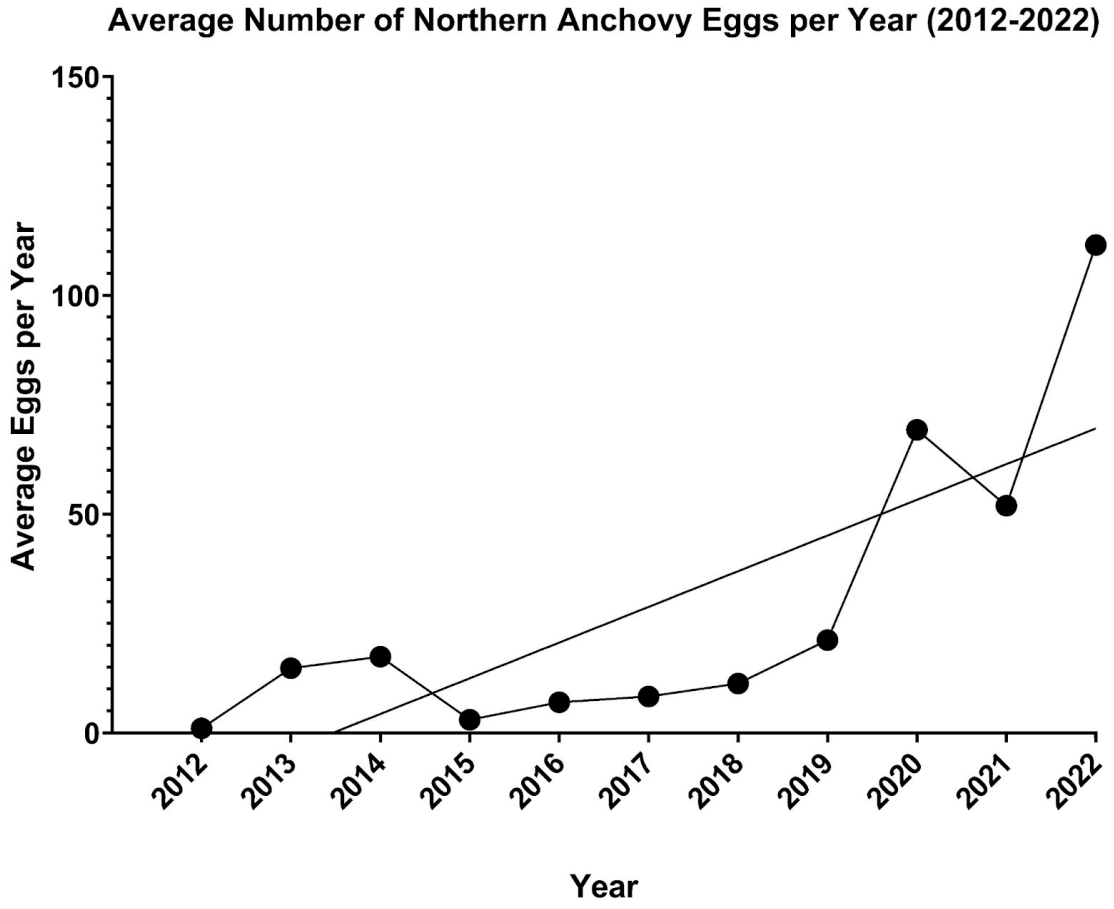

Figure 9. Average number of Northern anchovy (*Engraulis mordax*) eggs per collection each year (732 fish egg collections) from August 2012 to May 2022 ($p = 0.0046$, $R^2 = 0.6081$).

DISCUSSION

Comparison of methods

Concurrent eDNA metabarcoding and fish egg DNA barcoding found a substantial portion of the fish taxa inhabiting the waters surrounding Scripps Pier. The two methods obtained different but overlapping community compositions overall and within concurrent samples. Each method recovered nearly equivalent species richness overall and when comparing concurrent sampling. However, eDNA often failed to detect actively spawning fishes. This may indicate that the majority of the eDNA collected was from adults directly under the pier rather than fish eggs floating at the surface. These findings agree with a study conducted to detect the spawning of bigheaded carps, which found no relationship between eDNA and floating eggs (Erickson 2016). This highlights the need for more research on eDNA composition, shedding, and detection sensitivity during spawning events. Environmental DNA also showed pelagic spawning species present in the area but not actively spawning, which could indicate the fish are utilizing the area for habitat, foraging, or refuge from predators. For example, jack mackerel (*Trachurus symmetricus*), Pacific chub mackerel (*Scomber japonicus*), señorita (*Oxyjulis californica*), rock wrasse (*Haliochoeres semicinctus*), salema (*Brachygenys californiensis*), and California corbina (*Menticirrhus undulatus*) were detected in the eDNA prior to a spawning event. Because of this, frequent long-term sampling with both methods could be used to identify time frames when protected or commercially exploited species migrate to spawn or forage, which can lead to better conservation and management. Using these methods together in the long-term could also be used to understand documented inter-annual variation in species spawning off Scripps pier by determining if the species are present but not spawning or just not in the area.

Ecology

The months with the highest species richness across both methods (June, July, and August) coincide with the highest species richness found in previous fish egg surveys (Harada 2015, Duke 2018, Choi 2021). Taken together with prior research findings, this confirms that summer months tend to have the highest species richness overall. Additionally, co-detections occurred most often in the summer and late spring. This corresponds with the substantial number of fish species that migrate to these waters to forage and spawn during this timeframe (Allen 2006, McClatchie 2016, 2018, de Souza 2016).

Seasonal peaks in spawning were observed for the Northern anchovy in March and April, which are known to spawn in the winter and spring, according to the literature and the previous fish-egg surveys (Fishbase, Harada 2015, Duke 2018, Choi 2021). The speckled sanddab (*Citharichthys stigmaeus*) was seen to spawn throughout the sampling period, with peaks in egg abundance in May, June, July, and August, which corresponded with their year-round spawning. The speckled sanddab also had the greatest number of total fish eggs in the historical fish egg surveys and the second highest during our sampling period, as they are in high numbers throughout the California coastline (Harada 2015, Duke 2018, Choi 2021). This has been attributed to their small size limiting their commercial importance (Harada 2015, Duke 2018, Choi 2021). Seasonal trends in spawning are visible from spring to summer for the spotfin croaker (*Roncador stearnsii*), rock wrasse (*Haliochoeres semicinctus*), queenfish (*Serphius politus*), and California corbina (*Menticirrhus undulatus*) which again corresponds with their documented spawning seasons at SIO (Harada 2015, Duke 2018, Choi 2021).

Of the species co-detected using concurrent sampling as well as all fish egg samples, the majority were found to inhabit soft-bottom areas, followed by hard-bottom and the least utilizing

the pelagic zone. These fish are all found in coastal regions, with most in the neritic and/or epipelagic zone (Allen 2006, Hastings 2014). Species that were seen more often in the eDNA were those that are historically abundant and/or use pier pilings as habitat, such as the señorita (*Oxyjulis californica*), Northern anchovy (*Engraulis mordax*), Pacific sardine (*Sardinops sagax*), and jack mackerel (*Trachurus symmetricus*). These fish are also known to school (often together), which may lead to higher rates of eDNA shedding or greater density of eDNA shed due to more physical interaction and greater surface area (Thalinger 2021). The eDNA samples detected only a few elasmobranch taxa, which correlates lower amplification efficiencies (Miya 2015). Of all the pelagic spawning fish species detected from the concurrent samples, most species utilized soft-bottom habitats. This corresponded with the immediate habitat surrounding the pier being sandy bottom (Hastings 2014). Hard-bottom species were second-most prevalent due to the nearby rocky reefs. Species which utilize the pelagic zone are the least prevalent as the sampling location is a nearshore environment. This is in line with previous work that has shown pelagic species to be the least numerous (Hastings 2014, Allen 2006). Additionally, all the species are commonly observed in the area in fish collections and fish egg surveys (Hastings 2014, Harada 2015, Duke 2018, Choi 2021). This could result from larger populations, as eDNA has been associated with fish biomass (Willerslev 2016). As defined by Hastings et al. (2014), central range fishes (21) dominated the observed species, followed by Northern (11) and Southern (8). Traditional fish surveys have declined in this area since protections were placed on the area; therefore, continued surveying via eDNA and fish egg barcoding could fill in for monitoring the predicted increase of Southern species due to climate change and detection of invasive species (Hastings 2014).

Fishes of importance

Fishes of commercial and ecological importance, such as the Northern anchovy and Pacific sardine, were detected via eDNA and in the egg survey throughout the sampling period. These fishes are some of the most heavily harvested for human consumption, oil, and fish meal for aquaculture and agriculture (Hastings and Walker 2014). Looking specifically at the Northern anchovy, the population-specific to our sampling region (Central Stock of Northern Anchovy, CSNA)– which ranges from San Francisco to Central Baja California–, collapsed in 2015 (Sydeman 2020). The historical fish egg survey revealed that the average number of eggs increased over the last decade. There were greater total peaks in eggs in a single collection starting in 2019 and during our sampling period (Harada 2015, Duke 2018, Choi 2021). Current stock assessments also show the same increase. New fishery restrictions were put into place by the National Marine Fisheries Service in 2019, establishing new overfishing limits, acceptable biological catch limits, and annual catch limits for the CSNA with the intention to conserve and manage the stock (Department of Commerce 2019). These results suggest that the 2019 restrictions correlate with spawning increases in our sampling region. These findings provide motivation for frequent and rapid monitoring of the CSNA through ichthyoplankton surveys off La Jolla shores to complement the CalCOFI cruises as it is easily accessed and less costly and time intensive than the quarterly cruises. Fish eggs laid in this area spend an average of two to three days in the water column before hatching. Harada et al. (2015) were able to show with a high probability that most eggs collected off the SIO Pier were the result of spawning events within the MPA. Further, a surface transport model constructed by Harada et al. (2015) reported that the eggs laid within the MPAs drift outside them, displaying the potential for the MPAs to "seed" nearby areas and enhance fish stocks. The eDNA metabarcoding detected anchovy on

days without spawning, which may indicate the anchovy are present in the area for reasons besides reproduction, such as feeding. Over 70% of the (35/46) unique fish species identified were of recreational, commercial, or artisanal importance (Hastings 2014, Allen 2006). There were two fish species detected of conservation concern that are listed as vulnerable to extinction with declining populations due to commercial and recreational exploitation, the common thresher shark (*Alopias vulpinus*) and the California sheephead (*Semicossyphus pulcher*) (IUCN 2022). These results emphasize the importance of MPAs to serve as a refuge for reproduction, foraging, and habitat.

Detection limitations

Understanding the distribution and sensitivity of eDNA detection requires knowledge of how abiotic and biotic factors influence the concentration and distribution of eDNA (Andruszkiewicz 2017, Harrison 2019, Beng 2020). There were multiple instances of eDNA failing to detect a species found in the fish egg survey in the concurrently collected samples and samples within a month. Differences in sampling collection and effort can help explain the variance in species composition between methods and failed co-detections. A much larger volume of water was filtered during the plankton collections: 64,000-liters compared to the 3-liters collected for the eDNA (Choi 2021). For comparisons made within a month, failed detection in eDNA could be caused primarily by the greater sampling effort for fish egg collections (44 fish egg collections, 17 eDNA) as well as collection day and time. Moreover, fish egg tows sampled the entire water column while the eDNA was collected from only the surface waters, possibly limiting detection. A study on the vertical distribution of eDNA in the mesopelagic zone revealed that eDNA was found at depths within tens of meters of the source

(Allan 2021). Many flatfish species (Paralichthyidae and Pleuronectidae) found in the eggs were not always detected in the eDNA, potentially because they are bottom-dwelling species, limiting their eDNA at the surface (Hastings and Walker 2014). Environmental DNA can consist of intracellular DNA, including fish eggs and sperm (Harrison 2019). Pelagic fish eggs float to the surface slowly over time but have different buoyancy depending on the given species due to varied lipid/protein ratios and environmental variables such as vertical mixing and salinity (Sundby 2015). If we assume fish eggs are a significant component of the eDNA recovered during spawning peaks, it is possible that many of the eggs did not reach the surface or were not in a high enough density at the surface to be detected.

Fishes known to inhabit the waters surrounding SIO pier other than pelagic spawners from the fish egg survey were not detected in the eDNA, which could be due to limited sampling range. Horizontal dispersal of eDNA can also influence detection. Studies in aquatic environments have shown that horizontal distribution of eDNA decreases in concentration at >100 m from the organism, which is determined in part by current speed, and thus causes detection to be strongly affected by dilution. Distance from the sampling site would also lead to failed detection of fishes such as seabasses (Serranidae) which tend to aggregate near rocky reefs and historically have lower egg abundance in the fish egg surveys off Scripps Pier (Harada 2015, Duke 2018, Choi 2021). Environmental DNA can degrade relatively quickly; studies have shown that higher temperatures and UV exposure increase eDNA degradation rates, which are higher for extracellular DNA (Andrusakiewicz 2017, Saito 2021). Biotic factors such as microbial degradation of DNA and microbial biomass can also impact species detection (Maruyama 2019, Sassoubre 2016, Stewart 2019). Those species may primarily live in the nearby rocky reefs, kelp forests, eelgrass beds, and submarine canyons (Hastings 2014). Environmental DNA shed by

these fishes could have degraded, diluted by the time it reached Scripps Pier, or may not have been transported into the immediate vicinity to begin with. Seasonality can influence species detection by eDNA (de Souza 2016, Suzuki 2022). The sampling time frame was across six months, which would fail to detect species that spawn, migrate, or are generally more active in the fall and winter (de Souza 2016). The use of eDNA metabarcoding should be expanded across a greater geographical area, collected throughout the water column, and sampled at a higher frequency, as it has the potential to reveal seasonal patterns in presence, invasive species, and species range shifts due to climate change (Auth 2018, Suzuki 2022, Beng 2020). Overall, eDNA shedding rates, transport, dilution, degradation, and seasonality play a key role in species detection, so further analysis is needed to understand those variables and how they interplay.

Genetic marker choice has been shown to play a role in species detection and resolution (Polanco 2021). Pelagic spawning species composition differed between methods. This could be explained by the fact that we utilized different genetic markers for each method, which can bias detection of certain species (Wangensteen 2018, Polanco 2021). Rock wrasse (*Halichoeres semicinctus*) and spotfin croaker (*Roncador stearnsi*i), which were present at high levels in the fish eggs, were not seen in the eDNA but may have been the ASVs assigned to Labridae (wrasses) and Sciaenidae (drums/croakers). These families have limited differences within their mitochondrial genes between species (Gold 2021, Wainwright 2018). Environmental DNA also detected fish from a genus of surfperches (*Amphisticus*) common to the surf zone community. The family of surfperches (Embiotocidae) are a recent radiation that lack sufficient genetic differentiation to be resolved to lower taxonomic classifications (Gold 2021, Longo 2015). We also had low read counts due to the large portion of reads being assigned to bacteria which has been reported using the 12S gene, as the primer binding sites are not highly conserved, which

can lead to non-specific amplification of nearby regions, such as the 16S gene (Xiao 2022, Gold and Choi 2020, Gold 2020). While the two methods utilize different genetic markers for species identification, we can be confident in the taxonomic assignments due to the extensive barcoding of California Current fish by Gold et al. (2021) and Hastings and Burton et al. (2018). Fish egg barcoding was performed on the COI and 16S genes, widely used for fish species identification. These regions are longer and exhibit higher levels of differentiation leading to greater taxonomic resolution and higher confidence in species assignments. Over 90% of the eggs collected were successfully identified; eggs that failed to amplify with either barcode genes or did not pass the threshold for identification were likely a result of degraded DNA, failed DNA extraction, or PCR inhibition (Harada 2015). Continued efforts to barcode voucher species will lead to the discovery of fishes of conservation concern and invasive species in areas they were not previously cataloged.

Conclusion and future directions

This study reveals that eDNA metabarcoding has the potential to detect a wide array of fishes but can fail to capture a substantial portion of the actively spawning community. Comparisons of pelagic spawning fish species co-detection illuminate that eDNA metabarcoding should not be a replacement for fish egg DNA barcoding surveys and instead should be used to complement the method. Environmental DNA is likely to detect the adult fish community in the immediate area of the collection site. Fish egg monitoring provides essential information on reproduction that eDNA alone cannot. Tracking reproductive trends in response to anthropogenic stressors such as climate change, pollution, and overfishing is critical for managing fisheries and marine resources effectively. Concurrent environmental DNA metabarcoding and fish egg

barcoding detected a substantial proportion of a complex marine community identifying species of commercial, recreational, and ecological importance. While the eukaryotic eDNA read count is low for this study, the data can be used as a preliminary assessment of the combined use of the techniques. Future work includes resequencing these samples with modified PCR cycling conditions to reduce bacterial amplification. The archived portion of the filters can be extracted using alternative methods to increase eukaryotic DNA collection and limit breaking open bacterial cells. We can also utilize technical replicates to increase read counts and limit the occurrence of non-detections. Additionally, sequencing eDNA samples collected but not included in this study can provide more temporal information on species detection. Metabarcoding of eDNA should be used cautiously as it has the potential to capture a substantial portion of a pelagic spawning fish community but cannot replicate fish egg barcoding results. However, these methods can be used concomitantly to rapidly assess short- and long-term patterns in species presence and spawning, providing critical information for conservation and fisheries management on diversity, habitat use, and local population trends.

SUPPLEMENTAL TABLES AND FIGURES

Table S1. Environmental DNA read counts per species and sample.

Date	3/4/2021	3/11/2021	4/2/2021	4/23/2021	4/30/2021	5/7/2021	5/28/2021	6/2/2021	6/11/2021
Total reads	243	87	108	128	247	227	301	169	1967
Alopias vulpinus	0	0	0	0	0	0	0	0	2
Amphistichus rhodoterus	0	0	0	6	37	0	0	0	0
Anisotremus davidsonii	0	0	0	0	0	0	0	0	0
Atherinops affinis	17	6	0	8	42	11	16	22	0
Citharichthys sordidus	0	0	0	0	15	0	0	0	0
Citharichthys stigmaeus	0	0	0	0	23	0	0	3	59
Engraulis mordax	153	62	54	60	16	17	78	8	10
Fodiator acutus	0	0	0	0	0	0	0	0	19
Girella nigricans	0	0	0	0	0	0	0	7	0
Hermosilla azurea	0	0	0	0	0	0	0	0	0
Hypsoblennius gilberti	0	0	0	0	10	0	0	0	0
Hypsoblennius jenkinsi	0	0	0	19	0	0	6	0	0
Labridae	0	0	0	0	0	0	0	1	4
Leuresthes tenuis	11	0	0	0	0	0	0	0	7
Menticirrhus undulatus	0	0	0	0	0	0	0	11	0
Micrometrus minimus	0	0	0	0	0	0	0	0	0
Mugil cephalus	0	0	0	0	0	0	0	4	0
Myliobatis californica	18	0	0	16	10	0	7	8	0
Oxyjulis californica	30	0	18	5	49	85	0	29	1577
Paralabrax clathratus	0	0	0	0	0	0	0	0	0
Paralichthys californicus	0	0	0	0	0	0	0	0	0
Phoca vitulina	0	0	0	0	0	0	0	0	0
Pleuronichthys verticalis	0	0	0	0	0	0	0	0	0
Sardinops sagax	0	9	25	14	26	20	141	40	8
Sciaenidae1	0	0	0	0	0	0	8	0	10
Sciaenidae2	0	0	0	0	0	0	0	0	0
Sciaenidae3	0	0	0	0	0	0	0	0	16
Scomber japonicus	7	0	0	0	0	25	13	0	95
Sebastes melanops	0	0	0	0	0	18	0	0	0
Seriola lalandi	0	0	0	0	0	0	9	0	0
Seriphus politus	0	0	0	0	0	0	5	13	0
Squalus suckleyi	7	0	0	0	0	0	0	0	0
Strongylura exilis	0	0	0	0	0	0	0	8	14
Symphurus atricaudus	0	3	0	0	0	0	0	0	0
Synodus lucioceps	0	0	0	0	0	0	7	0	0
Trachurus symmetricus	0	7	0	0	0	51	7	14	0
Tursiops truncatus	0	0	0	0	19	0	4	0	10
Umbrina roncador	0	0	0	0	0	0	0	0	110
Urobatis halleri	0	0	0	0	0	0	0	1	0
Xenistius californiensis	0	0	6	0	0	0	0	0	26
Xystreurys liolepis	0	0	0	0	0	0	0	0	0
Zalophus californianus	0	0	5	0	0	0	0	0	0

Table S1. Environmental DNA read counts per species and sample. Continued.

Date	6/18/2021	6/24/2021	7/9/2021	7/23/2021	7/30/2021	8/5/2021	8/13/2021	8/20/2021
Total reads	1257	173	436	501	469	63	739	393
Alopias vulpinus	0	0	0	0	0	0	0	0
Amphistichus rhodoterus	0	0	0	0	0	0	0	0
Anisotremus davidsonii	72	14	0	34	0	0	0	35
Atherinops affinis	161	10	0	97	108	0	0	170
Citharichthys sordidus	0	0	0	14	0	0	0	0
Citharichthys stigmaeus	44	3	7	0	17	0	0	0
Engraulis mordax	228	0	10	0	0	0	0	0
Fodiator acutus	10	0	0	0	9	0	0	0
Girella nigricans	14	0	0	0	0	0	0	15
Hermosilla azurea	71	0	0	0	0	0	0	0
Hypsoblennius gilberti	0	0	0	13	0	0	0	0
Hypsoblennius jenkinsi	16	0	0	0	0	0	0	11
Labridae	1	0	0	0	0	0	0	0
Leuresthes tenuis	13	0	0	0	0	0	0	0
Menticirrhus undulatus	47	0	0	0	0	0	0	0
Micrometrus minimus	10	0	0	0	0	0	0	0
Mugil cephalus	0	0	0	0	0	0	0	0
Myliobatis californica	22	0	32	14	47	0	10	7
Oxyjulis californica	257	14	31	18	0	0	0	5
Paralabrax clathratus	47	0	0	0	0	0	0	0
Paralichthys californicus	0	0	0	0	17	0	0	11
Phoca vitulina	0	0	0	0	9	0	0	0
Pleuronichthys verticalis	32	0	0	0	0	0	0	0
Sardinops sagax	69	74	262	112	75	34	147	36
Sciaenidae1	34	0	0	0	62	0	0	21
Sciaenidae2	0	0	0	0	0	12	0	0
Sciaenidae3	0	0	0	0	0	0	0	0
Scomber japonicus	88	0	0	27	79	0	116	21
Sebastes melanops	0	0	0	0	0	0	0	0
Seriola lalandi	0	0	0	12	10	0	0	21
Seriphus politus	0	0	0	0	0	0	0	0
Squalus suckleyi	0	0	0	0	0	0	0	0
Strongylura exilis	0	0	0	0	12	0	11	0
Symphurus atricaudus	0	0	0	0	0	0	0	0
Synodus lucioceps	0	0	0	0	0	0	0	0
Trachurus symmetricus	0	58	94	146	24	14	455	36
Tursiops truncatus	0	0	0	0	0	0	0	4
Umbrina roncador	0	0	0	14	0	0	0	0
Urobatis halleri	11	0	0	0	0	0	0	0
Xenistius californiensis	10	0	0	0	0	0	0	0
Xystreurys liolepis	0	0	0	0	0	3	0	0
Zalophus californianus	0	0	0	0	0	0	0	0

Table S2. Raw Environmental DNA read counts per ASV and sample.

Raw Sum Taxonomy	3/4/2021	3/11/2021	4/2/2021	4/23/2021	4/30/2021	5/7/2021	5/28/2021	6/2/2021	6/11/2021
Eukaryota;Chordata;Actinopteri;Atheriniformes;Atherinopsidae;Atherinops;Atherinops affinis	17	6	0	8	42	11	16	22	0
Eukaryota;Chordata;Actinopteri;Atheriniformes;Atherinopsidae;Leuresthes;Leuresthes tenuis	11	0	0	0	0	0	0	0	7
Eukaryota;Chordata;Actinopteri;Aulopiformes;Synodontidae;Synodus;Synodus lucioceps	0	0	0	0	0	0	7	0	0
Eukaryota;Chordata;Actinopteri;Beloniformes;Belonidae;Strongylura;Strongylura exilis	0	0	0	0	0	0	0	8	14
Eukaryota;Chordata;Actinopteri;Beloniformes;Exocoetidae;Fodiator;Fodiator acutus	0	0	0	0	0	0	0	0	19
Eukaryota;Chordata;Actinopteri;Blenniiformes;Blenniidae;Hypsoblennius;Hypsoblennius gilberti	0	0	0	0	10	0	0	0	0
Eukaryota;Chordata;Actinopteri;Blenniiformes;Blenniidae;Hypsoblennius;Hypsoblennius jenkinsi	0	0	0	19	0	0	6	0	0
Eukaryota;Chordata;Actinopteri;Carangiformes;Carangidae;Seriola;Seriola lalandi	0	0	0	0	0	0	9	0	0
Eukaryota;Chordata;Actinopteri;Carangiformes;Carangidae;Trachurus;Trachurus symmetricus	0	7	0	0	0	51	7	14	0
Eukaryota;Chordata;Actinopteri;Centrarchiformes;Kyphosidae;Girella;Girella nigricans	0	0	0	0	0	0	0	7	0
Eukaryota;Chordata;Actinopteri;Centrarchiformes;Kyphosidae;Hermosilla;Hermosilla azurea	0	0	0	0	0	0	0	0	0
Eukaryota;Chordata;Actinopteri;Clupeiformes;Clupeidae;Sardinops;Sardinops sagax	0	9	25	14	26	20	141	40	8
Eukaryota;Chordata;Actinopteri;Clupeiformes;Engraulidae;Engraulis;Engraulis mordax	153	62	54	60	16	17	78	8	10
Eukaryota;Chordata;Actinopteri;Labriformes;Labridae;NA;	0	0	0	0	0	0	0	1	4
Eukaryota;Chordata;Actinopteri;Labriformes;Labridae;Oxyjulis;Oxyjulis californica	30	0	18	5	49	85	0	29	1577
Eukaryota;Chordata;Actinopteri;Lutjaniformes;Haemulidae;Anisotremus;Anisotremus davidsonii	0	0	0	0	0	0	0	0	0
Eukaryota;Chordata;Actinopteri;Lutjaniformes;Haemulidae;Brachygenys;Xenistius californiensis	0	0	6	0	0	0	0	0	26
Eukaryota;Chordata;Actinopteri;Mugiliformes;Mugilidae;Mugil;Mugil cephalus	0	0	0	0	0	0	0	4	0
Eukaryota;Chordata;Actinopteri;NA;Embiotocidae;Micrometrus;Micrometrus minimus	0	0	0	0	0	0	0	0	0
Eukaryota;Chordata;Actinopteri;NA;Embiotocidae;	0	0	0	6	37	0	0	0	0
Eukaryota;Chordata;Actinopteri;NA;Sciaenidae;Menticirrhus;Menticirrhus undulatus	0	0	0	0	0	0	0	11	0
Eukaryota;Chordata;Actinopteri;NA;Sciaenidae;NA;	0	0	0	0	0	0	8	0	10
Eukaryota;Chordata;Actinopteri;NA;Sciaenidae;Seriphus;Seriphus politus	0	0	0	0	0	0	5	13	0
Eukaryota;Chordata;Actinopteri;NA;Sciaenidae;Umbrina;Umbrina roncador	0	0	0	0	0	0	0	0	110
Eukaryota;Chordata;Actinopteri;Perciformes;Sebastidae;Sebastes;	0	0	0	0	0	18	0	0	0
Eukaryota;Chordata;Actinopteri;Perciformes;Serranidae;Paralabrax;Paralabrax clathratus	0	0	0	0	0	0	0	0	0
Eukaryota;Chordata;Actinopteri;Pleuronectiformes;Cynoglossidae;Symphurus;Symphurus atricaudus	0	3	0	0	0	0	0	0	0
Eukaryota;Chordata;Actinopteri;Pleuronectiformes;Paralichthyidae;Citharichthys;Citharichthys sordidus	0	0	0	0	15	0	0	0	0
Eukaryota;Chordata;Actinopteri;Pleuronectiformes;Paralichthyidae;Citharichthys;Citharichthys stigmaeus	0	0	0	0	23	0	0	3	59
Eukaryota;Chordata;Actinopteri;Pleuronectiformes;Paralichthyidae;Paralichthys;Paralichthys californicus	0	0	0	0	0	0	0	0	0
Eukaryota;Chordata;Actinopteri;Pleuronectiformes;Paralichthyidae;Xystreurys;Xystreurys liolepis	0	0	0	0	0	0	0	0	0
Eukaryota;Chordata;Actinopteri;Pleuronectiformes;Pleuronectidae;Pleuronichthys;Pleuronichthys verticalis	0	0	0	0	0	0	0	0	0
Eukaryota;Chordata;Actinopteri;Scombriformes;Scombridae;Scomber;Scomber japonicus	7	0	0	0	0	25	13	0	95
Eukaryota;Chordata;Chondrichthyes;Lamniformes;Alopiidae;Alopias;Alopias superciliosus	0	0	0	0	0	0	0	0	2
Eukaryota;Chordata;Chondrichthyes;Myliobatiformes;Myliobatidae;Myliobatis;Myliobatis californica	18	0	0	16	10	0	7	8	0
Eukaryota;Chordata;Chondrichthyes;Myliobatiformes;Urolophidae;Urolophus;Urolophus halleri	0	0	0	0	0	0	0	1	0
Eukaryota;Chordata;Chondrichthyes;Squaliformes;Squalidae;Squalus;Squalus acanthias	7	0	0	0	0	0	0	0	0
NA	34488	28308	26761	38671	43916	33603	36814	30397	36570

Table S2. Raw Environmental DNA read counts per ASV and sample. Continued.

Raw Sum Taxonomy	6/18/2021	6/24/2021	7/9/2021	7/23/2021	7/30/2021	8/5/2021	8/13/2021	8/20/2021
Eukaryota;Chordata;Actinopteri;Atheriniformes;Atherinopsidae;Atherinops;Atherinops affinis	161	10	0	97	108	0	0	170
Eukaryota;Chordata;Actinopteri;Atheriniformes;Atherinopsidae;Leuresthes;Leuresthes tenuis	13	0	0	0	0	0	0	0
Eukaryota;Chordata;Actinopteri;Aulopiformes;Synodontidae;Synodus;Synodus lucioceps	0	0	0	0	0	0	0	0
Eukaryota;Chordata;Actinopteri;Beloniformes;Belonidae;Strongylura;Strongylura exilis	0	0	0	0	12	0	11	0
Eukaryota;Chordata;Actinopteri;Beloniformes;Exocoetidae;Fodiator;Fodiator acutus	10	0	0	0	9	0	0	0
Eukaryota;Chordata;Actinopteri;Blenniiformes;Blenniidae;Hypsoblennius;Hypsoblennius gilberti	0	0	0	13	0	0	0	0
Eukaryota;Chordata;Actinopteri;Blenniiformes;Blenniidae;Hypsoblennius;Hypsoblennius jenkinsi	16	0	0	0	0	0	0	11
Eukaryota;Chordata;Actinopteri;Carangiformes;Carangidae;Seriola;Seriola lalandi	0	0	0	12	10	0	0	21
Eukaryota;Chordata;Actinopteri;Carangiformes;Carangidae;Trachurus;Trachurus symmetricus	0	58	94	146	24	14	455	36
Eukaryota;Chordata;Actinopteri;Centrarchiformes;Kyphosidae;Girella;Girella nigricans	14	0	0	0	0	0	0	15
Eukaryota;Chordata;Actinopteri;Centrarchiformes;Kyphosidae;Hermosilla;Hermosilla azurea	71	0	0	0	0	0	0	0
Eukaryota;Chordata;Actinopteri;Clupeiformes;Clupeidae;Sardinops;Sardinops sagax	69	74	262	112	75	34	147	36
Eukaryota;Chordata;Actinopteri;Clupeiformes;Engraulidae;Engraulis;Engraulis mordax	228	0	10	0	0	0	0	0
Eukaryota;Chordata;Actinopteri;Labriformes;Labridae;NA;	1	0	0	0	0	0	0	0
Eukaryota;Chordata;Actinopteri;Labriformes;Labridae;Oxyjulis;Oxyjulis californica	257	14	31	18	0	0	0	5
Eukaryota;Chordata;Actinopteri;Lutjaniformes;Haemulidae;Anisotremus;Anisotremus davidsonii	72	14	0	34	0	0	0	35
Eukaryota;Chordata;Actinopteri;Lutjaniformes;Haemulidae;Brachygenys;Xenistius californiensis	10	0	0	0	0	0	0	0
Eukaryota;Chordata;Actinopteri;Mugiliformes;Mugilidae;Mugil;Mugil cephalus	0	0	0	0	0	0	0	0
Eukaryota;Chordata;Actinopteri;NA;Embiotocidae;Micrometrus;Micrometrus minimus	10	0	0	0	0	0	0	0
Eukaryota;Chordata;Actinopteri;NA;Embiotocidae;	0	0	0	0	0	0	0	0
Eukaryota;Chordata;Actinopteri;NA;Sciaenidae;Menticirrhus;Menticirrhus undulatus	47	0	0	0	0	0	0	0
Eukaryota;Chordata;Actinopteri;NA;Sciaenidae;NA;	34	0	0	0	62	12	0	21
Eukaryota;Chordata;Actinopteri;NA;Sciaenidae;Seriphus;Seriphus politus	0	0	0	0	0	0	0	0
Eukaryota;Chordata;Actinopteri;NA;Sciaenidae;Umbrina;Umbrina roncador	0	0	0	14	0	0	0	0
Eukaryota;Chordata;Actinopteri;Perciformes;Sebastidae;Sebastes;	0	0	0	0	0	0	0	0
Eukaryota;Chordata;Actinopteri;Perciformes;Serranidae;Paralabrax;Paralabrax clathratus	47	0	0	0	0	0	0	0
Eukaryota;Chordata;Actinopteri;Pleuronectiformes;Cynoglossidae;Symphurus;Symphurus atricaudus	0	0	0	0	0	0	0	0
Eukaryota;Chordata;Actinopteri;Pleuronectiformes;Paralichthyidae;Citharichthys;Citharichthys sordidus	0	0	0	14	0	0	0	0
Eukaryota;Chordata;Actinopteri;Pleuronectiformes;Paralichthyidae;Citharichthys;Citharichthys stigmaeus	44	3	7	0	17	0	0	0
Eukaryota;Chordata;Actinopteri;Pleuronectiformes;Paralichthyidae;Paralichthys;Paralichthys californicus	0	0	0	0	17	0	0	11
Eukaryota;Chordata;Actinopteri;Pleuronectiformes;Paralichthyidae;Xystreurys;Xystreurys liolepis	0	0	0	0	0	3	0	0
Eukaryota;Chordata;Actinopteri;Pleuronectiformes;Pleuronectidae;Pleuronichthys;Pleuronichthys verticalis	32	0	0	0	0	0	0	0
Eukaryota;Chordata;Actinopteri;Scombriformes;Scombridae;Scomber;Scomber japonicus	88	0	0	27	79	0	116	21
Eukaryota;Chordata;Chondrichthyes;Lamniformes;Alopiidae;Alopias;Alopias superciliosus	0	0	0	0	0	0	0	0
Eukaryota;Chordata;Chondrichthyes;Myliobatiformes;Myliobatidae;Myliobatis;Myliobatis californica	22	0	32	14	47	0	10	7
Eukaryota;Chordata;Chondrichthyes;Myliobatiformes;Urolophidae;Urolophus;Urolophus halleri	11	0	0	0	0	0	0	0
Eukaryota;Chordata;Chondrichthyes;Squaliformes;Squalidae;Squalus;Squalus acanthias	0	0	0	0	0	0	0	0
NA	89518	26762	34626	32756	34425	35926	28992	32251

Table S3. Number of eggs per species, including all fish eggs collected and sequenced from March to August 2021. An asterisk denotes eDNA was sampled concurrently on this day.

Date	2/26/2021	3/4/2021	3/11/2021	3/18/2021	3/28/2021	3/31/2021	4/2/2021	4/7/2021	4/9/2021	4/14/2021	4/16/2021
eDNA collected		*	*				*				
Eggs collected	444	249	41	20	279	77	396	53	93	22	30
Eggs sequenced	431	230	41	19	284	71	398	48	65	20	28
Species richness	7	3	2	4	7	6	7	5	5	4	5
Anisotremus davidsonii	0	0	0	0	0	0	0	0	0	0	0
Brachygenys californiensis	0	0	0	0	0	0	0	0	0	0	0
Caulolatilus affinis	0	0	0	0	0	0	0	0	0	0	0
Caulolatilus princeps	0	0	0	0	0	0	0	0	0	0	0
Cheilotrema saturnum	0	0	0	0	0	0	0	0	0	0	0
Citharichthys sordidus	7	0	0	0	9	2	2	1	1	0	2
Citharichthys stigmaeus	90	1	5	15	190	16	5	32	14	3	21
Citharichthys xanthostigma	15	0	0	1	10	1	5	1	2	0	3
Cynoscion parvipinnis	0	0	0	0	0	0	0	0	0	0	0
Engraulis mordax	298	228	36	1	62	50	383	13	47	12	1
Fodiator acutus	0	0	0	0	0	0	0	0	0	0	0
Girella nigricans	0	0	0	0	0	0	0	0	0	1	0
Halichoeres semicinctus	0	0	0	0	0	0	0	0	0	0	0
Hypsopsetta guttulata	0	0	0	0	2	1	0	0	0	0	0
Kyphosus azureus	0	0	0	0	0	0	0	0	0	0	0
Menticirrhus undulatus	0	0	0	0	0	0	0	0	0	0	0
Oxyjulis californica	0	0	0	0	0	0	0	0	0	4	0
Paralabrax clathratus	0	0	0	0	0	0	0	0	0	0	0
Paralabrax maculatofasciatus	0	0	0	0	0	0	0	0	0	0	0
Paralabrax nebulifer	0	0	0	0	0	0	0	0	0	0	0
Paralichthys californicus	14	0	0	2	7	1	1	1	1	0	1
Pleuronichthys coenosus	0	0	0	0	0	0	1	0	0	0	0
Pleuronichthys verticalis	4	0	0	0	0	0	0	0	0	0	0
Roncador stearnsii	0	0	0	0	0	0	0	0	0	0	0
Sardinops sagax	3	1	0	0	4	0	1	0	0	0	0
Scomber japonicus	0	0	0	0	0	0	0	0	0	0	0
Semicossyphus pulcher	0	0	0	0	0	0	0	0	0	0	0
Seriphus politus	0	0	0	0	0	0	0	0	0	0	0
Symphurus atricaudus	0	0	0	0	0	0	0	0	0	0	0
Trachurus symmetricus	0	0	0	0	0	0	0	0	0	0	0
Umbrina roncador	0	0	0	0	0	0	0	0	0	0	0
Xystreurys liolepis	0	0	0	0	0	0	0	0	0	0	0

Table S3. Number of eggs per species, including all fish eggs collected and sequenced from March to August 2021. An asterisk denotes eDNA was sampled concurrently on this day. Continued.

Date	4/21/2021	4/23/2021	4/26/2021	4/30/2021	5/3/2021	5/7/2021	5/10/2021	5/14/2021	5/17/2021	5/21/2021	5/24/2021
eDNA collected		*		*		*					
Eggs collected	59	69	79	37	321	66	69	20	22	50	5
Eggs sequenced	57	71	19	32	255	62	89	20	38	32	5
Species richness	7	5	5	5	10	6	7	3	9	7	2
Anisotremus davidsonii	0	0	0	0	0	0	0	0	0	0	0
Brachygenys californiensis	0	0	0	0	0	0	0	0	0	0	0
Caulolatilus affinis	0	0	0	0	0	0	0	0	0	0	0
Caulolatilus princeps	0	0	0	0	0	0	0	0	0	0	0
Cheilotrema saturnum	0	0	0	0	1	0	0	0	3	0	0
Citharichthys sordidus	1	7	0	0	2	1	12	0	0	0	0
Citharichthys stigmaeus	4	33	14	2	51	18	45	16	20	13	4
Citharichthys xanthostigma	0	8	0	0	1	0	4	0	0	1	0
Cynoscion parvipinnis	0	0	0	0	0	0	0	0	0	0	0
Engraulis mordax	42	22	1	26	168	40	25	3	3	6	0
Fodiator acutus	0	0	0	0	2	0	0	0	0	0	0
Girella nigricans	0	0	0	0	0	0	1	0	0	0	0
Halichoeres semicinctus	0	0	0	0	0	0	0	0	0	2	0
Hypsopsetta guttulata	0	0	0	0	0	0	0	0	0	1	0
Kyphosus azureus	0	0	0	0	0	0	0	0	0	0	0
Menticirrhus undulatus	0	0	0	0	0	0	0	0	0	0	0
Oxyjulis californica	4	1	0	2	23	1	0	0	3	0	0
Paralabrax clathratus	0	0	0	0	0	0	0	0	0	0	0
Paralabrax maculatofasciatus	0	0	0	0	0	0	0	0	0	0	0
Paralabrax nebulifer	0	0	0	0	0	0	0	0	0	0	0
Paralichthys californicus	1	0	0	0	2	1	1	1	2	0	1
Pleuronichthys coenosus	0	0	0	0	0	0	0	0	0	0	0
Pleuronichthys verticalis	1	0	1	1	0	0	0	0	2	0	0
Roncador stearnsii	0	0	1	0	0	0	1	0	2	8	0
Sardinops sagax	0	0	2	1	0	0	0	0	0	0	0
Scomber japonicus	0	0	0	0	0	0	0	0	0	0	0
Semicossyphus pulcher	0	0	0	0	0	0	0	0	1	0	0
Seriphus politus	0	0	0	0	3	1	0	0	2	1	0
Symphurus atricaudus	0	0	0	0	0	0	0	0	0	0	0
Trachurus symmetricus	4	0	0	0	2	0	0	0	0	0	0
Umbrina roncador	0	0	0	0	0	0	0	0	0	0	0
Xystreurys liolepis	0	0	0	0	0	0	0	0	0	0	0

Table S3. Number of eggs per species, including all fish eggs collected and sequenced from March to August 2021. An asterisk denotes eDNA was sampled concurrently on this day. Continued.

Date	5/28/2021	6/2/2021	6/4/2021	6/9/2021	6/11/2021	6/16/2021	6/18/2021	6/21/2021	6/24/2021	6/29/2021	7/2/2021
eDNA collected	*	*			*		*		*		
Eggs collected	45	34	4	115	98	36	69	148	68	48	271
Eggs sequenced	31	16	4	102	80	30	64	142	63	48	267
Species richness	8	5	2	8	10	7	15	10	3	12	16
Anisotremus davidsonii	0	0	0	0	0	0	1	0	0	3	9
Brachygenys californiensis	0	0	0	0	0	0	1	0	0	0	0
Caulolatilus affinis	0	0	0	0	1	0	0	0	0	0	0
Caulolatilus princeps	0	0	0	0	0	0	0	0	0	0	1
Cheilotrema saturnum	0	0	0	0	0	0	1	0	0	0	0
Citharichthys sordidus	1	1	0	0	5	2	1	2	7	8	6
Citharichthys stigmaeus	16	11	0	71	62	21	23	91	55	13	203
Citharichthys xanthostigma	0	0	0	0	1	1	1	0	0	2	0
Cynoscion parvipinnis	0	0	0	0	0	0	1	0	0	0	0
Engraulis mordax	8	0	3	1	0	2	13	0	0	2	2
Fodiator acutus	0	0	0	0	0	0	0	0	0	0	0
Girella nigricans	0	0	0	0	0	0	0	0	0	0	0
Halichoeres semicinctus	1	1	1	0	2	0	0	17	1	6	15
Hypsopsetta guttulata	0	0	0	1	0	0	0	0	0	0	0
Kyphosus azureus	0	0	0	0	0	0	0	0	0	0	1
Menticirrhus undulatus	0	1	0	0	0	0	0	5	0	0	8
Oxyjulis californica	0	0	0	0	0	1	9	4	0	1	4
Paralabrax clathratus	0	0	0	0	0	0	1	0	0	1	1
Paralabrax maculatofasciatus	0	0	0	0	0	0	0	1	0	0	0
Paralabrax nebulifer	0	0	0	0	0	0	0	0	0	0	1
Paralichthys californicus	1	2	0	0	3	2	1	7	0	2	3
Pleuronichthys coenosus	0	0	0	1	0	0	0	0	0	0	0
Pleuronichthys verticalis	0	0	0	1	0	0	0	0	0	0	0
Roncador stearnsii	2	0	0	22	2	0	7	11	0	0	5
Sardinops sagax	1	0	0	0	2	1	0	0	0	0	0
Scomber japonicus	0	0	0	1	0	0	0	0	0	0	0
Semicossyphus pulcher	0	0	0	0	1	0	1	2	0	3	1
Seriphus politus	1	0	0	4	1	0	2	2	0	0	6
Symphurus atricaudus	0	0	0	0	0	0	0	0	0	2	0
Trachurus symmetricus	0	0	0	0	0	0	1	0	0	5	0
Umbrina roncador	0	0	0	0	0	0	0	0	0	0	1
Xystreurys liolepis	0	0	0	0	0	0	0	0	0	0	0

Table S3. Number of eggs per species, including all fish eggs collected and sequenced from March to August 2021. An asterisk denotes eDNA was sampled concurrently on this day. Continued.

Date	7/9/2021	7/14/2021	7/23/2021	7/30/2021	8/3/2021	8/5/2021	8/13/2021	8/16/2021	8/20/2021	8/24/2021	8/27/2021
eDNA collected	*		*	*		*	*		*		
Eggs collected	66	122	38	143	58	222	61	309	109	6	7
Eggs sequenced	58	101	33	133	58	212	60	300	113	6	7
Species richness	6	14	8	10	5	9	7	16	11	3	4
Anisotremus davidsonii	0	4	2	0	0	0	1	26	1	0	0
Brachygenys californiensis	0	23	0	0	0	0	0	6	12	0	0
Caulolatilus affinis	0	0	0	0	0	0	0	0	0	0	0
Caulolatilus princeps	0	0	0	0	0	0	0	0	0	0	0
Cheilotrema saturnum	0	5	0	0	0	0	0	0	0	0	0
Citharichthys sordidus	2	0	3	4	11	30	5	6	0	0	0
Citharichthys stigmaeus	43	20	7	104	40	151	39	47	83	2	4
Citharichthys xanthostigma	0	1	0	2	4	4	0	0	0	0	1
Cynoscion parvipinnis	0	0	0	0	0	0	0	0	0	0	0
Engraulis mordax	0	0	0	0	0	0	0	1	0	0	0
Fodiator acutus	0	0	0	0	0	0	0	0	0	0	0
Girella nigricans	0	2	0	0	0	0	0	0	0	0	0
Halichoeres semicinctus	0	5	4	13	2	10	10	147	3	0	1
Hypsopsetta guttulata	0	0	0	0	0	0	0	0	0	0	0
Kyphosus azureus	0	0	0	0	0	0	0	0	0	0	0
Menticirrhus undulatus	5	6	0	2	0	0	0	3	2	1	0
Oxyjulis californica	0	3	3	0	0	12	0	45	0	0	0
Paralabrax clathratus	0	1	1	0	1	1	3	3	0	0	0
Paralabrax maculatofasciatus	0	0	0	0	0	0	0	0	1	0	0
Paralabrax nebulifer	0	0	0	0	0	0	0	1	1	0	0
Paralichthys californicus	1	1	1	4	0	0	0	2	1	0	0
Pleuronichthys coenosus	0	0	0	0	0	0	0	0	0	0	0
Pleuronichthys verticalis	0	0	0	0	0	0	1	0	0	0	0
Roncador stearnsii	6	6	12	1	0	0	0	2	5	0	0
Sardinops sagax	0	0	0	0	0	0	0	0	0	0	0
Scomber japonicus	0	0	0	0	0	0	0	0	0	0	0
Semicossyphus pulcher	0	1	0	0	0	1	1	3	0	0	0
Seriphus politus	1	0	0	1	0	0	0	3	2	0	0
Symphurus atricaudus	0	0	0	0	0	2	0	1	0	3	1
Trachurus symmetricus	0	0	0	0	0	0	0	0	0	0	0
Umbrina roncador	0	23	0	1	0	0	0	3	2	0	0
Xystreurys liolepis	0	0	0	1	0	1	0	0	0	0	0

Table S3. Number of eggs per species, including all fish eggs collected and sequenced from March to August 2021. An asterisk denotes eDNA was sampled concurrently on this day. Continued.

Date	8/30/2021
eDNA collected	
Eggs collected	4
Eggs sequenced	4
Species richness	3
Anisotremus davidsonii	0
Brachygenys californiensis	0
Caulolatilus affinis	0
Caulolatilus princeps	0
Cheilotrema saturnum	0
Citharichthys sordidus	2
Citharichthys stigmaeus	1
Citharichthys xanthostigma	0
Cynoscion parvipinnis	0
Engraulis mordax	0
Fodiator acutus	0
Girella nigricans	0
Halichoeres semicinctus	0
Hypsopsetta guttulata	0
Kyphosus azureus	0
Menticirrhus undulatus	0
Oxyjulis californica	0
Paralabrax clathratus	0
Paralabrax maculatofasciatus	0
Paralabrax nebulifer	0
Paralichthys californicus	0
Pleuronichthys coenosus	0
Pleuronichthys verticalis	0
Roncador stearnsii	0
Sardinops sagax	0
Scomber japonicus	0
Semicossyphus pulcher	0
Seriphus politus	0
Symphurus atricaudus	1
Trachurus symmetricus	0
Umbrina roncador	0
Xystreurys liolepis	0

Table S4. Analysis of variance (ANOVA) on species richness of pelagic spawning fish from concurrent sampling events (including only taxa resolved to the family level) for eDNA and fish eggs. Species richness was not significantly different between methods.

ANOVA	DF	Sum of Squares	Mean Square	F-Value	P-Value
Method	1	1.1	1.059	0.091	0.765
Residuals	32	372.5	11.64		

Table S5. Species richness by month of pelagic spawning fish from concurrent eDNA metabarcoding and fish egg barcoding.

Month	Average eDNA richness	eDNA Standard Deviation	Average Fish Egg Richness	Fish Egg Standard Deviation
March	3.5	0.5	2.5	0.5
April	4	0.8	5.7	0.9
May	6.5	1.5	7	1
June	10.8	3.6	8.3	4.7
July	7.3	1.7	8	1.6
August	5.7	2.4	9	1.6

Table S6. Species richness by season of pelagic spawning fish from concurrent eDNA metabarcoding and fish egg barcoding.

Season	Average eDNA Richness	eDNA Standard Deviation	Average Fish Egg Richness	Fish Egg Standard Deviation
Winter	3.5	0.5	2.5	0.5
Spring	7.9	4.1	7.6	3.2
Summer	6.3	2.1	7.7	2.5

Table S7. Permutational analysis of variance (PERMANOVA) on Jaccard-binary dissimilarity indices generated using presence/absence data of pelagic spawning fish from concurrent collections. Sample community composition was significantly different between methods and months ($p < 0.001$, 999 permutations).

PERMANOVA	DF	Sum of Squares	R2	F-Value	Pr(>F)
Method	1	2.5177	0.31986	20.2799	0.001
Month	5	2.0448	0.25978	3.2942	0.001
Temperature	1	0.2614	0.0332	2.1052	0.074
Chlorophyll	1	0.1087	0.01381	0.8757	0.495
Salinity	1	0.0757	0.00961	0.6094	0.727
Current	1	0.0601	0.00764	0.4841	0.779
Season	1	0.0718	0.00912	0.5782	0.725
Residual	22	2.7313	0.34699		
Total	33	7.8714	1		

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