WATER RECLAMATION
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OF LOS ANGELES COUNTY

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GRACE ROBINSON HYDE
*Chief Engineer and General Manager*

February 15, 2018
File No: 31-300.25

***VIA ELECTRONIC MAIL***

Ms. Cris Morris, Executive Officer
California Regional Water Quality Control Board
Los Angeles Region
320 W. 4th St., Suite 200
Los Angeles, CA 90013

**Attn: Information Technology Unit**

**Joint Water Pollution Control Plant**
**CI No. 1758; Resolution R016-001; NPDES No. CA0053813**
**Special Study Final Report Submission**

As required under Resolution R016-001, please find enclosed the final report for the following special study:

Assessment of Ichthyoplankton Metabarcoding for Routine Monitoring (JWSS-16-003).

Unless otherwise instructed by the Regional Board or Regional Board staff, this will be the final submission associated with this Special Study. However, any other reports or peer-reviewed publications resulting from these studies will also be provided to Regional Board staff as they become available.

I certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief, true, accurate, and complete. I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations.

Very truly yours,

Naoko Munakata
Supervising Engineer
Reuse and Compliance Section

NM:SW:nm
Enclosure

DM#4453425

# Assessment of Ichthyoplankton Metabarcoding for Routine Monitoring

# (JWSS-16-003)

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**February 14, 2018**

**FINAL**

**DMS#4460072**

## *Introduction and Background*

The pelagic, demersal, and rocky reef-associated fauna of the Southern California Bight (SCB) are a significant ecological, economic, and cultural resource to the region. Routine monitoring programs established throughout the SCB are focused on the demersal and rocky reef/kelp-associated species; however, these taxa/habitats represent only a portion of the whole ecological landscape. Comprehensive ecosystem analysis would provide a more complete understanding of the condition of specific habitats and their ability to support diverse ecological communities. Pelagic ecosystems are particularly difficult to study due to their scale, vagility of their inhabitants, and a myriad of stochastic processes that define their dynamics. One approach to efficiently survey the pelagic fish community is to sample the early life history stages (i.e., ichthyoplankton) before they segregate as juveniles to their respective habitats (e.g., hard bottom, soft sediments, or water column) (Reviewed in Auth and Brodeur 2013). Quantifying the spatial and temporal distribution of planktonic fish stages is a useful tool for better understanding how anthropogenic and environmental forces impact the pelagic realm.

Traditionally, the study of ichthyoplankton has been a laborious and expensive process as it requires sorting and morphological identification of many small and difficult to differentiate fish larvae and eggs (Ahlstrom and Moser 1976, Thompson et al. 2014, McClatchie et al. 2016). The size and morphological similarity of many species at these life stages also makes ichthyoplankton analyses prone to identification errors. Over the past decade, DNA-based identification has been employed to more accurately identify morphologically indistinguishable species (Thompson et al. 2016, Hoffman et al. 2017). This approach, known as genetic barcoding, involves sequencing short regions of the genome that can be used to diagnostically identify taxa when compared to a reference database. Early approaches to genetic barcoding used Sanger sequencing, in which characteristic "genetic barcodes" were sequenced from one individual at a time. This process allows for direct connection between sequence data and the individual sources from which the sample tissue was collected. However, the time and effort of Sanger sequencing makes it difficult to scale up for robust regional surveys of pelagic ichthyoplankton. Recent technological advances in genetic sequencing, however, have the potential to increase the spatio-temporal coverage of ichthyoplankton surveys, potentially allowing for a better understanding of ecosystem dynamics (Coparaso et al. 2010).

New molecular taxonomic methods for barcoding (i.e., metabarcoding) of bulk environmental samples provide the ability to analyze barcodes from all species present in an ambient water or sediment sample at once. This approach holds promise for increasing the speed and taxonomic breadth of an assessment (Gray et al. 2006, Loh et al. 2014, Hubert et al. 2015). Although already proven effective in concept, this method still requires refinement to allow it to yield accurate, useful results, and be easily integrated into routine monitoring and assessment programs. Standardized, repeatable field protocols, lab methods, bioinformatics, and data regarding cost comparisons to traditional methods are necessary to allow routine use of metabarcoding in ambient monitoring, management, and regulatory applications.

The SCB pelagic fish community provides an excellent opportunity to refine metabarcoding methods for routine application. Many fish species associated with the different continental shelf habitats of the SCB have been collected as adults, morphologically identified (considerably easier as adults relative to larvae), and have had their mitochondrial cytochrome oxidase 1 (COI) gene (i.e., a commonly used DNA barcode) sequenced (Hebert et al. 2003, Harada et al. 2015). Consequently, an extensive COI reference

library already exists that can be used to match validated species identifications to sequences generated from the sampled ichthyoplankton (e.g., Harada et al. 2015). Additionally, there is existing infrastructure in the region to support routine sample collection by the local publicly-owned treatment works' (POTW) agencies (i.e., City of Los Angeles' Environmental Monitoring Division (CLAEMD), Los Angeles County Sanitation Districts (LACSD), Orange County Sanitation District, and the City of San Diego Public Utilities Department), as well as the Southern California Bight Regional Marine Monitoring Survey, and California Cooperative Oceanic Fisheries Investigations (CalCOFI) programs.

The overall goal of this project was to develop a framework for cost-effective and informative ichthyoplankton monitoring within the coastal waters of Santa Monica Bay (SMB) and beyond. The main objectives for this study were to increase the capacity of SCB POTWs to sample ichthyoplankton and compare traditional morphological and genetic barcoding approaches to metabarcoding results to determine whether metabarcoding is a viable alternative to traditional methods and could be used for a meaningful status and trends monitoring program in the SMB or SCB. The scope of this project is related to a larger regional effort involving the Southern California Coastal Water Research Project (SCCWRP), the National Oceanic and Atmospheric Administration's (NOAA) Southwest Fisheries Science Center (SWFSC), and the University of California at Santa Barbara Marine Biodiversity Observation Network (MBON) aimed at developing and evaluating molecular methods for ichthyoplankton assessment. As such, efforts under this project were closely coordinated and leveraged with the larger regional effort.

## *Methods*

Building field capacity among partners

An important component of this project was to build capacity and partnerships among regional monitoring agencies to sample ichthyoplankton and assess the relative health of pelagic ecosystems. All aspects of the larger regional project were a collaboration between SCCWRP and LACSD/CLAEMD (collectively referred to as POTW staff), or the University of California at Santa Barbara Marine Biodiversity Observation Network (MBON). POTW and MBON staff were trained in CalCOFI pairovet net tow sampling protocols, and staff from the CalCOFI field team, joined by scientists from SCCWRP and the National Oceanic and Atmospheric Administration's (NOAA) Southwest Fisheries Science Center (SWFSC), provided pairovet net equipment to the POTW and MBON groups that could be deployed on their monitoring vessels. The POTW and MBON staff were trained in assembling, deploying and recovering the equipment, processing the sample onboard the ship, and preserving the samples in ethanol for future lab work. These collection methods are not unique to metabarcoding, but were originally designed and intended for more traditional ichthyoplankton analyses. Subsequent laboratory analysis was done by staff from SCCWRP and SWFSC.

Sample collection and processing

Ichthyoplankton samples, including fish larvae and eggs, were collected from trawl monitoring stations by SWFSC and SCCWRP staff, in conjunction with MBON, LACSD, and CLAEMD. Samples were collected from 18 to 70 m deep via vertical tows through the water column with a 150-μm mesh pairovet net (25-cm diameter mouth). At each station, the pairovet was attached to a winch line and lowered to 70 m, or 10 m from the bottom on shallower stations. The net was then towed vertically to the surface, where all

the contents were gently washed into the cod end using a saltwater hose. The cod ends were then removed and washed into a mesh funnel, after which they were placed in a storage jar in 95% ethanol. Samples were then transported to SCCWRP where the ethanol was replaced with fresh ethanol after 24 hours to ensure proper preservation.

Sample sorting and morphological identification

Samples were transported to the SWFSC Ichthyoplankton Laboratory in La Jolla, CA, where all morphological, Sanger sequencing, and sample preparation was conducted by SWFSC/SCCWRP staff, unless noted otherwise. For the morphological identification, the contents of each sample jar were sorted under a dissecting microscope to separate fish eggs and larvae from other contents. Total ichthyoplankton counts per sample were recorded, while individual larva and eggs were placed into separate glass jars. Each individual fish egg and larva was then morphologically identified to the lowest possible taxonomic level by SWFSC staff, highly trained in ichthyoplankton taxonomy.

Sanger (individual) sequencing analyses

After morphological identification, a small amount of tissue was removed from each specimen under the dissecting microscope. For larvae, an eyeball was removed, when possible, otherwise a small piece of tail was used. Eggs, on the other hand, were torn and the smaller section was used for extraction. The DNA was extracted from each of those tissue samples using a Chelex protocol (Thompson et al. 2016). The extracted materials were then individually Sanger sequenced at both the COI and Cytochrome b (Cyt b) barcoding loci. The subsequent sequences were compared and matched to the closest related sequence in a reference library, by BLASTing (Basic Local Alignment Search Tool) against the National Center for Biotechnology Information (NCBI) Genbank nucleotide database for identification with an identity threshold of 0.97. COI was chosen because it is the most common locus used for categorizing animals (Hebert et al. 2003). Additionally, there is a robust reference library for fishes in the SCB available in Genbank (Harada et al. 2015). Unfortunately, the COI locus does not provide the resolution to differentiate among all the fish species within the SCB, particularly the rockfish assemblage, due to the conservative nature of the locus within closely related fish species. In such cases, Cyt b was used in concert with COI to improve taxonomic resolution, as it has been demonstrated to resolve some divergence undetected by COI alone (Thompson et al. 2016).

Metabarcoding (bulk tissue) molecular analysis

Each pairovet sample was reconstituted by adding the previously sorted and identified ichthyoplankton specimens back into the respective original sample jar, along with the entire original sample (ichthyoplankton and invertebrates). For the MBON samples, the ethanol was evaporated from the jars using an evaporating centrifuge. For the LACSD and CLAEMD samples, the ethanol was evaporated from the jars by removing the lids and placing them in a sterile fume hood due to the lack of access to an evaporating centrifuge. In both cases, the desiccated samples were transferred into test tubes, where they were homogenized using a power drill with a modified drill bit shaped to match the bottom of the test tube.

Homogenization was performed with the test tubes cooled by resting them in a bucket of ice to prevent the heat created by the friction with the drill bit from denaturing the DNA (**Figure 1**). One hundred microliters of each tissue homogenate were transferred into 2-mL microcentrifuge tubes where the DNA

was extracted using a Qiagen DNeasy Blood and Tissue Extraction Kit (Qiagen, Germany). DNA concentration and quality for each extracted sample was measured using a Qubit 3.0 Fluorometer (Invitrogen, Carlsbad, CA) and a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA).

**Figure 1**: Bulk tissue homogenization using a power drill and a modified drill bit.

Initial attempts to sequence the COI barcoding region were unsuccessful because the diagnostic region of the COI locus (i.e., the portion of the sequence that allows species to be differentiated) is too long to be sequenced on the Ilumina MiSeq sequencing platform (Illumina, San Diego, CA), which is the most commonly used high-throughput sequencer in ecological studies. Consequently, for the first phase of this study, sequences were processed for the 12S barcoding region, which does not suffer from the same length limitation as COI. This locus is a useful region because universal primers are available, and it is commonly used in studies on fishes (Miya et al. 2015). However, the libraries are not as well populated as for COI and hence taxa can often only be identified to family level.

To prepare for sequencing, the DNA was amplified using the MiFish Universal primers to amplify the 12S mitochondrial small-subunit ribosomal region (Miya et al. 2015). For each sample, three extractions were taken to test for extraction and amplification sensitivity. Little variability was observed among replicates; a more complete analysis of the variability is part of the larger project and is not discussed in this report. PCR amplification was evaluated using gel electrophoresis. Samples with successful amplification were sequenced on a MiSeq sequencing platform. The sequencing was conducted at Laragen, Inc. (Culver City, CA). Recovered sequences were then cleaned and processed using a customized bioinformatic pipeline, which includes clustering similar sequences, removing spurious sequences, and BLASTing the remaining sequences against the NCBI Genbank reference database (Kelly et al 2014, Amir et al. 2017). Concordance between metabarcoding and traditional approaches was then evaluated by comparing the number of taxa with matching identification to the total number

identified in each sample, and then averaged across all the samples in each group (MBON, LACSD, CLAEMD). Using the equation:

$$\frac{\text{\# of matching taxa}}{\text{total \# of taxa } \textit{identified} \text{ in the sample as determined by both Sanger and metabarcoding}}$$

It is important to note that the concordance value is based on a match of both the number and the identity of the taxa. Consequently, concordance values tend to decline with increasing taxonomic level (i.e. family → genus→ species). The lack of ichthyoplankton in a sample is a valid and important component of bioassessment. Therefore, samples with zero ichthyoplankton are considered a 100% match if the metabarcoding correctly identifies that the sample lacked fish eggs or larvae.

## *Results*

Capacity building, sample collection, and processing

Samples were collected at stations in the Santa Barbara Channel (SBC) with MBON, on the shelf and shelf break areas of the Palos Verdes Shelf (PVS) with LACSD, and in Santa Monica Bay (SMB) with CLAEMD (**Figure 2**, **Table 1, Appendix B**). Since pairovet nets are double hoop nets, each tow provided two samples, denoted in this report as the port and starboard side samples. Successful sampling suggests that local crews gained sufficient knowledge to master the CalCOFI pairovet protocol to a point where all stations for MBON and each POTW could be sampled in a single day, respectively.

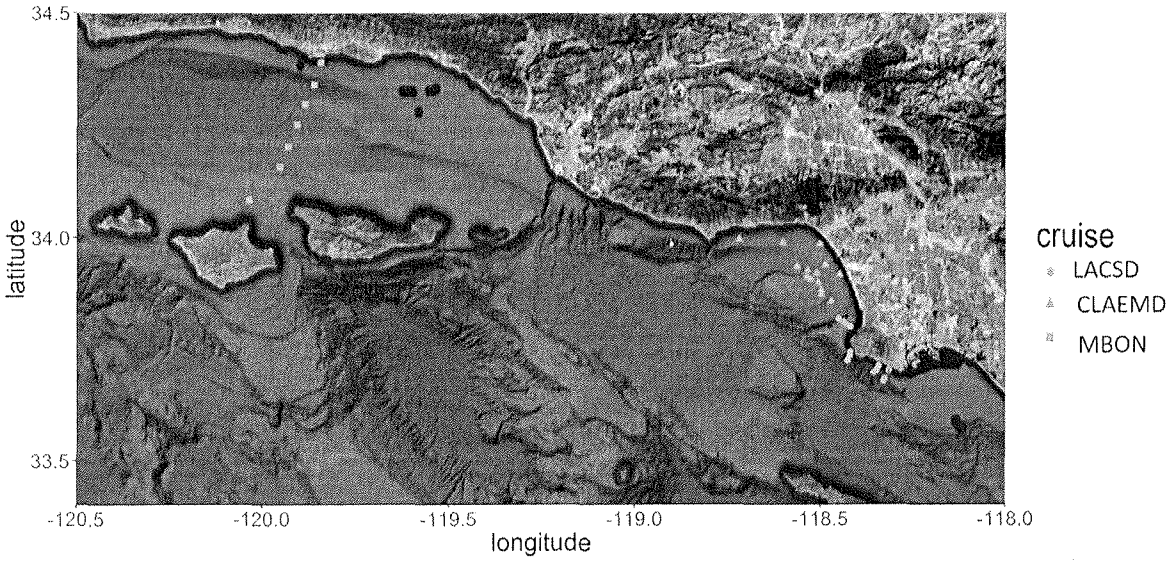


**Figure 2:** Map of the trawl stations on the Palos Verdes shelf, in Santa Monica Bay and Santa Barbara Channel.

**Table 1**. Sample collection metadata.

| Date | Partner | Location | Bottom Depth Range (m) | Number of Stations | Number of Samples |
|---|---|---|---|---|---|
| May 5, 2016 | MBON | SBC | 51-918 | 7 | 14 |
| June 8, 2016 | LACSD | PVS | 23-305 | 16 | 32 |
| June 28, 2016 | CLAEMD | SMB | 15-117 | 14 | 28 |

Morphological identification

Traditional morphological analysis showed that crustaceans, largely copepods and euphausids, comprised the majority of the plankton in each sample; **Table 2** summarizes the number of species identified and Appendix A provides a taxa summary. Samples ranged from zero to 21 individual ichthyoplankton, with a mean of 0.9 individuals in the MBON samples, 3.8 individuals in the LACSD samples, 6 individuals in the CLAEMD samples, and 4.1 individuals across all samples.

**Table 2**. Sample composition based on morphology and Sanger sequencing.

| | | | Quantity | | | |
|---|---|---|---|---|---|---|
| | **Identification Method** | **Parameter** | **MBON** | **LACSD** | **CLAEMD** | **Total** |
| **Larvae** | - | Total Collected | 3 | 66 | 65 | 134 |
| | Morphology | Individuals Identified | 3 | 66 | 62 | 131 |
| | | Species Identified | 2 | 8 | 10 | 14 |
| | Sanger Sequencing | Individuals Identified | 3 | 66 | 65 | 134 |
| | | Species Identified | 2 | 8 | 12 | 15 |
| **Eggs** | - | Total Collected | 10 | 55 | 103 | 168 |
| | Morphology | Individuals Identified | 0 | 18 | 31 | 49 |
| | | Species Identified | 0 | 4 | 5 | 8 |
| | Sanger Sequencing | Individuals Identified | 10 | 55 | 103 | 168 |
| | | Species Identified | 5 | 13 | 19 | 26 |
| **Total** | - | Total Collected | 13 | 121 | 168 | 302 |
| | Morphology | Individuals Identified | 3 | 84 | 93 | 153 |
| | | Species Identified | 2 | 9 | 13 | 18 |
| | Sanger Sequencing | Individuals Identified | 13 | 121 | 168 | 302 |
| | | Species Identified | 6 | 18 | 22 | 32 |

Sanger sequencing identification

**Table 2** summarizes the number of species identified through Sanger sequencing and Appendix A provides a taxa summary. In total, Sanger sequencing identified 29 different species of ichthyoplankton among the samples, comprising 13 families. Of the 180 individuals previously identified via morphology,

177 of them were corroborated with Sanger sequencing results, with increased resolution within the rockfish complex, which were only morphologically identified to *Sebastes* sp.

Metabarcoding as a method to identify ichthyoplankton

The primary goal of this study was to evaluate the use of a metabarcoding approach to identifying pelagic ichthyoplankton. Samples from all three sampling efforts yielded genomic sequence data, but the MBON samples had a much wider range of unique sequences per sample, as well as the highest success rate at amplifying the 12S locus and therefore producing the largest set of high quality sequence data. The MBON samples had the highest DNA yield with a mean of 26.1 ng/μL (range: 1.1 – 137.0 ng/μL), followed by LACSD with a mean of 5.7 ng/μL (range: 0 – 24.1 ng/μL) and then CLAEMD with a mean of 2.8 ng/μL (range: 0 - 9.0 ng/μL). Many of the CLAEMD and LACSD samples did not amplify successfully; the lower yield likely results from the different ethanol evaporation method (discussed below). Results from all samples are provided in **Table 3**, but with only a limited amount of high quality sequence data, we chose to focus our subsequent methodological comparison analyses on the MBON samples.

**Table 3.** Taxa identification data associated with each towed sample, including the DNA concentration for the bulk tissue extraction and the number of unique taxa identified per sample at three taxa levels, family, genus, and species. Each cell contains the taxa identification from the three identification methods, (morphology/Sanger/metabarcoding). An 'NA' indicates that metabarcoding results were not recovered for that sample.

| Region | Station | Side | DNA yield (ng/μL) | Family | Genus | Species |
|---|---|---|---|---|---|---|
| MBON | PB7 | Port | 71.9 | 1/2/2 | 1/2/2 | 1/2/2 |
| MBON | PB7 | Starboard | 18.4 | 1/3/3 | 1/3/3 | 1/3/3 |
| MBON | PB6 | Port | 51.4 | 0/1/1 | 0/1/1 | 0/1/1 |
| MBON | PB6 | Starboard | 36.2 | 1/2/NA | 1/2/NA | 1/2/NA |
| MBON | PB5 | Port | 8.9 | 0/0/0 | 0/0/0 | 0/0/0 |
| MBON | PB5 | Starboard | 25.1 | 0/0/0 | 0/0/0 | 0/0/0 |
| MBON | PB4 | Port | 64.6 | 0/0/0 | 0/0/0 | 0/0/0 |
| MBON | PB4 | Starboard | 35.2 | 0/0/0 | 0/0/0 | 0/0/0 |
| MBON | PB3 | Port | 5.0 | 0/1/2 | 0/1/2 | 0/1/2 |
| MBON | PB3 | Starboard | 26.6 | 0/0/0 | 0/0/0 | 0/0/0 |
| MBON | PB2 | Port | 18.0 | 1/1/1 | 1/1/1 | 1/1/1 |
| MBON | PB2 | Starboard | 13.1 | 0/0/0 | 0/0/0 | 0/0/0 |
| MBON | PB1 | Port | 1.2 | 0/0/0 | 0/0/0 | 0/0/0 |
| MBON | PB1 | Starboard | 2.1 | 0/1/1 | 0/1/1 | 0/1/1 |
| LACSD | T5-137 | Port | 14.2 | 1/2/1 | 1/2/2 | 1/2/2 |
| LACSD | T5-137 | Starboard | 2.9 | 0/0/NA | 0/0/NA | 0/0/NA |
| LACSD | T4-137 | Port | 10.0 | 0/1/1 | 0/1/1 | 0/1/1 |
| LACSD | T4-137 | Starboard | 8.8 | 3/3/2 | 3/3/2 | 3/3/2 |
| LACSD | T1-137 | Port | 1.8 | 3/3/1 | 3/3/1 | 3/3/1 |
| LACSD | T1-137 | Starboard | 4.7 | 0/1/NA | 0/1/NA | 0/1/NA |
| LACSD | T1-61 | Port | 1.9 | 1/3/NA | 1/3/NA | 1/4/NA |

Table 3 (continued).

| Region | Station | Side | DNA yield (ng/µL) | Family | Genus | Species |
|---|---|---|---|---|---|---|
| LACSD | T1-61 | Starboard | 0.5 | 2/4/NA | 2/4/NA | 2/4/NA |
| LACSD | T1-23 | Port | 0.0 | 1/2/NA | 1/2/NA | 1/2/NA |
| LACSD | T1-23 | Starboard | 3.0 | 0/1/NA | 0/1/NA | 0/1/NA |
| LACSD | T0-305 | Port | 5.0 | 0/0/NA | 0/0/NA | 0/0/NA |
| LACSD | T0-305 | Starboard | 6.7 | 0/0/NA | 0/0/NA | 0/0/NA |
| LACSD | T0-137 | Port | 1.6 | 0/0/NA | 0/0/NA | 0/0/NA |
| LACSD | T0-137 | Starboard | 14.3 | 0/0/NA | 0/0/NA | 0/0/NA |
| LACSD | T0-61 | Port | 10.3 | 2/5/3 | 2/5/4 | 2/5/4 |
| LACSD | T0-61 | Starboard | 13.0 | 0/1/NA | 0/1/NA | 0/1/NA |
| LACSD | T0-23 | Port | 0.8 | 0/2/NA | 0/2/NA | 0/2/NA |
| LACSD | T0-23 | Starboard | 1.4 | 1/1/NA | 1/1/NA | 1/1/NA |
| LACSD | T1-305 | Port | 4.5 | 1/1/NA | 1/1/NA | 1/1/NA |
| LACSD | T1-305 | Starboard | 2.7 | 1/1/NA | 1/1/NA | 1/1/NA |
| LACSD | T4-305 | Port | 21.8 | 2/3/NA | 2/3/NA | 2/3/NA |
| LACSD | T4-305 | Starboard | 9.3 | 1/1/NA | 1/1/NA | 1/1/NA |
| LACSD | T4-61 | Port | 6.7 | 1/2/NA | 1/2/NA | 1/2/NA |
| LACSD | T4-61 | Starboard | 8.4 | 1/2/NA | 1/2/NA | 1/2/NA |
| LACSD | T4-23 | Port | 1.3 | 2/3/NA | 2/3/NA | 2/3/NA |
| LACSD | T4-23 | Starboard | 0.4 | 2/2/NA | 2/2/NA | 2/2/NA |
| LACSD | T5-23 | Port | 1.3 | 0/1/NA | 0/1/NA | 0/1/NA |
| LACSD | T5-23 | Starboard | 2.4 | 0/2/NA | 0/2/NA | 0/2/NA |
| LACSD | T5-61 | Port | 3.2 | 1/1/NA | 1/1/NA | 1/1/NA |
| LACSD | T5-61 | Starboard | 3.0 | 2/2/NA | 2/2/NA | 2/2/NA |
| LACSD | T5-305 | Port | 9.1 | 1/2/2 | 1/2/2 | 1/2/2 |
| LACSD | T5-305 | Starboard | 7.3 | 0/0/NA | 0/0/NA | 0/0/NA |
| CLAEMD | C1 | Port | 4.4 | 1/1/2 | 1/1/2 | 1/1/2 |
| CLAEMD | C1 | Starboard | 2.4 | 2/4/NA | 2/4/NA | 2/4/NA |
| CLAEMD | C3 | Port | 8.1 | 0/1/NA | 0/1/NA | 0/1/NA |
| CLAEMD | C3 | Starboard | 2.4 | 1/2/NA | 1/2/NA | 1/2/NA |
| CLAEMD | 1B | Port | 6.4 | 0/2/NA | 0/2/NA | 0/2/NA |
| CLAEMD | 1B | Starboard | 6.2 | 2/2/NA | 2/2/NA | 2/3/NA |
| CLAEMD | C6 | Port | 2.6 | 2/2/NA | 2/3/NA | 2/3/NA |
| CLAEMD | C6 | Starboard | 5.0 | 2/3/NA | 2/3/NA | 2/3/NA |
| CLAEMD | D1T | Port | 0.0 | 2/2/NA | 2/2/NA | 2/2/NA |
| CLAEMD | D1T | Starboard | 2.7 | 1/1/NA | 1/1/NA | 1/1/NA |
| CLAEMD | Z2 | Port | 5.8 | 0/0/1 | 0/0/1 | 0/0/1 |
| CLAEMD | Z2 | Starboard | 2.5 | 2/2/NA | 2/2/NA | 2/2/NA |
| CLAEMD | Z3 | Port | 1.7 | 2/3/NA | 2/3/NA | 2/3/NA |
| CLAEMD | Z3 | Starboard | 1.6 | 2/2/NA | 2/2/NA | 2/2/NA |
| CLAEMD | 3B | Port | 2.5 | 2/4/NA | 2/5/NA | 2/5/NA |
| CLAEMD | 3B | Starboard | 3.4 | 4/5/NA | 4/6/NA | 4/7/NA |

**Table 3 (continued).**

| Region | Station | Side | DNA yield (ng/μL) | Family | Genus | Species |
|---|---|---|---|---|---|---|
| CLAEMD | 2A | Port | 2.5 | 2/2/NA | 2/3/NA | 2/4/NA |
| CLAEMD | 2A | Starboard | 1.2 | 4/4/NA | 4/5/NA | 4/6/NA |
| CLAEMD | 3A | Port | 0.3 | 3/6/NA | 4/7/NA | 4/7/NA |
| CLAEMD | 3A | Starboard | 0.0 | 2/3/NA | 2/3/NA | 2/4/NA |
| CLAEMD | A2 | Port | 1.1 | 2/3/NA | 2/4/NA | 2/4/NA |
| CLAEMD | A2 | Starboard | 0.4 | 1/2/NA | 1/3/NA | 1/4/NA |
| CLAEMD | 1A | Port | 2.2 | 2/3/NA | 2/3/NA | 2/5/NA |
| CLAEMD | 1A | Starboard | 1.7 | 3/3/NA | 3/6/NA | 3/6/NA |
| CLAEMD | Z4 | Port | 1.7 | 1/1/NA | 1/1/NA | 1/1/NA |
| CLAEMD | Z4 | Starboard | 3.0 | 2/3/NA | 2/3/NA | 2/3/NA |
| CLAEMD | 2B | Port | 1.8 | 2/5/NA | 2/6/NA | 2/8/NA |
| CLAEMD | 2B | Starboard | 1.5 | 1/3/NA | 2/4/NA | 2/4/NA |

Individual samples were compared to determine if the metabarcoding approach had similar resolving power to identify ichthyoplankton composition within a sample. We tested this resolving power at three taxonomic levels: species, genus, and family (**Figure 3, Table 4**). The samples exhibited high degrees of agreement, with over 96% concordance between metabarcoding and traditional methods at the family level, with a reduction in accuracy when comparing at the genus level (81%) and species (78%) levels. Overall, the metabarcoding approach was able to resolve all of the families identified in the morphological and Sanger sequencing analyses, but it was not able to resolve all of the same genera and species.

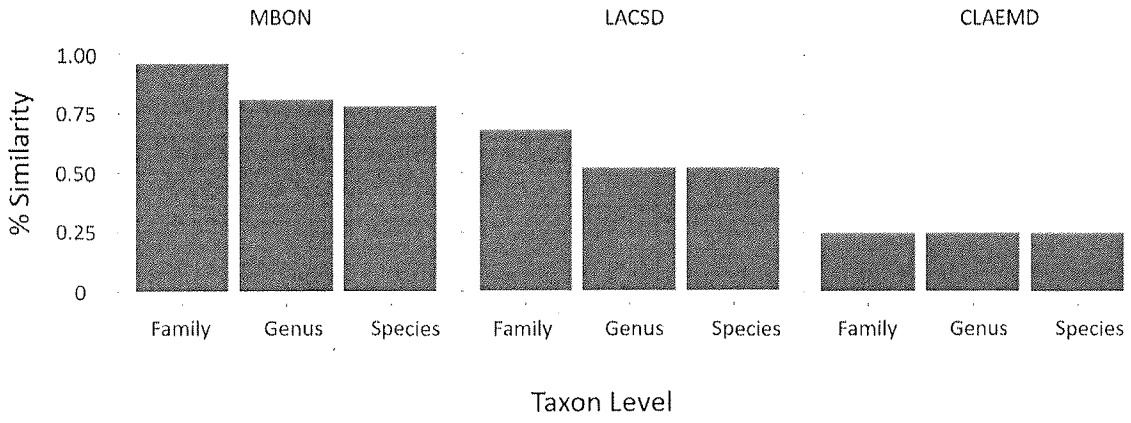


**Figure 3.** Bar graphs demonstrating the degree of mean similarity between metabarcoding results and those from traditional morphological and Sanger sequencing approaches for samples collected with MBON, LACSD, and CLAEMD. These comparisons were done at three taxonomic levels: family, genus, and species.

**Table 4.** Proportion concordance between the metabarcoding identification and the more traditional Sanger sequencing approach. Concordance is calculated by comparing the number and identity of taxa that match between methodologies.

| Region | Station | Side | Family | Genus | Species |
|---|---|---|---|---|---|
| | | | | | |
| MBON | PB7 | Port | 1.00 | 1.00 | 1.00 |
| MBON | PB7 | Starboard | 1.00 | 0.50 | 0.20 |
| MBON | PB6 | Port | 1.00 | 1.00 | 1.00 |
| MBON | PB5 | Port | 1.00 | 1.00 | 1.00 |
| MBON | PB5 | Starboard | 1.00 | 1.00 | 1.00 |
| MBON | PB4 | Port | 1.00 | 1.00 | 1.00 |
| MBON | PB4 | Starboard | 1.00 | 1.00 | 1.00 |
| MBON | PB3 | Port | 0.50 | 0.00 | 0.00 |
| MBON | PB3 | Starboard | 1.00 | 1.00 | 1.00 |
| MBON | PB2 | Port | 1.00 | 0.00 | 0.00 |
| MBON | PB2 | Starboard | 1.00 | 1.00 | 1.00 |
| MBON | PB1 | Port | 1.00 | 1.00 | 1.00 |
| MBON | PB1 | Starboard | 1.00 | 1.00 | 1.00 |
| | | | | | |
| **MBON** | **Average** | | **0.96** | **0.81** | **0.78** |
| | | | | | |
| LACSD | T5-137 | Port | 0.50 | 0.00 | 0.00 |
| LACSD | T4-137 | Port | 1.00 | 1.00 | 1.00 |
| LACSD | T4-137 | Starboard | 0.66 | 0.66 | 0.66 |
| LACSD | T1-137 | Port | 0.33 | 0.33 | 0.33 |
| LACSD | T0-61 | Port | 0.60 | 0.13 | 0.13 |
| LACSD | T5-305 | Port | 1.00 | 1.00 | 1.00 |
| | | | | | |
| **LACSD** | **Average** | | **0.68** | **0.52** | **0.52** |
| | | | | | |
| CLAEMD | C1 | Port | 0.50 | 0.50 | 0.50 |
| CLAEMD | Z2 | Port | 0.00 | 0.00 | 0.00 |
| | | | | | |
| **CLAEMD** | **Average** | | **0.25** | **0.25** | **0.25** |

The next test was to determine if the metabarcoding approach resolved a similar community composition across samples compared to traditional morphological and Sanger sequencing. The metabarcoding approach resolved a similar pattern of diversity at the family level to the Sanger approach. In contrast, the morphology alone yielded many unidentified specimens (**Figure 4**).

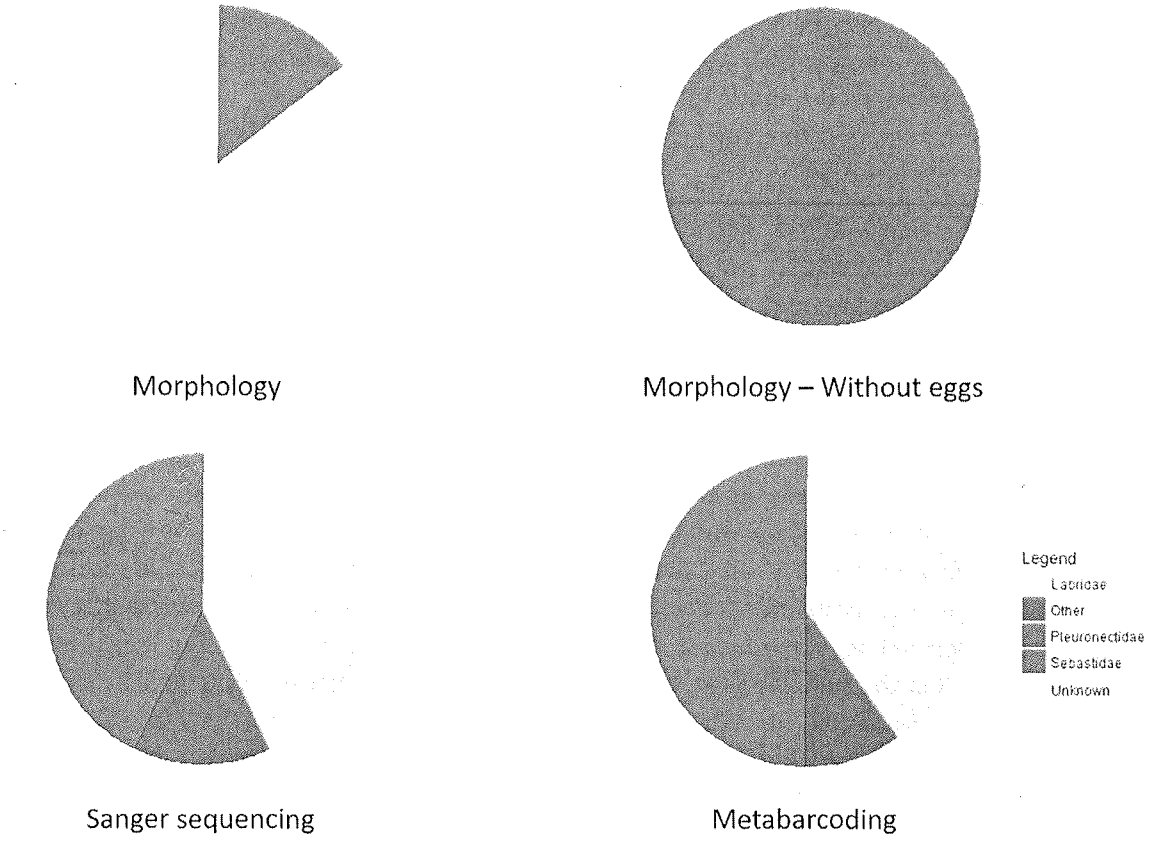


**Figure 4**. A series of pie charts showing the diversity of ichthyoplankton families detected by morphological identification of all samples, morphological identification of larvae only (excluding eggs), Sanger sequencing, and metabarcoding.

## *Discussion*

Sampling along the continental shelf yielded many fish eggs and larvae, with a variable level of taxa diversity among samples, both within and among regions (see Appendix A). This could possibly be a result of environmental patterns at the time of sampling, such as current flow, upwelling, proximity to rocky reef, and station depth. Also, sampling was conducted in the late spring, whereas it is possible that higher densities and greater diversity of ichthyoplankton may have been found if sampling was conducted earlier in the season. This supports previous work done by CalCOFi, which found that ichthyoplankton diversity and composition varied both spatially and temporally, with season being the main driver effecting diversity (Watson et al. 2002).

Sanger sequencing was a useful method in resolving species-level identifications. Morphological identification of ichthyoplankton is a slow and arduous task, and many of the individuals could not be identified morphologically. This is largely the result of the pairovet nets collecting many eggs, which were then preserved in ethanol to maintain integrity. Identification of fish eggs is a difficult task under

any condition. In the present study, the problem was exacerbated because ethanol does not preserve pigment as well as traditional formalin preservation, which made it even more difficult to observe many of the traits necessary to identify different taxa. In contrast, single-specimen Sanger sequencing proved effective at resolving species-level identification of the sampled ichthyoplankton beyond the morphological identification for many individual specimens. That being said, where both methods yielded a species-level identification, the high degree of agreement illustrates efficacy of molecular tools in ichthyoplankton identification.

The use of metabarcoding methods, however, encountered a critical problem with many of the samples – especially those from the LACSD and CLAEMD – was low quality and volume of DNA that could be extracted. Successful metabarcoding of these samples was further impaired by poor DNA amplification. This pattern of results is indicative of likely PCR inhibition - where the chemical processes involved in DNA amplification are interrupted by additional contaminants in the sample, including the ethanol used in preservation and extraction of the genetic material (Rossen et al. 1992) as well as other compounds such as excessive salt content (Foote et al. 2012) and body fluids from within the sample (Schrader et al. 2012). Since the general contents of the samples from the different regions were similar and the samples were collected and processed similarly with the exception of the evaporation step, it is likely that inhibition or degradation as a result of this step is responsible for the differences in results found among the regions. The fume hood evaporation approach takes longer than traditional filtering approaches and likely did not allow for full removal of ethanol from the samples. The longer exposure and residual ethanol in the samples likely reduced the efficacy of downstream PCR amplification. Also, the longer time period required for ethanol evaporation in the fume hood may have allowed for DNA degradation from exposure to microbes and UV radiation. Additional work is currently underway to test other ethanol evaporation and filtering protocols to avoid this issue in future work. These results also suggest that sample complexity may affect the efficacy of metabarcoding to identify taxa, as the concordance rates decreased in the regions as sample complexity increased. Previous work using metabarcoding and environmental DNA (eDNA) techniques, however, demonstrate that this is unlikely to be the case, as samples more complex than those used in this study have been resolved (Kelly et al. 2014, Hänfling 2016, Valentini et al. 2016, Yamamoto et al 2017). Ongoing work with more complex CalCOFI samples should elucidate this further as the larger validation project continues.

For those MBON samples from which DNA was successfully extracted and amplified, metabarcoding results closely matched traditional methods (i.e., morphological and Sanger sequencing) with regard to family level composition within a sample. However, at finer taxonomic levels, the metabarcoding results did not agree as closely to the traditional methodologies. Full use of metabarcoding requires robust, well curated reference libraries for as many species as possible. Moreover, use of multiple DNA markers (12S and COI for fish) allows for corroboration of identifications using two independent analyses. The first step to improve the resolution of metabarcoding methods is to improve the reference library for local fish species at the 12S locus. The current reference library for 12S in the SCB is sparse beyond family level and therefore the most closely matched taxa may be from studies on confamiliar species found elsewhere. Current efforts are focusing on Sanger sequencing fishes from CalCOFI to generate the necessary sequence library. Additional work is being conducted to develop new primers to sequence an abridged COI region. Although a thorough reference library already exists for this region, the sequence differences among fishes in the SCB are found throughout the COI locus, and a shorter sequence is needed that can provide enough resolving power for species identification.

The metabarcoding approach was demonstrated to resolve the same trends in family composition for the MBON samples as the traditional approach. Despite required troubleshooting in applying a new method to a new taxon, metabarcoding results may eventually prove useful as an effective replacement for traditional ichthyoplankton taxonomic family identification. Additional work is underway to demonstrate how both the traditional and metabarcoding approaches capture trends in pelagic communities.

This study represents the first attempt to apply metabarcoding to assess pelagic ichthyoplankton communities in the nearshore environment. Consequently, additional work is necessary before wholesale application of metabarcoding to pelagic bioassessment. Previous work has demonstrated that a metabarcoding approach is more efficient than a traditional morphological approach (Ji et al. 2013). The morphological approach often takes months, if not years, to process samples, requiring first to sort an entire sample under a dissection scope, where specimens can be overlooked. The sorted specimens then have to be identified by a highly trained taxonomist.

This study represents method development and validation, and as such, required time-consuming troubleshooting and refinement of the metabarcoding protocol. The metabarcoding approach, once optimized, can be processed much faster than traditional approaches and many samples can be processed simultaneously. The potential increase in identification efficiency provided by barcoding provides the opportunity to increase the capacity to collect more samples across a broader spatial/temporal range. Subsequently, a more robust assessment can be conducted to monitor the health of the system. Furthermore, samples run in different facilities can be directly compared with much higher comparability than morphological samples processed in different labs (ex. Arulandhu et al. 2017). Another benefit of the metabarcoding approach is that it does not require the same highly-trained taxonomic expertise, and can be processed in any lab with sequencing capability. As the technology becomes more common in bioassessment, the necessary laboratory and bioinformatic expertise are becoming more accessible, requiring far less nuanced training than traditional taxonomy.

Given these potential benefits, metabarcoding could be useful for assessing the pelagic condition of the SCB. However, before application, the method must perform reliably on samples across the region. Once a robust method is developed, it could be used in a larger scale study to describe ichthyoplankton composition across gradients of natural and anthropogenically varying environmental conditions. Community level indices that can be useful in monitoring various human impacts on pelagic environments can then be developed. This study demonstrated that following field training of the MBON and POTW staff and subsequent pairovet net sampling, it is logistically feasible to include ichthyoplankton tows into regular surveys in the SCB. By describing ichthyoplankton community dynamics across environmental gradients, we can improve our understanding of community composition and potential impacts of various anthropogenic stressors on pelagic communities. This work will require collaborations with local and regional organizations operating throughout the SCB. It is important to contextualize this study as being part of a larger collaboration with the POTW, MBON, SWFSC, and SCCWRP. As such additional samples are being processed to continue to validate metabarcoding as a tool to characterize ichthyoplankton for eventual management applications.

## *Conclusions*

In this study, MBON and POTW staff were trained to collect plankton samples using the CalCOFI pairovet net protocol. Ichthyoplankton within those samples were identified using morphological, Sanger sequencing, and metabarcoding methodologies. Sample processing for the metabarcoding is still in need of refinement, particularly in the ethanol evaporation/DNA extraction steps. Ongoing work is being conducted to improve the available reference library, primer sets, and analytical pipeline, all of which should improve the efficacy of metabarcoding for ichthyoplankton identification. When DNA was successfully extracted, the metabarcoding approach yielded similar results to the more traditional morphological and Sanger sequencing methods, but this may be affected by the complexity of the sample itself. Continued refinement of this new method will increase the utility of this high-throughput genetic approach for routine monitoring and assessment. As the larger validation study continues, the goal is to achieve comparable results in all samples processed from throughout the region.

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## Appendix A

Taxa summary table: Counts of each species identified in both fish eggs and larvae from the three sampled regions.

| Species | Family | Type | LACSD Morphology | LACSD Sanger | | CLAEMD Morphology | CLAEMD Sanger | | MBON Morphology | MBON Sanger | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Unknown | Unknown | Egg | 37 | 0 | | 72 | 0 | | 10 | 0 | | 119 | |
| | | Larvae | 0 | 0 | | 3 | 0 | | 0 | 0 | | 3 | 122 |
| *Bathylagus stilbius* | Bathylagidae | Egg | 0 | 0 | | 0 | 0 | | 0 | 1 | | 1 | |
| | | Larvae | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 1 |
| *Hypsoblennius jenkensi* | Blennidae | Egg | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | |
| | | Larvae | 0 | 0 | | 2 | 2 | | 0 | 0 | | 4 | 4 |
| *Sardinops sagax* | Clupeidae | Egg | 1 | 1 | | 0 | 0 | | 0 | 1 | | 3 | |
| | | Larvae | 1 | 1 | | 0 | 2 | | 0 | 0 | | 4 | 7 |
| *Ruscarius creaseri* | Cottidae | Egg | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | |
| | | Larvae | 0 | 1 | | 0 | 0 | | 0 | 0 | | 1 | 1 |
| *Symphurus atricaudus* | Cynoglossidae | Egg | 0 | 0 | | 0 | 1 | | 0 | 0 | | 1 | |
| | | Larvae | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 1 |
| *Engraulis mordax* | Engraulidae | Egg | 15 | 16 | | 8 | 8 | | 0 | 0 | | 47 | |
| | | Larvae | 56 | 56 | | 43 | 43 | | 0 | 0 | | 198 | 245 |
| *Lepidogobius lepidus* | Gobiidae | Egg | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | |
| | | Larvae | 0 | 0 | | 1 | 1 | | 0 | 0 | | 2 | 2 |
| *Rhinogobiops nicholsii* | Gobiidae | Egg | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | |
| | | Larvae | 1 | 1 | | 0 | 0 | | 1 | 1 | | 4 | 4 |
| *Girella nigricans* | Kyphosidae | Egg | 0 | 4 | | 0 | 0 | | 0 | 0 | | 4 | |
| | | Larvae | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 4 |
| *Hermosilla azurea* | Kyphosidae | Egg | 0 | 0 | | 0 | 4 | | 0 | 0 | | 4 | |
| | | Larvae | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 4 |
| *Oxyjulius californica* | Labridae | Egg | 0 | 12 | | 0 | 1 | | 0 | 6 | | 19 | |
| | | Larvae | 4 | 4 | | 4 | 4 | | 0 | 0 | | 16 | 35 |
| *Stenobrachius leucopsarus* | Myctophidae | Egg | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | |
| | | Larvae | 1 | 1 | | 0 | 0 | | 0 | 0 | | 2 | 2 |

| Species | Family | Type | LACSD Morphology | LACSD Sanger | | CLAEMD Morphology | CLAEMD Sanger | | MBON Morphology | MBON Sanger | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Citharichthys sordidus* | Paralichthydae | Egg | 0 | 8 | | 0 | 32 | | 0 | 0 | | 40 | |
| | | Larvae | 0 | 0 | | 2 | 1 | | 0 | 0 | | 3 | 43 |
| *Citharichthys stigmaeus* | Paralichthydae | Egg | 0 | 0 | | 17 | 19 | | 0 | 0 | | 36 | |
| | | Larvae | 1 | 1 | | 1 | 2 | | 0 | 0 | | 5 | 41 |
| *Citharichthys xanthostigma* | Paralichthydae | Egg | 0 | 0 | | 0 | 3 | | 0 | 0 | | 3 | |
| | | Larvae | 0 | 0 | | 0 | 1 | | 0 | 0 | | 1 | 4 |
| *Hippoglossina stomata* | Paralichthydae | Egg | 1 | 2 | | 0 | 0 | | 0 | 0 | | 3 | |
| | | Larvae | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 3 |
| *Paralichthys californicus* | Paralichthydae | Egg | 0 | 1 | | 0 | 4 | | 0 | 0 | | 5 | |
| | | Larvae | 0 | 0 | | 6 | 6 | | 0 | 0 | | 12 | 17 |
| *Xystreurys liolepis* | Paralichthydae | Egg | 0 | 0 | | 0 | 3 | | 0 | 0 | | 3 | |
| | | Larvae | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 3 |
| *Lyopsetta exilis* | Pleuronectidae | Egg | 1 | 2 | | 0 | 0 | | 0 | 1 | | 4 | |
| | | Larvae | 0 | 0 | | 1 | 1 | | 0 | 0 | | 2 | 6 |
| *Parophrys vetulus* | Pleuronectidae | Egg | 0 | 0 | | 0 | 3 | | 0 | 0 | | 3 | |
| | | Larvae | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 3 |
| *Psettichthys melanostictus* | Pleuronectidae | Egg | 0 | 0 | | 0 | 0 | | 0 | 1 | | 1 | |
| | | Larvae | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 1 |
| *Pleuronichthys verticalis* | Pleuronectidae | Egg | 0 | 0 | | 4 | 4 | | 0 | 0 | | 8 | |
| | | Larvae | 0 | 0 | | 1 | 1 | | 0 | 0 | | 2 | 10 |
| *Atractoscion nobilis* | Sciaenidae | Egg | 0 | 1 | | 0 | 1 | | 0 | 0 | | 2 | |
| | | Larvae | 1 | 1 | | 0 | 0 | | 0 | 0 | | 2 | 4 |
| *Menticirrhus undulatus* | Sciaenidae | Egg | 0 | 0 | | 0 | 4 | | 0 | 0 | | 4 | |
| | | Larvae | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 4 |
| *Seriphus politus* | Sciaenidae | Egg | 0 | 1 | | 0 | 2 | | 0 | 0 | | 3 | |
| | | Larvae | 0 | 0 | | 0 | 1 | | 0 | 0 | | 1 | 4 |
| *Genyonnemus lineatus* | Sciaenidae | Egg | 0 | 0 | | 1 | 1 | | 0 | 0 | | 2 | |
| | | Larvae | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 2 |
| *Scomber japonicus* | Scombridae | Egg | 0 | 0 | | 1 | 5 | | 0 | 0 | | 6 | |

| Species | Family | Type | LACSD Morphology | LACSD Sanger | | CLAEMD Morphology | CLAEMD Sanger | | MBON Morphology | MBON Sanger | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Larvae | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 6 |
| *Sebastes hopkinsi* | Sebastidae | Egg | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | |
| | | Larvae | 0 | 0 | | 0 | 0 | | 0 | 2 | | 2 | 2 |
| *Sebastes sp* | Sebastidae | Egg | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | |
| | | Larvae | 1 | 0 | | 1 | 0 | | 2 | 0 | | 4 | 4 |
| *Paralabrax clathratus* | Serranidae | Egg | 0 | 5 | | 0 | 0 | | 0 | 0 | | 5 | |
| | | Larvae | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 5 |
| *Paralabrax nebulifer* | Serranidae | Egg | 0 | 1 | | 0 | 5 | | 0 | 0 | | 6 | |
| | | Larvae | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 6 |
| *Sphyraena argentea* | Sphyraenidae | Egg | 0 | 0 | | 0 | 1 | | 0 | 0 | | 1 | |
| | | Larvae | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 1 |
| *Peprilus simillimus* | Stromateidae | Egg | 0 | 1 | | 0 | 2 | | 0 | 0 | | 3 | |
| | | Larvae | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 3 |
| | | | | | | | | | | | | | |
| Total | | Egg | 55 | 55 | | 103 | 103 | | 10 | 10 | | 336 | |
| | | Larvae | 66 | 66 | | 65 | 65 | | 3 | 3 | | 268 | |
| | | Total | 121 | 121 | | 168 | 168 | | 13 | 13 | | 604 | |

**Appendix B**

Pairovet tow information for each sample collection.

| Region | Station | Date | Time (PST) | Latitude (°N) | Longitude (°W) | Tow Type (150 µm) | Number of samples collected | Bottom depth (m) | Trawl depth (m) |
|---|---|---|---|---|---|---|---|---|---|
| MBON | PB7 | 5/5/2016 | 1015 | 34 5.0 | 120 2.1 | Pairovet | 2 | 82 | 70 |
| MBON | PB6 | 5/5/2016 | 1111 | 34 9.5 | 119 57.0 | Pairovet | 2 | 453 | 70 |
| MBON | PB5 | 5/5/2016 | 1209 | 34 12.2 | 119 55.7 | Pairovet | 2 | 535 | 70 |
| MBON | PB4 | 5/5/2016 | 1232 | 34 12.2 | 119 54.4 | Pairovet | 2 | 520 | 70 |
| MBON | PB3 | 5/5/2016 | 1259 | 34 17.8 | 119 53.1 | Pairovet | 2 | 918 | 70 |
| MBON | PB2 | 5/5/2016 | 1324 | 34 20.4 | 119 51.6 | Pairovet | 2 | 273 | 70 |
| MBON | PB1 | 5/5/2016 | 1351 | 34 23.4 | 119 50.5 | Pairovet | 2 | 51 | 40 |
| LACSD | T5-137 | 6/8/2016 | 834 | 33 41.1 | 118 19.6 | Pairovet | 2 | 135 | 70 |
| LACSD | T4-137 | 6/8/2016 | 858 | 33 42.1 | 118 21.0 | Pairovet | 2 | 144 | 70 |
| LACSD | T1-137 | 6/8/2016 | 930 | 33 43.8 | 118 25.3 | Pairovet | 2 | 141 | 70 |
| LACSD | T1-61 | 6/8/2016 | 947 | 33 44.2 | 118 25.2 | Pairovet | 2 | 57 | 50 |
| LACSD | T1-23 | 6/8/2016 | 1000 | 33 44.7 | 118 25.1 | Pairovet | 2 | 23 | 13 |
| LACSD | T0-305 | 6/8/2016 | 1040 | 33 49.2 | 118 27.0 | Pairovet | 2 | 317 | 70 |
| LACSD | T0-137 | 6/8/2016 | 1052 | 33 48.8 | 118 26.2 | Pairovet | 2 | 138 | 70 |
| LACSD | T0-61 | 6/8/2016 | 1105 | 33 48.5 | 118 25.8 | Pairovet | 2 | 67 | 55 |
| LACSD | T0-23 | 6/8/2016 | 1119 | 33 48.1 | 118 25.1 | Pairovet | 2 | 23 | 13 |
| LACSD | T1-305 | 6/8/2016 | 1153 | 33 43.5 | 118 25.6 | Pairovet | 2 | 310 | 70 |
| LACSD | T4-305 | 6/8/2016 | 1219 | 33 41.9 | 118 21.4 | Pairovet | 2 | 290 | 70 |
| LACSD | T4-61 | 6/8/2016 | 1229 | 33 42.3 | 118 20.9 | Pairovet | 2 | 60 | 50 |
| LACSD | T4-23 | 6/8/2016 | 1240 | 33 42.8 | 118 20.5 | Pairovet | 2 | 27 | 15 |
| LACSD | T5-23 | 6/8/2016 | 1252 | 33 42.2 | 118 18.9 | Pairovet | 2 | 23 | 13 |
| LACSD | T5-61 | 6/8/2016 | 1303 | 33 44.4 | 118 19.3 | Pairovet | 2 | 61 | 50 |
| LACSD | T5-305 | 6/8/2016 | 1313 | 33 40.8 | 118 19.8 | Pairovet | 2 | 300 | 70 |
| CLAEMD | C1 | 6/28/2106 | 924 | 33 59.8 | 118 43.1 | Pairovet | 2 | 60 | 50 |
| CLAEMD | C3 | 6/28/2106 | 1009 | 33 59.4 | 118 35.9 | Pairovet | 2 | 62 | 52 |
| CLAEMD | 1B | 6/28/2106 | 1050 | 33 56.2 | 118 33.8 | Pairovet | 2 | 115 | 70 |

| Region | Station | Date | Time (PST) | Latitude (°N) | Longitude (°W) | Tow Type (150 µm) | Number of samples collected | Bottom depth (m) | Trawl depth (m) |
|---|---|---|---|---|---|---|---|---|---|
| CLAEMD | C6 | 6/28/2106 | 1106 | 33 55.7 | 118 32.1 | Pairovet | 2 | 60 | 50 |
| CLAEMD | D1T | 6/28/2106 | 1120 | 33 54.8 | 118 32.1 | Pairovet | 2 | 62 | 52 |
| CLAEMD | Z2 | 6/28/2106 | 1135 | 33 54.5 | 118 31.4 | Pairovet | 2 | 59 | 50 |
| CLAEMD | Z3 | 6/28/2106 | 1147 | 33 54.0 | 118 30.3 | Pairovet | 2 | 55 | 45 |
| CLAEMD | 3B | 6/28/2106 | 1201 | 33 53.0 | 118 29.8 | Pairovet | 2 | 57 | 50 |
| CLAEMD | 2A | 6/28/2106 | 1215 | 33 52.4 | 118 29.8 | Pairovet | 2 | 58 | 50 |
| CLAEMD | 3A | 6/28/2106 | 1232 | 33 51.5 | 118 28.1 | Pairovet | 2 | 75 | 65 |
| CLAEMD | A2 | 6/28/2106 | 1305 | 33 55.1 | 118 26.9 | Pairovet | 2 | 15 | 10 |
| CLAEMD | 1A | 6/28/2106 | 1323 | 33 54.9 | 118 28.5 | Pairovet | 2 | 38 | 30 |
| CLAEMD | Z4 | 6/28/2106 | 1340 | 33 55.3 | 118 30.5 | Pairovet | 2 | 59 | 50 |
| CLAEMD | 2B | 6/28/2106 | 1356 | 33 56.4 | 118 29.2 | Pairovet | 2 | 33 | 25 |