Title

DNA sequencing of fish eggs and larvae reveals high species diversity and seasonal changes in spawning activity in the southeastern Gulf of California

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Using molecular identification of ichthyoplankton to monitor spawning activity in a subtropical no-take Marine Reserve

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ABSTRACT: Ichthyoplankton studies can provide valuable information on the species richness and spawning activity of fishes, complementing estimations done using trawls and diver surveys. Zooplankton samples were collected weekly between January and December 2014 in Cabo Pulmo National Park, Gulf of California, Mexico (n=48). Fish larvae and particularly eggs are difficult to identify morphologically, therefore the DNA barcoding method was employed to identify 4,388 specimens, resulting in 157 Operational Taxonomic Units (OTUs) corresponding to species. *Scarus* sp., *Halichoeres dispilus*, *Xyrichtys mundiceps*, *Euthynnus lineatus*, *Ammodytoides gilli*, *Synodus lacertinus*, *Etrumeus acuminatus*, *Chanos chanos*, *Haemulon flaviguttatum*, and *Vinciguerria lucetia* were the most abundant and frequent species recorded. Noteworthy species identified include rare mesopelagic species such as the giant oarfish (*Regalecus glesne*) and highly migratory and commercially important species such as black skipjack (*Euthynnus lineatus*) and yellowfin tuna (*Thunnus albacares*). Spawning activities showed distinct seasonal patterns with the highest abundance of ichthyoplankton recorded during spring, highest species richness during summer (90 OTUs) and lowest species richness during winter (28 OTUs). A total of seven OTUs were recorded throughout the year (4%), 11 OTUs during three seasons (7%), 36 OTUs in two seasons (23%) and 106 OTUs were recorded in only one season (66%). The study found eggs and/or larvae of 47 species that were not previously reported in Cabo Pulmo National Park. Results allow resource managers to compare shifting populations and spawning patterns of species that may be affected by both conservation efforts and broader oceanographic changes associated with climate change.

KEY WORDS: Marine protected area · DNA barcoding · molecular ecology · marine conservation · Cabo Pulmo National Park · Gulf of California · Mexico

INTRODUCTION

Cabo Pulmo National Park is a subtropical no-take marine reserve located on the southeast coast of Baja California Sur, Mexico in the Gulf of California (Fig. 1A, B). This national park is a unique example of a successfully managed marine protected area (Aburto-Oropeza et al. 2011). Established as a Mexican national marine park in 1995, Cabo Pulmo National Park is recognized as a UNESCO World Heritage Site. Since 1995, the community of Cabo Pulmo voluntarily chose to expand the no-take zone from an initial 35% to nearly 100% of the 27-square mile park (Aburto-Oropeza et al. 2011) (Fig. 1B). Although the community of Cabo Pulmo already supported a small tourism industry, it was bolstered as the biomass, abundance and diversity of charismatic and commercially important fish species as well as marine mammals increased. A ten-year study showed a 463% increase in total fish biomass and a 1070% increase in biomass of top predators since 1995, the largest ever measured in a marine reserve worldwide (Aburto-Oropeza et al. 2011, 2015).

While the biota of the rocky and coral reefs of Cabo Pulmo National Park are thriving, they are still vulnerable to a multitude of threats including coastal development (Arizpe & Covarrubias 2010), overfishing (Johnson et al. 2017), and climate change (Verutes et al. 2014, Robinson et al. 2013, 2016). As the area has garnered more public and academic attention, large international developers have proposed development projects in neighboring communities that could have negative effects for Cabo Pulmo National Park’s coastline and marine biota (Arizpe & Covarrubias 2010). As a consequence of global climate change, it is predicted the oceans will experience a significant increase in sea surface temperature in the next 100 years (Levitus et al. 2009), which could result in more frequent and intense El Niño Southern Oscillation (ENSO) events, many of which have had significant effects on the region in the past and recent years (Timmermann et al. 1999, Robinson et al. 2013, 2016). Since 2010 the Gulf of California has experienced an increase in sea surface temperature and decrease of wind speed, resulting in lower sea surface chlorophyll-*a* concentration than previous years (Robinson et al. 2016). The waters of Cabo Pulmo National Park will likely face increased fluctuations in temperature, dissolved oxygen concentrations, pH, nutrient content, and circulation that could negatively impact this delicate rocky and coral reef ecosystem (Doney et al. 2012).

Careful monitoring of vulnerable coastal marine ecosystems is needed to track biotic changes that may occur as a result of a changing climate and to inform marine resource management decisions (Harada et al. 2015). The abundance and species composition of ichthyoplankton collected from the water column provides valuable information concerning the broadcast spawning activities of fishes and plays a significant role in the assessment and management of marine ecosystems (Gleason & Burton 2012). Ichthyoplankton surveys can be used as a fisheries independent indicator of ecosystem health, estimating species-specific spawning biomass, reproductive periods, overall reproductive strategies and population dynamics as a function of environmental variability (Lo 2001, Aceves-Medina et al. 2003, 2004). They can also help identify the location of critical spawning habitat that should be protected in order to ensure the present and long-term sustainability of vulnerable fish populations (Sala et al. 2003).

Historically, scientists and fisheries managers have relied on morphological identification of ichthyoplankton (mostly larvae, occasionally eggs) to determine which species spawn in a particular area (Ahlstrom & Moser 1980, Aceves-Medina et al. 2003, 2004, Miller & Kendall 2009, Harada et al. 2015). A considerable drawback to this morphological method is the difficulty of telling species apart at early life stages; in fact, many species have virtually indistinguishable eggs and successful identification of fish eggs and preflexion fish larvae using morphological characteristics alone requires years of study (Ahlstrom and Moser 1980, Hyde et al. 2005). Even then, morphological experts can experience high uncertainty in fish egg identification (Ahlstrom & Moser 1980, Moser et al. 1974, 1993), which can prove costly when these data are used to determine population abundance and make management decisions for targeted species. Where traditional morphological analysis may have difficulty distinguishing species with similar morphological characteristics, molecular genetic analysis can provide accurate species identification to infer spawning strategies and determine the magnitude of reproductive efforts of fish assemblages (Burton 2009, Harada et al. 2015).

Molecular analysis of ichthyoplankton provides valuable information about temporal and geographic spawning activity and can be used for the purposes of stock assessments and monitoring ecosystem health (Perez et al. 2005, Harada et al. 2015). The use of molecular genetic tools to assist in conservation efforts is becoming increasingly affordable (NHGRI Genome Sequencing Program, http://www.genome.gov/sequencingcosts/). Other methods of monitoring fish populations include diver-conducted monitoring surveys, which until now have been the primary source of information about the abundance and diversity of fish species found in Cabo Pulmo National Park’s waters (Alvarez-Filip et al. 2006, Aburto-Oropeza et al. 2011, 2015). Many species reproduce at night when divers are unable to observe fish spawning events (Claro & Lindeman 2003, Erisman et al. 2014). Trawling is not only invasive to vulnerable fish populations and sensitive marine environments, but are often size and species selective. Additionally, trawling surveys are not allowed in most marine protected areas, making this method generally unsuitable. Video assessments can also be biased depending on the locations of camera traps and often suffer from the same daytime bias as diver surveys. Fish larvae with certain swimming capabilities and schooling behavior that are collected using nighttime light traps can be less diverse than daytime collections made with a plankton net over reefs in the Gulf of California, showing relevant differences in community structure (Brogan 1994). Ichthyoplankton surveys, especially those involving the analysis of eggs, provide better evidence of nearby spawning activity due to the short embryo development time in tropical and subtropical ecosystems (Pauly & Pullin 1988). Sampling zooplankton has a negligible impact on local biota and plankton nets sample any available pelagic eggs in the water column, reducing biases based on species size and juvenile and adult behavior or the habitat where mating and spawning events occur. Of course, it is important to note that just as visual species richness and abundance assessments have limitations, ichthyoplankton surveys under sample live-bearing species, as well as those with demersal eggs.

We monitored broadcast spawning activity of fish in Cabo Pulmo National Park through molecular identification of ichthyoplankton collected weekly within the marine protected area. This survey establishes a baseline for species richness and abundance that can be used to compare with data from annual diver-conducted monitoring surveys (Alvarez-Filip et al. 2006, Aburto-Oropeza et al. 2011, 2015, Harada et al. 2015). The goals of the present study were to use morphological and molecular identification of ichthyoplankton collected weekly within Cabo Pulmo National Park from January to December 2014 to: 1) estimate the spawning activity and species richness of fishes in Cabo Pulmo National Park, 2) identify which commercially and/or recreationally important species spawn in Cabo Pulmo National Park, and 3) uncover seasonal changes in broadcast spawning activity over the course of a single year.

MATERIALS AND METHODS

Sea surface temperature and chlorophyll-*a* concentration

Monthly mean night sea surface temperature (SST, °C) and concentration of chlorophyll-*a* (mg m^{-3}) data from 1999 to 2015 for the Cabo Pulmo region was obtained from NASA (http://podaac.jpl.nasa.gov and http://oceandata.sci.gsfc.nasa.gov/SeaWiFS) to infer seasonal environmental variability of sea surface temperature and chlorophyll-*a* concentration associated with fish spawning activity. The monthly dataset had a 4 km resolution from the composite Advanced Very High Resolution Radiometer (AVHRR) and a monthly, spatial resolution of 9 km from the composite Sea-viewing Wide Field-of-view Sensor (SeaWIFS). Monthly means and anomalies were calculated with the same method as satellite SST and Chl-*a* concentration time series were reported for the central and northern region of the Gulf of California (Robinson et al. 2013, 2016).

Zooplankton collection

Forty-eight weekly zooplankton samples were collected within Cabo Pulmo National Park, Baja California Sur, Mexico (23°27’ N, 109°25’W) from January to December 2014 using a conical zooplankton net (60 cm in diameter with a 330 µm mesh size) towed near the surface (<5 m depth) for ten minutes (Smith & Richardson 1977) (Fig. 1A, B). Zooplankton samples were collected during daytime hours (8:30–18:14 hr, 79% of which were collected before noon). The zooplankton net was equipped with a calibrated General Oceanics digital flowmeter (model 2030R6, Miami, USA) to estimate filtered seawater volume and estimate standardized abundance of fish eggs and larvae (ind. 1000 m^{-3}) (Smith & Richardson 1977). The zooplankton samples were sieved to remove seawater with a 200-μm sieve and preserved in 96% ethanol with an entire change of ethanol at the laboratory when biomass was measured using displacement volume method. Eggs and larvae were separated from the entire zooplankton sample (no aliquot), preliminary morphological identifications were made where possible and the number of eggs and larvae, identified to the most precise taxonomic level possible, were recorded following standard fish eggs and larvae identification keys (Chaudhuri 1977, Ahlstrom & Moser 1980, Nishikawa & Rimmer 1987, Moser 1996, Watson 1998, Saldierna-Martinez et al. 2005, Richards 2006a, b, Jiménez-Rosenberg et al. 2006, Kawakami et al. 2010 and González-Navarro et al. 2013). Digital photographs were taken of each specimen for a taxonomic record.

Ichthyoplankton specimens were stored at 4ºC in 96% ethanol until ready for genetic processing. Eggs and larvae were isolated and individually transferred to 0.2-mL PCR tubes. Any remaining ethanol was removed from the tubes and 15 µL of deionized H_2O was placed in each tube and then removed to rinse the specimens. Fifteen microliters (μL) of a mixture of two-thirds Qiagen AE Buffer and one-third water was added to the tube and a clean pipette tip was used to crush the specimen and release the DNA. No further extraction of DNA or purification was needed. Samples were stored at -20ºC prior to Polymerase Chain Reaction (PCR).

Molecular analysis of ichthyoplankton

Molecular analyses of the collected eggs and larvae were carried out using universal fish primers to amplify a 710 bp fragment of the mitochondrial gene, cytochrome oxidase c subunit 1 (COI) using COI VF1 forward primer (5′-TTCTCAACCAACCACAAAGACATTGG-3’) and COI VR1 reverse (5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′) (deWaard et al. 2007). If

COI did not amplify, a 570 bp fragment of the mitochondrial 16S ribosomal rRNA gene was amplified using forward primer 16Sar (5’-CGCCTGTTATCAAAAACAT-3’) and reverse primer 16Sbr (5’-CCGGTCTGAACTCAGATCACGT-3’) (Palumbi 1996). One µL of the extracted DNA solution was utilized for each PCR with 12.5 µL of Promega GoTaq Green Master Mix, 0.5 µL each of forward and reverse primers, and 10.5 µL of dH_2O. The thermal cycler profile for the PCR reaction was 95ºC for two min, 35 cycles of 95ºC for 30 s, 50ºC for 45 s, and 72ºC for one min, followed by 72ºC for five min. The PCR products were run on 1.5% agarose gels, stained with GelRed (Biotium, Inc., Fremont, CA) and visualized under UV light to verify successful amplification. Successfully amplified samples were then purified using G-50 Fine Sephadex (GE Healthcare) spin columns and sent offsite for sequencing (Retrogen, Inc., San Diego, CA) (Harada et al. 2015).

A DNA barcoding approach was used to identify the eggs and larvae. Once sequences were obtained, the software Geneious (http://www.geneious.com) and Sequencher (http://www.genecodes.com) were used to edit the sequenced fragment. COI sequences were then compared to sequences published in the Barcode of Life Data System (BOLD). We used the Barcode of Life Data System database first because sequences come from well-vouchered specimens and usually rely on multiple sequences. The Barcode of Life Data System is comprised of COI sequences only, therefore we could not compare our 16S sequences to this database. In the cases where no identification was obtained using the Barcode of Life Data System database or the gene sequenced was 16S, we used the Basic Local Alignment Search Tool (BLAST) in GenBank (National Center for Biotechnology Information) utilizing default parameters. For both COI and 16S sequences, we used a threshold of $\geq$ 97% to tentatively assign the sequence to a species. We then compared these molecular identifications with previous records from annual diver-conducted monitoring surveys (1995–2016) (Aburto-Oropeza et al. 2011, 2015) and lists of fish species reported from Cabo Pulmo National Park (Villarreal-Cavazos et al. 1999, Aburto-Oropeza et al. 2001, Alvarez-Filip et al. 2006, Reyes-Bonilla et al. 1999) and throughout the Gulf of California (Mascareñas-Osorio et al. 2011, Erisman et al. 2011, Cruz-Agüero et al. 1994, Del Moral-Flores et al. 2013, Villegas-Sánchez et al. 2009, Castro-Aguirre et al. 2002). Sequences that produced a ≥ 97% match to species that are not known to occur in the Gulf of California were considered "unidentified Operational Taxonomic Units (OTUs)." Additionally, sequences that failed to produce a ≥ 97% match were also considered unidentified OTUs.

Fish egg subsampling

Due to the large number of fish eggs (*n*=19,960) and larvae (*n*=1,184) collected between January and December 2014, it was cost and time-prohibitive to process all of them using molecular methods. For this reason, we employed a fixed-count sub-sampling method to determine the species richness within each ichthyoplankton sample collection date. If the collection included < 96 specimens (the number of wells in a standard PCR plate), we attempted to sequence all of the individuals from the ichthyoplankton sample. If a collection contained > 96 individuals, a minimum number of 96 specimens were randomly selected and sequencing was attempted. For collections with high numbers of individuals, rarefaction curves were created using PRIMER 6 (PRIMER-E Ltd) (Fig. S1, Supplemental material). If the curve reached an asymptote, indicating that additional analysis will likely not reveal additional species (or OTUs), analysis for that particular sampling date was halted (Gotelli & Colwell 2011). If an asymptote was not reached, a second round of subsampling was conducted with another set of 96 specimens randomly selected and analyzed genetically. This process was repeated until an asymptote was reached. In some zooplankton collections, amplification was minimally successful as a result of DNA degradation. In these cases, after two unsuccessful attempts at analyzing 96 specimens (amplification of <15%), further analysis was abandoned. This occurred in five ichthyoplankton samples, leaving 43 samples that produced successful results.

We determined the likely habitat of the adult fishes using data acquired from FishBase (http://www.fishbase.org). These data provided us with information about the possible origins of spawning events and we were able to indirectly infer which species are likely to inhabit and reproduce in Cabo Pulmo National Park (reef associated, demersal, pelagic neritic or bentho-pelagic species) or likely come from outside Cabo Pulmo National Park (pelagic oceanic, mesopelagic, bathypelagic, or bathy-demersal species).

RESULTS

Sea surface temperature and chlorophyll-*a* concentration

Monthly mean sea surface temperature in Cabo Pulmo National Park varied from 22.5° (Jan) to 30.4° C (Aug) during 2014. The Cabo Pulmo region has on average (2000–2015) a typical SST range between 21 and 29.6° C. Therefore, 2014 was an anomalously warm year, but showed the typical seasonality of a relatively cold period between December-May, a warm period between July-November, and two brief transition periods in May and November (Fig. 2A, B). Sea surface concentration of Chlorophyll-*a* recorded during 2014 was well below 2000-2015 monthly means with values between 0.17 and 0.24 mg/m^3 in the cold season (January-May), 0.11-0.15 mg/m^3 between July and September and between 0.19 and 0.49 mg/m^3 between October and December 2014 (Fig. 2C, D). Positive anomalies of SST and negative anomalies of Chl-*a* concentrations have been longer and more frequent in Cabo Pulmo National Park during 2010-2015 than during 2000-2009 (Fig. 2A–D).

Species composition

A total of 21,144 fish eggs and larvae were collected in Cabo Pulmo National Park between January 11th and December 25th, 2014 during 48 zooplankton collections. Sequencing >250 specimens per ichthyoplankton sample did not yield additional OTUs in any of the seasons (Fig. S1, Supplemental material). The maximum number of OTUs identified (40) in summer required analysis of <230 specimens while in fall 15 OTUs were observed from analyzing <150 specimens (Fig. S1, Supplemental material). Five ichthyoplankton samples produced no results due to poor sample preservation, leaving a total of 43 usable samples. After subsampling as described above, the target gene, either COI or 16S, was successfully amplified and sequenced for 2,589 specimens. A total of 6,883 eggs (n=6,422) and larvae (n=472) were analyzed using PCR. A total of 3,327 eggs (n=3,017) and larvae (n=310) successfully amplified the target gene, either COI or 16S (sequence data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.86fr4). The total PCR amplification success rate was 48%, with 47% of eggs and 65.7% of larvae successfully amplifying the target gene. 49.6% (n=2976) of COI and 39% (n=351) of 16S reactions resulted in successful amplification. The total sequencing success rate was 77.8%, with 2,354 (78%) eggs and 235 (77%) larvae successfully sequenced.

Due to their distinctive shape (see Fig. S3, Supplemental material), an additional 1,799 *Scarus* sp. eggs were identified morphologically to the genus level, bringing the total number of specimens analyzed to 4,388. Fifty *Scarus* sp. eggs were analyzed using DNA barcoding revealing three species: *S. ghobban* (26), *S. compressus* (23), and *S. rubroviolaceus* (1), all known to occur in Cabo Pulmo National Park. Figure 3A-C shows a time series of the number of ichthyoplankton specimens (eggs, larvae and total standardized abundance) collected during 2014.

A total of 4,388 fish eggs (4,153) and larvae (235) were identified consisting of 157 operational taxonomic units (103 identified to genus or species plus 54 unidentified OTUs). Of these, 105 and 31 OTUs were only detected in egg and larvae specimens, respectively, and the remaining 23 OTUs were detected in both stages. The majority of the specimens identified belong to species with pelagic broadcast spawning behavior. However, we also identified six species that are benthic broadcast spawners or open water/substratum egg scatterers (Tables 1 and 3, species indicated with the ⊙ symbol).

The ten most frequently identified fishes in order of relative abundance were: *Scarus* sp., *Halichoeres dispilus*, *Xyrichtys mundiceps*, *Euthynnus lineatus*, *Ammodytoides gilli*, *Synodus lacertinus*, *Etrumeus acuminatus*, *Chanos chanos*, *Haemulon flaviguttatum*, and *Vinciguerria lucetia*. Species identified both morphologically and genetically in order of relative abundance and the number of collections in which the eggs and larvae were present is shown in Table 1. This table was compared with species previously observed during diver-conducted monitoring surveys (1995-2016) and checklists of species reported in the Gulf of California (Aburto-Oropeza et al. 2011, 2015, Alvarez-Filip et al. 2006, Villareal-Cavazos et al. 2000) (Table 1). The total number of fish species reported from Cabo Pulmo National Park in previous studies is

270. This study revealed 47 species that were not previously reported in Cabo Pulmo National Park, increasing the known species diversity to 317 species (Table 1, indicated with a ▯).

Unidentified Operational Taxonomic Units (OTUs)

The sequences obtained were classified into 157 OTUs. Of the 157 unique sequences present in the study, 103 sequences produced a database match of ≥97%, enabling species level identification in 101 cases and genus level identification in two cases (Table 1). 43 sequences had hits that were below the 97% threshold, suggesting that they represent species that have not yet been entered into the COI or 16S online databases (updated November 2017). An additional 11 sequences produced a match of ≥97% to species that have not previously been known to occur in the GoC (*Caranx crysos, Hyporthodus niveatus, Kathetostoma laeve, Epinephelus clippertonensis, Assurger anzac, Syacium maculiferum, Hyporthodus niphobles, Genypterus maculatus, Kyphosus cinerascens, Paraconger ophichthys* and *Trachinotus goodei*). These sequences most likely belong to closely related species that do occur in the region, but have not yet been added to the online database or, less likely, they represent an occurrence of the matched species outside of its known distribution range. Table 2 shows the unidentified OTUs that did not produce a match of ≥97% in the online databases or are not known to occur in the Gulf of California (indicated with a *).

Species richness

A diverse fish species assemblage from 16 orders, 46 families and 84 genera was identified from eggs and larvae collected monthly from the zooplankton samples. Using habitat data of adults of each fish species inferred from FishBase.org, 63.9% of individual specimens identified were reef associated, 13.4% pelagic, 10.4% demersal, 7.5% unknown, 2.4% bentho-pelagic, 2.3% mesopelagic, 0.09% bathypelagic, and 0.05% bathy-demersal. The relative proportions of habitat of all of the specimens identified with molecular methods and the proportion of species from each habitat distribution identified throughout the year are shown in Fig. 4A, B. Reef associated, demersal, pelagic neritic and bentho-pelagic species dominated fish spawning events throughout the year with high abundance dominance during cold months (Jan-Mar and Nov-Dec). Pelagic oceanic species seem to spawn and enter Cabo Pulmo National Park from March to November but with relatively higher proportion during summer months (Jun-Sep) (Fig. 4B). Mesopelagic, bathypelagic and bathy-demersal species were observed mostly as larvae with low frequency and low abundance (albeit with sporadically large proportions in certain sampling weeks) primarily during the first six months of 2014 (Fig. 4B). Eggs from these species, including the giant oarfish *Regalecus glesne*, were only collected on rare occasions. To our knowledge this is the first record of *R. glesne* eggs in the Gulf of California. Due to the rarity of this deep-water species we compared the sequence we obtained against tissue from an adult *R. glesne* voucher specimen in the Scripps Institution of Oceanography Marine Vertebrates Collection (GenBank accession number HQ127659.1). The sequence provided a 99% match, confirming the identification of this egg as *R. glesne*.

Seasonal spawning structure

Weekly zooplankton samples revealed seasonal spawning patterns among the species (Table 3). Seven OTUs were recorded in all four seasons (4%), 11 OTUs in three seasons (7%), 36 OTUs in two seasons (23%), and the majority, 106 OTUs, in only one season (66%) (Table 3). *Synodus lacertinus* (lizardfish), *Halichoeres dispilus* (wrasse), *Vinciguerria lucetia* (lightfish) and *Ammodytoides gilli* (lance) specimens were found spawning throughout most of the year indicating a strategy of continuous reproduction, whereas *Etrumeus acuminatus* (herring) only appeared in six collections with 95% of the specimens appearing during the month of March, indicating a seasonally biased reproductive period. This low frequency spawning may illustrate a temporally delimited spawning season for *E. acuminatus,* or it may suggest that this species rarely spawns inside or in the vicinity of Cabo Pulmo National Park. Additionally, 96% of *Auxis rochei* specimens were found in winter and summer, 98% of *Euthynnus lineatus* specimens, 76% of *Lutjanidae* spp. specimens (5 species) and 78% of *Decapterus macarellus* specimens were found during the summer.

The highest abundance of ichthyoplankton was collected in the spring (8,824 specimens, 73 OTUs). The highest species richness (90 OTUs) with relatively low abundance (5,420 specimens) was found during the summer and the lowest species richness (28 OTUs) and lowest abundance (2,584 specimens) during autumn (Table 3, Fig. 3A-C). The highest number of OTUs on a single collection date occurred on September 10th (38 OTUs). On two occasions, peaks in abundance corresponded to spawning of a particular species: on February 16th, 99% of the specimens were *Scarus* spp. (parrotfish) and the collection with the lowest number of species (1), as well as the highest abundance (5,334), occurred on May 24th during a recent spawning event of *Chanos chanos* (milkfish). Peaks in spawning activity were observed in each month with the exception of November and December. Figure 3A-C illustrates the number of OTUs found in each sampling collection.

Sequencing using 16S rRNA primers

Initial sequencing was done using COI universal fish primers. If the reaction failed to amplify COI, then 16S rRNA primers were used. Sequences obtained from COI can identify closely related species as well as higher taxa in many animal phyla, whereas 16S has more difficulty discriminating between closely related species (Kochzius et al. 2010). We used COI primers on 5,996 samples and 16S primers on 887 samples. Of these, 49.6% (n=2976) of COI and 39% (n=351) of 16S reactions resulted in successful amplification. 16S reactions likely resulted in lower amplification success rates due to poor sample quality, since these attempts followed failure of COI amplification. Temperatures in Cabo Pulmo National Park can be quite high, especially in the summer months on sunny days, and DNA from many of the early zooplankton collections likely degraded due to poor sample preservation methods (e.g., leaving the sample in the sun before preserving in ethanol) that were subsequently corrected later in the study. Additionally, COI was preferred because the Barcode of Life Data System contains a large number of high quality COI sequences (Species Level Barcode Records: 2,929,775 Sequences/181,204 Species/69,400 Interim Species as of October 2017) with a minimum sequence length of 500bp. However, to date there has not been a concerted effort to barcode the fish of the Gulf of California, so our identifications relied on the available databases. Although the 16S gene database for fish is not as complete as that for COI, a GenBank search of the top 20 species in Table 1 found that 80% were represented by one or more 16S sequences, while 95%

were represented by COI sequences. Hence, one species in that top group (*Scarus compressus)* could only be identified by 16S sequencing. Further inspection of the list revealed that two other species (*Fistularia corneta and Pronotogrammus multifasciatus*) were not in the COI database but were identified by 16S sequencing.

DISCUSSION

In this study, ichthyoplankton collected from within Cabo Pulmo National Park over one year of weekly sampling were identified using DNA barcoding methods. This time series provides insight into fish spawning activity in and near Cabo Pulmo National Park including the presence of commercially and recreationally important species, seasonal changes in species composition, and evidence of high species richness. The study revealed information concerning local spawning of ecologically and economically valuable species that indicate the effectiveness of the marine protected area for preserving spawning habitat and conserving marine biodiversity, as well as contributing to health of the surrounding fisheries by acting as a potential source of population replenishment through spawning activity. The study enhances existing knowledge of fish assemblages in the park by finding 47 species not previously reported in systematic dive monitoring surveys from Cabo Pulmo National Park (Alvarez-Filip et al. 2006, Aburto-Oropeza et al. 2011, 201). The use of DNA barcoding to identify ichthyoplankton revealed three times more species richness than traditional morphological identification of ichthyoplankton. The results from the present study, in combination with data from standard diver-conducted monitoring surveys (Alvarez-Filip et al. 2006, Aburto-Oropeza et al. 2001, 2011, 2015, Ramirez-Valdez et al. 2014) and other data collection methods, can be used as a baseline to compare shifting populations and spawning patterns of species that may be affected by both the MPAs' protection and broader oceanographic changes associated with El Niño and recent warming in the Gulf of California (Robinson et al. 2013, 2016).

Fish reproduction and oceanic conditions

At temperatures ranging between 19 and 30°C through the year (Fig. 2), typical hatching time is between one and three days for fish eggs from most of the commercial and recreational species identified in this study (Harada et al. 2015, Pauly & Pullin 1988). Although planktonic eggs and larvae drift with marine currents, since most tropical and subtropical eggs hatch within 1-3 days of spawning, many of the collected eggs likely result from local spawning events in or around Cabo Pulmo National Park (Harada et al. 2015, Pauly & Pullin 1988). In contrast, larvae may have been adrift for several weeks and therefore only provide more regional and seasonal information. Explicit synoptic coastal current information is limited to 2010-2012 for Cabo Pulmo National Park (Tasviña-Castro et al. 2012), but a recent 3D numerical current model of particle (plankton) connectivity in the Gulf of California predicts that high dispersion occurs from the mainland coastal areas in the central and southern part of the Gulf of California to the rest of the gulf due to strong seasonal currents, implying that Cabo Pulmo National Park is in a region with relatively high connectivity (Marinone 2012). Peguero-Icaza et al. (2011) reported seasonal changes in connectivity routes among larval fish assemblages through particle tracking with a 3D baroclinic numerical model in the northern Gulf of California with seasonal circulation phases, cyclonic in summer with relatively larger particle retention than dispersion and anticyclonic in winter with relatively larger particle dispersion.

Trasviña-Castro et al. (2012) reported current information from Cabo Pulmo National Park using Acoustic Doppler Profiler (ADP), Acoustic Doppler Current Profiler (ADCP) and Global Positioning System (GPS) buoy observations from October 2010 to February 2012. Currents in Cabo Pulmo National Park are forced by tides, winds and the influence of mesoscale structures associated with circulation from the mouth of the Gulf of California. During winter and fall (and sometimes summer) the net flow is mostly toward the south associated with the predominance of intense and sustained northwest winds that cause current speeds up to 2 m s^{-1} on the surface and 0.5 m s^{-1} on the seafloor. During summer, weak southeast winds prevail with sporadic northward fluxes (observed in October 2011 when a southward-to-northward shift of current direction occurs). These weak wind conditions influence only near surface currents; thus, tides force most of the water column current circulation pattern (Trasviña-Castro et al. 2012).

We infer that a large proportion of ichtyoplankton from fish species that spawn in Cabo Pulmo National Park likely drift southward during fall and early winter with episodic, less intense northward fluxes during summer. Oceanic, mesopelagic, bathy-demersal and bathypelagic species (that as adults do not inhabit the shallow continental shelf of Cabo Pulmo National Park), observed primarily during the first six months of the year, most likely come from the northern regions of the park. Apango-Figueroa et al. (2015) studied fish larvae assemblages in mushroom shaped dipole eddies (eddies one cyclonic 50 km diameter and one anticyclonic 80 km diameter) that originate from the coast with a <0.25 m s^{-1} onshore-offshore central jet separating fish larvae assemblages in ocean waters from the mouth of the Gulf of California (southeast of the Baja California peninsula). Although these mesoscale features are sporadic, during their relatively brief existence they can promote large offshore transport of zooplankton in the southeast region of the Gulf of California.

Conditions in Cabo Pulmo National Park during 2014-2015 were atypically warm with low Chl-*a* concentrations (Fig. 1) associated with an anomalous warm region in the north Pacific (known as “the blob”) and the beginning of the 2015 El Niño that caused longer and more frequent warming events (known as El Niño 2015-2016). The anomalously warm 2014 conditions likely promoted two relevant ecological processes: fast embryonic and larval development rates and the presence of ichthyoplankton from a relatively large proportion of tropical and subtropical coral reef species (Figs. 2, 3). Because our sampling took place during an anomalously warm year (2014), all observed patterns of seasonal reproduction per species might change during anomalously cold conditions as would be expected during a strong La Niña event. Given these oceanographic limitations, this study provides a baseline for community structure of fishes from Cabo Pulmo National Park and how ichthyoplankton community structure varies over the course of an annual cycle during an anomalously warm year (Alvarez-Filip et al. 2006, Aburto-Oropeza & Balart 2001, Aburto-Oropeza et al. 2011, 2015). Additionally, the present study provides a more complete and integrated perspective about the state of fish species richness in this subtropical coastal marine ecosystem than would dive surveys alone.

The presence of fish eggs and larvae inside Cabo Pulmo National Park indicates that it is a potentially relevant source and/or spawning ground for the species identified in this survey. The long-term protection of spawning habitat for vulnerable, overfished species within marine protected areas can lead to spillover, or biomass export, to surrounding non-protected areas, with the potential of enhancing local fisheries (Gell & Roberts 2003) and improving ecosystem health indexes (Aburto-Oropeza et al. 2015). The presence of fish eggs and larvae in an area is a good indicator of the presence (or absence) of a species, and further monitoring of fish spawning behavior may lead to observations of changes in spawning behavior (Harada et al. 2015). Comparing future data with baseline studies such as this one may prove highly valuable and could suggest that the establishment of a no-take marine reserve or similar management actions can impact the health of important fish populations. We may also see an effect from increasing sea surface temperatures as more southerly species may begin to migrate northward as a result of global climate change.

Spawning activity of fishes in Cabo Pulmo National Park

We demonstrated the presence of nearby spawning activity for many species that are vital to both the commercial and recreational fisheries of the region such as *Nematistius pectoralis* (roosterfish), , *Coryphaena hippurus* (mahi-mahi) and *Euthynnus lineatus* (skipjack), providing evidence that suggests that Cabo Pulmo National Park may currently be an important spawning location for nearby commercially and recreationally valuable fish populations in the Los Cabos region to the south. Other commercially fished species of the region that appeared in the Cabo Pulmo National Park time series include *Euthynnus lineatus*, *Auxis rochei*, *Thunnus albacares*, *Auxis thazard*, *Katsuwonus pelamis*, *Micropogonias ectenes* and *Etrumeus acuminatus* (Ramírez-Rodriguez, 2013). These species form part of a Mexican fishery that has a relevant regional socio-economic impact, and understanding the reproductive biology of these key species is crucial in order to inform sound fisheries management regulations such as total allowable catch, seasonal closures and the establishment of marine protected areas (Sala et al. 2003). The local spawning of highly migratory species is valuable information for fisheries management to ensure the sustainable harvest of vulnerable populations.

Networks of marine protected areas that allow for the preservation of biodiversity and complement fisheries management should include areas for fish spawning to occur and should consider the location of spawning aggregations and connectivity among populations through larval dispersal to ensure biologically optimal performance (Sala et al. 2003). The results from this study, including the presence of a diverse assemblage of many commercially and ecologically important species, provides potential evidence of the success of the marine protected area in its ability to act as a refuge for fish spawning activity and can aid in persuading the public and policy makers of the value of setting aside critical spawning habitat for conservation, including its potential to contribute to increased commercial fishery catch sizes (Nemeth 2005).

Although ichthyoplankton studies are generally restricted to species with zooplanktonic eggs or larvae, this study detected higher species richness (16 Orders, 49 families, 94 genera, 159 species) than standard diver-conducted monitoring surveys of the same location (13 Orders, 38 families, 118 species) (Aburto-Oropeza et al. 2011, 2015, Ramirez-Valdez et al. 2014). The present study identified the early larval stages of five mesopelagic species, indicating that some of the ichthyoplankton species were advected into the park from outside its boundaries (likely drifting from the north with a predominant southward current pattern occurring in winter). Cabo Pulmo National Park has a narrow continental shelf, a deep canyon located in the south end of the park, and an abrupt continental slope that descends from 100 to 700 meters depth (Fig. 1B). The finding of mesopelagic species and benthic species that inhabit caves and crevices (and would be missed in diver monitoring surveys or other standard collection methods) point to connectivity between the reefs of Cabo Pulmo National Park and nearby regions, including deep submarine canyons. Notably, only larvae of three mesopelagic species (i.e., no eggs) were recovered in the Cabo Pulmo National Park samples. This observation suggests that transport of these mesopelagic species into Cabo Pulmo National Park waters likely took longer than the embryonic development time of the eggs or that the species spawns at a depth (well below the depth of our plankton tows) where egg transport time exceeds embryonic development time. The remaining mesopelagic species include the eggs and larvae of Panama lightfish, *Vinciguerria lucetia* (which is abundant and broadly distributed in the gulf) (Aceves-Medina et al. 2003, 2004), and eggs of the relatively rare giant oarfish *Regalecus glesne*. Oarfish eggs are larger than most planktonic fish eggs (> 2.0 mm diameter) (Kawakami et al. 2010); their embryonic developmental time is currently unknown but may well be longer and consistent with the apparently more extended transport time of other mesopelagic species. Adult *R. glesne* have been found stranded on the beach in the Gulf of California and a close relative, *Regalecus russelii,* has been recorded in Bahía de La Paz (Chávez et al. 1985) and Colima (Carrasco-Águila et al. 2014), Mexico. Eggs from *R. glesne* were reported from the southeast Yucatan Peninsula (Leyva-Cruz et al. 2016) and the Mariana Islands in the North Pacific (Kawakami et al. 2009), and an early larval stage was reported from the Adriatic Sea (Dragičević et al. 2011). This is the first record of *R. glesne* eggs in Cabo Pulmo National Park and indicates that the species likely occurs in oceanic waters of the Gulf of California or the submarine canyon located south of Cabo Pulmo National Park and that the species spawns in or near the Gulf of California.

A total of 22 fish species were identified with vertical distribution ranges to 200 m or deeper (mesopelagic or bathypelagic according to FishBase.org). Mesopelagic species such as *Vinciguerria lucetia* and others found in this study are strong vertical diel migrators and may provide a significant food source for deep-water fishery populations (Dransfeld et al. 2009). Larvae of *V. lucetia* are among the most frequent and abundant fish larvae in the Gulf of California (Moser et al.1974, Aceves-Medina et al. 2003, 2004). In fact Moser et al. (1974) was a pioneering study reporting eggs identified to species level using exclusively morphological criteria (*Scomber japonicus* and *Sardinops sagax*) in the Gulf of California. The present study represents the first report of *V. lucetia* eggs in the GoC, confirming that it spawns throughout most of the year.

The extent to which our study was biased due to the near-surface net tow method is unknown. For example, the most abundant, conspicuous reef species inside the park, like leopard groupers and snappers, were either sampled in very low numbers or not at all. Brogan (1984) found significant differences in species richness of fish larvae collected with night light traps (less diverse community) and daytime near-surface zooplankton nets (more diverse community) in the Gulf of California. Our sampling times (79% of samples were collected between 8:00-12:00 h) may have over represented mid-day spawners but likely under represented dusk and night spawners. While the majority of the species identified in our samples exhibit pelagic broadcast spawning behavior, one of the most common reproduction methods in the ocean, six of the species identified exhibit alternative reproductive strategies including benthic broadcast spawning, open water/substratum egg scattering, brood hiding, and guarding/nesting (Tables 1 and 3, species indicated with the ⊙ symbol). As a result of these strategies, the species in question were only identified from larval stages, as their eggs are not usually found in the upper water column where our plankton tows were conducted during 2014.

Comparing molecular and morphological identification methods

After sorting out the fish eggs and larvae from the rest of the zooplankton samples, fish eggs and larvae were identified to the most precise taxonomic level possible following the diagnostic morphological characteristics established in several specialized publications (Chaudhuri 1977, Ahlstrom & Moser 1980, Nishikawa & Rimmer 1987, Moser 1996, Watson 1998, Saldierna–Martinez et al. 2005, Richards 2006a, b, Jiménez-Rosenberg et al. 2006, Kawakami et al. 2010, and González-Navarro et al. 2013). An interesting result that emerged from the present study is that these initial identifications of fish eggs were generally inaccurate and underestimated the number of species present. Species level identification can be accurate for several species with distinctive morphology (Ahlstrom & Moser 1980, Kawakami et al. 2010, Moser et al. 1974, Hammann et al. 1998), but for most species, distinctive egg morphology is lacking because spherical shape is a generalized adaptive feature for pelagic fishes (Elgar 1990). For example, in one instance, molecular analysis of 81 eggs with similar egg size diameter and morphologically identified as Pacific red snapper *Lutjanus peru*, revealed eggs from eight separate species (Fig. S2). Among the eggs identified were species from two orders and seven families with adults ranging in size from 24 cm to 92 cm total length (Fig. S2). In other cases, specimens morphologically identified as belonging to a single species were found (by molecular analysis) to include eggs from up to 14 separate species. Overall, only 15.5% of the morphological identifications agreed with the results from molecular analysis (COI and 16S).

It is relevant to note that in this study, the difficulty of morphological identification was further compounded by the fact that the samples were preserved in ethanol (largely dehydrated) rather than formalin 5%, which better preserves the shape, transparency, pigments and morphology of the fish embryos. Preservation in ethanol shrinks fish egg size and obscures many of the characters traditionally used for morphological identification (Kawakami et al. 2010, Lewis et al. 2016). In a temperate ecosystem with relatively low species richness (21 species), Markle & Frost (1985) identified 12 species using chorion structure and egg diameter versus oil globule diameter scatter grams to completely or partially diagnose species identities. In tropical and subtropical ecosystems with diverse fish community structure this task is more complex. Spherical fish eggs are a successful and broadly observed feature in marine and fresh water fishes as an adaptive strategy to inhabit the relatively short transit of the pelagic life phase (Elgar 1990). There are some exceptions, such as parrotfish (genus *Scarus*) and anchovy (genus *Engraulidae*), which have oval, football shaped eggs and are easily identified at least to the genus level using morphological characters alone. Overall, our molecular identifications revealed three times as many species as the morphological identifications, including rare and unexpected species such as the giant oarfish *R. glesne*. The reliance on morphological identification alone could cause significant biases of species richness when used for making fisheries management decisions and these findings underscore the value of using molecular techniques to aid in marine ecological and conservation studies (Arinashi 2006, Teletchea 2009, Harada et al. 2015).

Conclusion

Future studies should take into account embryonic development time (dependent on seawater temperature) as well as synoptic ocean current patterns (speed and direction) to determine the approximate location of the spawning activity. High-resolution predictive current modeling or more extensive observational studies of regional currents at Cabo Pulmo National Park should help determine if eggs collected originated inside the park boundaries are retained, or spillover to areas outside of Cabo Pulmo National Park as predicted in the Gulf of California (Peguero-Icaza et al. 2008, 2011) or California coastal ecosystems (Harada et al. 2015). Future surveys that sample zooplankton just above the reef (perhaps with a net trawled by a diver with a scooter) might capture more reef-associated species increasing gamma species diversity. Similarly, additional sampling at night might increase fish egg and larvae species richness.

Based on DNA sequences obtained from the Cabo Pulmo National Park fish egg samples, we found 59 OTUs (~32%) that did not match sequences available for species known to inhabit the Gulf of California (Table 2). Successful DNA barcoding requires complete and reliable online sequence databases, so a primary limitation of DNA barcoding is that the sequence databases are still incomplete worldwide and particularly for species in the Gulf of California. Future research should sequence specimens from Cabo Pulmo National Park that have not yet been analyzed and compare them to sequences from eggs that did not find a sufficient match in the existing molecular database.

Evidence of spawning activity in the vicinity of Cabo Pulmo National Park during 2014 suggests that the reserve is currently functioning to protect spawning habitat for many commercially and ecologically important species and that continued monitoring may detect changes in spawning activity in future years as the environment changes in response to natural and anthropogenic activities (Robinson et al. 2013, 2016). Although Cabo Pulmo National Park is among the best-protected and healthiest MPAs in the Gulf of California (Aburto-Oropeza et al. 2001, 2011, 2015), it is still vulnerable in the face of increasing tourism, coastal development, overfishing, and climate change. Zooplankton monitoring surveys like this one, including molecular identification of ichthyoplankton, help us acquire a more complete understanding of the state of the ecosystem and can be used as a baseline to compare data with future ecological and taxonomic studies.

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List of Figures

Fig. 1. Area of study. Location of Cabo Pulmo National Park in the southeast region of Baja California peninsula (**A**) and bathymetry of the national park measured with 120 and 200 kHz echosounder showing the location of the weekly zooplankton time series (Jan–Dec 2014) (**B**)

Fig. 2. Satellite monthly anomaly and mean sea surface temperature (**A, B**) and satellite monthly anomaly and mean of surface chlorophyll-*a* concentration (**C, D**) recorded between 2000 and 2015 from the region of Cabo Pulmo National Park

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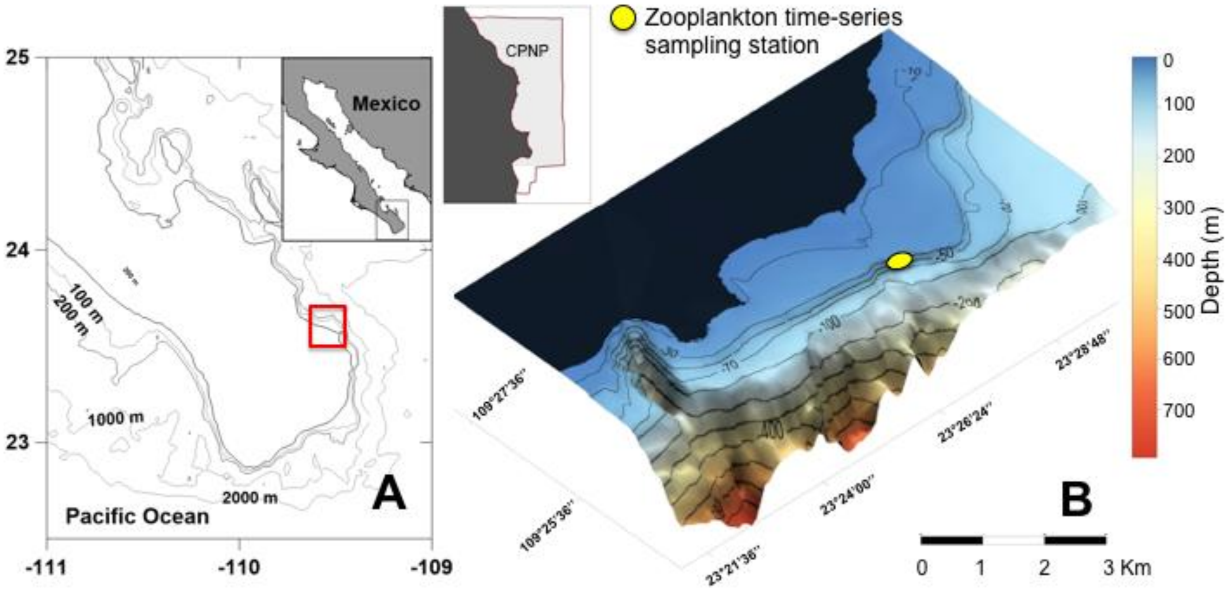
Supplemental Material

Fig. S1. Rarefaction curves of number of fish egg and larvae OTUs identified as a function of specimens analyzed per season.

Fig. S2. Example of morphological vs molecular fish egg identification: 81 eggs morphologically identified as *Lutjanus peru* (Pacific red snapper) revealed to be eggs from eight separate species. Scale bar represents 200 microns. The first two rows (capitalized letters) show the eggs that were identified molecularly as belonging to the species in the second two rows (non-capitalized letters).

Fig. S3. Composite photograph of multiple species of fish eggs found in the ichthyoplankton monitoring survey of Cabo Pulmo National Park in 2014 (not shown to scale). **A.** Morphologically distinct football shaped eggs belonging to *Scarus* spp. **B.** Egg of the giant oarfish, *Regalecus glesne*. Eggs of: C. *Synodus lucioceps* D. *Ammodytoides gilli* E. *Prionurus laticlavius* F. *Oxyporhamphus micropterus* G. *Pronotogrammus multifasciatus* H. *Vinciguerria lucetia*

Table S1. List of 157 Operational Taxonomic Units (OTU) found from fish eggs and larvae collections taken in 2014 from Cabo Pulmo National Park. The results from NCBI's GenBank and the Barcode of Life Database are shown as well as the number of identical sites for each sequence.



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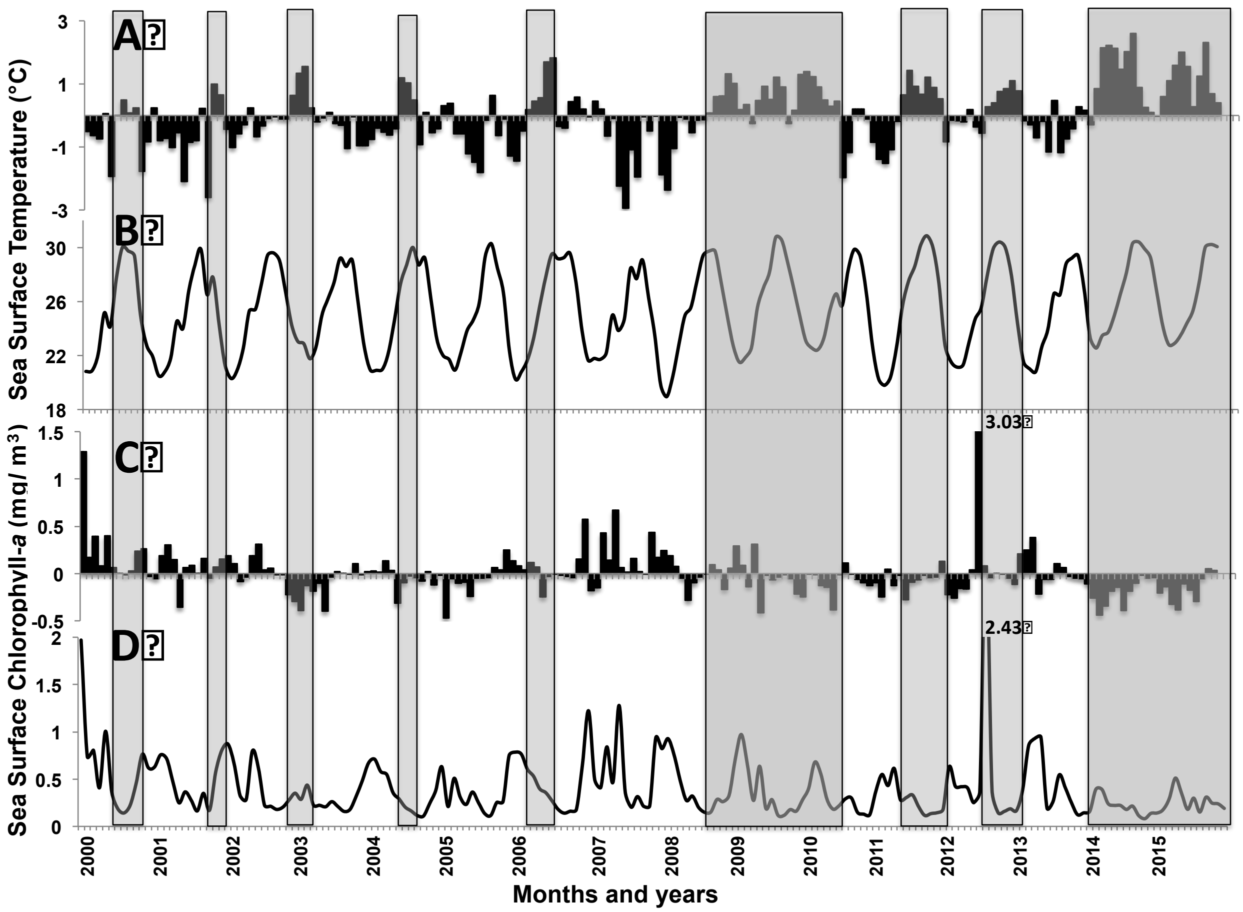

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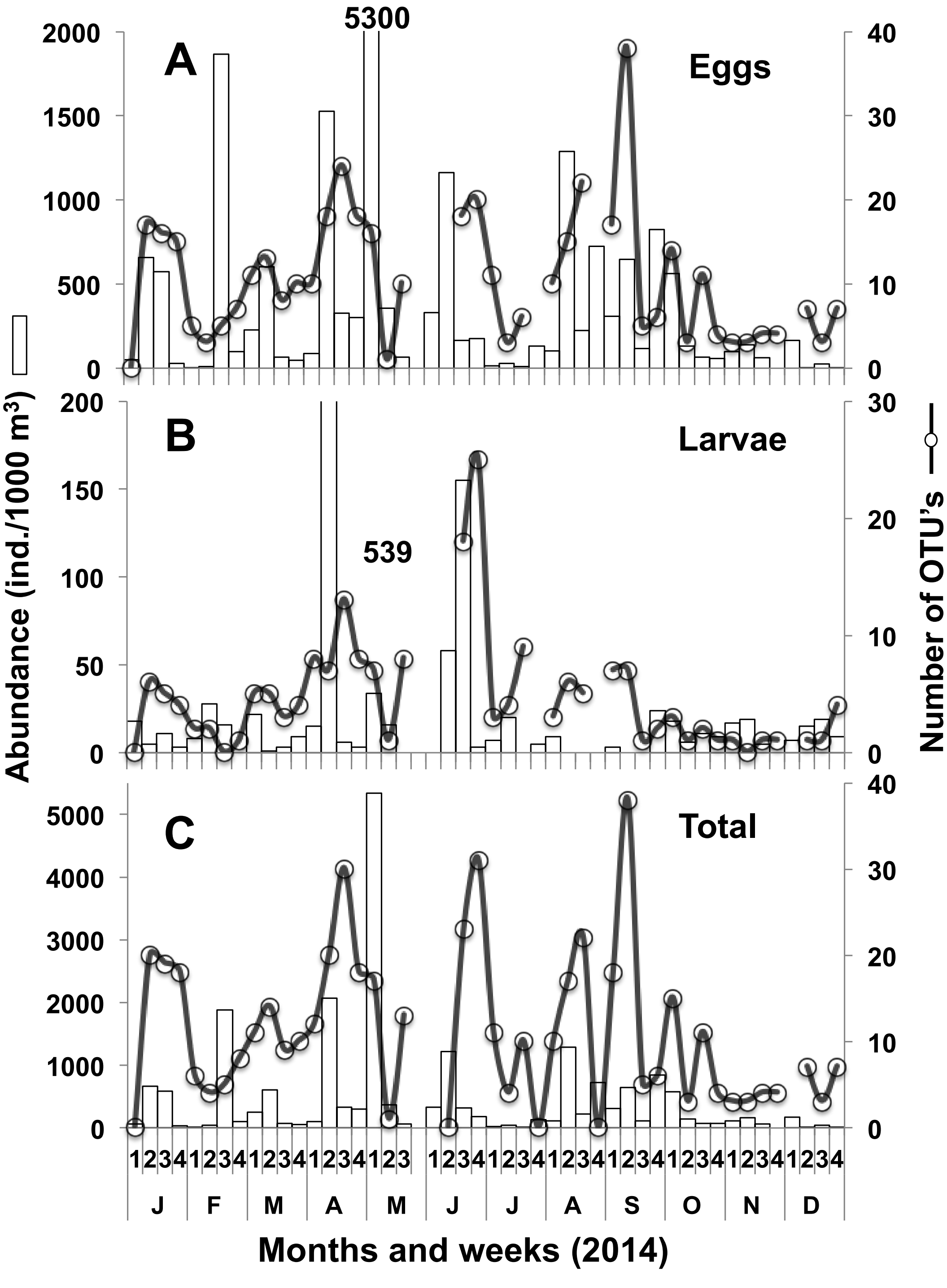

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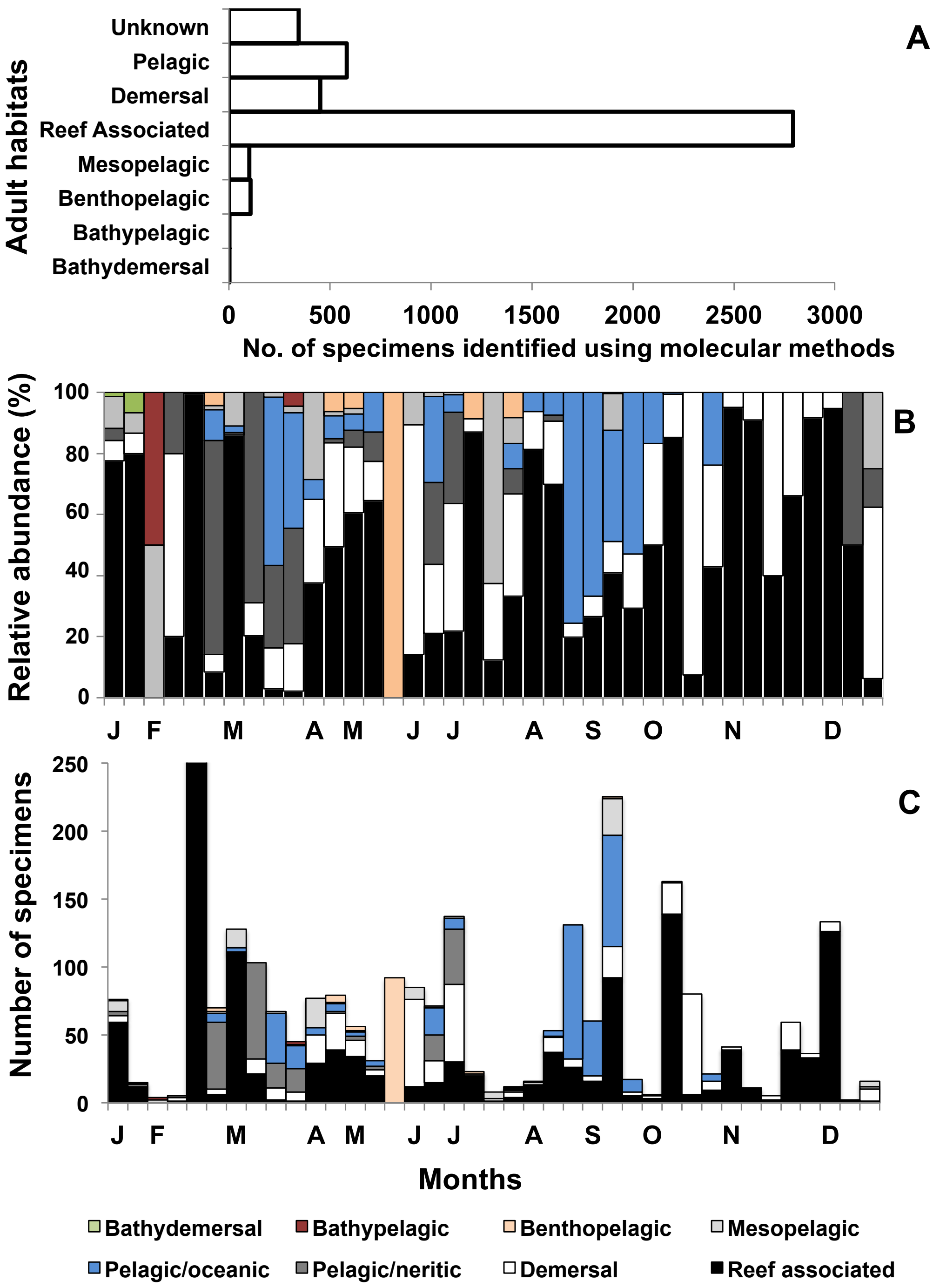

Fig. 4. A) Number of fish species (eggs and larvae) identified with molecular methods from ichthyoplankton collected in 2014 in the Cabo Pulmo National Park inferred per adult habitat distribution, **B)** relative abundance (%) and **C)** number of specimens analyzed of fish eggs and larvae classified by adult habitat distribution.

Note: 24th of June collection was taken after a known *Chanos chanos* (milkfish) spawning event

Table 1. List of all fish species in order of abundance (number of specimens identified) and number of specimens identified from eggs and larvae using molecular methods that produced a ≥97% match to sequences in GenBank and Barcode of Life Data System, number of zooplankton samples. Habitat of adults obtained from specialized literature is also shown.

▯ indicates species that have not been previously reported from Cabo Pulmo National Park

⊙ indicates species with demersal eggs that attach to substrate or a parent's body; the rest of the species have planktonic eggs

Species	Common name	Number of specimens identified	Number of collections	Number of larvae	Number of eggs	Gene used	Habitat
Scarus sp. (morphological ID)	Parrotfish	1799	7	0	1799	NA	Reef associated
Halichoeres dispilus	Chameleon wrasse	290	23	3	287	COI	Reef associated
Xyrichtys mundiceps ★	Cape razorfish	242	9	0	242	COI	Reef associated
Euthynnus lineatus	Black skipjack	213	8	3	210	COI	Pelagic/oceanic
Ammodytoides gilli ★	Gill's sand lance	123	13	0	123	COI	Demersal
Synodus lacertinus	Sauro lizardfish	118	26	0	118	COI	Demersal
Etrumeus acuminatus ★	Round herring	109	6	1	108	COI	Pelagic/neritic
Chanos chanos	Milkfish	96	3	1	95	COI	Benthopelagic
Haemulon flaviguttatum	Yellowspotted grunt	92	10	1	91	COI	Demersal
Vinciguerria lucetia ★	Panama lightfish	90	14	30	60	COI	Mesopelagic
Auxis rochei ★	Frigate tuna	71	8	14	57	COI	Pelagic/neritic
Haemulon sexfasciatum	Greybar grunt	67	10	1	66	COI	Reef associated
Caranx caninus ★	Pacific crevalle jack	61	8	9	52	COI	Pelagic/oceanic
Thalassoma lucasanum	Cortez rainbow wrasse	58	6	0	58	COI	Reef associated
Eucinostomus currani ★	Pacific flagfin mojarra	48	3	5	43	COI	Demersal
Decapterus macarellus	Mackerel Scad	40	10	8	32	COI	Pelagic/oceanic
Fistularia commersonii	Bluespotted cornetfish	33	7	0	33	COI	Reef associated
Sarda orientalis ★	Striped bonito	30	3	30	0	COI	Pelagic/neritic
Scarus ghobban	Bluebarred parrotfish	26	3	0	26	16S	Reef associated
Scarus compressus	Azure parrotfish	23	2	0	23	16S	Reef associated
Lutjanus guttatus	Spotted rose snapper	20	5	0	20	COI	Reef associated
Lutjanus argentiventris	Yellow snapper	17	7	5	12	COI	Reef associated
Umbrina xanti	Polla drum	16	4	0	16	COI	Reef associated
Cyclopsetta panamensis ★	God's flounder	15	8	0	15	COI	Demersal
Bothus leopardinus ★	Pacific leopard flounder	14	3	0	14	COI	Demersal
Paranthias colonus	Pacific creole-fish	14	7	0	14	COI	Reef associated
Sphyraena ensis ★	Mexican barracuda	14	3	8	6	COI	Pelagic/neritic
Pristigenys serrula ★	Popeye catalufa	13	2	0	13	COI	Reef associated
Cephalopholis panamensis	Pacific graysby	12	1	0	12	COI	Reef associated
Lutjanus novemfasciatus	Pacific dog snapper	12	4	7	5	COI	Reef associated
Rypticus bicolor	Mottled soapfish	12	3	0	12	COI	Reef associated
Acanthurus xanthopterus	Yellowfin surgeonfish	11	5	0	11	COI	Reef associated
Haemulon maculicauda	Spottail grunt	11	4	0	11	COI	Reef associated
Synodus evermanni ★	Inotted lizardfish	11	5	0	11	COI	Demersal
Hoplopagrus guentherii	Mexican barred snapper	10	4	0	10	COI	Reef associated
Myripristis leiognathus	Panamic soldierfish	10	4	0	10	COI	Reef associated

Paralabrax maculatofasciatus ★	Spotted sand bass	10	2	6	4	COI	Reef associated
Seriola rivoliana	Longfin yellowtail	10	2	0	10	COI	Pelagic/oceanic
Halichoeres melanotis	Golden wrasse	9	3	0	8	16S & COI	Reef associated
Lutjanus peru	Pacific red snapper	9	3	0	9	COI	Reef associated
Synodus scituliceps ★	Shorthead lizardfish	9	1	0	9	COI	Demersal
Calamus brachysomus	Pacific porgy	8	2	0	8	COI	Reef associated
Diodon holocanthus	Longspined porcupinefish	8	4	0	8	COI	Reef associated
Decapterus muroadsi	Amberstripe scad	7	2	6	1	COI	Pelagic/oceanic
Heteropriacanthus cruentatus	Glasseye	7	2	0	7	COI	Reef associated
Mulloidichthys dentatus	Mexican goatfish	7	4	0	7	COI	Reef associated
Prionurus punctatus	Yellowtail surgeonfish	7	2	0	7	COI	Reef associated
Selar crumenophthalmus	Bigeye scad	7	2	7	0	COI	Reef associated
Syacium ovale ★	Oval flounder	7	1	7	0	COI	Demersal
Trachinotus rhodopus	Gafftopsail pompano	7	2	0	7	COI	Pelagic/oceanic
Carangoides otrynter ★	Threadfin jack	6	3	0	6	COI	Benthopelagic
Coryphaena equiselis ★	Pompano dolphinfish	6	3	2	4	COI	Pelagic/oceanic
Coryphaena hippurus	Common dolphinfish	5	3	1	4	COI	Pelagic/neritic
Oxyporhamphus micropterus ★	Bigwing halfbeak	5	2	0	5	16S & COI	Pelagic/oceanic
Benthosema panamense ★	Panama lanternfish	4	4	4	0	COI	Mesopelagic
Cirrhitichthys oxycephalus	Coral hawkfish	4	3	0	4	COI	Reef associated
Nematistius pectoralis ★	Roosterfish	4	3	1	3	COI	Demersal
Plagiotremus azaleus ⦿	Sabertooth blenny	4	3	4	0	COI	Reef associated
Selene peruviana ★	Peruvian moonfish	4	1	0	4	COI	Benthopelagic
Balistes polylepis ⦿	Finescale triggerfish	3	1	3	0	COI	Reef associated
Bodianus diplotaenia	Mexican hogfish	3	1	0	3	COI	Reef associated
Regalecus glesne ★	Giant oarfish	3	1	0	3	16S	Mesopelagic
Alphestes immaculatus ★	Pacific mutton hamlet	2	1	0	2	COI	Demersal
Anisotremus taeniatus	Panama porkfish	2	1	0	2	COI	Demersal
Axoclinus storeyae ★ ⦿	Carmine triplefin	2	1	2	0	COI	Reef associated
Cheilopogon dorsomacula ★	Backspot flyingfish	2	2	0	2	COI	Pelagic/neritic
Diogenichthys laternatus ★	Diogenes lanternfish	2	2	2	0	COI	Mesopelagic
Fistularia corneta ★	Pacific cornetfish	2	2	0	2	16S	Pelagic/neritic
Gerres simillimus ★	Yellow fin mojarra	2	2	0	2	COI	Reef associated
Hygophum atratum ★	Thickhead lanternfish	2	1	2	0	COI	Bathypelagic
Labrisomus xanti ⦿	Largemouth blenny	2	2	2	0	COI	Reef associated
Liopropoma fasciatum ★	Wrasse ass bass	2	1	0	2	COI	Reef associated
Pontinus furcirhinus ★	Red scorpionfish	2	2	2	0	COI	Bathydemersal
Prionotus stephanophrys ★	Lumptail searobin	2	1	0	2	COI	Demersal
Acanthemblemaria macrospilus ⦿	Barnacle blenny	1	1	1	0	COI	Reef associated
Acanthurus triostegus	Convict surgeonfish	1	1	0	1	COI	Reef associated
Aulopus sp. ★	Flagfin	1	1	0	1	COI	Demersal
Bellator gymnostethus	Naked-belly searobin	1	1	0	1	COI	Demersal
Carangoides orthogrammus	Island trevally	1	1	0	1	COI	Reef associated
Caranx sexfasciatus	Bigeye trevally	1	1	1	0	COI	Reef associated
Carapus dubius	Pacific pearlfish	1	1	1	0	COI	Demersal
Cubiceps pauciradiatus ★	Bigeye cigarfish	1	1	1	0	COI	Bathypelagic
Engraulidae sp. ★	Anchovy	1	1	0	1	16S	Pelagic/neritic
Eucinostomus entomelas ★	Dark-spot mojarra	1	1	0	1	COI	Demersal
Gymnothorax castaneus	Panamic green moray	1	1	0	1	COI	Reef associated
Hemanthias signifer ★	Damsel bass	1	1	1	0	COI	Demersal
Katsuwonus pelamis	Skipjack tuna	1	1	0	1	COI	Pelagic/oceanic
Lampanyctus parvicauda ★	Slimtail lampfish	1	1	0	1	COI	Bathypelagic
Lutjanus colorado	Colorado snapper	1	1	0	1	COI	Reef associated
Microlepidotus inornatus	Wavyline grunt	1	1	0	1	COI	Reef associated
Micropogonias ectenes ★	Slender croaker	1	1	0	1	COI	Demersal
Mugil curema	White mullet	1	1	1	0	COI	Reef associated

Mycteroperca xenarcha	Broomtail grouper	1	1	0	1	COI	Demersal
Myrichthys tigrinus ★	Spotted snake eel	1	1	0	1	COI	Reef associated
Orthopristis reddingi ★	Bronze-striped grunt	1	1	1	0	COI	Demersal
Perissias taeniopterus ★	Striped-fin founder	1	1	0	1	COI	Demersal
Polydactylus approximans ★	Blue bobo	1	1	1	0	COI	Demersal
Polylepion cruentum ★	Bleeding wrasse	1	1	0	1	COI	Reef associated
Prognichthys sealei	Sailor flyingfish	1	1	0	1	COI	Pelagic/oceanic
Pronotogrammus multifasciatus ★	Threadfin bass	1	1	0	1	16S	Reef associated
Scarus rubroviolaceus	Ember parrotfish	1	1	0	1	16S	Reef associated
Stegastes rectifraenum ☉	Cortez damselfish	1	1	1	0	COI	Reef associated
Thunnus albacares	Yellowfin tuna	1	1	1	0	COI	Pelagic/oceanic
Triphoturus mexicanus ★	Mexican lampfish	1	1	1	0	COI	Mesopelagic

Table 2. List of 54 unidentified Operational Taxonomic Units (OTUs) (in order of abundance), total number of individuals collected, number of sampling collections (n = 48) in which individuals were found from eggs and larvae collected in Cabo Pulmo National Park in 2014 showing the closest match found in online databases and percentage sequence identity. Species with an asterisk (*) represent specimens that provided a match of ≥ 97% to a species that is not known to occur in the Gulf of California. They may represent a closely related species with sequences that are not present in GenBank or BOLD or, less likely, an occurrence of this species outside of its known distribution range.

OTUs	Number of specimens analyzed	Number of sampling collections	Gene used	Closest genus and species match	Identity (%)
OTU # 14	76	7	16S	*Ammodytes americanus*	94
OTU # 04	71	11	COI	*Bleekeria mitsukurii*	96
OTU # 54	26	5	COI	*Epinephelus clippertonensis**	99
OTU # 07	14	2	COI	*Syacium maculiferum*	85
OTU # 23	13	1	16S	*Xyrichths novacula*	96
OTU # 26	12	5	COI	*Abudefduf saxatilis*	96
OTU # 03	10	2	COI	*Cephalopholis cruentata*	95
OTU # 09	10	3	COI	*Diaphus watasei*	91
OTU # 53	8	1	16S	*Assurger anzac**	98
OTU # 58	8	4	COI	*Syacium maculiferum**	99
OTU # 11	7	2	COI	*Mycteroperca microlepis*	95
OTU # 08	6	2	COI	*Bothus robinsi*	90
OTU # 06	4	2	COI	*Synodus poeyi*	91
OTU # 15	4	2	COI	*Actinopterygii environmental sample*	90
OTU # 38	4	2	COI	*Caranx latus*	90
OTU # 02	3	2	COI	*Assurger anzac*	92
OTU # 12	3	3	COI	*Symphurus ginsburgi*	84
OTU # 40	3	2	COI	*Trachinotus goodei**	98
OTU # 48	3	1	COI	*Hyporthodus niveatus**	98
OTU # 50	3	3	16S	*Synodus lucioceps*	87
OTU # 01	2	2	COI	*Lampanyctus hubbsi*	94
OTU # 10	2	1	COI	*Uropterygius macularius*	90
OTU # 21	2	1	COI	*Synodus foetens*	88
OTU # 29	2	1	COI	*Opisthonema libertate*	94
OTU # 32	2	2	COI	*Bleekeria mitskurii*	91
OTU # 52	2	1	COI	*Genypterus maculatus**	99
OTU # 55	2	1	COI	*Hyporthodus niphobles**	100

OTU # 56	2	2	COI	*Kyphosus cinerascens**	99
OTU # 05	1	1	COI	*Tetragonorus cuvieri*	94
OTU # 13	1	1	COI	*Gillellus jacksoni*	84
OTU # 16	1	1	COI	*Gillellus jacksoni*	85
OTU # 17	1	1	COI	*Callechelys muraena*	93
OTU # 18	1	1	COI	*Caranx crysos**	99
OTU # 20	1	1	COI	*Synodus foetens*	86
OTU # 22	1	1	COI	*Siganus corallinus*	82
OTU # 24	1	1	COI	*Symphurus atricaudus*	83
OTU # 25	1	1	COI	*Polylepion russelli*	90
OTU # 27	1	1	COI	*Neoconger mucronatus*	93
OTU # 28	1	1	16S	*Prionotus scitulus*	95
OTU # 31	1	1	COI	*Kyphosus vaigiensis*	94
OTU # 33	1	1	COI	*Microdesmus carri*	86
OTU # 34	1	1	COI	*Ophichthus gomesii*	88
OTU # 35	1	1	COI	*Cypselurus poecilopterus*	82
OTU # 36	1	1	COI	*Trachipterus trachipterus*	82
OTU # 37	1	1	COI	*Anchoa hepsetus*	90
OTU # 39	1	1	COI	*Gymnothorax vicinus*	90
OTU # 41	1	1	COI	*Evoxymetopon taeniatus*	93
OTU # 42	1	1	COI	*Synodus poeyi*	91
OTU # 43	1	1	16S	*Kathetostoma laeve**	97
OTU # 44	1	1	COI	*Macruronus magellanicus*	81
OTU # 46	1	1	16S	*Ichthyapus ophioneus*	93
OTU # 47	1	1	COI	*Paralichthys lethostigma*	86
OTU # 51	1	1	16S	*Synodus lucioceps*	87
OTU # 57	1	1	COI	*Paraconger ophichthys**	99

Table 3. Seasonal number of specimens of fish egg and larvae species and Operational Taxonomic Units (OTUs) (pooled) observed in Cabo Pulmo National Park weekly time series (Jan-Dec 2014).

⊙ indicates species with demersal eggs attached to substrate or parent’s body; the rest of the species have planktonic eggs

Species	Winter	Spring	Summer	Autumn	# Seasons present
Acanthemblemaria macrospilus ⊙	0	1	0	0	1
Acanthurus triostegus	0	0	1	0	1
Acanthurus xanthopterus	0	0	11	0	1
Alphestes multiguttatus	0	0	2	0	1
Ammodytoides gilli	11	10	4	98	4
Anisotremus taeniatus	0	0	2	0	1
Aulopus sp.	1	0	0	0	1
Auxis rochei	48	2	20	1	4
Axoclinus storeyae ⊙	0	0	0	2	1
Balistes polylepis ⊙	0	0	3	0	1
Bellator gymnostethus	0	0	0	1	1
Benthosema panamense	2	1	1	0	3
Bodianus diplotaenia	0	3	0	0	1
Bothus leopardinus	0	0	10	4	2
Calamus brachysomus	3	5	0	0	2
Carangoides orthogrammus	0	0	0	1	1
Carangoides otrynter	0	3	3	0	2
Caranx caninus	0	0	1	0	1
Caranx sexfasciatus	1	54	5	1	4
Carapus dubius	0	0	1	0	1
Cephalopholis panamensis	0	0	12	0	1
Chanos chanos	3	92	1	0	3
Cheilopogon furcatus	0	2	0	0	1
Cirrhitichthys oxycephalus	0	2	1	1	3
Coryphaena equiselis	4	2	0	0	2
Coryphaena hippurus	5	0	0	0	1
Cubiceps pauciradiatus	0	1	0	0	1
Cyclopsetta panamensis	0	11	3	1	3
Decapterus macarellus	2	3	31	4	4
Decapterus muroadsi	0	6	1	0	2
Diodon holocanthus	0	0	5	3	2
Diogenichthys laternatus	1	1	0	0	2
Engraulidae sp.	1	0	0	0	1
Etrumeus acuminatus	71	38	0	0	2

Eucinostomus currani	0	1	47	0	2
Eucinostomus entomelas	0	0	1	0	1
Euthynnus lineatus	0	3	208	2	3
Fistularia commersonii	21	4	2	6	4
Fistularia corneta	2	0	0	0	1
Gerres simillimus	0	1	1	0	2
Gymnothorax castaneus	0	0	1	0	1
Haemulon flaviguttatum	0	68	24	0	2
Haemulon maculicauda	0	6	5	0	2
Haemulon sexfasciatum	0	3	64	0	2
Halichoeres dispilus	146	35	7	102	4
Halichoeres melanotis	2	0	0	7	2
Hemanthias signifer	1	0	0	0	1
Heteropriacanthus cruentatus	3	4	0	0	2
Hoplopagrus guentherii	0	0	10	0	1
Hygophum atratum	2	0	0	0	1
Katsuwonus pelamis	0	0	1	0	1
Labrisomus xanti ☉	1	1	0	0	2
Lampanyctus parvicauda	0	1	0	0	1
Liopropoma fasciatum	0	0	2	0	1
Lutjanus argentiventris	0	5	12	0	2
Lutjanus colorado	0	1	0	0	1
Lutjanus guttatus	0	0	20	0	1
Lutjanus novemfasciatus	0	0	12	0	1
Lutjanus peru	0	8	1	0	2
Microlepidotus inornatus	0	1	0	0	1
Micropogonias megalops	0	1	0	0	1
Mugil curema	0	0	1	0	1
Mulloidichthys dentatus	0	2	5	0	2
Mycteroperca xenarcha	0	0	1	0	1
Myrichthys tigrinus	0	0	1	0	1
Myripristis leiognathus	7	0	3	0	2
Nematistius pectoralis	0	1	3	0	2
Orthopristis reddingi	0	1	0	0	1
OTU # 01	0	1	1	0	2
OTU # 02	0	1	0	2	2
OTU # 03	0	0	10	0	1
OTU # 04	19	5	0	47	3
OTU # 05	0	1	0	0	1
OTU # 06	0	4	0	0	1
OTU # 07	0	0	14	0	1

OTU # 08	0	0	6	0	1
OTU # 09	0	5	5	0	2
OTU # 10	0	0	2	0	1
OTU # 11	0	7	0	0	1
OTU # 12	0	0	3	0	1
OTU # 13	0	1	0	0	1
OTU # 14	74	0	0	2	2
OTU # 15	0	3	0	1	2
OTU # 16	0	1	0	0	1
OTU # 17	0	0	0	1	1
OTU # 18	0	0	1	0	1
OTU # 20	0	1	0	0	1
OTU # 21	0	2	0	0	1
OTU # 22	1	0	0	0	1
OTU # 23	13	0	0	0	1
OTU # 24	0	0	1	0	1
OTU # 25	0	0	1	0	1
OTU # 26	1	1	10	0	3
OTU # 27	0	0	1	0	1
OTU # 28	1	0	0	0	1
OTU # 29	0	2	0	0	1
OTU # 31	0	0	1	0	1
OTU # 32	0	0	0	2	1
OTU # 33	0	0	1	0	1
OTU # 34	0	1	0	0	1
OTU # 35	0	1	0	0	1
OTU # 36	1	0	0	0	1
OTU # 37	0	0	1	0	1
OTU # 38	0	4	0	0	1
OTU # 39	0	0	1	0	1
OTU # 40	0	0	3	0	1
OTU # 41	0	0	0	1	1
OTU # 42	0	0	1	0	1
OTU # 43	1	0	0	0	1
OTU # 44	0	1	0	0	1
OTU # 46	1	0	0	0	1
OTU # 47	0	1	0	0	1
OTU # 48	0	0	3	0	1
OTU # 50	3	0	0	0	1
OTU # 51	1	0	0	0	1
OTU # 52	0	0	2	0	1

OTU #53	8	0	0	0	1
OTU #54	0	1	25	0	2
OTU #55	0	0	2	0	1
OTU #56	0	0	2	0	1
OTU #57	0	0	1	0	1
OTU #58	0	4	2	2	3
Oxyporhamphus micropterus	3	2	0	0	2
Paralabrax maculatofasciatus	0	10	0	0	1
Paranthias colonus	0	6	7	1	3
Perissias taeniopterus	0	0	1	0	1
Plagiotremus azaleus ☉	0	2	2	0	2
Polydactylus approximans	0	0	1	0	1
Polylepion cruentum	0	0	1	0	1
Pontinus furcirhinus	2	0	0	0	1
Prionotus stephanophrys	0	2	0	0	1
Prionurus punctatus	0	0	7	0	1
Pristigenys serrula	0	0	13	0	1
Prognichthys sealei	0	0	0	1	1
Pronotogrammus multifasciatus	1	0	0	0	1
Regalecus glesne	3	0	0	0	1
Rypticus bicolor	0	0	12	0	1
Sarda orientalis	0	1	29	0	2
Scarus compressus	23	0	0	0	1
Scarus ghobban	24	0	2	0	2
Scarus rubroviolaceus	0	0	1	0	1
Scarus sp. (morphological ID)	1737	0	19	43	3
Selar crumenophthalmus	0	0	7	0	1
Selene peruviana	0	4	0	0	1
Seriola rivoliana	0	0	10	0	1
Sphyraena ensis	0	1	13	0	2
Stegastes rectifraenum ☉	0	0	1	0	1
Syacium ovale	0	0	7	0	1
Synodus evermanni	2	9	0	0	2
Synodus lacertinus	24	32	23	39	4
Synodus scituliceps	0	9	0	0	1
Thalassoma lucasanum	31	27	0	0	2
Thunnus albacares	0	0	1	0	1
Trachinotus rhodopus	0	0	7	0	1
Triphoturus mexicanus	0	1	0	0	1
Umbrina xanti	0	10	6	0	2
Vinciguerria lucetia	24	32	34	0	3

Xyrichtys mundiceps	1	0	0	241	2
TOTAL Identified	**2337**	**580**	**854**	**617**	**4388**
Total Species	**46**	**73**	**90**	**28**	**104 species + 54 OTUs**
Total Collected	**4315**	**8824**	**5420**	**2584**	**21143**

Table S1. List of 157 Operational Taxonomic Units (OTU) found from ichthyoplankton collections taken in 2014 from Cabo Pulmo National Park. The results from NCBI's GenBank and the Barcode of Life Database are shown as well as the number of identical sites for each sequence.

OTU	**NCBI Result**	**Accession #**	**% Identical Sites**	**Barcode of Life result**	**Sample ID**	**% Identical Si**
Acanthemblemaria macrospilus	*Acanthemblemaria macrospilus*	FJ884556	100.0	NO MATCH		
Acanthurus triostegus	*Acanthurus triostegus*	HM034207	99.8	*Acanthurus triostegus*		100.0
Acanthurus xanthopterus	*Acanthurus xanthopterus*	KY570710	99.8	*Acanthurus xanthopterus*		100.0
Alphestes immaculatus	*Alphestes afer*	JQ840759	90.6	*Alphestes immaculatus*	gal90410a280	99.8
Ammodytoides gilli	*Bleekeria mitskurii*	KU944777	95.5	*Ammodytoides gilli*		100.0
Anisotremus taeniatus	*Anisotremus taeniatus*	EU697527	99.5	NO MATCH		
Aulopus sp.	*Aulopus sp.*	EU366559	97.0	NO MATCH		
Auxis rochei	*Auxis rochei*	KT074084	99.1	*Auxis rochei*	ADC08-L III A11.4	99.8
Auxis thazard	*Auxis thazard*	KP259551	99.8	NO MATCH		
Axoclinus storeyae	*Axoclinus storeyae*	KP636887	97.9	*Axoclinus storeyae*	mwb11e10	99.1
Balistes polylepis	*Balistes polylepis*	KF929641	100.0	NO MATCH		
Bellator gymnostethus	*Bellator gymnostethus*	KX810993	99.2	NO MATCH		
Benthosema panamense	*Benthosema panamense*	KJ555326	98.8	*Benthosema panamense*		99.7
Bodianus diplotaenia	*Bodianus diplotaenia*	KC684983	99.8	NO MATCH		
Bothus leopardinus	*Bothus robinsi*	KF929672	89.4	*Bothus leopardinus*	gv85310bo230	99.7
Calamus brachysomus	*Calamus brachysomus*	KJ012304	100.0	NO MATCH		
Carangoides orthogrammus	*Carangoides orthogrammus*	KU943780	99.0	NO MATCH		
Carangoides otrynter	*Caranx latus*	*JQ841100*	*90.3*	*Carangoides otrynter*	Co-29-IMARPE	100.0
Caranx caninus	*Caranx caninus*	EU752066	99.8	NO MATCH		
Caranx sexfasciatus	*Caranx sexfasciatus*	KU199209	100.0	NO MATCH		
Carapus dubius	*Halichoeres pictus*	JQ839789	82.2	*Carapus dubius*		100.0
Cephalopholis panamensis	*Cephalopholis cruentata*	GU225173	94.8	*Cephalopholis panamensis*		99.8
Chanos chanos	*Chanos chanos*	LT669927	100.0	NO MATCH		
Cheilopogon dorsomacula	*Cheilopogon furcatus*	KF489537	99.0	*Cheilopogon dorsomacula*		99.7
Cirrhitichthys oxycephalus	*Cirrhitichthys oxycephalus*	KR023554	98.4	*Cirrhitichthys oxycephalus*		99.4
Coryphaena equiselis	*Coryphaena equiselis*	KP266762	99.2	NO MATCH		
Coryphaena hippurus	*Coryphaena hippurus*	KY176439	98.5	NO MATCH		
Cubiceps pauciradiatus	*Cubiceps pauciradiatus*	KJ968014	99.0	*Cubiceps pauciradiatus*	DSLAR394-08.COI-5P	99.7
Cyclopsetta panamensis	*Cyclopsetta panamensis*	JX887475	100.0	NO MATCH		
Decapterus macarellus	*Decapterus macarellus*	KM986880	97.2	*Decapterus macarellus*	NOOR025-17.COI-5P	99.7

Decapterus muroadsi	*Decapterus macrosoma*	KC970467	99.6	*Decapterus muroadsi*	HQ010055	100.0
Diodon holocanthus	*Diodon holocanthus*	GU440304	100.0	NO MATCH		
Diogenichthys laternatus	*Diogenichthys laternatus*	HQ127668	99.8	*Diogenichthys laternatus*	gv12dl260	100.0
Engraulidae sp.	*Engraulidae sp.*	KC208625	98.8	NO MATCH		
Etrumeus acuminatus	*Etrumeus acuminatus*	KM116435	100.0	*Etrumeus sadina*	MFC231	99.1
Eucinostomus currani	*Eucinostomus currani*	KT067787	99.5	NO MATCH		
Eucinostomus entomelas	*Eucinostomus entomelas*	KJ622154	100.0	*Eucinostomus entomelas*	KJ622154	100.0
Euthynnus lineatus	*Euthynnus lineatus*	GU440322	99.8	NO MATCH		
Fistularia commersonii	*Fistularia commersonii*	KR861527	99.6	*Fistularia commersonii*	KP053209	100.0
Fistularia corneta	*Fistularia corneta*	HQ010105	99.6	NO MATCH		
Gerres simillimus	*Gerres simillimus*	KT005473	99.0	NO MATCH		
Gymnothorax castaneus	*Gymnothorax vicinus*	GU225293	89.8	*Gymnothorax castaneus*		99.7
Haemulon flaviguttatum	*Haemulon flaviguttatum*	GQ891092	98.6	*Haemulon flaviguttatum*	JQ741199	99.8
Haemulon maculicauda	*Haemulon maculicauda*	EU697537	99.4	NO MATCH		
Haemulon sexfasciatum	*Haemulon sexfasciatum*	JQ741255	100.0	NO MATCH		
Halichoeres dispilus	*Halichoeres dispilus*	JQ839467	99.0	*Halichoeres dispilus*		99.3
Halichoeres melanotis	*Halichoeres melanotis*	JQ839488	100.0	*Halichoeres melanotis*	pp96701hm160	100.0
Halichoeres melanotis (16S)	*Halichoeres melanotis*	KY815408	99.0	NO MATCH		
Hemanthias signifer	*Hemanthias signifer*	GU440335	99.6	*Hemanthias signifer*	MFC189	100.0
Heteropriacanthus cruentatus	*Heteropriacanthus cruentatus*	KT248793	100.0	*Heteropriacanthus cruentatus*		100.0
Hoplopagrus guentherii	*Hoplopagrus guentherii*	KJ557446	99.3	*Hoplopagrus guentherii*	KJ557446	99.7
Hygophum atratum	*Hygophum atratum*	GU440346	99.3	*Hygophum atratum*	MFC346	100.0
Katsuwonus pelamis	*Katsuwonus pelamis*	AB101290	99.5	NO MATCH		
Labrisomus xanti	*Labrisomus xanti*	HQ168599	99.6	NO MATCH		
Lampanyctus parvicauda	*Lampanyctus hubbsi*	KJ555411	93.6	*Lampanyctus parvicauda*		98.5
Liopropoma fasciatum	*Liopropoma fasciatum*	JX093903	99.4	NO MATCH		
Lutjanus argentiventris	*Lutjanus argentiventris*	KJ557432	100.0	*Lutjanus argentiventris*	MFC095	100.0
Lutjanus colorado	*Lutjanus colorado*	KJ557438	100.0	NO MATCH		
Lutjanus guttatus	*Lutjanus guttatus*	KT724723	100.0	NO MATCH		
Lutjanus novemfasciatus	*Lutjanus novemfasciatus*	KJ557444	100.0	NO MATCH		
Lutjanus peru	*Lutjanus peru*	KX119467	99.0	*Lutjanus peru*	HQ162412	99.8
Microlepidotus inornatus	*Microlepidotus inornatus*	JQ741282	99.8	NO MATCH		
Micropogonias ectenes	*Micropogonias ectenes*	KX401604	97.0	NO MATCH		
Mugil curema	*Mugil curema*	GU440409	100.0	NO MATCH		
Mulloidichthys dentatus	*Mulloidichthys dentatus*	JX390743	99.4	*Mulloidichthys dentatus*	gal490md850	99.7
Mycteroperca xenarcha	*Mycteroperca microlepis*	KF836490	93.1	*Mycteroperca xenarcha*		100.0
Myrichthys tigrinus	*Myrichthys ocellatus*	MF041112	92.9	*Myrichthys tigrinus*	gv125zop	100.0
Myripristis leiognathus	*Myripristis leiognathus*	JX390743	99.4	*Myripristis leiognathus*		100.0
Nematistius pectoralis	*Nematistius pectoralis*	DQ027998	99.8	NO MATCH		

Orthopristis reddingi	*Orthopristis reddingi*	JQ741300	99.8	NO MATCH		
OTU # 01	*Lampanyctus hubbsi*	KJ555411	94.5	NO MATCH		
OTU # 02	*Assurger anzac*	AP012508	91.7	NO MATCH		
OTU # 03	*Cephalopholis cruentata*	JQ841494	93.4	NO MATCH		
OTU # 04	*Bleekeria mitsukurii*	KU944777	96.0	NO MATCH		
OTU # 05	*Tetragonorus cuvieri*	KF489780	94.2	NO MATCH		
OTU # 06	*Synodus poeyi*	JX519399	91.4	NO MATCH		
OTU # 07	*Syacium maculiferum*	JX887478	85.6	NO MATCH		
OTU # 08	*Bothus robinsi*	KF929672	90.1	NO MATCH		
OTU # 09	*Diaphus watasei*	KP267585	91.3	NO MATCH		
OTU # 10	*Uropterygius macularius*	MF041358	0.9	NO MATCH		
OTU # 11	*Mycteroperca microlepis*	JQ842598	95.1	NO MATCH		
OTU # 12	*Symphurus ginsburgi*	JX124904	83.7	NO MATCH		
OTU # 13	*Gillellus jacksoni*	GU224859	84.3	NO MATCH		
OTU # 14	*Ammodytes americanus*	KT723027	93.5	NO MATCH		
OTU # 15	*Actinopterygii environmental sample*	KP111790	89.5	NO MATCH		
OTU # 16	*Gillellus jacksoni*	GU224859	86.0	NO MATCH		
OTU # 17	*Callechelys muraena*	MF041245	92.5	NO MATCH		
OTU # 18	*Caranx crysos*	MF041098	99.4	NO MATCH		
OTU # 20	*Actinopterygii environmental sample*	KY936605	88.5	NO MATCH		
OTU # 21	*Synodus foetens*	KF930488	88.1	NO MATCH		
OTU # 22	*Myripristis leiognathus*	JX390743	91.8	NO MATCH		
OTU # 23	*Xyrichthys novacula*	KY815468	96.9	NO MATCH		
OTU # 24	*Symphurus atricaudus*	GU440541	82.7	NO MATCH		
OTU # 25	*Polylepion russelli*	JF435093	90.2	NO MATCH		
OTU # 26	*Abudefduf saxatilis*	JQ839920	95.8	NO MATCH		
OTU # 27	*Neoconger mucronatus*	GU224984	92.9	NO MATCH		
OTU # 28	*Prionotus scitulus*	EU239810	94.6	NO MATCH		
OTU # 29	*Opisthonema libertate*	HQ010071	94.4	NO MATCH		
OTU # 31	*Kyphosus vaigiensis*	KP116935	93.8	NO MATCH		
OTU # 32	*Bleekeria mitskurii*	KU944777	91.1	NO MATCH		
OTU # 33	*Microdesmus carri*	JQ841721	86.0	NO MATCH		
OTU # 34	*Ophichthus gomesii*	KF461209	88.1	NO MATCH		
OTU # 35	*Cypselurus poecilopterus*	KU943243	82.0	NO MATCH		
OTU # 36	*Trachipterus trachipterus*	AP002925	81.8	NO MATCH		
OTU # 37	*Anchoa hepsetus*	JQ842003	90.0	NO MATCH		
OTU # 38	*Caranx latus*	JQ841100	90.5	NO MATCH		
OTU # 39	*Gymnothorax vicinus*	GU225293	89.5	NO MATCH		

OTU # 40	*Trachinotus goodei*	JQ841419	97.8	NO MATCH		
OTU # 41	*Evoxymetopon taeniatus*	KU945019	92.6	NO MATCH		
OTU # 42	*Synodus poeyi*	JX519399	90.6	NO MATCH		
OTU # 43	*Kathetostoma laeve*	KR153507	97.4	NO MATCH		
OTU # 44	*Macruronus magellanicus*	EU074458	81.1	NO MATCH		
OTU # 46	*Ichthyapus ophioneus*	AF455772	92.5	NO MATCH		
OTU # 47	*Paralichthys lethostigma*	KT896534	86.3	NO MATCH		
OTU # 48	*Hyporthodus niveatus*	KU739517	98.6	NO MATCH		
OTU # 50	*Synodus lucioceps*	KJ010667	87.3	NO MATCH		
OTU # 51	*Synodus lucioceps*	KJ010667	87.7	NO MATCH		
OTU # 52	*Brotula barbata*	KF461141	88.9	*Genypterus maculatus*		99.5
OTU # 53	*Assurger anzac*	AP012508	98.1	NO MATCH		
OTU # 54	*Epinephelus clippertonensis*	JX093914	99.5	NO MATCH		
OTU # 55	*Hyporthodus niveatus*	KF836483	96.8	*Hyporthodus niphobles*		100.0
OTU # 56	*Kyphosus cinerascens*	JQ350079	99.7	NO MATCH		
OTU # 57	*Paraconger caudilimbatus*	MF041623	91.6	*Paraconger ophichthys*	gv123po1888	99.5
OTU # 58	*Syacium maculiferum*	JX887478	99.6	*Syacium maculiferum*	gv85310sm60	99.6
Oxyporhamphus micropterus (16S)	*Oxyporhamphus micropterus*	AY693459	99.8	NO MATCH		
Oxyporhamphus micropterus	*Oxyporhamphus micropterus*	KX769054	99.6	*Oxyporhamphus micropterus*		99.8
Paralabrax maculatofasciatus	*Paralabrax maculatofasciatus*	GU440446	99.8	NO MATCH		
Paranthias colonus	*Paranthias colonus*	GU440449	99.0	NO MATCH		
Perissias taeniopterus	*Scleronema angustirostre*	KY857962	81.9	*Perissias taeniopterus*	gv85310es100	100.0
Plagiotremus azaleus	*Plagiotremus azaleus*	HQ168581	99.6	NO MATCH		
Polydactylus approximans	*Polydactylus approximans*	GU440471	99.6	NO MATCH		
Polylepion cruentum	*Polylepion russelli*	JQ432026	89.8	*Polylepion cruentum*		99.8
Pontinus furcirhinus	*Pontinus kuhlii*	JQ774695	95.9	*Pontinus furcirhinus*		99.7
Prionotus stephanophrys	*Prionotus stephanophrys*	GU440478	99.3	NO MATCH		
Prionurus punctatus	*Prionurus punctatus*	KP280490	100.0	*Prionurus punctatus*	KP280495	100.0
Pristigenys serrula	*Pristigenys serrula*	JQ741339	99.6	NO MATCH		
Prognichthys sealei	*Prognichthys sealei*	KX769050	97.6	NO MATCH		
Pronotogrammus multifasciatus	*Pronotogrammus multifasciatus*	FJ548774	100.0	NO MATCH		
Regalecus glesne	*Regalecus glesne*	DQ532951	99.8	NO MATCH		
Rypticus bicolor	*Rypticus saponaceus*	JN828108	97.4	*Rypticus bicolor*	gal98609r181	100.0
Scarus compressus	*Scarus compressus*	JX026478	100.0	NO MATCH		
Scarus ghobban	*Scarus ghobban*	JX026489	100.0	NO MATCH		
Scarus rubroviolaceus	*Scarus rubroviolaceus*	JX026509	99.5	NO MATCH		
Selar crumenophthalmus	*Selar crumenophthalmus*	KJ502071	99.8	NO MATCH		
Selene peruviana	*Selene peruviana*	EU752202	99.4	NO MATCH		

Seriola rivoliana	*Seriola rivoliana*	KP733847	98.8	NO MATCH		
Sphyraena ensis	*Sphyraena ensis*	GU440526	100.0	NO MATCH		
Stegastes rectifraenum	*Stegastes rectifraenum*	JQ729312	99.8	NO MATCH		
Syacium ovale	*Scarus psittacus*	KU944718	82.9	*Syacium ovale*	sio10018so	99.5
Synodus evermanni	*Synodus poeyi*	JX519399	91.4	*Synodus evermanni*		100.0
Synodus lacertinus	*Synodus lacertinus*	GU440545	99.4	NO MATCH		
Synodus scituliceps	*Synodus foetens*	KF930488	87.3	*Synodus scituliceps*		100.0
Thalassoma lucasanum	*Thalassoma lucasanum*	KY815460	100.0	NO MATCH		
Thalassoma lucasanum	*Thalassoma lucasanum*	JQ839621	100.0	NO MATCH		
Thunnus albacares	*Thunnus albacares*	LN908910	100.0	NO MATCH		
Trachinotus rhodopus	*Trachinodus goodei*	JQ843094	97.9	*Trachinodus rhodopus*		99.8
Triphoturus mexicanus	*Triphoturus mexicanus*	KJ555475	99.8	NO MATCH		
Umbrina xanti	*Umbrina xanti*	KP722787	98.6	NO MATCH		
Vinciguerria lucetia	*Vinciguerria lucetia*	HQ010067	100.0	*Vinciguerria lucetia*	HQ010067	100.0
Xyrichtys mundiceps	*Xyrichtys mundiceps*	JQ839662	99.8	NO MATCH		

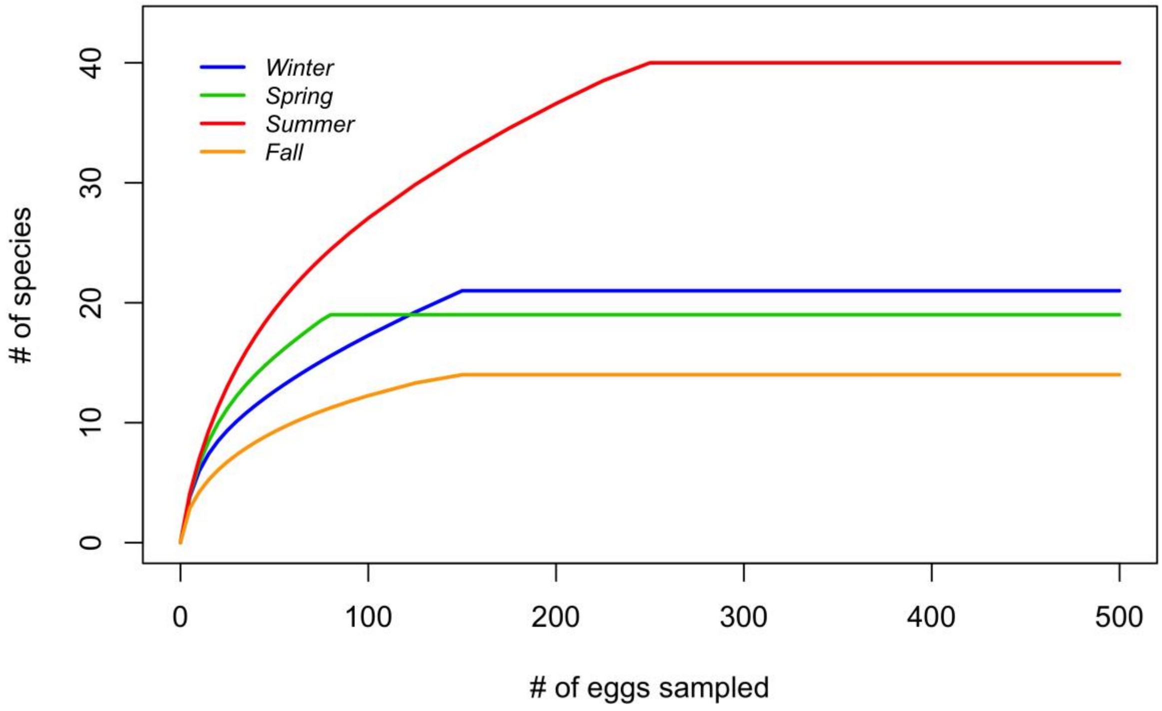

Fig. S1. Rarefaction curves of number of fish egg and larvae Operational Taxonomic Units (OTUs) identified as a function of specimens analyzed per season.

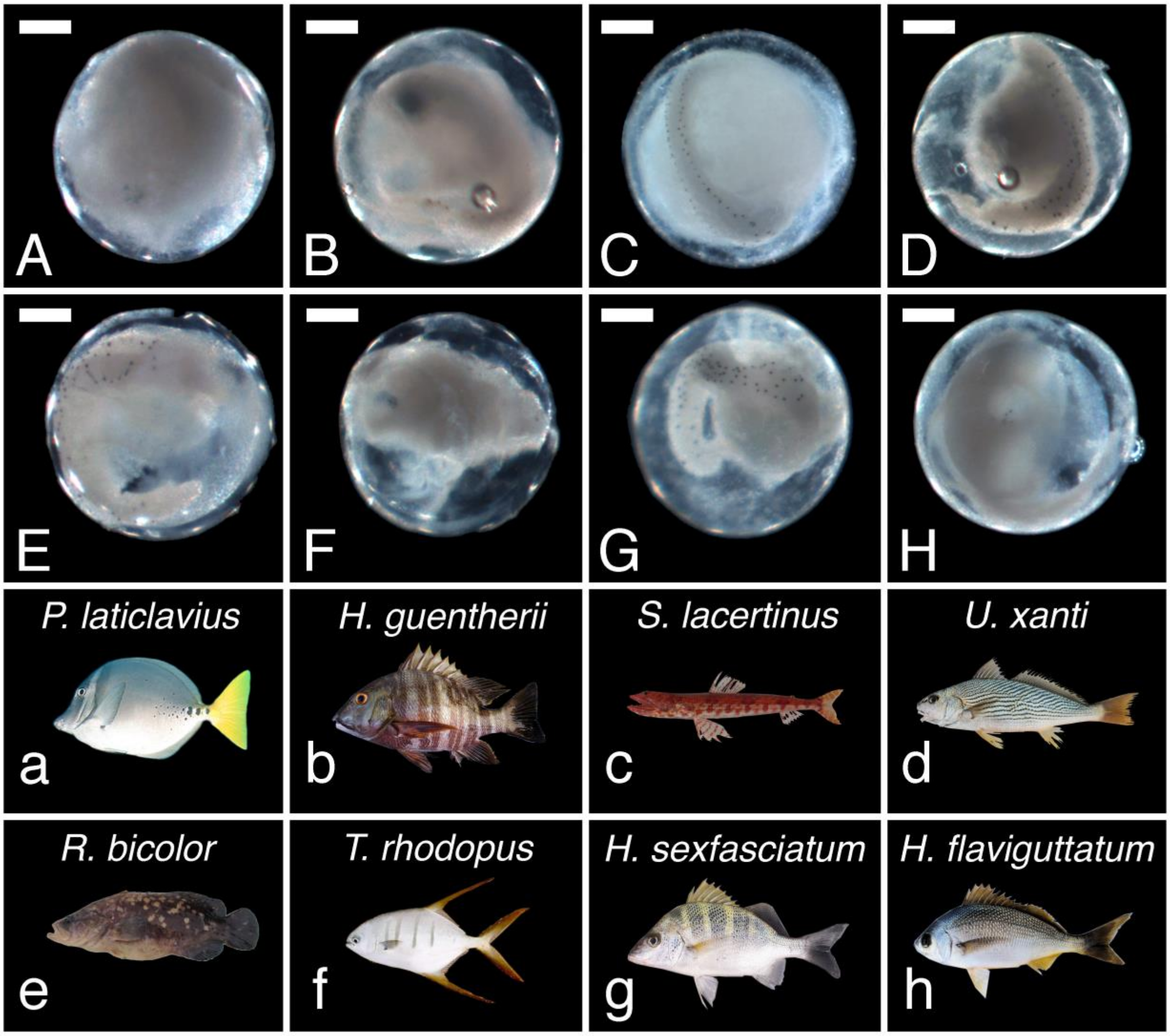

Fig. S2. Example of morphological vs molecular fish egg identification: 8 eggs morphologically identified as *Lutjanus peru* (Pacific red snapper) revealed to be eggs from eight separate species. Scale bar represents 200 microns. The first two rows (capitalized letters) show the eggs that were identified molecularly as belonging to the species in the second two rows (non-capitalized letters).

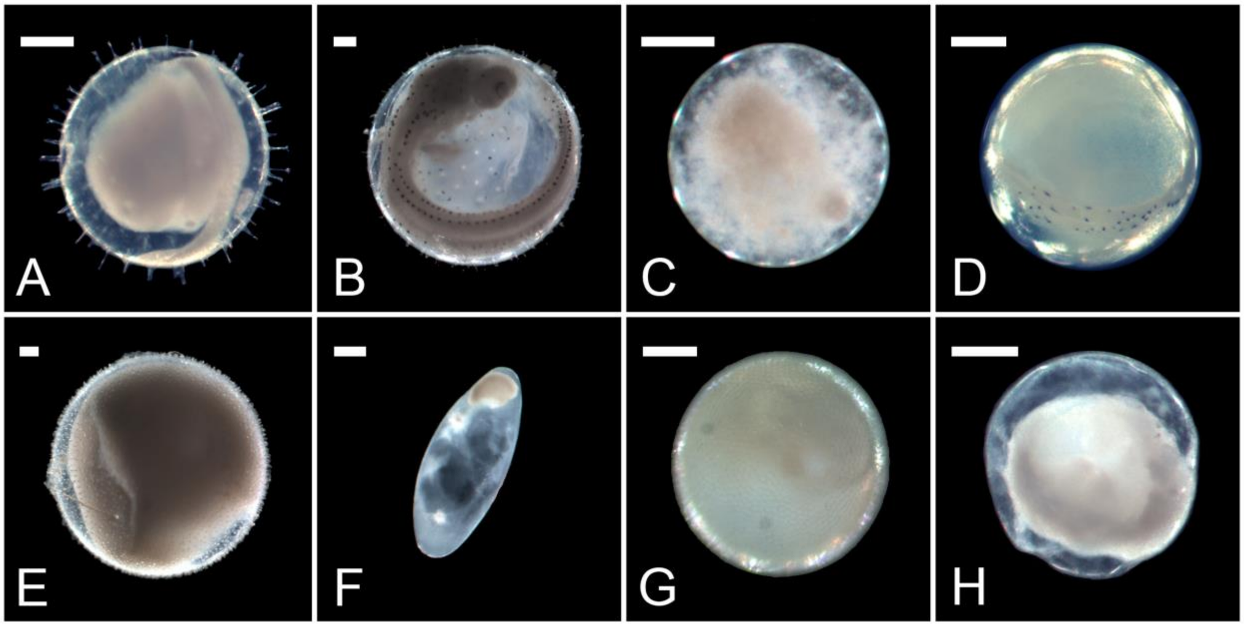

Fig. S3. Composite photograph of multiple species of fish eggs found in the ichthyoplankton monitoring survey of Cabo Pulmo National Park in 2014. Scale bar represents 200 microns. Eggs identified as: A. *Ammodytoides gilli* B. *Oxyporhamphus micropterus* C. *Prionurus laticlavius* D. *Pronotogrammus multifasciatus* E. *Regalecus glesne* F. *Scarus* spp. G. *Synodus lucioceps* H. *Vinciguerria lucetia*.