Vol. 592: 159–179, 2018
https://doi.org/10.3354/meps12446

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published March 29

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

Ana Luisa M. Ahern[1,*], Jaime Gómez-Gutiérrez[2], Octavio Aburto-Oropeza[1], Ricardo J. Saldierna-Martínez[2], Andrew F. Johnson[1], Alice E. Harada[1], Arturo R. Sánchez-Uvera[2], Brad Erisman[3], David I. Castro Arvizú[4], Ronald S. Burton[1]

[1]Marine Biology Research Division, Scripps Institution of Oceanography, University of California San Diego, La Jolla, California 92093, USA

[2]Departamento de Plancton y Ecología Marina, Centro Interdisciplinario de Ciencias Marinas, Instituto Politécnico Nacional, CP 23096, La Paz, Baja California Sur, Mexico

[3]Marine Science Institute, The University of Texas at Austin, Port Aransas, Texas 78373, USA

[4]Cabo Pulmo National Park, CP 23574, Los Cabos, Baja California Sur, Mexico

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). Ichthyoplankton is difficult to identify morphologically; therefore the DNA barcoding method was employed to identify 4388 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 7 OTUs were recorded throughout the year (4.5%), 10 OTUs during 3 seasons (6.5%), 36 OTUs in 2 seasons (23%) and 104 OTUs were recorded in 1 season (66%). The study found eggs and/or larvae of 47 species that were not previously reported in Cabo Pulmo National Park. The results will 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. 1). This national park is a unique example of a successfully managed marine protected area (Aburto-Oropeza et al. 2011, 2015). Established as a Mexican

*Corresponding author: ana.ahern@gmail.com

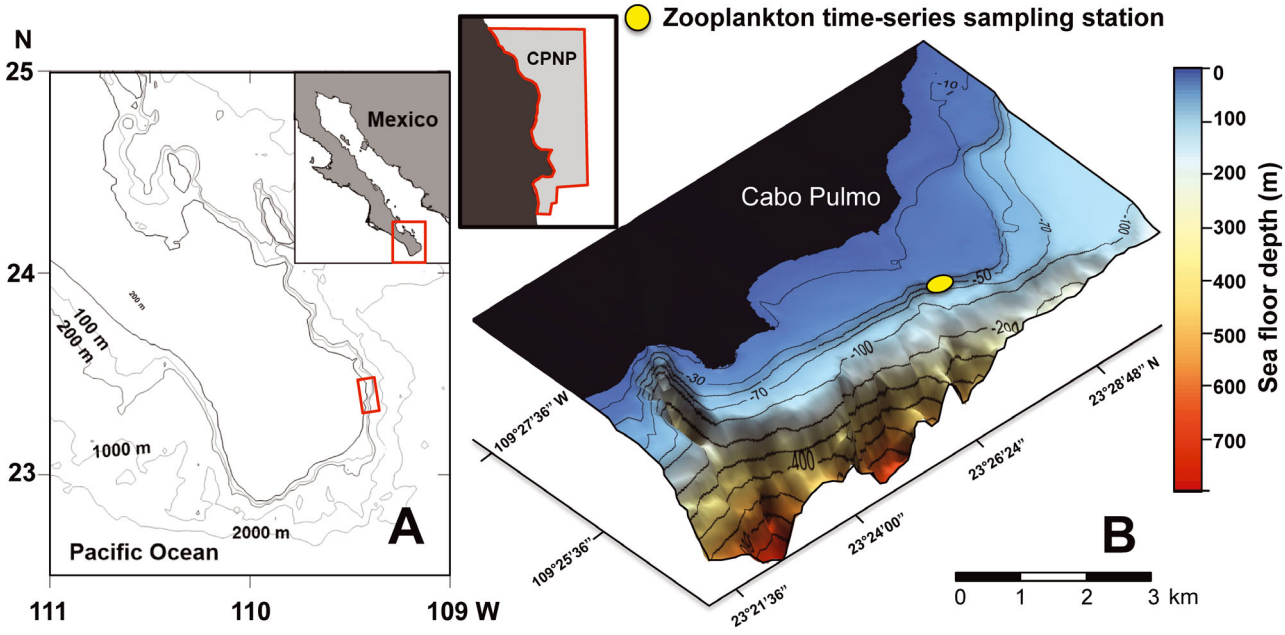

Fig. 1. (A) Cabo Pulmo National Park (CPNP) (red outline) in the southeast region of Baja California peninsula (inset) and (B) bathymetry of the national park measured with 120 and 200 kHz echosounder showing location of the weekly zooplankton time series (Jan–Dec 2014)

national marine park in 1995, Cabo Pulmo National Park is recognized as a UNESCO World Heritage Site. Since 1995, the community of Cabo Pulmo has voluntarily expanded the no-take zone from the initial 35% to 100% of the 27 square mile (71 km^2) park (Aburto-Oropeza et al. 2011) (our Fig. 1B). Although the community of Cabo Pulmo already supported a small tourism industry, this was bolstered as the biomass, abundance and diversity of charismatic and commercially important fish species, as well as marine mammals, increased. A 10 yr 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 (Robinson et al. 2013, 2016, Verutes et al. 2014). As the area has garnered more public and academic attention, large international developers have proposed tourism 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 that the oceans will experience a significant increase in sea surface temperature (SST) in the next 100 yr (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 SST and decrease in wind speed, resulting in lower mean sea surface chlorophyll *a* (chl *a*) concentrations than previous years (Robinson et al. 2013, 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 of species richness, 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 et al. 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. 2002, 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 & Moser 1980, Hyde et al. 2005). Even then, morphological experts can experience high uncertainty in fish egg identification (Moser et al. 1974, 1993, Ahlstrom & Moser 1980), which can prove costly when these data are used to determine population abundance and make management decisions for commercially targeted species. Where traditional morphological analysis may have difficulty distinguishing species with similar morphological characteristics, molecular genetic analysis from specimens identified in their adult phase 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 (National Human Genome Research Institute [NHGRI] Genome Sequencing Program, 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 is 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 ichthyoplankton 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 species that spawn adhesive 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 from Cabo Pulmo National Park (Alvarez-Filip et al. 2006, Aburto-Oropeza et al. 2011, 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 fish broadcast spawning activity over the course of a single year.

MATERIALS AND METHODS

SST and chl *a* concentration

Monthly mean night SST (°C) and concentration of chl *a* (mg m^{-3}) data from 1999 to 2015 for the Cabo Pulmo region were obtained from NASA (http://podaac.jpl.nasa.gov and https://oceandata.sci.gsfc.nasa.gov/SeaWiFS) to infer seasonal environmental

variability of SST and chl *a* concentration associated with fish spawning activity. The monthly datasets 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 SeaWiFS sensor. Monthly means and anomalies were calculated with the same analytical method as the satellite SST and chl *a* concentration time series 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 10 min (Smith & Richardson 1977) (our Fig. 1). Zooplankton samples were collected during daytime hours (08:30–18:14 h), 79% of which were collected before noon. The zooplankton net was equipped with a calibrated General Oceanics digital flowmeter (model 2030R6) 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 (300 µm) to remove seawater and preserved in 96% ethanol with an entire change of ethanol at the laboratory when biomass was measured using the ethanol displacement volume method. All 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 were counted. Fish eggs and larvae were identified to the most precise taxonomic level possible and were recorded following standard fish egg and larva identification keys (Chaudhuri et al. 1977, Ahlstrom & Moser 1980, Nishikawa & Rimmer 1987, Moser 1996, Watson 1998, Saldierna-Martínez et al. 2005, Jiménez-Rosenberg et al. 2006, Richards 2006a,b, Kawakami et al. 2010, González-Navarro et al. 2013). Digital photographs were taken of each specimen for a taxonomic record.

Ichthyoplankton specimens were stored at 4°C 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 µl of a mixture of two-thirds Qiagen AE Buffer and one-third H_2O 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 −20°C prior to 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 *c* oxidase subunit 1 (COI) using COI VF1 forward primer (5′-TTC TCA ACC AAC CAC AAA GAC ATT GG-3′) and COI VR1 reverse primer (5′-TAG ACT TCT GGG TGG CCA AAG AAT CA-3′) (deWaard et al. 2007). If COI did not amplify, a 570 bp fragment of the mitochondrial 16S rRNA gene was amplified using forward primer 16Sar (5′-CGC CTG TTA TCA AAA ACA T-3′) and reverse primer 16Sbr (5′-CCG GTC TGA ACT CAG ATC ACG T-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 95°C for 2 min, 35 cycles of 95°C for 30 s, 50°C for 45 s, and 72°C for 1 min, followed by 72°C for 5 min. The PCR products were run on 1.5% agarose gels, stained with GelRed (Biotium) 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) (Harada et al. 2015).

A DNA barcoding approach was used to identify the eggs and larvae. Once sequences were obtained, the software Geneious (www.geneious.com) and Sequencher (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 (www.boldsystems.org). 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 (www.ncbi.nlm.nih.gov/genbank/) utilizing default parameters. For both COI

and 16S sequences, we used a threshold of ≥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 (Reyes-Bonilla & Calderon Aguilera 1999, Villarreal-Cavazos et al. 1999, Aburto-Oropeza & Balart 2001, Alvarez-Filip et al. 2006) and throughout the Gulf of California (de la Cruz-Agüero et al. 1994, Castro-Aguirre & Balart 2002, Villegas-Sánchez et al. 2009, Erisman et al. 2011, Mascareñas-Osorio et al. 2011, Del Moral-Flores et al. 2013). Sequences that produced a ≥97% match to species that are not known to occur in the Gulf of California were considered to be unidentified operational taxonomic units (OTUs). Additionally, sequences that failed to produce a ≥97% match were also considered to be unidentified OTUs.

Fish egg subsampling

Due to the large number of fish eggs (n = 19960) and larvae (n = 1184) 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 subsampling 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 per season using PRIMER 6 (PRIMER-E) (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m592p159_supp.pdf). If the curve reached an asymptote, indicating that additional analysis would 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 2 unsuccessful attempts at analyzing 96 specimens (amplification of <15%), further analysis was abandoned. This occurred in 5 weekly ichthyoplankton samples, leaving 43 weekly samples that produced successful results.

We determined the likely habitat of the adult fishes using data acquired from FishBase (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 benthopelagic species) or likely come from outside Cabo Pulmo National Park (pelagic oceanic, mesopelagic, bathypelagic or bathy-demersal species).

RESULTS

SST and chl *a* concentration

Monthly mean SST in Cabo Pulmo National Park varied from 22.5 (January) to 30.4°C (August) during 2014. Between 2000 and 2015, the Cabo Pulmo region had on average 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 and May, a warm period between July and October and 2 brief transition periods in June and November (Fig. 2A,B). Sea surface concentration of chl *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 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 were longer and more frequent in Cabo Pulmo National Park during 2010–2015 than during 2000–2009 (Fig. 2).

Species composition

A total of 21144 fish eggs (n = 19960) and larvae (n = 1184) were collected in Cabo Pulmo National Park between January 11 and December 25, 2014 from 48 weekly zooplankton collections. Sequencing >250 specimens per ichthyoplankton sample did not yield additional OTUs in any of the seasons (Fig. S1 in the Supplement). The maximum number (40) of OTUs identified in summer required analysis of <230 specimens, while in fall, 15 OTUs were observed from analyzing <150 specimens (Fig. S1 in the Supplement). Five ichthyoplankton samples produced no

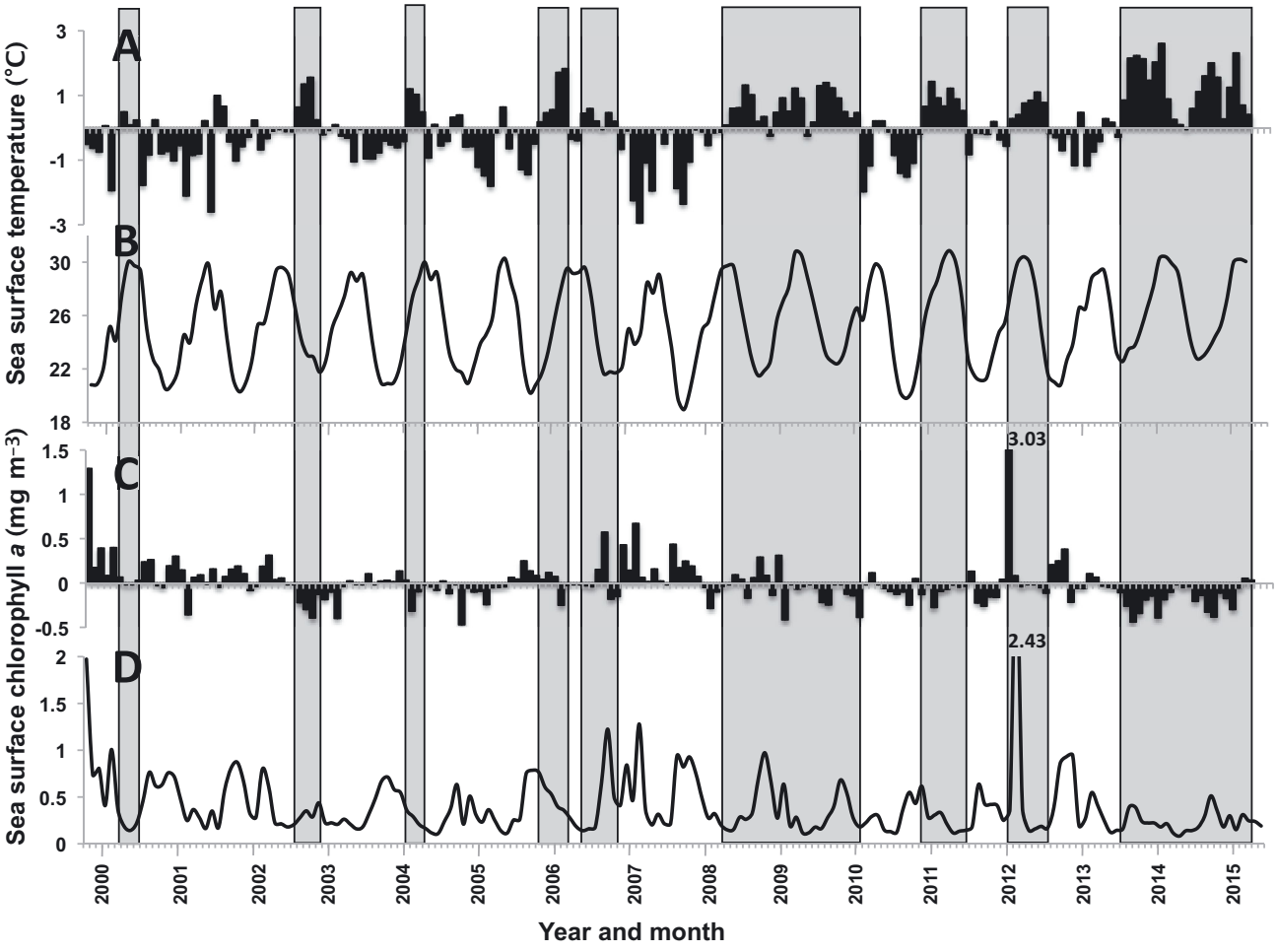

Fig. 2. (A) Satellite monthly anomaly and (B) mean sea surface temperature, and (C) satellite monthly anomaly and (D) mean of surface chl *a* concentration recorded between 2000 and 2015 from the region of Cabo Pulmo National Park. Grey shaded areas indicate periods with at least 3 consecutive months of positive SST anomalies

results due to poor sample preservation, leaving a total of 43 usable weekly samples. After subsampling as described above ('Materials and methods: Fish egg subsampling'), the target gene, either COI or 16S, was successfully amplified and sequenced for 2589 specimens. A total of 6894 eggs (n = 6422) and larvae (n = 472) were analyzed using PCR. A total of 3327 eggs (n = 3017) and larvae (n = 310) successfully amplified the target gene, either COI or 16S (sequence data available in the Dryad Digital Repository: doi: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. Also, 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% (n = 2589), with 2354 (78%) eggs and 235 (77%) larvae successfully sequenced. Due to their distinctive shape (Fig. 3), an additional 1799 *Scarus* sp. eggs were identified morphologically to the genus level, bringing the total number of specimens successfully analyzed to 4388. Fifty *Scarus* sp. eggs were analyzed using DNA barcoding, revealing 3 species: *S. ghobban* (26), *S. compressus* (23) and *S. rubroviolaceus* (1), all known to occur in Cabo Pulmo National Park. Fig. 4 shows a time series of total standardized abundance (ind. 1000 m^{-3}) and the number of OTUs (eggs and larvae) collected during 2014.

A total of 4388 fish eggs (n = 4153) and larvae (n = 235) were identified, consisting of 157 OTUs (103 identified to genus or species plus 54 unidentified OTUs) (Table S1 in the Supplement). 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 6 fish species that are benthic broadcast spawners or open-water/substratum egg scatterers (see footnotes under Tables 1 & 3).

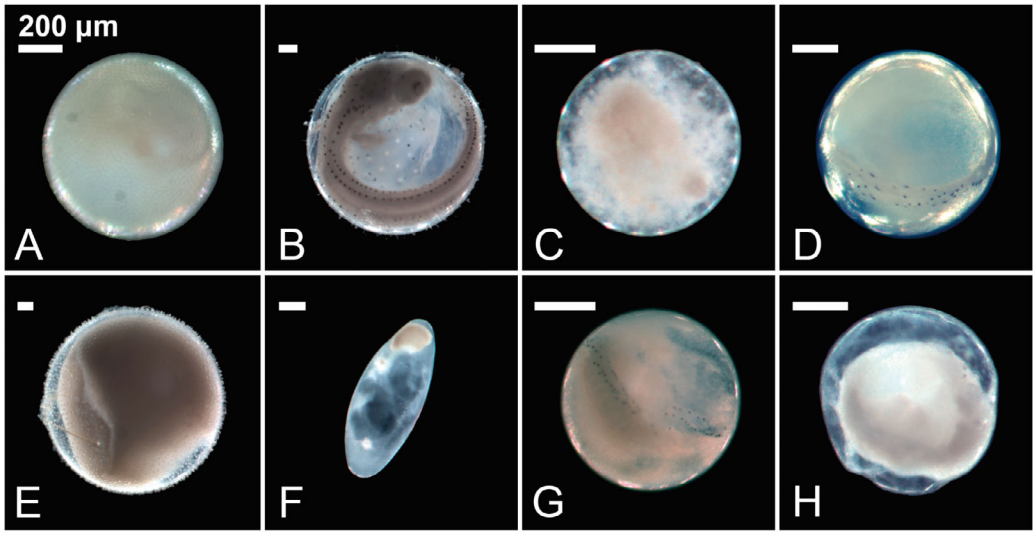

Fig. 3. Composite photograph of multiple species of fish eggs found in the ichthyoplankton monitoring survey of Cabo Pulmo National Park in 2014. Scale bar represents 200 microns. Eggs identified as: (A) *Synodus lucioceps*; (B) *Oxyporhamphus micropterus*; (C) *Prionurus laticlavius*; (D) *Pronotogrammus multifasciatus*; (E) *Regalecus glesne*; (F) *Scarus* sp.; (G) *Lutjanus guttatus*; (H) *Vinciguerria lucetia*

The 10 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 are 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 (Villarreal-Cavazos et al. 2000, Alvarez-Filip et al. 2006, Aburto-Oropeza et al. 2011, 2015). The total number of fish species (in juvenile or adult phases) reported from Cabo Pulmo National Park in previous studies is 270. The present study revealed 47 species that were not previously reported in Cabo Pulmo National Park, increasing the known species diversity to 317 species (Table 1, footnote 'a').

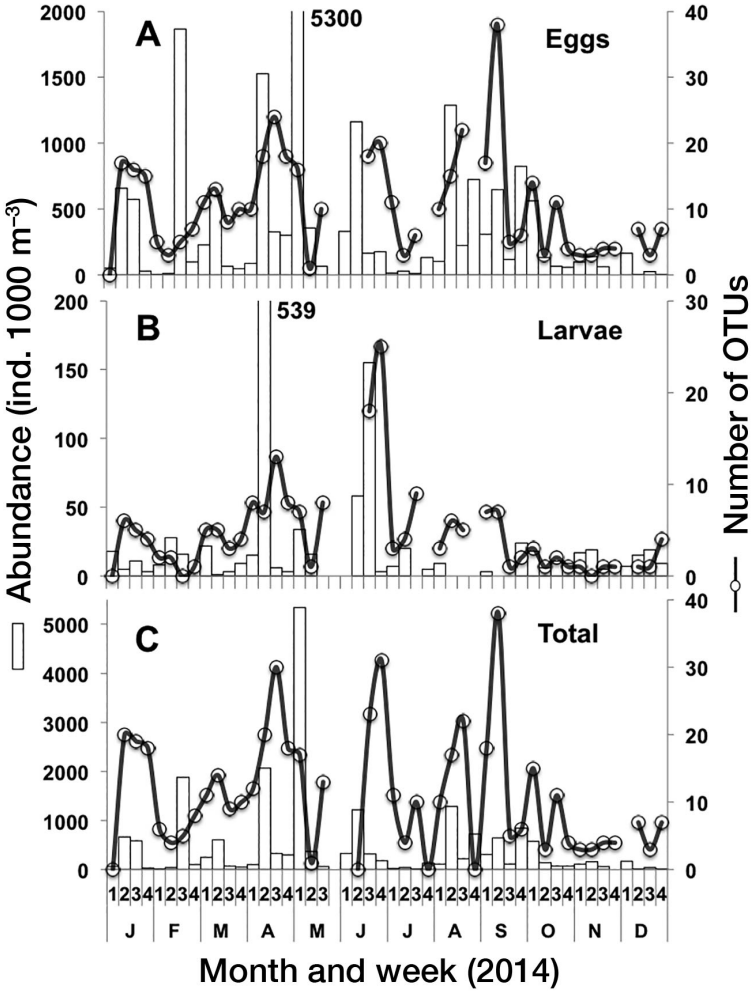

Fig. 4. Total standardized abundance and species richness as operational taxonomic units (OTUs) identified with molecular methods for (A) fish eggs, (B) larvae and (C) total (eggs and larvae) collected in Cabo Pulmo National Park, Jan–Dec 2014

Table 1. All fish species identified to genus or species (listed in order of abundance), with 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. Habitat of adults obtained from specialized literature is also shown. COI: a 710 bp fragment of the mitochondrial gene cytochrome *c* oxidase subunit 1; 16S: a 570 bp fragment of the mitochondrial 16S rRNA gene; na: not applicable. Species have planktonic eggs, unless marked otherwise (see footnote 'b')

Species	Common name	No. of specimens identified	No. of collections	No. of larvae	No. 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[a]	Cape razorfish	242	9	0	242	COI	Reef associated
Euthynnus lineatus	Black skipjack	213	8	3	210	COI	Pelagic/oceanic
Ammodytoides gilli[a]	Gill's sand lance	123	13	0	123	COI	Demersal
Synodus lacertinus	Sauro lizardfish	118	26	0	118	COI	Demersal
Etrumeus acuminatus[a]	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[a]	Panama lightfish	90	14	30	60	COI	Mesopelagic
Auxis rochei[a]	Frigate tuna	71	8	14	57	COI	Pelagic/neritic
Haemulon sexfasciatum	Greybar grunt	67	10	1	66	COI	Reef associated
Caranx caninus[a]	Pacific crevalle jack	61	8	9	52	COI	Pelagic/oceanic
Thalassoma lucasanum	Cortez rainbow wrasse	58	6	0	58	COI	Reef associated
Eucinostomus currani[a]	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[a]	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[a]	God's flounder	15	8	0	15	COI	Demersal
Bothus leopardinus[a]	Pacific leopard flounder	14	3	0	14	COI	Demersal
Paranthias colonus	Pacific creolefish	14	7	0	14	COI	Reef associated
Sphyraena ensis[a]	Mexican barracuda	14	3	8	6	COI	Pelagic/neritic
Pristigenys serrula[a]	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[a]	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[a]	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 and COI	Reef associated
Lutjanus peru	Pacific red snapper	9	3	0	9	COI	Reef associated
Synodus scituliceps[a]	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[a]	Oval flounder	7	1	7	0	COI	Demersal
Trachinotus rhodopus	Gafftopsail pompano	7	2	0	7	COI	Pelagic/oceanic
Carangoides otrynter[a]	Threadfin jack	6	3	0	6	COI	Benthopelagic

(continued on next page)

Table 1 (continued)

Species	Common name	No. of specimens identified	No. of collections	No. of larvae	No. of eggs	Gene used	Habitat
Coryphaena equiselis[a]	Pompano dolphinfish	6	3	2	4	COI	Pelagic/oceanic
Coryphaena hippurus	Common dolphinfish	5	3	1	4	COI	Pelagic/neritic
Oxyporhamphus micropterus[a]	Bigwing halfbeak	5	2	0	5	16S and COI	Pelagic/oceanic
Benthosema panamense[a]	Panama lanternfish	4	4	4	0	COI	Mesopelagic
Cirrhitichthys oxycephalus	Coral hawkfish	4	3	0	4	COI	Reef associated
Nematistius pectoralis[a]	Roosterfish	4	3	1	3	COI	Demersal
Plagiotremus azaleus[b]	Sabertooth blenny	4	3	4	0	COI	Reef associated
Selene peruviana[a]	Peruvian moonfish	4	1	0	4	COI	Benthopelagic
Balistes polylepis[b]	Finescale triggerfish	3	1	3	0	COI	Reef associated
Bodianus diplotaenia	Mexican hogfish	3	1	0	3	COI	Reef associated
Regalecus glesne[a]	Giant oarfish	3	1	0	3	16S	Mesopelagic
Alphestes immaculatus[a]	Pacific mutton hamlet	2	1	0	2	COI	Demersal
Anisotremus taeniatus	Panama porkfish	2	1	0	2	COI	Demersal
Axoclinus storeyae[a,b]	Carmine triplefin	2	1	2	0	COI	Reef associated
Cheilopogon dorsomacula[a]	Backspot flyingfish	2	2	0	2	COI	Pelagic/neritic
Diogenichthys laternatus[a]	Diogenes lanternfish	2	2	2	0	COI	Mesopelagic
Fistularia corneta[a]	Pacific cornetfish	2	2	0	2	16S	Pelagic/neritic
Gerres simillimus[a]	Yellow fin mojarra	2	2	0	2	COI	Reef associated
Hygophum atratum[a]	Thickhead lanternfish	2	1	2	0	COI	Bathypelagic
Labrisomus xanti[b]	Largemouth blenny	2	2	2	0	COI	Reef associated
Liopropoma fasciatum[a]	Wrasse ass bass	2	1	0	2	COI	Reef associated
Pontinus furcirhinus[a]	Red scorpionfish	2	2	2	0	COI	Bathydemersal
Prionotus stephanophrys[a]	Lumptail searobin	2	1	0	2	COI	Demersal
Acanthemblemaria macrospilus[b]	Barnacle blenny	1	1	1	0	COI	Reef associated
Acanthurus triostegus	Convict surgeonfish	1	1	0	1	COI	Reef associated
Aulopus sp.[a]	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[a]	Bigeye cigarfish	1	1	1	0	COI	Bathypelagic
Engraulidae sp.[a]	Anchovy	1	1	0	1	16S	Pelagic/neritic
Eucinostomus entomelas[a]	Dark-spot mojarra	1	1	0	1	COI	Demersal
Gymnothorax castaneus	Panamic green moray	1	1	0	1	COI	Reef associated
Hemanthias signifer[a]	Damsel bass	1	1	1	0	COI	Demersal
Katsuwonus pelamis	Skipjack tuna	1	1	0	1	COI	Pelagic/oceanic
Lampanyctus parvicauda[a]	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[a]	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[a]	Spotted snake eel	1	1	0	1	COI	Reef associated
Orthopristis reddingi[a]	Bronze-striped grunt	1	1	1	0	COI	Demersal
Perissias taeniopterus[a]	Striped-fin founder	1	1	0	1	COI	Demersal
Polydactylus approximans[a]	Blue bobo	1	1	1	0	COI	Demersal
Polylepion cruentum[a]	Bleeding wrasse	1	1	0	1	COI	Reef associated
Prognichthys sealei	Sailor flyingfish	1	1	0	1	COI	Pelagic/oceanic
Pronotogrammus multifasciatus[a]	Threadfin bass	1	1	0	1	16S	Reef associated
Scarus rubroviolaceus	Ember parrotfish	1	1	0	1	16S	Reef associated
Stegastes rectifraenum[b]	Cortez damselfish	1	1	1	0	COI	Reef associated
Thunnus albacares	Yellowfin tuna	1	1	1	0	COI	Pelagic/oceanic
Triphoturus mexicanus[a]	Mexican lampfish	1	1	1	0	COI	Mesopelagic

[a]Species not previously reported from Cabo Pulmo National Park; [b]Species with demersal eggs attached to substrate or parent's body

Unidentified OTUs

The sequences obtained were classified into 157 OTUs (Table S1 in the Supplement shows % match of each OTU to sequences in GenBank and the Barcode of Life Data System). 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 2 cases (Table 1). Forty-three 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 Gulf of California (*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.

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, 63.86% of individual specimens identified were reef-associated, 13.35% pelagic, 10.39% demersal,

Table 2. The 54 unidentified operational taxonomic units (OTUs) in order of abundance, showing the closest match found in online databases and percentage sequence identity. COI: a 710 bp fragment of the mitochondrial gene cytochrome *c* oxidase subunit 1; 16S: a 570 bp fragment of the mitochondrial 16S rRNA gene

OTU	No. of specimens analyzed	No. of sampling collections	Gene used	Closest genus and species match	Identity (%)
#14	76	7	16S	*Ammodytes americanus*	94
#04	71	11	COI	*Bleekeria mitsukurii*	96
#54	26	5	COI	*Epinephelus clippertonensis*[a]	99
#07	14	2	COI	*Syacium maculiferum*	85
#23	13	1	16S	*Xyrichths novacula*	96
#26	12	5	COI	*Abudefduf saxatilis*	96
#03	10	2	COI	*Cephalopholis cruentata*	95
#09	10	3	COI	*Diaphus watasei*	91
#53	8	1	16S	*Assurger anzac*[a]	98
#58	8	4	COI	*Syacium maculiferum*[a]	99
#11	7	2	COI	*Mycteroperca microlepis*	95
#08	6	2	COI	*Bothus robinsi*	90
#06	4	2	COI	*Synodus poeyi*	91
#15	4	2	COI	Actinopterygii environmental sample	90
#38	4	2	COI	*Caranx latus*	90
#02	3	2	COI	*Assurger anzac*	92
#12	3	3	COI	*Symphurus ginsburgi*	84
#40	3	2	COI	*Trachinotus goodei*[a]	98
#48	3	1	COI	*Hyporthodus niveatus*[a]	98
#50	3	3	16S	*Synodus lucioceps*	87
#01	2	2	COI	*Lampanyctus hubbsi*	94
#10	2	1	COI	*Uropterygius macularius*	90
#21	2	1	COI	*Synodus foetens*	88
#29	2	1	COI	*Opisthonema libertate*	94
#32	2	2	COI	*Bleekeria mitskurii*	91
#52	2	1	COI	*Genypterus maculatus*[a]	99
#55	2	1	COI	*Hyporthodus niphobles*[a]	100
#56	2	2	COI	*Kyphosus cinerascens*[a]	99
#05	1	1	COI	*Tetragonorus cuvieri*	94
#13	1	1	COI	*Gillellus jacksoni*	84
#16	1	1	COI	*Gillellus jacksoni*	85
#17	1	1	COI	*Callechelys muraena*	93
#18	1	1	COI	*Caranx crysos*[a]	99
#20	1	1	COI	*Synodus foetens*	86
#22	1	1	COI	*Siganus corallinus*	82
#24	1	1	COI	*Symphurus atricaudus*	83
#25	1	1	COI	*Polylepion russelli*	90
#27	1	1	COI	*Neoconger mucronatus*	93
#28	1	1	16S	*Prionotus scitulus*	95
#31	1	1	COI	*Kyphosus vaigiensis*	94
#33	1	1	COI	*Microdesmus carri*	86
#34	1	1	COI	*Ophichthus gomesii*	88
#35	1	1	COI	*Cypselurus poecilopterus*	82
#36	1	1	COI	*Trachipterus trachipterus*	82
#37	1	1	COI	*Anchoa hepsetus*	90
#39	1	1	COI	*Gymnothorax vicinus*	90
#41	1	1	COI	*Evoxymetopon taeniatus*	93
#42	1	1	COI	*Synodus poeyi*	91
#43	1	1	16S	*Kathetostoma laeve*[a]	97
#44	1	1	COI	*Macruronus magellanicus*	81
#46	1	1	16S	*Ichthyapus ophioneus*	93
#47	1	1	COI	*Paralichthys lethostigma*	86
#51	1	1	16S	*Synodus lucioceps*	87
#57	1	1	COI	*Paraconger ophichthys*[a]	99

[a]Specimens that provided a match of ≥97% to a species not known to occur in the Gulf of California. They may represent a closely related species with sequences not present in GenBank or the Barcode of Life Data System or, less likely, an occurrence of this species outside of its known distribution range

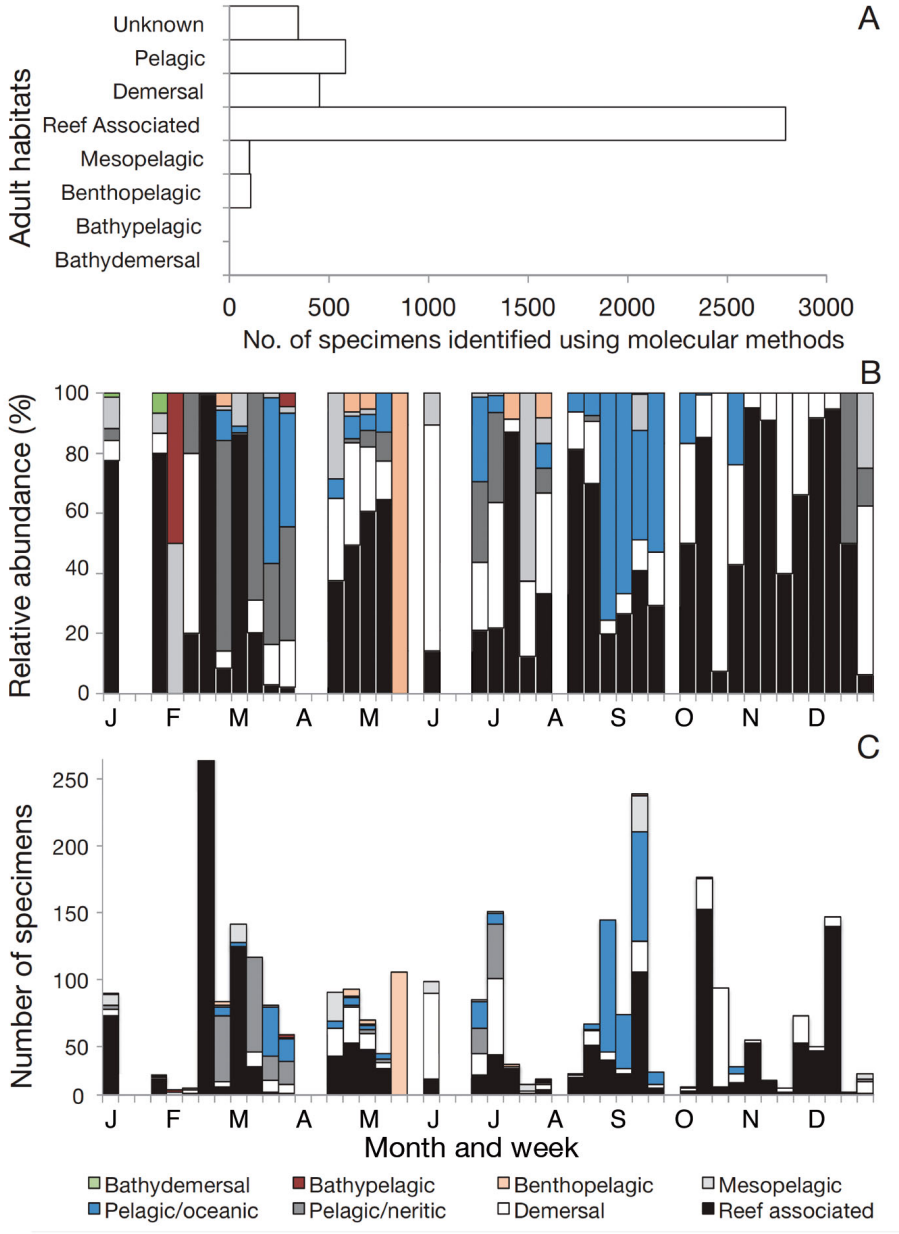

Fig. 5. (A) Number of fish species (eggs and larvae) identified with molecular methods from ichthyoplankton collected in 2014 in Cabo Pulmo National Park inferred per adult habitat distribution. (B) Relative abundance (%) and (C) number of specimens analyzed of fish eggs and larvae classified by adult habitat distribution. Note: May 24 collection was taken after a known *Chanos chanos* (milkfish) spawning event. Weeks with no data were not sampled

7.54% unknown, 2.44% bentho-pelagic, 2.28% meso-pelagic, 0.09% bathypelagic and 0.05% were bathy-demersal. The relative proportions of habitat of all of the specimens identified with molecular methods and the proportion of species and number of specimens from each habitat distribution identified throughout the year are shown in Fig. 5. Reef-associated, demersal, pelagic neritic and bentho-pelagic species dominated fish spawning events throughout the year, with high abundance dominance during cold months (January–March and November–December). Pelagic oceanic species seem to spawn and enter Cabo Pulmo National Park from March to November but with relatively higher proportion during summer months (June–September) (Fig. 5B). 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 cold season (first 6 mo of 2014) (Fig. 5B). 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 no. 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 4 seasons (4.5%), 10 OTUs in 3 seasons (6.5%), 36 OTUs in 2 seasons (23%), and the majority, 104 OTUs, in only 1 season (66%) (Table 3). Lizardfish *Synodus lacertinus*, wrasse *Halichoeres dispilus*, lightfish *Vinciguerria lucetia* and lance *Ammodytoides gilli* specimens were found spawning throughout most of the year, indicating a strategy of continuous reproduction, whereas herring *Etrumeus acuminatus* only appeared in 6 collections, with 95% of the specimens appearing during the

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). Species have planktonic eggs, unless marked otherwise (see footnote 'a')

Species or OTU	Winter	Spring	Summer	Autumn	No. of seasons present
Acanthemblemaria macrospilus[a]	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[a]	0	0	0	2	1
Balistes polylepis[a]	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[a]	1	1	0	0	2

(continued on next 2 pages)

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, and 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 (8824 specimens, 73 OTUs). The highest species richness (90 OTUs) with relatively low abundance (5420 specimens) was found during the summer, and the lowest species richness (28 OTUs) and lowest abundance (2584 specimens) was observed during autumn (Table 3, Fig. 4). The highest number of OTUs on a single collection date occurred on September 10 (38 OTUs). On 2 occasions, peaks in abundance corresponded to spawning of a particular species: on February 16, 99% of the specimens were parrotfish *Scarus* spp., and the collection with the lowest number of species (1), as well as the highest abundance (5334), occurred on May 24 during a recent spawning event of milkfish *Chanos chanos*. Peaks in spawning activity were observed in each month, with the exception of November and December. Fig. 4 illustrates the number of OTUs found in each weekly 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 5996 samples and

Table 3 (continued)

Species or OTU	Winter	Spring	Summer	Autumn	No. of seasons present
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

(continued on next page)

16S primers on 898 samples. Of these, 49.6% (n = 2976) of COI and 39% (n = 351) of 16S reactions resulted in successful amplification. The 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 time series 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 and 69 400 interim species as of October 2017) with a minimum sequence length of 500 bp. 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 1 or more 16S sequences, while 95% were represented by COI sequences. Hence, 1 species in that top group (*Scarus compressus*) could only be identified by 16S sequencing. Further inspection of the list revealed that 2 other species (*Fistularia corneta* and *Pronotogrammus multifasciatus*) were not in the COI database but were identified by 16S sequencing.

DISCUSSION

DNA barcoding of ichthyoplankton

In the present study, ichthyoplankton collected from within Cabo Pulmo National Park over 1 yr of weekly sampling were identified using DNA barcoding methods. This time series provides

Table 3 (continued)

Species or OTU	Winter	Spring	Summer	Autumn	No. of seasons present
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[a]	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[a]	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	103 species + 54 OTUs
Total collected	4316	8824	5420	2584	21144

[a]Species with demersal eggs attached to substrate or parent's body

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 present 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 the health of the surrounding fisheries by acting as a potential source of population replenishment through spawning activity. The present 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 (Aburto-Oropeza & Balart 2001, Alvarez-Filip et al. 2006, Aburto-Oropeza et al. 2011, 2015). The use of DNA barcoding to identify ichthyoplankton revealed 3 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 (Aburto-Oropeza & Balart 2001, Alvarez-Filip et al. 2006, Aburto-Oropeza et al. 2011, 2015, Ramírez-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 protection offered by marine protected areas and the 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 1 and 3 d for fish eggs from most of the commercial and recreational species identified in the study (Pauly & Pullin 1988, Harada et al.

2015). Although planktonic eggs and larvae drift with marine currents, since most tropical and subtropical eggs hatch within 1–3 d of spawning, many of the collected eggs likely result from local spawning events in or around Cabo Pulmo National Park (Pauly & Pullin 1988, Harada et al. 2015). This is true particularly during 2014, which was an anomalous warm year (Fig. 2A,B). 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 (Trasviña-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 southwestern 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 marine current information from Cabo Pulmo National Park using an acoustic Doppler profiler (ADP), acoustic Doppler current profiler (ADCP) and GPS buoy observations from October 2010 to February 2012. Sea 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 occurred; Trasviña-Castro et al. 2012). 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). With this information, we infer that a large proportion of ichthyoplankton 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 6 mo 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 of 1 cyclonic 50 km diameter and 1 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 southwest region of the Gulf of California.

Conditions in Cabo Pulmo National Park during 2014–2015 were atypically warm with considerably low chl *a* concentrations (Fig. 2) 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) (Cavole et al. 2016). The anomalously warm 2014 conditions likely promoted 2 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. 4 & 5). 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 (Aburto-Oropeza & Balart 2001, Alvarez-Filip et al. 2006, 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 SSTs as more southerly species may begin to migrate and extend 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 roosterfish *Nematistius pectoralis*, mahi-mahi *Coryphaena hippurus* and skipjack *Euthynnus lineatus*, 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 *Auxis rochei*, *Thunnus albacares*, *A. thazard*, *Katsuwonus pelamis*, *Micropogonias ectenes* and *Etrumeus acuminatus* (Ramírez-Rodríguez 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. 2002, 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 the present study, including the presence of a diverse assemblage of many commercially and ecologically important species, provide 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 policymakers 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, 46 families, 84 genera, 157 species) than solely visual monitoring surveys of the same location (13 orders, 38 families, 118 species) (Aburto-Oropeza et al. 2011, 2015, Ramírez-Valdez et al. 2014). The present study identified the early larval stages of 5 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 (Trasviña-Castro et al. 2012). 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 m 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 oceanic regions, including deep submarine canyons. Notably, only larvae of 3 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 highly 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, *R. 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. 2010), 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 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). Mesopelagic species such as *V. 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). 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 Gulf of California, 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, such as leopard groupers and snappers, were either sampled in very low numbers or not at all. Brogan (1994) 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 08:00 and 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, 6 of the species identified exhibit alternative reproductive strategies, including benthic broadcast spawning, open-water/substratum egg scattering, brood hiding, and guarding/nesting (see footnotes under Tables 1 & 3). 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 weekly 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 et al. 1977, Ahlstrom & Moser 1980, Nishikawa & Rimmer 1987, Moser 1996, Watson 1998, Saldierna-Martínez et al. 2005, Jiménez-Rosenberg et al. 2006, Richards 2006a,b, Kawakami et al. 2010, 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 in each sample. Species-level identification can be accurate for several species with distinctive morphology (Moser et al. 1974, Ahlstrom & Moser 1980, Hammann et al. 1998, Kawakami et al. 2010), 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 8 separate species (Fig. 6). Among the eggs identified were species from 2 orders and 7 families, with adults ranging in size from 24 to 92 cm total length (Fig. 6). 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 fish egg 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 scattergrams to completely or partially diagnose species identities. In tropical and subtropical ecosystems with diverse fish community structure, this task is considerably more complex. Spherical fish eggs are a

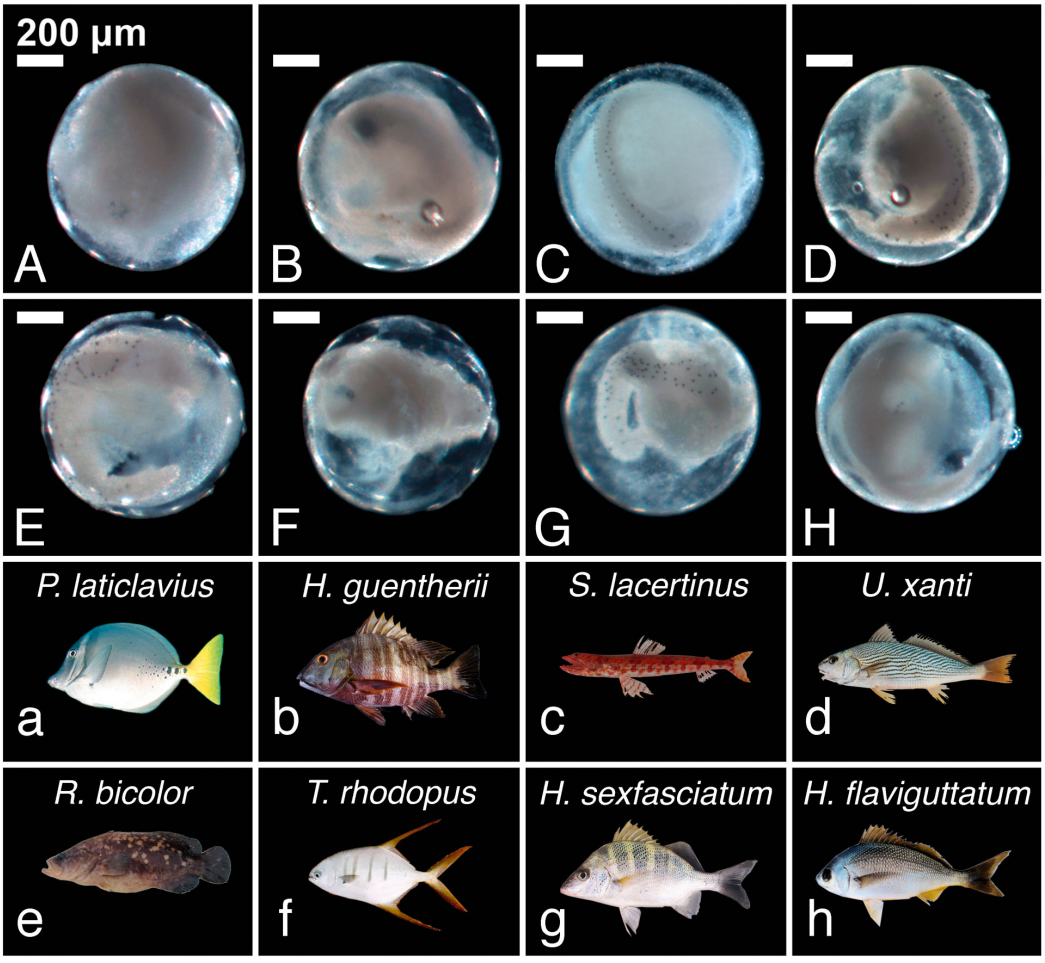

Fig. 6. Example of morphological vs. molecular fish egg identification: 8 eggs morphologically identified as *Lutjanus peru* (Pacific red snapper) revealed to be eggs from eight separate species. Scale bar represents 200 microns. The first two rows (capitalized letters) show the eggs that were identified molecularly as belonging to the species in the second two rows (non-capitalized letters)

successful and broadly observed feature in marine and freshwater 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 *Engraulis*), which have oval eggs and are easily identified at least to the genus level using morphological characters alone. Overall, our molecular identifications revealed 3 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 (Aranishi 2006, Teletchea 2009, Harada et al. 2015).

CONCLUSIONS

Future studies should take into account embryonic development time (inversely dependent on seawater temperature) as well as synoptic ocean current patterns (3D speed and direction) to determine the approximate location of spawning activity. High-resolution predictive current modeling or more extensive observational studies of regional currents at Cabo Pulmo National Park should help to determine whether the eggs that were collected originated inside or outside the park boundaries, and further, whether eggs are retained inside the park boundaries or drift to areas outside of park boundaries as predicted in multiple studies (Peguero-Icaza et al. 2008, 2011, Marinone 2012) 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 or immediately above the coral reef colonies 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, Cavole et al. 2016). Although Cabo Pulmo National Park is among the best-protected and healthiest marine protected areas in the Gulf of California (Aburto-Oropeza & Balart 2001, Aburto-Oropeza et al. 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 subtropical ecosystem and can be used as a baseline to compare data with future ecological and taxonomic studies.

Acknowledgements. We deeply thank the Castro family for their unconditional and invaluable help to collect weekly zooplankton samples at Cabo Pulmo National Park (January 2014 to 2017). We thank Carlos J. Robinson (Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México ICMyL-UNAM) for his satellite SST and chl *a* analyses from datasets available from NASA AVHRR and SeaWiFS. We thank the Comisión Nacional de Áreas Naturales Protegidas (CONANP), particularly Carlos Ramón Godínez-Reyes, for the permissions given for this research project, and members of the Gulf of California Marine Program for their help with this project, especially Juan José Cota Nieto, Arturo Ramírez-Valdez and Jose Alfredo Giron Nava. We also thank Phil Hastings and H. J. Walker for advice, suggestions, and access to the Scripps Institution of Oceanography Marine Vertebrate Collection. Photographs of adult fish (Fig. 6) were taken by John Snow. A.F.J. was supported by a National Science Foundation (NSF) grant DEB-1632648 (2016). J.G.-G. received support from the Centro Interdisciplinario de Ciencias Marinas, Instituto Politécnico Nacional (CICIMAR-IPN, Coordinación General de Posgrado e Investigación grants in 2013–2016), and a Secretaría de Educación Pública–Consejo Nacional de Ciencia y Tecnología (SEP-CONACYT) grant CB-2012-178615-01 and CB-2016-01-284201. J.G.-G. and R.J.S.-M. are fellows of the Comisión de Operación y Fomento de Actividades Académicas (COFAA-IPN) and Programa de Estímulos al Desempeño de los Investigadores (EDI-IPN) at the IPN, and the Sistema Nacional de Investigadores. Additional funding was provided by the Walton Family Foundation, Helmsley Charitable Trust, International Community Foundation, and David and Lucile Packard Foundation.

LITERATURE CITED

Aburto-Oropeza O, Balart EF (2001) Community structure of reef fish in several habitats of a rocky reef in the Gulf of California. Mar Ecol 22:283–305

Aburto-Oropeza O, Erisman B, Galland GR, Mascareñas-Osorio I, Sala E, Ezcurra E (2011) Large recovery of fish biomass in a no-take marine reserve. PLOS ONE 6: e23601

Aburto-Oropeza O, Ezcurra E, Moxley J, Sánchez-Rodríguez A and others (2015) A framework to assess the health of rocky reefs linking geomorphology, community assemblage, and fish biomass. Ecol Indic 52:353–361

Aceves-Medina G, Jiménez-Rosenberg SPA, Hinojosa-Medina A, Funes-Rodríguez R and others (2003) Fish larvae from the Gulf of California. Sci Mar 67:1–11

Aceves-Medina G, Jiménez-Rosenberg SPA, Hinojosa-Medina A, Funes-Rodríguez R, Saldierna-Martínez RJ, Smith PE (2004) Fish larvae assemblages in the Gulf of California. J Fish Biol 65:832–847

Ahlstrom EH, Moser HG (1980) Characters useful in identification of pelagic marine fish eggs. CalCOFI Rep 21: 121–131

Alvarez-Filip L, Reyes-Bonilla H, Calderon-Aguilera LE (2006) Community structure of fishes in Cabo Pulmo reef, Gulf of California. Mar Ecol 27:253–262

Apango-Figueroa E, Sánchez-Velasco L, Lavín MF, Godínez VM, Barton ED (2015) Larval fish habitats in a mesoscale dipole eddy in the gulf of California. Deep Sea Res I 103: 1–12

Aranishi F (2006) Single fish egg DNA extraction for PCR amplification. Conserv Genet 7:153–156

Arizpe O, Covarrubias M (2010) Sustainable tourism planning for the only coral reef in the Gulf of California: Cabo Pulmo National Park. WIT Trans Ecol Environ 139: 263–273

Brogan MW (1994) Two methods of sampling fish larvae over reefs: a comparison from the Gulf of California. Mar Biol 118:33–44

Burton RS (2009) Molecular markers, natural history, and conservation of marine animals. Bioscience 59:831–840

Carrasco-Águila MÁ, Miranda-Carrillo O, Salas-Maldonado M (2014) El rey de los arenques *Regalecus russelii*, segundo ejemplar registrado en Manzanillo, Colima.

Cienc Pesq 22:85–88
Castro-Aguirre JL, Balart EF (2002) The ichthyofauna of the Islas Revillagigedo and their zoogeographical relationships with comments about its origin and evolution. In: Lozano-Vilano ML (ed) Libro jubilar en honor al Dr. Salvador Contreras Balderas. UANL, Mexico, p 153–170
Cavole LM, Demko AM, Diner RE, Giddings A and others (2016) Biological impacts of the 2013–2015 warm-water anomaly in the Northeast Pacific: winners, losers, and the future. Oceanography 29:273–285
Chaudhuri H, Juario J, Primavera J, Mateo R and others (1977) Artificial fertilization of eggs and early development of the milkfish *Chanos chanos* (Forskal). Tech Rep No. 3, Aquacult Dep SEAFDEC, p 21–38
Chávez H, Galván-Magaña F, Torres-Villegas JR (1985) Primer registro de *Regalecus russellii* (Shaw) (Pisces: Regalecidae) de aguas mexicanas. Invest Mar CICIMAR 2:105–112
Claro R, Lindeman KC (2003) Spawning aggregation sites of snapper and grouper species (Lutjanidae and Serranidae) on the insular shelf of Cuba. Gulf Caribb Res 14: 91–106
de la Cruz-Agüero J, Galván-Magaña F, Abitia-Cárdenas LA, Rodriguez-Romero J, Gutiérrez-Sánchez FJ (1994) Systematic list of marine fishes from Bahía Magdalena, Baja California Sur (Mexico). Cienc Mar 20:17–31
Del Moral-Flores LF, González-Acosta AF, Espinosa-Pérez H, Ruiz-Campos G, Castro-Aguirre JL (2013) Annotated checklist of the ichthyofauna from the islands of the Gulf of California, with comments on its zoogeographic affinities. Rev Mex Biodivers 84:184–214
deWaard JR, Ivanova NV, Hajibabei M, Hebert PDN (2007) Assembling DNA barcodes. Environ Genomics, Methods Mol Biol 410:275–294
Doney SC, Ruckelshaus M, Emmett DJ, Barry JP and others (2012) Climate change impacts on marine ecosystems. Annu Rev Mar Sci 4:11–37
Dragičević B, Pallaoro A, Grgičević R, Lipej L, Dulčić J (2011) On the occurrence of early life stage of the king of herrings, *Regalecus glesne* (Actinopterygii: Lampriformes: Regalecidae), in the Adriatic Sea. Acta Ichthyol Piscat 41:251–253
Dransfeld L, Dwane O, Zuur AF (2009) Distribution patterns of ichthyoplankton communities in different ecosystems of the Northeast Atlantic. Fish Oceanogr 18:470–475
Elgar MA (1990) Evolutionary compromise between a few large and many small eggs: comparative evidence in teleost fish. Oikos 59:283–287
Erisman B, Galland GR, Mascareñas I, Moxley J and others (2011) List of coastal fishes of Islas Marías archipelago, Mexico, with comments on taxonomic composition, biogeography, and abundance. Zootaxa 2985:26–40
Erisman BE, Apel AM, MacCall AD, Román MJ, Fujita R (2014) The influence of gear selectivity and spawning behavior on a data-poor assessment of a spawning aggregation fishery. Fish Res 159:75–87
Gell FR, Roberts CM (2003) Benefits beyond boundaries: the fishery effects of marine reserves. Trends Ecol Evol 18: 448–455
Gleason LU, Burton RS (2012) High-throughput molecular identification of fish eggs using multiplex suspension bead arrays. Mol Ecol Resour 12:57–66
González-Navarro EA, Saldierna-Martínez RJ, Aceves-Medina G, Jiménez-Rosenberg SPA (2013) Atlas de identificación de larvas de peces de la subdivisión Elopomorpha del Pacífico mexicano. CICIMAR Oceánides 28: 7–40
Gotelli NJ, Colwell RK (2011) Chapter 4: Estimating species richness. In: Magurran A, McGill B (ed) Biological diversity: frontiers in measurement and assessment. Oxford University Press, Oxford, p 39–54
Hammann MG, Nevárez-Martínez MO, Green-Ruíz Y (1998) Spawning habitat of the Pacific sardine (*Sardinops sagax*) in the Gulf of California: egg and larval distribution 1956-1957 and 1971-1991. CalCOFI Rep 39: 169–179
Harada AE, Lindgren EA, Hermsmeier MC, Rogowski PA, Terrill E, Burton RS (2015) Monitoring spawning activity in a southern California marine protected area using molecular identification of fish eggs. PLOS ONE 10: e0134647
Hyde JR, Lynn E, Humphreys R Jr, Musyl M, West AP, Vetter R (2005) Shipboard identification of fish eggs and larvae by multiplex PCR, and description of fertilized eggs of blue marlin, shortbill spearfish, and wahoo. Mar Ecol Prog Ser 286:269–277
Jiménez-Rosenberg SPA, González Navarro E, Saldierna RJ (2006) Larval, prejuvenile and juvenile development of *Eucinostomus currani*. J Fish Biol 69:28–37
Johnson AF, Moreno-Báez M, Giron-Nava A, Corominas J, Erisman B, Ezcurra E, Aburto-Oropeza O (2017) A spatial method to calculate small-scale fisheries effort in data poor scenarios. PLOS ONE 12:e0174064
Kawakami T, Aoyama J, Tsukamoto K (2010) Morphology of pelagic fish eggs identified using mitochondrial DNA and their distribution in waters west of the Mariana Islands. Environ Biol Fishes 87:221–235
Kochzius M, Seidel C, Antoniou A, Botla SK and others (2010) Identifying fishes through DNA barcodes and microarrays. PLOS ONE 5(9):e12620
Levitus S, Antonov JI, Boyer TP, Locarnini RA, Garcia HE, Mishonov AV (2009) Global ocean heat content 1955–2008 in light of recently revealed instrumentation problems. Geophys Res Lett 36:L07608
Lewis LA, Richardson D, Zakharov E, Hanner R (2016) Integrating DNA barcoding of fish eggs into ichthyoplankton monitoring programs. Fish Bull 114:153–165
Leyva-Cruz E, Vásquez-Yeomans L, Carrillo L, Valdez-Moreno M (2016) Identifying pelagic fish eggs in southeast Yucatan Peninsula using DNA barcodes. Genome 59:1117–1129
Lo NCH, Hunter JR, Charter R (2001) Use of a continuous egg sampler for ichthyoplankton surveys: application to the estimation of daily egg production of Pacific sardine (*Sardinops sagax*) off California. Fish Bull 99:554–572
Marinone SG (2012) Seasonal surface connectivity in the Gulf of California. Estuar Coast Shelf Sci 100:133–141
Markle DF, Frost LA (1985) Comparative morphology, seasonality, and a key to planktonic fish eggs from the Nova Scotian shelf. Can J Zool 63:246–257
Mascareñas-Osorio I, Erisman B, Moxley J, Balart EF, Aburto-Oropeza O (2011) Checklist of conspicuous reef fishes of the Bahía de los Ángeles región, Baja California Norte, México, with comments on abundance and ecological biogeography. Zootaxa 2922:60–68
Miller B, Kendall AW (2009) Early life history of marine fishes. University of California Press, Berkeley
Moser HG (ed) (1996) The early stages of fishes in the California Current region. CalCOFI Atlas 33:1–1505
Moser HG, Ahlstrom EH, Kramer DA, Stevens EG (1974)

Distribution and abundance of fish eggs and larvae in the Gulf of California. CalCOFI Rep 17:112–128

Moser HG, Charter RL, Smith PE, Ambrose DA and others (1993) Distributional atlas of fish larvae and eggs in the California Current region: taxa with 1000 or more total larvae, 1951 through 1984. CalCOFI Atlas 31:1–233

Nemeth RS (2005) Population characteristics of a recovering US Virgin Islands red hind spawning aggregation following protection. Mar Ecol Prog Ser 286:81–97

Nishikawa Y, Rimmer DW (1987) Identification of larval tunas, billfishes and other scombroid fishes (suborder Scombroidei): an illustrated guide. CSIRO Mar Lab Rep 186

Palumbi SR (1996) Nucleic acids II: the polymerase chain reaction. In: Hillis DM, Moritz C, Mable BK (eds) Molecular systematics. Sinauer & Associates, Sunderland, MA, p 205–247

Pauly D, Pullin RSV (1988) Hatching time in spherical, pelagic, marine fish eggs in response to temperature and egg size. Environ Biol Fishes 22:261–271

Peguero-Icaza M, Sanchez-Velasco L, Lavín MF, Marinone SG (2008) Larval fish assemblages, environment and circulation in a semi enclosed sea (Gulf of California, Mexico). Estuar Coast Shelf Sci 79:277–288

Peguero-Icaza M, Sánchez-Velasco L, Lavín MF, Marinone SG, Beier E (2011) Seasonal changes in connectivity routes among larval fish assemblages in a semi-enclosed sea (Gulf of California). J Plankton Res 33:517–533

Perez J, Álvarez P, Martinez JL, Garcia-Vazquez E (2005) Genetic identification of hake and megrim eggs in formaldehyde-fixed plankton samples. ICES J Mar Sci 62: 908–914

Ramírez-Rodríguez M (2013) Especies de interés pesquero en el Pacífico Mexicano: nombres y claves para su registro. CICIMAR, IPN, Mexico City. http://catalogo.cicimar.ipn.mx

Ramírez-Valdez A, Johnson A, Aburto-Oropeza O, Giron-Nava A (2014) Mexico's reefs and underwater data. DataMares datasets, UC San Diego, La Jolla, CA

Reyes-Bonilla H, Calderon-Aguilera L (1999) Population density, distribution and consumption rates of three corallivores at Cabo Pulmo reef, Gulf of California, Mexico. Mar Ecol 20:347–357

Richards WJ (2006a) Early stages of Atlantic fishes. An identification guide for the Western Central North Atlantic, Vol I. CRC Press, Taylor and Francis Group, Boca Raton, FL

Richards WJ (2006b) Early stages of Atlantic fishes. An identification guide for the Western Central North Atlantic, Vol II. CRC Press, Taylor and Francis Group, Boca Raton, FL

Robinson CJ, Gómez-Gutiérrez J, Salas De León DA (2013) Jumbo squid (*Dosidicus gigas*) landings in the Gulf of California related to remotely sensed sea surface temperature and concentration of chlorophyll *a* (1998–2012). Fish Res 137:97–103

Robinson CJ, Gómez-Gutiérrez J, Markaida U, Gilly WF (2016) Prolonged decline of jumbo squid (*Dosidicus gigas*) landings in the Gulf of California is associated with chronically low wind stress and decreased chlorophyll *a* after El Niño 2009-2010. Fish Res 173:128–138

Sala E, Aburto-Oropeza O, Paredes G, Parra I, Barrera JC, Dayton PK (2002) A general model for designing networks of marine reserves. Science 298:1991–1993

Sala E, Aburto-Oropeza O, Paredes G, Thompson G (2003) Spawning aggregations and reproductive behavior of reef fishes in the Gulf of California. Bull Mar Sci 72:103–121

Saldierna-Martínez RJ, Gonzalez-Navarro E, Aceves-Medina G (2005) Larval development of *Symphurus atramentatus* (Cynoglossidae: Pleuronectiformes) from the Gulf of California. Zootaxa 1016:1–14

Smith PE, Richardson SL (1977) Standard techniques for pelagic fish egg and larva surveys. FAO Fish Tech Pap 175:1–100

Teletchea F (2009) Molecular identification methods of fish species: reassessment and possible applications. Rev Fish Biol Fish 19:265–293

Timmermann A, Oberhuber J, Bacher A, Esch M, Latif M, Roeckner E (1999) Increased El Niño frequency in a climate model forced by future greenhouse warming. Nature 398:694–697

Trasviña-Castro AT, Aburto-Oropeza O, Ezcurra E, Zaytsev O (2012) Observaciones de corrientes en el Parque Nacional de Cabo Pulmo, Baja California Sur: mediciones Eulerianas en verano, otoño e inicios del invierno. GEOS 32:323–341

Verutes GM, Huang C, Estrella RR, Loyd K (2014) Exploring scenarios of light pollution from coastal development reaching sea turtle nesting beaches near Cabo Pulmo, Mexico. Glob Ecol Conserv 2:170–180

Villarreal-Cavazos A, Reyes-Bonilla H, Bermúdez-Almada B, Arizpe-Covarrubias O (2000) Los peces del arrecife de Cabo Pulmo, Golfo de California, México: lista sistemática y aspectos de abundancia y biogeografía. Rev Biol Trop 48:413–424

Villegas-Sánchez CA, Abitia-Cárdenas LA, Gutiérrez-Sánchez FJ, Galván-Magaña F (2009) Rocky-reef fish assemblages at San José Island, Mexico. Rev Mex Biodivers 80:169–179

Watson W (1998) Early life history stages of the whitetip flyingfish, *Cheilopogon xenopterus* (Gilbert, 1890) (Pisces: Exocoetidae). Fish Bull 97:1031–1042

Editorial responsibility: Philippe Borsa, Montpellier, France

Submitted: May 24, 2017; Accepted: December 7, 2017
Proofs received from author(s): 15 February, 2018