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Identifying Pelagic Fish Eggs in the Southeast Yucatan Peninsula using DNA Barcodes

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Abstract

In the waters surrounding Banco Chinchorro in the Mexican Caribbean are spawning and nursery areas for many types of fish. In this natural environment, as opposed to under controlled lab conditions, it is almost impossible to link an individual egg to the adult that laid it. This makes identifying the species of the eggs difficult. However, DNA barcodes have made this easier. In the present study, 300 eggs were processed for molecular analysis, from which 139 sequences were obtained. We identified 42 taxa (33 species with their binomial names), 35 genera, and 24 families. The identified eggs included those from *Ariomma melanum*, which is the first recording of this species in the Mexican Caribbean. Eggs from economically important fish species were also identified, including frigate tuna (*Auxis thazard*), crevalle jack (*Caranx hippos*), common dolphinfish (*Coryphaena hippurus*), sailfish (*Istiophorus platypterus*), white marlin (*Kajikia albida*), skipjack tuna (*Katsuwonus pelamis*), blackfin tuna (*Thunnus atlanticus*), and swordfish (*Xiphias gladius*). We have also described new morphological characteristics and captured photographs for 21 species, as well as obtained new information about spawning locality and time for 16 species. This valuable information will provide the basis to develop more effective conservation measures for sustainable fisheries and protection of the Mesoamerican Barrier Reef System.

Key words: Early fish stages; Mesoamerican Reef; Fish spawning areas; Fish egg descriptions, BOLD database.

Introduction

The Mexican Caribbean lies within the Mesoamerican Barrier Reef System (MBRS), the second-largest reef in the world (Almada-Villela et al. 2003). This area provides a suitable habitat for many fish species, including jacks and bonefish (Perez-Cobb et al. 2014), snappers, and groupers (Graham et al. 2008; Heyman and Kjerfve 2008), all of which have both commercial and game value.

Overexploitation of fisheries and a decline in marine resources are global problems that require constant evaluation of the population dynamics of fish species (Arreguín-Sanchez and Arcos-Huitrón 2011). Several processes can influence the increase or decrease of fish populations, such as recruitment at different stages of the life cycle, dispersal, and retention of organisms (Gleason and Burton 2012; Cheng et al. 2013). In order to perform a detailed and reliable study of these phenomena, it is necessary to accurately identify specimens of these species at all stages of their life cycle (Richardson et al. 2007).

Morphological features that are routinely used to recognize adult fish species are not present at the early stages of development, making identification of younger fish more difficult. In the case of fish eggs, the most important characteristics used for identification are shape, size, perivitelline space, oil globules, inner membrane, chorion, embryo, yolk shape, and pigment pattern (Ahlstrom and Moser 1980; Matarese and Sandknop 1984).

Nevertheless, despite the limited number of diagnostic characters available for the identification of fish eggs, some progress has been made in obtaining descriptions and

producing identification guides for the early developmental stages of fish around the world (Leis and Carson-Ewart 2000; Richards 2006; Fahay 2007). However, in most cases, these guides are incomplete. For example, in the Western Central Atlantic (WCA, FAO area 31), which includes the Yucatan Peninsula, there are approximately 2235 known fish species, but only 9% of them have an identifiable egg stage (Richards 2006). In fact, it is estimated that only 10% of the world's fish species have been documented from the early stages (Richards 1985; Kendall and Matarese 1994).

Molecular characters, such as DNA barcodes, have been successfully used to identify most animal species (Hebert et al. 2003), including fish from different regions worldwide (Ward et al. 2005; Hubert et al. 2008; Rock et al. 2008; Lara et al. 2010; Valdez-Moreno et al. 2010; Oliveira-Ribeiro et al. 2012). The idea of identifying the early stages of fish development with DNA barcoding was first proposed about eight years ago (Pegg et al. 2006), but since then, it has only been used in a few studies (Valdez-Moreno et al. 2010; Burghart et al. 2014).

For this reason, the aim of this study was to use morphology and DNA barcodes to identify fish eggs from the south-central part of the Mesoamerican Reef. Our data on the species richness and geographical distribution of the eggs will provide a strong basis for environmentalists and developers to establish a sustainable environment within this important region.

Materials and methods

Sample collection and processing

Samples were collected during an oceanographic cruise supported by a collaborative effort between El Colegio de la Frontera Sur (ECOSUR), the National Oceanic and Atmospheric Administration (NOAA), and Miami University, and were processed on board the Research Vessel Gordon Gunter.

Sampling was conducted from the 20th to the 22nd of April 2011, from 17 offshore stations located in the waters surrounding the largest pseudo-atoll in the Caribbean, the Biosphere Reserve Banco Chinchorro (17°45'-19°30' N 85°40'-88°00' W). According to geographic coordinates, two stations (82 and 83) were in the Belize jurisdiction, and the remaining fifteen (78-81 and 84 to 95, there is not number 92) were in Quintana Roo State, Yucatan Peninsula, Mexico (Fig. 1).

Two types of nets were used to collect the zooplankton: Neuston with a 0.947 mm mesh cloth size fitted on a 1 × 2 m rectangular aluminum frame, and a new sampling implement, Spanish Neuston S-10 with a 0.505 mm mesh size fitted on a 1 x 2 m rectangular aluminum frame (Habtes et al. 2014). Tows conditions for both nets were: duration 10 min, at a speed of two knots (3.7 m/h). The neuston net was used at a depth 0.5 m, and hence half of the frame was out of the water. Therefore, we cannot estimate the volume of filtered water. The Spanish Neuston net was used at 10 m depth. Samples were fixed in 96% ethanol, to replace the seawater.

Laboratory analyses

Eggs were extracted from plankton samples, counted, and sorted under a stereomicroscope according to recognizable morphotypes. Observations were made using 4x, 5x, and 10x magnifications. The main characteristics used were size, shape (spherical, ellipsoidal), width of the perivitelline space (narrow, wide, medium), presence or absence of oil globules, presence or absence of an inner membrane, pigmentation patterns and yolk characteristics (colour, segmentation), chorion (ornamentation, texture), and embryo (formation, pigments) (Ahlstrom and Moser 1980; Matarese and Sandknop 1984; Richards 2006).

Depending on the number of eggs available that displayed a particular morphotype, between one and five specimens per morphotype for each sampling station were selected for the DNA barcode analysis. Each specimen was transferred into water before photography to avoid drying during this process. All of the eggs were photographed using Nikon SMZ745T and Canon EOS Rebel T31 cameras, both of which were connected to a stereomicroscope. Between 100 and 250 photographs were stacked into one image using Helicon Focus 6.2.0 software (Kozub et al. 2008) to represent the entire egg. Once photographed, the eggs were placed in a 96-well plate for molecular analysis. All tools were flame sterilized before and after use with each sample to prevent contamination.

Extraction, amplification, and sequencing of the COI gene

DNA extraction, amplification, and sequencing were performed according to published protocols (Valdez-Moreno 2010). Before DNA extraction, ethanol was evaporated at 56°C. PCR amplification was performed in the barcode laboratory at ECOSUR (Chetumal). Fish primers used were Fish F1, Fish R1, Fish F2, and Fish R2 (Ward et al. 2005). Sequencing was performed using an M13-tailed fish primer-cocktail (Ivanova et al. 2007). Sequences were aligned using Codon Code 5.0.1 software. Primer details, trace files, and sequences are available under the project “Fish Eggs of the Caribbean” (code MXFEC) in the Barcode of Life Data Systems database (BOLD, www.boldsystems.org; Ratnasingham and Hebert 2007) and GenBank (<http://www.ncbi.nlm.nih.gov>).

Data analysis

Sequences were compared with previously published sequences in BOLD using the specimen identification tool provided in the system (Ratnasingham and Hebert 2007). Neighbor–Joining (NJ) trees (Saitou and Nei 1987) were constructed using pairwise genetic distances based upon the Kimura two-parameter model (K2P) (Kimura 1980) and pairwise deletion of missing data using MEGA 3 software (Kumar et al. 2004). Terminal nodes were collapsed to represent species-level groupings for visualization purposes. The height of each triangle represents the number of specimens sequenced while the depth represents the genetic diversity present within each cluster. BOLD also assigned a Barcode Index Number (BIN) to each specimen (see Ratnasingham and Hebert 2013).

The criteria to obtain species-level identifications using the barcode database were as follows: specimens with $> 98.4\%$ sequence similarity to reference sequences and within the same BIN (Ratnasingham & Hebert, 2013) were assigned a species-level identification. When the similarity values to reference sequences were between 95% and 98.3%, eggs were assigned to genus, family, and order. When genetic similarity was $< 95\%$, specimens were considered unidentified.

Species richness of all sequenced eggs at each sampling station, as well as the distributions of the three most broadly distributed species, were mapped using ArcView GIS^R 3.2 software. For the richness values, we included those eggs identified to a Linnaean species as well as those eggs that were recognized as being likely separate species on the basis of the BIN criterion, but which currently lack a binomial.

Results

Species identification

A total of 1391 eggs was collected. All of them were recognized within 94 different morphotypes, but it was only possible to identify the species for 10 of them using morphology: *Auxis thazard*, *Cheilopogon exsiliens*, *Gempylus serpens*, *Lachnolaimus maximus*, *Oxyporhamphus micropterus*, *Sparisoma viride*, *Diplospinus multistriatus*, *Lactoprhyx trigonus*, *Synodus synodus*, and *Xiphias gladius*. The remaining 84 morphotypes could not be assigned to a particular species on the basis of morphology.

For the molecular analyses, we processed 300 eggs, which represented all of the morphotypes with at least one specimen (see methods). From these, we obtained 139 sequences, representing a 46.3% sequencing success rate. Sequence length ranged from 188 to 665 base pairs (bp). Short sequences were used as mini-barcodes for identification (*sensu* Meusnier et al. 2008). No insertions, deletions, or stop codons were observed. The average K2P distance between barcode sequences within species was 0.64%, whereas interspecific divergences among species within genera 13.3%. All species, except for the bigeye (*Priacanthus arenatus*) (451 bp) and *Peristedion* sp. (265 bp), were assigned to a BIN number (Ratnasingham and Hebert 2013) within the BOLD system (Fig. S1).¹ DNA sequences have been submitted to GenBank (Accession No. KR086792-KR086931).

Of the 139 sequences, 85.7% matched sequences in the BOLD reference library with >98.4% similarity, allowing for species-level identification (Ratnasingham and Hebert 2013). The remaining 14.3% could be identified at only the genus, family, or order level. Seven orders, 24 families, 35 genera, and 42 taxa (33 species with their binomial names) were identified (see project “Fish Eggs of the Caribbean” (code MXFEC) in www.boldsystems.org). The families with the most species were Gempylidae and Stomiidae (four species), followed by Carangidae, Scombridae, and Synodontidae (each with three); Bramidae, Coryphaenidae, and Istiophoridae (two species each); and Acropomatidae, Ariommidae, Diodontidae, Echeneidae, Exocoetidae, Lampridae,

¹ Fig.S1. Full neighbor-joining tree based on genetic distances (K2P) from the COI gene, with their respective name and BIN.

Lophotidae, Ostraciidae, Peristediidae, Priacanthidae, Regalecidae, Sparidae, Trachipteridae, Trichiuridae, and Xiphiidae (one species each) (Fig. 2).

Sixteen eggs were identified only at the genus level: *Benthodesmus*, *Brama*, *Chauliodus*, *Decapterus*, *Leptostomias*, *Peristedion*, and *Trachipterus*; one egg was identified as belonging to the family Exocoetidae, and four were identified as belonging to the order Perciformes.

New observations on morphological features of the eggs

All species displayed a spherical-shaped egg, although the morphology in some cases was damaged during collection and storage. Egg diameters ranged from 0.4 mm to 2.1 mm, which falls within the range that had been previously reported for marine fish (0.5-5.5 mm) (Ahlstrom and Moser 1980; Matarese and Sandknop 1984). The smallest eggs were from Sailfish (*Istiophorus platypterus*), and the largest were from Giant oarfish (*Regalecus glesne*) and *Chauliodus* sp. Most of the eggs were smaller than those reported in the literature (Richards 2006; Fahay 2007); however, this was likely due to slight dehydration of the specimen from the alcohol fixation, because the diameter increased slightly when the eggs were introduced into water.

Here, we report the first morphological descriptions of egg stages for twenty-one different species. The number of individuals analyzed, diameter of the eggs, and all characteristics are summarized in Table 1 (images are available in Fig. S2-S8).²

The morphologies of the remaining twelve species reported here have been described previously (Richards, 2006; Fahay, 2007), although we did observe some differences. We could not find oil globules in the eggs of frigate tuna (*Auxis thazard*), pompano dolphinfish (*Coryphaena equiselis*), common dolphinfish (*Coryphaena hippurus*), sailfish (*I. platypterus*), and skipjack tuna (*Katsuwonus pelamis*), but it is likely that they were dissolved during alcohol fixation and storage. An intussusception in the chorion was observed in the eggs of *I. platypterus* and *K. pelamis* (for a summary of dissimilarities, see Fig. S9).³

²Fig. S2. Photographs of unknown egg stages of *Calamus calamus* (A, B, C), *Caranx hippos* (D), *Chauliodus danae* (E), *Decapterus punctatus* (F).

Fig. S3. Photographs of unknown egg stages of *Diplospinus multistriatus* (A, B, C), *Gempylus serpens* (D), *Kajikia albida* (E, F).

Fig. S4. Photographs of unknown egg stages of *Lactophrys trigonus* (A, B, C), *Nesiarchus nasutus* (D, E, F).

Fig. S5. Photographs of unknown egg stages of *Priacanthus arenatus* (A, B), *Prognichthys occidentalis* (C), *Promethichthys prometheus* (D, E, F).

Fig. S6. Photographs of unknown egg stages of *Pterycombus brama* (A, B, C), *Remora osteochir* (D, E).

Fig. S7. Photographs of unknown egg stages of *Saurida normani* (A), *Scombrobrax heterolepis* (B), *Synagrops bellus* (C) and *Synodus synodus* (D, E, F).

Fig. S8. Photographs of unknown egg stages of *Thunnus atlanticus* (A), *Trachinotus falcatus* (B, C) and *Ariomma melanum* (D).

³Fig. S9. Differences from previous descriptions are highlighted for *Auxis thazard*, *Coryphaena equiselis*, *Coryphaena hippurus*, *Istiophorus platypterus*, and *Katsuwonus pelamis*.

The eggs of Sloane's viperfish *Chauliodus sloani* lacked the double membrane and narrow perivitelline space that had been previously reported (Fahay 2007); in fact, these had been reported as having a wide perivitelline space (Ahlstrom and Moser 1980; Matarese and Sandknop 1984). No tiny spines were observed in eggs from the crested oarfish (*Lophotus lacepede*). Oil globules and pigments were not observed in the eggs of *R. glesne*, possibly because they were unfertilized. In contrast, we observed small projections in the chorion, as reported by Kawakami and Aoyama (2010). The eggs of the sand diver (*Synodus intermedius*) did not have a sculptured chorion, and we observed pigmentation in the embryo head (Fig. S10).⁴

It is possible that we were able to observe more detailed characteristics than had previously been reported because we performed our analyses at higher magnifications. Here, we used 4x, 5x, and 10x magnification, whereas the original descriptions did not indicate the magnifications at which the observations were made.

Egg richness

Stations located near to Chinchorro Bank contained the highest richest of eggs: station 91 had ten taxa, followed by stations 88 and 90, which both had nine taxa. The southern stations (78, 81 and 82) located north of Belize contained the fewest, with only three taxa in each one (Fig. 3).

⁴Fig. S10. Differences from previous descriptions are highlighted for *Chauliodus sloani*, *Lophotus lacepede*, *Regalecus glesne* and *Synodus intermedius*.

The most broadly distributed species were *Diplospinus multistriatus* and *R. glesne*, which were found in eight stations each, followed by *N. nasutus*, which was found in six stations. The eggs of *D. multistriatus* were present in the south and central stations; *R. glesne* was recorded in the north and central stations; and *N. nasutus* was more common in the southernmost stations (Fig. 4).

Discussion

In the current study, we used the mitochondrial COI gene to identify a number of species from fish eggs (42 taxa), more than was found in previous reports. Burghart et al. (2014) found 14 taxa, from Terra Ceia Bay in Florida. When other genetic markers were used, a smaller number of species was found. For example, sixteen species were identified from 2698 eggs from the Mariana Islands using 16S ribosomal RNA (Kawakami et al. 2010), and five species were recognized from 52 eggs using a shorter version of 16S ribosomal RNA (Saitoh et al. 2008). Other work has focused on the identification of particular species; for example, Hyde et al. (2005) recognized six species: swordfish (*Xiphias gladius*), shortbill spearfish (*Tetrapturus angustirostris*), common dolphinfish (*Coryphaena hippurus*), wahoo (*Acanthocybium solandri*), blue marlin (*Makaira nigricans*) and pompano dolphinfish (*Coryphaena equiselis*), and Carreon-Martínez (2010) recognized four species: (*Menticirrhus americanus*, *Menticirrhus littoralis*, *Pogonias cromis* and *Sciaenops ocellatus*) from the family Sciaenidae

When we compared the barcodes (42 taxa as determined using the BOLD system tools) against the morphology (10 taxa morphologically identified to a Linnaean species name), only four species were the same: *X. gladius*, *D. multistriatus*, *A. thazard*, and *S. synodus*.

Of the ninety-four morphotypes that we identified, thirty two did not have sequence. Thirty could be grouped into ten species. This means that *K. albida*, *K. pelamis*, and *R. osteochir* each were comprised of four morphotypes; *D. multistriatus* was represented by six morphotypes; and *A. thazard*, *C. sloani*, *C. equiselis*, *I. platypterus*, *N. nasutus*, and *Leptostomias* sp. each contained two morphotypes. The different morphotypes within species each represented a different stage of development of the same species. The remaining thirty-two morphotypes were species represented by only one stage (or morphotype), giving a total of 42 taxa that were recognized by the DNA barcodes. These results confirm the value of this technique for the identification of fish eggs.

In this work, we reported a 46.6% success rate in sequencing. This reflects the added difficulties in obtaining amplicons from eggs, as opposed to adults or larvae. These problems could be caused by several possible factors, such as poor fixation, storage time, egg handling, or the presence of oil globules that could interfere with the polymerase chain reaction. The exact cause will require further investigation.

Of the 33 Linnaean species reported here, there were no existing data about the early developmental stages in the Mexican Caribbean for nine species: *C. danae*, *C. sloani*, *D. multistriatus*, *L. guttatus*, *L. lacepede*, *P. occidentalis*, *P. prometheus*, *R. osteochir*, and

Synagrops bellus (Schmitter-Soto et al. 2000; Lavett 2003; Valdez-Moreno et al. 2010; Muhling et al. 2013).

The eggs that were identified as belonging to *Benthodesmus* using the BOLD system showed 100% sequence identity to Simony's frostfish (*B. simony*), elongate frostfish (*B. elongates*), and slender frostfish (*B. tenuis*). The latter is the only species that had been previously recorded in the Western Central Atlantic (WCA) (Carpenter 2002). Recently, however, *B. simonyi* was recorded from Panama, Nicaragua, Portugal, and one larva was collected from near Punta Allen, Quintana Roo, as determined using sequences from BOLD (Valdez-Moreno et al. 2010). The confusion with the taxonomy of these three species means that at present, it is not possible to assign these eggs to any species, and doing so will require a more detailed analysis of the entire genus.

A similar situation exists with *Brama* eggs that matched two different taxa (with 98.89% similarity or more), bigtooth pomfret (*B. orcini*) and lesser bream (*B. dussumieri*). The latter is distributed in the WCA (Carpenter 2002). Furthermore, while it was also recently recorded in BOLD for the Mexican Caribbean, these sequences came from larvae not adults; therefore, identification of individuals using these data may not be reliable.

One egg, identified as the driftfish (*Ariomma melanum*), was found at station 90, close to Mahahual. In 2006, we barcoded a larva (MFLVI3570) from this species collected from an area close to Isla Mujeres, north of the Yucatan Peninsula, confirming the presence of this fish in this area (see project "Fish larvae of Mexico VI" in www.boldsystems.org).

A. melanum is listed as having been identified in the Western Atlantic from New York, the northern Gulf of Mexico to Panama (Robins and Ray 1986), extending up to Uruguay (Figueiredo et al. 2002). Although its presence is expected in the Mexican Caribbean, it has not been reported here before, and thus our work presents a new record for this region. The discovery of this egg suggests that the south of the Mexican Caribbean was once a spawning area for this species.

Eggs identified as belonging to *Decapterus* matched the species mackerel scad (*D. macarellus*) (99.54-100%) and roughear scad (*D. tabl*) (99.54-99.69%). However, in order to establish their correct taxonomic identity, it will be necessary to review all the specimens from both species in more detail.

Three specimens were identified as belonging to the genus *Leptostomias*. Twelve *Leptostomias* species have currently been reported worldwide (Froese and Pauly 2014). In BOLD, there are sequences for scaleless dragonfish (*L. gladiator*, *L. longibarba*), and nine more specimens not identified to species level. Six species are known to exist in the WCA: *L. gladiator*, *L. longibarba*, *L. analis*, *L. bermudensis*, *L. bilobatus*, and *L. haplocaulus* (Carpenter 2002). Our specimens most likely belong to one of the latter four that still have no sequence.

To date, twenty-three species of *Peristedion* have been described (Froese and Pauly 2014); eleven are distributed in the WCA (Carpenter 2002), seven of which are represented in BOLD. The remaining four, longnose armored searobin (*P. antillarum*) and flathead

searobin (*P. brevirostre*, *P. longispatha*, and *P. unicuspis*), are not present in the database, and therefore, our sequence could belong to one of these species.

The *Trachipterus* egg showed sequence identity with dealfish (*T. arcticus*) and blackflash ribbonfish (*T. jacksonensi*) (with 98.4-99.8% similarity). The latter is distributed in the WCA (Carpenter 2002), Western Indian Ocean, Southern Australia, Southwest Pacific, and Southwest Atlantic: Brazil and Argentina (Froese and Pauly 2014; Robins and Ray 1986; Ward et al. 2008). The former is distributed in the Northeast Atlantic. According to Fish Base, the Western Atlantic populations are most likely to be a separate species (Froese and Pauly 2014). For this reason, we were only able to assign our sample to a genus.

We found six eggs belonging to *Chauliodus*. After barcode analysis, they could be divided into two groups with greater than 3% divergence. The first group comprised five eggs (MXFEC52, MXFEC95, MXFEC183, MXFEC110, and MXFEC201) that were identified as *C. sloani*. The second group, represented by a single specimen (MXFEC022), was identified as *Chauliodus* sp. The species *C. sloani* is broadly distributed, and BOLD data indicate 16 published records are forming three BINs. These findings indicate that there are more than one species of *Chauliodus*, which was not previously known.

Five species were identified at the egg stage, all of which were previously identified in MBRS (Valdez-Moreno et al. 2010): yellow jack (*Carangoides bartholomaei*), white grunt (*Haemulon plumieri*), hogfish (*Lachnolaimus maximus*), flathead grey mullet (*Mugil cephalus*), and ocean surgeon (*Acanthurus bahianus*). These species, plus the 33 species

reported here, making a total of 38 species, were confirmed by DNA barcoding to spawn in the Mexican Caribbean and surrounding waters. This represents approximately 6.7% of the 552 fish species reported in this area (Schmitter-Soto et al. 2000).

At the time of collection, some eggs were unfertilized, and others were fertilized. The majority were approximately at the same developmental stage, except for three species, *K. albida* (Fig. S11)⁵, *K. pelamis* (Fig. S12)⁶, and *R. osteochir* (Fig. S13)⁷. For these species, we observed differences in embryo morphology, as well as the tone and distribution of pigments, between the different stages.

We recorded the developmental stage of these three species, as well as the day of collection and the station from which the eggs were collected. We found that the less-developed stages were found in the southern stations (78, 79, 82 and 83), whereas the most-developed stages were found in the northern stations (90, 93 and 95). We were able to draw some interesting conclusions from these data. First, it seems likely that spawning occurred in Belizean waters, and the eggs were transported northward by strong currents in this area (Muhling et al. 2013; Carrillo et al. 2015). Another possibility is that the spawning zones were in the coastal area of Xcalak and the vicinity of Banco Chinchorro, where spawning aggregations sites have been documented (Aguilar-Perera and Aguilar-Dávila 1996; Sosa et

⁵ Fig. S11. Photographs showing differences in the morphology of eggs *Kajikia albida* collected from different stations.

⁶ Fig. S12. Photographs showing differences in the morphology of eggs of *Katsuwonus pelamis* collected from different stations.

⁷ Fig. S13. Photographs showing differences in the morphology of *Remora osteochir* collected from different stations.

al. 2009). In both cases, egg hatching most likely would occur in the northern part of the Yucatan Peninsula. The timing of egg release and the likely trajectories caused by the currents is outside the scope of the present work; however, it would be an interesting subject for future investigation.

New information about spawning locality and time was obtained from egg records (see www.boldsystems.org) for saucereye porgy (*Calamus calamus*), dana viperfish (*Chauliodus danae*, *C. sloani*, *D. multistriatus*), snake mackerel (*Gempylus serpens*), buffalo trunkfish (*Lactophrys trigonus*), opah (*Lampris guttatus*, *L. lacepede*, *N. nasutus*, *P. arenatus*), bluntnose flyingfish (*Prognichthys occidentalis*), roudi escolar (*Promethichthys prometheus*), Atlantic fanfish (*Pterycombus brama*), marlin sucker (*Remora osteochir*), short jaw lizardfish (*Saurida normani*), and diamond lizardfish (*Synodus synodus*).

These data are critical for the protection of nurseries and spawning areas, especially for species of economic or game value (Richards 1985; Kawakami et al. 2010; Gleason and Burton 2012), such as *A. thazard*, *Caranx hippos*, *C. hippurus*, *I. platypterus*, *Kajikia albida*, *K. pelamis*, *Thunnus atlanticus*, and *Xiphias gladius* (Herrera-Pavon 2011; Muhling et al. 2013; Perez-Cobb 2014). This information is important because if we take into account the fact that most eggs remain in the water column for less than 24-48 hours after spawning (Richards et al. 2006), we will have a better idea about the closed seasons for these species. Nonetheless, further studies will be required to determine if these fish spawn during more than one season of the year.

The sampling area used in the present study is influenced by the Caribbean circulation system (Muhling et al. 2013; Carrillo et al. 2015), and it has been suggested that there may be connectivity among all the MBRS (Muhling et al. 2013; Heyman and Kjerfve 2008). Therefore, the presence of eggs from species that had not previously been recorded in this region could be caused either by advection from currents or by adults migrating to these places to spawn. Deeper and more systematic oceanographic studies will be required to track the course of the eggs.

Our results provide fundamental evidence for fisheries management, allowing for more focus on conservation efforts in breeding areas. The information on egg distribution and possible spawning time will contribute to dispersal and connectivity studies that will support the sustainable use of the Mesoamerican Reef System. This information is crucial because these species could be more vulnerable at early developmental stages than at other stages, something that should be tested with more fieldwork and continuing research in the area.

Finally, it is important to mention that these types of studies will reduce the complexity of laboratory work as it would no longer be necessary to reproduce natural conditions to hatch eggs and rear larvae to identifiable stages.

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Figure captions

Figure 1. Location of sample stations.

Figure 2. Neighbor-joining tree. The tree is based on genetic distances (K2P) of the COI gene. The height of each triangle represents the number of specimens sequenced, while the depth represents the genetic diversity present within each cluster.

Figure 3. Egg species richness by station. Circle size indicates number of taxa per station. The number of taxa was estimated based in the identifications made in BOLD, including species bearing a Linnaean name and those currently recognized only by a BIN and higher-level identification.

Figure 4. Distribution of fish eggs from the most common species, A) *Nesiarchus nasutus*, B) *Diplospinus multistriatus*, and C) *Regalecus glesne*.

Table

Table I. **Morphological features of the studied eggs for 21 species.**

* Species with eggs with different stage.

Species	Sample size	Shape	Diam (mm)	Chorion	Perivitelline space	Yolk	Oil globule	Pigmentation	Embryo	Observations
<i>Ariomma melanum</i>	4	Spherical	1	Smooth	Narrow	Homogeneous	Absent	None	None	There is a circle in the chorion.
<i>Calamus calamus</i>	8	Spherical	1	Smooth and transparent	Narrow	Homogeneous	Absent	Black spots in the head of the embryo.	Large, with a wide head.	Fertilized eggs, with an intussusception in the yolk sac.
<i>Caranx hippos</i>	2	Spherical	0.5	Smooth and transparent	Narrow	Homogeneous	Absent	None	None	Unfertilized egg
<i>Chauliodus danae</i>	2	Spherical	1.7	Smooth and transparent	Undefined	Undefined	Absent	None	None	Unfertilized egg
<i>Decapterus punctatus</i>	7	Spherical	0.5	Smooth and transparent	Narrow	Homogeneous	Absent	None	None	Unfertilized egg
<i>Diplospinus multistriatus</i>	54	Spherical	1-1.1	Smooth and transparent	Narrow	Homogeneous	Some	Stains in the head and some around. 1 spot more in the tail.	Slim, in some cases with caudal fin forming.	Fertilized eggs, with an intussusception in the yolk sac with pigments inside.
<i>Gempylus serpens</i>	11	Spherical	0.8	Smooth and transparent	Narrow	Homogeneous	Absent	None	None	Unfertilized eggs
<i>Kajikia albida</i> *	3	Spherical	0.6-1	Smooth and transparent	Narrow	Homogeneous	Some	In the dorsal part of the embryo, and the yolk sac. The pigments are tenuous.	Short and thin, head just being formed. Still without eyes formation.	Fertilized eggs with an intussusception in the yolk sac, with pigments inside.

<i>Katsuwonus pelamis</i> *	6	Spherical	0.9-1.1	Smooth and transparent	Narrow	Homogeneous	Some	Dark, asterisk-shaped pigments, focus on the dorsal part of the embryo.	Could not appreciate the head. Thicker body which tapers towards the tail.	With an intussusception in the yolk sac, with pigments inside.
	2	Spherical	0.9-1	Smooth and transparent	Medium	Homogeneous	Absent	None	None	No fertilized
	5	Spherical	0.9	Smooth and transparent	Medium	Homogeneous	Absent	Black and circular pigments in the dorsal part of the embryo.	Could not appreciate the head and tail because all body have almost the same thickness.	The eggs have an intussusception in the yolk sac with black pigments inside.
	124	Spherical	0.8	Smooth and transparent	Medium	Homogeneous	Absent	The pigments bind forming spots in the dorsal part of the embryo.	Large, the head and tail almost touch. Head slightly thicker than the rest of the body.	The eggs have an intussusception in the yolk sac with black pigments inside.
<i>Lactophrys trigonus</i>	4	Spherical	1.3	Smooth and transparent	Narrow	Homogeneous with black spots	Absent	Pigments brown color in the embryo and the yolk.	Wide head with eyes is forming.	Fertilized egg, with an intussusception in the yolk sac.
<i>Nesiarchus nasutus</i>	451	Spherical	0.5-0.7	Transparent and wrinkled	Narrow	Homogeneous	Absent	None	Embryo short and with the same thickness along of the	Fertilized and unfertilized eggs, with an intussusception in the yolk sac.

									body.	
<i>Priacanthus arenatus</i>	1	Spherical	0.6	Transparent and wrinkled	Medium	Homogeneous	Absent	Black spots in the dorsal part of the embryo.	Newly formed, is not enough to distinguish the head.	Fertilized egg, with an intussusception in the yolk sac with pigments inside. There is a circle in the chorion with black spots.
<i>Prognichthys occidentalis</i>	6	Spherical	1.8-2	Transparent, with short cilia scattered	Narrow	Homogeneous	Absent	None	Wide and a big head with eyes forming.	Fertilized and unfertilized eggs.
<i>Promethichthys prometheus</i>	4	Spherical	0.8	Transparent and wrinkled	Medium	Homogeneous	Absent	Some in the head of the embryo.	Slim, with the head forming.	Fertilized eggs, Apparently with a double membrane.
<i>Pterycombus brama</i>	3	Spherical	1	Clear and wrinkled	Narrow	Homogeneous	Some	None	Slim, with the head forming.	Fertilized eggs
<i>Remora osteochir*</i>	2	Spherical	1.2-1.3	Smooth and clear	Narrow	Homogeneous	Some	Faint brown points mainly in the dorsal part of the embryo.	Thin, just being forming.	Invagination not yet seen in the yolk sac.
	2	Spherical	1.2	Smooth and clear	Narrow	Homogeneous	Some	Faint black points mainly in the dorsal part of the embryo and some in the yolk sac.	Thin, the head and eyes begin to show.	There is an intussusception in the yolk sac with faint black points around.
	2	Spherical	1-1.2	Smooth and clear	Narrow	Homogeneous	Some	Points black in the dorsal part of the embryo and around in the yolk sac.	Large, the big head and tail meet. The head and eyes are	There is an intussusception in the yolk sac, with black points inside.

									formed.	
<i>Saurida normani</i>	1	Spherical	1.2	Smooth and whitish	Narrow	Homogeneous	Absent	None	Thin, just forming	Fertilized egg
<i>Scombrobrax heterolepis</i>	1	Spherical	0.8	Clear and wrinkled	Narrow	Homogeneous	Absent	None	None	Unfertilized egg
<i>Synagrops bellus</i>	1	Spherical	0.7	Smooth and transparent	Narrow	Homogeneous	Absent	None	None	Unfertilized egg
<i>Synodus synodus</i>	34	Spherical	0.8-0.9	Ornate with hexagon shaped figures	Narrow	Homogeneous	Absent	None	Frontal part (head and neck) thickness and the rest of embryo thin.	Fertilized eggs, Embryo similar to <i>S. intermedius</i> .
<i>Thunnus atlanticus</i>	14	Spherical	0.7-1.5	Smooth and unclear	Narrow	Homogeneous	Some	None	Embryo is just forming.	Mostly unfertilized eggs, only 1 fertilized egg.
<i>Trachinotus falcatus</i>	1	Spherical	1	Smooth and unclear	Narrow	Homogeneous	Absent	Faint pigments brown color in the dorsal part of the embryo.	Eyes and head forming, body and head with the same thickness.	Fertilized egg, with an intussusception in the yolk sac, with brown, faint spots inside.

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Figure 1. Location of sample stations.
180x124mm (300 x 300 DPI)

Figure 2. Neighbor-joining tree. The tree is based on genetic distances (K2P) of the COI gene. The height of each triangle represents the number of specimens sequenced while the depth represents the genetic diversity present within each cluster.
226x283mm (300 x 300 DPI)

Figure 3. Egg species richness by station. Circle size indicates number of taxa per station. The number of taxa was estimated was based in the identifications made in BOLD, including all, not only the species with Linnean name.
180x124mm (300 x 300 DPI)

Figure 4. Distribution of fish eggs from the most common species, A) *Nesiarchus nasutus*, B) *Diplospinus multistriatus*, and C) *Regalecus glesne*.
180x381mm (300 x 300 DPI)

Figure S1. Neighbour-joining tree (K2P distance) of 139 COI sequences from 42 marine fish eggs species.

Figure S2. *Calamus calamus* (A, B, C) *Caranx hippos* (D), *Chauliodus danae* (E), *Decapterus punctatus* (F). In the pictures with increase in 4x, measuring slide equals 0.95 mm and with increase of 10x equals 0.37 mm.

279x361mm (300 x 300 DPI)

Figure S3. *Diplospinus multistriatus* (A, B, C), *Gempylus serpens* (D), *Kajikia albida* (E, F). In figures with increase 4x, the measuring slide equals 0.95 mm.

215x279mm (300 x 300 DPI)

Figure S4. *Lactophrys trigonus* (A, B, C), *Nesiarchus nasutus* (D, E, F). In figures with increase of 4x, the measuring slide equals 0.95 mm and with an increase of 10x equals 0.37 mm.

215x279mm (300 x 300 DPI)

Figure S5. *Priacanthus arenatus* (A, B), *Prognichthys occidentalis* (C) *Promethichthys prometheus* (D, E, F). In figures with increase 4x, the measuring slide equals 0.95 mm.

215x279mm (300 x 300 DPI)

Figure S6. *Pterycombus brama* (A, B, C) and *Remora osteochir* (D, E). In images with increase 4x, the measuring slide equals 0.95 mm.

215x279mm (300 x 300 DPI)

Figure S7. *Saurida normani* (A), *Scombrobrax heterolepis* (B), *Synagrops bellus* (C) and *Synodus synodus* (D, E, F). In images with increase 4x, the measuring slide equals 0.95 mm and an increase of 10x equals 0.37 mm.

215x279mm (300 x 300 DPI)

Figure S8. *Thunnus atlanticus* (A), *Trachinotus falcatus* (B, C) *Ariomma melanum* (D). In figures with increase 4x the measuring slide equals 0.95 mm.

147x190mm (300 x 300 DPI)

Figure S9. *Auxis thazard* (A), *Coryphaena equiselis* (B), *Coryphaena hippurus* (C), *Istiophorus platypterus* (D) and *Katsuwonus pelamis* (E, F) without oil globules and the two latter species with an intussusception in the yolk sac.

215x279mm (300 x 300 DPI)

Figure S10. *Chauliodus sloani* (A) with wide perivitelline space and without double membrane. *Lophotus lacepede* (B) without spines, *Regalecus glesne* (C, D) with small projections and without oil globules and pigments. *Synodus intermedius* (E, F) with pigments in the head and without ornate corion.

215x279mm (300 x 300 DPI)

Figure S11. The images are from species *Kajikia albida*, pictures show the embryo with different level of development. Egg in picture A was found in station 78, B and C in station 88 and D in station 79.

215x279mm (300 x 300 DPI)

Figure S12. The figures are eggs from species *Katsuwonus pelamis*, each image shows the embryo with different level of development. Egg in the figure A was found in station 79, egg in the figure B in station 95, C and D in station 82, and E and F in station 93

215x279mm (300 x 300 DPI)

Figure S13. The figures are from the species *Remora osteochir*, each image shows the eggs with different level of development. Figures A, B, C are from station 83, D is from the station 84, E is from station 95 and F from station 90.

215x279mm (300 x 300 DPI)