Check for updates

Received: 6 December 2019 | Revised: 10 April 2020 | Accepted: 13 April 2020

DOI: 10.1111/fog.12475

ORIGINAL ARTICLE

FISHERIES OCEANOGRAPHY WILEY

DNA barcoding of fish eggs collected off northwestern Cuba and across the Florida Straits demonstrates egg transport by mesoscale eddies

Makenzie Kerr[1] | Jeremy Browning[1] | Eva-Maria Bønnelycke[1] | Yingjun Zhang[1] | Chuanmin Hu[1] | Maickel Armenteros[2,3] | Steven Murawski[1] | Ernst Peebles[1] | Mya Breitbart[1]

[1]College of Marine Science, University of South Florida, Saint Petersburg, Florida

[2]Centro de Investigaciones Marinas, Universidad de La Habana, Habana, Cuba

[3]Universidad Nacional Autónoma de México, Ciudad de México, México

Correspondence
Makenzie Kerr, College of Marine Science, University of South Florida, Saint Petersburg, FL, USA.
Email: burrowsm@usf.edu

Funding information
The Gulf of Mexico Research Initiative/C-IMAGE II, Grant/Award Number: No. SA 15-16; Florida RESTORE Act Centers of Excellence Program, Grant/Award Number: 4710-1126-02 and 4710-1126-03; National Aeronautics and Space Administration, Grant/Award Number: FINESST, 80NSSC19K1358

Abstract

Identifying spawning sites for broadcast spawning fish species is a key element of delineating critical habitat for managing and regulating marine fisheries. Genetic barcoding has enabled accurate taxonomic identification of individual fish eggs, overcoming limitations of morphological classification techniques. In this study, planktonic fish eggs were collected at 23 stations along the northwestern coast of Cuba and across the Florida Straits to United States waters. A total of 564 fish eggs were successfully identified to 89 taxa within 30 families, with the majority of taxa resolved to species. We provide new spawning information for *Luvarus imperialis* (Louvar), *Bothus lunatus* (Plate Fish), *Eumegistus illustris* (Brilliant Pomfret), and many economically important species. Data from most sites supported previously established patterns of eggs from neritic fish species being found on continental shelves and oceanic species spawning over deeper waters. However, some sites deviated from this pattern, with eggs from reef-associated fish species detected in the deep waters of the Florida Straits and pelagic species detected in the shallow, continental shelf waters off the coast of northwestern Cuba. Further investigation using satellite imagery revealed the presence of a mesoscale cyclonic eddy that likely entrained neritic fish eggs and transported them into the Florida Straits. The technique of combining DNA-based fish egg identification with remotely-sensed hydrodynamics provides an important new tool for assessing the interplay of regional oceanography with fish spawning strategies.

KEYWORDS

barcoding, Cuba, fish egg, Florida Straits, genetic, reef-associated, spawning

1 | INTRODUCTION

Understanding the life cycle of fish species, including their spawning locations and early life history, is necessary for identifying critical habitats and supporting future fisheries management (Claro, Lindeman, & Parenti, 2014). Due to the difficulty in accurately identifying fish eggs based on morphology, many studies have inferred spawning locations of a given species based on the presence of larvae (Limouzy-Paris, McGowan, Richards, Umaran, & Cha, 1994; Peebles & Tolley, 1988; Sassa, Konishi, & Mori, 2006). However, fish larvae can be days to months old before capture (Cowen & Sponaugle, 2009); therefore, using larval-fish collection locations to hindcast spawning locations may result in considerable uncertainty. Variation in pelagic larval duration and larval behaviors,

including vertical migration (Vikebø, Jørgensen, Kristiansen, & Fiksen, 2007) along with variation in surface-water transport, contribute to this uncertainty. Alternatively, fish eggs are typically only hours old before hatching into larvae, and eggs behave as relatively passive particles once they have floated to surface waters, reducing the error in predicting spawning location using hydrodynamic models (Harada et al., 2015). The only study to directly compare the species composition of fish eggs and larvae from the same location (Terra Ceia Bay, Florida, USA) demonstrated different species in each of these early life stages, weakening the value of using larvae for spawning site prediction (Burghart et al., 2014).

Broadcast spawning is the most common reproductive strategy among reef fishes (Thresher, 1984), resulting in egg dispersal in the water column, where the majority of eggs float to the surface due to positive buoyancy (Fabra, Raldúa, Power, Deen, & Cerda, 2005). Identification of drifting fish eggs collected with plankton nets, combined with hydrographic modeling of surface currents, can therefore be used to hindcast spawning locations of fish taxa. Historically, fish eggs have been identified based on color, size of perivitelline space, oil globules, yolk, and shape. But there are very few distinguishable characteristics and continuous ontogenetic changes make visual discrimination among taxa ambiguous (Kawakami, Aoyama, & Tsukamoto, 2010). One exception is for clupeoid (anchovy, herring, and sardine) eggs, which are easily distinguished from percomorph (spiny-finned fishes) eggs based on their shape and size. Additionally, some visual features used for identification, including coloration, can be lost during the preservation process (Smith, McVeagh, Allain, & Sanchez, 2005). Previous studies comparing visual identifications of percomorph eggs with results from genetic barcoding have shown that visual identification is unreliable (Larson et al., 2016). In contrast, genetic barcoding of the mitochondrial cytochrome *c* oxidase I (COI) gene allows for the identification of fish eggs, often to species (Ahern et al., 2018; Burrows, Browning, Breitbart, Murawski, & Peebles, 2019; Duke, Harada, & Burton, 2018). The Barcode of Life Database (BOLD; http://www.boldsystems.org/) contains reference sequences for over 20,000 ray-finned fish species (Actinopterygii), serving as an excellent community-driven resource for the identification of fish sequences (Ward, Hanner, & Hebert, 2009). Furthermore, DNA barcoding of individual fish eggs allows for a quantitative measure of the relative abundance of eggs contributed by each species, from a given multispecies sample (Burrows et al., 2019).

We used DNA barcoding to determine which broadcast-spawning fish species spawn in Cuban (all stations south of station F6) and United States (U.S.) waters of the southeastern Gulf of Mexico (GoM). The northwestern Cuba region has a broad continental shelf in the western part (Gulf of Guanahacabibes) and a very narrow shelf in the eastern part (Punta Gobernadora to Habana bay). The continental slope extends from the shelf break (~35 m depth) to about 1000 m with a very steep slope and then to about 2000 meters with a gentle slope (Ionin, Pavlidis, & Avello, 1977). There are well-developed tropical habitats (e.g., mangrove, seagrass meadows, and shallow and mesophotic reefs) supporting over 1,000 fish species or subspecies (Claro et al., 2014). Cuba also has many commercially important finfish species including tunas (e.g., *Thunnus atlanticus*, *Katsuwonus pelamis*), Swordfish (*Xiphias gladius*), snappers (Lutjanidae), grunts (Haemulidae), jacks (Carangidae), and groupers (Serranidae). One experiment in the Florida Straits observed the spawning of *Istiophorus platypterus* (Sailfish) to study the relationship between physical processes and larval development (Richardson et al., 2009). In another study, the species composition of fish eggs found on and off the continental shelves of the GoM showed a clear delineation of neritic species spawning on the continental shelves and oceanic species spawning in deeper waters (Burrows et al., 2019). The present study aims to add new spawning information for species in the southeastern GoM. This area has a very steep shelf slope, dynamic physical processes, and acts as the major exit area for the surface circulation of the entire GoM through the Florida Straits, making it an interesting study area for observing fish egg distributions.

2 | METHODS

2.1 | Study site and sample collection

Planktonic fish eggs were collected at 23 stations across the Florida Straits and along the northwestern coast of Cuba with a 333 μm mesh bongo net, towed at the surface for 15 min from the *RV Weatherbird II* (http://www.fio.usf.edu/vessels/rv-weatherbird) in May 2017 (Figure 1, Table 1). One of the two bongo net samples was preserved immediately with 30% isopropanol and returned to the laboratory.[1] Plankton samples from sites F1-F9, C7, C8, C10, C11, and C14 were held in the original preservative (30% isopropanol) for two months, while sites C1-C6, C9, C12, and C13 were held in the original preservative for over a year. The percomorph eggs were then picked from samples with forceps under a stereomicroscope at 9–108X magnification. Egg density for each site was calculated as the total number of eggs in the sample divided by the volume of water filtered by the plankton net (determined via flowmeter). A subsample of ≥96 percomorph eggs from each collection was placed into a glass vial with 50% isopropanol until DNA extractions were performed on individual eggs.

2.2 | Genetic identification

Using a sterile pipette tip, individual fish eggs were placed in 0.2 ml polymerase chain reaction (PCR) tubes, and excess isopropanol was removed. DNA extractions were performed using the HotSHOT method (Truett et al., 2000). To lyse the eggs, 50 μl of alkaline lysis buffer (0.2 mM disodium EDTA, 25 mM NaOH, pH 12) was added to each tube and each egg was crushed with a sterile toothpick.

[1]The intended method of preservation was 70% isopropanol, but there was a miscommunication in the protocol.

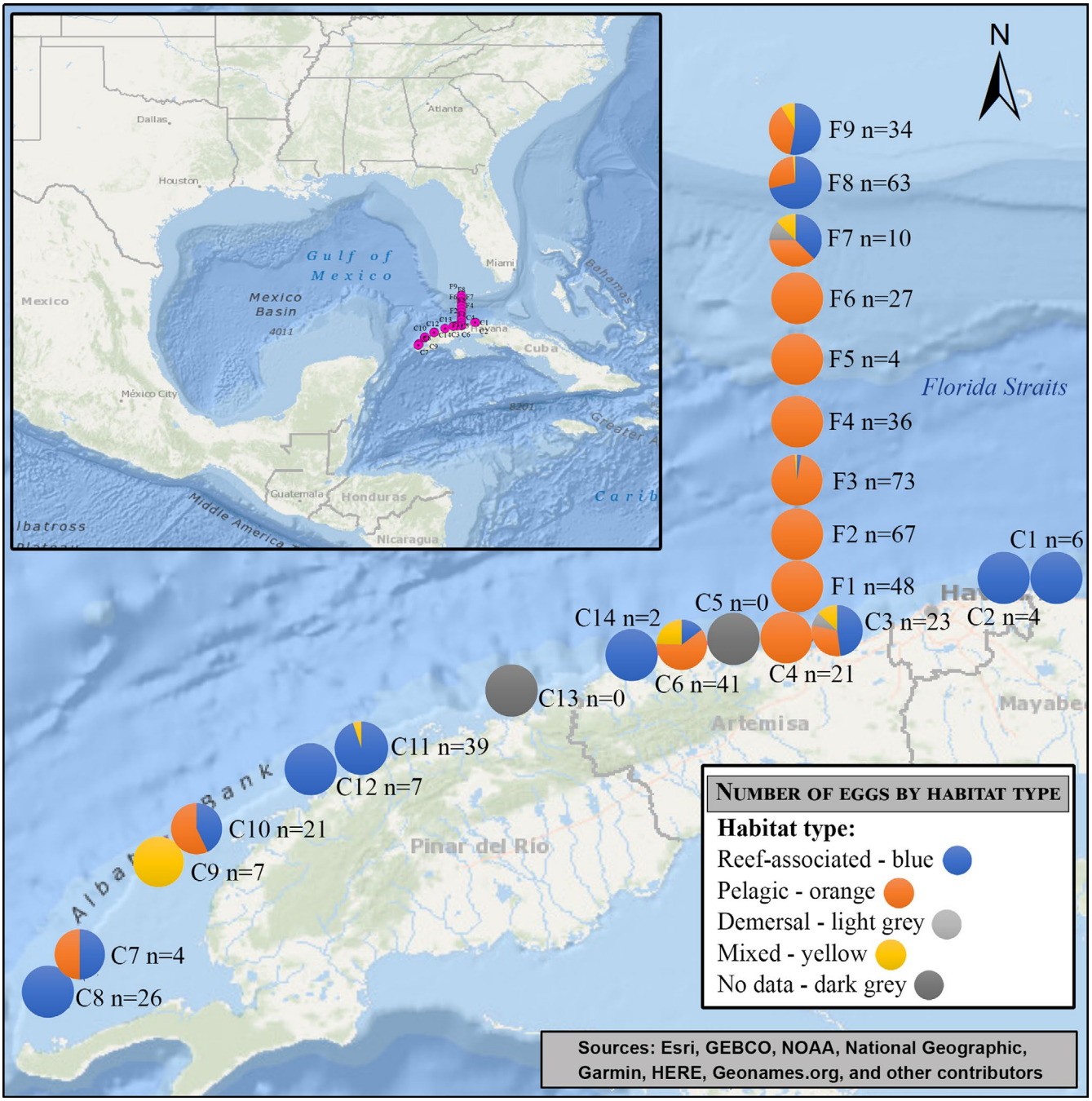

FIGURE 1 Map of bongo net deployment stations with number of fish eggs by habitat type from May 2017. C, Cuba; F, Florida Straits. Raw data are available in Table 1 and Table S1. Mixed stations are those with taxa not identified down to species level, so their habitats are either pelagic, reef-associated, or demersal. The total number of eggs per station represents the amount of eggs successfully barcoded. Map made in ArcGIS [Colour figure can be viewed at wileyonlinelibrary.com]

All PCR tubes were set in a thermocycler at 95°C for 30 min and then moved onto ice for three minutes to cool to room temperature. To complete the extraction, 50 µl of neutralization buffer (40 mM Tris-HCL, pH 5) was added and the samples were vortexed to mix thoroughly.

The PCR technique was used to amplify a portion of the mitochondrial COI gene with the COI-3 universal fish primer cocktail (Ivanova, Zemlak, Hanner, & Hebert, 2007). Each 50 µl PCR reaction contained 2 µl of DNA template and final concentrations of 1X Apex NH_4 buffer, 1.5 mM Apex $MgCl_2$, 10 µg/µl bovine serum albumin (New England BioLabs Inc.), 0.2 µM Apex dNTPs, 0.2 µM primer cocktail, and 1 U Apex RedTaq® (Genesee Scientific). The thermocycling protocol consisted of heating to 94°C for 2 min, 45 cycles of (94°C for 30 s, 52°C for 40 s, 72°C for 1 min), followed by extension at 72°C for 10 min. To confirm successful amplification, the PCR products were run on a 1.5% agarose gel (60 min, 120 V) and stained with ethidium bromide for visualization. Successful PCR products were sent to TACGen (tacgen.com) for purification

TABLE 1 Cruise information (sampling date, depth, latitude, and longitude) of the 23 sampled stations and the total egg density at each station

Station	Date (YYMMDD)	Depth (m)	Latitude	Longitude	Total egg density (eggs/m^3)
C1	170513	223	23.19°N	82.08°W	7.36
C2	170513	292	23.19°N	82.11°W	4.52
C3	170514	165	23.03°N	82.76°W	0.62
C4	170514	464	23.04°N	82.75°W	0.23
C5	170515	416	23.02°N	83.02°W	3.38
C6	170515	268	23.03°N	82.97°W	0.33
C7	170518	782	22.16°N	84.81°W	0.02
C8	170518	252	22.10°N	84.85°W	Not calculated*
C9	170519	255	22.45°N	84.53°W	0.07
C10	170519	660	22.49°N	84.53°W	0.12
C11	170521	501	22.73°N	84.07°W	9.44
C12	170521	122	22.71°N	84.09°W	2.22
C13	170522	286	22.91°N	83.56°W	6.76
C14	170523	350	23.00°N	83.16°W	3.51
F1	170524	1665	23.17°N	82.77°W	0.18
F2	170524	1996	23.34°N	82.77°W	0.34
F3	170524	1764	23.50°N	82.77°W	0.22
F4	170524	1594	23.67°N	82.77°W	0.07
F5	170524	1774	23.83°N	82.77°W	0.02
F6	170524	865	23.99°N	82.77°W	0.09
F7	170525	667	24.17°N	82.76°W	0.06
F8	170525	113	24.35°N	82.75°W	4.70
F9	170525	19	24.50°N	82.76° W	1.90

**Note:* The total egg density in sample C8 could not be calculated because there was excessive *Sargassum* in the sample.

Abbreviations: C, Cuba, F, Florida Straits.

and Sanger sequencing using the M13 forward primer (Ivanova et al., 2007). In order to differentiate between certain economically important species that are closely related, a second PCR was performed using the same PCR mixture described above with different primers. Primers L8562 and H9432 were used to differentiate between *Thunnus thynnus* and *Katsuwonus pelamis* (72 eggs), with the following thermocycler conditions: 2 min at 94°C, followed by 45 cycles of (94°C for 30 s, 50°C for 40 s, 72°C for 1.5 min), and 10 min at 72°C (Chow & Inoue, 1993). Primers LCOI 121 and HCOI 1199 were used to differentiate between *Scomberomorus cavalla* and *Acanthocybium solandri* (five eggs), with the following thermocycler conditions: 2 min at 94°C, followed by 45 cycles of (94°C for 30 s, 57°C for 40 s, 72°C for 2 min), and 10 min at 72°C (Paine, McDowell, & Graves, 2007). All PCR products were cleaned with a Zymo Clean & Concentrator −25 kit and sent for bidirectional Sanger sequencing at TACGen (tacgen.com).

2.3 | Data analysis

Sequencher™ 5.3 (Genecodes) was used to trim the DNA sequences for quality. Poor quality sequences were removed from further processing and considered unidentified. Trimmed sequences were compared with the species-level records on the Barcode of Life Database (BOLD; http://www.boldsystems.org/) for assignment to the lowest taxonomic level possible. If there was no match in BOLD, sequences were compared with the GenBank nucleotide database using the Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences with less than 97% identity to the GenBank database were considered unidentified. Information on fish common names, habitat, and economic importance was taken from FishBase (Froese, 2019).

2.4 | Satellite imagery

To provide context for the results, satellite imagery from the closest possible dates was examined for the region of interest. MODIS/A chlorophyll *a* data from May 10, 2017, were obtained from NASA (https://oceancolor.gsfc.nasa.gov) (Figure 2a). These 1-km resolution data were generated using the NASA standard algorithms for atmospheric correction and bio-optical inversion and were used in this study to visualize color patterns to infer circulations. The geostrophic velocity anomaly data with a spatial resolution of 1/4 × 1/4°

for May 24, 2017, (Figure 2b) and April 10, 2017 through July 25, 2017 (Supplemental Animation Appendix S1) were derived from the altimetry sea level anomalies and distributed by Copernicus Marine Environment Monitoring Service (CMEMS; http://marine.copernicus.eu/).

3 | RESULTS

3.1 | Fish eggs

The density of fish eggs at stations along the cruise transect ranged from 0.02 to 9.44 eggs/m^3 (Table 1). A total of 1,562 eggs from 23 sites were processed for DNA barcoding. Due to poor sample preservation (see Discussion), a large number of these eggs did not yield PCR products (n = 832) or high-quality sequences (n = 101). In addition, 65 sequences were most similar to invertebrates or had <97% identity to sequences in the BOLD or GenBank databases. The 564 fish eggs successfully identified included 89 taxa within 30 families. Fifty-eight of the taxa were identified to species level, 21 to genus, and the remaining 10 to family or higher.

Of the 89 fish taxa identified, 51 were associated with reefs or shallow water (reef-associated). Some of the most frequently encountered reef-associated species (Table S1) included *Acanthurus* spp. (surgeonfishes), *Haemulon* spp. (grunts), *Halichoeres* spp. (wrasses), *Lactophrys* spp. (trunkfishes), *Lutjanus* spp. (snappers), *Ocyurus chrysurus* (Yellowtail Snapper), *Sparisoma viride* (Stoplight Parrotfish), *Syacium papillosum* (Dusky Flounder), *Synodus* spp. (lizardfishes), *Thalassoma bifasciatum* (Bluehead Wrasse), and *Xyrichtys novacula* (Pearly Razorfish). Some of the most frequently detected oceanic species (Table S1) included *Decapterus* sp. (scad), *Katsuwonus pelamis* (Skipjack Tuna), *Psenes* spp. (driftfish), and *Thunnus* spp. (tunas). In general, reef-associated species dominated the fish eggs detected in nearshore waters, while pelagic species were found in deeper waters (Figure 1). A notable exception to this trend was station F7, which had eggs from a few reef-associated species in an area of deep water (667 m), leading to further investigation of oceanographic conditions.

3.2 | Oceanographic conditions

Satellite imagery was examined to determine whether any physical oceanographic features were associated with site F7, which yielded a mixture of eggs from reef-associated and pelagic species, despite being over deep waters. MODIS chlorophyll *a* concentration (May 10, 2017) and the geostrophic velocity anomaly field derived from sea level anomaly data (May 24, 2017) revealed a mesoscale cyclonic eddy in the Florida Straits near the time of egg collection. The eddy was persistent for 3 months from 18 April to 19 July 2017, which likely entrained waters from the West Florida Shelf (WFS) or northwestern coast of Cuba and moved drifting eggs offshore and into the Florida Straits south of the WFS (Figure 2b).

4 | DISCUSSION

This study presents genetic identification of fish eggs in the waters surrounding Cuba and across the Florida Straits, providing valuable spawning information for many species of commercial, recreational, and subsistence importance, including *Auxis* spp. (tunas), *Coryphaena* sp. (dolphinfish), *Decapterus* sp. (scad), *Epinephelus adscensionis* (Rock Hind), *Euthynnus alletteratus* (Little Tunny), *Istiophorus albicans* (Sailfish), *Katsuwonus pelamis* (Skipjack Tuna), *Thunnus* spp. (tunas), *Lutjanus* spp. (snappers), *Makaira nigricans* (Blue Marlin), *Scomberomorus cavalla* (King Mackerel), *Ocyurus chrysurus* (Yellowtail Snapper), *Trachinotus falcatus* (Permit), and *Xiphias gladius* (Swordfish). Several of the species that eggs were identified from were also captured as adults in a companion longline survey conducted on the same cruise and stations along the coast of Cuba, including *Gymnothorax moringa* (Spotted Moray), *Thunnus atlanticus* (Blackfin Tuna), *Ocyurus chrysurus* (Yellowtail Snapper), *Lutjanus analis* (Mutton Snapper), *Epinephelus adscensionis* (Rock Hind), and *Haemulon plumierii* (White Grunt) (Murawski, Peebles, Gracia, Tunnell, & Armenteros, 2018).

Additionally, this study identified eggs from species not previously known to spawn in this area, including *Luvarus imperialis* (Louvar; detected at station F9), *Bothus lunatus* (Plate Fish; detected

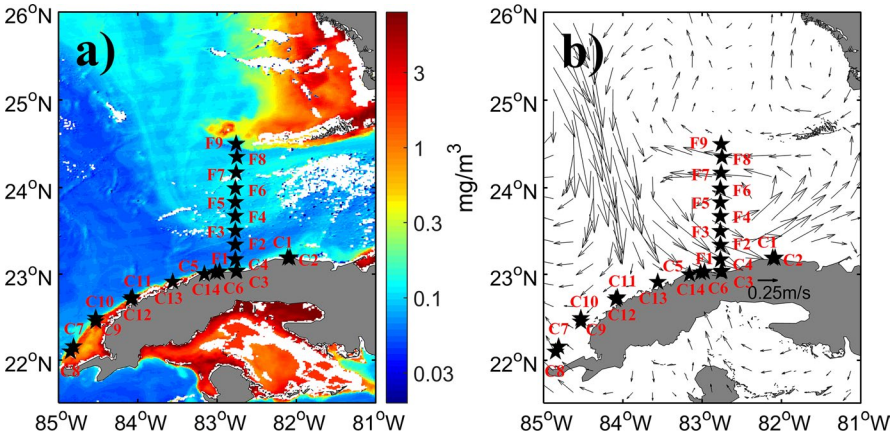

FIGURE 2 Mesoscale cyclonic eddy in the Florida Straits. (a) A snapshot of MODIS/Aqua chlorophyll *a* concentration on May 10, 2017 (white color means no data). (b) The geostrophic velocity anomaly field derived from sea level anomaly data on May 24, 2017. Fish egg collection stations are marked with black stars. Note that Figure 2b represents the time of collection (see Table 1) better than Figure 2a [Colour figure can be viewed at wileyonlinelibrary.com]

at station C6), and *Eumegistus illustris* (Brilliant Pomfret; detected at stations C4 and C6). There is little information on the spawning habits or the duration of larval development in each of these species; therefore, their detection in this region is of importance in evaluating regional biodiversity. Only adult specimens of Louvar have been documented in the GoM (Topp & Girardin, 1971), all from the western coast of Florida. Although gravid females were among the three specimens, our study is the first documented spawning location of Louvar in the GoM. *L. imperialis* larvae have been identified in the northwestern Pacific ocean near Japan (Nishikawa, 1987), and larvae, juveniles, and a single adult specimen with mature gonads were collected in the southwestern Atlantic ocean, near southern Brazil (Domingues, Montealegre-Quijano, Soto, & Amorim, 2015). Larval development in this species can take months, and larvae can thus travel great distances from spawning locations (Domingues et al., 2015). While little is known about the early life stages of *Bothus lunatus* (van der Veer, Cardoso, Mateo, Witte, & van Duyl, 2018), spawning has been observed near Bonaire, Netherlands Antilles (Konstantinou & Shen, 1995). Juvenile *Eumegistus illustris* of various sizes have been collected and studied from the Line Islands in Kiribati and waters near Japan and New Guinea (Moteki & Mundy, 2005; Okiyawa, 1989).

Determination of the location and timing of spawning for species is valuable for designing conservation strategies, including the creation of Marine Protected Areas (MPAs) and setting fishing strategies to minimize targeting of spawning aggregations. Temporary and permanent MPAs are chosen based on their conservation value and the presence of marine species of ecological and economic importance (Valderrama et al., 2018); protecting spawning sites can be a key component of ensuring sufficient protection of spawning aggregations. For example, one of the species we recovered eggs from, *Lutjanus analis* (Mutton Snapper), aggregates in May and June in Cuban waters (Claro et al., 2014). Lutjanidae is the most economically important fish family in Cuba, comprising 21% of total fish catches (Salas, Chuenpagdee, Charles, & Seijo, 2011). Many of these catches occur during spawning season because spawning aggregations can result in high catch rates as compared with non-spawning periods (Salas et al., 2011). Therefore, closing fishing seasons during times of spawning could decrease mortality of spawning adults and aid in fishery conservation (van Overzee & Rijnsdorp, 2015). Thus, a strategy for monitoring fish egg abundance and species composition seasonally and regionally may aid in identifying persistent spawning aggregations.

This study had a higher failure rate (64%) of DNA barcoding compared with previous studies (Ahern et al., 2018; Burrows et al., 2019; Harada et al., 2015; Lewis, Richardson, Zakharov, & Hanner, 2016; Leyva-Cruz, Vásquez-Yeomans, Carrillo, & Valdez-Moreno, 2016). This low success rate was most likely due to poor preservation technique, since the plankton tow biomass was stored in 30% isopropanol instead of the intended 70% isopropanol. The low isopropanol concentration and long-term storage prior to processing is known to negatively affect the long-term stability of DNA (Michaud & Foran, 2011). This degradation led to the higher failure rate (86%) in the second batch of samples processed (stations C1-C6, C9, C12 and C13; which were not extracted for over a year) compared with the 42% failure rate of the first batch of samples processed (stations F1-F9, C7, C8, C10, C11 and C14; which were extracted within two months of collection). A previous study showed that DNA degradation accelerates after six months of storage in alcohol solutions (Michaud & Foran, 2011). In future studies, proper preservation and rapid processing (no longer than 6 months after collection) should be considered a high priority. Due to the low success rates of DNA barcoding in the present study, emphasis should be placed on the positive detection of particular taxa, but the lack of detection does not imply absence of spawning of a given taxon.

In our previous work in the GoM, there was a clear delineation between neritic (largely reef-associated) fish species spawning on continental shelves and oceanic species spawning in deeper waters (Burrows et al., 2019). While this observation generally applied in this study as well, eggs from some reef-associated species were found in the deep water of the Florida Straits and eggs from pelagic-associated species were found on or near continental shelves, as seen in Figure 1. For example, fish eggs found at station C3 (165 m) were from pelagic, reef-associated, demersal, and mixed (taxa either pelagic or reef-associated) species and the eggs found at station C6 (near the shelf break; 268 m) were mostly pelagic species, possibly due to the steepness of the continental slope off the northwestern coast of Cuba (Claro et al., 2014) and onshore flow from the deep GoM (Figure 2). On the other hand, station C10 (660 m) had eggs from a few reef-associated species. Many eggs identified at station F7 belonged to reef-associated species but occurred in deeper waters (667 m) off the Florida coast (Figure 1). This finding is likely due to the presence of a cyclonic eddy off the coast of the Florida Keys at the time of egg collection (Figure 2). Based on the species observed at station F7, which was located toward the distal end of the jet of water, this eddy entrained eggs of *Lutjanus* sp. (snapper), *Diplectrum formosum* (Sand Perch), *Xyrichtys novacula* (Pearly Razorfish), and *Haemulon aurolineatum* (Tomtate Grunt), all species that are typically found in shallow Cuban waters (Claro et al., 2014) and other shallow GoM waters (Burrows et al., 2019).

The Florida Straits is a relatively narrow channel linking the GoM to the Atlantic Ocean through the Florida Current, which transports a large volume of seawater, ~30 Sv per year, at high speeds (Richardson, 2001). In addition to the fast-moving Florida Current, both mesoscale and sub-mesoscale eddies frequently occur in the area (Kourafalou & Kang, 2012; Lee, Leaman, Williams, Berger, & Atkinson, 1995; Shay, Lee, Williams, Graber, & Rooth, 1998). Based on long-term satellite ocean color measurements, Zhang, Hu, Liu, Weisberg, and Kourafalou (2019) revealed strong seasonality of mesoscale cyclonic eddy occurrence in the Florida Straits with the highest occurrence in the summer and lowest occurrence in the winter. These cyclonic eddies are highly productive ecosystems that are rich in nutrients, phytoplankton, and copepods (Hitchcock et al., 2005; Lee, Clarke, Williams, Szmant, & Berger, 1994) and can influence cross-shelf transport of fish larvae (Lane, Smith, Graber, & Hitchcock, 2003; Lee et al., 1992; Limouzy-Paris, Graber, Jones,

Röpke, & Richards, 1997; Shulzitski, Sponaugle, Hauff, Walter, & Cowen, 2016; Shulzitski et al., 2017; Sponaugle, Lee, Kourafalou, & Pinkard, 2005). Sponaugle et al. (2005) showed that larvae can be concentrated by mesoscale eddies and transported from the lower to upper Florida Keys. Our findings extend this observation by providing evidence of entrainment of eggs spawned by reef fishes on the WFS into the deep Florida Straits via a mesoscale eddy. Calculations based on an average egg longevity of 24 hr (Pauly & Pullin, 1988) and assuming an average speed of 1 m/s for the Florida Current (Kourafalou & Kang, 2012) estimate that fish eggs could travel 86 km prior to hatching into larvae. Therefore, it is feasible that the fish eggs identified at station F7 could have been transported from the WFS (about 81 km away following the inner circle of the eddy seen in Figure 2) by the mesoscale eddy observed in this region. In a second scenario, fish eggs could have been transported via the same eddy seen in Figure 2 from the northwestern Cuba coast (about 140 km away from station F7) if the eggs did not hatch for about 39 hr. Based on altimetry measurements, the cyclonic eddy observed in this study was present for about 3 months (from April 18, 2017 through July 19, 2017). The formation and disappearance of this eddy can be seen in the Supplementary Animation Appendix S1. In these scenarios, after the fish eggs hatch to larvae, they could be transported back to the continental shelf of southern Florida (Florida Keys) or be swept up closer to the eastern coast of Florida.

5 | CONCLUSION

This study provided novel information about reproduction of commercially and ecologically important species along the southwestern Florida coast, northwestern Cuban coast, and in the Florida Straits. For most stations, eggs from reef-associated species were found in relatively shallow waters close to continental shelves, while eggs from pelagic species were found in relatively deep waters off continental shelves. However, the mesoscale eddy observed in the Florida Straits suggested that oceanographic processes were able to transport reef-associated fish eggs away from spawning locations and into deeper waters, revealing a possible explanation for the detection of reef-associated species in deep waters. Such information can allow fisheries scientists to combine knowledge of biological and physical processes to better estimate the spawning locations and fate of fish early life stages. This pilot study demonstrates the power of these techniques for identifying critical habitats to conserve species of interest and defining the timing, locations, and interrelationships of fish spawning in the waters surrounding Florida and Cuba.

ACKNOWLEDGEMENTS

We gratefully acknowledge funds provided by a grant from The Gulf of Mexico Research Initiative/C-IMAGE II (No. SA 15-16) supporting the expedition of the R/V Weatherbird II to Cuba. The captain and crew of the ship provided invaluable logistical support. Additionally, the Ministerio de Relaciones Exteriores de Cuba (MINREX) and the U.S. Departments of Commerce, Treasury, and State provided export and import licenses for the expedition. The collection of samples in Cuban waters was made under the authorization AN(17)/2017 granted by the Centro de Inspección y Control Ambiental (CICA) from the Ministerio de Ciencia, Tecnología y Medioambiente (CITMA) of the República de Cuba. DNA barcoding was supported by grants 4710-1126-02 and 4710-1126-03 from the Florida RESTORE Act Centers of Excellence Program, with oversight by the US Treasury. M.K. was funded by the William T. Hogarth Fellowship in Marine Mammals and the Women in Leadership and Philanthropy Dorothy L. Morgan Endowed Fellowship for Marine Science, and Y.Z. was supported by a NASA student fellowship (FINESST, 80NSSC19K1358). Additionally, we would like to thank Julie Vecchio for her expert advice.

CONFLICT OF INTERESTS

The authors have no affiliation with organizations or entities that have financial or non-financial interest in the subject matter discussed in this manuscript.

AUTHOR CONTRIBUTIONS

S.M., E.P., M.B. and M.A. developed the project, S.M. and M.A. collected the samples, J.B. picked the eggs, M.K. and E-M.B. performed DNA barcoding, Y.Z. and C.H. analyzed satellite data, M.K. and Y.Z. made the figures, all authors discussed the results and contributed to the final manuscript.

ETHICAL APPROVAL

Consent was received for all research conducted in this manuscript, with all participants voluntarily involved. We ensure quality and integrity of the research involved, and we are independent and impartial to the results.

DATA AVAILABILITY STATEMENT

The sequence data have been deposited into GenBank under the accession numbers MN811696-MN812161 (www.ncbi.nlm.nih.gov/genbank).

ORCID

Makenzie Kerr https://orcid.org/0000-0003-0519-6076
Eva-Maria Bønnelycke https://orcid.org/0000-0002-6084-9378
Yingjun Zhang https://orcid.org/0000-0003-2079-5157
Chuanmin Hu https://orcid.org/0000-0003-3949-6560
Maickel Armenteros https://orcid.org/0000-0002-9736-9247
Steven Murawski https://orcid.org/0000-0001-7002-4356
Ernst Peebles https://orcid.org/0000-0003-1256-2345
Mya Breitbart http://orcid.org/0000-0003-3210-2899

REFERENCES

Ahern, A., Gómez-Gutiérrez, J., Aburto-Oropeza, O., Saldierna-Martínez, R. J., Johnson, A. F., Harada, A. E., … Burton, R. S. (2018). DNA sequencing of fish eggs and larvae reveals high species diversity and seasonal changes in spawning activity in the southeastern Gulf of

California. *Marine Ecology Progress Series*, *592*, 159–179. https://doi.org/10.3354/meps12446

Burghart, S. E., Van Woudenberg, L., Daniels, C. A., Meyers, S. D., Peebles, E. B., & Breitbart, M. (2014). Disparity between planktonic fish egg and larval communities as indicated by DNA barcoding. *Marine Ecology Progress Series*, *503*, 195–204. https://doi.org/10.3354/meps10752

Burrows, M., Browning, J. S., Breitbart, M., Murawski, S. A., & Peebles, E. B. (2019). DNA barcoding reveals clear delineation between spawning sites for neritic versus oceanic fishes in the Gulf of Mexico. *Fisheries Oceanography*, *28*(2), 228–239. https://doi.org/10.1111/fog.12404

Chow, S., & Inoue, S. (1993). Intra-and interspecific restriction fragment length polymorphism in mitochondrial genes of *Thunnus* tuna species. *Bulletin of National Research Institute Far Seas Fisheries*, *30*, 207–225.

Claro, R., Lindeman, K. C., & Parenti, L. R. (2014). *Ecology of the marine fishes of Cuba*. Washington, DC: Smithsonian Institution.

Cowen, R. K., & Sponaugle, S. (2009). Larval dispersal and marine population connectivity. *Annual Review of Marine Science*, *1*, 443–466. https://doi.org/10.1146/annurev.marine.010908.163757

Domingues, R., Montealegre-Quijano, S., Soto, J., & Amorim, A. (2015). Five new records of *Luvarus imperialis* (Acanthuroidei: Luvaridae) in the south-west Atlantic Ocean. *Journal of Fish Biology*, *86*(3), 1203–1208. https://doi.org/10.1111/jfb.12629

Duke, E. M., Harada, A. E., & Burton, R. S. (2018). Large interannual variation in spawning in San Diego marine protected areas captured by molecular identification of fish eggs. *Marine Ecology Progress Series*, *604*, 199–210. https://doi.org/10.3354/meps12738

Fabra, M., Raldúa, D., Power, D. M., Deen, P. M., & Cerda, J. (2005). Marine fish egg hydration is aquaporin-mediated. *Science*, *307*(5709), 545–545. https://doi.org/10.1126/science.1106305

Froese, R. & Pauly, D. (Eds.) (2019). FishBase. World Wide Web electronic publication. www.fishbase.org, (12/2019).

Harada, A. E., Lindgren, E. A., Hermsmeier, M. C., Rogowski, P. A., Terrill, E., & Burton, R. S. (2015). Monitoring spawning activity in a southern California Marine Protected Area using molecular identification of fish eggs. *PLoS ONE*, *10*(8), e0134647. https://doi.org/10.1371/journal.pone.0134647

Hitchcock, G. L., Lee, T. N., Ortner, P. B., Cummings, S., Kelble, C., & Williams, E. (2005). Property fields in a Tortugas Eddy in the southern Straits of Florida. *Deep Sea Research Part I: Oceanographic Research Papers*, *52*(12), 2195–2213. https://doi.org/10.1016/j.dsr.2005.08.006

Ionin, A., Pavlidis, Y., & Avello, O. (1977). *Geología de la plataforma marina de Cuba* (p. 364). Moscú: Editorial Nauka.

Ivanova, N. V., Zemlak, T. S., Hanner, R. H., & Hebert, P. D. (2007). Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Resources*, *7*(4), 544–548. https://doi.org/10.1111/j.1471-8286.2007.01748.x

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. *Environmental Biology of Fishes*, *87*(3), 221–235. https://doi.org/10.1007/s10641-010-9592-2

Konstantinou, H., & Shen, D. C. (1995). The social and reproductive behavior of the eyed flounder, *Bothus ocellatus*, with notes on the spawning of *Bothus lunatus* and *Bothus ellipticus*. *Environmental Biology of Fishes*, *44*(4), 311–324. https://doi.org/10.1007/BF00008245

Kourafalou, V. H., & Kang, H. (2012). Florida Current meandering and evolution of cyclonic eddies along the Florida Keys Reef Tract: Are they interconnected? *Journal of Geophysical Research*, *Oceans*, 117(C5). https://doi.org/10.1029/2011JC007383

Lane, P. V., Smith, S. L., Graber, H. C., & Hitchcock, G. L. (2003). Mesoscale circulation and the surface distribution of copepods near the south Florida Keys. *Bulletin of Marine Science*, *72*(1), 1–18.

Larson, J. H., Grace McCalla, S., Chapman, D. C., Rees, C., Knights, B. C., Vallazza, J. M., … Amberg, J. (2016). Genetic analysis shows that morphology alone cannot distinguish Asian Carp eggs from those of other cyprinid species. *North American Journal of Fisheries Management*, *36*(5), 1053–1058. https://doi.org/10.1080/02755947.2016.1185057

Lee, T. N., Clarke, M., Williams, E., Szmant, A. F., & Berger, T. (1994). Evolution of the Tortugas Gyre and its influence on recruitment in the Florida Keys. *Bulletin of Marine Science*, *54*(3), 621–646.

Lee, T. N., Leaman, K., Williams, E., Berger, T., & Atkinson, L. (1995). Florida Current meanders and gyre formation in the southern Straits of Florida. *Journal of Geophysical Research: Oceans*, *100*(C5), 8607–8620. https://doi.org/10.1029/94JC02795

Lee, T. N., Rooth, C., Williams, E., McGowan, M., Szmant, A. F., & Clarke, M. (1992). Influence of Florida Current, gyres and wind-driven circulation on transport of larvae and recruitment in the Florida Keys coral reefs. *Continental Shelf Research*, *12*(7–8), 971–1002. https://doi.org/10.1016/0278-4343(92)90055-O

Lewis, L. A., Richardson, D. E., Zakharov, E. V., & Hanner, R. (2016). Integrating DNA barcoding of fish eggs into ichthyoplankton monitoring programs. *Fishery Bulletin*, *114*(2), 153–165. https://doi.org/10.7755/FB.114.2.3

Leyva-Cruz, E., Vásquez-Yeomans, L., Carrillo, L., & Valdez-Moreno, M. (2016). Identifying pelagic fish eggs in the southeast Yucatan Peninsula using DNA barcodes. *Genome*, *59*(12), 1117–1129. https://doi.org/10.1139/gen-2015-0151

Limouzy-Paris, C., Graber, H. C., Jones, D. L., Röpke, A. W., & Richards, W. J. (1997). Translocation of larval coral reef fishes via sub-mesoscale spin-off eddies from the Florida Current. *Bulletin of Marine Science*, *60*(3), 966–983.

Limouzy-Paris, C., McGowan, M., Richards, W., Umaran, J., & Cha, S. (1994). Diversity of fish larvae in the Florida Keys: Results from SEFCAR. *Bulletin of Marine Science*, *54*(3), 857–870.

Michaud, C. L., & Foran, D. R. (2011). Simplified field preservation of tissues for subsequent DNA analyses. *Journal of Forensic Sciences*, *56*(4), 846–852. https://doi.org/10.1111/j.1556-4029.2011.01771.x

Moteki, M., & Mundy, B. C. (2005). Notes on the morphology of the early stage of the rare bramid genus *Eumegistus*. *Ichthyological Research*, *52*(4), 401–405. https://doi.org/10.1007/s10228-005-0294-2

Murawski, S. A., Peebles, E. B., Gracia, A., Tunnell, J. W. Jr, & Armenteros, M. (2018). Comparative abundance, species composition, and demographics of continental shelf fish assemblages throughout the Gulf of Mexico. *Marine and Coastal Fisheries*, *10*(3), 325–346. https://doi.org/10.1002/mcf2.10033

Nishikawa, Y. (1987). Larval morphology and occurrence of the Louvar, *Luvarus imperialis* (Luvaridae). *Japanese Journal of Ichthyology*, *34*(2), 215–221. https://doi.org/10.1007/bf02912417

Okiyawa, M. (1989). *An atlas of the early stage fishes in Japan*. Tokai University Press. Retrieved from https://escholarship.org/uc/item/9284j82s

Paine, M. A., McDowell, J. R., & Graves, J. E. (2007). Specific identification of western Atlantic Ocean Scombrids using mitochondrial DNA cytochrome c oxidase subunit I (COI) gene region sequences. *Bulletin of Marine Science*, *80*(2), 353–367.

Pauly, D., & Pullin, R. S. (1988). Hatching time in spherical, pelagic, marine fish eggs in response to temperature and egg size. *Environmental Biology of Fishes*, *22*(4), 261–271. https://doi.org/10.1007/BF00004892

Peebles, E., & Tolley, S. (1988). Distribution, growth and mortality of larval Spotted Seatrout, *Cynoscion nebulosus*: A comparison between two adjacent estuarine areas of southwest Florida. *Bulletin of Marine Science*, *42*(3), 397–410.

Perera Valderrama, S., Hernández Ávila, A., González Méndez, J., Moreno Martínez, O., Cobián Rojas, D., Ferro Azcona, H., … Rodríguez Farrat,

L. F. (2018). Marine protected areas in Cuba. *Bulletin of Marine Science*, *94*(2), 423–442. https://doi.org/10.5343/bms.2016.1129

Richardson, D. E., Llopiz, J. K., Leaman, K. D., Vertes, P. S., Muller-Karger, F. E., & Cowen, R. K. (2009). Sailfish (*Istiophorus platypterus*) spawning and larval environment in a Florida Current frontal eddy. *Progress in Oceanography*, *82*(4), 252–264. https://doi.org/10.1016/j.pocean.2009.07.003

Richardson, P. L. (2001). Florida current, gulf stream, and labrador current. In J. H. Steele, S. A. Thorpe, & K. K. Turekian (Eds). *Encyclopedia of Ocean Sciences*, Vol. *2* (pp. 1054–1064). Amsterdam, the Netherlands: Elsevier Ltd.

Salas, S., Chuenpagdee, R., Charles, A. T., & Seijo, J. C. (2011). *Coastal fisheries of Latin America and the Caribbean* (Vol. *544*): Food and Agriculture Organization of the United Nations Rome.

Sassa, C., Konishi, Y., & Mori, K. (2006). Distribution of Jack Mackerel (*Trachurus japonicus*) larvae and juveniles in the East China Sea, with special reference to the larval transport by the Kuroshio Current. *Fisheries Oceanography*, *15*(6), 508–518. https://doi.org/10.1111/j.1365-2419.2006.00417.x

Shay, L. K., Lee, T. N., Williams, E. J., Graber, H. C., & Rooth, C. G. (1998). Effects of low-frequency current variability on near-inertial submesoscale vortices. *Journal of Geophysical Research: Oceans*, *103*(C9), 18691–18714. https://doi.org/10.1029/98JC01007

Shulzitski, K., Sponaugle, S., Hauff, M., Walter, K. D., & Cowen, R. K. (2016). Encounter with mesoscale eddies enhances survival to settlement in larval coral reef fishes. *Proceedings of the National Academy of Sciences*, *113*(25), 6928–6933. https://doi.org/10.1073/pnas.1601606113

Shulzitski, K., Sponaugle, S., Hauff, M., Walter, K., D'Alessandro, E., & Cowen, R. (2017). Patterns in larval reef fish distributions and assemblages, with implications for local retention in mesoscale eddies. *Canadian Journal of Fisheries and Aquatic Sciences*, *75*(2), 180–192. https://doi.org/10.1139/cjfas-2016-0304

Smith, P., McVeagh, S. M., Allain, V., & Sanchez, C. (2005). DNA identification of gut contents of large pelagic fishes. *Journal of Fish Biology*, *67*(4), 1178–1183. https://doi.org/10.1111/j.0022-1112.2005.00804.x

Sponaugle, S., Lee, T., Kourafalou, V., & Pinkard, D. (2005). Florida Current frontal eddies and the settlement of coral reef fishes. *Limnology and Oceanography*, *50*(4), 1033–1048. https://doi.org/10.4319/lo.2005.50.4.1033

Thresher, R. E. (1984). *Reproduction in reef fishes*. Neptune City, NJ: T.F.H. Publications Inc.

Topp, R. W., & Girardin, D. L. (1971). An adult louvar, *Luvarus imperialis* (Pisces, Luvaridae), from the Gulf of Mexico. *Copeia*, *1971*(1), 181–182. https://doi.org/10.2307/1441628

Truett, G., Heeger, P., Mynatt, R., Truett, A., Walker, J., & Warman, M. (2000). Preparation of PCR-quality mouse genomic DNA with hot Sodium Hydroxide and Tris (HotSHOT). *BioTechniques*, *29*(1), 52–54. https://doi.org/10.2144/00291bm09

van der Veer, H. W., Cardoso, J. F., Mateo, I., Witte, J. I., & van Duyl, F. C. (2018). Occurrence and life history characteristics of tropical flatfishes at the coral reefs of Curaçao, Dutch Caribbean. *Journal of Sea Research*, *142*, 157–166. https://doi.org/10.1016/j.seares.2018.09.010

van Overzee, H. M., & Rijnsdorp, A. D. (2015). Effects of fishing during the spawning period: Implications for sustainable management. *Reviews in Fish Biology and Fisheries*, *25*(1), 65–83. https://doi.org/10.1007/s11160-014-9370-x

Vikebø, F., Jørgensen, C., Kristiansen, T., & Fiksen, Ø. (2007). Drift, growth, and survival of larval Northeast Arctic cod with simple rules of behaviour. *Marine Ecology Progress Series*, *347*, 207–219. https://doi.org/10.3354/meps06979

Ward, R. D., Hanner, R., & Hebert, P. D. (2009). The campaign to DNA barcode all fishes, FISH-BOL. *Journal of Fish Biology*, *74*(2), 329–356. https://doi.org/10.1111/j.1095-8649.2008.02080.x

Zhang, Y., Hu, C., Liu, Y., Weisberg, R. H., & Kourafalou, V. H. (2019). Submesoscale and mesoscale eddies in the Florida Straits: Observations from satellite ocean color measurements. *Geophysical Research Letters*, *46*(22), 13262–13270. https://doi.org/10.1029/2019GL083999

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Kerr M, Browning J, Bønnelycke E-M, et al. DNA barcoding of fish eggs collected off northwestern Cuba and across the Florida Straits demonstrates egg transport by mesoscale eddies. *Fish Oceanogr.* 2020;29:340–348. https://doi.org/10.1111/fog.12475