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ORIGINAL ARTICLE

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DNA barcoding reveals clear delineation between spawning sites for neritic versus oceanic fishes in the Gulf of Mexico

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Abstract

We combined research-vessel cruises of opportunity with DNA barcoding to survey planktonic, percomorph fish eggs at 40 stations distributed across and around the Gulf of Mexico (GoM). The objectives were (a) to determine whether eggs of fishes that are potential candidates for the daily egg production method (DEPM) can be readily barcoded, (b) to identify taxa that are spawning in the GoM, (c) to determine encounter rates for eggs of economically valuable taxa, and (d) to characterize individual egg taxa as being primarily neritic, primarily oceanic, or primarily mixed (i.e., both neritic and oceanic). Of the 1,144 eggs that were individually barcoded, 709 (62%) were definitively identified to species (62 species from 42 families), with an additional 20 taxa identified to genus or subfamily level. The eggs of 15 economically important species were identified, most of which had intermediate encounter rates and moderately dispersed spatial distributions, as indicated by an index of aggregation. SIMPROF analysis of stationwise cluster analysis identified eight significant groups within the 35 stations that yielded percomorph eggs; a corresponding specieswise analysis identified six groups of stations as having a neritic egg community and two groups as having an oceanic community, with a community transition located at the shelf break. Although the neritic and oceanic stations did not share important species, it remains possible that coastal pelagic species have mixed neritic–oceanic distributions. Together, these results indicate DEPM fish-egg surveys based on DNA barcoding are feasible at the large marine ecosystem scale.

KEYWORDS
community transition, daily egg production method, DNA barcoding, fish eggs, fish spawning, Gulf of Mexico, large marine ecosystems

1 | INTRODUCTION

Using the abundance of planktonic fish eggs to estimate the biomass of the parent fish stocks (daily egg production method, DEPM) has been hindered by practical limitations, including difficulty with visually identifying drifting eggs to species, and not knowing the appropriate spatial range for egg surveys that are required by DEPM (Borchers, Buckland, Priede, & Ahmadi, 1997; Lo, Ruiz, Cervantes, Moser, & Lynn, 1996; Stratoudakis, Bernal, Ganias, & Uriarte, 2006). Fish larvae are usually more visually distinctive than fish eggs, and thus, larval distributions have been used to identify spawning grounds (Peebles & Tolley, 1988; Sassa, Konishi, & Mori, 2006). However, the capture locations of planktonic larvae are not ideal proxies for spawning habitat because the larvae can drift long distances over days or weeks prior to capture, resulting in substantial spatial offsets from natal spawning grounds (Cowen & Sponaugle, 2009; Muhling et al., 2017); this drift is often behaviorally modulated rather than passive (Vikebo, Jorgensen, Kristiansen, & Fiksen, 2007).

The net effect of these spatial offsets can be egg and larval species compositions that are highly dissimilar (Burghart et al., 2014). In contrast, the progeny of live-bearing species (e.g., rockfishes) may live in close proximity to their locations of parturition.

In contrast to larvae, the eggs of broadcast-spawning fishes are often only hours old when collected and are passive particles (except for often being positively buoyant), and these two traits reduce error when hydrodynamic models are used to locate spawning areas (Burghart et al., 2014). Genetic barcoding has been used extensively to identify adult fishes (Ward, Zemlak, Innes, Last, & Hebert, 2005), fish stomach contents (Smith, McVeagh, Allain, & Sanchez, 2005), and fish larvae (Hubert, Delrieu-Trottin, Irisson, Meyer, & Planes, 2010), but is less commonly applied to individual fish eggs from plankton samples (e.g., Burghart et al., 2014; Harada et al., 2015). Previous applications of genetics-based approaches have either used specific primers to search samples of fish eggs for species of interest (Chow & Inoue, 1993) or used degenerate primers and DNA barcoding to identify individual fish eggs to species level (Ivanova, Zemlak, Hanner, & Hebert, 2007). Degenerate primers are mixtures of oligonucleotide sequences (primers) that produce a larger range of possible nucleotide matches during PCR amplification (Iserte et al., 2013). Studies that have compared visual identifications of percomorph eggs with identifications obtained through DNA barcoding have indicated visual identifications can be highly unreliable (Larson et al., 2016).

DNA barcoding of individual fish eggs alleviates DEPM limitations by allowing the spatial ranges of the drifting eggs to be identified with greater confidence (Burghart et al., 2014; Stratoudakis et al., 2006). In cases where fish eggs can be identified directly (whether visually or by barcoding), it has been observed that the eggs of some species occur on both the continental shelf and over deep, ocean waters, invalidating the shelf break as a natural boundary for egg surveys (Borchers et al., 1997). For other species, it has been suggested that spawning near the shelf break facilitates population connectivity (sensu Cowen & Sponaugle, 2009) via dispersion of eggs and larvae by ocean currents. The literature supporting dispersion-based reproductive success is described by Karnauskas, Cherubin, and Paris (2011), who challenged this idea after their hydrodynamic models indicated predominant particle trajectories resulted in retention on the continental shelf, rather than offshore dispersion. Various other studies have also described biophysical interactions that retain the eggs and larvae of continental shelf (neritic) species on the continental shelf or near the upper slope (Hutchings et al., 2002; Muhling et al., 2017; Weisberg, Zheng, & Peebles, 2014). It thus appears that while some species have eggs and larvae that are widely dispersed in both neritic and deep-oceanic waters, other species have eggs and larvae that are generally retained either in the deep sea or on the continental shelf, inclusive of the upper slope. However, retention is not perfect, and the process may be accompanied by spillover of eggs and larvae into adjacent waters. In the case of neritic retention, spillover is not always aberrant in regard to survival, as offshore-displaced larvae may remain competent enough to reach advanced larval stages (Velez & Moore, 2018). In general, research activities that document spawning locations and then compare these locations with oceanographic features (currents, remotely sensed data, bottom physiography, etc.) are likely to provide insight regarding the factors that influence spawning locations, including factors such as the likelihood of retention.

The objective of this study was to combine DNA barcoding of individual fish eggs within plankton samples from cruises of opportunity in order to do the following:

1. determine whether eggs of fishes that are potential candidates for DEPM (i.e., species with economic value) can be readily barcoded from large batches containing multiple species;
2. identify economically valuable taxa that have the potential to be self-recruiting in the Gulf of Mexico (GoM), as opposed to being dependent on connectivity with populations outside the GoM;
3. determine encounter rates for eggs of economically valuable taxa; and
4. characterize the fish-egg taxa as being primarily neritic, primarily oceanic, or primarily mixed (i.e., both neritic and oceanic).

This effort intentionally addressed the large marine ecosystem (LME) scale and thus examines coarse scales of distribution.

Because DNA barcoding of individual fish eggs is a relatively new research activity that has not been widely applied geographically, the results of genetics-based egg surveys also have fundamental exploratory value and relevance to biogeographic studies. Collecting passively drifting fish eggs is one of the least biased methods of collecting fishes. It can be equally effective at collecting small, cryptic species as it is for large, evasive species; most economically valuable species are at least moderately large and moderately evasive. The fourth objective provides linkages between biodiversity studies of fishes in continental shelf (Murawski, Peebles, Gracia, Tunnell, & Armenteros, 2018), deep benthic (Wei, Rowe, Haedrich, & Boland, 2012), mesopelagic (Sutton et al., 2017), and epipelagic (Habtes, Muller-Karger, Roffer, Lamkin, & Muhling, 2014) habitats of the GoM.

2 | METHODS

2.1 | Study site and sample collection

Planktonic fish eggs were collected during three cruises by the R/V *Weatherbird II* during 2015–2016 (Figure 1). The first cruise crossed the GoM from Tuxpan, Mexico to St. Petersburg, Florida (USA), during fall 2015 (September 27–October 1). The second cruise focused on a smaller region of the northeastern GoM during spring 2016 (April 8–12), and the third cruise circumnavigated the GoM during late summer 2016 (August 4–September 10). Egg sampling was conducted on cruises where the primary activity was collecting adult fishes using demersal long lines (Murawski et al., 2018), and thus, plankton tows were only conducted at times when other shipboard research activities were not ongoing and long steaming periods were anticipated. As a result, potential transects were preselected during

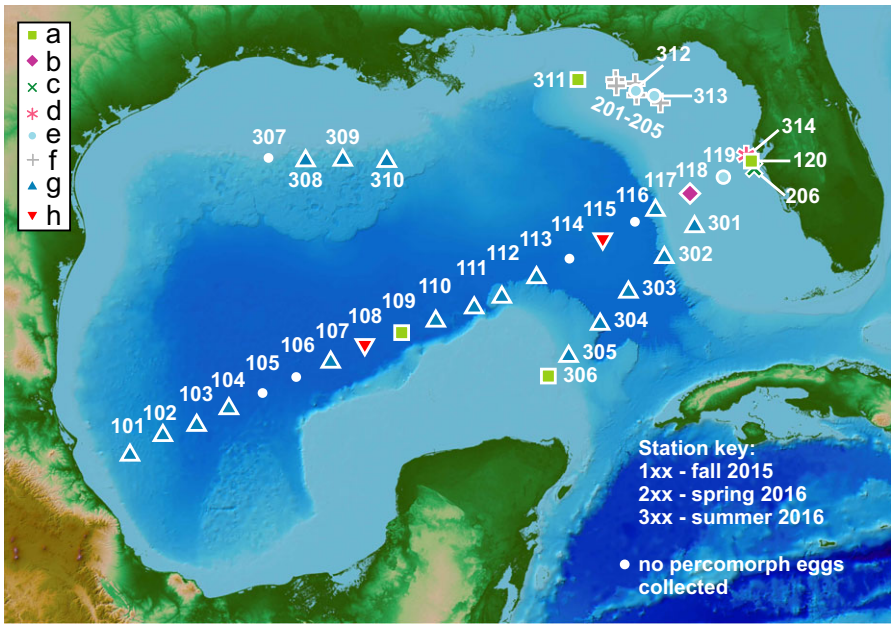

FIGURE 1 Map of plankton stations, identified by SIMPROF group. The symbols for stations 206 and 314 were jittered for clarity, but were much closer to station 120 than depicted here [Colour figure can be viewed at wileyonlinelibrary.com]

the cruise planning stage. Given objective 4, effort was made to include stations that either crossed the shelf break or ran parallel to it. All stations (n = 40, Figure 1) were sampled using a bongo-type (double conical) plankton net (333 μm mesh, 61 cm mouth diameter) equipped with General Oceanics 2030R mechanical flowmeters and 1-liter plastic cod-end jars. The net was towed obliquely, starting at depth (~100 m) followed by haulback to the surface by hydraulic winch while the vessel was underway; tow duration was 15 min, which resulted in a mean filtered volume of 310 m^3. After retrieval and net washdown, one of the two bongo samples was preserved in 50% isopropanol in ambient seawater (the other sample was used in a different study). Upon return to the laboratory, percomorph eggs (eggs of spiny-finned fishes) were visually distinguished from clupeoid eggs (eggs of anchovies, herrings, and sardines) via stereomicroscopy at 9-108X. All percomorph eggs from each plankton sample were transferred to 70% isopropanol in a glass vial, except when the total number of percomorph eggs was >96; in such cases, a subsample of 96 eggs was transferred. The total number of percomorph eggs was recorded for all samples.

2.2 | Genetic identification of fish eggs

Individual eggs were placed in 0.2-ml polymerase chain reaction (PCR) tubes with a sterile pipette tip, and all excess isopropanol was removed. The Chelex DNA extraction method adapted from Hyde et al. (2005) was used for stations 101–120 and 201, but HotSHOT DNA extraction (Truett et al., 2000) was used for all subsequent samples. The change was based on a previous study that demonstrated that DNA extracted from zooplankton eggs with the HotSHOT method was stable for longer periods of time than DNA extracted using the Chelex method (Montero-Pau, Gómez, & Muñoz, 2008). No noticeable differences in the overall success of DNA barcoding were noted between the methods.

For Chelex extraction, 150 μl of 10% Chelex 100 molecular biology grade resin (Bio-Rad Laboratories) was added to each tube containing an individual fish egg, followed by crushing the egg in the resin with a sterile toothpick. The tubes were then incubated in a thermocycler (Eppendorf 6321) at 60°C for 20 min, 99°C for 25 min, 37°C for 1 min, and 99°C for 15 min. For the HotSHOT extraction method, 50 μl of alkaline lysis buffer (25 mM NaOH, 0.2 mM disodium EDTA, pH 12) was added to each tube and the fish egg was crushed in the buffer using a sterile toothpick. Tubes were then heated in a thermocycler for 30 min and cooled on ice for 3 min. Finally, 50 μl of neutralization buffer (40 mM Tris–HCl, pH 5) was added and the sample was vortexed quickly to complete the extraction.

Each fish egg was then genetically identified by PCR amplification and Sanger sequencing (DNA barcoding) of the mitochondrial cytochrome c oxidase I (COI) gene using the COI-3 universal fish primer cocktail described by Ivanova et al. (2007). Each 50 μl PCR contained final concentrations of 1x Apex NH_4 buffer, 1.5 mM Apex $MgCl_2$, 0.2 μM Apex dNTPs, 1 U Apex RedTaq (Genesee Scientific), 0.2 μM primer cocktail, 10 μg/μl bovine serum albumin (New England BioLabs Inc.), and 2-5 μl of target DNA. At first, 2 μl of target DNA was used; if the PCR failed, then 5 μl of target DNA was used in a second reaction. The PCR was heated to 94°C for 2 min, followed by 45 cycles of (94°C for 30 s, 52°C for 40 s, 72°C for 1 min) and 72°C for 10 min. Successful PCR amplification was confirmed by running products on a 1.5% agarose gel stained with ethidium bromide. Successful PCR products were sent to TACGen (tacgen.com) for purification and Sanger sequencing using the M13 forward primer, which is contained within flanking tails of the primer cocktail (Ivanova et al., 2007). Sequences were then trimmed for quality using Sequencher™ 5.3 (Genecodes) and compared against the species-level records in the Barcode of Life Database (BOLD; http://www.boldsystems.org/) for identification. The lowest level of

taxonomic assignment confidently predicted by BOLD is reported here. Barcodes ranged from 608 to 645 base pairs in length (Ivanova et al., 2007).

In several cases, the COI-3 region did not provide sufficient resolution for discriminating between related species, only allowing identification to genus. To achieve definitive species-level identifications for eggs belonging to economically important groups, additional PCRs were performed. The ATCO region between the ATPase6 and COI-3 genes was amplified from the DNA of 23 fish eggs originally identified as either *Thunnus thynnus* or *Katsuwonus pelamis* (Chow & Inoue, 1993). With the exception of different primers (L8562 and H9432), the reaction mixture was the same as above, with cycling conditions of heating to 94°C for 2 min, followed by 45 cycles of (94°C for 30 s, 50°C for 40 s, 72°C for 1.5 min) and 72°C for 10 min. Another PCR was performed on DNA from a single fish egg initially identified as either *Scomberomorus cavalla* or *Acanthocybium solandri* to achieve a definitive identification. The same PCR composition from above was used with different primers to amplify a longer section of the COI gene (Paine, McDowell, & Graves, 2007) and was heated to 94°C for 2 min, followed by 45 cycles of (94°C for 30 s, 57°C for 40 s, 72°C for 2 min) and 72°C for 10 min. A total of 24 products were cleaned with a Clean & Concentrator-25 kit (Zymo) and sent to TACGen for bidirectional Sanger sequencing.

2.3 | Statistical analyses

Several multivariate community analyses were conducted to identify taxonomic communities, and the collection locations of the resulting communities were plotted on a map of the survey area; the objective was to determine the degree of spatial overlap between neritic and oceanic communities. For each station, the density (effort-corrected abundance) of eggs in the water column was calculated by dividing the total number of percomorph eggs in the sample by the volume filtered by the plankton net, as determined from flowmeter readings. The total egg density at each station was then apportioned to individual taxa according to proportional representations in the sample of barcoded eggs relative to the total number of percomorph eggs in the sample.

The resulting catch table was analyzed using PRIMER 7 software (v. 7.0.13, PRIMER-E, Auckland, New Zealand), wherein the density values were square-root-transformed and used to compute a Bray–Curtis similarity matrix. Stations and taxa were independently grouped using hierarchical cluster analysis based on the group-average cluster mode, with the resulting dendrogram for stations being subdivided into statistically significant clusters (groups of stations) via SIMPROF analysis (Clarke, Somerfield, & Gorley, 2008); SIMPROF-group identities were then plotted on the map of stations. To describe major taxonomic trends in community structure, dendrograms for both stations and taxa were arranged into a seriated heatmap (*shade plot* routine in PRIMER 7), with abundance represented by the square root of density. Note that PRIMER 7 limits heatmap depictions to the most "important" taxa, which are taxa that have the highest percentage contributions to any of the samples, with a maximum depiction of 50 important taxa.

The similarity matrix was also used to generate a nonmetric multidimensional scaling (nMDS) plot for station associations, which included an overlay of SIMPROF groups. SIMPROF groups were classified as being neritic or (deep) oceanic by examining taxon-specific native distribution maps in FishBase (http://www.fishbase.org/search.php) and by comparing these with the species-wise cluster analysis. The nMDS plot was used to compare relative station similarities.

A species accumulation curve was generated to gauge the extent to which the surveys represented the fish-egg species richness of the GoM. Stochastic species accumulation curves were created using the *specaccum* function in the *vegan* package implemented in R (Oksanen et al., 2017); the jackknife procedure (n = 1,000) used selection of stations without replacement. These results provide a rarefaction curve that depicts the number of cumulative species encountered as a function of the number of stations sampled. Egg patchiness was described using Bez's (2000) index of aggregation (I_a) to characterize the relative spatial dispersion of individual egg taxa.

3 | RESULTS

Five of the 40 samples (13%) did not contain percomorph eggs. Of the 1,144 successfully barcoded eggs, the BOLD database definitively identified (>97% certainty) 62 species from 42 families. An additional 18 taxa were identified at genus level, and two taxa (one species of scad and one species of tuna) were identified at the subfamily level. The species accumulation curve (Figure 2) was nonasymptotic, indicating substantially more taxa would have been encountered with additional sampling.

Summary statistics for the egg catch are presented in Table 1. The eggs of 15 economically important species were identified; in decreasing order of mean abundance (eggs 10^3 m^{-3}), these were *Sciaenops ocellatus* (red drum), *Katsuwonus pelamis* (skipjack tuna), *Thunnus atlanticus* (blackfin tuna), *Mycteroperca phenax* (scamp), *Rhomboplites aurorubens* (vermilion snapper), *Lutjanus campechanus* (northern red snapper), *Euthynnus alletteratus* (little tunny), *Mycteroperca microlepis* (gag), *Pagrus pagrus* (red porgy), *Coryphaena hippurus* (common dolphinfish), *Thunnus albacares* (yellowfin tuna), *Auxis thazard* (frigate tuna), *Caulolatilus cyanops* (blackline tilefish), *Scomberomorus cavalla* (king mackerel), and *Istiophorus albicans* (Atlantic sailfish).

The index of aggregation (I_a, Figure 3) was negatively correlated with encounter frequency (Pearson's r = –0.81, n = 82, $p < 0.0001$). By mathematical definition, taxa with a frequency of encounter of 1 have high I_a values. At the other extreme, the most frequently encountered taxon, *Diplospinus* sp. (escolar), had the lowest I_a value, indicating its spawning was broadly dispersed relative to other taxa. Many of the economically valuable species (listed above) had intermediate I_a values (Figure 3), indicating their spawning was moderately dispersed.

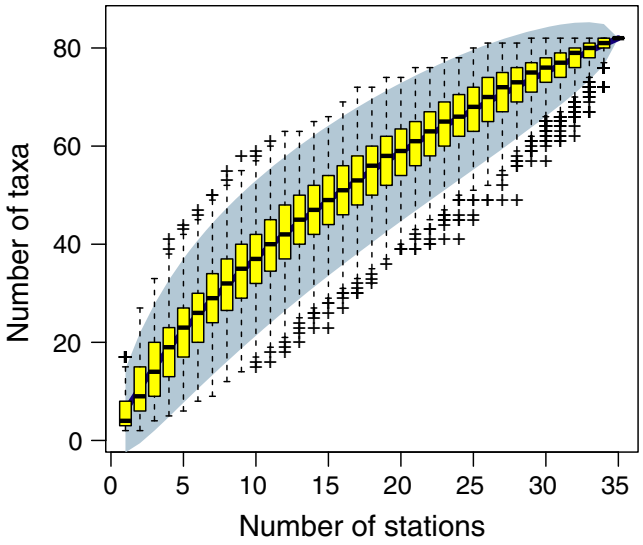

FIGURE 2 Stochastic species accumulation results for fish-egg sampling in the Gulf of Mexico during 2015 and 2016 based on 1,000 jackknifed iterations that sampled the station data without replacement. Yellow bars are the interquartile ranges of species richness from each experiment; light blue is the confidence interval for the mean species richness as a function of the number of stations sampled, and crosses and "Ts" are the ranges from jackknife iterations [Colour figure can be viewed at wileyonlinelibrary.com]

The SIMPROF analysis produced eight significant groups within the 35 stations that yielded percomorph eggs (Figure 1). The compositions of these groups (top 50 most important taxa) are presented in Figure 4, which includes a species-wise dendrogram that has a major division that corresponds with the interface between neritic and oceanic station-wise (SIMPROF) groups. A minor oceanic group, group *h*, consisted of the eggs of two mesopelagic species at two stations. Together, Figures 1 and 4 indicate a community transition at the shelf break. The nMDS plot (Figure 5) and Figure 4 indicate SIMPROF group *a* was most similar to the major oceanic group (group *g*), but there was no overlap between these groups in low-stress 2D nMDS space, and they did not share important species.

4 | DISCUSSION

4.1 | Factors that affect egg distribution

The distribution of planktonic fish eggs is influenced by the biogeography of broadcast spawners, the spatial patchiness of spawning habitats, temporal variation in spawning activity, and advection of eggs after spawning. Regarding biogeography, the fundamental distinction among egg communities was the separation of neritic species from deep-ocean species (Figures 1, 4, and 5). This distinction is not entirely explained by position in the water column, as both groups included a mixture of pelagic and demersal species. The oceanic group included epipelagic species (e.g., *C. hippurus* and *Istiophorus albicans*), mesopelagic species (e.g., Günther's lanternfish, *Lepidophanes guentheri*, and prickly fanfish, *Pterycombus petersii*), and demersal species that live near the shelf break (e.g., *Pristipomoides aquilonaris* and blackbar drum, *Pareques iwamotoi*). In general, however, the neritic group included far more demersal species, including cryptic burrowers (e.g., the eels *Paraconger caudilimbatus* and *Echiophis intertinctus*).

Although detailed biogeographic histories are not available for most of the fish families in the GoM, demersal neritic species such as lutjanids (snappers) appear to be derived from eastern Pacific ancestors that became isolated after the Panamanian Gateway closed 4.5 million years ago (Gold, Voelker, & Renshaw, 2011). Since then, vicariant and ecological speciation has produced a large number of species that are now endemic to the tropical and temperate waters of the western Atlantic, inclusive of the GoM. This contrasts with the oceanic egg group, which was dominated by species that have much larger ranges, including ranges that are circumglobal within tropical and temperate waters (e.g., *C. hippurus* and *K. pelamis*) and ranges that extend across large areas of both the North and South Atlantic Oceans (e.g., *Lepidophanes guentheri* and *T. atlanticus*).

To some extent, the patchiness of spawning habitat is indicated by the percent of stations at which a given taxon occurred (Figure 3). Frequently encountered taxa (e.g., *Diplospinus* sp. and driftfish, *Cubiceps* sp.) appear to be more general in their spawning habitat than taxa that were encountered in large numbers at relatively few locations (e.g., *K. pelamis* and rough scad, *Trachurus lathami*). Higher levels of spawning-habitat selectivity may relate to the geography of egg and larval transport (Cowen & Sponaugle, 2009; Weisberg et al., 2014) or to orientation with dynamic physical processes that support biological productivity (Peebles, 2002; Peebles, Hall, & Tolley, 1996; Reglero, Tittensor, Alvarez-Berastegui, Aparicio-Gonzalez, & Worm, 2014).

The species–accumulation curve for the present survey was not asymptotic (Figure 2), indicating that adding stations to the survey (more potential spawning sites) would have added many more species to the overall catch. There are more than 1,500 identified fish species in the GoM (McEachran, 2009), and a large proportion of these are broadcast spawners with buoyant eggs. Our study identified 82 egg taxa, and thus, there are a considerable number of additional species known from the continental shelves and deep ocean that were not collected. This pilot study, however, did not systematically sample the spatial or temporal (seasonal) range of fish spawning in the GoM. Nevertheless, the success of the method indicates that a systematic survey of the entire GoM using these methods would provide a more comprehensive data set with which to evaluate spatial and temporal patterns in fish biodiversity. Such a study could also evaluate population connectivity among different continental shelf areas and the deep-oceanic GoM using particle (fish eggs and larvae) tracking studies by seeding models with identified fish-egg densities. Furthermore, a more comprehensive survey would provide genetic materials for discerning potential subpopulation connectivity.

Temporal variation in spawning season is widespread among species, but tends to be consistent within species (Cushing, 1969). Phenological studies indicate species-specific spawning seasons are

TABLE 1 Habitat, economic importance, catch statistics, and Bez's (2000) index of aggregation (I_a) for all fish-egg taxa encountered during the survey

Taxon	FishBase common name	Habitat	Economic importance	Encounter frequency	Mean density	Maximum density	Mean nonzero density	Aggregation (I_a)
Acanthostracion quadricornis	Scrawled cowfish	Neritic	No	1	0.08	2.96	2.96	1.00
Auxis sp.	Frigate tuna	Oceanic	Yes	5	5.24	98.10	36.69	0.35
Auxis thazard	Frigate tuna	Oceanic	Yes	1	0.18	6.23	6.23	1.00
Bellator militaris	Horned searobin	Neritic	No	1	0.84	29.52	29.52	1.00
Bothus robinsi	Twospot flounder	Neritic	No	1	0.18	6.23	6.23	1.00
Brama sp.	Pomfret	Oceanic	No	2	0.40	8.18	6.96	0.52
Calamus sp.	Porgy	Neritic	Yes	1	0.69	24.15	24.15	1.00
Caranx crysos	Blue runner	Both	No	1	0.08	2.64	2.64	1.00
Caulolatilus cyanops	Blackline tilefish	Neritic	Yes	1	0.13	4.63	4.63	1.00
Centropristis ocyurus	Bank seabass	Neritic	No	2	0.37	7.03	6.47	0.50
Coryphaena hippurus	Common dolphinfish	Oceanic	Yes	2	0.34	9.50	5.97	0.67
Cubiceps sp.	Driftfish	Oceanic	No	8	10.58	286.87	46.28	0.62
Cyclopsetta fimbriata	Spotfin flounder	Neritic	No	2	0.62	17.76	10.78	0.71
Cyclopsetta sp.	Flounder	Neritic	No	1	0.18	6.23	6.23	1.00
Decapterus sp.	Scad	Both	Minor	8	11.27	216.12	49.32	0.35
Diplectrum formosum	Sand perch	Neritic	No	1	1.38	48.31	48.31	1.00
Diplospinus sp.	Escolar	Oceanic	No	11	4.47	26.88	14.23	0.12
Echeneis naucrates	Live sharksucker	Both	No	1	0.08	2.96	2.96	1.00
Echeneis sp.	Remora	Both	No	3	0.28	3.71	3.21	0.34
Echiophis intertinctus	Spotted spoon-nose eel	Neritic	No	1	0.17	5.92	5.92	1.00
Etrumeus sadina	Red-eye round herring	Both	No	2	1.50	47.23	26.27	0.82
Eucinostomus sp.	Mojarra	Neritic	No	1	0.69	24.15	24.15	1.00
Euthynnus alletteratus	Little tunny	Both	Yes	2	0.43	12.46	7.55	0.71
Fistularia tabacaria	Cornetfish	Neritic	No	1	0.08	2.96	2.96	1.00
Gempylid	Snake mackerel	Oceanic	No	1	0.49	17.29	17.29	1.00
Gymnachirus sp.	Sole	Neritic	No	1	0.11	3.71	3.71	1.00
Haemulon aurolineatum	Tomtate	Neritic	No	1	0.20	7.03	7.03	1.00
Haemulon sp.	Grunt	Neritic	Unknown	1	13.80	483.08	483.08	1.00
Halichoeres bathyphilus	Greenband wrasse	Neritic	No	1	0.80	28.10	28.10	1.00
Halichoeres bivittatus	Slippery dick	Neritic	No	1	0.69	24.15	24.15	1.00
Istiophorus albicans	Atlantic sailfish	Oceanic	Yes	1	0.08	2.88	2.88	1.00
Katsuwonus pelamis	Skipjack tuna	Oceanic	Yes	4	18.50	570.79	161.86	0.79
Lepidophanes guentheri	Günther's lanternfish	Oceanic	No	1	0.54	18.75	18.75	1.00
Lutjanus campechanus	Northern red snapper	Neritic	Yes	1	0.59	20.57	20.57	1.00
Malacanthus plumieri	Sand tilefish	Neritic	No	1	0.18	6.23	6.23	1.00
Menticirrhus littoralis	Gulf kingcroaker	Neritic	Minor	1	0.69	24.15	24.15	1.00
Menticirrhus saxatilis	Northern kingfish	Neritic	Minor	1	0.69	24.15	24.15	1.00
Mugil sp.	Mullet	Neritic	Yes	1	0.40	14.06	14.06	1.00
Mullus auratus	Red goatfish	Neritic	No	2	1.84	59.05	32.18	0.85
Mycteroperca microlepis	Gag	Neritic	Yes	2	0.38	7.03	6.63	0.50
Mycteroperca phenax	Scamp	Neritic	Yes	2	0.70	14.06	12.33	0.51
Nesiarchus nasutus	Black gemfish	Oceanic	No	2	0.69	15.12	11.99	0.53
Ophichthus gomesii	Shrimp eel	Neritic	No	1	0.08	2.96	2.96	1.00
Orthopristis chrysoptera	Pigfish	Neritic	Minor	1	1.38	48.31	48.31	1.00

(Continues)

TABLE 1 (Continued)

Taxon	FishBase common name	Habitat	Economic importance	Encounter frequency	Mean density	Maximum density	Mean nonzero density	Aggregation (I_a)
Oxyporhamphus micropterus	Bigwing halfbeak	Oceanic	No	2	0.26	6.22	4.62	0.56
Pagrus pagrus	Red porgy	Neritic	Yes	1	0.36	12.46	12.46	1.00
Paraconger caudilimbatus	Margintail conger	Neritic	No	1	0.08	2.96	2.96	1.00
Pareques iwamotoi	Blackbar drum	Neritic	No	1	0.13	4.48	4.48	1.00
Prionotus martis	GoM barred searobin	Neritic	No	2	1.89	62.37	33.04	0.89
Prionotus ophryas	Bandtail searobin	Neritic	No	1	0.69	24.15	24.15	1.00
Prionotus sp.	Searobin	Neritic	No	5	3.85	56.21	26.94	0.26
Pristipomoides aquilonaris	Wenchman	Neritic	Minor	2	1.99	67.25	34.85	0.93
Pterycombus petersii	Prickly fanfish	Oceanic	No	1	0.14	4.78	4.78	1.00
Remora osteochir	Marlin sucker	Oceanic	No	1	0.09	3.05	3.05	1.00
Rhomboplites aurorubens	Vermilion snapper	Neritic	Yes	2	0.63	14.80	11.11	0.56
Rypticus bistrispinus	Freckled soapfish	Neritic	No	1	0.08	2.64	2.64	1.00
Rypticus maculatus	Whitespotted soapfish	Neritic	No	1	0.22	7.57	7.57	1.00
Rypticus sp.	Soapfish	Neritic	No	1	1.78	62.37	62.37	1.00
Saurida brasiliensis	Brazilian lizardfish	Neritic	No	4	3.48	98.37	30.43	0.67
Saurida normani	Shortjaw lizardfish	Neritic	No	1	0.90	31.40	31.40	1.00
Scad	Scad	Both	Minor	2	0.20	4.48	3.46	0.54
Sciaenops ocellatus	Red drum	Neritic	Yes	1	19.60	686.09	686.09	1.00
Scomberomorus cavalla	King mackerel	Neritic	Yes	1	0.11	3.71	3.71	1.00
Serranus notospilus	Saddle bass	Neritic	No	1	0.19	6.52	6.52	1.00
Sphyraena borealis	Northern sennet	Neritic	No	1	0.34	11.81	11.81	1.00
Stomias sp.	Dragonfish	Oceanic	No	1	0.89	31.09	31.09	1.00
Syacium papillosum	Dusky flounder	Neritic	No	8	14.95	194.84	65.39	0.23
Symphurus plagiusa	Blackcheek tonguefish	Neritic	No	1	0.69	24.15	24.15	1.00
Symphurus urospilus	Spottail tonguefish	Neritic	No	1	0.11	3.79	3.79	1.00
Synodus foetens	Inshore lizardfish	Neritic	No	4	2.37	62.37	20.77	0.60
Synodus sp.	Lizardfish	Neritic	No	7	6.11	89.17	30.53	0.26
Thunnus albacares	Yellowfin tuna	Oceanic	Yes	2	0.28	6.22	4.97	0.53
Thunnus atlanticus	Blackfin tuna	Both	Yes	6	13.68	260.02	79.81	0.37
Trachinocephalus myops	Snakefish	Neritic	No	8	13.22	156.96	57.83	0.22
Trachurus lathami	Rough scad	Neritic	Minor	3	10.66	360.06	124.33	0.93
Tuna	Tuna	Oceanic	Yes	4	2.74	57.46	23.99	0.44
Urophycis floridana	Southern codling	Neritic	No	1	0.15	5.30	5.30	1.00
Vinciguerria sp.	Bristlemouth	Oceanic	No	2	0.68	18.66	11.96	0.66
Xyrichtys novacula	Pearly razorfish	Neritic	No	5	19.38	267.86	135.66	0.32

Note. Encounter frequency is number of stations. Densities are eggs 10^3 m^{-3}, with the mean calculated from 35 stations with positive catches. GoM, Gulf of Mexico. Columns 2–4 are derived from FishBase (http://www.fishbase.org/search.php).

responsive to climate change, generally starting earlier in the year when sea surface temperatures are warmer (Jansen & Gislason, 2011). In the present survey, cruises at different times of year collected the eggs of spring spawners (e.g., *M. microlepis* and *M. phenax*), summer spawners (e.g., *L. campechanus* and *R. aurorubens*), and fall spawners (e.g., *S. ocellatus*). Within spawning seasons, there can be substantial variation in egg production in association with changing moon phases, and the same species may spawn more heavily during different moon phases at different locations (Farmer et al., 2017). Note that we arranged for each of the three cruises to visit a common location during different seasons (stations 120, 206, 314, Figure 1), and this common location was classified as having different egg communities during each of the three seasons. When spawning season is targeted by egg cruises, encounter rates can be substantially higher than indicated in Table 1. For example, we collected eggs of each of the spring-spawning groupers *M. microlepis* and *M. phenax* in two of the six spring samples (33% encounter rate).

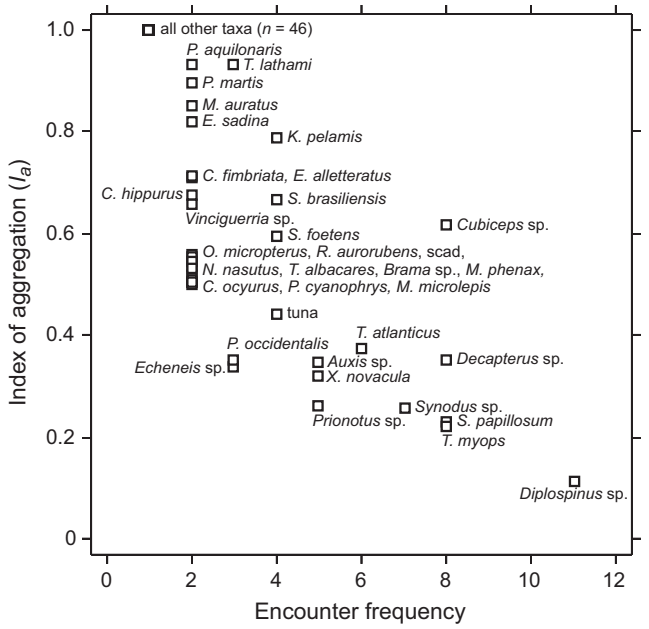

FIGURE 3 Comparison of fish-egg encounter frequency (number of stations) with Bez's (2000) index of aggregation, I_a. The two metrics were strongly correlated (Pearson's $r = -0.81$, $n = 82$, $p < 0.0001$)

4.2 | Geographic delineation of the neritic and oceanic egg communities

Within the observed fish-egg distributions, there was little evidence of a community gradient (coenocline) associated with the rapid increase in depth that occurs beyond the shelf break. The fish-egg community transition thus resembled the abrupt transition of an ecotone (DiCastri, Hansen, & Holland, 1988; Figures 1, 4 and 5), rather than the gradual transition of an ecocline (Kent, Gill, Weaver, & Armitage, 1997). However, this perception is partly attributable to the scale of the survey and its sampling resolution, as the benthic fish communities of the GoM clearly exhibit depth zonation on the continental slope (Wei et al., 2012) that would have been difficult to resolve with the widely spaced stations used in this study (Figure 1).

On the continental shelf, an economically valuable assemblage of reef fishes known as the "grouper–snapper complex" (e.g., serranids, lutjanids, sparids, haemulids; sensu Coleman et al., 2000) is one potential target for DEPM. Reef fishes adhered to the abrupt neritic–oceanic transition at the shelf break, with minor exceptions. The circumglobal snapper genus *Pristipomoides*, which favors deeper water (upper slope to depths > 500 m) more than most other snapper species (Allen, 1985), was one of two reef-associated taxa to be

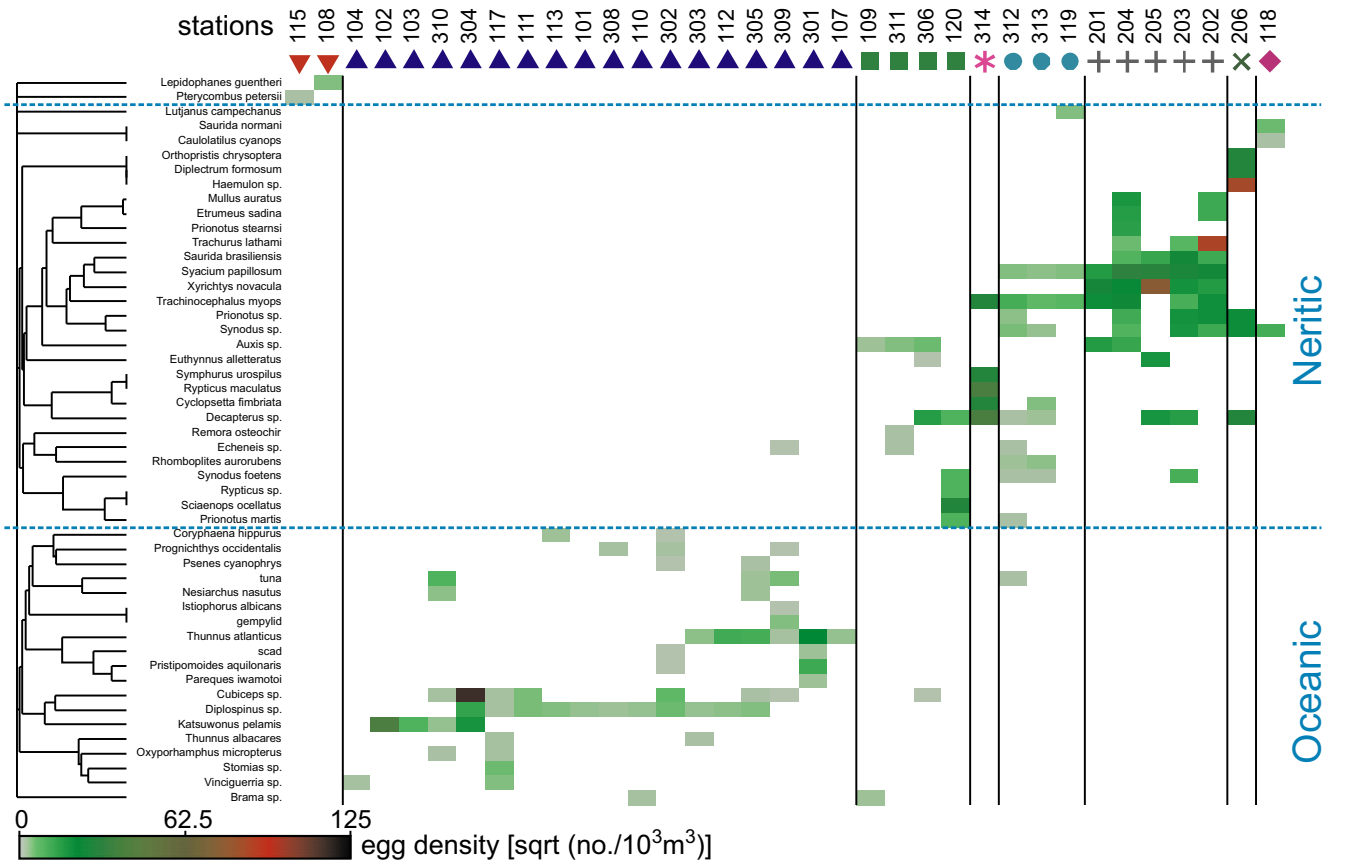

FIGURE 4 Heatmap of the 50 most important fish-egg taxa, with a dendrogram indicating species associations and vertical lines identifying statistically significant station associations (SIMPROF groups). For clarity, the station-wise dendrogram is not shown [Colour figure can be viewed at wileyonlinelibrary.com]

classified within the oceanic fish-egg community (Figure 5; the second species was a poorly known species, *Pareques iwamotoi*). Leis and Lee (1994) describe the genus *Pristipomoides* as favoring rocky bottoms of the upper continental slope, while also being semipelagic. This genus is ancestral and zooplanktivorous, in contrast to more derived snappers that eat larger prey (Frédérich & Santini, 2017). *Pristipomoides* larvae attain relatively large sizes (>2 cm) and become fully scaled, while remaining translucent, before developing the dense body pigmentation that is associated with settlement from the water column onto benthic habitats (Leis & Lee, 1994). This genus and several other snapper species, including some that are considered to be neritic species (e.g., *L. campechanus*), are present as relatively large, translucent larvae within the water column seaward of the GoM's shelf break (Velez & Moore, 2018); the large size of these presettlement individuals and their position in the water column suggest some reef fish larvae remain competent even while existing in a deep, pelagic environment. It is unclear whether the presence of these advanced-stage larvae in deep waters adjacent to the slope is the result of involuntary spillover or active habitat selection. Among groupers, there are a number of species that occupy slope waters (e.g., the diverse group of anthias serranids), although the eggs of this group were not identified during the present survey. Thus, while the eggs of reef fishes were essentially confined to the continental shelf, it is also known that reef fishes use habitats on the upper slope and in the adjacent water column seaward of the upper slope.

One important consideration when examining the biogeography of species associated with the neritic–oceanic comparison is the occurrence of ocean currents, warm- and cold-core eddies, and associated fronts (Schmitz, 2005). During the 2015 and 2016 cruises, oceanic conditions in the GoM were substantially different (Figure 6). In 2015, the Loop Current was well-established, with intense eddies present in the western GoM. The eastern boundary of the Loop Current often interacts with the outer shelf, potentially providing a mixing zone for neritic and oceanic species and transport of coastal species from the northern GoM southeast to the Florida Keys. The cross-Gulf transect occupied during 2015 crossed the Loop Current, which extended deep into the GoM to the northwest. During the 2016 sampling, however, the Loop Current was weak and was eventually cut off, and thus, there was not a sharp water column boundary among faunas. There was, however, a cold-core eddy at the center of the Florida–Yucatan transect, potentially upwelling cold, nutrient laden water, yet there were no apparent egg community trends associated with this feature. In general, ocean circulation did not appear to be responsible for the community transition at the shelf break (Figure 6).

None of the 50 important taxa (as defined in statistical analyses section) that occurred in both the oceanic and neritic groups (Figure 5) were reef fishes. The (relatively few) overlapping taxa were *Echeneis* sp. (a remora that travels while attached to much larger nekton), unidentified tunas, a species of driftfish (*Cubiceps* sp.), and

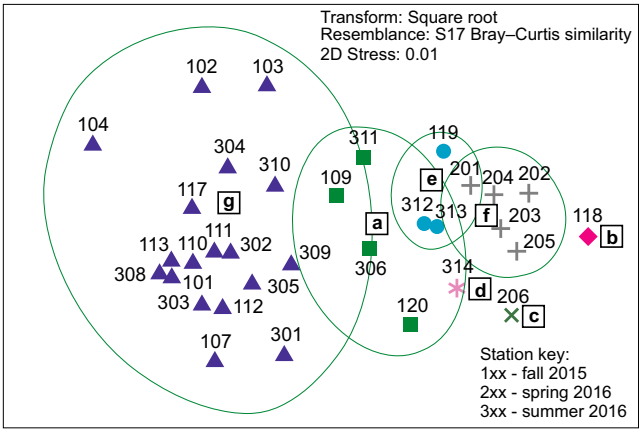

FIGURE 5 nMDS plot of station similarities, with ellipses drawn around SIMPROF groups, which are labeled with letters within boxes. The minor oceanic group, group *h* (two mesopelagic species), is not shown because the two stations in this group were spatial outliers that made the overall scale illegible for the other groups [Colour figure can be viewed at wileyonlinelibrary.com]

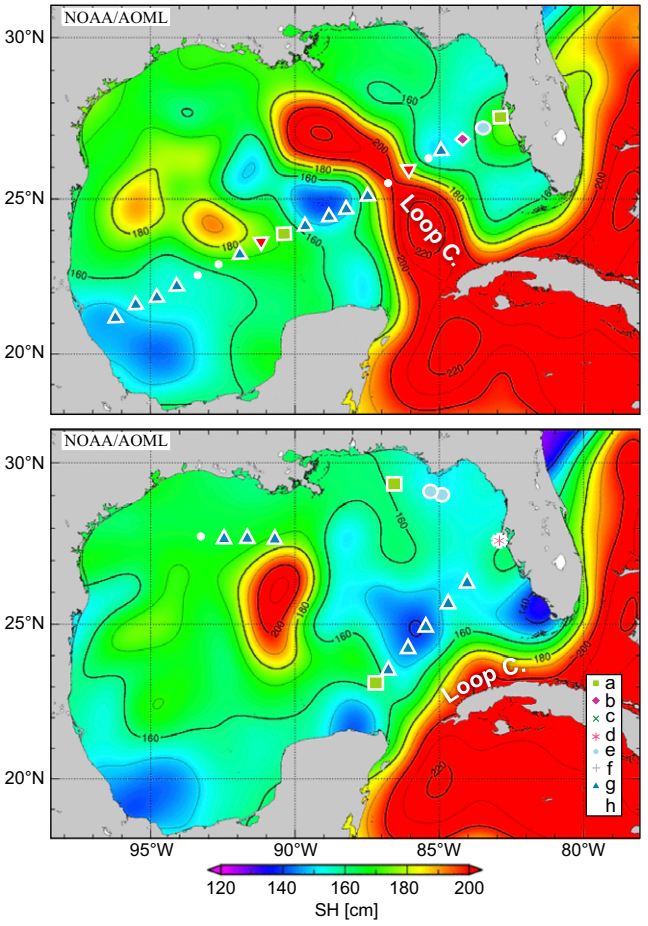

FIGURE 6 Sea heights (SH) from satellite altimetry during the fall (upper panel; September 9, 2015) and summer 2016 (lower panel; August 22, 2016) cruises. Data indicate strong northward intrusion of the Loop Current (Loop C.) into the GoM during 2015, with warm- and cold-core eddies present in the western Gulf. During 2016, the Loop Current was essentially cut off and flowed more directly through the Florida Straits. Graphics modified from http://www.aoml.noaa.gov/phod/dhos/altimetry.php#SHA [Colour figure can be viewed at wileyonlinelibrary.com]

an oceanic pomfret (*Brama* sp.) that was collected near the Yucatan Shelf at the only deepwater station that was classified as neritic (station 109). Both of the mixed neritic–oceanic egg taxa described by Borchers et al. (1997) were coastal pelagics (sensu Klima & Wickham, 1971; Bakun & Parrish, 1991), which include zooplanktivorous clupeids, engraulids, carangids, and scombrids. The Borchers et al. taxa were a scad (carangid) and a mackerel (scombrid). While we did not identify any mixed neritic–oceanic SIMPROF groups or observe substantive taxonomic overlap between the neritic and oceanic groups, there was taxonomic uncertainty within the scad genera *Trachurus* and *Decapterus*, and also among the tunas (inclusive of the genus *Auxis*), and so it is possible that some coastal pelagics spawned in both neritic and oceanic waters (note that clupeid and engraulid eggs were visually identified and were excluded from DNA barcoding, with the exception of *Etrumeus sadina* eggs, which were mistaken for percomorph eggs).

4.3 | DEPM and egg mortality

Daily egg production method requires consideration of egg mortality when estimating the abundance of eggs at the time of spawning (vs. the time of collection). Microscopy can be used to visually stage the eggs of a given species, and the abundances of successive egg stages can be fit to catch curves to estimate egg mortality for that species (e.g., Lo et al., 1996). Because the DNA barcoding process destroys the eggs, the eggs must be visually staged before barcoding, and a protocol for assigning species proportions to egg-stage distributions in the entire sample must be developed. Visual staging, however, may be more difficult for eggs that have been directly preserved in alcohol (ethanol or isopropanol) than for eggs that have been fixed in formalin first. Formalin cannot be used in conjunction with barcoding because it damages DNA by fragmentation, base modification, and by cross-linking the DNA with itself or proteins (Hykin, Bi, & McGuire, 2015). Fragmentation, in particular, interferes with the Sanger sequencing method. We observed variation in the preservation quality of eggs preserved in isopropanol. While many of the isopropanol-preserved eggs still contained detailed anatomical features that would allow staging, others did not. Additional research into the cause of these variable preservation results is needed.

5 | CONCLUSION

We found the eggs of fishes that are potential candidates for DEPM were readily barcoded and were encountered at high enough rates to make egg surveys practical for many species. Eggs from 15 economically important fish species were definitively identified by DNA barcoding. These and other taxa that spawn within the GoM are likely self-recruiting to some extent. Population connectivity may subsidize these GoM populations, but the taxa encountered in the egg survey are less likely to be dependent on such connectivity. At the LME scale, the eggs reflected spatial variation in the community structure of spawners, which had a distinct community transition at the shelf break. Together, these results indicate DEPM fish-egg surveys based on DNA barcoding are feasible at the LME scale.

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CONFLICT OF INTEREST

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

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REFERENCES

Allen, G. R. (1985). Snappers of the world. FAO Species Catalog 6, 1–208.

Bakun, A., & Parrish, R. H. (1991). Comparative studies of coastal pelagic fish reproductive habitats - The anchovy (*Engraulis anchoita*) of the southwestern Atlantic. *ICES Journal of Marine Science*, *48*, 343–361. https://doi.org/10.1093/icesjms/48.3.343

Bez, N. (2000). On the use of Lloyd's index of patchiness. *Fisheries Oceanography*, *9*, 372–376. https://doi.org/10.1046/j.1365-2419.2000.00148.x

Borchers, D. L., Buckland, S. T., Priede, I. G., & Ahmadi, S. (1997). Improving the precision of the daily egg production method using generalized additive models. *Canadian Journal of Fisheries and Aquatic Sciences*, *54*, 2727–2742. https://doi.org/10.1139/f97-134

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

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

Clarke, K. R., Somerfield, P. J., & Gorley, R. N. (2008). Testing null hypotheses in exploratory community analyses: Similarity profiles and biota-environmental linkage. *Journal of Experimental Marine Biology and Ecology*, *366*, 56–69. https://doi.org/10.1016/j.jembe.2008.07.009

Coleman, F. C., Koenig, C. C., Huntsman, G. R., Musick, J. A., Eklund, A. M., McGovern, J. C., … Grimes, C. B. (2000). Long-lived reef fishes: The grouper-snapper complex. *Fisheries*, *25*, 14–21. https://doi.org/10.1577/1548-8446(2000) 025<0014:LRF>2.0.CO;2

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

Cushing, D. H. (1969). Regularity of spawning season of some fishes. *Journal Du Conseil*, *33*, 81–92. https://doi.org/10.1093/icesjms/33.1.81

DiCastri, F., Hansen, A., & Holland, M. M. (Eds.) (1988). *A New Look At Ecotones: Emerging international projects on landscape boundaries. Special Issue 17 Biology International* (pp. 1–163). Paris, France: International Union of Biological Sciences.

Farmer, N. A., Heyman, W. D., Karnauskas, M., Kobara, S., Smart, T. I., Ballenger, J. C., & … Sedberry, G. R., (2017). Timing and locations of reef fish spawning off the southeastern United States. *PLoS ONE*, *12*, e0172968. https://doi.org/10.1371/journal.pone.0172968

Frédérich, B., & Santini, F. (2017). Macroevolutionary analysis of the tempo of diversification in snappers and fusiliers (Percomorpha: Lutjanidae). *Belgian Journal of Zoology*, *147*, 17–35.

Gold, J. R., Voelker, G., & Renshaw, M. (2011). Phylogenetic relationships of tropical western Atlantic snappers in subfamily Lutjaninae (Lutjanidae: Perciformes) inferred from mitochondrial DNA sequences. *Biological Journal of the Linnean Society*, *102*, 915–929. https://doi.org/10.1111/j.1095-8312.2011.01621.x

Habtes, S., Muller-Karger, F. E., Roffer, M. A., Lamkin, J. T., & Muhling, B. A. (2014). A comparison of sampling methods for larvae of medium and large epipelagic fish species during spring SEAMAP ichthyoplankton surveys in the Gulf of Mexico. *Limnology and Oceanography Methods*, *12*, 86–101. https://doi.org/10.4319/lom.2014.12.86

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*, e0134647. https://doi.org/10.1371/journal.pone.0134647

Hubert, N., Delrieu-Trottin, E., Irisson, J.-O., Meyer, C., & Planes, S. (2010). Identifying coral reef fish larvae through DNA barcoding: A test case with the families Acanthuridae and Holocentridae. *Molecular Phylogenetics and Evolution*, *55*, 1195–1203. https://doi.org/10.1016/j.ympev.2010.02.023

Hutchings, L., Beckley, L. E., Griffiths, M. H., Roberts, M. J., Sundby, S., & van der Lingen, C. (2002). Spawning on the edge: Spawning grounds and nursery areas around the southern African coastline. *Marine and Freshwater Research*, *53*, 307–318. https://doi.org/10.1071/MF01147

Hyde, J. R., Lynn, E. A., Humphreys, R. Jr, Musyl, M. K., West, A. P., & 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. *Marine Ecology Progress Series*, *286*, 269–277. https://doi.org/10.3354/meps286269

Hykin, S. M., Bi, K., & McGuire, J. A. (2015). Fixing formalin: A method to recover genomic-scale DNA sequence data from formalin-fixed museum specimens using high-throughput sequencing. *PLoS ONE*, *10*, e0141579. https://doi.org/10.1371/journal.pone.0141579

Iserte, J. A., Stephan, B. I., Goni, S. E., Borio, C. S., Ghiringhelli, P. D., & Lozano, M. E. (2013). Family-specific degenerate primer design: A tool to design consensus degenerated oligonucleotides. *Biotechnology Research International*, *2013*, 38364.

Ivanova, N. V., Zemlak, T. S., Hanner, R. H., & Hebert, P. D. (2007). Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Resources*, *7*, 544–548.

Jansen, T., & Gislason, H. (2011). Temperature affects the timing of spawning and migration of North Sea mackerel. *Continental Shelf Research*, *31*, 64–72. https://doi.org/10.1016/j.csr.2010.11.003

Karnauskas, M., Cherubin, L. M., & Paris, C. B. (2011). Adaptive significance of the formation of multi-species fish spawning aggregations near submerged capes. *PLoS ONE*, *6*, e22067. https://doi.org/10.1371/journal.pone.0022067

Kent, M., Gill, W. J., Weaver, R. E., & Armitage, R. P. (1997). Landscape and plant community boundaries in biogeography. *Progress in Physical Geography*, *21*, 315–353. https://doi.org/10.1177/030913339702100301

Klima, E. F., & Wickham, D. A. (1971). Attraction of coastal pelagic fishes with artificial structures. *Transactions of the American Fisheries Society*, *100*, 86–99. https://doi.org/10.1577/1548-8659(1971) 100<86:AOCPFW>2.0.CO;2

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*, 1053–1058. https://doi.org/10.1080/02755947.2016.1185057

Leis, J. M., & Lee, K. (1994). Larval development in the lutjanid subfamily Etelinae (Pisces) – The Genera *Aphareus*, *Aprion*, *Etelis* and *Pristipomoides*. *Bulletin of Marine Science*, *55*, 46–125.

Lo, N. C. H., Ruiz, Y. A. G., Cervantes, M. J., Moser, H. G., & Lynn, R. J. (1996). Egg production and spawning biomass of Pacific sardine (*Sardinops sagax*) in 1994, determined by the daily egg production method. *California Cooperative Oceanic Fisheries Investigations Reports*, *37*, 160–174.

McEachran, J. D. (2009). Fishes (Vertebrata: Pisces) of the Gulf of Mexico. In D. L. Felder, & D. K. Camp (Eds.), *Gulf of Mexico–origins, waters, and biota* (Vol. *1*: Biodiversity, pp. 1223–1316). College Station, TX: Texas A&M University Press.

Montero-Pau, J., Gómez, A., & Muñoz, J. (2008). Application of an inexpensive and high-throughput genomic DNA extraction method for the molecular ecology of zooplanktonic diapausing eggs. *Limnology and Oceanography: Methods*, *6*, 218–222.

Muhling, B. A., Lamkin, J. T., Alemany, F., García, A., Farley, J., Ingram, G. W., … Carrion, R. L. (2017). Reproduction and larval biology in tunas, and the importance of restricted area spawning grounds. *Reviews in Fish Biology and Fisheries*, *27*, 697–732. https://doi.org/10.1007/s11160-017-9471-4

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*, 325–346. https://doi.org/10.1002/mcf2.10033

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., … Wagner, H. (2017). Package 'vegan'. CRAN R documentation. Retrieved from https://github.com/vegandevs/vegan, 292 pp.

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*, 353–367.

Peebles, E. B. (2002). Temporal resolution of biological and physical influences on bay anchovy (*Anchoa mitchilli*) egg abundance near a river-plume frontal zone. *Marine Ecology Progress Series*, *237*, 257–269. https://doi.org/10.3354/meps237257

Peebles, E. B., Hall, J. R., & Tolley, S. G. (1996). Egg production by the bay anchovy *Anchoa mitchilli* in relation to adult and larval prey fields. *Marine Ecology Progress Series*, *131*, 61–73. https://doi.org/10.3354/meps131061

Peebles, E. B., & Tolley, S. G. (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*, 397–410.

Reglero, P., Tittensor, D. P., Alvarez-Berastegui, D., Aparicio-Gonzalez, A., & Worm, B. (2014). Worldwide distributions of tuna larvae: Revisiting hypotheses on environmental requirements for spawning habitats. *Marine Ecology Progress Series*, *501*, 207–224. https://doi.org/10.3354/meps10666

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*, 508–518. https://doi.org/10.1111/j.1365-2419.2006.00417.x

Schmitz Jr, W. J.. (2005). Cyclones and westward propagation in the shedding of anticyclonic rings from the Loop Current. In W. Sturges & A. Lugo-Fernandez (Eds.), *Circulation in the Gulf of Mexico: Observations and models* (pp. 241–261). Geophysical Monograph Series 161.

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

Stratoudakis, Y., Bernal, M., Ganias, K., & Uriarte, A. (2006). The daily egg production method: Recent advances, current applications and future challenges. *Fish and Fisheries*, *7*, 35–57. https://doi.org/10.1111/j.1467-2979.2006.00206.x

Sutton, T. T., Clark, M. R., Dunn, D. C., Halpin, P. N., Rogers, A. D., Guinotte, J., … Heino, M. (2017). A global biogeographic classification of the mesopelagic zone. *Deep-Sea Research Part I*, *126*, 85–102. https://doi.org/10.1016/j.dsr.2017.05.006

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*, 52–54.

Velez, S., & Moore, J. A. (2018). Juvenile assemblages of families Lutjanidae and Serranidae in the Gulf of Mexico, with respect to the Loop Current and other hydrographic features. 2018 Gulf of Mexico Oil Spill and Ecosystem Science Conference, February 5–8, New Orleans, LA, USA (Abstract).

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

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. N. (2005). DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *360*, 1847–1857. https://doi.org/10.1098/rstb.2005.1716

Wei, C. L., Rowe, G. T., Haedrich, R. L., & Boland, G. S. (2012). Long-term observations of epibenthic fish zonation in the deep northern Gulf of Mexico. *PLoS ONE*, *10*, e46707. https://doi.org/10.1371/journal.pone.0046707

Weisberg, R. H., Zheng, L., & Peebles, E. B. (2014). Gag grouper larvae pathways on the West Florida Shelf. *Continental Shelf Research*, *88*, 11–23. https://doi.org/10.1016/j.csr.2014.06.003

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