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Spatial and Temporal Heterogeneity of Fish Spawning Dynamics on the West Florida Shelf During Fall

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ABSTRACT

Identifying spawning areas for economically and ecologically important fishes is critical for fisheries conservation and ecosystem-based management. We used genetic barcoding to identify fish eggs collected across the West Florida Shelf (WFS) during September of 2013, 2014, and 2019. Fish eggs were collected on Southeast Area Monitoring and Assessment Program (SEAMAP) ichthyoplankton cruises using a Continuous Underway Fish Egg Sampler. Analysis of 4833 fish eggs from the 3 years resulted in the identification of 82 species within 35 families. A 78% DNA barcoding success rate was achieved, with 46% of all identifications being at the species level. PERMANOVA results revealed significant differences in fish egg beta-diversity across time (the 3 years sampled) and space (preassigned levels of both depth class and regional strata). Our findings generally aligned with known adult fish distributions and spawning patterns, and we found that water-column depth played a more important role than regional strata in structuring the fish egg assemblages. Eggs from several economically important species were collected and observed at relatively high frequencies, including red snapper, lane snapper, vermilion snapper, yellowedge grouper, and king mackerel. We used the West Florida Coastal Ocean Model to hindcast the trajectories of all fish eggs and trace them back to possible spawning locations. We conducted backward tracking over a span of 36 h based on the assumption that most fish eggs on the WFS undergo hatching within this time window. The model estimated egg transport distances ranging from 1 to 79 km (mean distance of ~21 km), with greater transport distances estimated on the outer shelf in comparison to the middle and inner shelf. These results further our understanding of the spatial and interannual variation of fish spawning dynamics on the WFS and mark the beginning of a long-term monitoring effort.

1 | Introduction

Understanding the characteristics of fish spawning events is essential for effective fisheries management. Studying fish spawning and early-life stages provides a powerful tool for assessing population and ecosystem health, allows for the identification of critical habitats needed for successful reproduction, increases our understanding of dispersal patterns and population connectivity, aids in the estimation of population reproductive capacity, and allows for assessment of the effects of human activities and environmental changes on reproductive success (Fuiman and Werner 2009; Pankhurst and Munday 2011; Wright and Trippel 2009). This knowledge is especially critical on the West Florida Shelf (WFS), which supports dozens of fish species of

economic importance but where spawning information for most remains unknown. Addressing this knowledge gap is pivotal, as understanding spawning dynamics is useful for estimating the success of ensuing generations and ensuring the sustainability of fisheries.

Approximately 75% of marine teleosts broadcast spawn pelagic buoyant eggs that float near the surface due to their lipid-rich content (Miller and Kendall 2009). This includes most fish species of commercial and recreational importance that spawn in the eastern Gulf of Mexico, such as groupers, snappers, drums, grunts, porgies, tunas, and mackerels (Allen 1985; Collete 1983; Heemstra and Randall 1993). Collection of pelagic fish eggs allows for sampling across trophic levels and is also effective for species that are cryptic or tend to be elusive when using other capture methods. Egg surveys provide valuable insights into defining spawning areas (Burrows et al. 2019; Harada et al. 2015; Kerr et al. 2020; Lewis et al. 2016), assessing faunal diversity (Ahern et al. 2018), determining dispersal patterns (Nguyen et al. 2024), and understanding reproductive processes critical for estimating future stock sizes (Armstrong and Witthames 2012; Rothschild 2000). Despite the myriad of motivations for understanding reproductive output of fishes, the planktonic early-life stages of most fishes are understudied (Zhang et al. 2022).

The primary reason that fish eggs have been understudied is because they are challenging to identify based on morphology. It has been estimated that eggs from less than 10% of broadcast-spawning fishes can be reliably identified to the species level using visual methods (Shao et al. 2002) and comparative analyses have shown that visual identification of eggs and larvae is unreliable when compared to molecular methods (Larson et al. 2016). DNA barcoding of the cytochrome c oxidase I (COI) gene has become a widely used method for taxonomic identification of ichthyoplankton and has proven to be an effective way to successfully identify large numbers of fish eggs and identify spawning sites for fishes that broadcast spawn pelagic eggs (Burrows et al. 2019; Duke et al. 2018; Harada et al. 2015; Kerr et al. 2020; Lewis et al. 2016). The Barcode of Life Database (BOLD) contains over 24,000 species barcodes for Actinopterygii (ray-finned fishes), making DNA barcoding an effective way to identify most known ray-finned fish species (Ratnasingham and Hebert 2007).

Historically, challenges in identifying fish eggs have necessitated the use of larvae for spawning area identification (Peebles and Tolley 1988; Sassa et al. 2006). However, the use of fish eggs enables a more accurate approach to identifying spawning locations. In the eastern Gulf of Mexico, fish eggs typically hatch within 36 h after being spawned during summer/early fall temperatures, as warmer water accelerates their development (Pauly and Pullin 1988; Barón-Aguilar et al. 2015). In contrast, fish larvae can be up to several weeks old (Houde and Chitty 1976). Larvae therefore are exposed to oceanic currents for a longer duration of time than fish eggs, adding uncertainty when trying to backtrack their trajectories to the location where they were spawned. Additionally, postflexion fish larvae can behaviorally control their movement through vertical migration and directed horizontal swimming (Cowen and Sponaugle 2009). The vertical distribution behavior of larvae can greatly influence their dispersal, even early in development, and as larvae develop and enter the postflexion stage, they gain the ability to actively swim at speeds faster than typical currents for extended periods, allowing them to travel up to tens of kilometers (Leis 2006). Additionally, larval and egg assemblages have been shown to have large disparities in composition in both estuarine (Burghart et al. 2014) and oceanic environments (Lin et al. 2016), further demonstrating that relying solely on larvae for identifying spawning areas may be misleading. In contrast to larvae, fish eggs are younger, tend to behave as passive particles, and are positively buoyant, which makes them better candidates than fish larvae for identifying spawning locations.

To monitor fish spawning, we analyzed planktonic fish eggs collected with a Continuous Underway Fish Egg Sampler (CUFES), a proven tool for sampling high quantities of fish eggs over large-scale geographic areas (Checkley et al. 1997, 2000). The CUFES consists of a submersible pump attached to the ship's hull that draws in water from a 3-m depth while a research vessel is underway. The water then flows to a concentrator that uses an oscillating net to capture egg-sized particles. Finally, the concentrated sample is collected on a mesh filter. Although CUFES samples may have lower precision compared to vertically integrated bongo nets (Pepin et al. 2005), its ability to collect fish eggs continuously while underway provides improved spatial resolution compared to the use of towed nets. Furthermore, CUFES samples contain a higher proportion of fish eggs relative to nontarget material compared to traditional net tows, which facilitates faster sample processing. The CUFES is an ideal method for mapping egg distributions for broadcast-spawning fishes because their eggs typically float to the surface early in development, where they remain abundant even during turbulent mixing (Conway et al. 1997; Lelievre et al. 2012).

To address knowledge gaps in the reproductive ecology of the WFS fish community, we used DNA barcoding to identify archived fish egg samples collected using a CUFES on National Oceanic and Atmospheric Administration (NOAA) Southeast Area Monitoring and Assessment Program (SEAMAP) cruises in September of 2013, 2014, and 2019. The WFS is a 170,000-km^2 carbonate shelf that extends off the west coast of Florida in the northeastern Gulf of Mexico and contains a rich assemblage of fish species that support a multibillion-dollar fishery (NMFS 2024). Here, we aimed to expand the knowledge base for fisheries ecology on the WFS by examining the primary spawning areas for fish species on the WFS, particularly those of economic and ecological importance, and exploring the interannual and spatial variability in fish spawning assemblages.

2 | Methods

2.1 | Fish Egg Collections

Samples were collected on NOAA SEAMAP oceanographic cruises in September of 2013, 2014, and 2019 using the CUFES. The 2013 and 2019 cruises were on the NOAA Research Vessel (R/V) Pisces, and the 2014 cruise was on the NOAA R/V Gordon Gunter. Samples were collected on the WFS from 81°00′ W to 87°50′ W and 25°00′ N to 30°30′ N (Figure 1). The submersible pump associated with the CUFES was located at a depth of three meters and the concentrator net and sample collector had

a mesh size of 500 μm. The pump ran continuously during the surveys along with the ship's flow-through system so that temperature and salinity data were collected simultaneously with the CUFES samples.

CUFES samples were collected in 30-min intervals while transiting between SEAMAP stations. These stations were part of a systematic grid spaced approximately 30 nautical miles apart. Given the vessel's average speed of 10 knots, each CUFES sample covered a distance of 8–10 km along a transect. Several CUFES samples were taken while transiting between each pair of SEAMAP stations. At the end of each 30-min interval along the transect (referred to as a "site" and shown by the location of the starting position), all material from the CUFES sample was rinsed into an individual ~20-mL collection vial and preserved with 95% ethanol. CUFES samples were collected from 775 sites across the 3 years of sampling, of which we processed 199 for this study (63 sites in 2013, 73 sites in 2014, and 63 sites in 2019; Table 1). Figure 1 shows the 199 processed sites used for this study. Sampling locations and dates were not identical across the 3 years but were consistent in geographic extent (Figure 1). To select these 199 processed sites while ensuring spatial balance, we employed the Generalized Random Tessellation Stratified (GRTS) method (Stevens and Olsen 1999) using the *spsurvey* package in R (Dumelle et al. 2023). This approach treats the

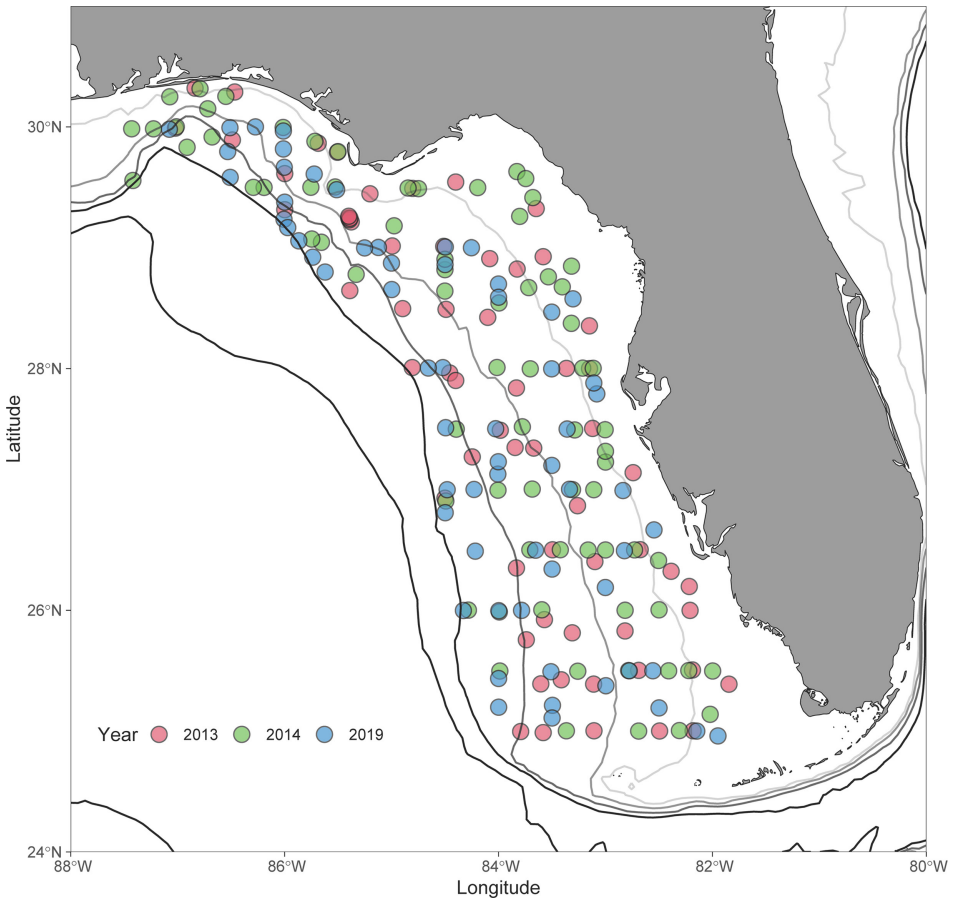

FIGURE 1 | Map of processed fish egg collection locations for 2013, 2014, and 2019. Red circles represent 2013 sites, green circles represent 2014 sites, and blue circles represent 2019 sites. The start point of the collection transects are used. Gray and black lines represent 25-, 50-, 100-, 200-, and 1000-m isobaths.

TABLE 1 | Summary of fish egg collection information for each sampling year.

Year (number of sites processed)	Number of sites where fish eggs were present	Number of sites where fish eggs were successfully barcoded	Total fish eggs collected	Maximum number of eggs at a site	Number of fish eggs collected per site (mean ± standard error)
2013 ($n = 63$)	60 (95%)	53 (84%)	2840	222	45.1 ± 6.0
2014 ($n = 73$)	70 (96%)	59 (81%)	448	39	6.1 ± 0.7
2019 ($n = 63$)	61 (97%)	58 (92%)	2204	285	35.0 ± 5.6

TABLE 2 | Annual sampling information for NOAA SEAMAP cruises on the WFS.

Year	Range of dates sampled	Water-column depth sampled (mean ± standard error)	Surface water temperature (mean ± standard error)	Surface water salinity (mean ± standard error)
2013	9/13–9/26	52.7 ± 4.8	29.8 ± 0.05	34.1 ± 0.3
2014	9/7–9/27	53.4 ± 5.3	29.6 ± 0.05	34.5 ± 0.1
2019	9/11–9/24	76.3 ± 6.9	29.7 ± 0.09	33.4 ± 0.3

entire WFS as the sampling domain, aiming for unbiased spatial coverage across the area and minimizing potential biases from consistently sampling the same locations. Although randomized year-to-year site selection may have introduced additional variation in observed species assemblages among years, GRTS helps control for spatial bias and allows for a more robust analysis of long-term trends across the entire WFS. There was variation in average water-column depth at sampling sites across the sampling years (Table 2), but eggs were only processed at sites where depth was 200 m or less.

2.2 | Molecular Analysis of Fish Eggs

We poured each CUFES sample into a gridded dish and picked individual fish eggs under a dissecting microscope following the pattern of the grid until either 96 or all eggs were removed, whichever came first. If more than 96 eggs were present in a sample, the rest of the eggs were counted but not removed for further processing because 96 is the maximum number of wells in the thermocycler. We placed the individual fish eggs into individual polymerase chain reaction (PCR) tubes before performing a DNA extraction using the HotSHOT method (Truett et al. 2000). We performed the HotSHOT DNA extraction method by adding 50 μL of alkaline lysis buffer (0.2-mM disodium EDTA, 25-mM NaOH, pH 12) to each PCR tube and pulverizing each egg against the walls of the tube using an autoclaved toothpick. Next, we placed the PCR tubes in a thermocycler for 30 min at 95°C, then onto ice for 3 min to cool. After cooling, we added 50 μL of neutralization buffer (40-mM Tris–HCL, pH 5) and vortexed the samples. We stored the extracted DNA at −20°C until PCR was performed to amplify a portion of the mitochondrial COI gene with the COI-3 universal fish primer cocktail (Ivanova et al. 2007). The 50-μL PCR for each fish egg contained 2 μL of extracted DNA and final concentrations of 1× Apex NH_4 buffer, 1.5-mM Apex $MgCl_2$, 10-μg/μL bovine serum albumin, 0.2-μM Apex dNTPs, 0.2-μM primer cocktail, and 1-U Apex RedTaq (Genesee Scientific). The PCR cycling conditions were as follows: heating to 94°C for 2 min, 45 cycles of (94°C for 30 s, 52°C for 40 s, and 72°C for 1 min), and a final extension at 72°C for 10 min. We checked for positive PCR amplification for each sample using gel electrophoresis on a 1.5% agarose gel stained with ethidium bromide. We purified and Sanger sequenced the successful PCR products using the M13 forward primer by TACGen (tacgen.com).

We used the Geneious bioinformatics software (Kearse et al. 2012) to trim and assemble the DNA sequences. Trimming parameters were set to trim primers allowing five mismatches, 5′ and 3′ ends were also trimmed with an error probability of 0.05 and trimming at least three base pairs. We considered trimmed sequences shorter than 80 base pairs to be unidentified and not processed further. We used the BOLD (Ratnasingham and Hebert 2007) to identify sequences to the lowest taxonomic level possible using a 97% match threshold for identification. When sequences had no match in BOLD, we used the Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990) against the GenBank nucleotide database for sequence identification. We considered sequences that did not have matches meeting the 97% identification threshold in both BOLD and GenBank to be unidentified. Detailed collection and identification data, including the fish egg DNA sequences, can be found in the Gulf Science Data Repository (GRIIDC) (https://doi.org/10.7266/dmp83r1m; https://doi.org/10.7266/j7qzhk64).

2.3 | Taxonomic Classification

To minimize bias in fish egg assemblage composition analyses, we used the most resolved taxonomic identifications possible. For example, we combined taxonomic groups consistently identifiable only to the genus level (e.g., *Synodus* spp. and *Prionotus* spp.) into single taxonomic units. However, some identifications occurred at the family level (e.g., Synodontidae, unable to distinguish between *Synodus poeyi*/*Saurida normani* within these genera). We excluded these family-level identifications from assemblage composition analyses as both *Synodus* spp. and *Saurida* sp. existed as separate lowest taxonomic groups. Inclusion would have resulted in either artificially decreasing diversity by assigning all *Synodus* spp. and *Saurida* sp. identifications to the coarser taxonomic level of family (Synodontidae) or artificially inflating diversity by counting this identification as distinct from either *Synodus* spp. or *Saurida* sp. These cases were rare and only accounted for 19 out of the 3778 successful identifications. Additionally, we treated taxa as a singular taxonomic unit in cases where identifications were typically unresolved between two closely related species within the same genus (e.g., *Decapterus punctatus*/*Decapterus tabl*), but a few specimens of one of the taxa (e.g., *D. punctatus*) were identified to the species level. This approach aimed to avoid the artificial inflation of diversity by not counting the unresolved taxa as separate entities when there was limited confidence in their distinction.

2.4 | Statistical Analyses

To estimate the total taxon richness present in the study area, we created a taxon accumulation curve using the *specaccum* function within the vegan package (Oksanen et al. 2022). We used the

accumulation curve to model how the number of taxa detected increased with the number of samples collected, thus providing estimated total taxon richness in a given area. Specifically, we used the nonlinear, self-starting "lomolino" model, to estimate the asymptote of the curve. This model provides initial estimates that are further refined through the fitting process. The asymptote of this curve represents the total fish egg taxon richness expected in the study area for the sampling period, based on our collection and identification methods.

To objectively identify distinct groups within the fish egg assemblage based on their dissimilarity, we conducted a dissimilarity profile (DISPROF) analysis. We used the *ClustDISPROF* (Jones et al. 2023) package to perform DISPROF analysis with agglomerative, hierarchical clustering. A DISPROF analysis creates a dissimilarity profile for a set of objects and compares it to profiles generated from permuted data to test for multivariate structure; this approach has proven to be effective as a clustering decision criterion (Clarke et al. 2008; Kilborn et al. 2017). DISPROF was used to interpret a dendrogram created from an unweighted pair group method with arithmetic mean (UPGMA) clustering analysis. A series of DISPROF tests objectively identified the number of statistically significant groups (1000 permutations, $\alpha = 0.05$) within the UPGMA-created dendrogram based upon fish egg assemblage similarity. We mapped the groups identified via DISPROF to visualize spatial and temporal patterns in fish egg assemblage structure across the study area.

We used permutational multivariate analysis of variance (PERMANOVA) to assess whether assemblage composition differed among years, depths, and regions, including tests for potential interactions among these factors. PERMANOVA is a robust nonparametric statistical method designed for analyzing composition and abundance data in multivariate datasets (Anderson 2014). PERMANOVA uses permutation tests on dissimilarity matrices to determine whether a priori-assigned groups differ (i.e., constrained analysis). Distance-based methods, such as those based on Bray–Curtis dissimilarity, are used to compare fish egg assemblage composition by quantifying pairwise dissimilarities between samples. We performed post hoc pairwise tests to examine differences between years, depths, and regions. We used Holm *p*-value adjustment for pairwise tests to account for multiple comparisons (Holm 1979). For all permutational analyses, *p* values were calculated using 10,000 permutations and significance was assessed with $\alpha = 0.05$ unless otherwise specified. Following PERMANOVA, we visually verified the results using canonical analysis of principal coordinates (CAP), a constrained ordination method (Anderson and Willis 2003). We used leave-one-out cross-validation (LOO-CV) to identify group overlap and to quantify separation among groups. Additionally, to determine which taxa were indicative of particular groups, we used an indicator value analysis (IndVal) (Dufrêne and Legendre 1997). These analyses and all subsequent statistical analyses were performed using R version 4.1.2 (R Core Team 2021).

Before PERMANOVA, CAP, and IndVal analyses, we classified sites by water-column depth and by regional strata following definitions adapted from Switzer et al. (2023) (Table 3). This classification allowed us to test whether fish egg assemblages differed among groups and determine if the assemblages aligned with expectations based on previous research.

TABLE 3 | Classification of sampling sites by region and water-column depth. Sample size refers to the number of sites (*n*) from which eggs were successfully identified for each category.

Classification	Description	Sample size (*n*)
Region		
South Florida	Sites south of 27° N latitude	55
Big Bend	Sites north of 27° N latitude and east of 84.9° W longitude (including 27° N and 84.9°)	60
Panhandle	Sites north of 27° N latitude and west of 84.9° W longitude	55
Depth (m)		
25 and less	Water-column depth of 25 m or less	43
25–50	Water-column depth between 25 and 50 m	65
50–100	Water-column depth between 50 and 100 m	37
100–200	Water-column depth between 100 and 200 m	25

We performed square-root transformations of the fish egg data before analyses to downweigh highly abundant taxa. The square-root transformation was chosen based on the interpretation of a shade plot (Clarke et al. 2014) (Figure S1). We used Bray–Curtis dissimilarity matrices for DISPROF, PERMANOVA, and CAP analyses.

We used generalized linear mixed models (GLMM; Poisson family) to examine the relationships between total egg abundance and taxon richness (as individual response variables) with the effects of sampling year, water-column depth, and latitude (as fixed explanatory variables) and with site included as a random variable. For explanatory variables, we used the mean of the start and end values of water-column depth and latitude recorded during each sampling transect. We scaled the explanatory variables to have a mean of zero and a standard deviation of one. We performed these analyses using the *lme4* package (Bates et al. 2014). We calculated the total egg abundance based on the raw counts of eggs from a site whereas taxon richness was based on the number of unique taxonomic identifications from a maximum of 96 eggs processed per site. We removed sites where none of the processed eggs were successfully barcoded from the analysis to avoid assigning them a false richness of zero.

2.5 | Fish Egg Trajectory Modeling

The West Florida Coastal Ocean Model (WFCOM) is a high-resolution ocean circulation model that downscales from

deep ocean, across the WFS and into the estuaries (Weisberg et al. 2014a; Zheng and Weisberg 2012). Configured for realistic simulation of the ocean circulation on the shelf, it has been validated with observations in previous studies (Liu et al. 2014, 2020). The model has been used in various applications, such as estimating fish larvae and fish egg transport pathways (Nguyen et al. 2024; Weisberg et al. 2014b), and red tide tracking (Liu et al. 2023; Weisberg et al. 2019). Based on the WFCOM hindcast, we used a Lagrangian trajectory model to track the fish eggs backward for 36 h. We used surface currents for tracking because we assumed that the buoyant fish eggs floated passively at the surface layer and were advected by the currents. We chose 36 h for the trajectory time based on fish egg hatching times described in Pauly and Pullin (1988) for mean water temperature values measured during the sampling period. To quantify the dispersal of the fish eggs, we calculated the distance between the point of collection and the estimated point of origin for each 36-h trajectory. We only modeled sites that had successful identifications.

3 | Results

3.1 | Fish Egg Identifications

Across the 3 years of sampling, we processed 4833 fish eggs (5492 fish eggs were collected; a maximum of 96 fish eggs were processed from each site). Of the 4833 fish eggs processed, 3778 (78.2%) resulted in successful identification (Table 4). The unidentified eggs ($n = 1055$) most commonly failed to amplify during the PCR; however, in some cases, we obtained poor-quality sequences from amplified DNA, or the resulting sequence did not match to a reference sequence in BOLD or NCBI. Of the 3778 fish eggs identified, 1737 fish eggs (46%) were matched to a single species and represent the highest taxonomic resolution of identifications. The remaining 2041 fish eggs (54%) showed more complex outcomes. This included 1837 eggs (49%) matching two species within the same genus and 204 eggs (5%) with matches to either more than two species within a genus or a combination of species from different genera within the same family.

TABLE 4 | DNA barcoding success rate and percentage of total successful identifications matched to a single species, two species in the same genus, or more than two species. *Decapterus* spp. accounted for 54% of IDs that contained two species in the same genus.

Barcoding success and resolution ($n = 4833$ eggs processed)	
Barcoding success rate (one or more ID with a > 97% match)	78.2% (3778 eggs)
Single species ID	35.9% (1737 eggs)
Two species-level IDs in the same genus	38.0% (1837 eggs)
More than two species in the same genus or two or more species from different genera	4.2% (204 eggs)

3.2 | Assemblage Composition

Across all 3 years, we observed 82 species, 70 genera, and 35 families from the 3778 identifications (Table 5). We observed a total of 90 unique taxa including those identified to the genus but not species level. The taxon accumulation curve (Figure 2) did not reach an asymptote, suggesting that a considerable number of additional taxa could be identified with additional sampling. Most often, there were only a few eggs for each taxon found at a particular site (median = 2) and cases where there were large quantities of eggs from a single taxon at a site were generally rare. There were 43 instances where 20 or more eggs were collected from a single taxon at a site, with six of these involving 50 or more eggs. In total, 12 taxa had at least one occurrence of 20 or more eggs collected at a site, and, of these, only two taxa had an instance of 50 or more eggs (Table S1).

The eight most abundant families by total number of eggs collected were, in order of greatest to least, Carangidae, Triglidae, Synodontidae, Lutjanidae, Cyclopsettidae, Scombridae, Stromateidae, and Serranidae (Figure 3). These eight families accounted for 88.4% of the fish eggs identified. Although there were notably fewer eggs collected in 2014, the relative abundance

TABLE 5 | The total number of fish species, genera, and families represented in successful fish egg identifications for each year.

	2013	2014	2019	Total
Species	53	32	50	82
Genera	50	33	44	70
Families	24	21	25	35

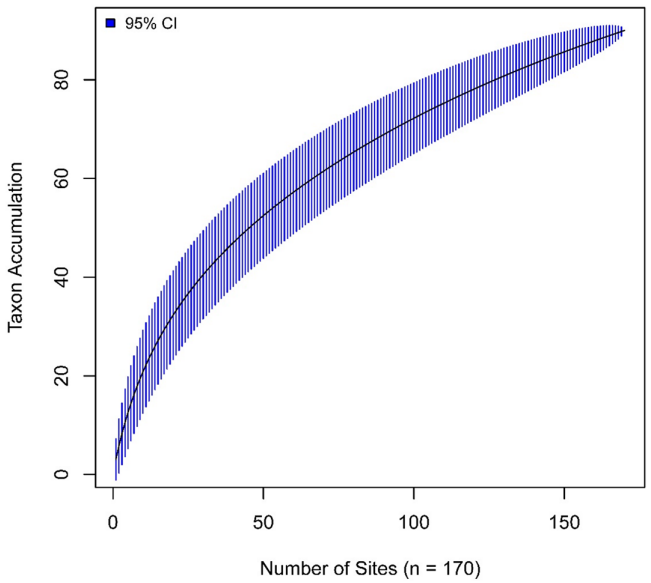

FIGURE 2 | Taxon accumulation curve for fish egg sampling using a sample-based rarefaction method. The *x* axis represents the cumulative number of samples processed across the three sampling years, while the *y* axis depicts the cumulative number of identified fish species. Blue lines represent confidence intervals for the mean taxon richness as a function of the number of sites sampled.

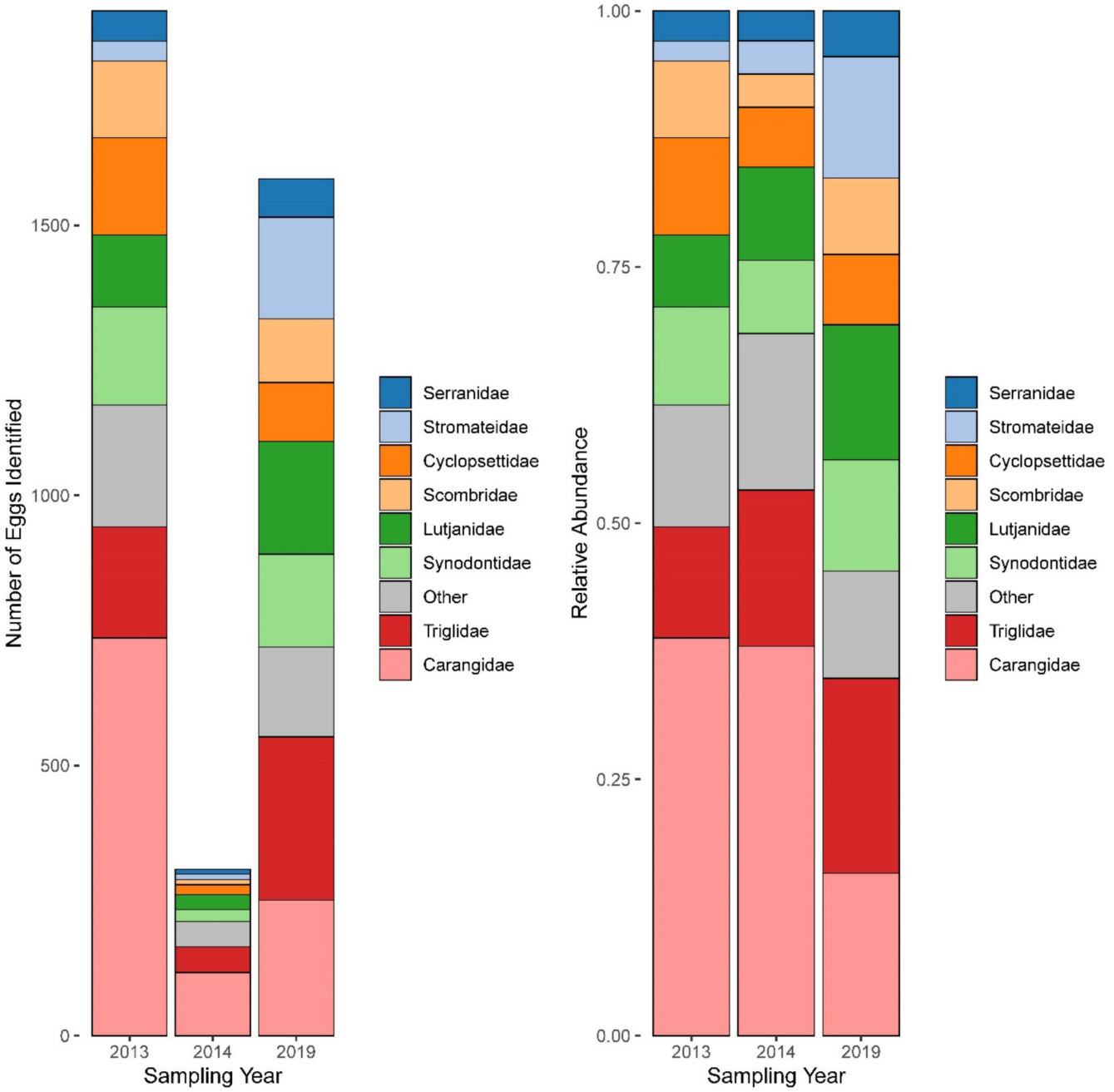

FIGURE 3 | Stacked bar charts showing (left) total abundance and (right) relative abundance of eggs identified for the eight most abundant fish families. The "Other" category includes eggs from all other fish families present in the study ($n = 28$).

of each of these families was consistent across the 3 years. The carangids, which includes the genus *Decapterus*, was the most abundant family in terms of total egg abundance.

All the most highly represented genera, *Decapterus* (Carangidae), *Prionotus* (Triglidae), *Syacium* (Cyclopsettidae), and *Synodus* (Synodontidae), belonged to one of the eight most abundant families. These four genera accounted for 55.7% of all identifications. There were only two taxa identified from the *Decapterus* genus, and the identifications were rarely resolved at the species level between *D. punctatus* (round scad) and *D. tabl* (roughear scad). Overall, *D. punctatus/tabl* was the most abundant taxon in our study, accounting for 94% of carangid identifications and 27% of all egg identifications.

3.3 | Federally Managed Species

Fish eggs from multiple federally managed species were identified, including *Lutjanus analis* (mutton snapper), *Lutjanus campechanus* (red snapper), *Lutjanus griseus* (gray snapper), *Lutjanus synagris* (lane snapper), *Rhomboplites aurorubens* (vermilion snapper), *Pristipomoides aquilonaris* (wenchman), *Hyporthodus flavolimbatus* (yellowedge grouper),

Scomberomorus cavalla (king mackerel), *Scomberomorus maculatus* (Spanish mackerel), *Katsuwonus pelamis* (skipjack tuna), and *Istiophorus platypterus* (sailfish). Fish eggs from federally managed taxa accounted for 568 (15.0%) of all successful identifications. Maps of egg distributions for all federally managed taxa are shown in Figures S2–S14. These maps display the distinct spatial distributions of the eggs from these taxa. For example, eggs from some taxa, such as yellowedge grouper and wenchman, were consistently found in deeper offshore waters, while Spanish mackerel and lane snapper eggs were predominantly found in nearshore areas. Additionally, some taxa exhibited a wide distribution of their eggs, whereas others were confined to a smaller spatial range. The total number of eggs identified, encounter frequency (number of sites present), and mean geographical and environmental parameters at the collection site are shown for 10 of the most abundant of these species in Table 6. There was notable variation in the years some taxa were present, the geographic location of the eggs, and the water-column depth at the location where the eggs were collected, but there were no large variations observed in mean temperature or salinity where eggs were found for each of these species (Table 6).

Of the federally managed species whose eggs were identified, vermilion snapper was encountered at the highest number of sites (19.4%; $n = 33$), followed by wenchman (12.9%; $n = 22$) and red snapper (12.9%; $n = 22$). Wenchman and vermilion snapper had the highest and second highest total egg counts from this group ($n = 145$ and $n = 122$, respectively), while red snapper had the third highest ($n = 70$). Generally, only a few eggs of federally managed taxa were collected from each site; however, wenchman eggs were the exception, with two instances each of over 30 eggs being found at a site.

3.4 | Spatial and Interannual Trends

The DISPROF analysis produced 16 groups within the 170 sampling sites that contained fish eggs with successful identifications (Figure 4). Five of the 16 groups were present in all 3 years of sampling. Nearly all the groups exhibited a large spatial extent, particularly with respect to latitude. However, several groups exhibited a spatial organization that aligned with their respective water-column depths. For instance, it was common for sites within a group to be widely dispersed but occur within similar isobathic ranges. IndVal analysis resulted in eight significant indicators: *Prionotus* spp., *Synodus* spp., *Myrophis punctatus*, *Xyrichtys novacula*, *Selar crumenophthalmus*, *Prognichthys occidentalis*, *Saurida* sp., and *D. punctatus/tabl* (Table S2).

All interaction effects in the PERMANOVA analysis were significant, indicating that the effects of year, region, and depth on fish egg beta-diversity were not independent of one another (Table 7). Despite this, we explored the individual factors to provide a more detailed understanding of how each variable was related to the observed patterns in fish egg beta-diversity. However, it is important to note that the effects of individual factors were likely overestimated due to the significant interaction terms, which suggests that these factors are interdependent and cannot be fully understood in isolation.

There were significant differences in the fish egg beta-diversity among the four assigned depth classes (PERMANOVA $F_{3,138} = 9.74$, $R^2 = 0.13$, $p = 0.0001$; Table 7). Pairwise tests indicated that all four of the assigned depth classes were significantly different from one another (Holm's adjusted $p < 0.001$ for all six pairwise comparisons). The PERMANOVA results were supported by the CAP ordination (Figure 5), which shows that the four depth classes plotted in clusters that form a gradient with neighboring depth classes overlapping. This gradient was further evidenced by the confusion matrix from the LOO-CV, which showed that when sites from a group were misclassified, they were most likely to be misclassified with adjacent depth classes (Table S3). For example, when sites from the 25–50 m group were held out and reclassified, they were correctly classified 64.6% of the time, and misclassified as 0–25 m and 50–100 m 18.5% and 16.9% of the time, respectively, and were never misclassified as 100–200 m. The CAP model had an overall classification success rate of 69.41% (118/170 sites were classified correctly) for the depth grouping factor. IndVal analysis for water-column depth resulted in 25 significant indicators with all depth classes having at least one taxon that was a significant indicator (0–25 $n = 14$; 25–50 $n = 1$; 50–100 $n = 4$; 100–200 $n = 6$; see Table S4 for full list of indicators).

There were significant differences in fish egg beta-diversity among the three sampled regions (PERMANOVA $F_{2,138} = 2.64$, $R^2 = 0.02$, $p = 0.0001$; Table 7). Pairwise tests indicated that the Panhandle region had significantly different fish egg beta-diversity than the Big Bend and the South Florida regions of the WFS (Holm's adjusted $p = 0.0020$ and 0.0071). However, no significant differences were found between the beta-diversity of the fish egg assemblage for the Big Bend and South Florida region (Holm's adjusted $p = 0.3209$). The confusion matrix from the LOO-CV showed that there was substantial overlap between nearly all pairs of regions when sites were held out and reclassified (Table S5), with sites from the Big Bend and South Florida regions most often misclassified as each other. The CAP model had an overall classification success rate of 46.47% for the region grouping factor, which was the lowest among the three grouping tests. IndVal analysis for region resulted in 10 significant indicators with all regions having at least one significant indicator (Big Bend $n = 5$; Panhandle $n = 4$; South Florida $n = 1$; see Table S6 for full list of indicators).

Fish egg beta-diversity differed among the 3 years of sampling (PERMANOVA $F_{2,138} = 5.99$, $p = 0.0001$; Table 7). Pairwise tests indicated that all 3 years were significantly different from one another (Holm's adjusted $p < 0.005$ for all three pairwise comparisons). The PERMANOVA results were supported by the CAP ordination (Figure 6), which showed the 3 years formed discrete clusters with minimal overlap. However, there was a greater amount of overlap between the 2013 and 2019 clusters in the CAP ordination than in the other pairs. To further quantify group overlap, we generated a LOO-CV confusion matrix (Table S7). The confusion matrix from the LOO-CV showed that 2014 sites were most likely to be accurately classified (91.5%) when sites were held out and reclassified, and sites from 2013 and 2019 were more frequently misclassified as each other. Specifically, 2013 sites were misclassified as 2019 26.4% of the time. Similarly, 2019 sites were misclassified as 2013 34.5% of the time. The CAP model had an overall classification success

TABLE 6 | Sampling information for federally managed taxa whose eggs were collected and identified. Encounter frequency refers to the number of sites where eggs were collected. Mean depth refers to the water-column depth where eggs were collected (samples were all collected from 3-m depth).

Taxon	Number of eggs identified	Encounter frequency	Mean latitude ± standard error	Mean depth ± standard error (m)	Mean temperature ± standard error (°C)	Mean salinity ± standard error
Pristipomoides aquilonaris	145	22	28.27 ± 0.29	115.1 ± 10.2	29.7 ± 0.1	33.1 ± 0.6
Rhomboplites aurorubens	122	33	27.55 ± 0.25	58.8 ± 6.3	29.7 ± 0.1	34.2 ± 0.3
Lutjanus campechanus	70	22	29.09 ± 0.22	47.9 ± 4.6	29.6 ± 0.1	32.0 ± 0.7
Scomberomorus cavalla	68	19	28.17 ± 0.38	49.1 ± 2.8	29.7 ± 0.1	33.1 ± 0.8
Scomberomorus maculatus	59	13	28.30 ± 0.29	22.0 ± 1.4	30.0 ± 0.1	33.9 ± 0.5
Lutjanus synagris	20	12	26.77 ± 0.44	29.4 ± 3.6	29.8 ± 0.1	35.2 ± 0.2
Hyporthodus flavolimbatus	10	4	26.25 ± 0.43	152.5 ± 17.7	30.4 ± 0.1	35.7 ± 0.6
Lutjanus analis	10	2	25.00 ± 0.01	68.3 ± 8.3	29.3 ± 0.0	35.3 ± 0.1
Lutjanus griseus	2	2	29.50 ± 0.72	35.3 ± 5.6	29.7 ± 0.3	33.8 ± 1.7

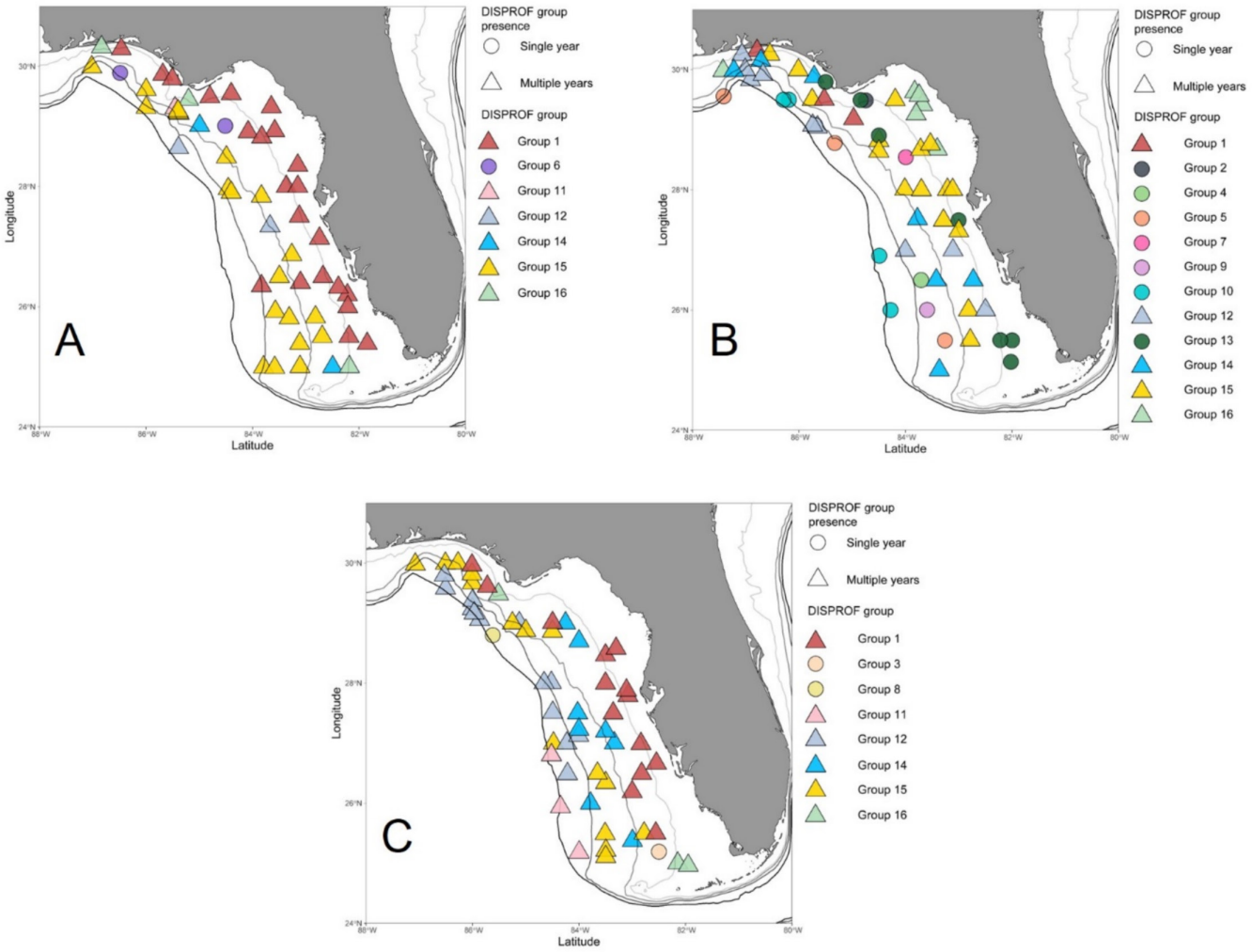

FIGURE 4 | Maps of fish egg collection locations that were selected by the GRTS method, colored by their assigned DISPROF groups. Sampling locations where no eggs were identified are not shown. Triangles represent DISPROF groups present in multiple years while circles represent DISPROF groups unique to a single year. Gray and black lines represent 25-, 50-, 100-, and 200-m isobaths (A: 2013, B: 2014, C: 2019).

TABLE 7 | PERMANOVA results of fish egg assemblages for the factors year, region, water-column depth, and their interactions.

Factor	df	SumOfSqs	R^2	Pseudo-F	Pr(>F)
Year	2	3.513	0.0541	5.9874	0.0001
Region	2	1.547	0.02383	2.637	0.0001
Depth group	3	8.573	0.13202	9.7401	0.0001
Year:Region	4	2.135	0.03287	1.8189	0.0006
Year:Depth group	6	2.97	0.04573	1.6871	0.0001
Region:Depth group	6	2.787	0.04292	1.5832	0.001
Year:Region:Depth group	8	2.925	0.04505	1.2463	0.0401
Residual	138	40.486	0.62348		
Total	169	64.936	1		

rate of 69.41% (118/170 sites were classified correctly) for the year grouping factor. IndVal analysis for sampling year resulted in 15 significant indicators. Only 2013 ($n=9$) and 2019 ($n=6$) had taxa that were significant indicators (see Table S8 for full list of indicators).

Fish egg taxon richness was not related to latitude (coefficient = 0.039, $se=0.049$, $z=0.796$, $p=0.426$) but was negatively related with depth (coefficient = −0.286, $se=0.056$, $z=-5.080$, $p<0.001$). Additionally, taxon richness was positively related with year (coefficient = 0.103, $se=0.049$, $z=2.117$, $p=0.034$).

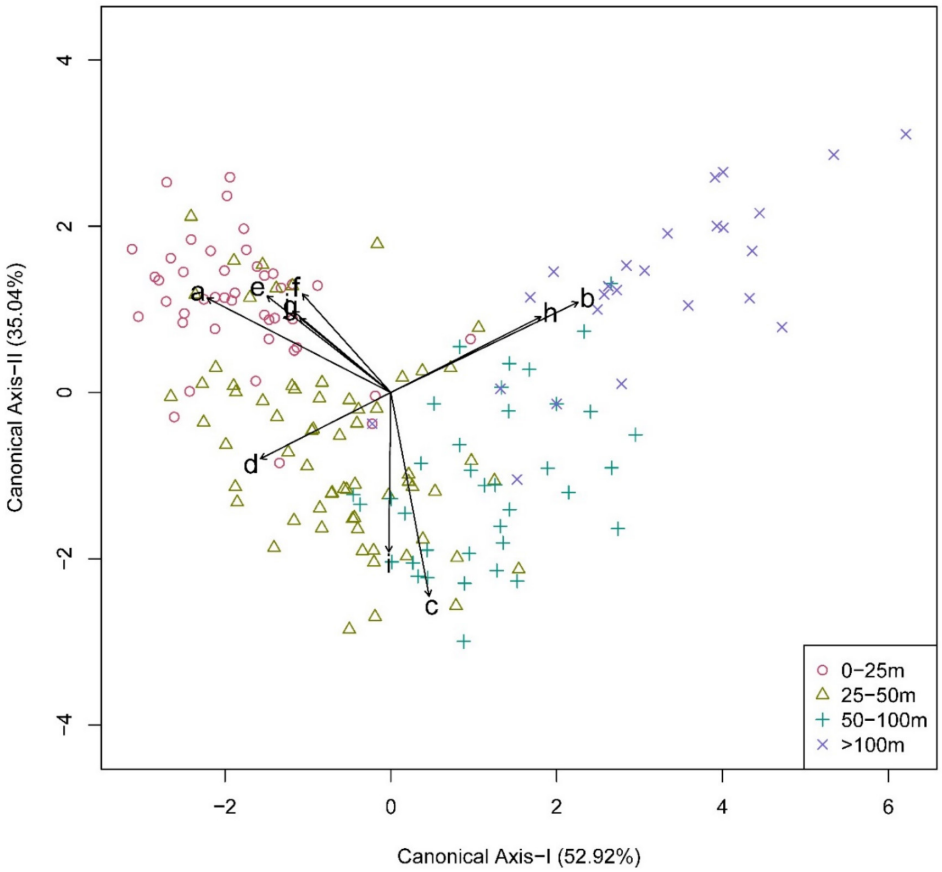

FIGURE 5 | Canonical analysis of principal coordinates (CAP) ordination diagram that includes all 3 years of fish egg data and depicts differences among depth classes with respect to fish egg beta-diversity. The canonical axes I and II explain 50.6% and 38.14% of the total variability between each depth class's group of objects (sampling sites). There is also an unvisualized axis that explains the remaining 11.26% of variability. The axes are drawn to maximize variation among groups, so while proximity reflects similarity, it is influenced by the grouping structure. Arrows represent the correlation between taxon abundances and the canonical axes' scores for the 10 taxa with the highest indicator values (IndVal). They illustrate each taxon's contribution to the observed group differences, with the direction indicating the taxon's influence on the ordination space. Longer arrows signify a stronger correlation with the separation of groups. Taxon vectors: (a) *Decapterus punctatus/tabl*, (b) *Synodus* spp., (c) *Syacium papillosum/gunteri*, (d) *Prionotus* spp., (e) *Rhomboplites aurorubens*, (f) *Cyclopsetta fimbriata*, (g) *Pristipomoides aquilonaris*, (h) *Auxis thazard/rochei*, (i) *Haemulon aurolineatum/striatum*, and (j) Hyporthodus flavolimbatus.

Total egg abundance was positively related with both latitude (coefficient = 0.292, *se* = 0.097, $z = 3.020$, $p = 0.003$) and year (coefficient = 0.312, *se* = 0.098, $z = 3.181$, $p = 0.001$), and negatively related with depth (coefficient = −0.435, *se* = 0.102, $z = -4.279$, $p < 0.001$).

3.5 | Fish Egg Trajectories

The modeled trajectories using surface currents showed variation in the potential dispersal distances and trajectory shapes in which fish eggs may have traveled, depending on the oceanographic conditions at the time and location they were spawned. Modeled trajectory distances ranged from less than 1 to greater than 50 km, and trajectory paths included linear, arced, and looping shapes. The mean and maximum estimated distances of dispersal were comparable in 2013 and 2014, while generally larger distances were estimated in 2019 (Figure 7). Additionally, we typically estimated greater transport distances on the outer shelf compared to the inner shelf. The results of the model simulation are summarized in Table 8, which shows the mean and maximum estimated dispersal distances for fish eggs spawned in 2013, 2014, and 2019. The dispersal distances in Table 8 represent the potential distance a fish egg could have traveled within 36 h if it was collected just before hatching. It is important to acknowledge that actual dispersal distances may be shorter, especially for eggs collected shortly after they were spawned. The values in the "maximum estimated dispersal distance" (Table 8) represent the site that had the farthest potential dispersal distance.

4 | Discussion

4.1 | Assemblage Composition

In this study, we genetically identified fish eggs collected in 2013, 2014, and 2019 to examine the assemblage composition and abundance of spawning fishes on the WFS. We gathered spawning information for 82 species of broadcast spawners, many for which we previously lacked basic spawning data, including economically important and federally managed species. Our results underscore the power of using a CUFES and DNA barcoding to monitor fish spawning on a large ecosystem scale,

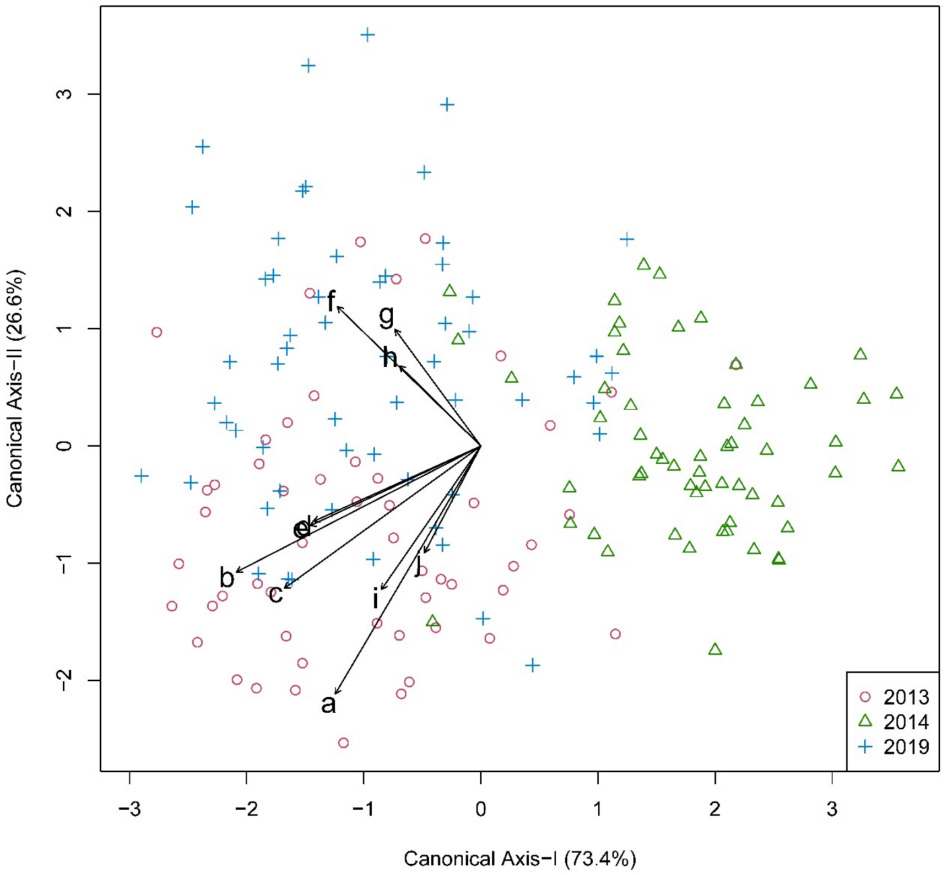

FIGURE 6 | Canonical analysis of principal coordinates (CAP) ordination diagram depicting differences among years with respect to fish egg beta-diversity in 2013, 2014, and 2019. The canonical axes I and II explain 75.62% and 24.38% of the total variability between each year's group of objects (sampling sites). The axes are drawn to maximize variation among groups, so while proximity reflects similarity, it is influenced by the grouping structure. Arrows represent the correlation between taxon abundances and the canonical axes' scores for the 10 taxa with the highest indicator values (IndVal). They illustrate each taxon's contribution to the observed group differences, with the direction indicating the taxon's influence on the ordination space. Longer arrows signify a stronger correlation with the separation of groups. Taxon vectors: (a) *Prionotus* spp., (b) *Syacium papillosum/gunteri*, (c) *Synodus* spp., (d) *Haemulon aurolineatum/striatum*, (e) *Decapterus punctatus/tabl*, (f) *Serraniculus pumilio*, (g) *Rypticus saponaceus/maculatus*, (h) *Katsuwonus pelamis*, (i) *Trachinocephalus myops*, and (j) *Scomberomorus maculatus*.

with eggs recovered from demersal, reef-associated, highly migratory pelagic, and cryptic fish species (see Table S1 for a list of all taxa identified).

The eastern Gulf of Mexico, defined as Florida Bay to Pensacola, Florida, includes the entire WFS and has a rich fish assemblage of approximately 1259 actinopterygians (ray-finned fishes) (Chen 2017; McEachran and Fechhelm 2010). Our study successfully identified fish eggs from 90 unique taxa on the WFS, and the taxon accumulation curve (Figure 2) did not reach an asymptote, suggesting that a considerable number of additional taxa could be observed with additional sampling. Based on the calculated asymptote, the taxon accumulation curve predicted that around 193 fish taxa actively spawn pelagic eggs in September on the WFS. This estimate represents roughly 20% of the broadcast-spawning fishes in the eastern Gulf of Mexico. Our estimate of 20% of broadcast-spawning fishes spawning in September provides new insights into the temporal dynamics of spawning activities in this region. This percentage suggests a substantial concentration of spawning activity within a single month, which could have important ecological implications. It is important to acknowledge the limitations and assumptions of our calculation. The assumption that about 75% of marine teleosts are broadcast spawners (Miller and Kendall 2009) is based on general knowledge and might not accurately reflect the specific composition of the WFS fish assemblage. Additionally, the asymptote of our taxon accumulation curve is likely an underestimation because biases introduced by the sampling design and identification method may cause us to underestimate the true richness. Focusing only on eggs collected at 3 m by the CUFES may have resulted in missing those that hatched before reaching the surface or that achieved neutral buoyancy deeper in the water column. Limitations of COI barcoding may also contribute to underestimation of richness by failing to distinguish closely related species, by potentially failing to identify certain taxa, and by the absence of reference sequences for some species in barcoding databases.

The most abundant genera were *Decapterus*, *Prionotus*, *Syacium*, and *Synodus*. It is likely that nearly all the *Decapterus* spp. eggs observed in our study that were only identified to

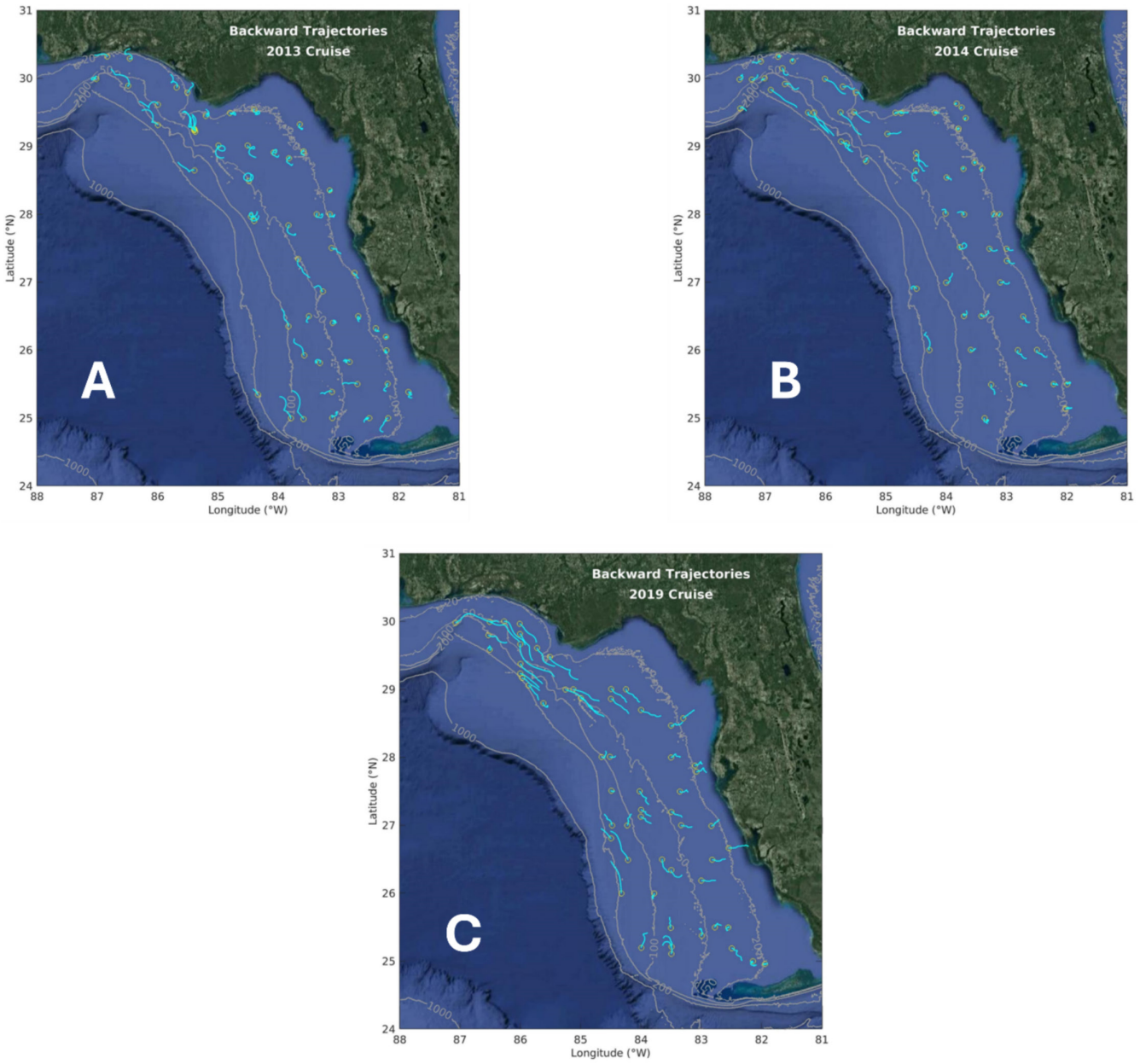

FIGURE 7 | Maps depicting backward Lagrangian tracking for 36 h starting from the sample locations based on WFCOM hindcasted surface currents. Yellow circles represent sampling locations while blue lines represent the 36-h estimated backward trajectories. Gray lines represent 20-, 50-, 100-, 200-, and 1000-m isobaths (A: 2013, B: 2014, C: 2019).

TABLE 8 | Summary of estimated fish egg dispersal distances from the West Florida Coastal Ocean Model. The maximum and minimum values were determined from the sites with the highest and lowest estimated dispersal distances.

Year	Mean estimated dispersal distance (km)	Maximum estimated dispersal distance (km)	Minimum estimated dispersal distance (km)
2013	18.1	50.2	1.0
2014	15.3	52.1	0.7
2019	29.2	79.4	3.7

the genus level were from *D. punctatus*. Some *Decapterus* spp. eggs collected may belong to *D. tabl*, albeit they likely only account for a small proportion, if they are present at all, as *D. tabl* is only rarely present in the Gulf of Mexico (Berry 1968). The dominance of *D. punctatus* aligns with its historical status as the most abundant carangid in larval surveys in the eastern Gulf of Mexico, with peak abundances occurring in summer and fall (Ditty et al. 2004; Leak 1981). Additionally, *D. punctatus* is an important forage fish on the WFS and serves as a food source for a variety of predatory fishes (Naughton et al. 1986). Monitoring the spawning patterns of *D. punctatus* therefore may aid in the understanding of the broader ecosystem health of the region. Furthermore, all of these genera (*Decapterus*, *Prionotus*, *Syacium*, and *Synodus*) are known to be abundant on the WFS based on trawl surveys (Matheson et al. 2017); therefore, their

high representation in the egg assemblage reflects their established abundance in the region.

4.2 | Federally Managed Species

The successful barcoding of eggs from federally managed fish species expands upon existing spawning information that has primarily been focused on adult and larval distributions. The knowledge of spawning locations, timing, and environmental factors is useful for the management of these economically valuable species. Overall, our findings support established spawning patterns, reveal potential spawning site preferences, provide new insights into spawning behaviors of federally managed species, and highlight the potential of our methodology for monitoring spawning locations and timings for various federally managed species at a large spatial scale.

The majority of the lutjanid eggs in our study were located on the mid-to-outer shelf. A prominent reproductive characteristic exhibited by many inshore-dwelling lutjanid species is a migration to offshore regions to establish seasonal spawning aggregations (Thresher 1984; Farmer et al. 2017). Furthermore, red snapper eggs (Figure 8) were mainly found close to known and estimated spawning aggregations (Coleman et al. 2011; Grüss et al. 2018).

Yellowedge grouper eggs were found along the shelf edge in the southern portion of the WFS. This area is a potential spawning aggregation hotspot for yellowedge grouper based on species distribution models by Grüss et al. (2018). Our observation of yellowedge grouper eggs in the region supports this hypothesis. However, no yellowedge grouper eggs were collected from the other estimated spawning hotspot that is in the northern region of the WFS. It is possible that spawning in the northern region occurs at a different time than in the south or that we did not collect in that area during active spawning (i.e., we may have sampled at an inappropriate time of day or lunar phase).

Spanish mackerel and king mackerel were both found to spawn across a large latitudinal range on the WFS, and their eggs were found in areas of expected water-column depths based on previous research (Collins and Stender 1987). Spanish mackerel eggs occurred nearshore at depths less than 40 m, and king mackerel eggs occurred farther offshore at depths usually greater than 40 m.

The eggs collected from federally managed species in this study agree with known spawning times, with the exception of mutton snapper, whose spawning period was assumed to end in August (Biggs et al. 2018). Available information on the timing of spawning for mutton snapper comes from Cuba and the

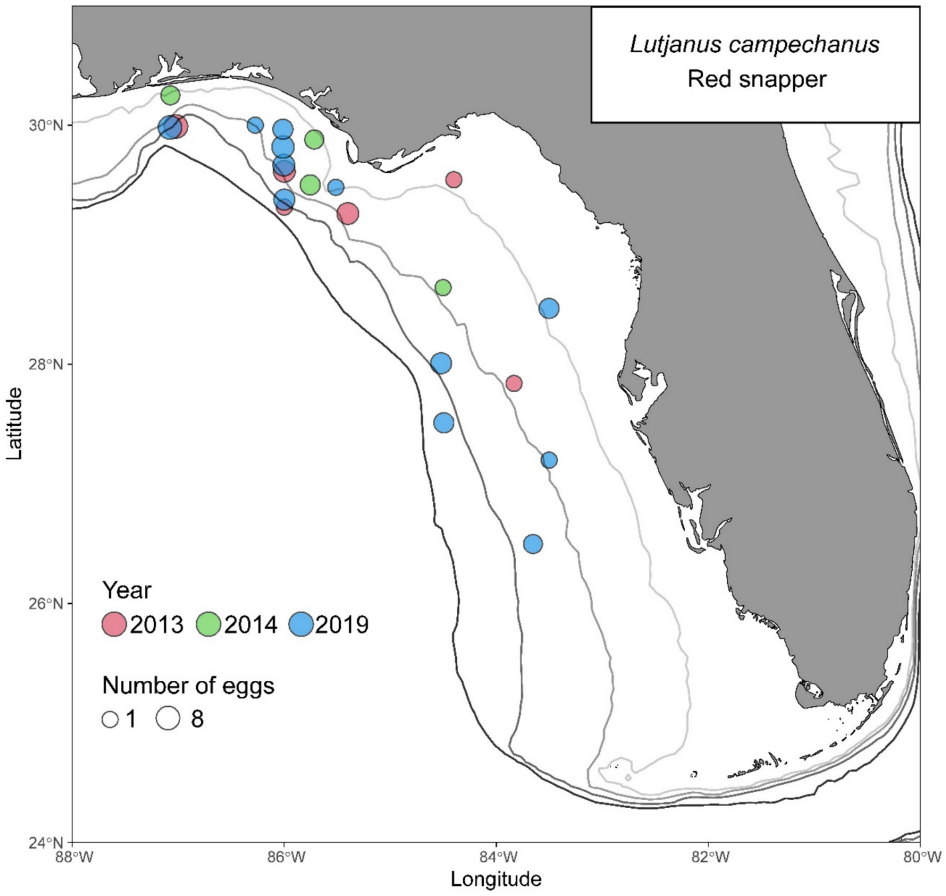

FIGURE 8 | Map of sites where *Lutjanus campechanus* (red snapper) eggs were collected. Circle size is scaled to the number of eggs collected at each site, with larger circles representing higher counts. Gray and black lines represent 25-, 50-, 100-, and 200-m isobaths.

Florida Keys (Burton et al. 2005; Claro and Lindeman 2003). Although no previous studies have documented mutton snapper spawning in September, the limited presence of mutton snapper eggs at only two sites over our 3 years of sampling suggests that spawning in this month may be rare on the WFS. Given that mutton snapper is an aggregating species, their spawning is likely concentrated in specific areas rather than being widespread across the shelf.

Additionally, spawning aggregations are key aspects in the life history of some of the economically important and federally managed fish species on the WFS (Grüss et al. 2014). Despite their importance, and recent efforts that have filled previous knowledge gaps and identified priority areas where information is needed, the locations and dynamics of spawning aggregations on the WFS are poorly understood (Erisman et al. 2018). Of the managed fish species for which we collected and identified eggs, several are known to exhibit aggregative spawning behavior, including mutton snapper, red drum, and yellowedge grouper. During peak spawning periods the concentrated abundances of these species can change from no more than a few individuals to hundreds (yellowedge grouper), thousands (red drum), and even tens of thousands (mutton snapper) (Biggs et al. 2018).

In general, the number of eggs for any particular taxon collected at a site was relatively low. It is possible that egg dispersal between spawning events and collection caused egg concentrations to become widely diffused in the surrounding water, even during large spawning events, such as aggregations, thereby limiting the number we collected. Additionally, our sampling method, which involved conducting straight-line transects at ~10 kts, may have caused us to quickly pass over spawning hotspots. Sampling in a more confined area, such as using a grid pattern, might have allowed us to detect higher egg densities if we had sampled near a large spawning aggregation.

The identification of fish eggs from species known to form spawning aggregations is a step towards identifying potentially new spawning aggregations and further understanding known aggregations. Our findings highlight the potential for egg-based monitoring to provide valuable insights into the timing and location of spawning aggregations, which can inform targeted conservation and management efforts (Erisman et al. 2017; Sadovy and Domeier 2005).

4.3 | Spatial Trends

Our findings on fish egg distribution generally aligned with established knowledge of adult fish zonation on the WFS (Darcy and Gutherz 1984; Saul et al. 2013; Switzer et al. 2023). The spatial distribution of reef fish composition and abundance across the WFS is strongly associated with depth and latitude (Saul et al. 2013). The influence of depth on both fish assemblage composition and population-level characteristics such as abundance and size distribution is well documented on the WFS (Darcy and Gutherz 1984; Saul et al. 2013; Switzer et al. 2023). Additionally, latitudinal variations are known to influence ichthyofaunal communities in the eastern Gulf of Mexico (Matheson et al. 2017; Saul et al. 2013; Switzer et al. 2023). Furthermore, Spalding et al. (2007) classified the WFS as being divided into two distinct ecoregions; the tropical Floridian ecoregion in the southern half and the temperate Northern Gulf of Mexico region in the northern half of the WFS. Our findings were fairly consistent with this south to north demarcation.

The DISPROF analysis revealed distinct spatial groups representing shallow, mid-shelf, and offshore assemblages. These groups exhibited a wider range in latitude compared to water-column depth, suggesting a stronger influence of depth on fish egg assemblage structure. Notably, specific DISPROF groups consistently represented shallow (1, 16), mid-shelf (14, 15), and offshore (12) assemblages across all 3 years (Figure 4). The DISPROF groups outside of those five were primarily grouped due to the sole presence of one taxon and thus contained only a few sites or just one site. The most abundant taxa in our study (*Decapterus* sp., *Prionotus* spp., and *Synodus* spp.) had the strongest influence on the structure of the DISPROF groups based on our IndVal analysis. The consistency of these groups across years indicates distinct ecological niches along a depth gradient. This pattern highlights the importance of depth in structuring fish egg assemblages on the WFS (Murawski et al. 2018).

We adopted similar depth strata and regional divisions defined by Switzer et al. (2023) and although we observed significant differences in fish egg beta-diversity between all depth strata, we did not find differences between each pair of regional strata. Our results indicate that there was a distinct shift in assemblage composition between the panhandle and peninsular Florida (near 85° W) with respect to September-spawned fish eggs. We did not observe a significant difference in assemblage composition between the northern half of the peninsular section of the WFS and the southern half, which deviates from the known split between ecoregions that occurs on the WFS around the Tampa Bay area (Spalding et al. 2007; Walker et al. 2020) and that was observed by Switzer et al. (2023), who found that there were distinct assemblages of reef fishes between the Big Bend and South Florida regions. This discrepancy might be attributed to our focus on the broader egg assemblage, encompassing fishes from all habitats, compared to Switzer et al. (2023) who focused solely on reef fishes. Additionally, our sampling being limited to a single month may have contributed to the observed deviations from previous studies. Despite these variations, our egg survey's zonation patterns generally reflected known biogeographical trends of adult fishes on the WFS.

Although both water-column depth and regional strata were found to be associated with variability in fish egg beta-diversity, our DISPROF analysis and PERMANOVA results suggest water-column depth explained more of the variability based on its larger F-statistic and R^2 values. However, the PERMANOVA results indicated that all interaction terms were statistically significant, suggesting that the combined effects of year, depth, and region were important in shaping the fish egg assemblage structure on the WFS and the effects of the individual factors were likely overestimated.

4.4 | Interannual Trends

Far fewer eggs were collected during 2014 compared to the other 2 years of sampling. Despite the > 80% decline in the

mean number of fish eggs collected per site in 2014, the relative abundances of the eight most common families were similar to the other 2 years, being nearly identical to the relative abundances in 2013 (Figure 3). This makes it unlikely that the lower egg abundance resulted from ecological factors, which would be expected to affect different fish families disproportionately. Additionally, sea surface temperature, bottom temperature, and salinity on the WFS during late summer 2014 were consistent with average values typically observed during late summer. The only noteworthy environmental perturbation that occurred in 2014 was a large and intense *Karenia brevis* bloom in the Big Bend region that was associated with localized hypoxia and mass fish mortality (Driggers et al. 2016; Turley et al. 2022). Although the effects of the 2014 *K. brevis* bloom were intense, its spatial extent (~10,000 km^2) was limited compared to our broader study area (170,000 km^2) and therefore does not fully explain the reduced egg abundances observed across the entire WFS. Therefore, the reduced egg abundance observed throughout the WFS in 2014 was likely an artifact of the CUFES being operated on a different vessel in 2014 (NOAA Ship Gordon Gunter). It is speculated that the pumping mechanism had a lower flow rate in 2014 than during the other years when it was operated on the NOAA Ship Pisces. Notably, CUFES samples collected during the September 2022 SEAMAP survey on the NOAA Ship Gordon Gunter revealed similarly low egg abundances as observed in 2014 (Keel, unpublished data). While we did not measure the flow rate during our sampling, doing so in future surveys will be essential to determine standardized egg abundances, allowing for more reliable assessments of interannual variability.

Observed differences in fish egg taxon richness and beta-diversity across the 3 years of sampling were likely related to a combination of methodological and ecological factors. The sampling locations each year were not identical because they were chosen using GRTS to minimize spatial bias, which could introduce year-to-year variation. In addition, lower egg abundance in 2014 explains reduced richness and differences in beta-diversity (Gotelli and Colwell 2001). Differences in beta-diversity between 2013 and 2019 suggest there were additional influences beyond egg abundance because those years had similar egg abundances. In 2013, *D. punctatus/tabl* was a significant indicator taxon (IndVal = 0.39) and was substantially more abundant in 2013 than in 2019. The increased abundance of *D. punctatus/tabl* in 2013 could be attributed to various factors, such as favorable ecological conditions that were not detected in this study. For example, basin-scale forcing by the North Atlantic Oscillation and El Niño Southern Oscillation Indices are related to subdecadal population trends in other forage fishes in the eastern Gulf of Mexico (Peake et al. 2022). In 2019, two of the significant indicator taxa that were identified, *P. aquilonaris* (IndVal = 0.16) and *Peprilus triacanthus/burti* (IndVal = 0.16), were also indicative of the deepest water-column depths (100–200 m). The presence of these indicator species in 2019 is likely because the average water-column depth during sampling was greater in 2019 than in 2013.

Given that these results are based on only three nonconsecutive years of data, broader conclusions about interannual spawning variability cannot be made. Expanding on this study to better monitor interannual spawning activity would require a more systematic sampling approach. Future efforts should focus on standardizing egg abundances by continually measuring the flow rate of the CUFES, conducting sampling annually and at more consistent sites to capture year-to-year variability and improve spatial comparability, and collecting additional environmental data (e.g., chlorophyll-a) to better understand factors influencing spawning activity.

4.5 | Fish Egg Trajectories

Using models to backtrack the possible trajectories of fish eggs based on ocean currents allowed for identification of their likely location of origin, aiding in the identification of spawning grounds. Trajectory distances displayed substantial interannual and intra-annual variability. The variability in trajectory distance can be explained by temporal and spatial differences in weather and current patterns. WFS currents are driven by both local and offshore forcing and exhibit distinct spatial and temporal variations (Weisberg and Liu 2022). Circulation on the inner shelf is primarily influenced by wind patterns and displays stronger seasonal variations compared to the outer shelf, which is dominated by the Loop Current, its eddies, and their interaction with the shelf slope (Liu et al. 2016; Weisberg and He 2003). We estimated greater transport distances on the outer shelf due to the influence of the Loop Current and its associated eddies. These findings align with previous research by Nguyen et al. (2024) that suggested shallow-water spawning on the WFS likely leads to retention, and deep-water spawning leads to export off the WFS. Additionally, the trajectory patterns suggested minimal inshore-to-offshore transport within the 200-m isobath.

Surface currents were used for trajectory modeling, as we assumed that fish eggs rise relatively quickly to the surface layer and then passively float there where they were advected by surface currents. The depth at which the identified eggs were spawned, as well as the upward speed of fish eggs is unknown for species on the WFS. The modeled trajectories using surface currents will therefore be the most accurate for relatively shallow sites and may be misleading for eggs spawned at depth at sites on the outer shelf as the egg would have to spend much of its prehatching time below the surface layer. Additionally, although the choice to use 36 h of backtracking encompasses the likely time spent in the embryonic stage before hatching for most of the taxa in our study, there are differences in this timing for various taxa (Pauly and Pullin 1988). If known, the specific hatching times for each taxon should be considered when interpreting the trajectories and attempting to estimate the original location where the fish egg was spawned. Furthermore, the modeled trajectories represent the maximum potential distance most fish eggs could have traveled if collected just before hatching, given the 36-h backtracking period. The actual dispersal distances were likely shorter, especially for eggs collected shortly after they were spawned or those that have a shorter incubation time. Future studies aiming to further

constrain spawning locations using similar methods may attempt developmental staging of fish eggs prior to barcoding to better estimate how long to backtrack specific fish egg trajectories based on age and species-specific hatching times.

5 | Conclusions

We used a CUFES and DNA barcoding to examine the fish spawning assemblage on the WFS during September of 2013, 2014, and 2019. We identified eggs from 82 fish species and successfully identified spawning locations for several federally managed fishes. Our findings generally aligned with known adult fish distributions and spawning patterns, and we found that water-column depth played a more important role than regional strata in structuring the fish egg assemblage. We observed distinct shallow, mid-shelf, and offshore communities. Our results demonstrated that our methodology can be used to monitor fish spawning activity over large spatial and temporal scales and for a wide taxonomic range of fish taxa. Future research should prioritize refining the sampling approach, broadening seasonal coverage, exploring environmental factors that may drive interannual variation, expanding trajectory modeling to incorporate egg-specific buoyancy and development times, and detailed examination of spawning areas of interest identified in this study.

Author Contributions

Keith Keel, Glenn Zapfe, Ernst B. Peebles, Christopher D. Stallings, and Mya Breitbart designed the study. Glenn Zapfe led the fish egg collection efforts. Makenzie Kerr and Keith Keel conducted the DNA barcoding of fish eggs under the guidance of Mya Breitbart. Keith Keel performed the statistical analyses under the guidance of Joshua P. Kilborn and Christopher D. Stallings. Yonggang Liu and Laura Y. Liu performed the fish egg trajectory modeling. Keith Keel interpreted the data and prepared the first draft of the manuscript as part of his master's thesis. Glenn Zapfe, Mya Breitbart, and Christopher D. Stallings edited the first draft to prepare it for journal publication. All authors edited intermediate drafts. All authors reviewed and approved the final version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in GRIIDC at https://doi.org/10.7266/dmp83r1m and https://doi.org/10.7266/j7qzhk64.

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Supporting Information

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