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Exploring the Dynamics of September Fish Spawning on the West Florida Shelf Through the DNA Barcoding of Fish Eggs

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by

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ABSTRACT

Identifying spawning areas for economically and ecologically important fishes is critical for fisheries conservation and ecosystem-based management. Additionally, monitoring the spawning dynamics of fishes is increasingly important as temperatures and oceanic conditions change. I 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 National Oceanic and Atmospheric Administration (NOAA) Southeast Area Monitoring and Assessment Program (SEAMAP) ichthyoplankton cruises using a Continuous Underway Fish Egg Sampler (CUFES). Analysis of 4,400 fish eggs from the three years resulted in the identification of 83 unique species within 36 families. A 79% DNA barcoding success rate was achieved, with 47% of all identifications being at the species level. PERMANOVA results revealed significant differences in fish egg beta-diversity across the three years sampled, four preassigned depth classes, and within pre-assigned regional strata. My findings generally aligned with known adult fish distributions and spawning patterns, and I found that water column depth played a more significant role than regional strata in structuring the fish egg community. Eggs from several economically important species were collected and observed at relatively high frequencies, including *Lutjanus campechanus* (red snapper), *Lutjanus synagris* (lane snapper), *Rhomboplites aurorubens* (vermillion snapper), *Hyporthodus flavolimbatus* (yellowedge grouper), and *Scomberomorus cavalla* (king mackerel). The West Florida Coastal Ocean Model was used to hindcast the trajectories of the fish eggs and predict possible spawning locations. Backward tracking over a span of 36 hours was conducted, based on the assumption that most fish eggs on

the WFS undergo hatching within this time window. The model predicted egg transport distances ranging from 7-79 km (mean distance of ~21 km), with greater transport distances observed 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.

CHAPTER ONE: EXPLORING THE DYNAMICS OF SEPTEMBER FISH SPAWNING ON THE WEST FLORIDA SHELF THROUGH THE DNA BARCODING OF FISH EGGS

Introduction

Understanding the characteristics of fish spawning events is essential for effective fisheries management, and studying fish reproduction and their early life stages provides a powerful tool for assessing population and ecosystem health (Fuiman & Werner, 2009). Fish spawning, eggs, and larvae are sensitive to environmental changes, allowing for the monitoring of the impact of human activities and environmental changes on reproductive success (Pankhurst & Munday, 2011; Wright & Trippel, 2009). Additionally, studying fish spawning and early life stages allows for the identification of critical habitats needed for successful reproduction, increases our understanding of dispersal patterns and population connectivity, and aids in the estimation of population reproductive capacity (Fuiman & Werner, 2009). This knowledge is especially critical on the West Florida Shelf (hereafter, WFS), where spawning information for most fish species remains unknown. Addressing this knowledge gap is pivotal, as understanding spawning dynamics is essential for predicting the next generation's success and ensuring the sustainability of fisheries.

Approximately 75% of marine teleosts broadcast spawn pelagic buoyant eggs that float near the surface (Miller & 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; Collette, 1983; Heemstra & Randall, 1993; Sedberry et al., 2006). Collection of pelagic fish eggs therefore allows for the sampling of many different 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; Sedberry et al., 2006), assessing faunal diversity (Ahern et al., 2018), determining dispersal patterns (Nguyen et al., 2024), and understanding reproductive processes critical for predicting future stock sizes (Armstrong & Witthames, 2012; Rothschild, 2000). Despite this, 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 to the species level based on morphology. It has been estimated that less than 10% of fish eggs can be reliably identified to the species level using visual methods (Shao et al., 2002). In response to the challenges of studying spawning patterns in marine fishes, scientists have applied DNA barcoding techniques to planktonic fish eggs that previously could not be reliably identified (Saitoh et al., 2009; Shao et al., 2002). This method has proven to be effective for identifying spawning sites for fishes that broadcast spawn pelagic eggs (Burrows et al., 2019; Harada et al., 2015; Kerr et al., 2020; Lewis 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 (Burrows et al., 2019; Duke et al., 2018; Harada et al., 2015; Kerr et al., 2020; Lewis et al., 2016). DNA barcoding has become an ever more powerful tool as genetic sequences of voucher specimens are added to community-driven databases such as The Barcode of Life Database (BOLD) (Lira et al., 2023; Ratnasingham & Hebert, 2007). Currently, BOLD has

accumulated 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 & Hebert, 2007). Comparative analyses have shown that visual identification of eggs and larvae is unreliable when compared to molecular methods (Larson et al., 2016).

Historically, challenges in identifying fish eggs have necessitated the use of larvae for spawning area identification (Peebles & 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 hours after being spawned during summer/early fall temperatures, as warmer water accelerates their development (Pauly & Pullin, 1988). In contrast, fish larvae can be up to several weeks old (Houde & 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, post-flexion fish larvae can behaviorally control their movement through vertical migration and directed horizontal swimming (Cowen & Sponaugle, 2009). The vertical distribution behavior of larvae can greatly influence their dispersal, even early in development, and as larvae develop and enter the post-flexion 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 (Lin et al., 2016) environments, further demonstrating that relying solely on larvae for identifying spawning areas may miss crucial information or yield misleading conclusions. In contrast to larvae, fish eggs are younger, tend to be passive, and are positively buoyant, which make them better candidates than fish larvae for identifying spawning locations.

To monitor fish spawning, I 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 Jr et al., 1997; Checkley Jr. et al., 2000). The CUFES continuously filters seawater at a 3-meter depth while a research vessel is underway. It consists of a submersible pump attached to the ship's hull that draws in water. 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. While CUFES samples may have lower precision compared to vertically integrated bongo nets (Pepin et al., 2005) the CUFES' ability to collect fish eggs continuously while underway provides improved spatial resolution compared to the use of towed nets. Furthermore, CUFES samples exhibit significantly higher fish egg enrichment compared to traditional net tows due to lower recovery of zooplankton and other non-target material, which facilitates faster sample processing. A drawback when using a CUFES is that the total water column egg abundance is difficult to estimate because the CUFES samples at a fixed depth (3 meters) and therefore may not be a suitable method for measuring absolute egg production (Lelievre et al., 2012). However, the use of a CUFES is an ideal method for mapping egg distribution for broadcast-spawning fishes as, while their eggs are not evenly distributed through the water column, they typically float to the surface early in development and remain abundant at the surface even during turbulent mixing (Conway et al., 1997; Lelievre et al., 2012). Although all eggs are collected 3 m below the surface, the water column depth at the collection site provides an indirect measure of the maximum potential spawning depth.

To address knowledge gaps in the reproductive ecology of the WFS fish community, I 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, 2019. The WFS is a 170,000 km^2 bedrock 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 multi-billion-dollar fishery (NMFS, 2022). This study aimed to expand the knowledge base for fisheries ecology on the West Florida Shelf (WFS) by addressing the following questions:

1. Where are the primary spawning areas for fish species on the WFS, particularly those of economic and ecological significance?
2. How are fish spawning assemblages structured across the WFS in terms of space and time, considering both broad spatial scales and taxonomic levels?

An additional objective was to establish a time series of fish spawning data that will allow researchers to track changes in spawning patterns over time.

Methods

Fish Egg Collection

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 Ship Pisces, and the 2014 cruise was on the NOAA Ship Gordon Gunter. Samples were collected in the northeastern Gulf of Mexico 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 the mesh sample collectors had a mesh size of 500 μm. The pump ran continuously during the survey 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-minute intervals while transiting between SEAMAP stations with

an average speed of 10 kts (corresponding to a distance of 8-10 km). SEAMAP sampling is conducted based on a predefined cruise track of stations, approximately 30 nautical miles apart in a systematic grid. CUFES samples were not collected while stationary. Each CUFES sample was stored in individual ~20 ml collection vials and preserved with 95% ethanol. CUFES samples were collected from 775 sites across the three years of sampling and a total of 218 sites were processed (70 sites in 2013, 75 sites in 2014, and 73 sites in 2019; Table 1). Sampling locations and dates were not identical across the three years but were consistent in geographic extent (Figure 1). However, the range of water column depth at sampling sites varied across the sampling years (Table 2).

Molecular Analysis of Fish Eggs

To ensure that a spatially balanced subset of CUFES samples were selected for DNA barcoding each year, I employed the Generalized Random Tessellation Stratified (GRTS) method (Stevens Jr & Olsen, 1999) using the spsurvey package in R (Dumelle et al., 2023). This approach treats the entire WFS as the sampling domain, aiming for unbiased spatial coverage across the area and minimizing potential biases from consistently sampling the same locations. While year-to-year site variation likely introduces additional variation in species assemblages between years, GRTS helps control for spatial bias and allows for a more robust analysis of long-term trends across the entire WFS. To separate the fish eggs from invertebrate zooplankton, each CUFES sample was poured into a gridded dish and placed under a dissecting microscope. Fish eggs were picked out of the dish following the pattern of the grid until all fish eggs were removed or 96 eggs were removed, whichever came first. If >96 eggs were present in a sample the rest of the eggs were counted but not removed for further processing. The eggs from each station were then placed in 70% isopropyl alcohol in a 1.5 ml tube and kept at room temperature.

Individual fish eggs were then placed into individual polymerase chain reaction (PCR) tubes before performing a DNA extraction using the HotSHOT method (Truett et al., 2000). The HotSHOT DNA extraction method was performed by adding 50 µl of alkaline lysis buffer (0.2 mM disodium EDTA, 25 mM NaOH, pH 12) to each PCR tube and mashing each egg against the walls of the tube using an autoclaved toothpick. The PCR tubes containing a mashed fish egg and alkaline lysis buffer were then placed in a thermocycler for 30 minutes at 95°C, after which they were moved onto ice for three minutes to cool. After cooling, 50 µl of neutralization buffer (40 mM Tris-HCL, pH 5) was added and the samples were vortexed. Extracted DNA was stored 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 1X 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, 72°C for 1 min), and a final extension at 72°C for 10 min. Positive PCR amplification was checked for each sample using gel electrophoresis on a 1.5% agarose gel stained with ethidium bromide. The successful PCR products were then purified and Sanger sequenced using the M13 forward primer by TACGen (tacgen.com) (Ivanova et al., 2007).

Geneious bioinformatics software was used to trim and assemble the DNA sequences (Kearse et al., 2012). Trimming parameters were set to trim primers allowing 5 mismatches, 5’ and 3’ ends were also trimmed with an error probability of 0.05 and trimming at least 3 base pairs. Trimmed sequences that were shorter than 80 base pairs were considered unidentified and not processed further. The Barcode of Life Database (BOLD) was used to identify sequences to

the lowest taxonomic level possible using a 97% match threshold for identification (Ratnasingham & Hebert, 2007). The Basic Local Alignment Search Tool (BLAST) in the GenBank nucleotide database was used for sequence identification when sequences had no match in BOLD (Altschul et al., 1990). Sequences that did not have matches meeting the 97% identification threshold in both BOLD and GenBank were considered 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).

Taxonomic Classification

To minimize bias in fish egg assemblage composition analyses, the most resolved taxonomic identifications possible were used. For example, taxonomic groups consistently identifiable only to the genus level (e.g., *Synodus* spp., *Saurida* spp.) were combined 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). These family level identifications were excluded from community composition analyses as both *Synodus* spp. and *Saurida* spp. existed as separate lowest taxonomic groups. Inclusion would have resulted in either artificially decreasing diversity by assigning all *Synodus* spp. and *Saurida* spp. 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* spp. These cases were rare and only accounted for 24 out of the 3964 successful identifications. Additionally, 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., *Decapterus punctatus*) were identified to the species level, the

taxa were treated as a singular taxonomic unit. 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. Because of the focus on using the most resolved taxonomic units achievable for analysis, taxon richness, which includes all unique taxonomic units identified (including those not identified to the species level), was used as a measure of diversity instead of species richness.

Statistical Analyses

For data analysis focused on fish egg abundance, richness, Shannon diversity, and beta-diversity, including the Dissimilarity profile analysis (DISPROF) analysis, sites were classified by water column depth and by regional strata following categories adapted from Switzer et al. (2023) (Table 3). Additionally, total egg abundance was calculated based on the raw counts of eggs from a site, taxon abundances were based on the number of identifications from a maximum of 96 eggs processed per site, and taxon relative abundances were calculated as the proportion of identifications out of all successful identifications.

Analysis of Variance (ANOVA) was used to assess whether mean fish egg abundance, richness, and Shannon diversity differed among years, depths, and regions. Post-hoc Tukey's honest significant difference (HSD) tests were employed to identify specific pairwise differences between groups.

Permutational multivariate analysis of variance (PERMANOVA) was used to assess whether assemblage composition differed among years, depths, and regions. PERMANOVA is a non-parametric statistical method used to determine the significance of differences among groups in multivariate datasets by employing permutation tests on dissimilarity matrices. The

data were cube-root transformed prior to analyses to downweigh highly abundant taxa and Bray-Curtis dissimilarities were used. Post-hoc pairwise tests were performed to test differences between years, depths, and regions. Holm p-value adjustment was used for pairwise significance tests to account for multiple testing. A canonical analysis of principal coordinates (CAP) ordination was used to visually compare fish egg beta-diversity among years and depths.

The ClustDISPROF package (R software) was used to perform a DISPROF analysis with agglomerative, hierarchical clustering. A DISPROF analysis was performed on Bray-Curtis dissimilarities calculated from cube-root transformed data to assess the overall presence of multivariate structure in the fish egg community composition data. Following the confirmation of the presence of multivariate structure in the data by DISPROF, an unweighted pair group method with arithmetic mean (UPGMA) clustering analysis was performed to partition the samples into distinct groups based on their resemblance. To objectively identify the number of statistically significant groups based on fish egg assemblage similarity, the UPGMA clustering analysis was followed by a second DISPROF analysis. Finally, the groups identified via DISPROF were plotted to visualize spatial and temporal patterns in fish egg community structure across the study area.

To estimate the total taxon richness present in the study area, a taxon accumulation curve was created using the specaccum function in R within the vegan package (Oksanen et al., 2022). A non-linear self-starting model was then used to estimate the asymptote of the curve. This asymptote represents the total fish egg taxon richness expected on the WFS for the sampling period using my collection and identification methodology.

Fish Egg Trajectory Modeling

The West Florida Coastal Ocean Model (WFCOM) (Zheng & Weisberg, 2012) is a Lagrangian trajectory model that was used to track the fish eggs backward in time for 36 hours based on the surface currents to study their potential origins. Surface currents were used for tracking as it was assumed that the fish eggs passively float at the surface layer and are advected by the currents. WFCOM is a WFS-specific application of the Finite Volume Coastal Ocean Model (FVCOM; Chen et al., 2003) that is nested into the Global Hybrid Coordinate Model (HYCOM; Chassignet et al., 2009). The trajectory time of 36 hours was chosen based on fish egg hatching times described in Pauly and Pullin (1988) for mean water temperature values measured during the sampling period. For the 2013 cruise, there were three stations located outside of the WFCOM domain, thus, they were excluded from the trajectory model simulation. For the 2019 cruise, there were six such stations excluded. All these points are located outside the 200 m isobath. To quantify the dispersal of the fish eggs, I calculated the distance between the point of collection and the estimated point of origin for each 36-hour trajectory.

Results

Fish Egg Identifications

Across the three years of sampling, 4991 fish eggs were processed (5640 fish eggs were collected; a maximum of 96 fish eggs were processed from each site). Of the 4991 fish eggs processed, 3964 (79.4%) resulted in successful identification (Table 4). Successful identification was defined as an egg's DNA sequence being at least 97% identical to one or more reference sequences in the BOLD or NCBI databases. Unsuccessful identifications (n=1297) occurred due to one of three possible causes. By far, the most common cause of failure in the barcoding

process was DNA failing to amplify during the PCR. The second most common cause of failure was obtaining poor-quality sequences from amplified DNA. The third, and least common, cause of failure was not obtaining a match to a reference sequence in BOLD or NCBI. Of the 3964 fish eggs that had successful DNA amplification and produced high-quality sequences with matches to reference sequences in BOLD, 1845 fish eggs (47%) were matched to a single species and represent the highest resolution of identifications. The remaining 2099 fish eggs (53%) showed more complex outcomes. This includes 1914 eggs (48%) matching two species within the same genus and 205 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. These identifications were unresolved at the species level.

Most often, there were only a few eggs for each taxon found at a particular site (median = 2). Cases where there were large quantities of eggs from a singular taxon at a site were generally rare. There were 45 instances where 20 or more eggs were collected from a single taxon at a site and eight instances where 50 or more eggs were collected. Fourteen different taxa had at least one occurrence of 20 or more eggs being collected at a site and only 4 different taxa had an occurrence of 50 or more eggs (see Appendix A).

Community Composition

Across all three years, 83 species, 71 genera, and 36 families were represented from the 3964 identifications (Table 5). A total of 91 unique taxa were identified, 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. The asymptote was estimated to occur at 199 taxa.

The eight most abundant families by total number of eggs collected were, in order of greatest to least, Carangidae, Triglidae, Synodontidae, Lutjanidae, Scombridae, Cyclopsettidae, Stromateidae, and Serranidae (Figure 3). These eight families accounted for 87.2% of the fish eggs identified. Although there were notably fewer eggs collected in 2014, the relative abundance of each of these families was consistent across the three years. The Carangidae family, 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) – belong to one of the eight most abundant families. The four mentioned genera accounted for 42.5% of all identifications. There were only two taxa identified from the *Decapterus* genus, and the identifications were virtually always unresolved at the species level between the species *Decapterus punctatus* (round scad) and *Decapterus tabl* (roughear scad). Overall, *Decapterus* sp. was the most abundant taxon in my study, accounting for 85% of Carangidae identifications and 20.7% of all egg identifications.

Federally Managed Species

Fish eggs from multiple federally managed species were identified. These included *Lutjanus analis* (mutton snapper), *Lutjanus campechanus* (red snapper), *Lutjanus griseus* (grey snapper), *Lutjanus synagris* (lane snapper), *Rhomboplites aurorubens* (vermillion snapper), *Pristipomoides aquilonaris* (wenchman), *Hyporthodus flavolimbatus* (yellowedge grouper), *Sciaenops ocellatus* (red drum), *Scomberomorus cavalla* (king mackerel), *Scomberomorus maculatus* (Spanish mackerel), *Katsuwonus pelamis* (skipjack tuna), and *Istiophorus platypterus* (sailfish). 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 the ten

most abundant of these species in Table 6. For maps of egg distributions for all federally managed taxa see Appendix B.

Of the federally managed species whose eggs were identified, vermillion snapper was encountered at the greatest number of sites (15.1%; n = 33) followed by wenchman (10.6%; n = 23) and red snapper (10.1%; n = 22). Wenchman and vermillion snapper had the first and second highest numbers of total eggs collected from this group (n = 146 and n = 122 respectively) and red snapper had the fourth highest number of eggs collected from federally managed taxa (n = 70) with only red drum having a greater amount than red snapper (n = 87). Nearly all 87 (98.9%; n = 86) red drum eggs were collected from two sites. For the fish eggs from federally managed species collected in this study, much like most of the non-federally managed taxa, there were generally only a few eggs collected from a site when they were present, with red drum and wenchman being exceptions, as both taxa had two instances of >30 eggs being found at a site.

There was also 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. There were no large variations observed in mean temperature or salinity where eggs were found for each of these species (Table 6).

Mutton snapper (*Lutjanus analis*):

Mutton snapper eggs were found at two sites with five eggs collected at each. Both instances where mutton snapper eggs were collected occurred in 2013, with both sites located in the southernmost portion of the WFS. The water column depths of the sites were 60.0 m and 76.6 m. (Refer to Figure B1 for the spatial distribution of sites where mutton snapper eggs were collected.)

Red snapper (*Lutjanus campechanus*):

Red snapper eggs were found at 22 sites, and a total of 70 eggs were identified. Red snapper eggs were found at six sites in 2013, four sites in 2014, and 12 sites in 2019. Most of the sites where red snapper eggs were present were between Pensacola Bay and Apalachicola Bay and north of 29 degrees latitude. 2019 exhibited the greatest spatial extent in terms of sites where red snapper eggs were found, as 2019 had the largest proportion of sites below 29N and the site with the most southernly latitude, 26.5N. red snapper eggs were found where water column depths ranged from 21.5 to 123.1 m (mean = 47.9 m). (Refer to Figure B2 for the spatial distribution of sites where red snapper eggs were collected.)

Lane snapper (*Lutjanus synagris*):

Lane snapper eggs were found at 12 sites, and 20 eggs were identified. Eggs were found in all three years of sampling and all but two sites were in the southern half of the WFS (i.e., at or below 27.5N) in relatively shallow depths ranging from 18.6 m to 59.7 m. The two exceptions were sites located nearshore in the Apalachicola area in less than 25 m of water. (Refer to Figure B4 for the spatial distribution of sites where lane snapper eggs were collected.)

Vermillion snapper (*Rhomboplites aurorubens*):

Vermillion snapper eggs were found at 33 sites, and 122 eggs were identified. Vermillion snapper eggs were found at 16 sites in 2013, three sites in 2014, and 14 sites in 2019. Vermillion snapper egg locations had a broad latitudinal distribution and were found from the far northern extent of the WFS to approaching the most southernly extent of the WFS (30N-25N). Vermillion snapper eggs were generally found in the mid-shelf region. Water column depths at sites where

vermillion snapper eggs were found ranged from 15.4 to 198 m (mean = 58.8 m). (Refer to Figure B5 for the spatial distribution of sites where vermillion snapper eggs were collected.)

Wenchman (*Pristipomoides aquilonaris*):

Wenchman eggs were found at 23 sites, and 146 eggs were identified. Wenchman eggs were found at five sites in 2013, five sites in 2014, and 13 sites in 2019. Wenchman egg locations had a broad latitudinal distribution and were found from the far northern extent of the WFS to around 25.9N, being absent from only the most southern part of the WFS. Wenchman eggs were generally found near the outer shelf. Water column depths at sites where wenchman eggs were found ranged from 33.7 to 205 m (mean = 119.1 m). (Refer to Figure B6 for the spatial distribution of sites where wenchman eggs were collected.)

Yellowedge grouper (*Hyporthodus flavolimbatus*):

Yellowedge grouper eggs were found at seven sites, and 24 eggs were identified. Yellowedge grouper eggs were only present in the 2019 sampling year. All yellowedge grouper eggs were found in the southwest portion of the WFS in areas where the water column depth ranged from 114.5 m to 336 m (mean = 195.1 m). The eggs were found in the vicinity of Pulley Ridge Habitat Area of Particular Concern. (Refer to Figure B8 for the spatial distribution of sites where yellowedge grouper eggs were collected.)

Red drum (*Sciaenops ocellatus*):

Red drum eggs were found at three sites, and 87 eggs were identified. Red drum eggs were found at one site in 2013 and two sites in 2019. The 2013 site was located ~7 km off the coast near Navarre Beach in the Florida panhandle in 22.7 m of water and 32 eggs were collected. One of the sites in 2019 was located ~25 km offshore of Pinellas County in 18.2 m of

water and 54 eggs were collected. The other site in 2019 was in the far southern portion of the WFS, ~70 km NNE of the Dry Tortugas, in 34.9 m of water and only one egg was collected there. (Refer to Figure B9 for the spatial distribution of sites where red drum eggs were collected.)

King mackerel (*Scomberomorus cavalla*):

King mackerel eggs were found at 19 sites, and 68 eggs were identified. King mackerel eggs were found at 10 sites in 2013, four sites in 2014, and five sites in 2019. King mackerel egg locations had a broad latitudinal distribution and were found from the far northern extent of the WFS to approaching the most southernly extent of the WFS (30N-25N). King mackerel eggs were generally found near the mid-shelf region. Water column depths at sites where king mackerel eggs were found ranged from 28.4 to 72.8 m (mean = 49.1 m). (Refer to Figure B10 for the spatial distribution of sites where king mackerel eggs were collected.)

Spanish mackerel (*Scomberomorus maculatus*):

Spanish mackerel eggs were found at 13 sites, and 60 eggs were identified. Spanish mackerel eggs were found at seven sites in 2013 and six sites in 2019. Spanish mackerel egg locations had a broad latitudinal distribution and were generally found near the inner parts of the shelf in relatively shallow water. Water column depths at sites where Spanish mackerel eggs were found ranged from 15.7 to 33.1 m (mean = 22.0 m). (Refer to Figure B11 for the spatial distribution of sites where Spanish mackerel eggs were collected.)

Skipjack tuna (*Katsuwonus pelamis*):

Skipjack tuna eggs were found at eight sites, and 30 eggs were identified. Skipjack tuna eggs were found at four sites in 2013 and four sites in 2019. Skipjack tuna eggs were found in

the southern half of the WFS along the outer edge of the shelf. Water column depths at sites where skipjack tuna eggs were found ranged from 130.3 to 1080 m (mean = 316.2 m). (Refer to Figure B12 for the spatial distribution of sites where skipjack tuna eggs were collected.)

Annual Trends

Egg abundance varied across the three years of sampling and was notably much lower in 2014 compared to the other two years (Table 1). ANOVA results indicated that there were significant differences among years in mean egg abundance ($p < 0.001$, $F = 29.69$). Pairwise tests using Tukey's honest significance test indicated that egg abundance was lower in 2014 than in the other two years ($p < 0.001$) but did not differ between 2013 and 2019 ($p > 0.1$).

There were also notable differences in taxon richness and diversity among sampling years (Table 7). ANOVA results indicated that there were significant differences among years in both mean taxon richness ($p < 0.001$, $F = 21.87$) and mean Shannon diversity index ($p < 0.001$, $F = 12.58$). Pairwise tests using Tukey's honest significance test indicated that taxon richness differed between all three pairs of years (2013 > 2019 > 2014; $p < 0.05$ for all three pairwise comparisons). Additionally, Shannon diversity was lower in 2014 compared to both 2013 ($p < 0.001$) and 2019 ($p < 0.01$).

Fish egg beta-diversity differed among the three years of sampling (PERMANOVA $p = 0.0001$, $F = 5.43$). Pairwise tests indicated that all three years were significantly different from one another (Holm's adjusted $p < 0.01$ for all three pairwise comparisons). The PERMANOVA results were supported by the CAP ordination (Figure 4), which shows the three years form discrete clusters with only 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.

Spatial Trends

Egg abundance varied across water column depths with a trend suggesting a possible inverse relationship (Table 8). However, ANOVA results indicated that there were no significant differences among water column depths in terms of mean egg abundance ($p > 0.05$, $F = 2.14$). There were significant differences in taxon richness ($p < 0.001$, $F = 10.10$) and Shannon diversity ($p < 0.001$, $F = 9.64$) among the different depth classes (Table 8). Pairwise tests using Tukey's honest significance test indicated that shallower sites (0-25 m and 25-50 m) displayed significantly greater taxon richness compared to the deepest sites (>100 m) ($p < 0.05$), and similarly, the deepest sites exhibited significantly lower Shannon diversity compared to all other sites (0-25 m, 25-50 m, 50-100 m) ($p < 0.05$ for all comparisons). There were no significant differences between any other pairs of depth classes for mean taxon richness or mean Shannon diversity index. These results can be visualized in Figure 5.

There were significant differences in the fish egg beta-diversity among the four assigned depth classes (PERMANOVA $p = 0.0001$, $F = 11.47$, $R^2 = 0.14$). Pairwise tests indicated that all four of the assigned depth classes were significantly different from one another (Holm's adjusted $p < 0.01$ for all six pairwise comparisons). The PERMANOVA results were supported by the CAP ordination (Figure 6), which shows that the four depth classes were plotted in clusters that form a gradient with neighboring depth bins overlapping.

There were significant differences in fish egg beta-diversity among the three sampled regions (PERMANOVA $p = 0.0001$, $F = 3.31$, $R^2 = 0.03$). Pairwise tests indicated that the Panhandle region had significantly different fish egg beta-diversity than the Big Bend and the South regions of the WFS (Holm's adjusted $p < 0.01$). However, no significant differences were

found between the beta-diversity of the fish egg community for the Big Bend and South region (Holm's adjusted p >0.05). Full PERMANOVA statistics can be found in Table 9.

The DISPROF analysis produced 14 significant groups within the 184 stations that contained fish eggs with successful identifications (Figure 7). Six of the 14 groups were present in all three 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.*

Fish Egg Trajectories

The mean and maximum predicted distances of dispersal were similar in 2013 and 2014 but were much larger in 2019 (Figure 8). The results of the model simulation are summarized in Table 10, which shows the mean and maximum predicted dispersal distances for fish eggs spawned in 2013, 2014, and 2019. The dispersal distances in Table 10 represent the potential distance a fish egg could have traveled within 36 hours 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 Predicted Dispersal Distance' column of Table 10 represent the site that had the farthest potential dispersal distance. Sites outside of the 200 m isobath were excluded from this analysis as that is the extent of the model's domain.

Discussion

Community Composition

In this study, I genetically identified fish eggs that were collected in 2013, 2014, and 2019 to examine the community composition and abundance of spawning fishes on the WFS. Spawning information was collected for 83 species of broadcast spawning fishes, including a variety of economically important and federally managed fish species. This study highlights the efficacy of using a CUFES and DNA barcoding to monitor fish spawning at a large ecosystem scale.

Fish egg surveys offer a broader taxonomic representation of spawning activity compared to traditional methods, such as those reliant on capturing gravid females. Using fish eggs allows for the capture of a wide range of taxa across diverse trophic levels and ecological niches. Egg surveys can provide a broader picture of spawning activity within a shorter timeframe and with potentially less taxonomic bias compared to methods targeting adult fish. This study exemplifies this advantage, as eggs were collected from demersal, reef-associated, highly migratory pelagic, and cryptic fish species (Appendix A).

My study successfully identified 91 unique taxa, including those identified to genus but not species level, and the taxon accumulation curve did not reach an asymptote, suggesting that a considerable number of additional taxa could be discovered with additional sampling. Based on the calculated asymptote, the taxon accumulation curve predicted that around 199 fish taxa actively spawn pelagic eggs in September on the WFS. However, this is likely an underestimation because biases introduced by the sampling design and identification method may cause us to underestimate the true diversity. Focusing only on eggs collected at 3 m by the

CUFES may have resulted in missing eggs that hatch before reaching the surface or eggs that achieve neutral buoyancy deeper in the water column. Limitations of COI barcoding may also contribute to underestimating diversity by failing to distinguish closely related species and by potentially failing to identify certain taxa.

The eastern Gulf of Mexico, defined as Florida Bay to Pensacola, Florida, includes the entire WFS and has a rich fish community of around 1,259 actinopterygians (ray-finned fishes) (Chen, 2017; McEachran & Fechhelm, 2010). Using the estimate of 199 from the taxon accumulation curve asymptote, and assuming that 75% of ray-finned fishes in the eastern Gulf broadcast spawn pelagic eggs (Miller & Kendall, 2009), I estimate that at least 21% of the broadcast spawning fishes on the WFS spawn during September. It is critical to acknowledge that this value is an approximation based on assumptions and may be influenced by the limitations of the study design and the uncertainty regarding the percentage of broadcast spawners on the WFS.

The most abundant genera were *Decapterus*, *Prionotus*, *Syacium*, and *Synodus*. It is likely that nearly all the *Decapterus* spp. eggs observed in my study were from *Decapterus punctatus*. Some *Decapterus* spp. eggs collected may belong to *Decapterus 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). Furthermore, all of these genera (*Decapterus*, *Prionotus*, *Syacium*, and *Synodus*) are known to be abundant on the WFS based on trawl surveys (Matheson Jr et al., 2017); therefore, their high representation in the egg community reflects their established abundance in the region.

The fish family abundance generated from my analysis of the fish egg assemblages on the WFS differed from that reported in a multi-decadal study of larval fish assemblages in the wider northern Gulf of Mexico (including the WFS) by Muhling et al. (2012). While both studies found that Carangidae, Synodontidae, Scombridae, and Cyclopsettidae made up a substantial portion of their respective assemblages, specimens from Triglidae, Lutjanidae, Stromattidae, and Serranidae made up a larger portion of total abundance in my study relative to Muhling et al. (2012). Several factors could explain this discrepancy. Firstly, Muhling et al. (2012) encompassed a larger spatial area that included the north-central and western Gulf of Mexico, which contain different benthic habitats and biotic communities (Ward & Tunnell, 2017). Secondly, Muhling et al. (2012) included multiple seasons, while my sampling was performed exclusively in September. The families that were more prevalent in my study may spawn more on the WFS compared to other regions of the Gulf of Mexico, their spawning intensity may be greater in September, there may be discrepancies between fish egg and larval communities, or a combination of these factors may have influenced the results.

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. This approach provides valuable new data for many commercially, recreationally, and culturally important species. The knowledge of spawning locations, timing, and environmental factors is useful for the management of these economically valuable species. In general, the presence and spatial distributions of the fish eggs collected in this study are consistent with previously recognized trends in spawning locations and timing for the federally managed species collected.

The majority of the lutjanid eggs in my 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). Furthermore, red snapper eggs were mainly found close to known and predicted spawning aggregations (Coleman et al., 2011; Grüss et al., 2018), supporting my results.

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 (Grüss et al., 2018). However, no yellowedge grouper eggs were collected from the other predicted 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 I did not collect in that area during active spawning. Because little spawning information exists for the yellowedge grouper, future studies may aim to focus on the southwest portion of the WFS where water column depths range from 100 m- 400 m, as this is where all yellowedge grouper eggs were found in my sampling.

Red drum eggs were found in high abundance at two sites that are predicted to be spawning aggregation hotspots (Grüss et al., 2018). This may suggest a very high specificity to these spawning areas. However, a single egg was found ~70 km NNE of the Dry Tortugas where spawning aggregations were not predicted for red drum. It may be worth investigating further if there is a spawning aggregation in the southern part of the WFS for red drum.

Spanish mackerel and king mackerel were both found to spawn in a large range of latitudes on the WFS and their eggs were found in areas of expected water column depth (Collins

& Stender, 1987), with Spanish mackerel eggs occurring where water depths were less than 40 m and king mackerel eggs occurring where water depths were usually greater than 40 m.

All the spawning times for the eggs collected from federally managed species in this study agree with known spawning times, with the exception that eggs were collected from mutton snapper, whose spawning period is assumed to end in August (Biggs et al., 2018). Available information on the timing of spawning for mutton snapper comes from Cuba and the Florida Keys (Burton et al., 2005; Claro & Lindeman, 2003). It is possible that mutton snapper in more northerly parts of their range exhibit a delayed spawning time. While no previous studies have documented mutton snapper spawning in September, it is unlikely that spawning in September is ubiquitous on the WFS as mutton snapper eggs were found at only two sites across my three years of sampling. Overall, my findings support established spawning patterns while revealing potential site preferences and highlight the reliability of my methodology in monitoring spawning locations for various federally managed species at a large spatial scale.

Annual Trends

Far fewer eggs were collected during 2014 when compared to the other two years of sampling. I documented a decline of over 80% in the mean number of fish eggs collected per site in 2014 compared to 2013 and 2019. The reduced egg abundance observed in 2014 could be the result of ecological factors, however, it is far more likely that this result is 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 the other years when it was operated on the NOAA Ship Gordon Gunter instead of 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. Further evidence for this is that while fewer eggs were collected in 2014, the relative abundances of the eight most common families were similar to the other two years, being nearly identical to the relative abundances in 2013. If environmental factors were the cause of the decline in egg abundance it would have likely affected 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 III et al., 2016; Turley et al., 2022). In addition to causing mass fish mortality and hypoxia, *Karenia brevis* blooms can hinder the development of ichthyoplankton (Colman & Ramsdell, 2003), displace spawning aggregations (Walters et al., 2013), and severely impact entire benthic communities (Smith, 1975); however, it is unclear how *Karenia brevis* blooms and the brevetoxins they release affect fish fecundity. Although the impacts from the 2014 *Karenia brevis* bloom were intense and spatially extensive (approximately 10,000 km^2), it does not explain the reduced egg abundances I observed across the entire WFS (170,000 km^2). While it is unlikely that the *Karenia brevis* bloom was the main driver of the reduced egg abundances, I did observe below average taxon richness and egg abundances in three out of four of my 2014 sites that were in the region where the bloom was present. Future egg surveys may attempt to further our understanding of how *Karenia brevis* blooms influence fish spawning dynamics by conducting sampling inside and outside of bloom-affected areas. Additionally, in future sampling efforts, the volume of water pumped from the CUFES should be measured so fish egg abundances can be standardized.

Observed differences in fish egg taxon richness, Shannon diversity, and beta-diversity across the three years of sampling are likely related to a combination of methodological and ecological factors. The sampling locations each year were not identical but were chosen using GRTS to minimize spatial bias, which could introduce year-to-year variation. Meanwhile, significantly lower egg abundance in 2014 explains reduced richness, diversity, and differences in beta-diversity. Differences in beta-diversity between 2013 and 2019 suggest there are additional influences beyond egg abundance as 2013 and 2019 had similar egg abundance. Further investigations into spatial patterns, additional environmental variables, and species-specific population dynamics are necessary for understanding the drivers of interannual variation in fish spawning on the WFS. Additionally, future studies may choose to use more consistent sampling locations across years and remove sites that are off the shelf to reduce variability introduced by inconsistent sampling.

Spatial Trends

My findings on fish egg distribution generally aligned with established knowledge of adult fish zonation on the WFS (Darcy & Gutherz, 1984; Saul et al., 2013; Switzer et al., 2023). While both water column depth and regional strata were found to be associated with variability in fish egg beta-diversity, my PERMANOVA results suggest water column depth explains more of the variability in fish egg beta-diversity for the September egg community on the WFS based on its larger F-statistic and R-squared values.

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 & 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 Jr et al., 2017; Saul et al., 2013; Switzer et al., 2023).

I adopted similar depth strata and regional divisions defined by Switzer et al. (2023) and while I observed significant differences in fish egg beta-diversity across all depth strata, I did not find significant differences between all regional strata. This discrepancy might be attributed to my focus on the broader egg community, encompassing fishes from all habitats, compared to Switzer et al. (2023) who focused solely on reef fish. Additionally, my sampling being limited to a single month may have contributed to the observed differences. Despite these variations, my egg survey's zonation patterns generally reflected known biogeographical trends of adult fishes on the WFS.

Further supporting the primary role of water column depth in structuring the fish egg community on the WFS, the DISPROF analysis revealed distinct spatial groups representing shallow, mid-shelf, and offshore communities. 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 (12, 14), mid-shelf (1), and offshore (9, 10) communities across all three years. 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.

Fish Egg Trajectories

Using models to back-track the possible trajectories of fish eggs based on ocean currents allows for increased accuracy in predicting their likely location of origin, aiding in the identification of spawning grounds. Trajectory distances displayed substantial inter- 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 & 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 & He, 2003). I observed 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 suggests shallow water spawning on the WFS likely leads to retention, while deep water spawning leads to export. Additionally, the trajectory patterns suggest minimal inshore-to-offshore transport within the 200 m isobath.

Surface currents were used for modeling, as it was assumed that fish eggs rise relatively quickly to the surface layer and then passively float at the surface where they are advected by surface currents. The upward speed of pelagically spawned fish eggs likely varies among taxa but has been estimated to be 3.6–7.2 for *Sardinops melanostictus* (Japanese sardine) and 4.9–8.6 m/h for *Gadus chalcogrammus* (walleye pollock) eggs respectively (Nakatani, 1988; Tanaka & Franks, 2008). The modeled trajectories using surface currents will therefore be the most accurate for relatively shallow sites. The trajectories for eggs spawned at depth at sites on the outer shelf may be misleading as the egg will spend much of its pre-hatching time below the

surface layer. For these cases, the trajectory would be more accurate if a model considering the egg's original spawning depth and time spent at various depths was used. Additionally, while the choice to use 36 hours of backtracking encompasses the likely time spent in the embryonic stage before hatching for most of the taxa in my study, there are differences in this timing for various taxa (Pauly & Pullin, 1988). The specific hatching times for each taxon should be considered when interpreting the trajectories and attempting to predict the original location where the fish egg was spawned. Additionally, the modeled trajectories represent the maximum potential distance most fish eggs could have traveled if collected just before hatching, given the 36-hour backtracking period. The actual dispersal distances are likely shorter, especially for eggs collected shortly after they were spawned or 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. While this study provides insights into the use of oceanographic models for back-tracking fish egg transport, further refinement and exploration are needed, particularly regarding the consideration of vertically-structured currents, the upward velocity of fish eggs for taxa on the WFS, and the range of embryonic development times. These considerations will allow for improved accuracy in the attempt to identify spawning locations across different species and habitats.

DNA Barcoding

A substantial proportion (approximately 21%) of the collected fish eggs did not yield DNA sequences. Barcoding failure was linked to a few different issues that occurred at various steps from collection to sequencing, with the greatest proportion of failures arising from amplification failures during PCR. It is possible that the COI-3 universal fish primer cocktail

failed to amplify DNA from a small portion of the fish egg community due to a primer mismatch. However, it is unlikely that a substantial amount of taxa were missed. Harada et al. (2015) also experienced a high amplification failure rate when using the COI-3 universal fish primer cocktail but found that using the 16S rRNA gene instead of COI-3 for fish egg DNA amplification yielded similar species identifications. In their study, there was evidence of only a single additional species found using 16S rRNA primers. Thus, it is likely that the PCR failures are primarily the result of poor template quality (insufficient DNA quantity, degraded DNA, or PCR inhibitors being present). There were several instances where the DNA from all eggs at a station failed to amplify, which may reflect storage conditions as all eggs from a given collection station were stored in the same vial. Additionally, DNA barcoding of the samples occurred 2-8 years after the eggs were collected and while DNA is typically stable when stored properly, it is not always feasible to have optimal storage conditions. In future studies, it may be beneficial to attempt to determine the exact causes of failures during the barcoding process. This would allow for targeted approaches to improve success rates in future studies.

It was common for my method, using the COI-3 universal fish primer cocktail, to fail to determine the species-level identification of a sequence. Identifications that included two or more closely related species made up a significant portion of the total identifications. These instances of unresolved species-level identifications were more pronounced in certain fish families. For instance, fish eggs from the tuna and mackerel (Scombridae) family, and particularly those in the *Thunnus* genus, were seldom identified at the species-level, while snappers (Lutjanidae) were virtually always identified at the species-level. For future studies that are interested in barcoding eggs or larvae for a particular taxonomic group, such as tunas, it may

be advantageous to use different primers targeting a less conserved region for DNA amplification so that identifications can be resolved to the species-level more reliably.

Conclusions

This study used a CUFES and DNA barcoding to examine the fish spawning community on the WFS during September of 2013, 2014, and 2019. I identified eggs from 83 fish species and successfully identified spawning locations for several federally managed species. My findings generally aligned with known adult fish distributions and spawning patterns, and I found that water column depth played a more significant role than regional strata in structuring the fish egg community. I observed distinct shallow, mid-shelf, and offshore communities. My results demonstrated that this 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 broadening seasonal coverage, exploring environmental factors that may drive interannual variation, expanding and refining trajectory modeling to incorporate egg-specific buoyancy and development times, and detailed examination of spawning areas of interest identified in this study.

Tables and Figures

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

Year	Percentage of sites with fish eggs present	Total fish eggs collected	Maximum number of eggs at a site	Number of fish eggs collected per site (mean ± standard error)
2013 (n = 70)	94%	2882	222	41 ± 5.6
2014 (n = 75)	96%	450	39	6 ± 0.7
2019 (n = 73)	97%	2308	285	32 ± 5.0

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	9/11 – 9/24	76.9 ± 19.3	29.7 ± 0.05	34.1 ± 0.3
2014	9/7 – 9/27	55.6 ± 6.4	29.6 ± 0.05	34.4 ± 0.1
2019	9/13 – 9/26	123.2 ± 22.6	29.8 ± 0.09	33.5 ± 0.3

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	54
Big Bend	Sites north of 27° N latitude and east of 84.9° W longitude (including 27° N and 84.9°)	61
Panhandle	Sites north of 27° N latitude and west of 84.9° W longitude	69
Depth (m)		
25 and less	Water column depth of 25 meters or less	44
25-50	Water column depth between 25 and 50 meters	65
50-100	Water column depth between 50 and 100 meters	37
> 100	Water column depth greater than 100 meters	38

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* sp. accounted for 54% of IDs that contained two species in the same genus.

Barcoding success and resolution (n = 4991 eggs processed)	
Barcoding success rate (one or more ID with a >97% match)	79.4% (3964 eggs)
Single species ID	47% (1845 eggs)
Two species-level IDs in the same genus	48% (1914 eggs)
More than two species in the same genus or two or more species from different genera	5% (205 eggs)

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	54	32	51	83
Genera	51	33	45	71
Families	24	21	26	36

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**
Lutjanus analis	10	2	25.0 ± 0	68.3 ± 8.3	29.3 ± 0	35.3 ± 0.1
Lutjanus campechanus	70	22	29.1 ± 0.2	47.9 ± 4.6	29.6 ± 0.1	32.0 ± 0.1
Lutjanus synagris	20	12	26.8 ± 0.4	29.4 ± 3.6	29.8 ± 0.1	35.2 ± 0.2
Rhomboplites aurorubens	122	33	27.5 ± 0.3	58.8 ± 6.3	29.7 ± 0.1	34.2 ± 0.3
Pristipomoides aquilonaris	146	23	28.3 ± 0.3	119.1 ± 10.5	29.7 ± 0.1	33.2 ± 0.6
Hyporthodus flavolimbatus	24	7	26.2 ± 0.3	195.1 ± 27.4	30.3 ± 0.1	36.0 ± 0.3
Sciaenops ocellatus	87	3	27.8 ± 1.5	25.2 ± 5	30.4 ± 0.1	33.9 ± 1.5
Scomberomorus cavalla	68	19	28.2 ± 0.4	49.1 ± 2.8	29.7 ± 0.1	33.1 ± 0.8
Scomberomorus maculatus	60	13	28.3 ± 0.3	22.0 ± 1.4	30.0 ± 0.1	33.9 ± 0.5
Katsuwonus pelamis	30	8	25.9 ± 0.3	316.2 ± 111	29.8 ± 0.2	35.7 ± 0.3

Table 7. Mean taxon richness and Shannon diversity index per site by year. Different superscripts indicate statistically significant differences ($p < 0.05$).

Year	Taxon richness (mean ± standard error)	Shannon diversity index (mean ± standard error)	Total taxon richness
2013	5.4 ± 0.4^{a}	1.18 ± 0.1^{a}	58
2014	2.6 ± 0.2^{b}	0.67 ± 0.1^{b}	38
2019	4.4 ± 0.3^{c}	0.98 ± 0.1^{a}	50

Table 8. Mean egg abundance, taxon richness, and Shannon diversity index per site by water column depth. Different superscripts indicate statistically significant differences ($p < 0.05$).

Depth (m)	Number of fish eggs collected (mean ± standard error)	Taxon richness (mean ± standard error)	Shannon diversity index (mean ± standard error)	Total taxon richness
0-25	27.4 ± 7.6^{a}	5.2 ± 0.5^{a}	1.11 ± 0.1^{a}	45
25-50	24.8 ± 3.9^{a}	4.7 ± 0.3^{a}	1.1 ± 0.1^{a}	47
50-100	21.5 ± 5.3^{a}	3.8 ± 0.4^{ab}	1 ± 0.1^{a}	29
>100	14.2 ± 3.1^{a}	2.3 ± 0.2^{b}	0.64 ± 0.1^{b}	28

Table 9. PERMANOVA results of fish egg assemblages for the factors year, region, water column depth, and their interactions.

Factor	df	R^2	Pseudo-F	p-value
Year	2	0.04393	5.4049	0.0001
Region	2	0.02679	3.2959	0.0001
Depth	3	0.14474	11.8716	0.0001
Year x Region	4	0.03198	1.9671	0.0001
Year x Depth	6	0.04492	1.8420	0.0001
Region x Depth	6	0.04670	1.9150	0.0001
Year x Region x Depth	9	0.04729	1.2930	0.0172
Residual	151	0.61366		
Total	183	1.00000		

Table 10. Summary of predicted fish egg dispersal distances from the West Florida Coastal Ocean Model.

Year	Mean predicted dispersal distance (km)	Maximum predicted dispersal distance (km)
2013	17.7	50.2
2014	15.8	52.1
2019	28.5	79.4

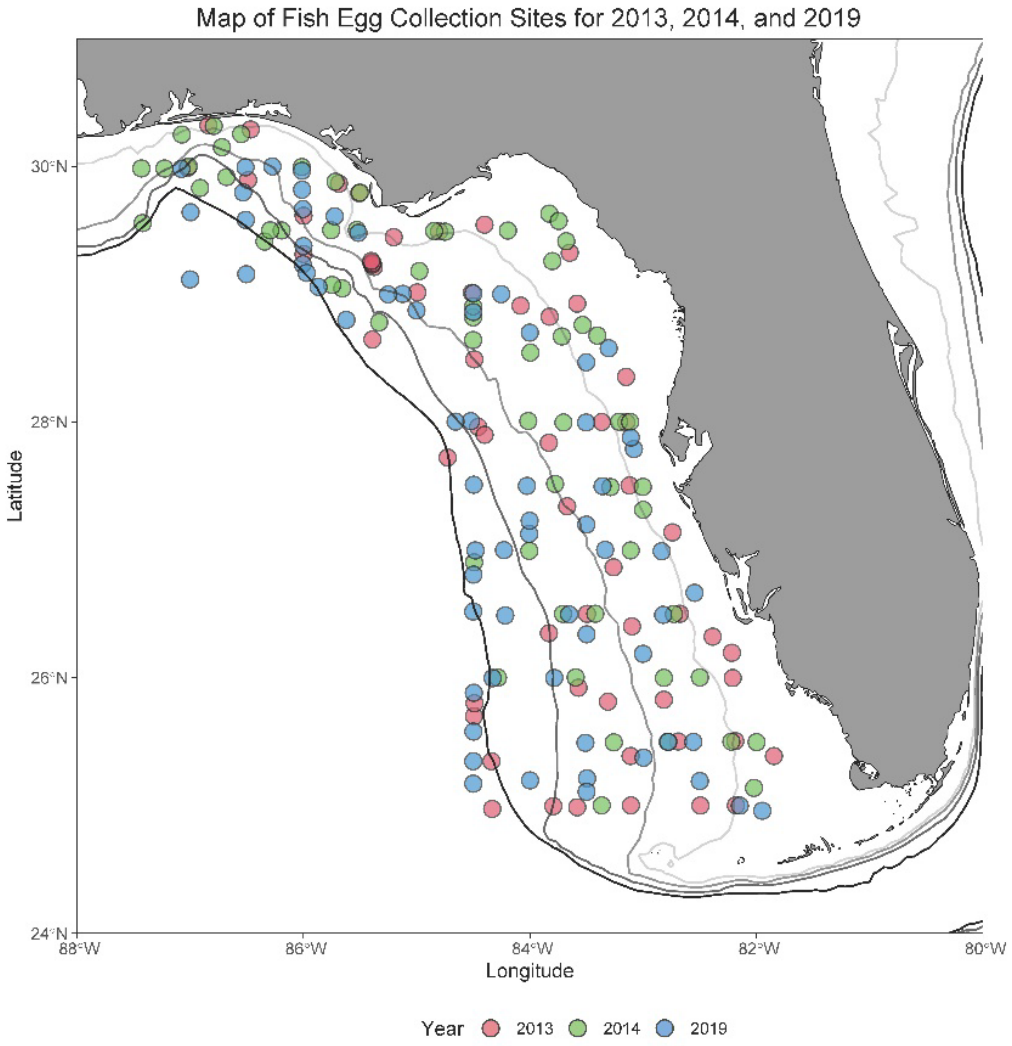

Figure 1. Map of fish egg collection locations for 2013, 2014, and 2019. Red circles represent 2013 sites, green circles represent 2014 sites, and blue circles represent 2019 sites. The start point of the collection transects are used. Grey and black lines represent 25 m, 50 m, 100 m, and 200 m isobaths.

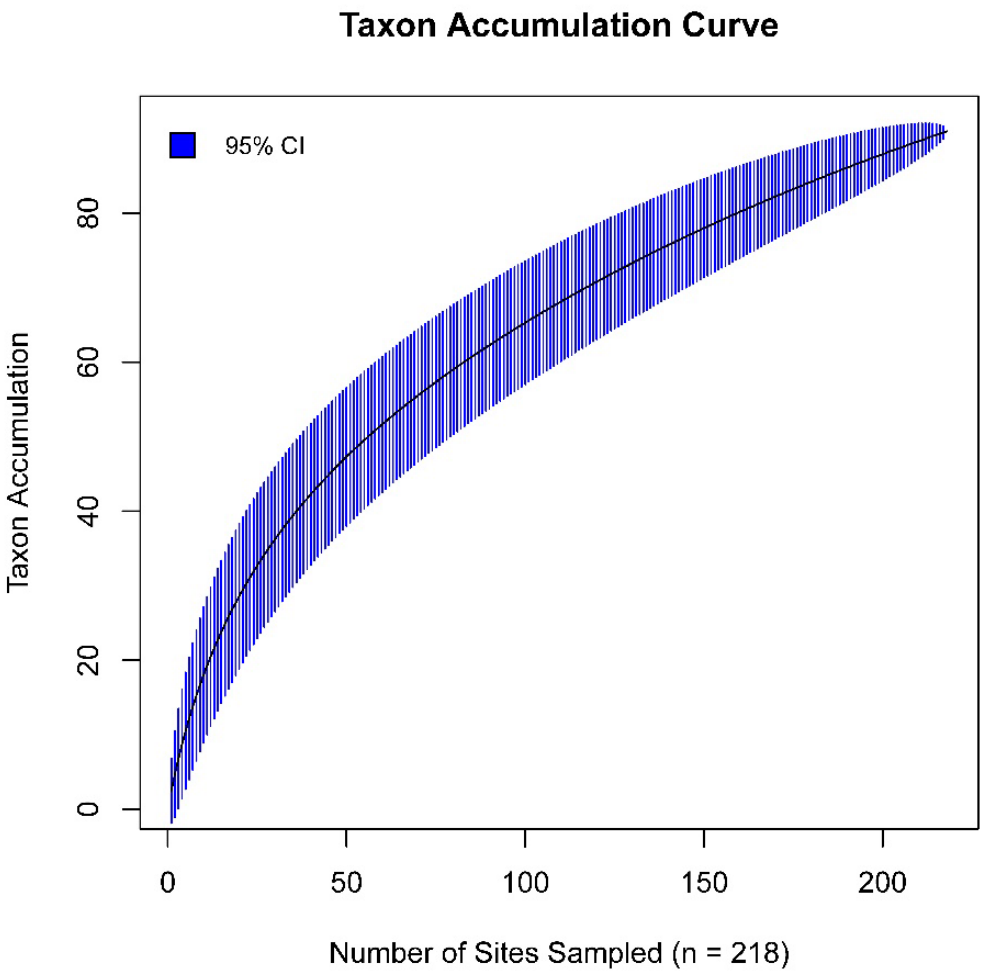

Figure 2. Taxon accumulation curve for fish egg sampling using a sample-based rarefaction method. The x-axis represents the cumulative number of samples processed across the three sampling years, while the y-axis depicts the cumulative number of identified fish species. Blue lines represent confidence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

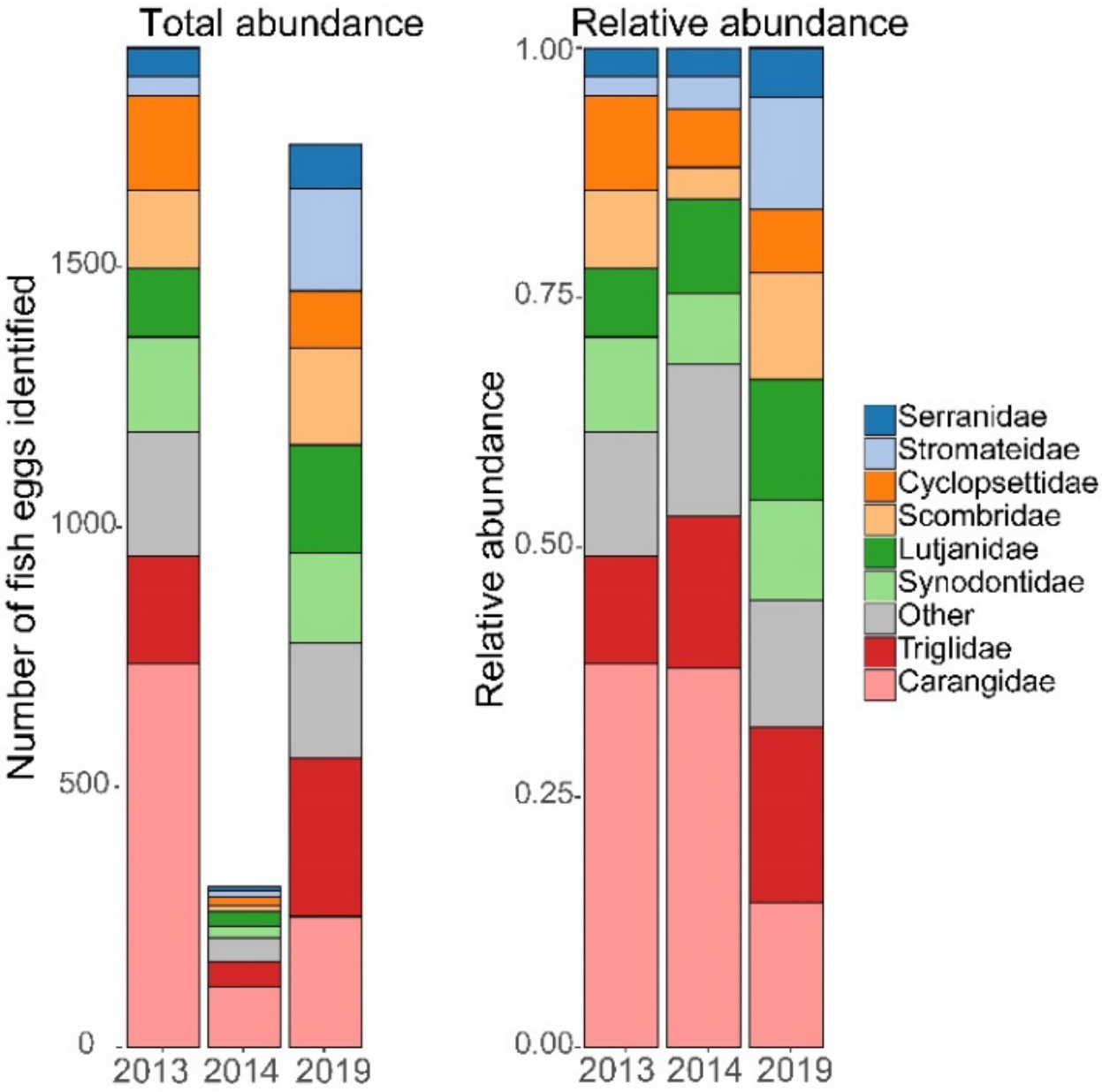

Figure 3. Stacked bar charts showing (left) total abundance and (right) relative abundance of eggs identified for the eight most abundant fish families. The "Other" category includes eggs from all other fish families present in the study (n = 28).

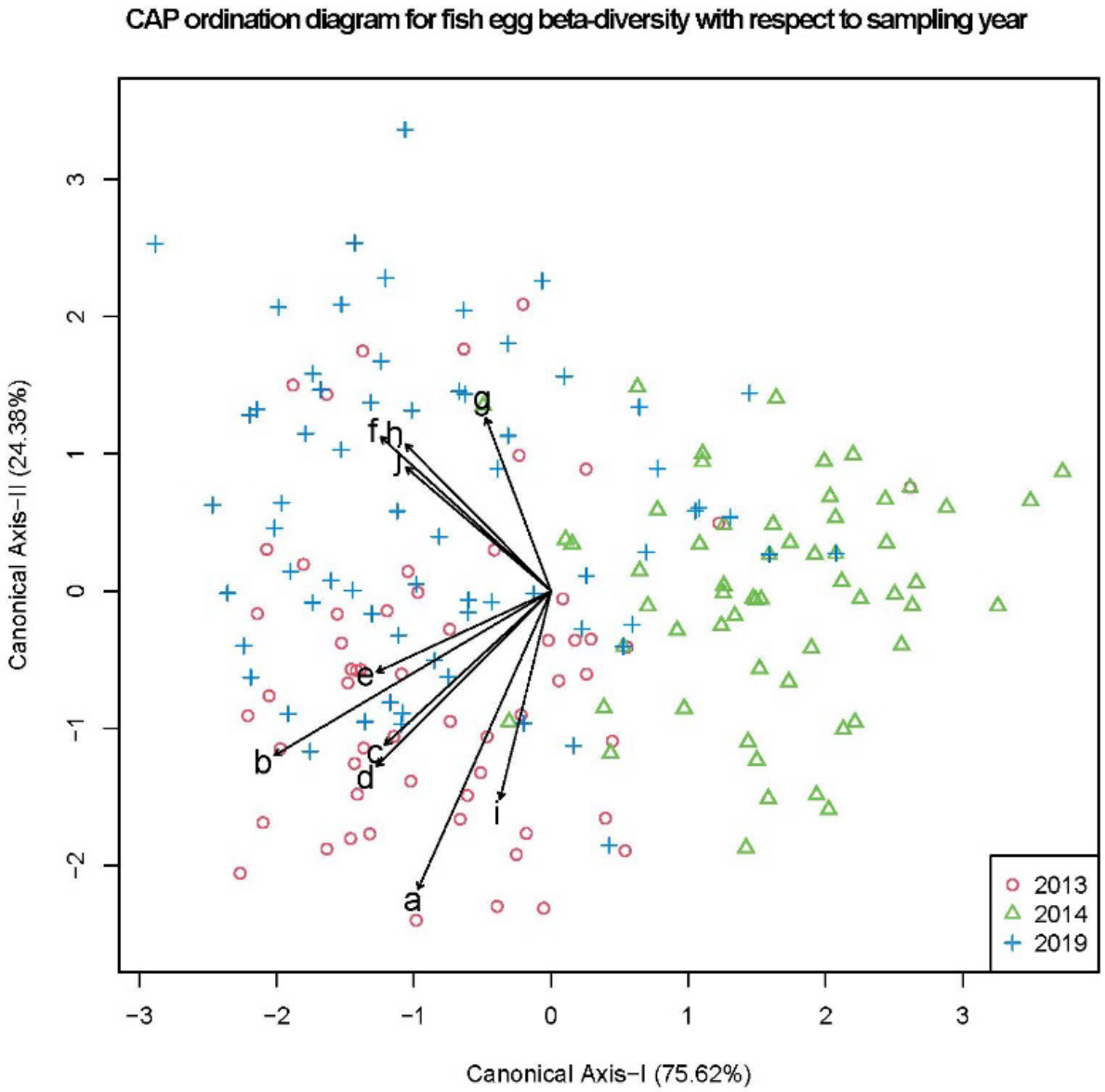

Figure 4. Canonical Analysis of Principal coordinates (CAP) ordination diagram depicting differences among years with respect to fish egg beta-diversity in 2013, 2014, and 2019. The canonical axes I and II explain 75.62% and 24.38% of the total variability between each year's group of objects (sampling sites). Proximity between two objects indicates likeness, with closer objects being more alike. Arrows represent taxon scores for the taxon that had the ten largest indicator values as determined by an indicator value analysis (IndVal). These scores depict the contribution of each taxon to the observed differences between groups. Arrows point in the direction of the taxon's influence on the ordination space and longer arrows indicate a stronger influence by the taxon on 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*, j) *Scomberomorus maculatus*.

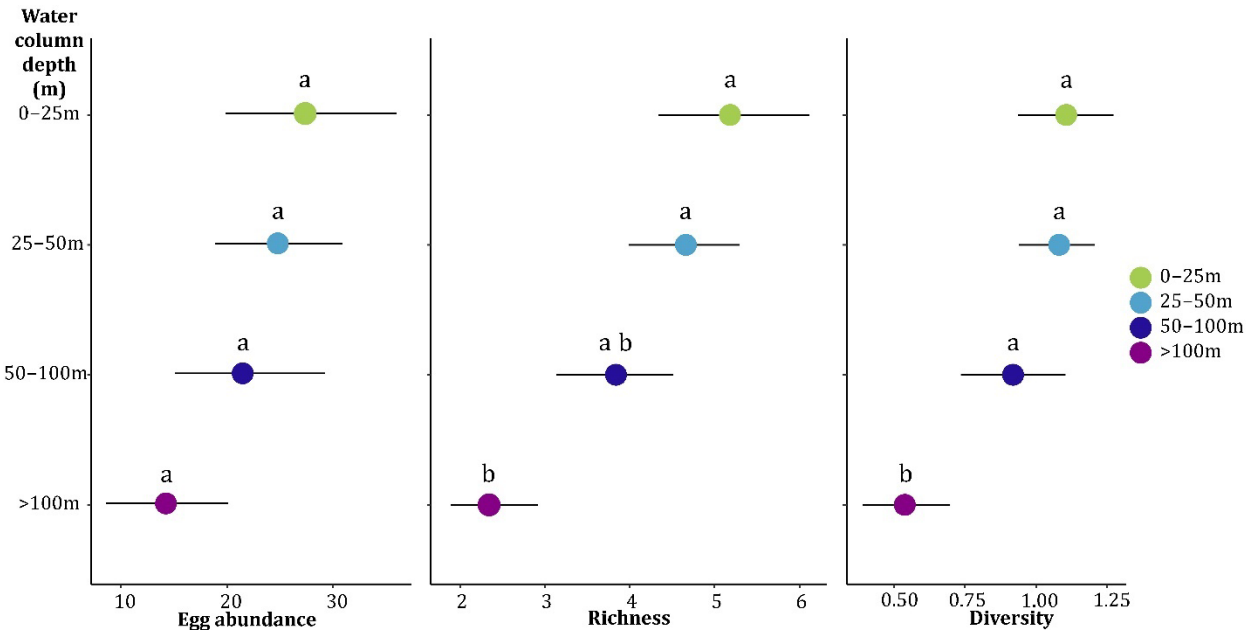

Figure 5. Variation in (left) mean number of eggs collected per site, (middle) mean taxon richness per site, and (right) mean Shannon diversity index per site among depth classes. Points represent mean values and horizontal bars represent 95% confidence intervals. Different letters above points indicate statistically significant differences ($p < 0.05$).

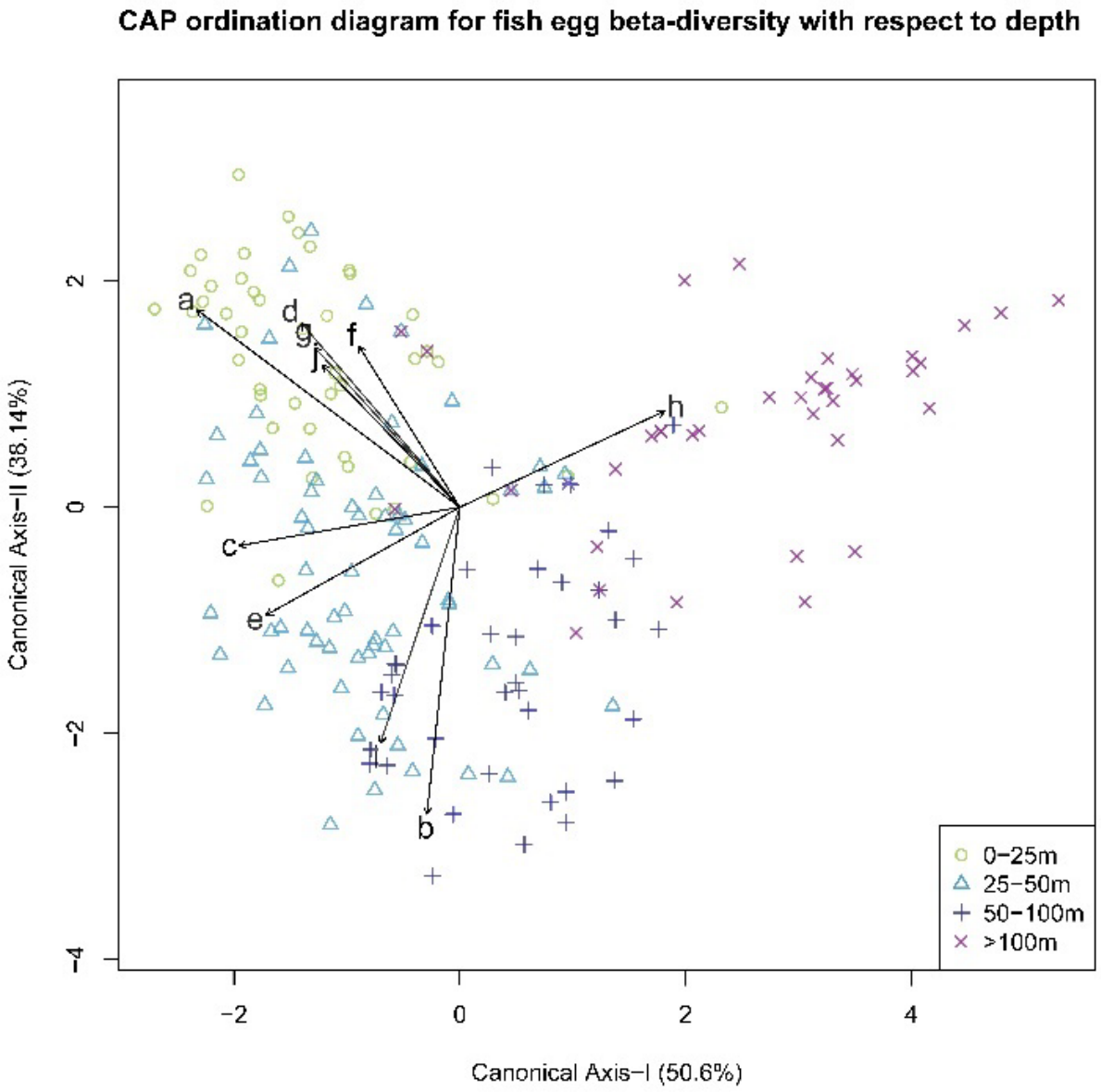

Figure 6. Canonical Analysis of Principal coordinates (CAP) ordination diagram that includes all three years of fish egg data and depicts differences among depth classes with respect to fish egg beta-diversity. The canonical axes I and II explain 50.6% and 38.14% of the total variability between each depth class's group of objects (sampling sites). Proximity between two objects indicates likeness, with closer objects being more alike. Arrows represent taxon scores for the taxon that had the ten largest indicator values as determined by an indicator value analysis (IndVal). These scores depict the contribution of each taxon to the observed differences between groups. Arrows point in the direction of the taxon's influence on the ordination space and longer arrows indicate a stronger influence by the taxon on 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*, j) *Hyporthodus flavolimbatus*.

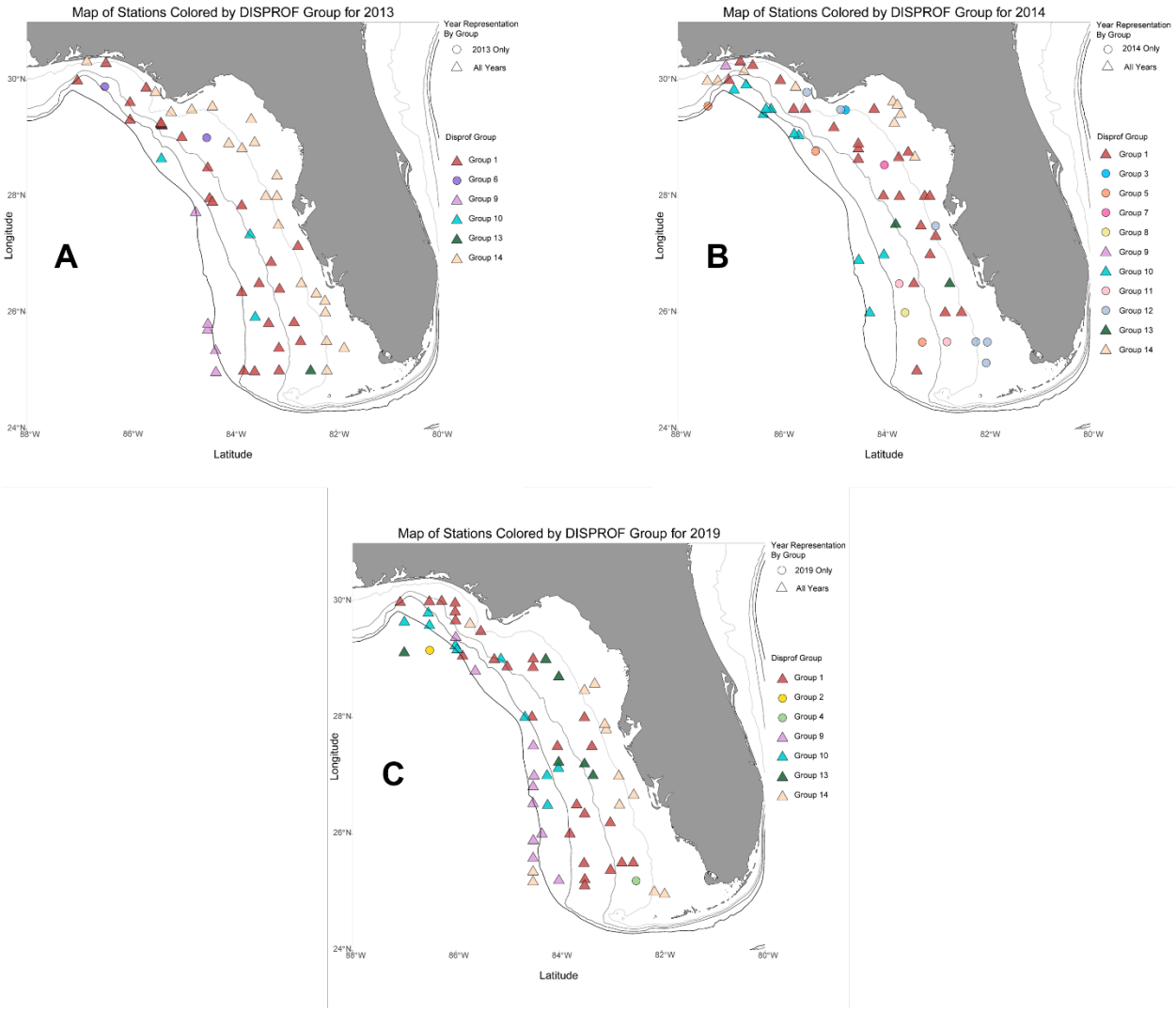

Figure 7. Maps of fish egg collection locations, colored by their assigned DISPROF groups. Triangles represent DISPROF groups present in all years while circles represent DISPROF groups unique to a single year. Grey and black lines represent 25 m, 50 m, 100 m, and 200 m isobaths (A: 2013, B: 2014, C: 2019).

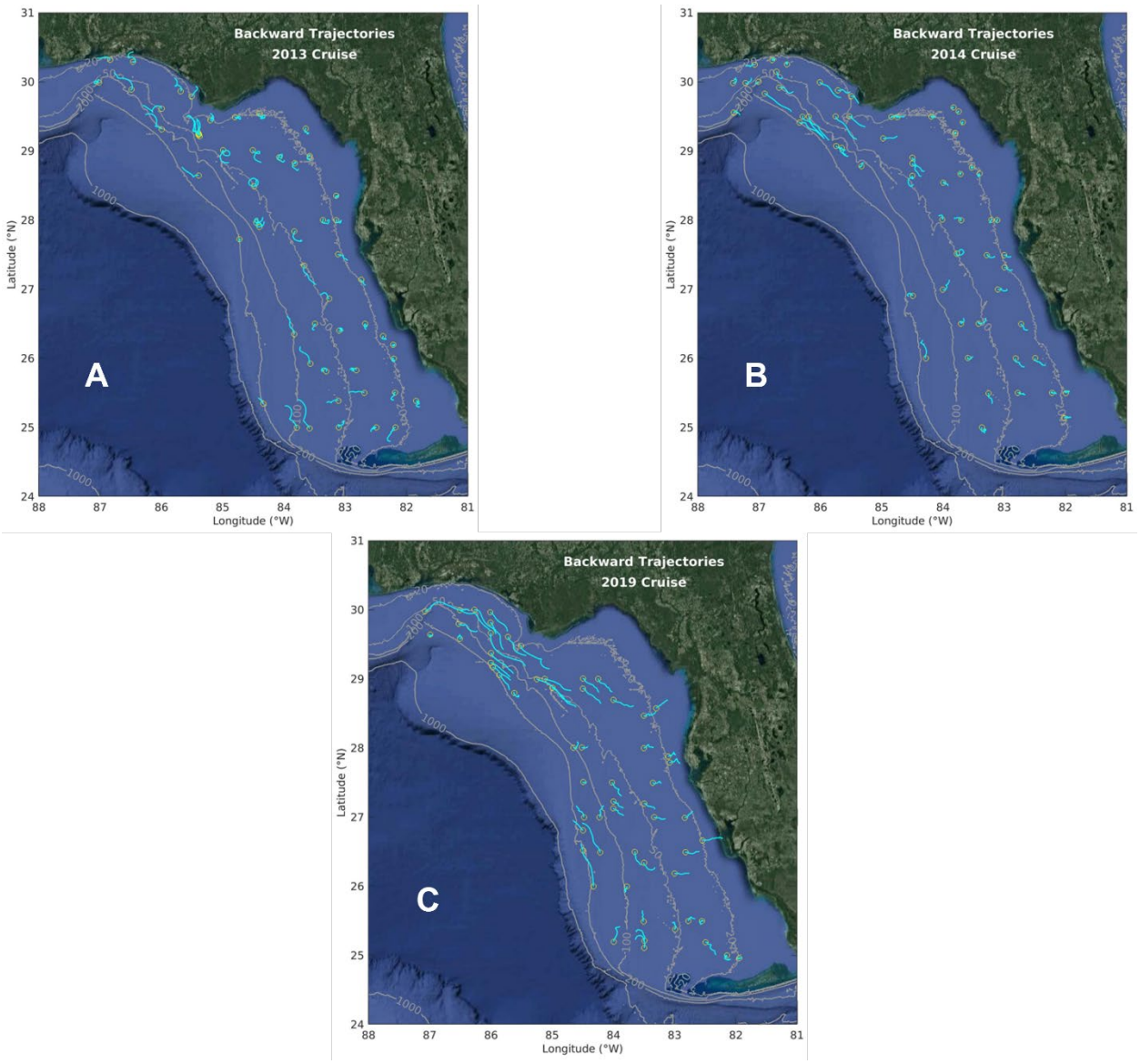

Figure 8. Maps depicting backward Lagrangian tracking for 36 hours starting from the sample locations based on WFCOM hindcasted surface currents. Yellow circles represent sampling locations while blue lines represent the 36-hour predicted backward trajectories. Grey lines represent 20 m, 50 m, 100 m, 200 m, and 1000 m isobaths (A: 2013, B: 2014, C: 2019).

REFERENCES

Ahern, A. L. M., Gómez-Gutiérrez, J., Aburto-Oropeza, O., Saldierna-Martínez, R. J., Johnson, A. F., Harada, A. E., Sánchez-Uvera, A. R., Erisman, B., Arvizú, D. I. C., & Burton, R. S. (2018). DNA sequencing of fish eggs and larvae reveals high species diversity and seasonal changes in spawning activity in the southeastern Gulf of California. *Marine Ecology Progress Series*, *592*, 159-179.

Allen, G.-R. (1985). *FAO Species Catalogue: Vol. 6. Snappers of the World: An Annotated and Illustrated Catalogue of Lutjanid Species Known to Date*.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403-410.

Armstrong, M. J., & Witthames, P. R. (2012). Developments in understanding of fecundity of fish stocks in relation to egg production methods for estimating spawning stock biomass. *Fisheries Research*, *117*, 35-47.

Berry, F. H. (1968). A new species of carangid fish (Decapterus tabl) from the western Atlantic. *Contributions in Marine Science*, *13*, 145-167.

Biggs, C., Erisman, B., Heyman, W., Kobara, S., Farmer, N., Lowerre-Barbieri, S., & Brenner, J. (2018). Cooperative monitoring program for spawning aggregations in the Gulf of Mexico: Life History and Spawning Behavior. Version 2018.07. Retrieved from GCOOS Web site: http://geo.gcoos.org/restore

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.

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

Burton, M. L., Brennan, K. J., Muñoz, R. C., & Parker Jr, R. (2005). Preliminary evidence of increased spawning aggregations of mutton snapper (*Lutjanus analis*) at Riley's Hump two years after establishment of the Tortugas South Ecological Reserve. *Fishery Bulletin*, *103*(2), 404-410.

Chassignet, E. P., Hurlburt, H. E., Metzger, E. J., Smedstad, O. M., Cummings, J. A., Halliwell, G. R., Bleck, R., Baraille, R., Wallcraft, A. J., & Lozano, C. (2009). US GODAE: global ocean prediction with the HYbrid Coordinate Ocean Model (HYCOM). *Oceanography*, *22*(2), 64-75.

Checkley Jr, D. M., Ortner, P. B., Settle, L. R., & Cummings, S. R. (1997). A continuous, underway fish egg sampler. *Fisheries Oceanography*, *6*(2), 58-73.

Checkley Jr., D. M., Dotson, R., & Griffith, D. A. (2000). Continuous, underway sampling of eggs of Pacific sardine (*Sardinops sagax*) and northern anchovy (*Engraulis mordax*) in spring 1996 and 1997 off southern and central California. *Deep Sea Research Part II: Topical Studies in Oceanography*, *47*(5), 1139-1155.

Chen, C., Liu, H., & Beardsley, R. C. (2003). An unstructured grid, finite-volume, three-dimensional, primitive equations ocean model: application to coastal ocean and estuaries. *Journal of Atmospheric and Oceanic Technology*, *20*(1), 159-186.

Chen, Y. (2017). Fish Resources of the Gulf of Mexico. In C. H. Ward (Ed.), *Habitats and Biota of the Gulf of Mexico: Before the Deepwater Horizon Oil Spill: Volume 2: Fish Resources, Fisheries, Sea Turtles, Avian Resources, Marine Mammals, Diseases and Mortalities* (pp. 869-1038). Springer New York.

Claro, R., & Lindeman, K. C. (2003). Spawning aggregation sites of snapper and grouper species (Lutjanidae and Serranidae) on the insular shelf of Cuba. *Gulf and Caribbean Research*, *14*(2), 91-106.

Coleman, F. C., Scanlon, K. M., & Koenig, C. C. (2011). Groupers on the edge: Shelf edge spawning habitat in and around marine reserves of the northeastern gulf of mexico. *The Professional Geographer*, *63*(4), 456-474.

Collette, B. B. (1983). FAO species catalogue, Vol 2. Scombrids of the world: an annotated and illustrated catalogue of tunas, mackerels, bonitos and related species known to date. *FAO Fisheries Synopsis*, *125*, 1-137.

Collins, M. R., & Stender, B. W. (1987). Larval king mackerel (*Scomberomorus cavalla*), Spanish mackerel (*S. maculatus*), and bluefish (*Pomatomus saltatrix*) off the southeast coast of the United States, 1973–1980. *Bulletin of Marine Science*, *41*(3), 822-834.

Colman, J. R., & Ramsdell, J. S. (2003). The type B brevetoxin (PbTx-3) adversely affects development, cardiovascular function, and survival in Medaka (*Oryzias latipes*) embryos. *Environmental Health Perspectives*, *111*(16), 1920-1925.

Conway, D. V. P., Coombs, S. H., & Smith, C. (1997). Vertical distribution of fish eggs and larvae in the Irish Sea and southern North Sea. *ICES Journal of Marine Science*, *54*(1), 136-147.

Cowen, R. K., & Sponaugle, S. (2009). Larval dispersal and marine population connectivity. *Annual Review of Marine Science*, *1*(1), 443-466.

Darcy, G. H., & Gutherz, E. J. (1984). Abundance and density of demersal fishes on the west Florida shelf, January 1978. *Bulletin of Marine Science*, *34*(1), 81-105.

Ditty, J. G., Shaw, R. F., & Cope, J. S. (2004). Distribution of carangid larvae (Teleostei: Carangidae) and concentrations of zooplankton in the northern Gulf of Mexico, with illustrations of early *Hemicaranx amblyrhynchus* and *Caranx* spp. larvae. *Marine Biology*, *145*, 1001-1014.

Driggers III, W., Campbell, M., Debose, A., Hannan, K., Hendon, M., Martin, T., & Nichols, C. (2016). Environmental conditions and catch rates of predatory fishes associated with a mass mortality on the West Florida Shelf. *Estuarine, Coastal and Shelf Science*, *168*, 40-49.

Duke, E., Harada, A., & Burton, R. (2018). Large interannual variation in spawning in San Diego marine protected areas captured by molecular identification of fish eggs. *Marine Ecology Progress Series*, *604*, 199-210.

Dumelle, M., Kincaid, T., Olsen, A. R., & Weber, M. (2023). spsurvey: spatial sampling design and analysis in R. *Journal of Statistical Software*, *105*(3), 1.

Fuiman, L. A., & Werner, R. G. (2009). *Fishery Science: the Unique Contributions of Early Life Stages*. John Wiley & Sons.

Grüss, A., Biggs, C., Heyman, W. D., & Erisman, B. (2018). Prioritizing monitoring and conservation efforts for fish spawning aggregations in the US Gulf of Mexico. *Scientific Reports*, *8*(1), 8473.

Harada, A. E., Lindgren, E. A., Hermsmeier, M. C., Rogowski, P. A., Terrill, E., & Burton, R. S. (2015). Monitoring spawning activity in a southern California marine protected area using molecular identification of fish eggs. *PLOS ONE*, *10*(8), e0134647.

Heemstra, P. C., & Randall, J. E. (1993). Groupers of the world. *FAO Fisheries Synopsis*, *16*(125).

Houde, E. D., & Chitty, N. (1976). *Seasonal abundance and distribution of zooplankton, fish eggs, and fish larvae in the eastern Gulf of Mexico, 1972-74*. NOAA Technical Report, NMFS SSRF-701

Ivanova, N. V., Zemlak, T. S., Hanner, R. H., & Hebert, P. D. N. (2007). Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes*, *7*(4), 544-548.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., & Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, *28*(12), 1647-1649.

Kerr, M., Browning, J., Bønnelycke, E. M., Zhang, Y., Hu, C., Armenteros, M., Murawski, S., Peebles, E., & Breitbart, M. (2020). DNA barcoding of fish eggs collected off northwestern Cuba and across the Florida Straits demonstrates egg transport by Mesoscale eddies. *Fisheries Oceanography*, *29*(4), 340-348.

Larson, J. H., Grace McCalla, S., Chapman, D. C., Rees, C., Knights, B. C., Vallazza, J. M., George, A. E., Richardson, W. B., & Amberg, J. (2016). Genetic analysis shows that morphology alone cannot distinguish Asian carp eggs from those of other cyprinid species. *North American Journal of Fisheries Management*, *36*(5), 1053-1058.

Leak, J. C. (1981). Distribution and abundance of carangid fish larvae in the eastern Gulf of Mexico, 1971–1974. *Biological Oceanography*, *1*(1), 1-28.

Leis, J. M. (2006). Are larvae of demersal fishes plankton or nekton? *Advances in Marine Biology*, *51*, 57-141.

Lelievre, S., Vaz, S., & Fox, C. (2012). The effectiveness of the continuous underway fish egg sampler compared with standard vertical plankton hauls for sampling the eggs of demersal species in the southern North Sea. *Fisheries Oceanography*, *21*(2-3), 109-124.

Lewis, L. A., Richardson, D. E., Zakharov, E. V., & Hanner, R. (2016). Integrating DNA barcoding of fish eggs into ichthyoplankton monitoring programs. *Fishery Bulletin*, *114*(2).

Lin, H.-Y., Chiu, M.-Y., Shih, Y.-M., Chen, I.-S., Lee, M.-A., & Shao, K.-T. (2016). Species composition and assemblages of ichthyoplankton during summer in the East China Sea. *Continental Shelf Research*, *126*, 64-78.

Lira, N. L., Tonello, S., Lui, R. L., Traldi, J. B., Brandão, H., Oliveira, C., & Blanco, D. R. (2023). Identifying fish eggs and larvae: from classic methodologies to DNA metabarcoding. *Molecular Biology Reports*, *50*(2), 1713-1726.

Liu, Y., Weisberg, R. H., Vignudelli, S., & Mitchum, G. T. (2016). Patterns of the loop current system and regions of sea surface height variability in the eastern Gulf of Mexico revealed by the self-organizing maps. *Journal of Geophysical Research: Oceans*, *121*(4), 2347-2366.

Matheson Jr, R. E., Flaherty-Walia, K. E., Switzer, T. S., & McMichael Jr, R. H. (2017). The importance of time of day in structuring demersal ichthyofaunal assemblages on the West Florida Shelf. *Bulletin of Marine Science*, *93*(2), 407-437.

McEachran, J. D., & Fechhelm, J. D. (2010). *Fishes of the Gulf of Mexico, volume 2: Scorpaeniformes to Tetraodontiformes*. University of Texas Press.

Miller, B. S., & Kendall, A. W. (2009). *Early life history of marine fishes*. University of California Press.

Muhling, B. A., Lamkin, J. T., & Richards, W. J. (2012). Decadal-scale responses of larval fish assemblages to multiple ecosystem processes in the northern Gulf of Mexico. *Marine Ecology Progress Series*, *450*, 37-53.

Nakatani, T. (1988). Studies on the early life history of walleye pollock *Theragra chalcogramma* in Funka Bay and vicinity, Hokkaido. *Memoirs of the Faculty of Fisheries Hokkaido University*, *35*(1), 1-46.

Nguyen, B. V. V., Liu, Y., Stallings, C. D., Breitbart, M., Murawski, S. A., Weisberg, R. H., Kerr, M., Bønnelycke, E. M. S., & Peebles, E. B. (2024). Retention and export of planktonic fish eggs in the northeastern Gulf of Mexico. *Fisheries Oceanography*, *33*(1), e12655.

NMFS. (2022). *Fisheries Economics of the United States, 2019*.

Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., . . . Weedon, J. (2022). vegan: Community Ecology Package. In (Vol. R package version 2.6-4.).

Pankhurst, N. W., & Munday, P. L. (2011). Effects of climate change on fish reproduction and early life history stages. *Marine and Freshwater Research*, *62*(9), 1015-1026.

Pauly, D., & Pullin, R. S. (1988). Hatching time in spherical, pelagic, marine fish eggs in response to temperature and egg size. *Environmental Biology of Fishes*, *22*, 261-271.

Peebles, E. B., & Tolley, S. (1988). Distribution, growth and mortality of larval spotted seatrout, *Cynoscion nebulosus*: a comparison between two adjacent estuarine areas of southwest Florida. *Bulletin of Marine Science*, *42*(3), 397-410.

Pepin, P., Snelgrove, P. V. R., & Carter, K. P. (2005). Accuracy and precision of the continuous underway fish egg sampler (CUFES) and bongo nets: a comparison of three species of temperate fish. *Fisheries Oceanography*, *14*(6), 432-447.

Ratnasingham, S., & Hebert, P. D. (2007). bold: The Barcode of Life Data System (http://www.barcodinglife.org). *Molecular Ecology Notes*, *7*(3), 355-364.

Rothschild, B. J. (2000). “Fish stocks and recruitment”: the past thirty years. *ICES Journal of Marine Science*, *57*(2), 191-201.

Saitoh, K., Uehara, S., & Tega, T. (2009). Genetic identification of fish eggs collected in Sendai Bay and off Johban, Japan. *Ichthyological Research*, *56*, 200-203.

Sassa, C., Konishi, Y., & Mori, K. (2006). Distribution of jack mackerel (*Trachurus japonicus*) larvae and juveniles in the East China Sea, with special reference to the larval transport by the Kuroshio Current. *Fisheries Oceanography*, *15*(6), 508-518.

Saul, S., Walter III, J., Die, D., Naar, D., & Donahue, B. (2013). Modeling the spatial distribution of commercially important reef fishes on the West Florida Shelf. *Fisheries Research*, *143*, 12-20.

Sedberry, G. R., Pashuk, O., Wyanski, D., Stephen, J., & Weinbach, P. (2006). Spawning locations for Atlantic reef fishes off the southeastern US. *Proceedings of the Gulf and Caribbean Fisheries Institute*(57), 463–514.

Shao, K.-T., Chen, K.-C., & Wu, J.-H. (2002). Identification of marine fish eggs in Taiwan using light microscopy, scanning electric microscopy and mtDNA sequencing. *Marine and Freshwater Research*, *53*(2), 355-365.

Smith, G. B. (1975). The 1971 red tide and its impact on certain reef communities in the mid-eastern Gulf of Mexico. *Environmental Letters*, *9*(2), 141-152.

Stevens Jr, L., & Olsen, A. R. (1999). Spatially restricted surveys over time for aquatic resources. *Journal of Agricultural, Biological, and Environmental Statistics*, 415-428.

Switzer, T. S., Keenan, S. F., Thompson, K. A., Shea, C. P., Knapp, A. R., Campbell, M. D., Noble, B., Gardner, C., & Christman, M. C. (2023). Integrating assemblage structure and habitat mapping data into the design of a multispecies reef fish survey. *Marine and Coastal Fisheries*, *15*(4), e10245.

Tanaka, Y., & Franks, P. J. (2008). Vertical distributions of Japanese sardine (Sardinops melanostictus) eggs: comparison of observations and a wind-forced Lagrangian mixing model. *Fisheries Oceanography*, *17*(2), 89-100.

Thresher, R. E. (1984). *Reproduction in reef fishes*. T.F.H. Publications.

Truett, G. E., Heeger, P., Mynatt, R., Truett, A., Walker, J., & Warman, M. (2000). Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). *Biotechniques*, *29*(1), 52-54.

Turley, B. D., Karnauskas, M., Campbell, M. D., Hanisko, D. S., & Kelble, C. R. (2022). Relationships between blooms of *Karenia brevis* and hypoxia across the West Florida Shelf. *Harmful Algae*, *114*, 102223.

Walters, S., Lowerre-Barbieri, S., Bickford, J., Tustison, J., & Landsberg, J. H. (2013). Effects of Karenia brevis red tide on the spatial distribution of spawning aggregations of sand seatrout *Cynoscion arenarius* in Tampa Bay, Florida. *Marine Ecology Progress Series*, *479*, 191-202.

Ward, C. H., & Tunnell, J. W. (2017). Habitats and Biota of the Gulf of Mexico: An overview. In C. H. Ward (Ed.), *Habitats and Biota of the Gulf of Mexico: Before the Deepwater Horizon Oil Spill: Volume 1: Water Quality, Sediments, Sediment Contaminants, Oil and Gas Seeps, Coastal Habitats, Offshore Plankton and Benthos, and Shellfish* (pp. 1-54). Springer New York.

Weisberg, R. H., & He, R. (2003). Local and deep-ocean forcing contributions to anomalous water properties on the West Florida Shelf. *Journal of Geophysical Research: Oceans*, *108*(C6), 3184.

Weisberg, R. H., & Liu, Y. (2022). Local and deep-ocean forcing effects on the West Florida continental shelf circulation and ecology. *Frontiers in Marine Science*, *9*, 863227.

Wright, P. J., & Trippel, E. A. (2009). Fishery-induced demographic changes in the timing of spawning: consequences for reproductive success. *Fish and Fisheries*, *10*(3), 283-304.

Zhang, H., Jiang, C., & Li, Y. (2022). Biological and ecological studies on marine ichthyoplankton. *Frontiers in Marine Science*, *9*(948521).

Zheng, L., & Weisberg, R. H. (2012). Modeling the west Florida coastal ocean by downscaling from the deep ocean, across the continental shelf and into the estuaries. *Ocean Modelling*, *48*, 10-29.

APPENDIX A: TABLE OF ALL FISH EGG TAXA IDENTIFIED DURING THE STUDY

Table A1. Common names, families, known habitat of adults, economic importance, total number of specimens identified from eggs, and encounter frequency for all fish taxa identified (listed in alphabetical order). Habitat and economic importance information was gathered from FishBase (**http://www.fishbase.org/search.php**).

Legend: Bold = Federally managed taxa

X = Taxa that had at least one occurrence of 20 or more eggs being collected at a site.

Y = taxa that had at least one occurrence of 50 or more eggs being collected at a site.

Taxon	Family	Common Name	Habitat	Economic Importance	Total Number of Eggs Identified	Encounter Frequency (n = 218)
Acanthostracion quadricornis	Ostraciidae	Scrawled cowfish	Reef-associated	minor commercial; aquarium	2	2
Alectis ciliaris	Carangidae	African pompano	Reef-associated	minor commercial; gamefish; aquarium	3	2
Anisotremus virginicus/surinamensis	Haemulidae	Porkfish/Black margate	Reef-associated	minor commercial; gamefish; aquarium	2	1
Antigonia capros Y	Caproidae	Deepbody boarfish	Demersal	minor commercial	94	8
Antigonia combatia	Caproidae	Shortspine boarfish	Bathydemersal	minor commercial	9	1
Auxis thazard/rochei Y	Scombridae	Frigate tuna/Bullet tuna	Pelagic-neritic	highly commercial; gamefish	106	14
Bascanichthys scuticaris	Ophichthidae	Whip eel	Reef-associated	no	16	8
Calamus penna	Sparidae	Sheepshead porgy	Reef-associated	minor commercial	1	1
Callechelys guineensis	Ophichthidae	Shorttail snake eel	Demersal burrower	no	1	1
Chaetodon ocellatus	Chaetodontidae	Spotfin butterflyfish	Reef-associated	aquarium	1	1
Chloroscombrus chrysurus X	Carangidae	Atlantic bumper	Pelagic-neritic	commercial	44	6
Citharichthys macrops	Cyclopsettidae	Spotted whiff	Reef-associated	no	3	3

Table A1. (Continued)

Cubiceps baxteri/pauciradiatus	Nomeidae	Black fathead/Bigeye cigarfish	Pelagic-oceanic/bathypelagic	no	1	1
Cyclopsetta fimbriata	Cyclopsettidae	Spotfin flounder	Reef-associated	minor commercial	32	18
Cynoscion nothus/arenarius	Sciaenidae	Silver seatrout/Sand weakfish	Demersal	minor commercial/commercial	2	2
Decapterus punctatus/tabl [Y]	Carangidae	Round scad/Roughear scad	Reef-associated/Demersal	minor commercial; bait	1034	87
Diplectrum formosum	Serranidae	Sand perch	Reef-associated	commercial; gamefish	29	12
Echeneis/Remora sp.	Echeneidae	Remoras	Reef-associated	minor commercial	5	4
Echiophis intertinctus	Ophichthidae	Spotted spoon-nose eel	Demersal	no	1	1
Equetus/Pareques sp.	Sciaenidae	Drums (Jacknifes/Highats)	Reef-associated	minor commercial; aquarium	5	4
Etropus crossotus	Cyclopsettidae	Fringed flounder	Demersal	minor commercial	1	1
Eucinostomus sp.	Gerreidae	Mojarras	Reef-associated/Demersal	minor commercial	22	10
Euthynnus alletteratus [X]	Scombridae	Little tunny	Pelagic-oceanic	commercial; gamefish	44	10
Exocoetidae sp.	Exocoetidae	Flyingfish	Pelagic-neritic/Pelagic-oceanic	no/minor commercial; bait	2	2
Gordiichthys irretitus	Ophichthidae	Horsehair eel	Demersal	no	1	1
Gymnachirus sp.	Achiridae	Soles	Demersal	commercial	7	6
Gymnothorax saxicola	Muraenidae	Honeycomb moray	Demersal	no	1	1
Haemulon aurolineatum/striatum	Haemulidae	Tomtate grunt/Striped grunt	Reef-associated	minor commercial; aquarium	76	24
Haemulon plumierii	Haemulidae	White grunt	Reef-associated	minor commercial; gamefish; aquarium	18	10
Hemicaranx amblyrhynchus	Carangidae	Bluntnose jack	Pelagic-neritic	minor commercial	3	2
Heteroconger luteolus	Congridae	Yellow garden eel	Demersal burrower	no	1	1
Hyporhamphus unifasciatus	Hemiramphidae	Common halfbeak	Reef-associated	minor commercial; bait	1	1
Hyporthodus flavolimbatus	Serranidae	Yellowedge grouper	Demersal	commercial; gamefish	24	7
Istiophorus platypterus	Istiophoridae	Sailfish	Pelagic-oceanic	commercial; gamefish	3	3
Katsuwonus pelamis	Scombridae	Skipjack tuna	Pelagic-oceanic	highly commercial; gamefish	30	8
Katsuwonus pelamis*/Thunnus atlanticus*	Scombridae	Skipjack tuna/Blackfin tuna	Pelagic-oceanic	highly commercial; gamefish	4	1

Table A1. (Continued)

Lepophidium brevibarbe/Ophidion holbrooki X	Ophidiidae	Shortbeard/Band cusk-eel	Demersal/Reef-associated	minor commercial	28	1
Letharchus velifer	Ophicthidae	Sailfin eel	Demersal	no	19	11
Liopropoma eukrines	Serranidae	Wrasse bass	Reef-associated	no	1	1
Lobotes surinamensis	Lobotidae	Tripletail	Pelagic	commercial; gamefish; aquarium	1	1
Lutjanus analis	Lutjanidae	Mutton snapper	Reef-associated	highly commercial; gamefish; aquarium	10	2
Lutjanus campechanus	Lutjanidae	northern red snapper	Reef-associated	commercial; gamefish	70	22
Lutjanus griseus	Lutjanidae	Grey snapper	Reef-associated	commercial; gamefish; aquarium	2	2
Lutjanus synagris	Lutjanidae	Lane snapper	Reef-associated	commercial; gamefish; aquarium	20	12
Malacanthus plumieri	Malacanthidae	Sand tilefish	Reef-associated	minor commercial	1	1
Micropogonias undulatus	Sciaenidae	Atlantic croaker	Demersal	commercial; aquaculture; gamefish	10	4
Myrophis punctatus	Ophichthidae	Speckled worm-eel	Reef-associated	commercial; bait	2	2
Ocyurus chrysurus	Lutjanidae	Yellowtail snapper	Reef-associated	commercial; aquaculture; gamefish; aquarium	1	1
Ophidion selenops	Ophidiidae	Mooneye cusk-eel	Reef-associated	no	3	2
Opisthonema oglinum	Clupeidae	Atlantic thread herring	Reef-associated	commercial; bait	1	1
Otophidium omostigma	Ophidiidae	Polka-dot cusk-eel	Demersal	no	2	1
Paraconger caudilimbatus	Congridae	Margintail conger	Reef-associated burrower	no	1	1
Parahollardia lineata	Triacanthodidae	Jambeau	Demersal	no	1	1
Pareques iwamotoi	Sciaenidae	Blackbar drum	Demersal	no	1	1
Peprilus paru	Stromateidae	American harvestfish	Benthopelagic	minor commercial	30	13
Peprilus triacanthus/burti X	Stromateidae	Atlantic butterfish/Gulf butterfish	Benthopelagic	commercial; gamefish/no	213	16
Peristedion gracile	Triglidae	Slender searobin	Bathydemersal	no	2	1
Pomacanthus arcuatus	Pomacanthidae	Gray angelfish	Reef-associated	minor commercial; aquarium	2	2
Pomatomus saltatrix	Pomotomidae	Bluefish	Pelagic-oceanic	highly commercial; aquaculture; gamefish; bait	6	2
Priacanthus arenatus	Priacanthidae	Atlantic bigeye	Reef-associated	minor commercial; gamefish; aquarium	8	4
Prionotus sp. X	Triglidae	Searobins	Demersal	no	557	71
Pristipomoides aquilonaris X	Lutjanidae	Wenchman	Demersal	minor commercial	146	23

Table A1. (Continued)

Prognichthys occidentalis	Exoceotidae	Western bluntnose flyingfish	Pelagic-neritic	no	2	2
Pseudupeneus maculatus	Mullidae	Spotted goatfish	Reef-associated	commercial; aquarium	1	1
Pterois sp.	Scorpaenidae	Lionfishes	Reef-associated	Invasive	1	1
Rhomboplites aurorubens	Lutjanidae	Vermillion snapper	Demersal	minor commercial	122	33
Rypticus bistrispinus	Serranidae	Freckled soapfish	Reef-associated	no	1	1
Rypticus saponaceus/maculatus [X]	Serranidae	Greater soapfish/Whitespotted soapfish	Reef-associated/Demersal	subsistence; aquarium/no	84	18
Sardinella aurita [X]	Clupeidae	Round sardinella	Pelagic-neritic	highly commercial; bait	53	10
Saurida sp.	Synodontidae	Lizardfishes	Reef-associated/Demersal	no	3	2
Sciaenops ocellatus [Y]	Sciaenidae	Red drum	Demersal	minor commercial; aquaculture; gamefish; aquarium	87	3
Scomberomorus cavalla	Scombridae	King mackerel	Pelagic-neritic	commercial; gamefish	68	19
Scomberomorus cavalla/Acanyhocybium solandri	Scombridae	King mackerel/Wahoo	Pelagic-neritic/Pelagic-oceanic	commercial; gamefish	15	6
Scomberomorus maculatus [X]	Scombridae	Atlantic Spanish mackerel	Pelagic-neritic/Reef-associated	highly commercial; gamefish/minor commercial; gamefish	60	13
Selar crumenophthalmus	Carangidae	Bigeye scad	Reef-associated	highly commercial; bait	7	6
Selene setapinnis/vomer	Caranngidae	Atlantic moonfish/Lookdown	Benthopelagic/Demersal	highly commercial; aquarium/minor commercial; gamefish; aquarium	13	4
Serraniculus pumilio	Serranidae	Pygmy sea bass	Reef-associated	no	11	10
Syacium papillosum/gunteri	Cyclopsettidae	Dusky flounder	Reef-associated	minor commercial	271	56
Symphurus diomedeanus	Cynoglossidae	Spotted tonguefish	Reef-associated	no	1	1
Symphurus plagiusa	Cynoglossidae	Blackcheek tonguefish	Demersal	minor commercial	1	1
Synodus sp. [X]	Synodontidae	Lizardfishes	Reef-associated/Demersal	no	256	67
Synodus poeyi/Saurida normani	Synodontidae	Offshore lizardfish/Shortjaw lizardfish	Reef-associated	no	3	2
Thunnus spp.	Scombridae	Tunas	Pelagic-oceanic	highly commercial; gamefish	23	11

Table A1. (Continued)

Trachinocephalus myops	Synodontidae	Snakefish	Reef-associated	minor commercial	116	40
Xyrichtys novacula	Labridae	Pearly razorfish	Reef-associated	minor commercial; aquarium	4	3

APPENDIX B: EGG DISTRIBUTION MAPS OF FEDERALLY MANAGED TAXA

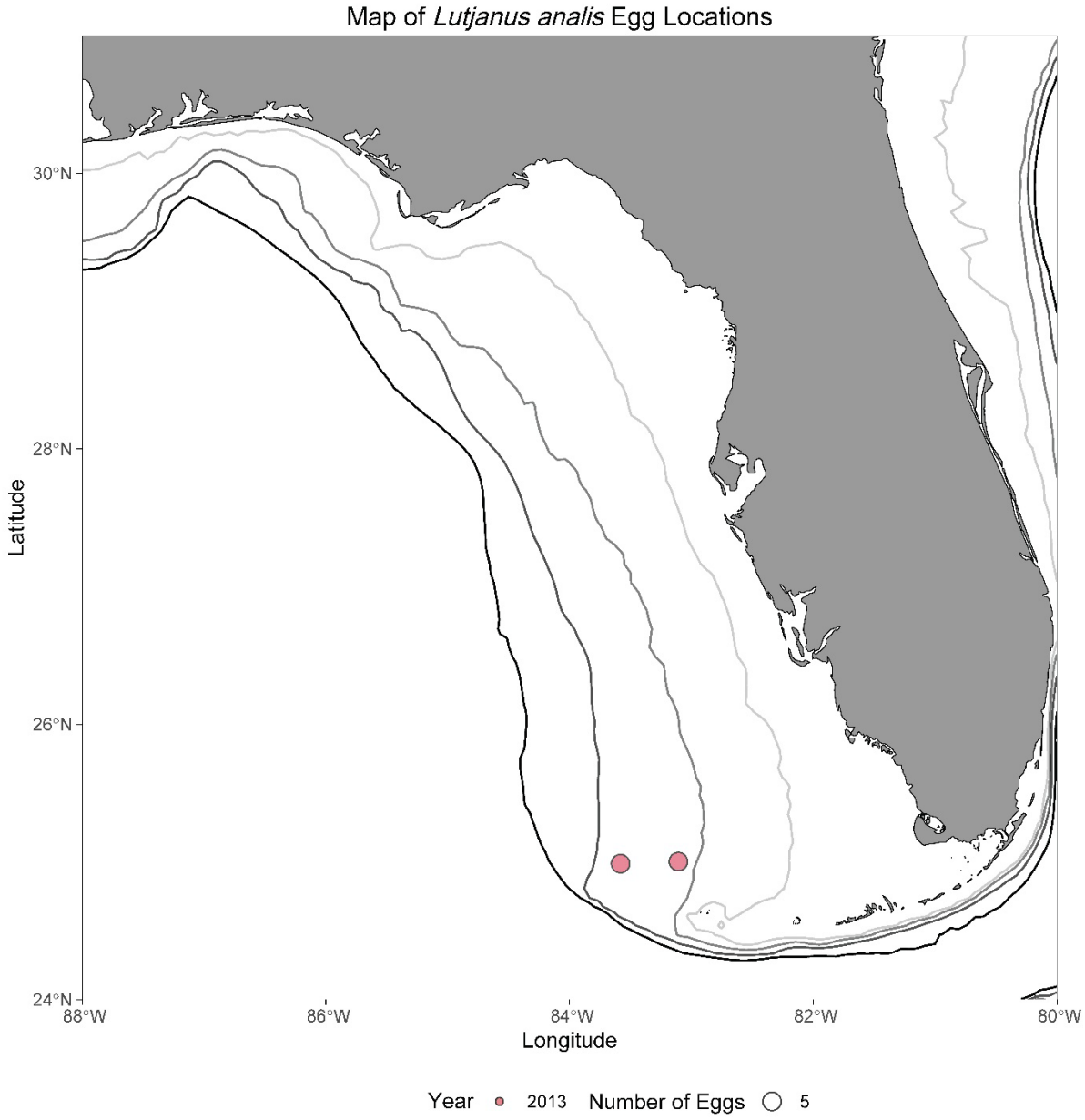

Figure B1. Map of sites where mutton snapper eggs were collected. Grey and black lines represent 25 m, 50 m, 100 m, and 200 m isobaths.

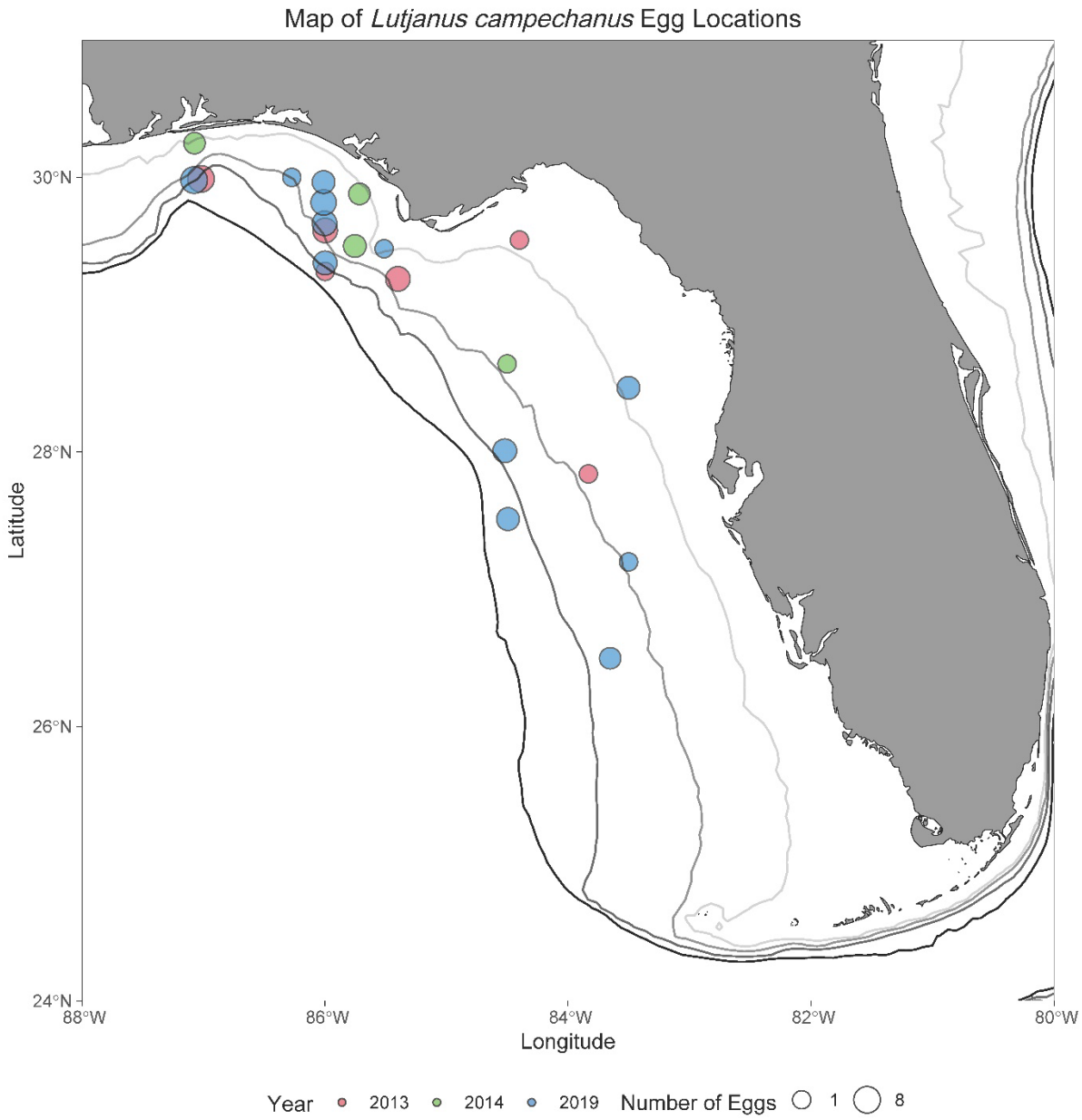

Figure B2. Map of sites where red snapper eggs were collected. Grey and black lines represent 25 m, 50 m, 100 m, and 200 m isobaths.

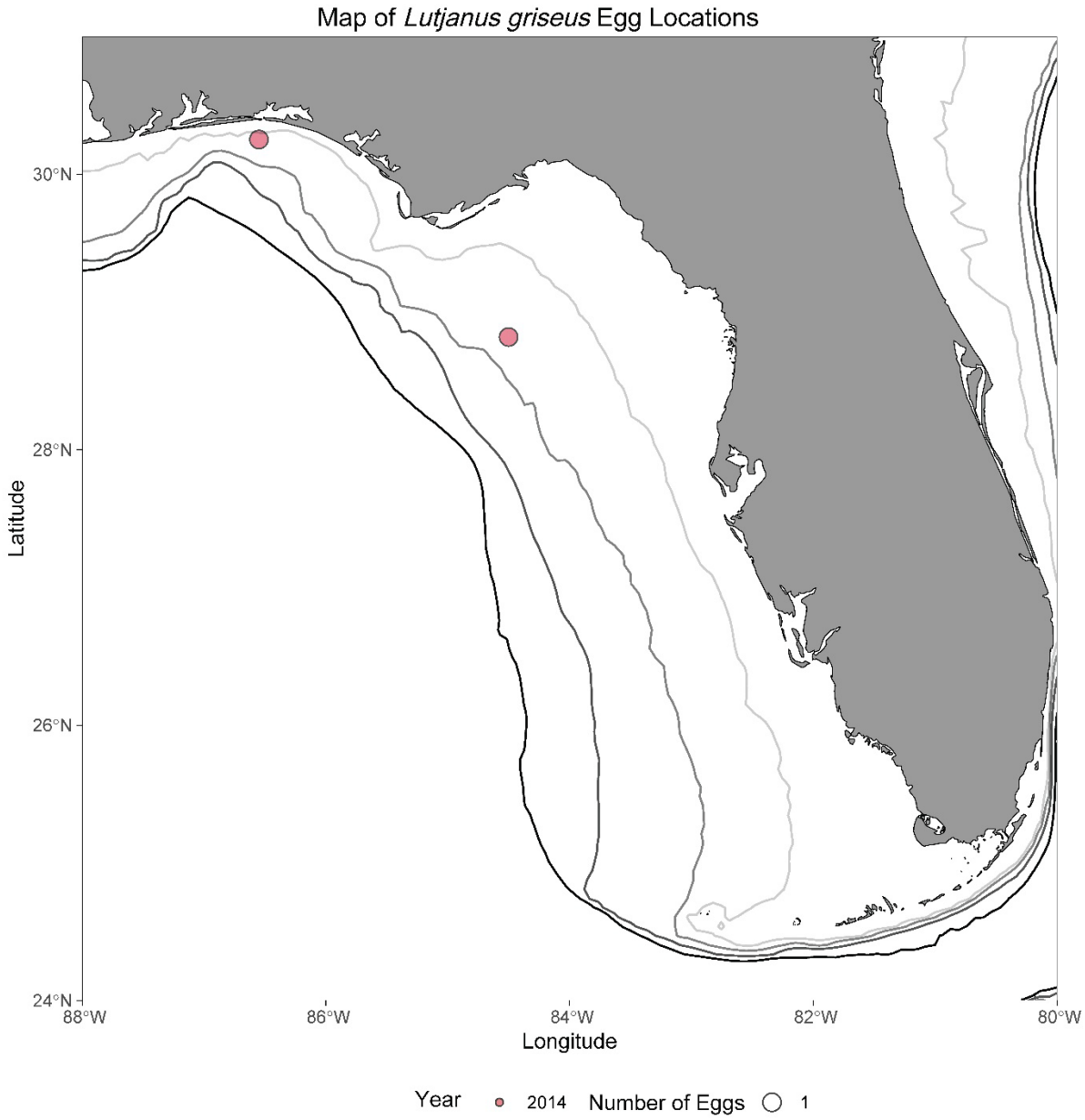

Figure B3. Map of sites where grey snapper eggs were collected. Grey and black lines represent 25 m, 50 m, 100 m, and 200 m isobaths.

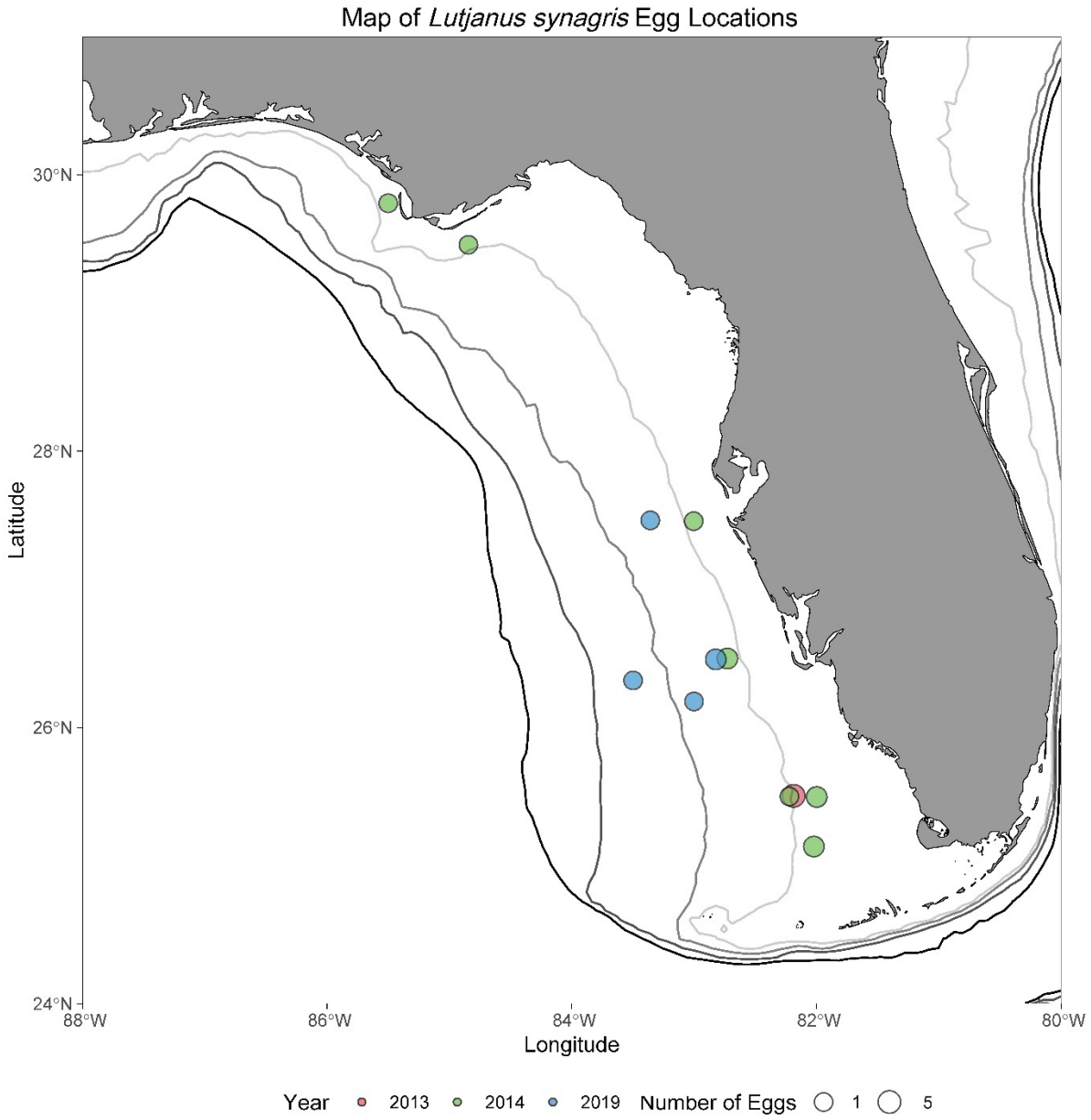

Figure B4. Map of sites where lane snapper eggs were collected. Grey and black lines represent 25 m, 50 m, 100 m, and 200 m isobaths.

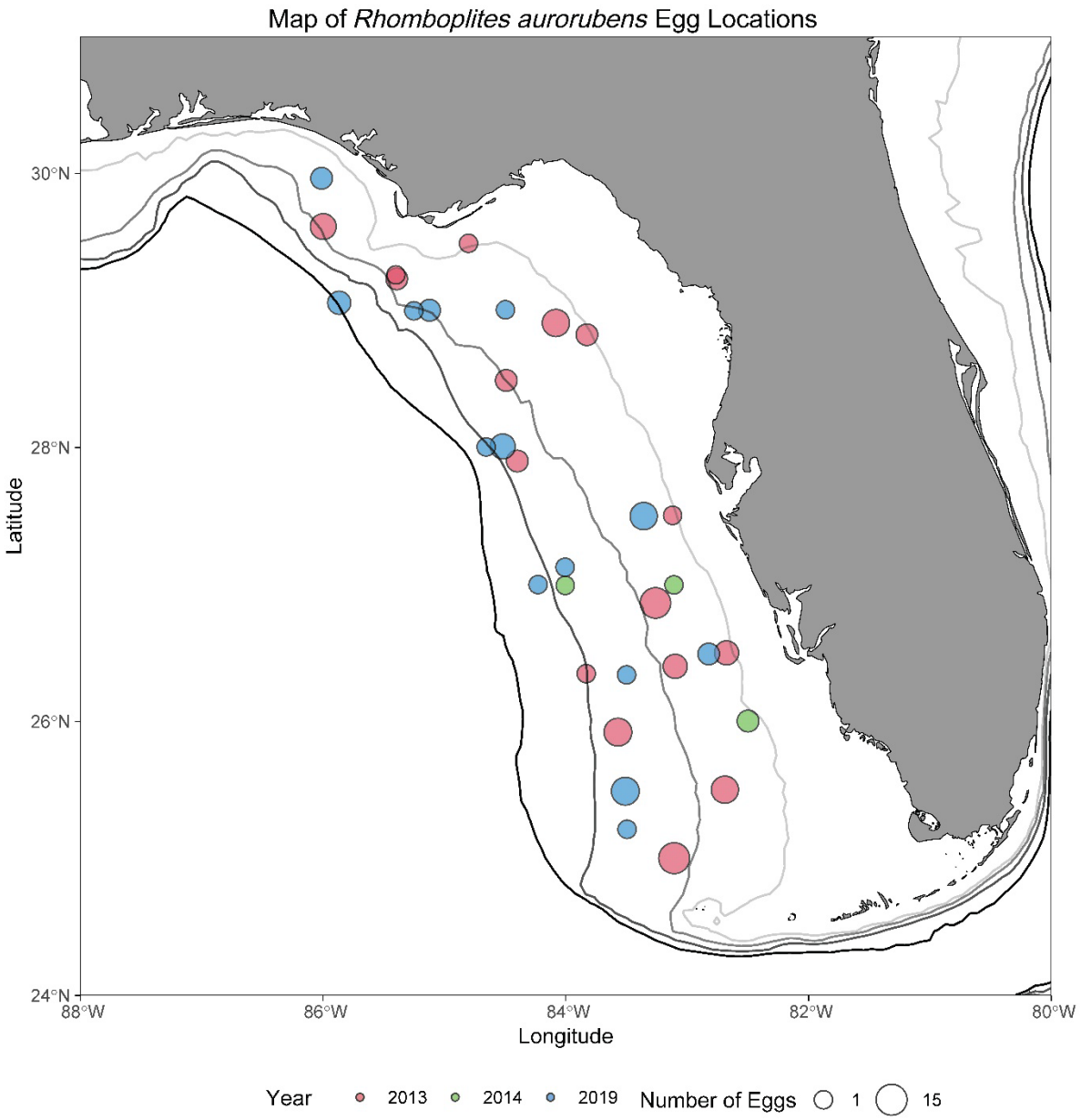

Figure B5. Map of sites where vermillion snapper eggs were collected. Grey and black lines represent 25 m, 50 m, 100 m, and 200 m isobaths.

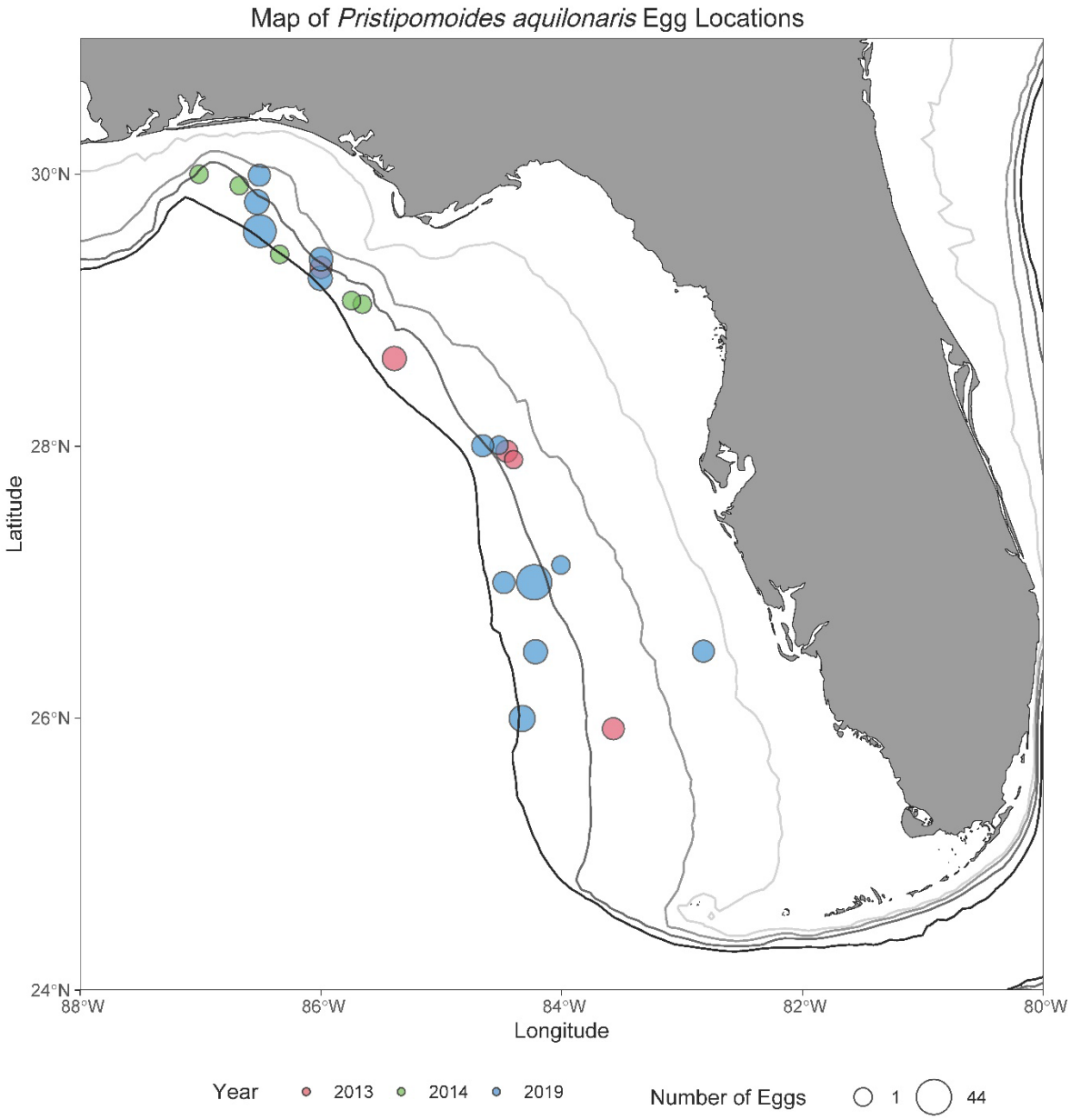

Figure B6. Map of sites where wenchman eggs were collected. Grey and black lines represent 25 m, 50 m, 100 m, and 200 m isobaths.

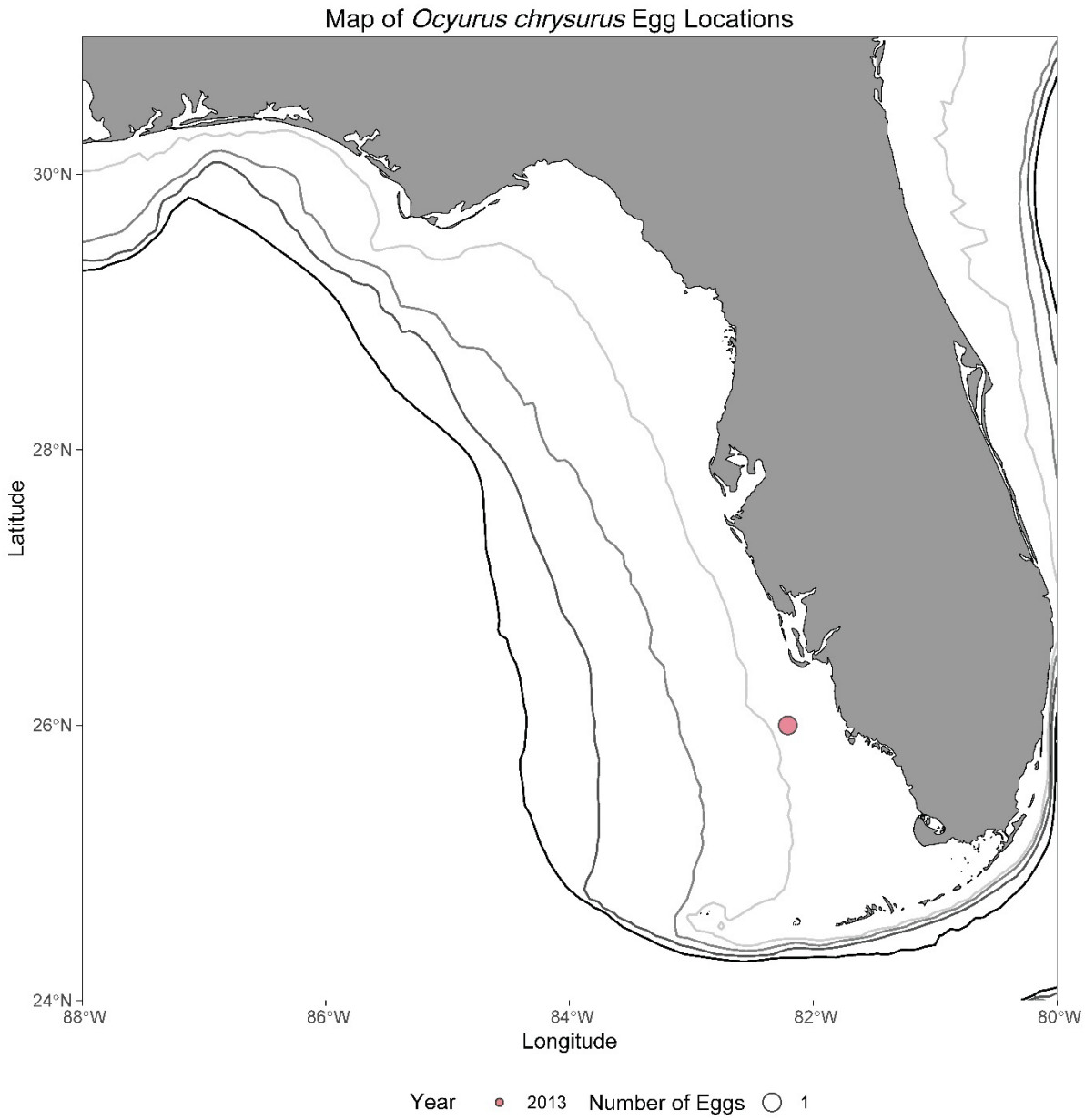

Figure B7. Map of sites where yellowtail snapper eggs were collected. Grey and black lines represent 25 m, 50 m, 100 m, and 200 m isobaths.

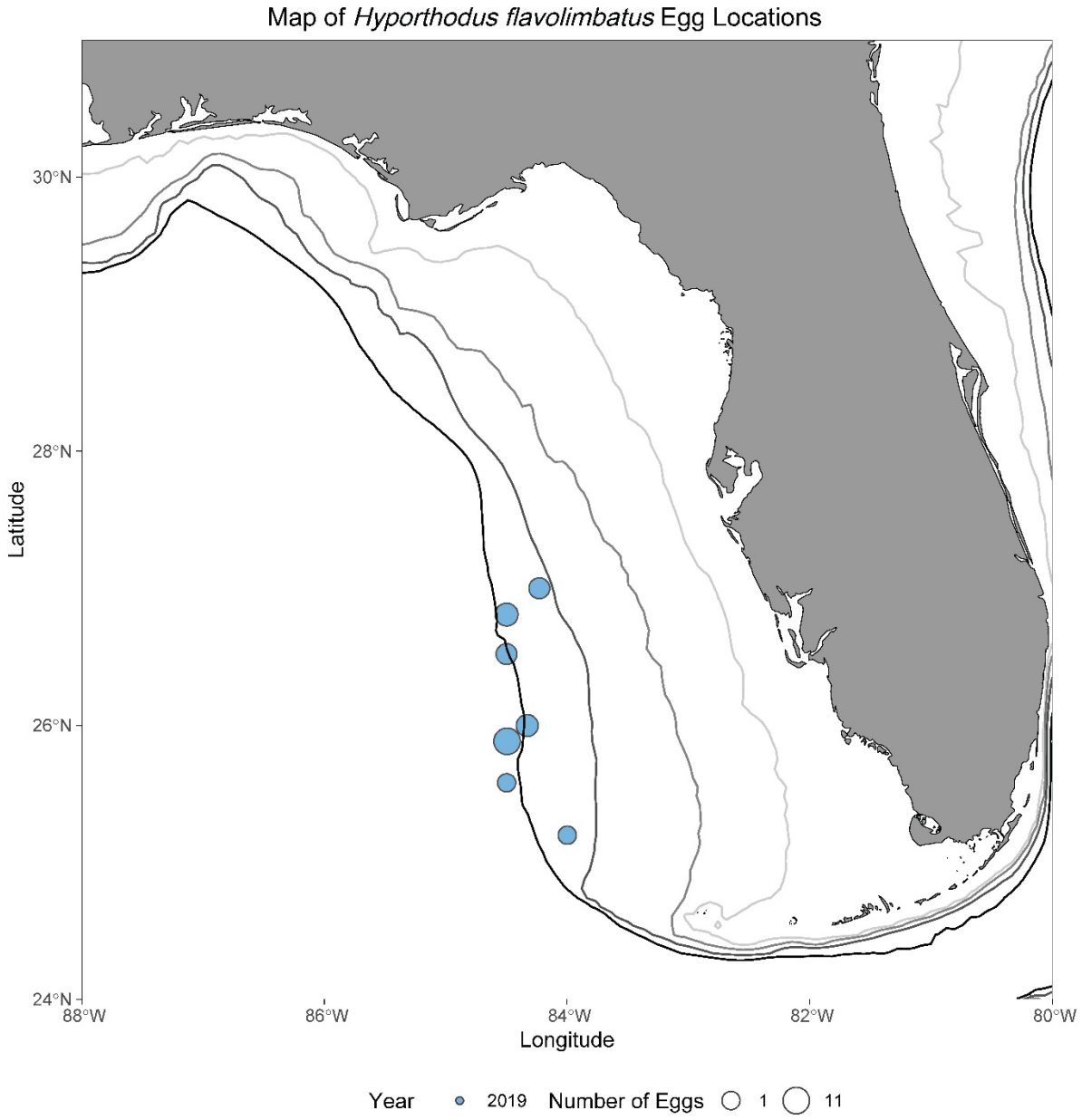

Figure B8. Map of sites where yellowedge grouper eggs were collected. Grey and black lines represent 25 m, 50 m, 100 m, and 200 m isobaths.

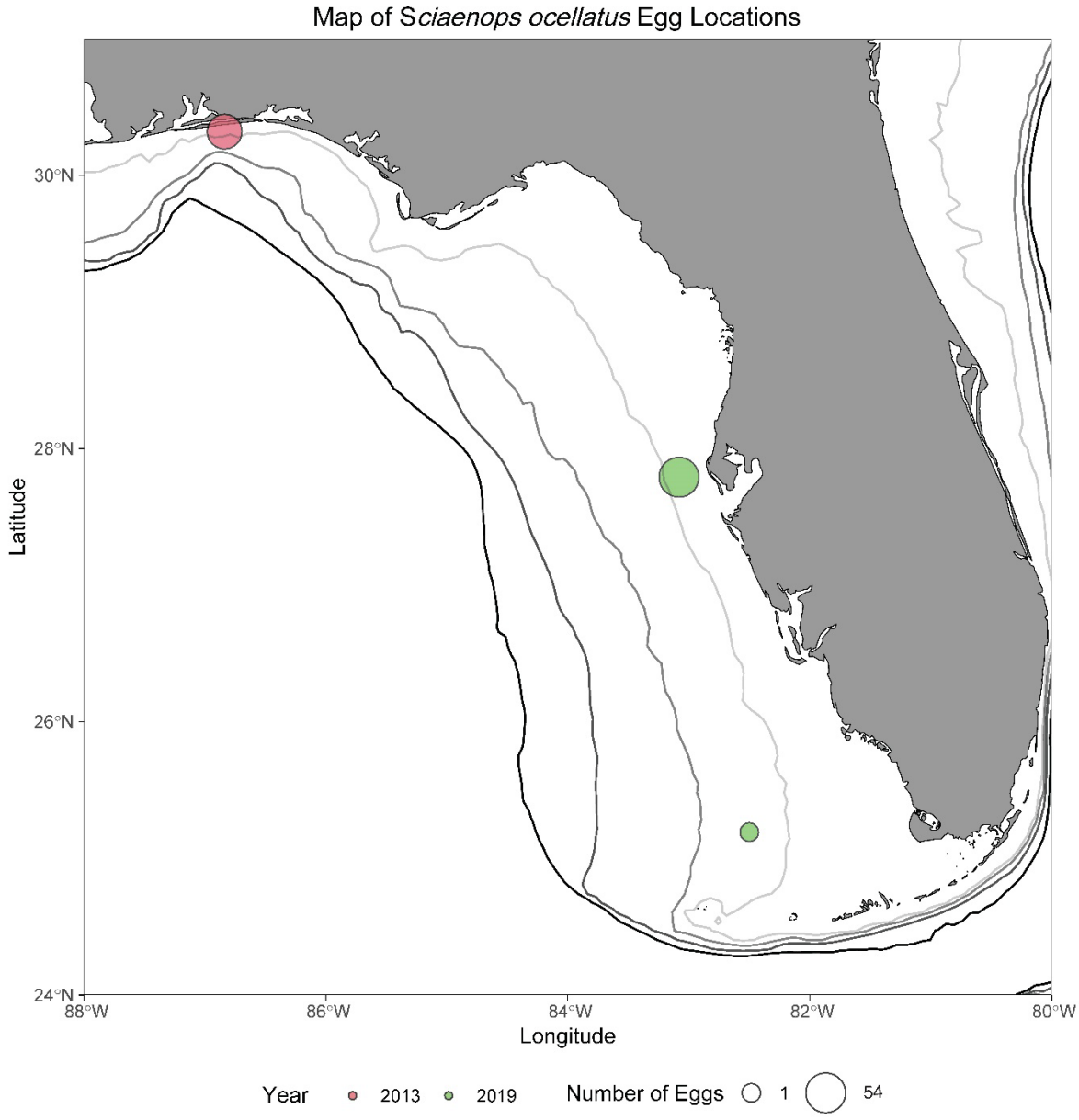

Figure B9. Map of sites where red drum eggs were collected. Grey and black lines represent 25 m, 50 m, 100 m, and 200 m isobaths.

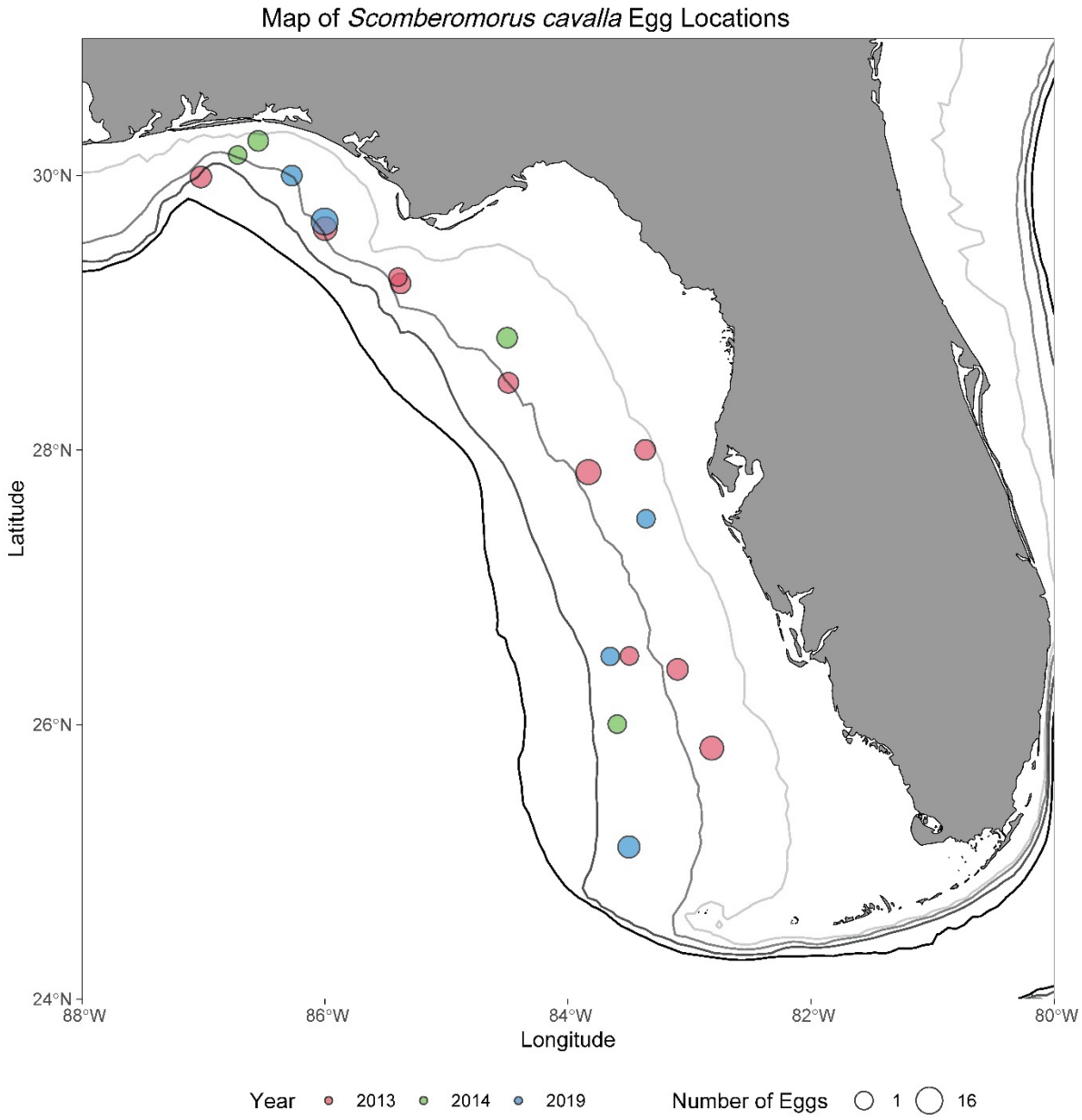

Figure B10. Map of sites where king mackerel eggs were collected. Grey and black lines represent 25 m, 50 m, 100 m, and 200 m isobaths.

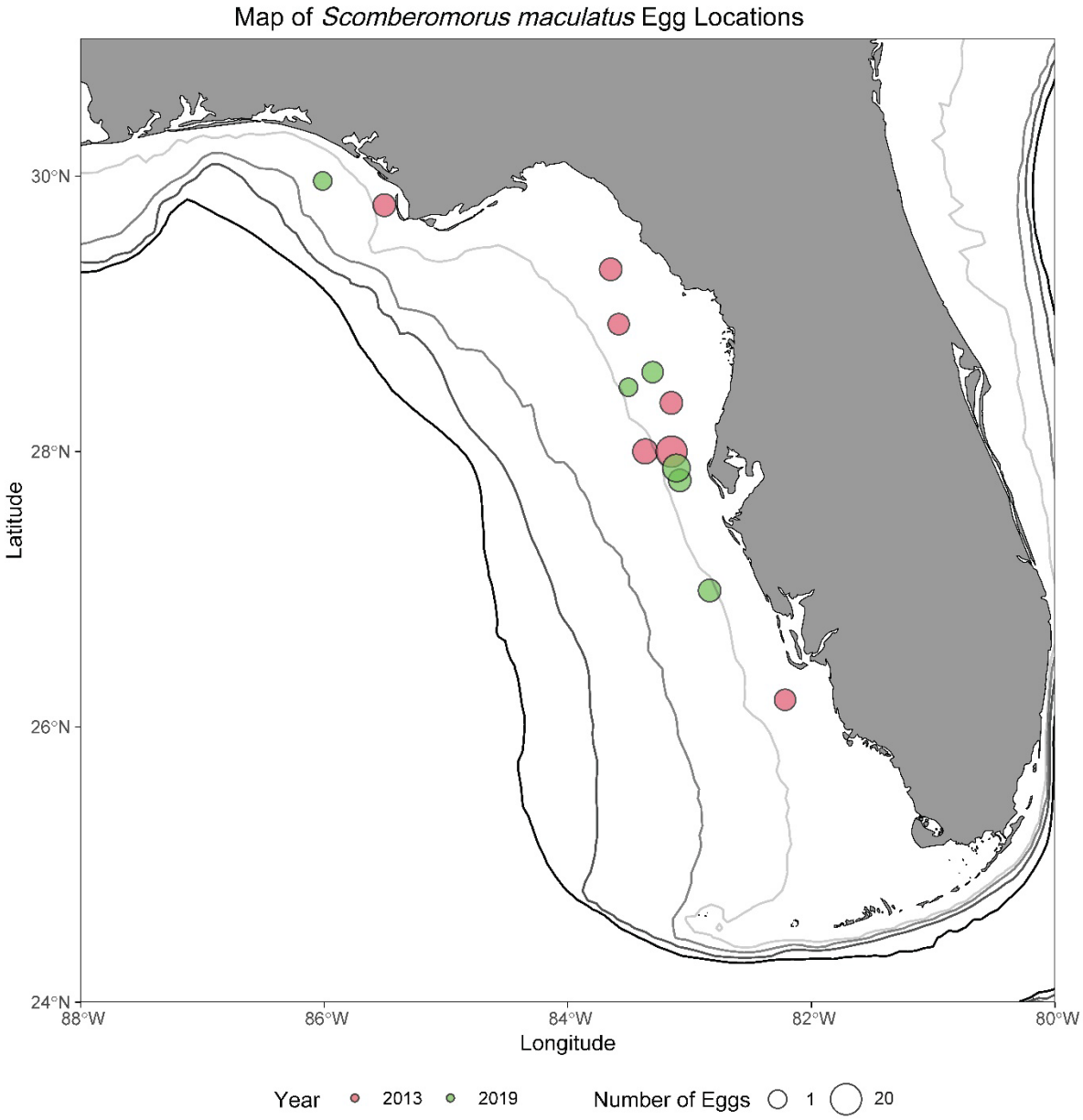

Figure B11. Map of sites where Atlantic Spanish mackerel eggs were collected. Grey and black lines represent 25 m, 50 m, 100 m, and 200 m isobaths.

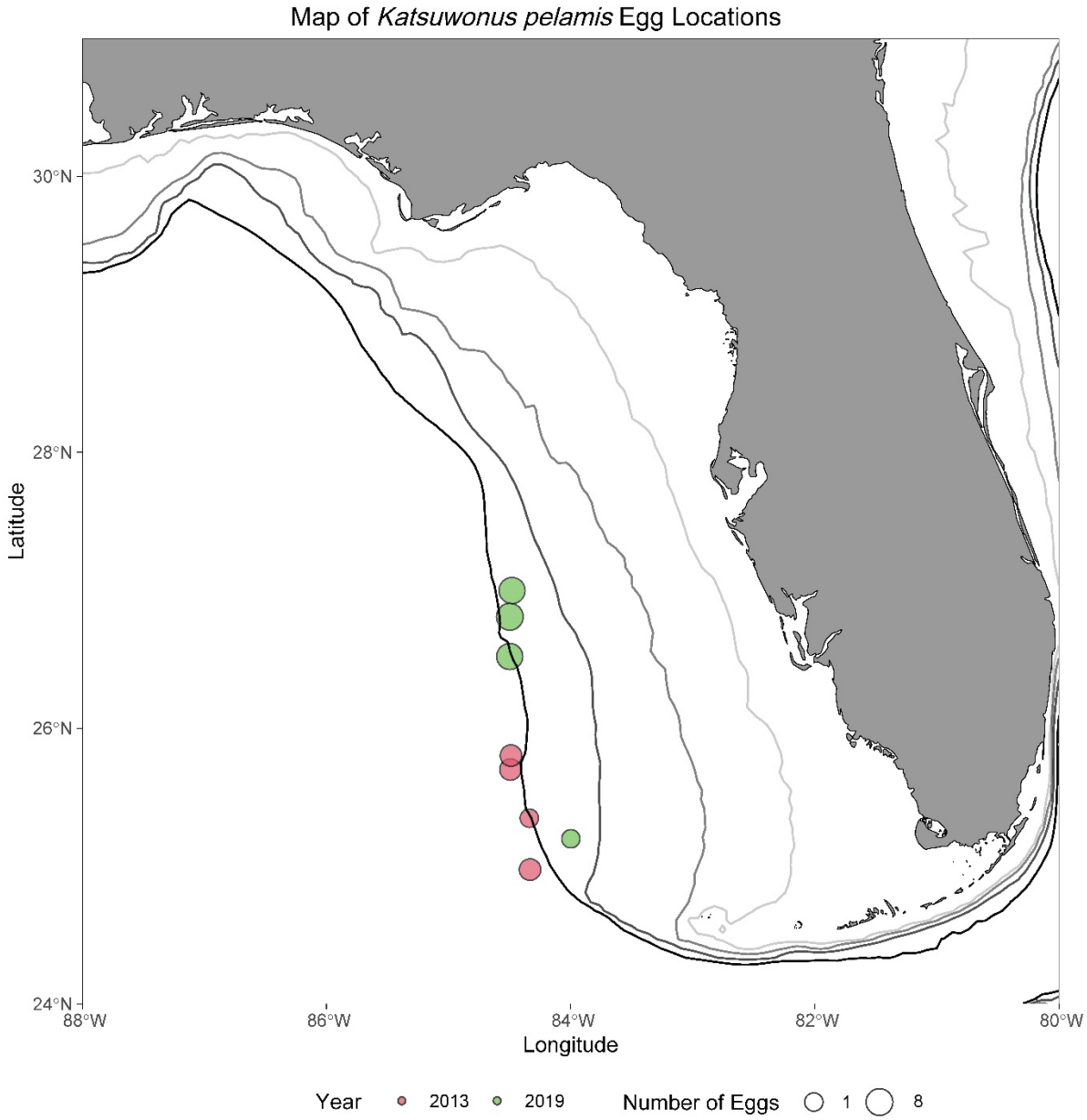

Figure B12. Map of sites where skipjack tuna eggs were collected. Grey and black lines represent 25 m, 50 m, 100 m, and 200 m isobaths.

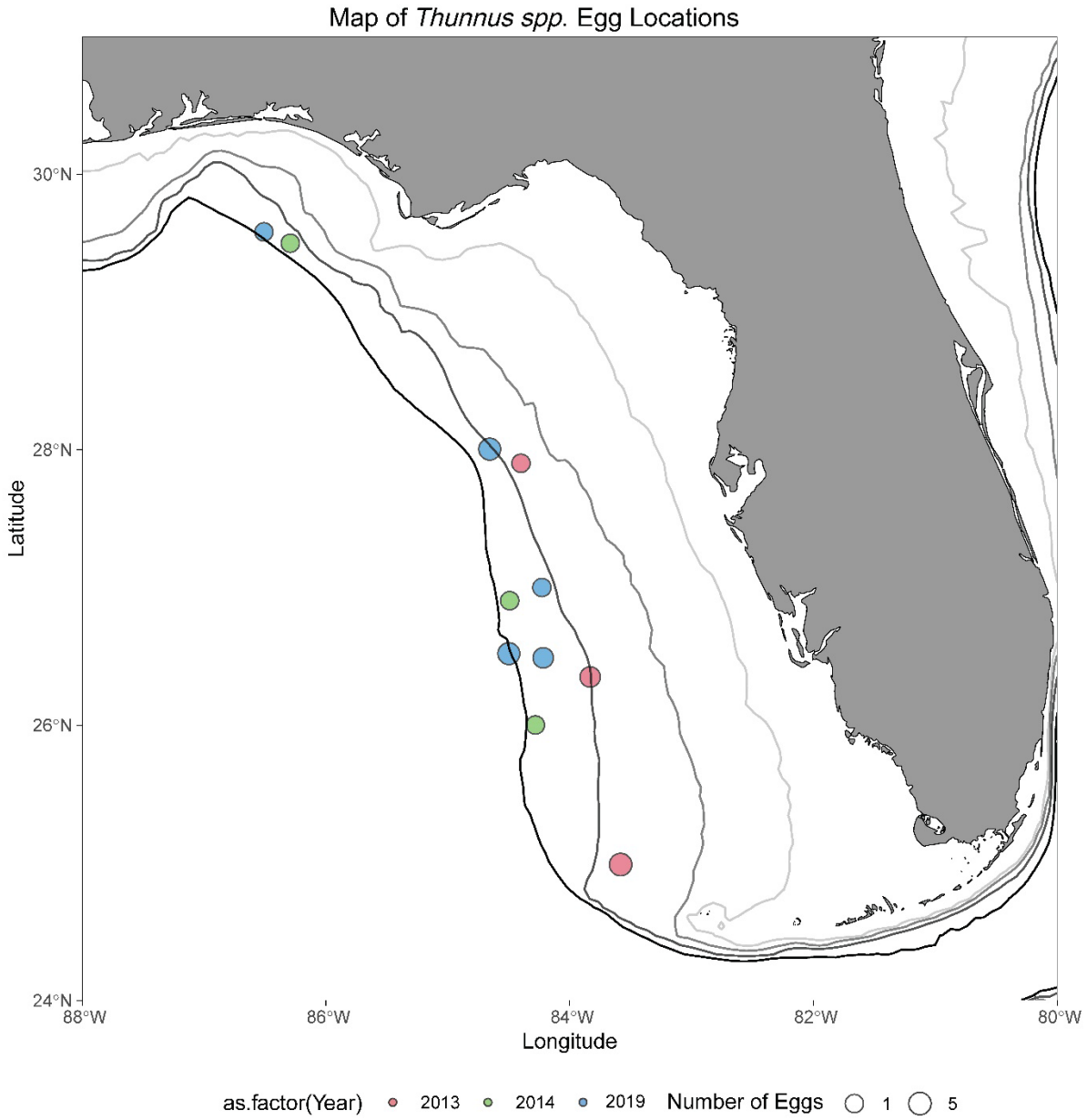

Figure B13. Map of sites where *Thunnus* spp. eggs were collected. Grey and black lines represent 25 m, 50 m, 100 m, and 200 m isobaths.

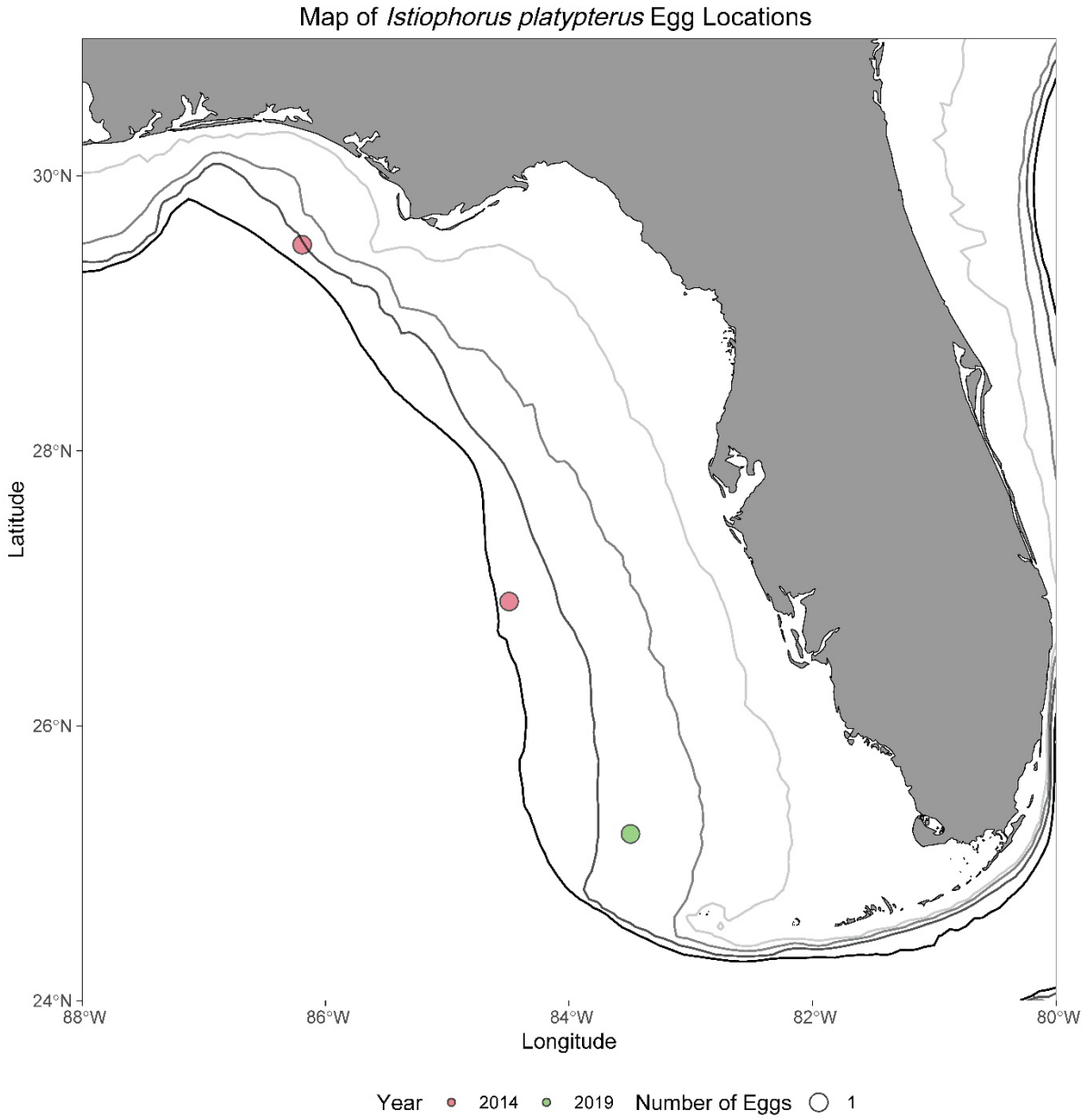

Figure B14. Map of sites where sailfish eggs were collected. Grey and black lines represent 25 m, 50 m, 100 m, and 200 m isobaths.