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Investigation of Retention Versus Export of Planktonic Fish Eggs in the Northeastern Gulf of Mexico

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Investigation of Retention Versus Export of Planktonic Fish Eggs in the Northeastern Gulf of Mexico

by

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ABSTRACT

Planktonic fish eggs can be reliably identified with DNA barcoding, and their distribution and abundance can be monitored. Passive drifting fish eggs can be advected by ocean currents and as a result, can either be locally retained or exported away from the West Florida Shelf (WFS). Investigating their retention or export helps in the interpretation of egg abundance trends and in understanding their distribution in long-term surveys. The present investigation was performed in two steps using a combination of biological and physical oceanographic methods. First, fish fecundity of three species (Red Snapper, Vermilion Snapper, and Sand Perch) was assessed, first, through hydrated egg counts leading to batch fecundity estimates, and second, through histological analysis of post-ovulatory follicles. Both approaches were used to find evidence of imminent or recent spawning and the results allowed the identification of spawning sites. In the second step, coordinates of the spawning and fish-egg collection sites were put into the West Florida Coastal Ocean Model (WFCOM). Trajectories of the drifting eggs were then simulated over two weeks at three depths (surface, mid-water, and bottom). The results indicated that there were two groups of trajectories: one nearshore group indicating retention and one offshore group resulting in long-distance dispersal and potential export. In addition, the nearshore stations were associated with higher fish-egg abundance. This study also found evidence of a relationship between retention and higher fish-egg abundance. This was hypothesized to be representative of increased spawning in those areas, increased drift convergence, or both processes acting together. Batch fecundity appeared to be higher nearshore, although no relationship between batch fecundity and retention was found. After fish eggs were

identified to species via DNA barcoding, a community analysis using SIMPROF was performed and indicated the presence of a depth-related structure in the community. Fish eggs species were also categorized as pelagics or non-pelagics. No evidence of whether pelagic species are more likely to be exported was found. This study is a starting point for the creation of an index of egg abundance that will be fine-tuned over time with recurrent surveys. Ultimately, the index can be useful to fisheries management.

INTRODUCTION

OBJECTIVES

Advances in DNA barcoding have allowed monitoring of planktonic fish eggs that previously could not be reliably identified (e.g., Burghart et al. 2014, Burrows et al., 2018). As part of the Florida Restore Act Centers of Excellence Program (FLRACEP), planktonic fish-egg distributions on the entire West Florida Shelf (WFS) will be monitored annually for a period of up to 15 years.

Egg distributions observed by the FLRACEP egg-monitoring program can potentially be modified by subsequent, variable egg retention on the WFS after spawning, which would interfere with the use of data from the egg-survey as a fisheries management index. Thus, the purpose of the present effort is to develop preliminary methods for investigating egg retention on the WFS. More specifically, the primary objective is to determine whether planktonic eggs are being retained on the WFS or exported away from it by local currents. The first part of this process is to definitively locate fish spawning sites in order to provide a basis for initial geographic drift coordinates. The second part is to use numerical models to simulate the drift of planktonic fish eggs and early larvae after spawning has occurred at these locations.

Spawning sites were identified using two methods. First, locations where spawning females are present were identified. This required detection of impending spawning by individual females through a combination of microscope-based egg staging and histological

examination of ovaries. As part of this effort, the relative intensity of spawning was also represented by the number of eggs that were pending release (i.e., determination of batch fecundity).

A second method was provided by detection of drifting fish eggs after spawning, which was only possible via DNA barcoding; note that the barcoding process itself was being conducted as part of other tasks within the FLRACEP project, and its methods were outside the present scope of work. Although detection of the drifting eggs of a given species provided definitive evidence that spawning has occurred, advection caused an unknown spatial offset between spawning and subsequent collection of the drifting eggs.

One advantage of the second approach was that it was a more practical means of comparing the egg trajectories of different species. The first method was more logistically difficult and time-consuming to implement and could only be applied to a few species with the resources available to us. The reason for comparing species was to determine whether certain types of fish were more likely to have their eggs retained on the WFS than others. For example, it might be expected that pelagic species such as tunas would be less adapted to retention than reef-related fishes such as snappers.

To accomplish these objectives, the following null hypotheses were investigated in this study:

1. Fish eggs and larvae on the WFS are not likely to be exported.
2. Sites with higher batch fecundity are not more likely to result in retention than sites with lower batch fecundity.
3. Sites with higher fish-egg abundance are not more likely to result in retention than sites with lower egg abundance.

4. Eggs and larvae of pelagic fish species are not more likely to be exported away from the WFS than eggs and larvae of non-pelagic species.
5. There is no depth related community structure in fish eggs on the WFS.

REGION OF INTEREST

This study focused on the northern West Florida Shelf, which was surveyed as part of a pilot study of the FLRACEP egg-monitoring project (Figure 1). The WFS is a large continental shelf in the eastern Gulf of Mexico (GOM) with a width from 25 to 250 km and a length of about 900 km. It contains a variety of bottom features, including open sand, hard bottoms, and low-relief, exposed rock ledges and paleoshorelines (Hine and Locker 2011).

The Loop Current is the large-scale circulation feature that dominates the GOM beyond the continental shelf (Hurlburt and Thompson 1980; Ohlmann and Niiler 2005; Romanou et al. 2004). It is a deep ocean current that enters the GOM through the Yucatan Channel, flows northward to various extents at various times, and exits through the Florida Straits between Cuba and the Florida Keys (Ohlmann and Niiler 2005; Romanou et al. 2004; Vukovich et al. 1979). The Loop Current does not pass directly over the WFS, yet it influences the circulation on the WFS (Hine and Locker 2011; Weisberg et al. 2005; Weisberg and He 2003).

The circulation on the WFS is driven by multiple physical features. It is mainly influenced by winds, and also by the interaction between the Loop Current and the shelf slope (Weisberg et al. 2014). The long-term mean circulation pattern is upwelling with seasonality and interannual variability (Liu and Weisberg 2012). Additionally, in shallow waters, the circulation in the inner shelf is mainly driven by wind forcings and is more subject to seasonal variations.

More specifically, in the summer, the southerly winds drive the current in shallow water and create a downwelling-favorable system. From fall to spring, the northerly winds generate an upwelling system (Liu and Weisberg 2012). The outer-shelf circulation is mainly influenced by the Loop Current, its eddies, and their interaction with the shelf slope; it is less likely to vary seasonally and rather to vary with the flow variations of the Loop Current. Along with the generalized seasonal variability, there are instances when Loop Current interactions with the shelf slope near the Dry Tortugas can set the entire shelf in an upwelling circulation (Weisberg and He, 2003; Liu et al., 2016a) profoundly influencing shelf ecology.

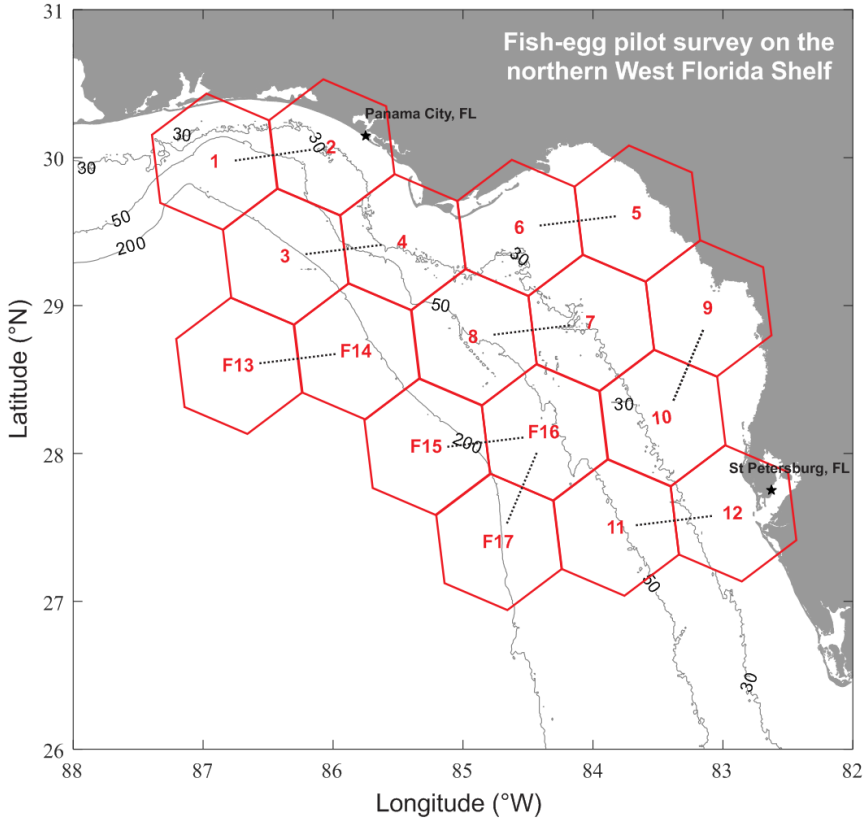

Figure 1. Location of drifting-egg collection stations for the pilot study for the egg-monitoring project. Boats operating out of Panama City and Indian Shores were used. Each hexagon represents the area in which boats had to sample. The dashed lines are cruise tracks between two stations (they are not transects with multiple stations). Stations starting with an F indicated research vessels from FIO.

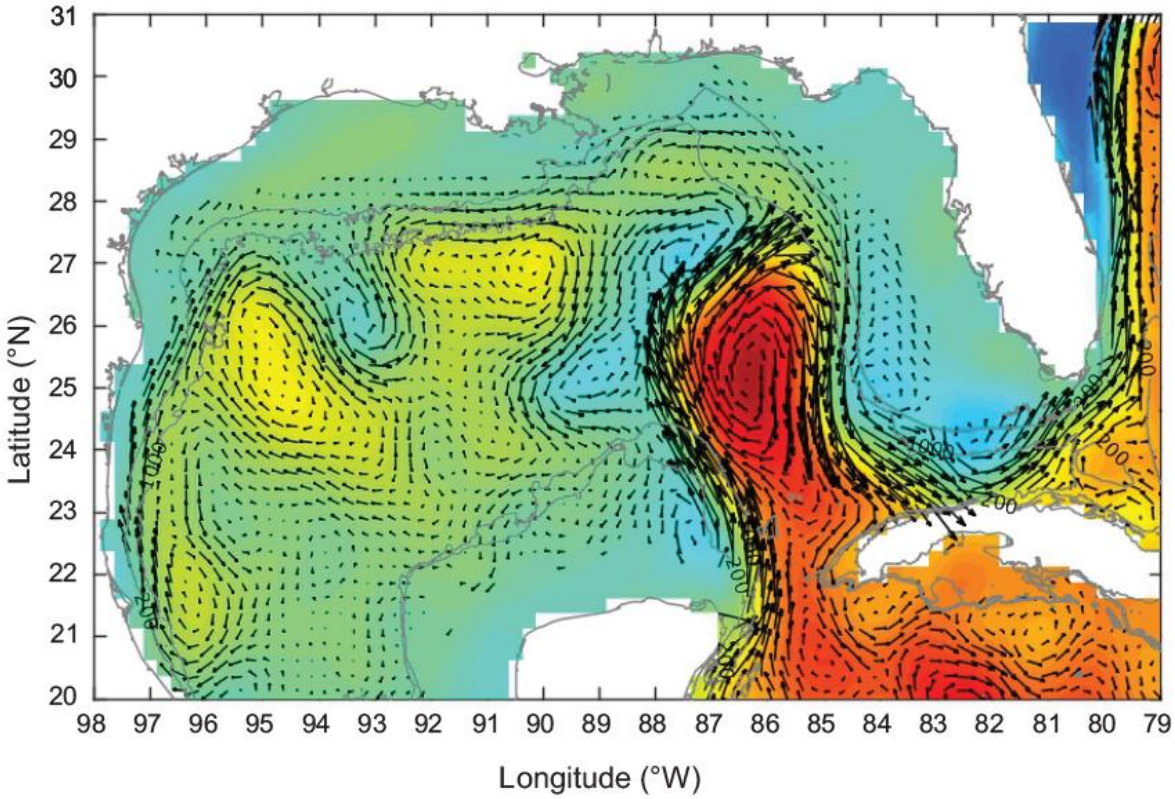

Figure 2. Altimetry-derived surface geostrophic currents showing the GOM Loop Current intrusion onto the outer shelf during July 2017. The altimetry product is generated following a procedure described in Liu et al. (2016a), Liu et al. (2016b), and Weisberg and Liu (2017).

CHAPTER 1 ABSTRACT

Knowing where fish spawn is valuable information for tracking the movement of their eggs and larvae. On July 11, 2017, six commercial and two research vessels synoptically fished on the northern West Florida Shelf. Three species (Red Snapper, Vermilion Snapper, and Sand Perch) were retained for this study. Spawning sites on the WFS were identified via two approaches. The first approach consisted of finding females that were hours from spawning by macroscopically examining their ovaries for the presence of hydrated oocytes. Oocytes go through a hydration process in the hours that precede a spawning event and their presence indicate the location of a spawning site. The second approach consisted in finding females that have recently spawned by analyzing the histology of ovary tissue for the presence of recent post-ovulatory follicles (POFs). POFs are envelopes of cells that remain in the ovary after hydrated eggs are released during spawning. The histological method allowed identification and aging of those POFs and therefore indicated the location of a spawning site. In the process, the batch fecundity (e.g., number of eggs produced by a batch spawner during one spawning event) for each species of interest was estimated using a volumetric method for hydrated-egg count. Results of batch fecundity (number of hydrated eggs per batch ± standard error) are as follows: 101,293 ± 55,787 for Vermilion Snapper, 75,119 ± 19,802 for Red Snapper, and 8,272 ± 1,869 for Sand Perch. The combination of macroscopic examination for hydrated eggs and the histological observation of ovary tissue for presence of recent POFs provided the location of spawning sites.

CHAPTER 1: LOCATING SPAWNING SITES ON THE NORTHERN WEST FLORIDA SHELF

1.1. INTRODUCTION

Batch spawning fish species release eggs at multiple times during their respective spawning seasons (Brown-Peterson et al. 2011). Many batch spawning species also display indeterminate fecundity in which oocytes are recruited to secondary growth multiple times so they can be released at various times during the spawning season (Ganias et al. 2015; Hunter et al. 1992; Lowerre-Barbieri et al. 2011). The production of eggs in a female fish depends on her energetic state (Peebles et al. 1996), which dictates the amount and the quality of the eggs and their survival once released from the female (Berkeley et al. 2004; McCormick 2006). Many fish species spawn in the late afternoon or during the first half of the night. This timing protects the adults and the eggs against visual predation, and maximizes the time that eggs spend in darkness (Smejkal et al. 2018). The adult collection method was designed based on this knowledge. We investigated three species for this first chapter.

Species of interest

For the first part of this research, adult females were sampled, with the species of interest being *Lutjanus campechanus* (Red Snapper), *Rhomboplites aurorubens* (Vermilion Snapper), and *Diplectrum formosum* (Sand Perch). These species were chosen because of their higher abundance in the catches of adult fish and high numbers of gravid females.

The Red Snapper is a reef fish that lives on the continental shelf, over deep-reefs, banks, and rocky bottoms (Patterson et al. 2001). It has a maximum length of about 100 cm and is an asynchronous batch spawner with an indeterminate fecundity for which each individual continuously develops oocytes at different stages at the same time (Lowerre-Barbieri et al. 2011; Porch et al. 2007). It reaches maturity after 2 years (Lowerre-Barbieri et al. 2011). This species spawns multiple times from April to September (Bradley and Bryan 1975; Gallaway et al. 2009). It feeds on demersal crustaceans, fish, and pelagic zooplankton. It is an important species for commercial and recreational fisheries, and according to the 2018 Red Snapper Allocation report by the Gulf of Mexico Fishery Management Council and the National Oceanic and Atmospheric Administration (NOAA), close to 7 million pounds of this species are caught commercially in US waters each year and 4 to 8 million pounds are caught recreationally.

The Vermilion Snapper is another reef fish that lives in the GOM over substrates of sand, reef, gravel, or rock. It grows to an average of 35 cm in length and reaches a maximum total length of about 60 cm. Females reach maturity between 3 and 4 years old and spawn in batches between April to September. This species feeds on small pelagic crustaceans, cephalopods, pelagic gastropods, and fish (Grimes 1979). Vermilion Snapper is not as important as other snappers to local commercial fisheries, but is sometimes sold as Red Snapper (Grimes et al. 1982).

The Sand Perch is a reef-associated, warm-water, fish that is commonly associated with sandy bottoms adjacent to wrecks, reefs, and other bottom structures. Smaller individuals can also be found in shallow bays and seagrass beds; older individuals range more seaward out to depths of at least 80 m. This species digs holes in sandy bottoms or under rocks. It has a life span to up to 8 years old and reaches a maximum total length of approximately 30 cm. It is a

simultaneous hermaphrodite and batch spawner that reaches 50% sexual maturity at around 12 months and spawns throughout the year, with a peak during spring and early summer. Its diet is mainly composed of benthic crustaceans and small fishes. This species is often used as bait to catch groupers, snappers, and sharks, and has ecological significance to commercially important fish even though it does not have a commercial importance (Bubley and Pashuk 2010). Some particularities of its reproductive system will be covered in the discussion.

Comparing methods to estimate spawning intensity

To gain more insight into the relative intensity of spawning at different locations, I needed to determine average batch fecundity at different locations. For batch spawners, batch fecundity is the number of mature hydrated oocytes a female fish produces in one spawning event (Hunter et al. 1985). In reviewing the literature, I found no consensus on the best method for egg enumeration, and so I conducted a study to investigate the accuracy and precision of the two dominant methods. The first commonly used method is the volumetric method, which consists of estimating the number of eggs in an ovary by suspending all eggs into a fluid and counting eggs within subsamples of known volume. The second method is the gravimetric method, which consists in weighing the entire egg sample and then counting eggs in subsamples of known mass (Hunter et al. 1985). The published literature suggests both methods have similar usage, yet the details and level of complexity are variable within each approach. Hunter et al. (1985) first described the use of the volumetric method by suspending the ovary tissue in Gilson's fluid to break down connective tissue and release the eggs which are then transferred to water. This latter step was accomplished mechanically with hydraulic pressure instead of chemically, as in our study. The use of Gilson's fluid is inadequate for the volumetric method

because it destroys hydrated eggs. Another volumetric method is the displacement method, which consists of evaluating the volume of water that is displaced once samples of eggs of known number and size are added. I decided to simplify both primary methods (gravimetric and volumetric) and test their accuracy and precision.

Hydration of oocytes and ovulation

Ovulation is the event during which mature, hydrated oocytes are produced and released from their follicular envelope by a female fish (Goetz and Garczynski 1997). Prior to ovulation, the follicular cells start dissociating and weakening the layer of envelope cells until the rupture of the envelope occurs. The process could be facilitated by the hydration process that destroys the follicular envelope because of the increase in internal pressure from the oocyte itself. Ovulation is independent of non-follicular ovarian smooth–muscle contractions; indeed, the phenomenon can occur even in isolated follicles *in vitro* under the influence of hormones, and is likely to be dependent on an intra-follicular mechanism and possibly contractile cells that appear in the few hours preceding ovulation (Pendergrass and Schroeder 1976; Skoblina 2010). Also, it is assumed that when spawning occurs, all and only the hydrated eggs are released from the ovary.

This information is pertinent in that I counted hydrated eggs to get an estimate of batch fecundity. Understanding that the presence of hydrated eggs indicates that spawning is going to occur in a near future (next few hours) allowed me to locate the spawning sites.

Objectives

The objectives for this chapter were to (1) locate the spawning sites on the WFS to be seeded in a hydrodynamic model, (2) estimate batch fecundity, which is data that could also be relevant to future stock assessments, and (3) compare two methods for estimating batch fecundity for accuracy, precision, and processing time, with one method based on volume and the other on weight. These goals were accomplished by capturing adult fish, identifying spawning females, and quantifying the total number of hydrated eggs within ovaries.

1.2. METHODS

Fish collection and sampling

Adult fish were concurrently collected at various locations (to cover a larger sampling area) during a single day. On July 11, 2017, six hired commercial fishing boats fished between 1100 and 2000h EDT, using bandit gear (electric or hydraulic vertical lines with multiple hooks) or hand lines (rod and reel). Each boat was designated to fish in specific hexagonal grid cells (Figure 1). Sampled fishes were tagged with uniquely labeled zip-tie tags, iced whole, and returned to port. Times and locations of collection for individual fish were recorded. All fish were processed within 24 h of collection. Specimens were identified to species, weighed (±1g), measured for standard length (SL) (±1mm) and total length (TL) (±1mm), and dissected. The fishes were sexed and maturity stages were assigned macroscopically (Table 1). For mature females (F4 and F5), the gonads were weighed (±0.01g) and separated into right and left lobes.

Batch fecundity & POF identification

For batch-fecundity enumeration, the left lobe was washed using the hydraulic pressure of tap water (Figure 3) (Lowerre-Barbieri and Barbieri 1993; Stallings et al. 2016). The collected oocytes were then preserved in 2.5% buffered formalin and the ovarian membrane was then weighed. I used two approaches to verify the presence of spawning females at potential spawning locations. The first approach was to find females that were hours away from spawning, as evidenced by the presence of hydrated oocytes in their ovaries. The second approach was to find females that had spawned within the previous 24 hours, as indicated by the presence of recent post-ovulatory follicles (POFs) within the ovary (Hunter et al. 1985). My intent in combining the two approaches was to maximize the number of individuals that I could integrate into the process of locating spawning locations.

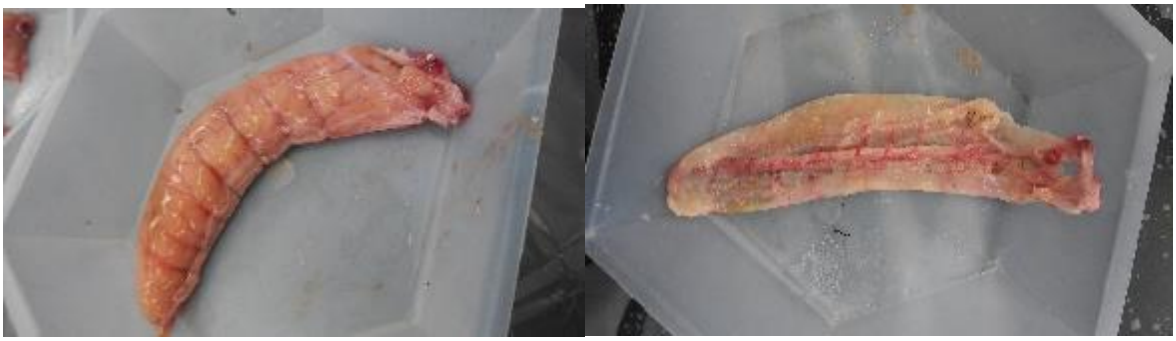

Figure 3. A Red Snapper left ovary before (left) and after (right) rinsing with tap water, leaving only the ovarian membrane behind. The eggs were separated from the tissue by hydraulic pressure.

Table 1. Gonad maturity stages and their developmental characteristics based on macroscopic examination (Stahl and Kruse 2008).

Stage	Class	Gonad developmental characteristics
F1	Immature unsexed female	No differentiation of the gonad. Immature gonads. Ovary transparent with no visible eggs.
F2	Differentiated/ immature female	Small ovaries, pinkish to translucent in color. Oocytes are not visible.
F3	Developing/ maturing female	Flattened ovaries with pink color Oocytes are not visible externally. Vascularization starts to show.
F4	Pre-spawning/ spawning-capable/mature female	Ovaries have started swelling. Vascularized Yellow in color Small, individual oocytes are visible through the ovarian membrane.
F5	Spawning female	Large, swollen, yellow ovaries with visible individual oocytes. Blood capillaries are visible. Hydrated, clear oocytes/eggs may be released when pressure is applied to ovary.
F6	Spent female	Ovaries are shrunken, flaccid, they are not as inflated or swollen; line going through lumen. Post-ovulatory follicles of less than 24 hours are present (histological examination).
M	Male	Non-staged males
M1	Non-Spawning male	No differentiation of the gonad Immature gonads
M2	Spawning male	Milt may be released when pressure is applied to testes

Histology

For histological analysis, a 2-3 cm section from the right ovary was preserved in buffered formalin (10% in de-ionized water) and was later transferred to 70% ethanol and shipped to Mass Histology Service (Worcester, MA) for staining with hematoxylin and eosin-y and mounting as thin sections on slides. Two slides were prepared for each fish based on samples that were spaced approximately 2-3 mm apart within the ovary section. This was done to identify individual females that had already partially released their eggs, as evidenced by recent POFs (Figures 4 and 5); inclusion of such individuals in the estimation of batch fecundity would have biased the estimates of batch fecundity. Batch fecundity was thus measured as the number of hydrated eggs (Figures 6 and 7) from specimens without recent POFs, with POF presence being determined from the histology slides (Ganias et al. 2014; Hunter and Macewicz 1985; Murua et al. 2009). PAX-it™ imaging software, in combination with a PAXcam™ camera, were used to record images of egg subsamples, which were used with J-Image software to generate oocyte size distributions (see *Appendix A).*

These methods also revealed females that had spawned within the past 24 hours, thus not presenting with hydrated oocytes but having recent POFs. For each slide, hydrated eggs were identified and POFs were aged. Ovaries containing hydrated eggs and recent POFs were excluded from batch-fecundity estimation, whereas those containing hydrated eggs one- or two-day-old POFs were included. The histological identification of oocyte stages was based on Kulaw et al. (2017).

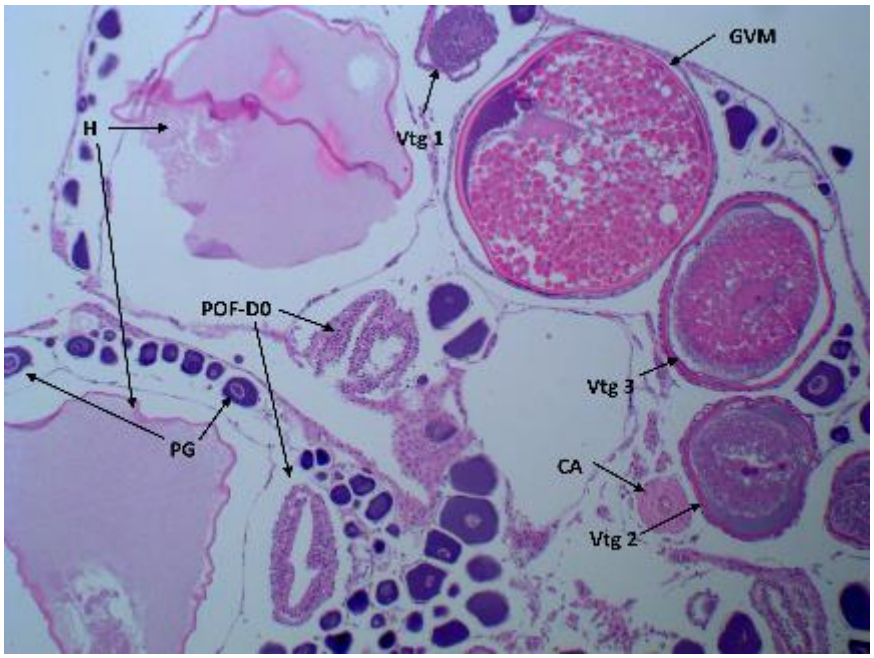

Figure 4. Photomicrograph of oocytes within Sand Perch ovary, where H: hydrated oocyte, POF-D0: recent POF (<24 hours), PG: primary growth, CA: cortical alveolus, Vtg 1: primary vitellogenic oocyte, Vtg 2: secondary vitellogenic oocyte, Vtg 3: tertiary vitellogenic oocytes, and GVM: germinal vesicle migration.

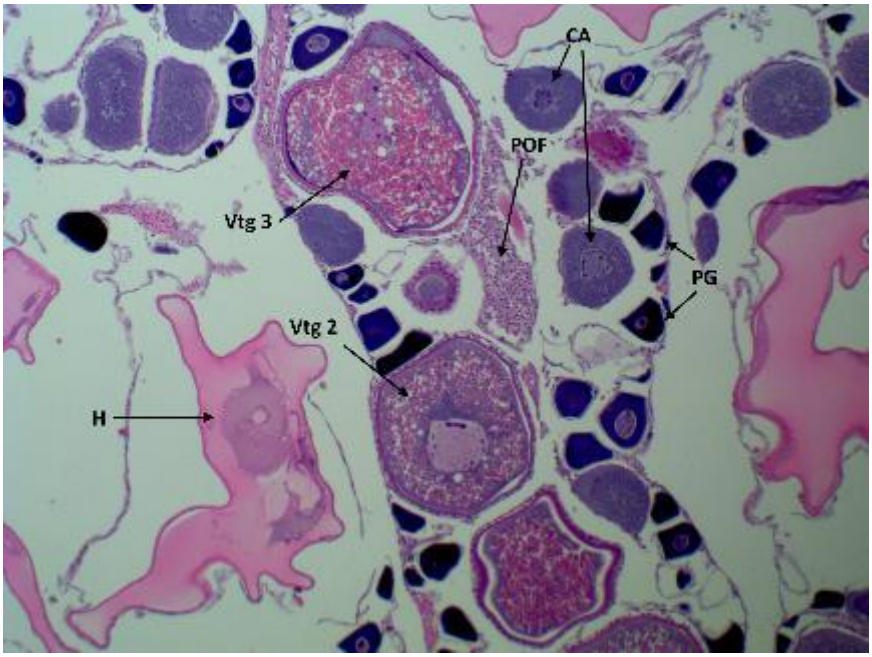

Figure 5. Photomicrograph of oocytes within Red Snapper ovary where H: hydrated oocyte, POF: old POF > 24 hours, PG: primary growth. CA: cortical alveolus, Vtg 2: secondary vitellogenic oocyte, and Vtg 3: tertiary vitellogenic oocyte.

Hydrated egg identification and batch fecundity estimation method

Because the reef fishes that were caught are batch spawners with indeterminate annual fecundity, batch fecundity was measured as the number of eggs produced in a single spawning event, as determined via counting hydrated oocytes (Hunter et al. 1985). Hydrated oocytes can be easily differentiated from the other stages and counted under a stereomicroscope (Figures 6 and 7).

Comparing methods of enumeration

Before enumerating eggs rinsed from fish with no recent POFs, both the volumetric and the gravimetric methods of enumeration were evaluated. The objective was to compare accuracy and precision using direct counts with tally meters for comparison.

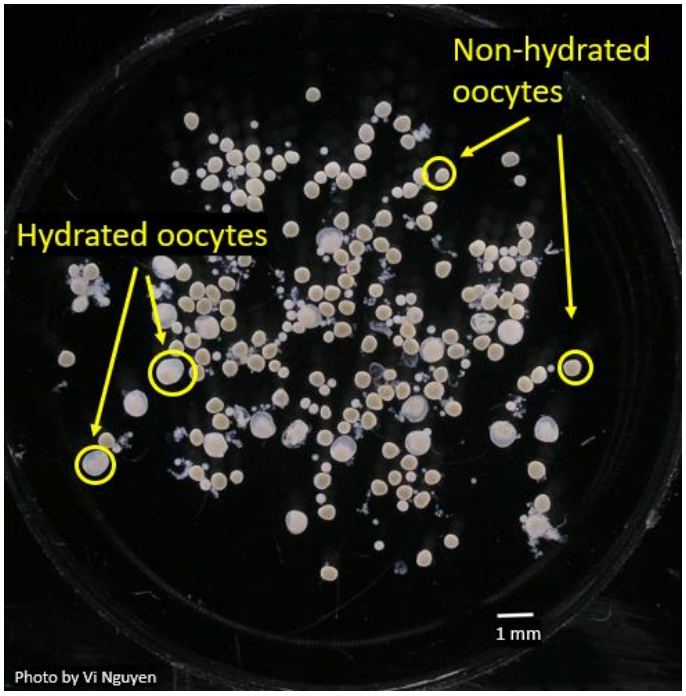

Figure 6. Hydrated and non-hydrated oocytes of Red Snapper under stereomicroscope.

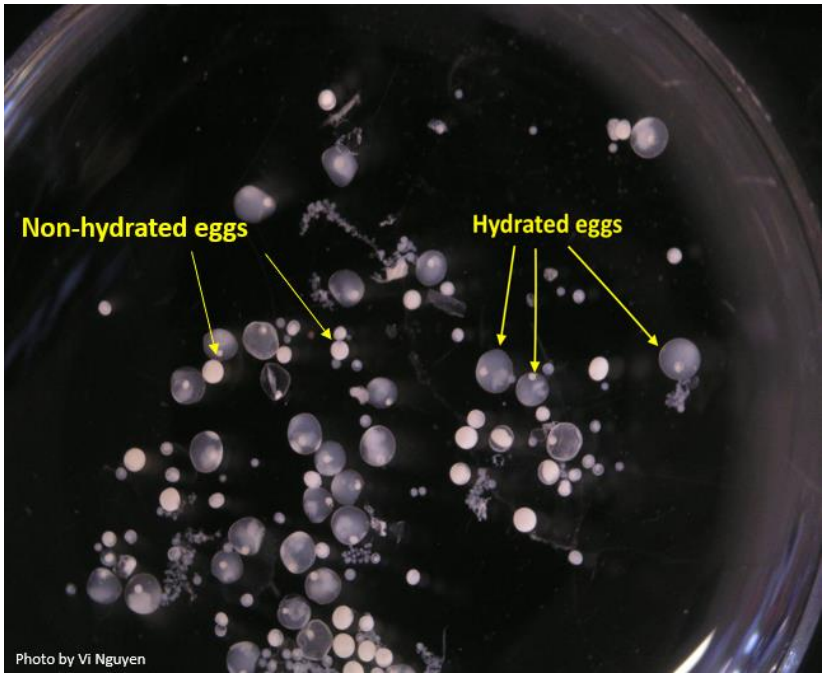

Figure 7. Hydrated and non-hydrated oocytes of Sand perch under stereomicroscope.

The speed of sample processing was also considered. Nine egg samples were selected, and for each of them, a total count of hydrated eggs (using tally meters) was conducted along with estimates from the volumetric and gravimetric methods.

For the volumetric method, the eggs were transferred to a known volume of water in a graduated cylinder, with the amount of water depending on the amount of eggs in the sample. The graduated cylinder was then inverted repeatedly in an attempt to distribute the eggs evenly within the cylinder. Aliquots of known volume (between 8 and 20 ml) were quickly poured into a gridded petri dish, where hydrated eggs were counted by tally meter under a stereomicroscope (broken eggs were still identifiable as hydrated eggs by their size and were counted as hydrated eggs). The unprocessed volume was then recorded, and the number of hydrated eggs was prorated to the entire sample volume (Holden and Raitt 1974; Hunter et al. 1985). The eggs in the aliquot were returned to the sample, and the process was repeated until five estimates were

obtained for each of the ten egg samples. The five estimates were averaged, producing a single estimate of total number for each egg sample.

The gravimetric method followed a similar design, wherein the total mass of eggs was weighed, and then a subsample of eggs was weighed; weights were measured after blotting preservative fluid with a paper towel. The mass of the subsample (0.02 to 0.03 g) was recorded along with the total number of hydrated eggs tallied from it, and the total number was prorated (Hunter and Macewicz 1985). The subsampling was repeated five times for each of the nine egg samples.

These enumerations and first estimates were results for right ovary lobes only. To generate the total batch fecundity estimates for each individual (2 ovary lobes), numbers were corrected for the weight of the pair of lobes and for the mass of the ovarian membrane. The mass of the membrane of the left lobe was not weighed but was considered proportional to the mass of the membrane for the right lobe.

The batch fecundity estimates for a species were obtained by summing the total number of batch fecundity estimates of individuals of the same species and dividing by the total number of individuals for that species:

$$Batch\ fecundity = \frac{Sum\ of\ batch\ fecundity\ estimates\ for\ one\ species}{n}$$

with n being the number of individuals for one species.

To compare the two methods, 10 samples were randomly selected and for each, the entire sample was enumerated to obtain the total count of eggs. The error for each sample was determined for the volumetric and the gravimetric method following the ratio:

$$\%\ error = \frac{[Total\ count\ of\ eggs - Estimated\ count\ of\ eggs]}{Total\ count\ of\ eggs} \times 100$$

The average error for each method was then calculated with standard error:

$$Average\ error = \frac{\sum\ errors\ for\ one\ method}{N}$$

with N being the number of samples.

Spawning time window

Within the fishing time window of 1100 to 2000 hours EDT, coupled with the analysis of hydrated eggs inside female ovaries, it is possible to determine a spawning time window by considering the first hydrated female captured as the start time and the last hydrated female captured as the end time.

1.3. RESULTS

A total of 519 adult fish were collected, including 312 females and 207 males. We collected 12 species of which only three [Vermilion Snapper (n = 123), Red Snapper (n = 251), and Sand Perch (n = 44)] had a number of individuals high enough for processing (Figure 8).

Table 2. Summary of the number of fish per fecundity state and with presence of recent or day-0 POFs (POF-D0) after histological identification.

	Vermilion Snapper	Red Snapper	Sand Perch
Total number of F4 females	24	60	14
Total number of F5 hydrated females	8	32	21
Number of F4 females with POF-D0	0	16	2
Number of F5 hydrated females with POF-D0	0	2	21

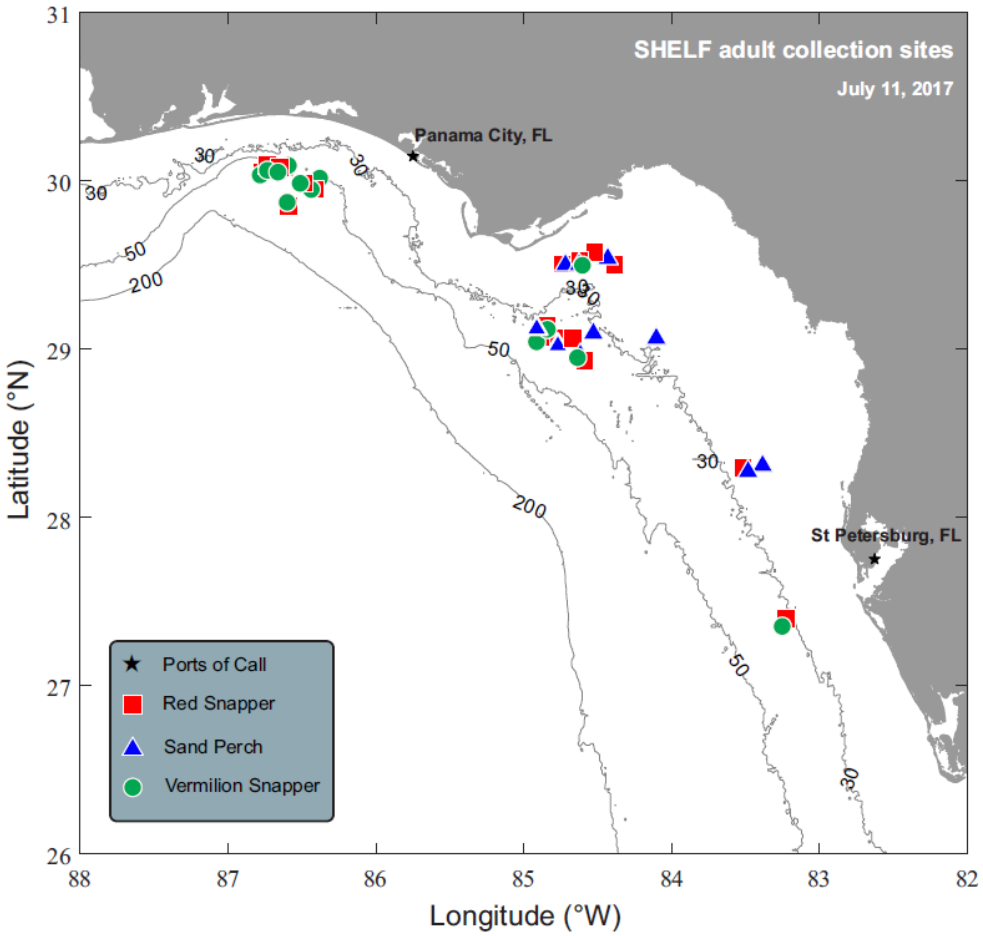

Figure 8. Collection sites where adult Red Snapper, Sand Perch, and Vermilion Snapper were caught on the northern West Florida Shelf on July 11, 2017.

Histology

Histological analysis allowed the identification of hydrated females and the aging of the POFs. Females with hydrated oocytes were indicative of spawning (n = 61). An additional 18 female fish had no visible hydrated eggs but presented with recent POFs (D0-POF) which indicated that they had finished spawning for that day or had spawned within the past 24 hours (Table 2). Those also contributed to locating spawning sites. Spawning females (stage F5) that presented with D0-POFs were considered for locating spawning sites but could not be incorporated in batch fecundity estimates that I established in the following sections.

Comparison of volumetric versus gravimetric estimates

When evaluating the 9 samples for which total counts of eggs were available, the average volumetric estimates underestimated the directly tallied count by -29.8 ± 4.8%, with variation ranging between underestimates of -9.8 and -56.6%. In contrast, the gravimetric method always overestimated the directly tallied count except for one sample, with an average overestimate of 63.8 ± 15.9% and a range between -9.1% and 140%. The volumetric method was therefore chosen for the estimation of the batch fecundity for most specimens because of its superior accuracy and precision (Table 3).

In the gravimetric method, the weight of interstitial water, which varied with time due to evaporation, appeared to increase as the total volume of eggs increased. However, for a few samples with a right lobe weight >23 g, the gravimetric method was employed because the volumetric method required an initial volume of more than 700 ml, which was difficult to homogenize by inversion.

Table 3. Results of comparison between the volumetric and the gravimetric method.

	Volumetric	Gravimetric
Number of samples	9	9
Average error ± standard error	29.8 ± 4.8%	63.8 ± 15.9%
Range of error	9.8–56.6%	-9.1–140%.

Batch fecundity estimates and morphometric data

Batch fecundity estimates and adult morphometric data are summarized for all three species in Table 4 and plotted on Figure 9. The batch fecundity of Sand Perch was estimated even though all hydrated females presented with recent POFs. The average batch fecundity per site was also calculated (Table 5).

Spawning sites

Spawning and non-spawning females were generally captured at the same locations. A higher number of fish was collected at the Florida Middle Ground and near the Florida Panhandle compared to other locations on the WFS (Figures 9 and 10). The spawning sites of the fish appeared to be closer to shore than to the shelf break and in shallow waters.

Table 4. Batch fecundity and adult morphometric data for Red Snapper, Sand Perch, and Vermilion Snapper.

		Vermilion Snapper	Red Snapper	Sand Perch
For all individuals (male and female)	Total number of individuals	153	251	44
	Total length average (mm)	308	427.3	257
	Total Length Range (mm)	192–496	311–833	208–322
	Weight average (g)	370.7	1159	155.3
	Weight range (g)	85–1,415	407–8,140	85–250
For fecund females	Total length average (mm)	318	429	258
	Total length range (mm)	265–455	364–750	218–303
	Weight average (g)	430	1191	155.9
	Weight range (g)	235–1,235	635–5,875	90–250
	Number of females for batch fecundity estimates	8	28	21
	Average batch fecundity (eggs per batch) ± standard error	101,293 ± 55,787	75,119 ± 19,802	8,272 ± 1,869
	Batch fecundity range (eggs per batch)	27,999–490,064	4,685–411,905	1,146–39,528

Table 5. Batch fecundity average per species per station.

	Average of batch fecundity estimates		
Station	Vermilion Snapper	Red Snapper	Sand Perch
1	490,064	-	-
6	-	-	3,816
7	-	-	6,989
8	21,633	50,618	2,308
10	-	21,824	14,157
12	-	26,210	-

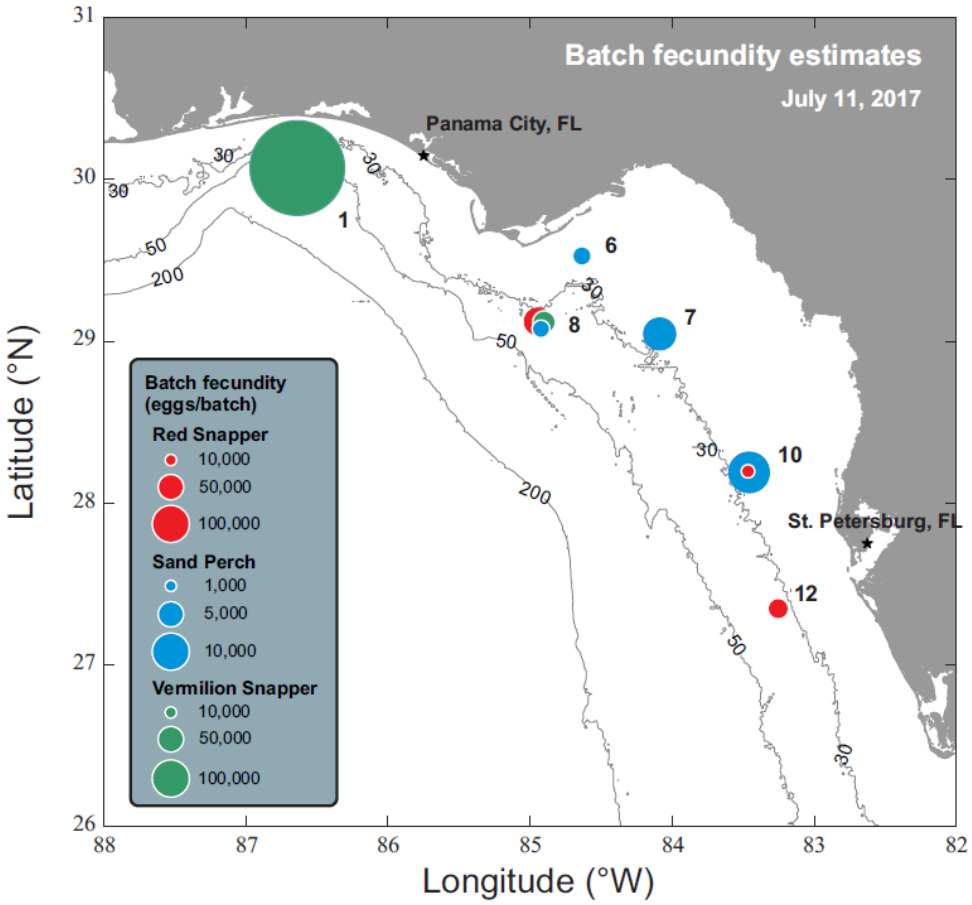

Figure 9. Fishing stations with batch fecundity estimates of adult female fish on the northern West Florida Shelf. The size of the circles is proportional to the average batch fecundity estimates for each species at each location. The three species of interest Red Snapper, Vermilion Snapper, and Sand Perch are represented.

Table 6. Adult female size data.

	Vermilion Snapper	Red Snapper	Sand Perch
Average ovaries mass (g)	24.52	24.73	2.49
Average female body mass (g)	430.00	1190.58	148.90
Average ovary mass as a percentage of body mass (%)	4.43	2.08	1.61

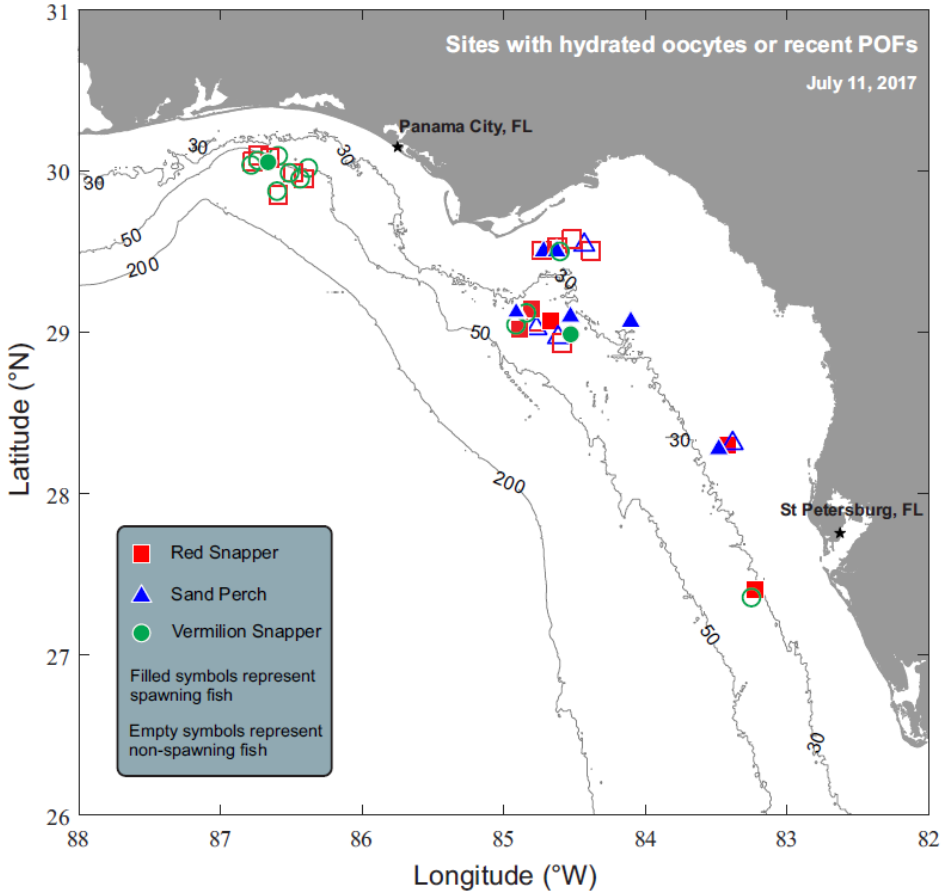

Figure 10. Spawning sites where females with hydrated oocytes or recent POFs were found. The three species of interest Red Snapper, Vermilion Snapper, and Sand Perch are represented.

Spawning time windows

The fishing time window during which collection occurred was 1100 to 2000 h EDT (Figure 11). For Red Snapper, the spawning time window was determined to be 1400 to 1730 hours. The end-boundary for the time window for this species is relatively well defined; however, it is not certain that the start time for spawning was 1400, as too few individuals were collected between 1100 and 1400 hours. For Vermilion Snapper, the spawning time window started at 1630 and went past our designated sampling end time; therefore, the end time for this species is uncertain. For Sand Perch, no time window was determined due to all females presenting recent POFs. The females started spawning before 1100 and stopped after 2000 hours, or else they prepared for spawning and started isolating hydrated eggs in their accessory structure for future use (refer to discussion for details).

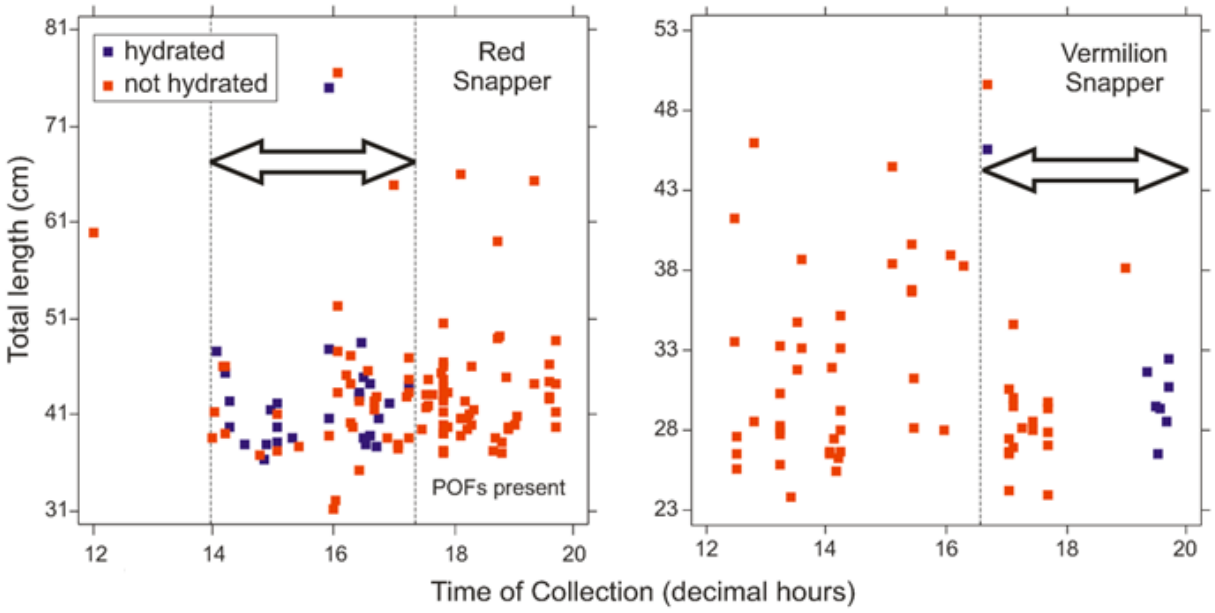

Figure 11. Spawning time window for Red Snapper and Vermilion Snapper (total length was primarily plotted as the y-axis to create separation among individuals).

Egg mass in relation to body mass

The average mass of ovaries and the average mass of body for each species was calculated. For each species, the average ovary mass was expressed as a percentage of body mass (Table 6).

From this table, it is notable that the mass of ovaries is not proportional to the body mass. Indeed, Red Snappers have an average body mass that is 2.77 times greater than Vermilion Snappers yet these species have approximately the same average mass of ovaries.

1.4. DISCUSSION

The objective of this chapter was to locate the spawning sites of three reef-associated fishes on the northern WFS. Those spawning sites were successfully located with the use of macroscopic examination and histology-based methods. The histological work investigated the presence of recent POFs that suggested that recent spawning had occurred in the previous 24 hours, therefore indicating the location of spawning sites. The batch fecundity work, which was based on hydrated egg counts, showed the progress of the hydration process and the preparation by fish for ovulation and spawning. This method also indicated the location of a spawning sites since the fish were only hours away from spawning. The hydrated-egg count work concurrently allowed the estimation of batch fecundity.

In estimating the batch fecundity, two methods were compared for accuracy and precision. It was concluded from this study that the volumetric method was more accurate and precise for batch fecundity estimates because of the sample handling process that was facilitated by the use of water. The technique also did not require taking into account the interstitial fluid

and its evaporation as the oocytes were maintained in water throughout the sample processing. This volumetric method was different from the displacement method in that it required fewer steps and had less opportunity for bias from potential changes to the eggs due to preservation technique (i.e., the hydrated eggs may lose size and shape when preserved in formalin or other preservatives). Evaluation of individual oocyte volume was not necessary. This volumetric method clearly had a cost advantage compared to previously used methods, was a much-simplified procedure, and had a better outcome than the gravimetric method. It also allowed the conservation of the oocytes for a long period of time without concerns about sample degradation and bias in individual egg volumes.

It is interesting to note that batch fecundity is specific to each species and that large fish do not necessarily have proportionally larger batch sizes. There is no cross-species relationship between the batch fecundity and the length or weight of the females. Red Snapper individuals were longer and heavier than Vermilion Snapper on average and both have similar average ovary weights, yet the batch fecundity for Vermilion Snapper estimated to be a third higher than for Red Snapper.

All female Sand Perch with mature gonads and hydrated oocytes presented with recent POFs. The average batch fecundity was calculated for Sand Perch despite this fact. For this particular simultaneous hermaphroditic species, Bubley et al. (2010) observed that hydrated oocytes were migrated to an anatomical feature known as the “accessory structure” before spawning. Those females with hydrated oocytes in this accessory structure were ready to ovulate but had oocytes within ovarian tissue that ranged from actively spawning to resting stages. This explains the presence of recent POFs in all of the females processed. Indeed, the recent POFs were presumably from the hydrated oocytes that had already migrated to the accessory structure.

Such POFs do not necessarily indicate that spawning has already started. It is not possible to confirm whether ovulation has started because this simultaneous hermaphroditic species has an organ (the accessory structure) that is not present in gonochoristic species. The role of the accessory structure was initially unclear. It was originally thought to be used for storage of hydrated oocytes until the encounter of a suitable mate or for resorption of hydrated oocytes that would not be spawned (Bortone 1977). This indicates that what would be determined to be average batch fecundity for this species could be biased by the fact that the entire oocyte batch is not always ovulated. Bubley et al. (2010) mentioned that whenever hydrated oocytes were present in the accessory structure and the gonads were staged as spawning, the hydrated oocytes were less than 36 hours old due to the degeneration of POFs that can require up to 36 hours after spawning (Hunter et al. 1986). The accessory structure also has the capacity to maintain hydrated oocytes for a period of time longer than 36 hours, which is evidence that this structure is used for storage. However, another deduction is that the assumption that hydration generally occurs only hours from spawning is not valid for all fish species. Future studies should thus consider the hydration process as species-specific. Additionally, Sand Perch is a territorial fish that tends to have small individual spatial ranges. Therefore, for this chapter, we considered that the individuals did not likely travel far or live far from the sites where they were caught, and it can be assumed that females with hydrated eggs or with recent POFs indicated spawning sites, if not times.

The spawning-time windows provided here suggest ideal catch times for future studies of these species' reproduction. The time windows were not definite and entirely conclusive, but helped define a start and end time for fishing in future surveys. Particularly, for Sand Perch, it can be speculated that the females started spawning before and after the fishing time window or

that they prepared for spawning, isolating some of the hydrated oocytes in their accessory structure, and waited for the right moment and the right mate to spawn, which could have been within a few hours to more than 36 hours afterwards (Bubley and Pashuk 2010).

Expanding adult collections over space and time should identify more spawning sites or confirm the already known ones. The successful use of biological methods allowed the identification of spawning sites that were used to initiate the trajectory simulations in a numerical model discussed in chapter 2.

CHAPTER 2 ABSTRACT

Planktonic fish eggs and larvae can be advected by ocean currents. Knowing whether newly spawned fish-eggs are retained or exported away from the West Florida Shelf (WFS) has a significance for the interpretation of fish-egg abundance trends from annual egg surveys. Movement of fish eggs and larvae on the WFS can be investigated via numerical simulations of their trajectories in a hydrodynamic model. We used the West Florida Coastal Ocean Model (WFCOM) to track the movement of fish eggs over space and time. Fish eggs and larvae were seeded into the model as passive particles at three depths (surface, mid-water, and bottom) over a period of two weeks. Coordinates for the trajectories were from two sources: 1) spawning sites identified previously via macroscopic examination and histological-based methods and 2) fish-egg collection sites. The results suggested that trajectories initiated from nearshore stations mostly resulted in retention, whereas trajectories initiated from offshore stations mostly resulted in export away from the WFS. The trajectories were generally consistent with the physical attributes of the WFS circulation. The study indicated that fish-egg abundance was higher nearshore. It was also found that stations with higher fish-egg abundance were more likely to result in retention. Batch fecundity estimated previously was not found to be a good indicator of where the eggs and larvae will end up, but it was observed that spawning sites appeared to be closer to shore than to the shelf break. Also, DNA barcoding was used to identify the fish eggs to species. A fish-egg community analysis, using SIMPROF and based on taxonomic composition, identified geographic station groupings from west to east (from deep to shallow water). Egg

species were also categorized as pelagics or non-pelagics. Pelagic species were not found to be more likely exported than non-pelagics. This study serves as a starting point for future egg-monitoring projects by merging ocean circulation on the WFS with fish-egg abundance data.

CHAPTER 2: INVESTIGATING RETENTION OF FISH EGGS ON THE WFS

2.1. INTRODUCTION

Ocean circulation modeling

Ocean circulation is known to influence the chemical composition of seawater, its physical properties, geological features, and many biological processes that occur in the marine environment. In particular, coastal processes, such as frontal convergences or sub-mesoscale eddies (Bassin et al. 2005; Sponaugle et al. 2005), can have direct and indirect influences on the patterns of egg and larval retention (i.e., fish eggs and larvae originate and remain within the region of interest) or connectivity (i.e., export to viable habitats at other locations). These coastal processes are responsible for influencing egg transport, larval growth, and survival.

As an example of numerical simulation, Liu et al. (2011) employed a biophysical model to track the Deepwater Horizon (DWH) oil spill. Virtual particles were released in the model from the oil-spill site and their movement was forecasted. The results indicated that the accuracy of the models was variable, but that such models can be useful for future forecasting work, including forecasting of trajectories of fish eggs and larvae.

To investigate the movements of larvae and eggs, a variety of techniques are now being employed such as larval tagging (i.e., incorporation of isotopes or chemicals by the embryo from the mothers or via incubation), DNA sequencing (i.e., genetic analysis leading to identification of species), or biophysical circulation models (i.e., numerical simulation of trajectories) (Jones et al. 2009; Thorrold et al. 2002). Weisberg et al. (2014) used a numerical circulation model, the

West Florida coastal ocean model (WFCOM), which is similar to the one employed for the DWH oil spill, to explain the movement of Gag (*Mycteroperca microlepis*) larvae on the WFS. The authors compared surface and near-bottom trajectories to determine which pathway led to known locations of pre-settlement fish and how the larvae are transported to settlement sites. This study found that Gag most likely use bottom currents to move from spawning locations to juvenile habitats.

This approach has also been used in other regions of the world. Grinson et al. (2011) investigated the larval dispersal of fish in the Gulf of Kachchh, on the west coast of India, using a two-dimensional numerical model and confirmed the retention of fish larvae in that region.

Buoyancy, pelagic larval duration and drift characteristics of fish eggs and larvae

The buoyancy of pelagic fish eggs and larvae depends on several internal characteristics such as lipid content in oil globules or the large quantities of aqueous fluid in the egg (Craik and M. Harvey 1987). These pelagic eggs float in the water column as plankton. Aside from being buoyant, planktonic fish eggs are considered to be passive particles that move with the current (Paris and Cowen 2004). The pelagic larval duration is a developmental period that larvae spend in the water column (Kendall et al. 2013). This period differs among species, ranging from days to months until the larvae become juveniles and start settling in schools for pelagic species or in habitats for non-pelagic species (Shanks 2009). Reef fish in the Gulf of Mexico have a relatively short larval duration. For example, Red Snapper egg incubation period is 20 to 27 hours before hatching into larvae. The total pelagic larval duration (PLD) of this species is approximately 26 days (Hernandez et al. 2016). More generally, the average PLD for marine fishes is 36 days (Fuiman and Werner 2009).

Flexion is a development stage or process during which fish larvae go through morphological transformations that involve the flexion of the notochord and the development of the caudal fin and behavioral changes that involve swimming and potential schooling. In the postflexion larval stage, fish change morphologically and become better able to swim. Multiple studies have published evidence of changes that occur in association with reaching the postflexion larval stage. These developmental changes generally occur rapidly. To name a few, these changes include allometric growth, changes in swimming mode, inflation of the swim-bladder, the onset of schooling behavior, increased vertical migration, advances in internal organ growth, or changes in feeding behavior (Somarakis and Nikolioudakis 2010).

Export and retention

During the pelagic phase, the passive planktonic larvae are the most likely to be dispersed by ocean currents. Larval dispersal can result in two main outcomes: export or retention. Export is the rapid movement of eggs and larvae away from a region of interest (Jones et al. 2009). In this study, the region of interest is the WFS. Export can result in aberrant drift or connectivity (Jones et al. 2009). Aberrant drift is defined as the dispersal of eggs and larvae away from essential larval and juvenile habitat, resulting in loss or mortality (Hjort 1926). Connectivity is the movement of eggs or larvae to viable habitats outside a region of interest (Cowen and Sponaugle 2009). In contrast, when fish eggs and larvae are found to both originate and remain within the region of interest, the process is referred to as retention or self-recruitment (Cowen and Sponaugle 2009; Jones et al. 2009). Thus, information about the fate of early-life history stages of fish is important for proper management of fisheries.

Objectives

Integrating biological features into an ocean model is becoming more common and can be expanded and used for fish-egg trajectories. Aside from the Weisberg (2014) study, studies of fish egg trajectories in the eastern GOM have not been previously reported, even though the WFS is home to many important fisheries species. The overall goal of this chapter is to investigate the movement of fish eggs and larvae on the WFS using high-resolution hydrodynamic models (Aiken et al. 2007; Cowen et al. 2006; James et al. 2002), similar to how Weisberg et al. (2014) tracked Gag larval movement. We used the WFCOM to predict the location of larvae after two weeks in the plankton by:

1) using the spawning sites for Red Snapper, Vermilion Snapper, and Sand Perch identified in the previous chapter as start locations,
2) using the locations of collected fish eggs from all species that were sampled via plankton nets.

2.2. METHODS

The West Florida Coastal Ocean Circulation Model

In 2012, Zheng and Weisberg developed an application of the Finite Volume Coastal Ocean Model (FVCOM) that they called the West Florida Coastal Ocean Circulation Model (WFCOM) (Figure 12). This model is a numerical model that combines local forcing with remote forcing acting upon coastal ocean circulation and it is a nesting of the FVCOM (Chen et al. 2003) into the Global Hybrid Coordinate Model (HYCOM) (Chassignet et al. 2009). By virtue of nesting FVCOM in HYCOM, the WFCOM attains increasingly higher resolution upon

approaching the shoreline. In that way WFCOM downscales from the deep ocean, across the shelf and into the estuaries (Zheng and Weisberg 2012).

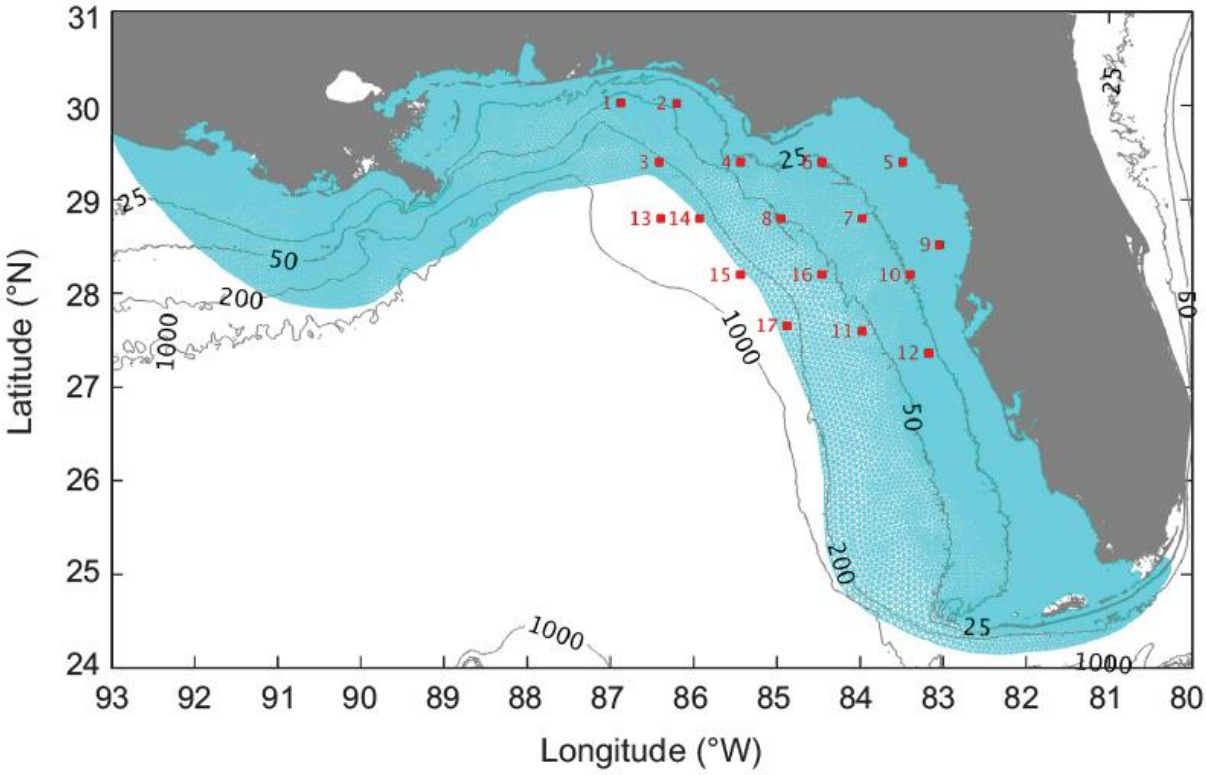

Figure 12. WFCOM domain and grid system (blue) with fish-egg locations, modified from Figure 1 of Liu et al. (2019).

Fish-egg data and collection

During the morning hours of July 12, 2017, at 0600 and 1200 h EDT, two FIO vessels and six commercial fishing vessels sampled drifting eggs via plankton net tows within designated hexagonal grid cells on the northern WFS (Figures 1 and 13). Each vessel sampled two stations, with the first station sampled at 0600 h EDT and the second station sampled at 1200 h EDT. At each station, two types of plankton net tows were conducted: (1) a single, horizontal tow and (2) three replicate vertical tows.

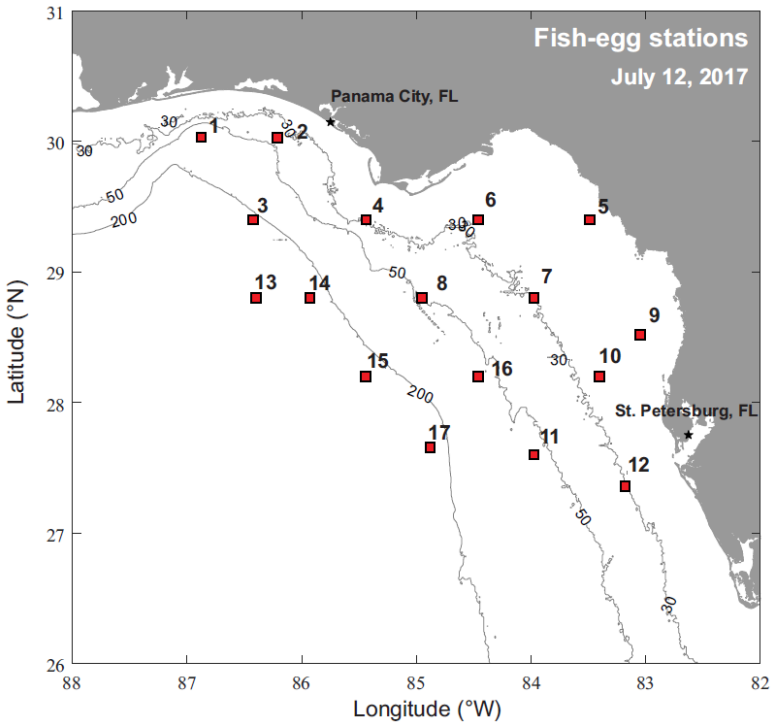

Figure 13. Fish-egg stations on the northern WFS for the pilot survey on July 12, 2017.

For the horizontal tow, a 335-µm mesh plankton was towed in one single passage for 15 minutes at idle speed. The conical net had a 0.73-meter mouth and was equipped with a flowmeter. The net was attached to the rear of the vessel, with the net ring maintained at the surface with flotation. The net was towed close to the vessel but away from the prop wash. Vertical tows used identical gear with the exception of the flotation on the ring. Instead, a 0.9 kg weight was hung from the cod-end. The vertical net was lowered by hand, cod-end-first, to 30 m depth or the bottom, whichever was shallower. Once retrieved, time of day was recorded for each type of net. Flowmeter readings for the horizontal tows were also recorded. The net was rinsed on board with seawater. Samples were collected and preserved in 70% isopropanol 30% ambient seawater. The exact location of each sampling event was recorded. For the purpose of this study, the egg abundances for the three vertical tows were averaged. DNA barcoding of fish eggs

(Burrows et al, 2018) was primarily performed on eggs collected in horizontal tows. Whenever the eggs had preservation issues or barcoding was not successful, eggs from vertical tows were also used for the DNA sequencing. Three stations did not provide information for the eggs that were barcoded due to poor preservation of genetic material.

Fish-egg trajectories

As in Weisberg et al. (2014), trajectories of fish eggs and larvae were simulated at different depths. The fish eggs were seeded as passive particles into the model for July 11, 2017. We assumed that the eggs were spawned in the evening, which would make the drifting eggs only a few hours old when they were collected the following morning.

Each modeled trajectory originated from (1) the spawning sites that were identified from the adult-fish fecundity study (Figure 10) and (2) the sites of fish-egg collection during plankton net sampling (Figure 13).

All eggs and larvae were considered neutrally buoyant and were not species-specific. Drifts at three depths (surface, mid-water, and bottom) were modeled because the depths at which the eggs drifted were unknown.

We decided to average the fish egg and larvae tracking time period to 15 days. This time period is about half of the average PLD of marine species (36 days) and represents a generalized time between the spawning of eggs and the larval flexion stage. We consider this period to be a largely passive stage for larvae during which they live in the plankton, before the development of stronger swimming abilities. The movement of fish eggs and larvae was, therefore, forecasted from July 11, 2017 to July 25, 2017.

Only the horizontal direction of planktonic fish eggs and larvae was considered in the trajectory simulations. We did not integrate vertical migrations or other biological responses to

environmental factors (e.g., salinity, temperature, light, food availability, currents) because of the limited information available regarding behavior during these life stages; this lack of information largely exists due to the lack of in-situ observation and experimental constraints on observing wild larvae after capture (Paris and Cowen 2004; Somarakis and Nikolioudakis 2010). For the forecasting of fish-eggs trajectories from the known spawning sites, retention and export from sites with high or low fecundity was also assessed using data generated from the histological and batch-fecundity analyses.

In combination with simulation of fish-egg trajectories from the fish-egg collection sites, we used the DNA barcoding identification to categorize species as being either pelagic or non-pelagic (Table 7) and visually compared their trajectories. We decided to define pelagic species as species that do not have any connection with a substrate throughout life and non-pelagic species as all other species that use or relate to substrate at one or more times during their lifetime. This gave insight into whether certain types of fish are more or less likely to have their eggs retained on the WFS.

Interpretation of trajectories

The model outputs are in the form of maps with trajectories. Categorizing the trajectories as resulting in retention or export can be complex because the spatial scale over which they are interpreted should be considered. Here, we consider retention on and export from the WFS. Trajectories can indicate short- or long-distance movement and can have different direction (e.g., toward the coast, along the coast, toward the open ocean, etc.).

Table 7. Categorization of pelagic and non-pelagic species.

Family	Species	Common name	Pelagic vs. non-pelagic
Achiridae	*Achirus lineatus*	Lined Sole	non-pelagic
Carangidae	*Selene setapinnis*	Atlantic Moonfish	non-pelagic
Carangidae	*Selene vomer*	Lookdown	non-pelagic
Carangidae	*Syacium papillosum*	Dusky Flounder	non-pelagic
Chaetodontidae	*Chaetodon ocellatus*	Spotfin Butterflyfish	non-pelagic
Ephippidae	*Chaetodipterus faber*	Atlantic Spadefish	non-pelagic
Gerreidae	*Eucinostomus argenteus/E. gula*	Spotfin Mojarra/Silver Jenny	non-pelagic
Gerreidae	*Eucinostomus spp.*	Mojarra	non-pelagic
Haemulidae	*Haemulon aurolineatum*	Tomtate	non-pelagic
Lutjanidae	*Lutjanus apodus*	Schoolmaster	non-pelagic
Lutjanidae	*Lutjanus griseus*	Mangrove Snapper	non-pelagic
Lutjanidae	*Pristipomoides aquilonaris*	Wenchman	non-pelagic
Lutjanidae	*Rhomboplites aurorubens*	Vermillion Snapper	non-pelagic
Ophidiidae	*Ophidion selenops*	Mooneye Cusk-eel	non-pelagic
Paralichthyidae	*Cyclopsetta fimbriata*	Spotfin Flounder	non-pelagic
Rachycentridae	*Rachycentron canadum*	Cobia	non-pelagic
Sciaenidae	*Equetus lanceolatus*	Jackknife Fish	non-pelagic
Serranidae	*Rypticus bistrispinus*	Freckled Soapfish	non-pelagic
Serranidae	*Rypticus maculatus/saponaceus*	Whitespotted Soapfish/Greater Soapfish	non-pelagic
Serranidae	*Rypticus sp.*	Soapfish	non-pelagic
Serranidae	*Serraniculus pumilio*	Pygmy Sea Bass	non-pelagic
Synodontidae	*Saurida normani*	Shortjaw Lizardfish	non-pelagic
Synodontidae	*Synodus foetens/macrostigmus*	Inshore Lizardfish/Largespot Lizardfish	non-pelagic
Synodontidae	*Synodus intermedius*	Sand Diver	non-pelagic
Synodontidae	*Trachinocephalus myops*	Bluntnose Lizardfish	non-pelagic
Triglidae	*Prionotus martis*	Gulf of Mexico Barred Searobin	non-pelagic
Triglidae	*Prionotus ophryas*	Bandtail Searobin	non-pelagic
Triglidae	*Prionotus punctatus/Prionotus rubio*	Bluewing Searobin/Blackwing Searobin	non-pelagic
Triglidae	*Prionotus rubio*	Blackwing searobin	non-pelagic
Carangidae	*Chloroscombrus chrysurus*	Atlantic Bumper	pelagic
Carangidae	*Decapterus punctatus/D. tabl*	Round Scad/Roughear Scad	pelagic
Carangiformes	*Caranx crysos*	Blue Runner	pelagic
Scombridae	*Euthynnus alletteratus*	Little Tunny	pelagic
Scombridae	*Scomberomorus cavalla*	King Mackerel	pelagic
Scombridae	*Scomberomorus maculatus*	Atlantic Spanish Mackerel	pelagic
Scombridae	*Thunnus atlanticus*	Blackfin Tuna	pelagic

Additionally, the trajectories cannot be precisely qualified as resulting in aberrant drift or connectivity because we do not have information regarding the outcome of the drift (e.g., mortality rate, proportion of settlers, exact settlement site). On the WFS, trajectories that are bringing the egg and larvae toward the coast or along the coast but remaining on the shelf can be considered retained. The trajectories that are toward the open ocean, away from the coast, and away from the WFS can be considered as export (Figure 14).

The distance of dispersal was calculated as the distance from the initial station coordinates (day 1, first day of tracking) to the final coordinates (day 15, final tracking day). The haversine formula was used to calculate the great-circle distance between two points using coordinate inputs. The distances were generated through an online calculator using that formula.

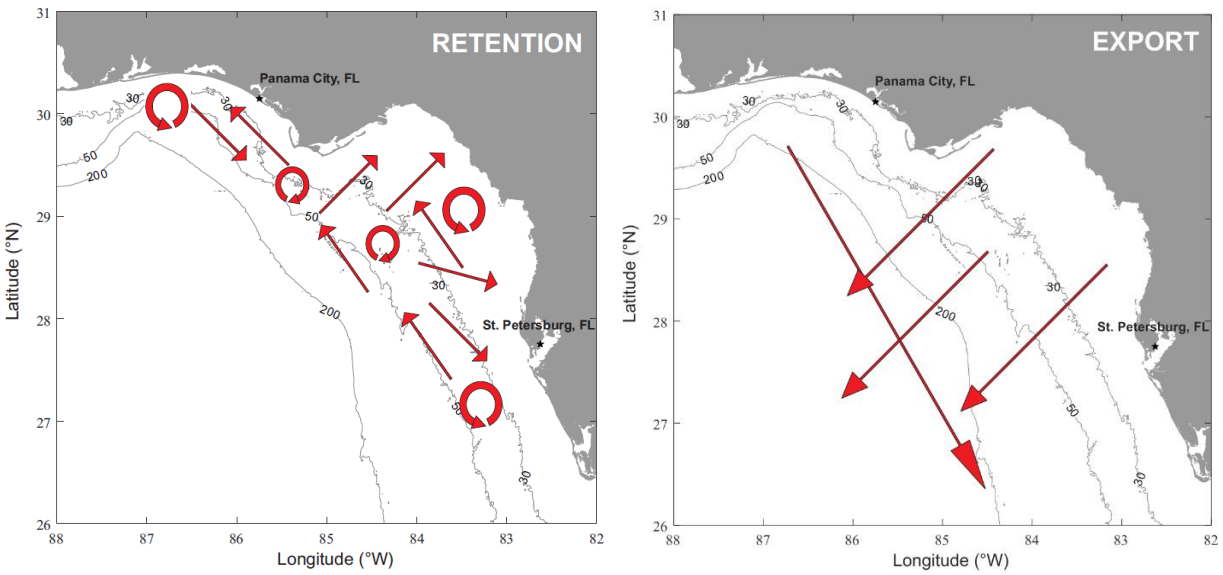

Figure 14. Schematic for interpretation of drifting fish egg trajectories on the northern West Florida Shelf. On the left, movement towards the coast or along the coast on the WFS is considered retention. On the right, movement towards the open ocean and away from the coast is considered export.

Three stations (13, 14, and 15) were outside the WFCOM geographic domain, and thus no trajectories were generated. These stations were identified with a distinct symbol from the other stations on the trajectory maps (Figure 16; this lack of trajectory does not indicate a lack of movement).

Community analysis

Multivariate community analyses were conducted (1) to determine the distribution of pelagic species eggs and larvae amongst the stations and their likelihood to be exported, and (2) to determine if species grouped by location on the WFS. For each station, the density of fish eggs was calculated from the vertical tows as the number of eggs under one square meter of water surface (number of eggs.m^{-2}). This approach avoids the problem of egg abundance varying with depth (i.e., due to differences in buoyancy or variation in vertical mixing of eggs from station to station). The horizontal tows filtered more water and collected far more eggs than the vertical tows and were used to identify the proportion of each fish species at each station. The abundance of eggs for each fish species was then calculated as the proportion of the average total egg abundance (n = 3 vertical tows) at each station according to the formula

$$Abundance\ of\ eggs\ of\ species\ A = Proportion\ of\ species\ A_{horizontal} \times Mean\ total\ egg\ abundance_{vertical}$$

PRIMER 7 software (v. 7.0.13, PRIMER-E, Auckland, New Zealand) was used to analyze fish-egg community structure. Egg abundance was square-root transformed and Bray-Curtis similarity was calculated for all possible station pairs. Cluster analysis was performed

using these Bray-Curtis similarities, and SIMPROF analysis was used to identify statistically significant groupings of stations within the results of the cluster analysis. A seriated heatmap was generated to allow simultaneous visual comparisons of (1) station compositional similarity and (2) species associations. The PRIMER 7 heatmap algorithm re-arranges both axes (station similarities and species associations) to maximize diagonal trends in the heatmap without changing quantitative relationships within the cluster-analysis results (i.e., by re-arranging the horizontal connectors in the cluster-analysis dendrograms). SIMPROF groups were geographically mapped.

2.3. RESULTS

For the following description and observations, the trajectories will be named after the number of the hexagonal region from which they were initiated. Dispersal distances are summarized in Table 8.

Tracking based on known spawning locations

The larval dispersal from spawning locations followed two types of trajectories (Figure 15). At the surface (Figure 15B and Table 9), we observed that the trajectories from stations 6, 7, 8, 10, and 12 had a northwest direction. Trajectories from stations 7, 10, and 12 diverted northeast toward the coast whereas trajectories from stations 6 and 8 continued their course northwest along the coast. Those trajectories were categorized as representing retention on the WFS. For station 1, the direction of the trajectory was southeast before turning northwest toward the coast; these trajectories were also categorized as representing retention the WFS. It is

interesting to note that the trajectories initialized from station 6 and 8 appeared to converge toward the same coastal area near St. Joseph Bay. Overall, at the surface, trajectories from spawning sites resulted in retention.

At mid-water (Figure 15C and Table 10), we observed that the trajectories from station 1 went southeast. They seemed to follow the shape of the 50-meter isobath for 11 days before continuing their course farther out on the WFS and parallel to the 50-meter isobath over a maximum of 375 km. At station 8, there were two groups of trajectories. Some trajectories took a northwest direction and indicated retention. Some trajectories went west toward deeper waters until they reached the 50-meter isobath and joined the southeast trajectories that originated from station 1 and reached a distance of 292 km. Trajectories from station 1 and the long-distance ones from station 8 could potentially be qualified as export away from the WFS. At station 6, the trajectories went southwest toward deeper water and reached the 30-meter isobath before taking a northwest direction along that isobath. The direction was northwest for the trajectories from station 7 and then turned to the east toward the coast. The direction was northwest and then southwest for the trajectories from station 12. Movement at stations 7, 10, and 12 was nominal over short distances (between 11 and 19 km) and was qualified as retention on the WFS.

Near the bottom of the water column (Figure 15D and Table 11) at station 1, the observed trajectories had an east and southeast direction, following the shape of the 50-meter isobath for 92 to 94 km. For station 6, the trajectories went north toward the coast. The trajectories from station 8 initially had a southwest direction that diverted northward toward the coast after a few days. At stations 7 and 10, the movement was nominal, with a direction toward the coast. At station 12, the trajectories had a southeast direction along the coast. Trajectories from those

stations (6, 7, 8, 10, and 12) occurred over an average distance of 24 km. All trajectories from adult spawning sites near the bottom were categorized as retention on the WFS.

Overall, the trajectories from stations in nearshore areas resulted mostly in short distance movement with retention on the WFS. Only at mid-water, two trajectories from a deep-water station (station 1) indicted potential export.

Fish fecundity and retention

Spawning females were found at seven of the twelve sampled sites (Figure 15A). I compared the sites with nominally larger batch fecundity to the ones with smaller batch fecundity. It might be expected that stations with higher batch fecundity would be more likely to result in retention (i.e., assuming natural selection for retention). When looking at the panel of trajectories at three depths for each spawning location concurrently, with the estimates of batch fecundity for those locations, no difference is evident in terms of retention or export between those with nominally higher batch fecundity and those with lower batch fecundity. At station 1, Vermilion Snapper batch fecundity was the highest (Table 5), and this site was categorized as resulting in retention on the WFS at the surface and near bottom and potentially exported at mid-water. Vermilion Snapper batch fecundity was almost 23 times lower at station 8, and that station was considered to result in retention on the WFS with potential export for a few trajectories at mid-water. For Sand Perch, the batch fecundity was 3.7 times lower at station 6 compared to station 10, with both stations 6 and 10 considered to result in retention at all depths on the WFS. From these observations, there was no consistent relationship between the sites with higher or lower batch fecundity and likelihood of retention or export.

Table 8. Distance of dispersal from the initial coordinates of sites to the last coordinates of trajectory after two weeks of tracking from the fish-egg collection sites.

	Dispersal distance (km)		
Station	Surface	Mid-water	Bottom
1	38.1	385.3	109.4
2	57.6	24.6	84.0
3	438.2	458.6	191.1
4	86.0	38.4	56.9
5	31.1	5.6	6.3
6	24.4	57.8	16.1
7	71.6	15.0	13.5
8	292.4	144.5	67.8
9	67.8	11.3	10.6
10	76.9	11.1	13.0
11	128.5	5.7	50.1
12	107.6	20.5	28.5
16	46.6	38.2	90.4
17	60.2	66.5	28.2
Average dispersal distance (km)	109.1	91.6	54.7
Range of dispersal distance (km)	24.4–438.2	5.6–458.6	6.3–191.1

Table 9. Characteristics of fish-egg surface trajectories from spawning sites.

Station	Description of trajectory	Categorization of movement
1	southeast then northwest towards the coast	retention
6	northwest along the coast	retention
7	north towards the coast	retention
8	northwest along the coast	retention
10	northwest then southeast and east towards the coast	retention
12	northwest then west and northeast towards the coast	retention

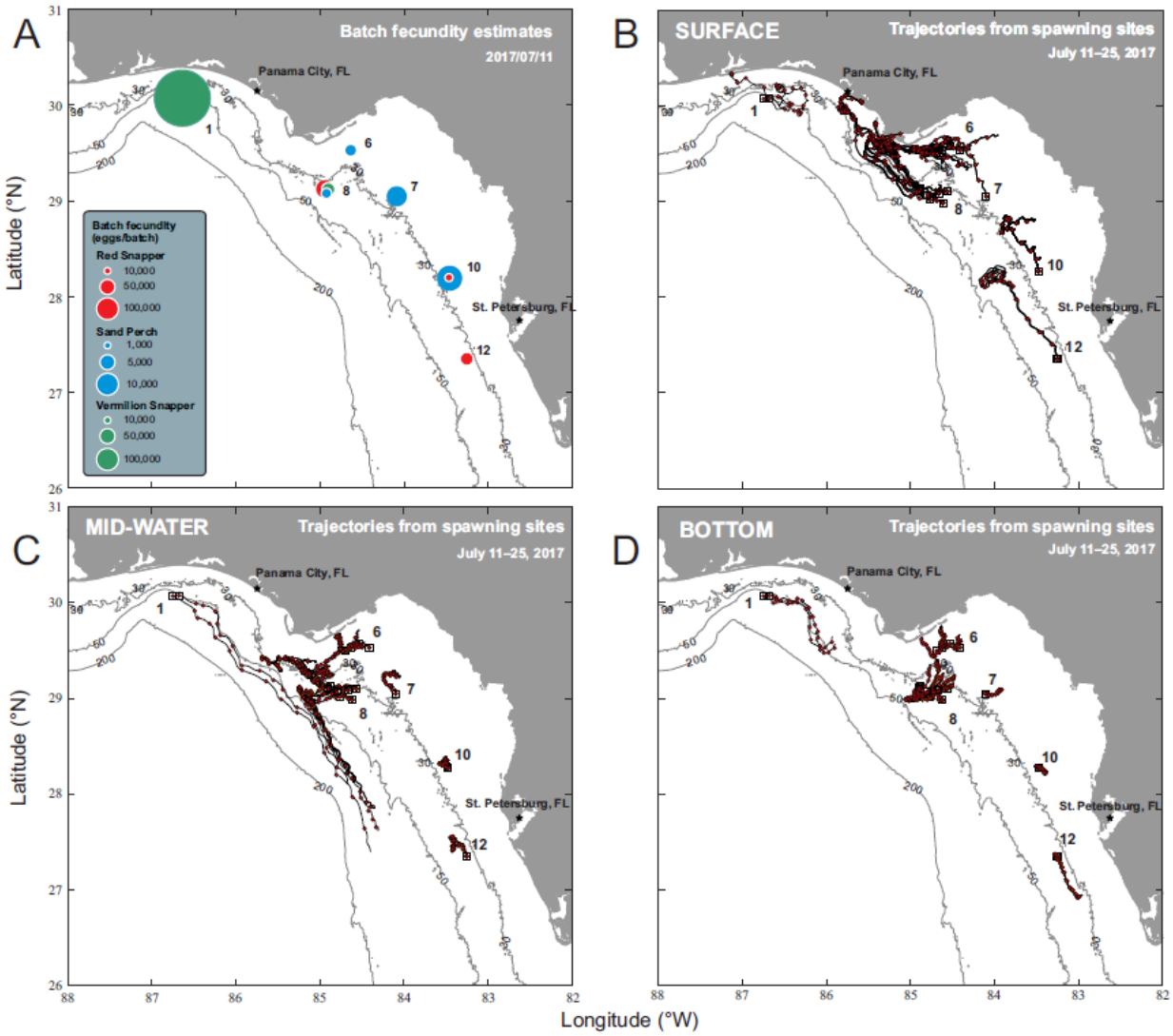

Figure 15. Fish-egg trajectories generated by the West Florida coastal ocean model (WFCOM). The trajectories were initialized from the adult spawning sites. The trajectories suggest a retention pattern toward and along the coast. A. Batch fecundity estimates per site of adult female fish on the northern WFS. B. Surface trajectories. C. Mid-water column trajectories. D. Bottom trajectories.

Table 10. Characteristics of fish-egg mid-water trajectories from spawning sites.

Station	Description of trajectory	Categorization of movement
1	east and southeast along 50-meter isobath	potential export
6	north or southwest	retention
7	northwest then east	retention
8	northwest or east and southeast	retention
10	nominal movement	retention
12	northwest then south	retention

Table 11. Characteristics of fish-egg bottom trajectories from spawning sites.

Station	Description of trajectory	Categorization of movement
1	east and southeast along 50-meter isobath	potential export
6	north towards the coast	retention
7	nominal northeast movement towards the coast	retention
8	southwest then northeast toward the coast	retention
10	nominal movement	retention
12	southeast along the coast	retention

Tracking based on fish-egg collection sites

Trajectories from sites that were closer to the coast on the inner shelf (on the shallower side of the 50-meter isobath) seemed to result in retention near the originating stations and on the WFS, compared to trajectories from sites that were farther out on the WFS (where the ocean floor was deeper than the 50-meter isobath) that appeared to be exported away from the WFS.

At the surface (Figure 16B and Table 12), we observed that the trajectories from stations 2 to 7, 9 to 12, and 16 had a northwest direction for the first 7 to 10 days. The trajectories then took a direction toward the coast whether it was northward, northeastward or eastward. Trajectories from those stations indicated fish eggs and larvae collected from those stations would have most likely been retained close to the areas where they were spawned. For station 1, the direction of the trajectories was southwest for three days and then went northeast toward the

coast. Those trajectories are considered retained near station 1 and on the WFS. Trajectories from stations 3 and 8 were southeastward and could result in export from the WFS because of the long-distance transport of 292 to 438 km. The trajectory from station 17 was tracked over six days before leaving the WFCOM domain. This trajectory most likely resulted in export.

At mid-water (Figure 16C and Table 13), we observed that the trajectories from stations 1, 3, and 8 had a southeast direction along the WFS, parallel to the coast. Those trajectories indicated passive particles could have traveled long distances (as long as 458 km) over a two-week period. Fish eggs and larvae that followed those trajectories would be considered exported from the WFS because of the long-distance they traveled in a short period. Trajectories from stations 2, 4, to 7, 9 to 12, and 16 had nominal movement. The observed trajectory from station 4 had a northwest then southeast and west direction. At station 8, the trajectory had a southwest direction and followed the shape of 30-meter isobath. The trajectory from station 16 had a northward direction. Fish eggs and larvae from those stations were more likely to have been spawned and have remained in the same areas. They can be considered to have been retained on the WFS. The trajectory from station 17 was tracked over four days before leaving the WFCOM domain. The trajectory most likely resulted in export.

Near the bottom (Figure 16D and Table 14), the observed trajectories from stations 2, 4, 8, and 11 had a southeast direction where the fish eggs and larvae seemed to be transported over short distances. The movement appeared to result in retention on the WFS. Trajectories initialized from stations 5, 6, 7, 9, 10, and 12 had very nominal movement, and eggs and larvae collected from those stations were considered to have been likely retained on the WFS; the larvae traveled only short distances of 15 km on average. At stations 1 and 3, the trajectories could potentially indicate export from the WFS, with the trajectory from station 1 having a

direction toward the open Gulf of Mexico over 109 km and trajectory from station 3 having a length of 191 km. At station 16, the trajectory appears to indicate export. The trajectory from station 17 was tracked over six days before leaving the WFCOM domain. That trajectory most likely resulted in export.

Overall, at all depths, the trajectories simulated from inshore, shallow-water stations appeared to result in retention on the WFS. In contrast, trajectories that were generated from offshore, deep-water stations appeared to result in potential export from the WFS. The strongest potential export of fish eggs and larvae away from the WFS was trajectories from the most offshore stations at the surface and at mid-water.

Table 12. Characteristics of fish-egg surface trajectories from fish-egg collection sites.

Station	Depth (m)	Description of trajectory	Categorization of movement
1	120	southwest for 3 days then northeast towards the coast	retention
2	34	northwest for 7 to 10 days then northeast towards the coast	retention
3	298	southeast along the WFS	potential export
4	30	northwest for 7 to 10 days then northeast towards the coast	retention
5	10	northwest for 7 to 10 days then northeast towards the coast	retention
6	22	northwest for 7 to 10 days then southeast	retention
7	21	northwest for 7 to 10 days then northeast towards the coast	retention
8	43	northwest for 3 days then southeast along the coast	potential export
9	16	northwest for 7 to 10 days then northeast towards the coast	retention
10	19	northwest for 7 to 10 days then northeast towards the coast	retention
11	39	northwest for 7 to 10 days then northeast towards the coast	retention
12	29	northwest for 7 to 10 days then remains in the same area going southwest and northeast	retention

Table 12 (Continued)

Station	Depth (m)	Description of trajectory	Categorization of movement
13	397	outside domain of WFCOM	N/A
14	285	outside domain of WFCOM	N/A
15	323	outside domain of WFCOM	N/A
16	59	northwest for 7 to 10 days then southeast along the coast	retention
17	412	southeast along the WFS for 3 days before going off domain of WFCOM	potential export

Table 13. Characteristics of fish-egg mid-water trajectories from fish-egg collection sites.

Station	Depth (m)	Description of trajectory	Categorization of movement
1	120	southeast along the WFS	potential export
2	34	southeast then northwest	retention
3	298	southeast along the WFS	potential export
4	30	northwest for 7 days then southeast	retention
5	10	nominal movement	retention
6	22	southwest movement	retention
7	21	nominal movement	retention
8	43	southeast along the WFS	potential export
9	16	nominal movement	retention
10	19	nominal movement	retention
11	39	nominal movement	retention
12	29	nominal movement	retention
13	397	outside domain of WFCOM	N/A
14	285	outside domain of WFCOM	N/A
15	323	outside domain of WFCOM	N/A
16	59	northwest movement	retention
17	412	southeast along the WFS for 4 days before going off domain of WFCOM	potential export

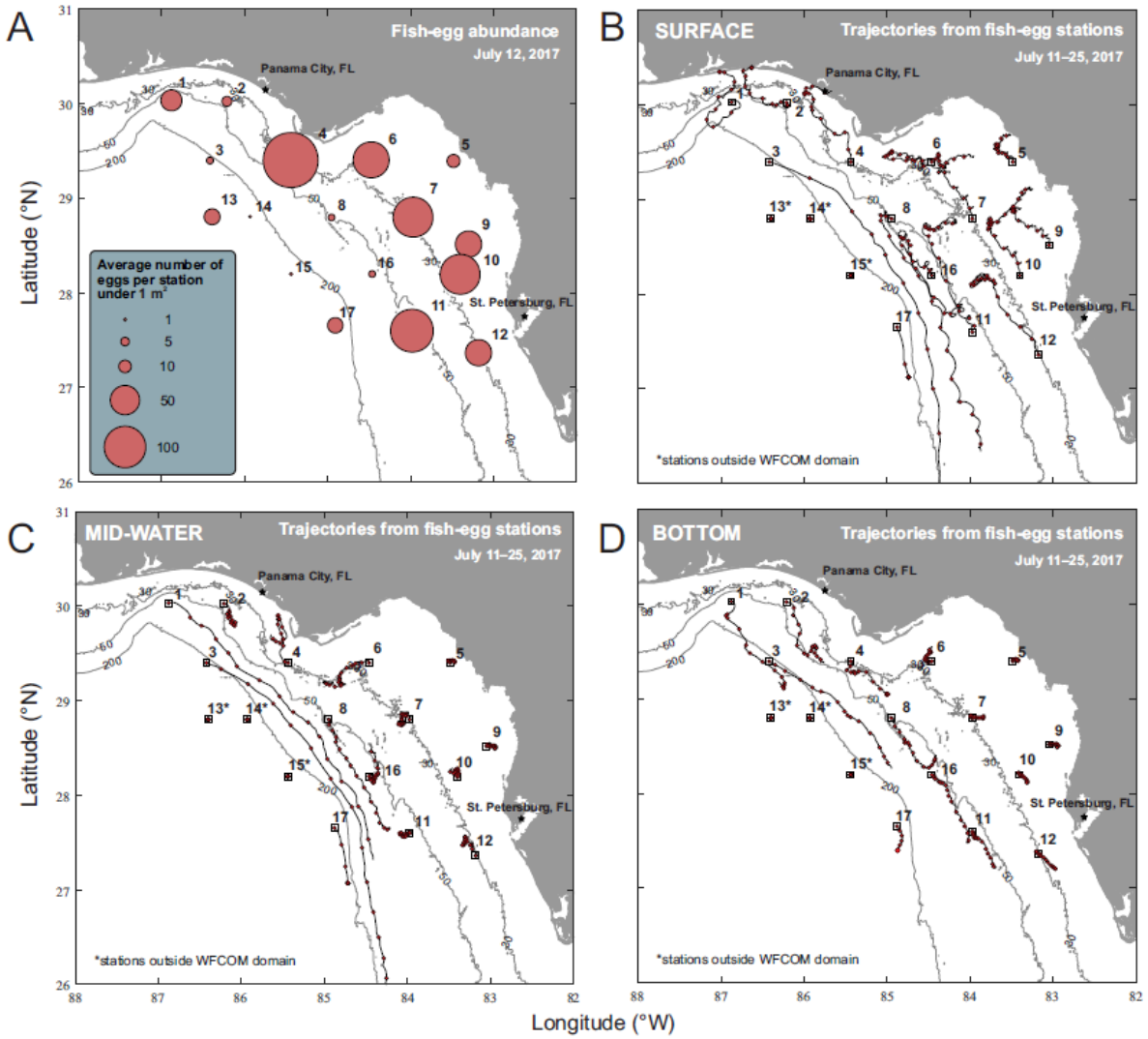

Figure 16. Fish-egg trajectories generated by the West Florida coastal ocean model (WFCOM). The trajectories were initialized from the fish egg collection sites. The trajectories suggest a retention pattern toward and along the coast. A. Fish-egg abundance per site on the northern WFS. B. Surface trajectories. C. Mid-water column trajectories. D. Bottom trajectories.

Table 14. Characteristics of fish-egg bottom trajectories from fish-egg collection sites.

Station	Depth (m)	Description of trajectory	Categorization of movement
1	120	southeast towards open ocean	potential export
2	34	southeast along the coast	retention
3	298	southeast along the coast	Potential export
4	30	southeast along the coast	retention
5	10	nominal movement	retention
6	22	nominal movement	retention
7	21	nominal movement	retention
8	43	southeast along the coast	retention
9	16	nominal movement	retention
10	19	nominal movement	retention
11	39	southeast along the coast	retention
12	29	southeast towards the coast	retention
13	397	outside domain of WFCOM	N/A
14	285	outside domain of WFCOM	N/A
15	323	outside domain of WFCOM	N/A
16	59	southeast along the coast	potential export
17	412	southwest for 6 days before going off domain of WFCOM	potential export

Fish-egg abundance and retention

From Figure 16A and Table 15, fish-egg abundance appeared higher closer to shore on the inner shelf. At station 4, fish-egg abundance was the highest (130 eggs under 1 m^2) and interestingly, when concurrently looking at the trajectories at all depths from stations 6 and 8 that are in the vicinity of station 4 (Figure 15 and 16), it is apparent that they converged to locations near station 4. Generally, stations with nominally higher fish-egg abundance (6, 7, 9, 10, 11, and 12) had trajectories with nominal movement at mid-water and near the bottom and were considered to result in retention. Also, the surface trajectories from those stations, for the majority, had a northwest and then toward-the-coast direction, where fish eggs and larvae would also be considered to be retained. Stations with lower fish-egg abundance (1, 2, 3, 8, 16, 17)

were farther out on the WFS and corresponded with trajectories that resulted in apparent export. This suggests a relationship exist between retention and locations with higher fish-egg abundance.

Table 15. Number of fish species identified per station and average number of eggs under 1 m2 per station.

Station	Number of species identified per station	Average number of eggs per station under 1 m^2
1	4	46.99
2	5	21.50
3	4	12.74
4	7	130.61
5	7	31.86
6	13	82.83
7	10	87.61
8	9	16.72
9	10	59.73
10	7	92.38
11	11	99.55
12	5	55.75
13	1	35.84
14	0	3.98
15	0	7.17
16	2	14.34
17	0	40.62

Pelagic versus non-pelagic species

The results for the SIMPROF analysis are summarized in Table 16 and Figures 17, 18, and 19. Figure 17 is an nMDS plot that shows the groupings of stations according to their Bray-Curtis similarity. Figure 18 is a heatmap of the fish-egg taxa, with a dendrogram indicating species associations and vertical lines identifying statistically significant station associations

(SIMPROF groups). Magenta rectangles indicate pelagic species. Pelagic species were found in all four groups of stations and were found at 12 stations out of 14.

Table 16. Grouping output from SIMPROF analysis in PRIMER 7 with the number of species per group.

Group	Stations	Number of species per group
a	6, 12	14
b	1, 2, 3, 4, 7, 8, 10, 11, 13, 16	25
c	5, 9	16

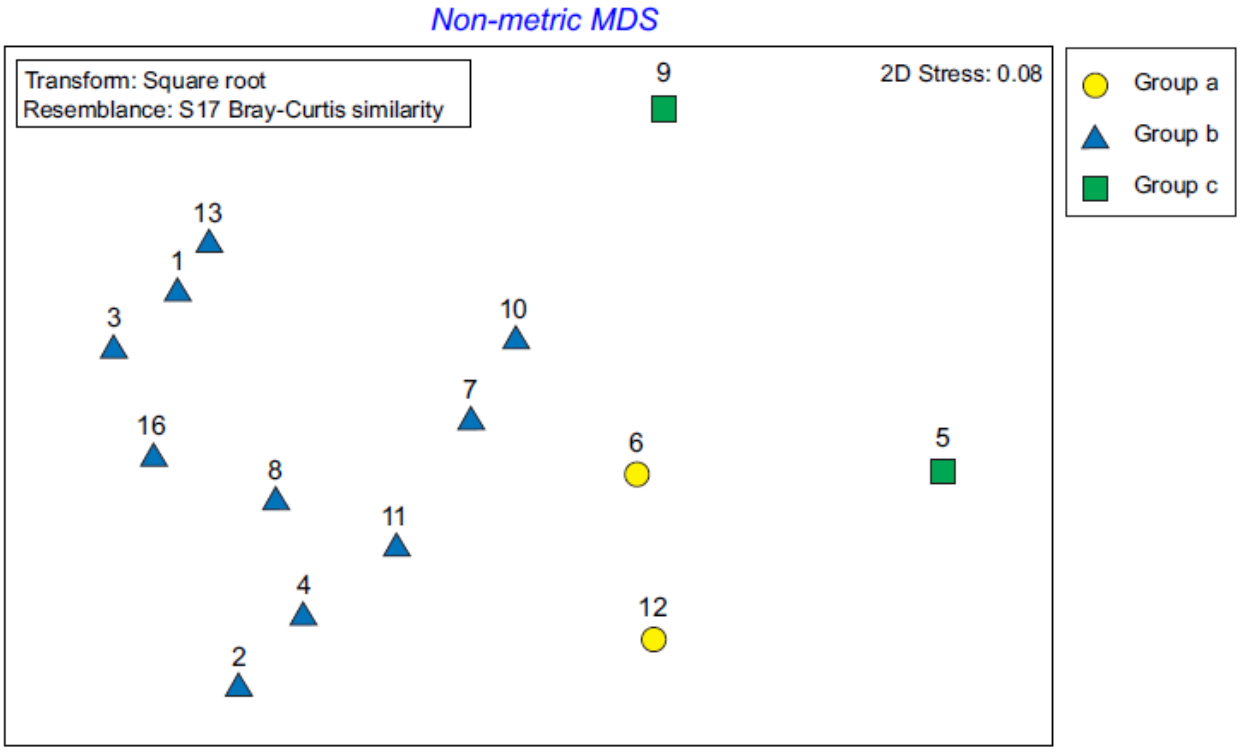

Figure 17. A non-metric multidimensional scaling (nMDS) ordination of fish egg species distribution by collection sites on the northern West Florida Shelf. The fish species were grouped by abundances, square-root transformed and standardized, and a Bray-Curtis similarity calculated on the resulting fish species composition per collection site.

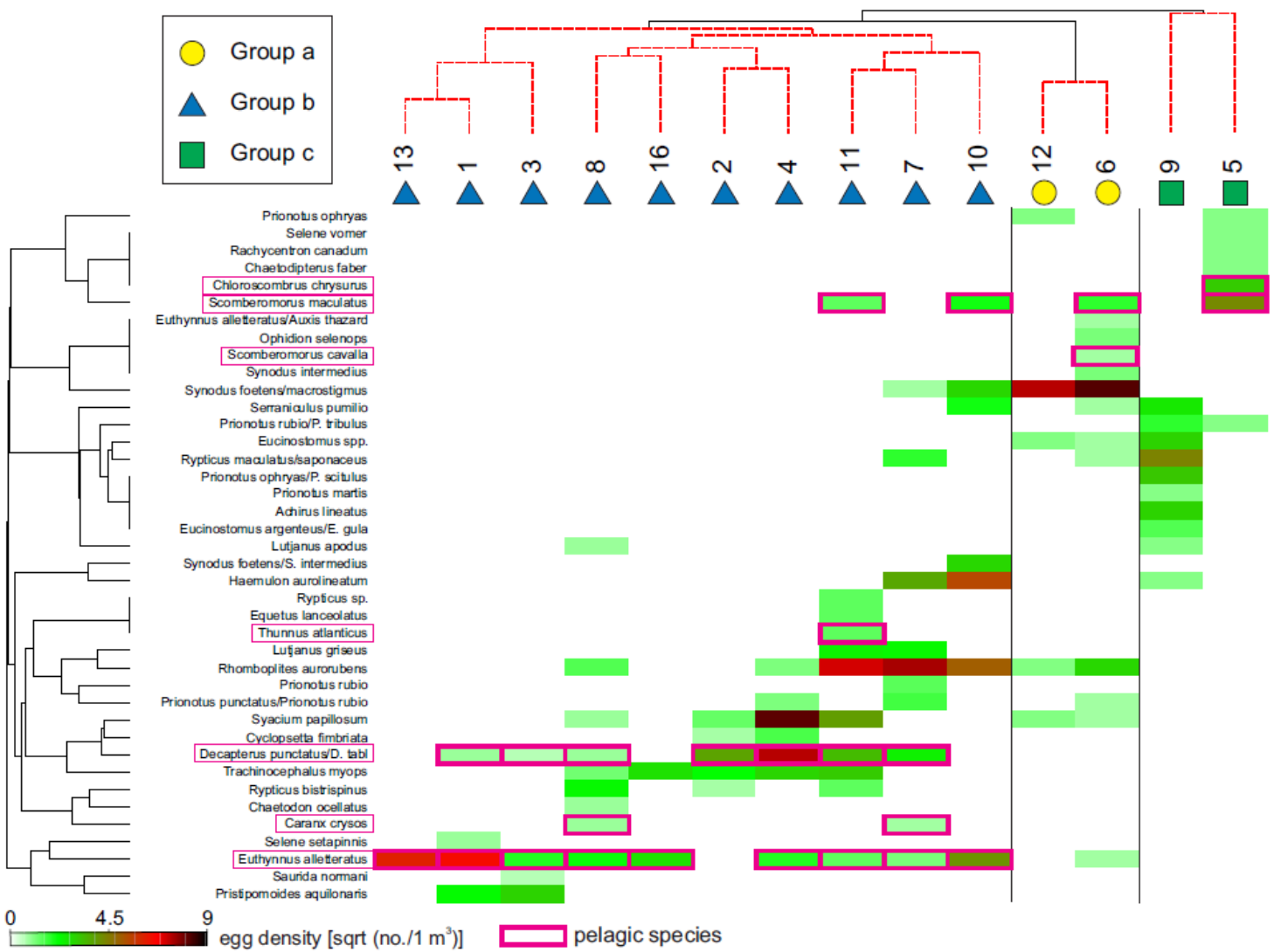

Figure 18. Seriated heatmap of the fish-egg taxa, with a dendrogram indicating species associations (y-axis) and vertical lines identifying statistically significant station associations (SIMPROF groups). On the x-axis, the dendrogram indicated station associations and distinguished four groups. Magenta rectangles indicate pelagic species.

Community structure

The SIMPROF analysis produced 7 groups within the 17 stations. Three stations (14, 15, 17) did not have identifiable fish eggs from the DNA sequencing, and we removed these stations from the analysis. The analysis was therefore reduced to three groups within 14 stations. For each of the three groups, the stations were mapped with a unique symbol in Figure 19. From the representation, a geographic grouping can be observed from west to east and from deep to shallow waters, with group *a* being the farthest west and in deeper water than groups *b* and *c*, in that order.

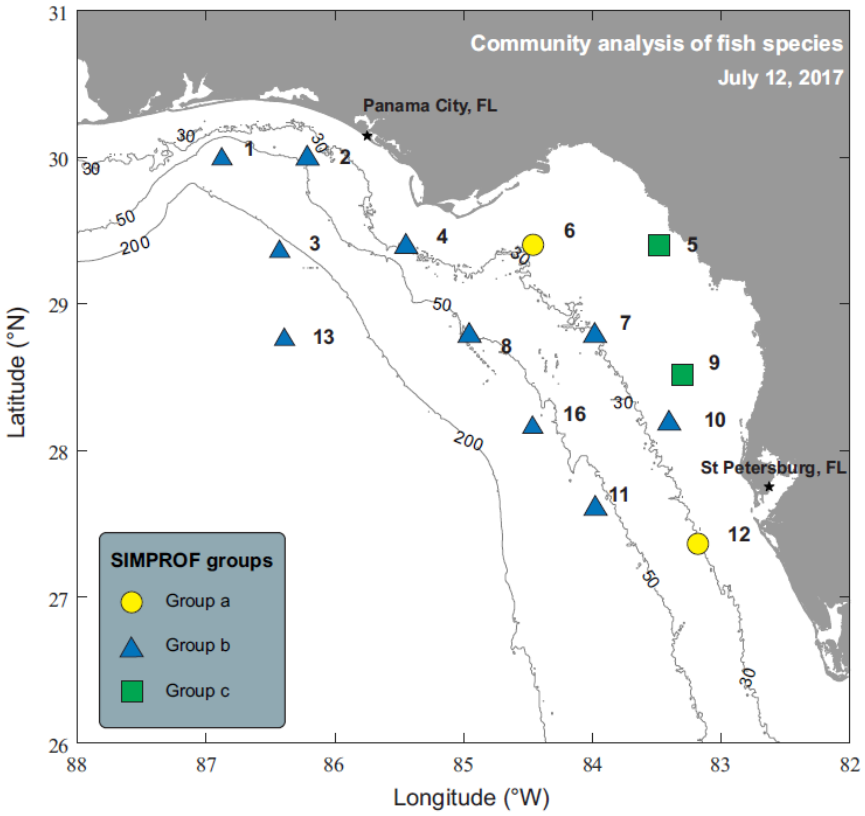

Figure 19. Geographical representation of community analysis of fish species identified via DNA barcoding of fish eggs. The station grouping emerged from Bray-Curtis resemblance (SIMPROF groups). The stations with similar species were grouped together. A depth-related association of stations can be observed in this representation.

2.4 DISCUSSION

Two patterns of trajectories were observed on the WFS: trajectories initiated from stations that were on the inner shelf and shallower waters (inshore of 50-meter isobath) more likely resulted in retention on the WFS, whereas trajectories initiated from stations that were on the outer shelf and deeper waters (offshore of 50-meter isobath) more likely resulted in export away from the WFS. Trajectories with two types of origins were simulated at three different depths (surface, mid-water, and bottom) by WFCOM: the ones initiated from the spawning sites that were identified in chapter 1 and the ones initiated from the fish-egg stations nearby the spawning sites. From maps of trajectories, we analyzed the relationship between ocean circulation, batch fecundity, fish-egg abundance, and community composition.

The first hypothesis examined the potential retention and export of fish eggs. Evidence of export of fish eggs and larvae away from the WFS were found. There were two dominant trends in the trajectories depending on the depth of water. In areas with a depth of less than about 50 m, the trend was retention on the WFS; and in areas with depth of more than 50 m, the trend was export away from the WFS. The null hypothesis, stating that fish eggs and larvae on the WFS are not likely to be exported, was rejected.

It is important to consider the general ocean circulation features of the WFS when interpreting those trajectories. In general, the WFS is upwelling-favorable, with seasonal variability. The circulation on the WFS is known to be influenced by multiple hydrodynamic features. In particular, the circulation in shallow water is influenced by wind forcings, whereas the circulation in deeper waters on the outer part of the WFS is influenced by the Loop Current and its eddies. To be more specific, in the summer months, the southerly winds have a stronger influence on the surface currents, especially in shallow water (Liu and Weisberg 2012). With

Ekman transport, the deflection of the surface current in shallow water should be 45-90° (depending on depth) to the right of the current toward the coast. From this, water circulation would result in downwelling. However, from the trajectories in shallow water, at mid-water, and near the bottom, there was no strong evidence of downwelling and movement away from the coast and toward the open ocean during time periods considered here. Two explanations are plausible in this case: the winds were not strong enough to drag surface water and create a surface current strong enough to result in downwelling of water, or the influence of the Loop Current was stronger than the downwelling process and countered it, slowing the water flow at shallow depths. Daily-averaged winds for our observation time period had the same patterns as the surface trajectories and therefore confirmed that surface water flow was influenced primarily by winds (Figure 20).

On the outer part of the WFS, the circulation is influenced by several factors such as winds, eddies, and the Loop Current (Weisberg et al. 2005). The latter has the strongest influence on the outer shelf, even though its influence varies annually depending on how northward the Loop Current penetrates into the Gulf of Mexico. The trajectories that were initiated from stations at deep water were highly influenced by the Loop Current.

Overall, fish eggs and larvae are more likely to be dispersed and transported over long distanced by large-scale circulation features and they are more likely to be concentrated locally by small-scale processes and the interaction of those processes and currents with bathymetry (Paris and Cowen 2004). In general, the observed trajectories in shallow and deep water are consistent with the physical attributes of the WFS circulation.

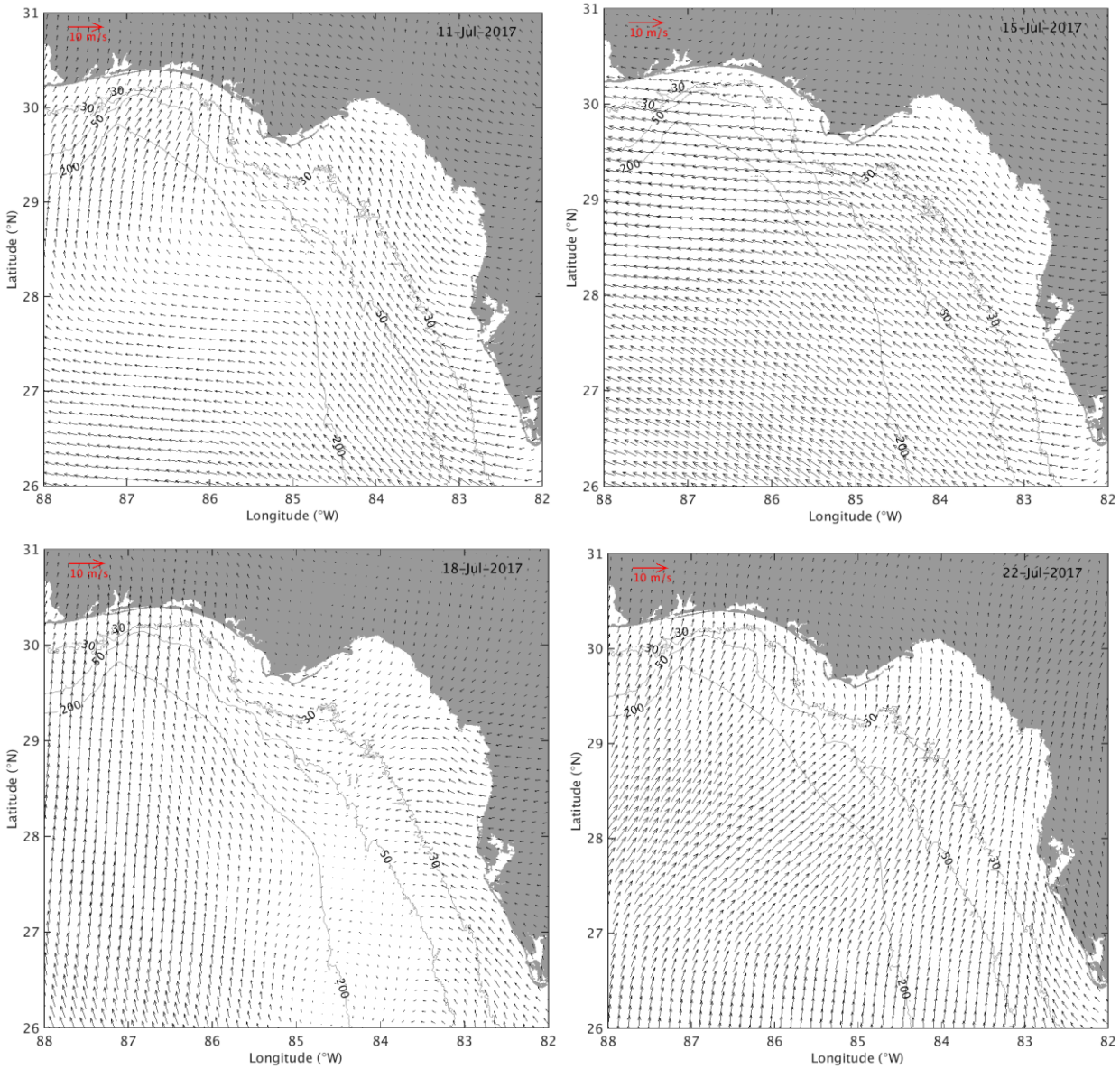

Figure 20. Daily-averaged winds over the northern WFS for July 11, 15, 18, and 22, 2017.

Moreover, it is important to note that categorizing the trajectories cannot be solely based on hydrodynamic models. In this study, the trajectories were categorized according to relative retention versus export, and not to the further level of aberrant drift and connectivity that are sub-categories of export. More specifically, to make inferences on connectivity, biophysical models are often used because of the incorporation of hydrodynamic data from ocean circulation (e.g.,

current and other environmental parameters) and biological data (e.g., pelagic larval duration and larval behavior; Abesamis et al. (2016)). In addition, establishing connectivity would require larger-scale modeling to include potentially connected areas such as North and South Carolina's continental shelf.

The offshore trajectories indicated a strong advection of fish eggs and larvae. Indeed, the distance of dispersal was up to 458 km, reaching the southern half of the WFS, in a period of two weeks. In the study of gag larval transport by Weisberg et al. (2014), trajectories were simulated over 45 days, accounting for the approximate age of individuals approaching coastal nursery habitats. This suggests that the long-distance offshore movement of fish eggs potentially resulting in export could possibly reach the Florida Keys and be entrained in the Florida Current and continue its course in the Gulf Stream up to the Carolinas where similar fish assemblages occur. The Carolinas are known to be a harvest region of the snapper-grouper complex (Overton et al. 2008). Connectivity between the WFS snapper-grouper complex and the one from the coast of North and South Carolina could exist because of the possible export of the fish eggs and larvae away from the WFS.

Recently, through trajectory simulation of particles on the WFS at the surface layer, Weisberg et al. (2019) identified the process that was responsible for the presence of *K. brevis* on the east coast of Florida. Strong continuous upwelling after bloom initiation ensured transport to the nearshore along the bottom followed by subsequent export offshore at the surface and eventual transport around the Florida Keys and into the Florida Current to end up in the Gulf Stream and along Florida's east coast.

Specifically, for July of 2017, the penetration of the Loop Current into the Gulf of Mexico was far northward, and the northern part of the loop was in close contact with the shelf

break. Based on the trajectories originating from station 17, we observed that only three days at the surface and mid-water and six days at the bottom were represented before the trajectory went outside of the WFCOM domain and was not trackable anymore. Observation of the shape and movement of the Loop Current for that summer indicated advection of water from the WFS into the Loop Current and potential export of fish eggs and larvae that were spawned close to the shelf break. Those eggs and larvae could then be entrained in the strong currents described above (i.e., Florida Current, Gulf Stream) and brought to the Carolinas in a few weeks, where they could settle. This time period can be estimated by considering the average velocity of 0.8 $m.s^{-1}$ for the Loop Current (Milliman and Imamura 1992), 1.9 $m.s^{-1}$ for the Florida Current (Niiler and Richardson 1973), and 2.5 $m.s^{-1}$ for the Gulf Stream (Shanmugam 2011). The distance between the point of contact between the Loop Current and the shelf break and an area on the shelf off the coast of North Carolina can also be estimated. The calculations resulted in an approximate period of transport of two weeks. This time period is reasonable for hypothesizing that, in the summer of 2017, when the Loop Current came into close contact with the WFS break, fish eggs and larvae that were spawned in that area could have been advected into the strong current and transported passively to North Carolinas where they would eventually settle. This phenomenon of potential export and connectivity is evidently highly variable depending on the years and seasons because it is associated with the shape and intrusion of the Loop Current into the Gulf of Mexico.

Furthermore, the 15-year egg monitoring project would cover the entire WFS. Water on the WFS can be transported southward through an exit area west to the Dry Tortugas, in the Florida Keys (Weisberg et al. 2019). This location would be another way for WFS fish eggs and larvae to exit the shelf and end up in the strong Florida Current, which would then entrain and

export them away from areas on the shelf where they were spawned. Again, this transport of water through the exit zone is likely to be highly variable both interannually and seasonally, and thus ocean circulation in this area should be closely monitored.

Other evidence of this potential "shelf exit" in the Florida Keys was described by Burrows et al. (in review). DNA barcoding of fish eggs collected along a transect from the WFS to Cuba (i.e., across the Straits of Florida) distinguished reef-associated fish species from the pelagic-associated species. The results indicated the presence of reef-associated species commonly found on the WFS in deeper water within the Straits of Florida. This was due to the presence of a mesoscale cyclonic eddy that introduced water from the WFS into the Florida Current. That study is an additional demonstration of how considering ocean circulation in combination with biological data is fundamental to understanding how all processes are working together and would be another hypothesis regarding connectivity of the WFS and the Carolinas. In addition to creating an index for fisheries management over time, the annual FLRACEP egg survey could help answer questions regarding export and connectivity.

The second hypothesis inspected the relationship between the batch fecundity and retention. There is no apparent relationship between batch fecundity and whether the eggs are retained on or exported off the WFS. Batch fecundity was not found to be a good indicator of where the eggs and larvae will end up. However, from Chapter 1, we observed that the spawning sites of the fish appeared to be closer to shore than to the shelf break. There is evidence that females have a tendency of not spawning in areas where export away from the shelf is common, but rather spawn in locations that result in retention. This knowledge can be taken into consideration for future interpretation of trends in fish eggs survey and fine-tuned over the years, with repeated simulations of trajectories from the collection sites.

The third hypothesis investigated fish-egg abundance in relation to retention. Fish-egg abundance appeared higher closer to shore, where the majority of fish egg collection sites resulted in retention. The fish-egg abundance was nominally lower at sites in deeper water where trajectories were more likely to result in export. Also, there was a convergence of retention trajectories from spawning sites (stations 6 and 8) to the area near station 4 where the highest number of fish eggs was found. There is an apparent relationship between egg abundance and retention, and we can reject the null hypothesis stating that sites with higher egg abundance were not more likely to result in retention. Note that this could represent increased spawning in these areas, increased drift convergence, or both processes acting together. This finding is consistent with the concept of self-recruitment that is common in the ecology literature. An increase in self-recruitment is generally associated with retention zones that are found adjacent to the coast. It has been suggested that these zones tend to retain eggs and larvae because of interactions between circulation and topography (e.g., bays, reefs and other bottom features; Gawarkiewicz et al. (2007)).

The fourth hypothesis was related to pelagic versus non-pelagic species. Pelagic species were thought more likely to be exported than non-pelagic fish species. No evidence of this was found during the present study. The pelagic species were found at most of the stations whether they were retained or exported, and the SIMPROF analysis found pelagics to be represented in all community groups.

Lastly, the fifth hypothesis inquired about an eventual structure in the community of fish egg species. The SIMPROF analysis brought insight in the form of geographic station groupings based on taxonomic composition. The analysis indicated a geographical grouping from west to east (from deep to shallow water). Even though physical and chemical properties were relatively

non-changing, the topography or other currently unidentified factors seemed to influence the species composition at the different stations on the WFS. Species found in deeper water were not likely to be found in shallow water and vice versa. The sites that were geographically closer together were home to similar species. Because there was depth-related variation in community structure, the fifth null hypothesis was rejected. This is consistent with findings by Huelster (2015) and Huelster and Peebles (2019). From stable-isotopic values from muscle tissue, these authors found a separation between nearshore and offshore energy pathways that coincided with changes in community structure. As in the present study, the Huelster (2015) SIMPROF analysis, which was based on SEAMAP trawl data, also separated fish communities into shallow and deep components. The reef-fish component (snappers, grunts, porgies) favored the inner WFS. The analysis was repeated for 11 years from 2008 to 2018, and a similar shallow-deep division in community structure was found in 10 years out of 11 (Huelster and Peebles 2019). In some years, it appeared that the shallow-water species extended the spatial area over which they were found, farther toward deeper water. Other years, the reef-fish community was narrowly restricted to shallower waters. It is interesting to note that similar patterns were found with adults and fish eggs.

In summary, the main findings of this chapter are:

1. Shallow-water trajectories resulted in retention and deep-water trajectories resulted in potential export.
2. There was no apparent relationship between high batch fecundity and retention.
3. There was higher egg abundance in shallow water and there was also a relationship between higher egg abundance and retention. There was a convergence of trajectories

to a region with higher fish-egg abundance, which was due to either retention or more spawning occurring in those areas.

4. Pelagic species were not found to have their eggs more likely to be exported than non-pelagics.
5. The SIMPROF analysis generated groups that indicated a depth-related community structure, similar to that observed in SEAMAP trawl communities.

This study serves as a starting point by merging ocean circulation on the WFS with fish-egg abundance data. However, it was conducted using data collected during just one day. Repeated efforts at different times would be needed to increase certainty in the generality of the results presented here.

Over the years, multiple studies have acknowledged that oceanographic processes and physical features can potentially have an influence on the recruitment success of fish stocks (Hinrichsen et al. 1997). DNA barcoding is highly reliable in species identification of fish eggs (Burghart et al. 2014; Burrows et al. 2018; Ward et al. 2009), and tracking of movement via numerical models can be done efficiently in a timely manner once initial coordinates are identified and put into the model. The use of a novel approach combining DNA barcoding with trajectory simulations in a numerical model confirms that integrating multiple disciplines of marine science and using diverse methods may improve the utility of future surveys. The present approach provides a good starting point for future, interdisciplinary egg-monitoring projects.

CONCLUSIONS

The goal of chapter 1 was to use biological methods to investigate fish fecundity and definitively locate spawning sites. Adult female parameters were studied and brought new data that could be useful as future reference for fisheries managers. In chapter 2, the goal was to use the data generated from chapter 1 along with DNA barcoding species identification as input in a numerical model to predict the drift trajectories of fish eggs and larvae of fish species found on the WFS and aid in the interpretation of future abundance surveys. This research contributed to the identification of patterns of larval dispersal: retention or export from the WFS.

The research allowed us to have a first reference point for creating a time series that can be used as an index to assist in future stock assessments. Additionally, the combination of DNA barcoding with modeling of trajectory of drifting fish-eggs provides a new investigation method for fish-egg surveys; it allows accurate species identification and the trajectory modeling is a practical technique for tracking the exportation of drifting eggs, thus providing a level of certainty regarding egg abundance estimates and trends over time. Batch fecundity data, generated along with the completion of the first objective of locating spawning sites, were not a good indicator of larval movement but are relevant to fisheries management for calculation of parameters related to stock assessment.

In Chapter 1, the macroscopic examination for presence of hydrated oocytes and the histological analysis of ovary tissue for the presence of recent POFs allowed to identify spawning sites. The hydrated occytes were counted and batch fecundity was estimated via the

volumetric method, which was found to be more accurate and more precise than the gravimetric method. Batch fecundity (number of hydrated eggs per batch ± standard error) results were as follows: 101,293 ± 55,787 for Vermilion Snapper, 75,119 ± 19,802 for Red Snapper, and 8,272 ± 1,869 for Sand Perch. Spawning time windows were partially determined and can be useful for ideal fishing times in future surveys. Sand Perch fecundity was examined in more detail due to the presence of recent POFs in all the females that were caught. This observation was explained by the presence of an accessory structure in the anatomy of this simultaneous hermaphrodite species that is used for storage of hydrated oocytes and for resorption of unused eggs. It was deduced that the hydration process should be considered as species-specific and that the presence of recent POFs does not necessarily indicate recent spawning. Although with the Sand Perch being a territorial species, it could be assumed that the spawning sites were at or close to the location where the individual with recent POFs was caught. The spawning sites were successfully located and were used as initial coordinates for trajectory simulations.

In Chapter 2, the findings were:

1) WFCOM-simulated trajectories originating from nearshore (shallow water) stations generally resulted in retention on the WFS, whereas the trajectories originating from offshore (deep water) stations generally resulted in export over long distances.

2) Egg abundance appeared to be higher nearshore. A relationship between a higher egg abundance and retention was apparent and could be attributed to increased spawning in those areas, increased drift convergence, or both processes acting together. A region of trajectory convergence was identified, and it corresponded to the region with the highest egg abundance.

Females appeared to spawn preferentially nearshore where eggs would be retained rather than offshore where they could be exported by ocean currents.

3) However, batch fecundity was not found to be a good indicator of eggs and larvae destination.

4) With DNA barcoding, the fish eggs were identified to species and categorized as pelagics or non-pelagics. No evidence of whether pelagic species are more likely to be exported was found.

5) The SIMPROF analysis indicated a geographical grouping of the stations from west to east (from deep to shallow water) based on taxonomic composition. This result is consistent with previous findings by Huelster (2015) who did a similar analysis with SEAMP trawl data and found a shallow versus deep division in community structure.

FUTURE STUDIES

Although this research provides a model of methods combination involving biological and physical data and gives an initial set of data on a fish-egg survey, the work should be reiterated in future egg surveys with retention and export investigated each time because of changes in ocean circulation. In the long term, general patterns of retention or export would become clearer and provide more certainty to abundance trends. Given that fish spawn at different seasons, depending on species, sampling at different times of year (spring, summer, early fall) during future years could be beneficial by including a wider range of species. Our pilot study was focused on the northern WFS, whereas the survey will be extended to the entire WFS and conducted for up to 15 years.

The techniques employed in the present research have been previously established, but their combination is quite novel. The identification of fish egg species can be simplified by analyzing multiple fish eggs or all fish eggs collected at a station in one run of DNA barcoding (i.e., DNA metabarcoding). This method could provide identification of all the fish species encountered in a sample; however, it would result in presence-absence data and would thus lose information on the proportion that each species represented in that sample.

A practical feature of ocean modeling is the repeatability over time and space of the tracking. Projecting trajectories over entire seasons and over years can provide an idea of the patterns of retention or export in a region. These simulations alone are not sufficient for interpretation of connectivity patterns because they are modeled solely based on physical

oceanographic data. The simulations should be repeatedly compared to biological data and field sampling. Therefore, it would be informative to combine the modeled trajectories with field data for young recruits (as in Weisberg et al. 2014) and to repeat this effort over seasons and years. In other words, what was presented here may be considered to be a single ensemble member subject to a specific set of wind and Loop Current conditions and certain behavioral assumptions on water column location. Broadening the ensemble to include numerous start times (each with different wind and Loop Current conditions) and various behavioral attributes would result in a more robust set of conclusions.

If the concept of connectivity were to be thoroughly investigated, involving biophysical models that simultaneously integrate the two types of data (physics and biological behavior) would be ideal. Including DNA barcoding in the process would increase the level of specificity brought to the model and would provide better insight into the larval transport and connectivity of species of management concern.

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APPENDIX A: EGG COUNT METHOD WITH IMAGING SOFTWARE AND SIZE FREQUENCY ANALYSIS

Estimation of batch fecundity and size distribution of fish eggs with processing of egg images in ImageJ was attempted. Similar to the volumetric method, egg samples were suspended in water in a graduated cylinder. The cylinder was inverted to homogenize the distribution of eggs and a subsample of known volume was poured into a petri dish. Images of the eggs were taken and analyzed with ImageJ.

The procedure was time consuming and the software was unable to distinguish some of the oocyte stages. For example, some fish species presented with mature hydrated oocytes that had the same diameter as immature non-hydrated oocytes. Those hydrated oocytes can also appear opaque with a clear space between the yolk and the chorion making it challenging for the software to distinguish between hydrated and non-hydrated oocytes. The human eye is more adept at separating elements displaying only slight differences.

The egg size distribution for a single Vermilion Snapper is reported in figure 21. The distribution revealed three modes, indicating the presence of three size classes of eggs within the ovaries. A multiple size class distribution is typical for a batch spawner (Crampton 2008).

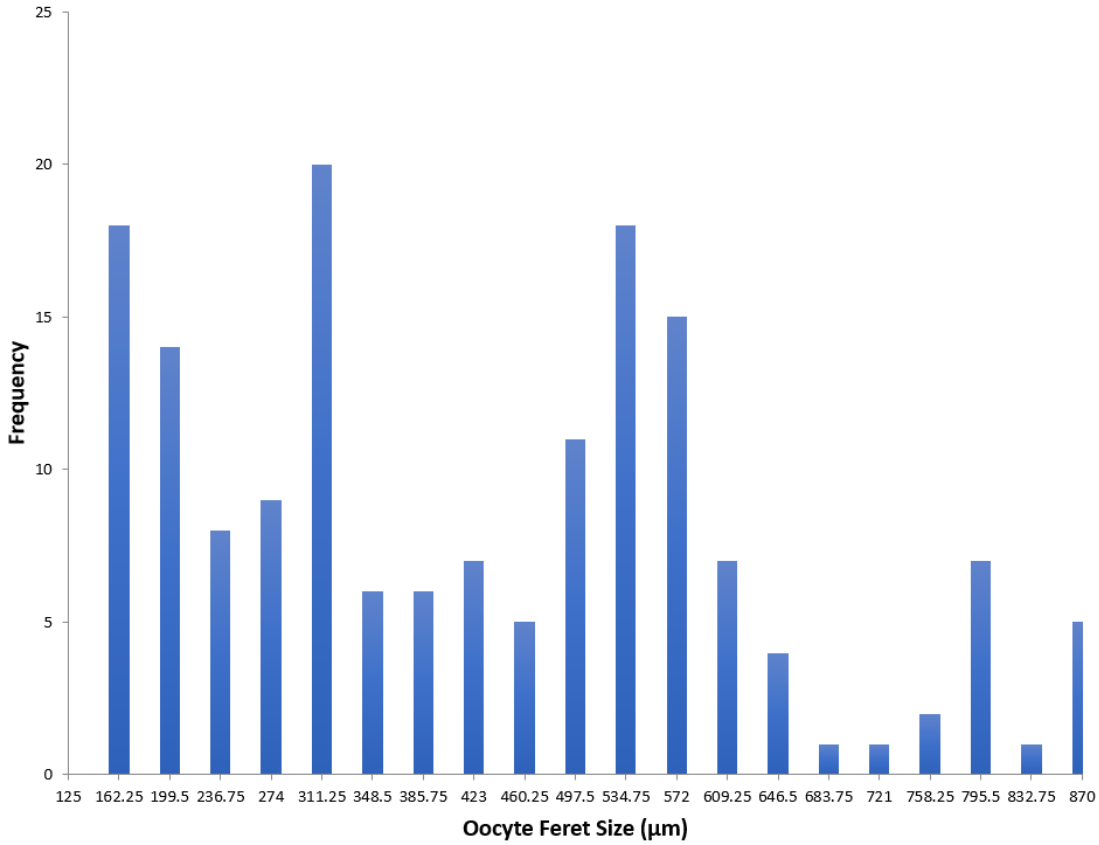

Figure A1. Vermilion Snapper egg size distribution via processing of egg images in ImageJ.

APPENDIX B: FRONTAL DENSITY MAPS

Maps of ocean fronts from color-indexed images of monthly average frontal density for June-July 2017 were obtained (Figures B1 to B6). Satellite images were produced from data collected by MODIS Aqua from NASA (https://oceancolor.gsfc.nasa.gov). Novel color index (CI) data products were derived following the approaches described in Hu (2011). Ocean fronts were then detected from CI satellite imagery using a modified Canny edge-detection method (Canny 1986; Zhang et al. 2019). Figures were finally plotted using MATLAB R2017a (http://www.mathworks.com/) with the mapping package M Map (http://www.eos.ubc.ca/~rich/map.html). Adult fish collection sites were plotted for each species of interest. The sites where the average batch fecundity was higher and where there was a higher abundance of fish eggs were similar and were located closer to shore. When overlaying maps of chlorophyll *a* concentration and ocean fronts, the most abundant sites for fish eggs and batch fecundity tended to be closer to areas with high chlorophyll *a* and close to ocean fronts. An area with high chlorophyll *a* is an indication of convergence or else high nutrient levels. Moving up the food web, small fishes and crustaceans would also gather closer to the food sources and become, in turn, sources of food for the reef-fish. As Peebles et al. (1996) established, fish fecundity is dependent on the female energetic state. The better females feed and the more energy they have, the more fecund they will be and the more eggs they will produce and release. With this knowledge, it can be speculated that the patterns of higher batch fecundity and higher fish-egg abundance closer to the coast correspond to higher and more nutritive food sources present in shallow waters. From figures B5 and B6, with an overlay of batch fecundity and egg

abundance respectively, it can be observed that there is a higher fish-egg abundance closer to ocean fronts. However, this relationship was not observed for batch fecundity. Generating maps of ocean fronts repeatedly for the periods of time that cover surveys could give more insight into the locations of spawning sites and indicate whether fish would tend to spawn near ocean fronts.

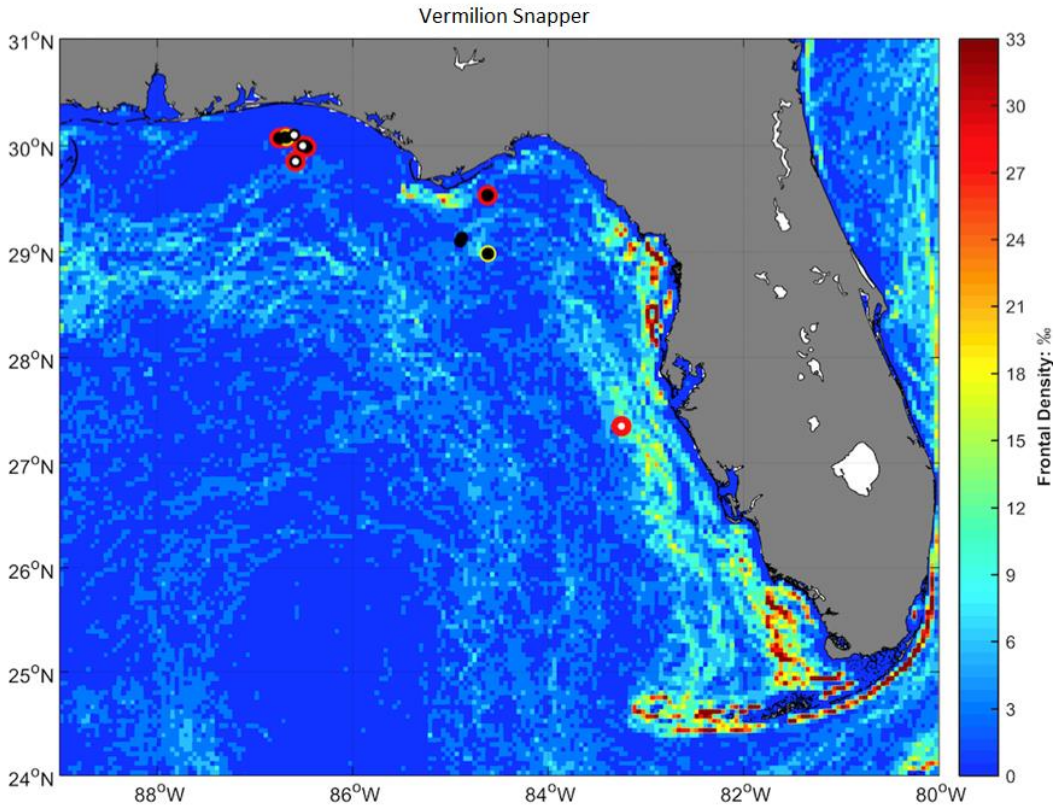

Figure B1. Ocean fronts from color-indexed images of monthly average frontal density for June-July 2017 with Vermilion Snapper represented. Sites where adult fish were caught are indicated with colored circles. Red circles: spawning females, yellow circles: non-spawning females, black circles: spawning males, white circles: non-spawning males.

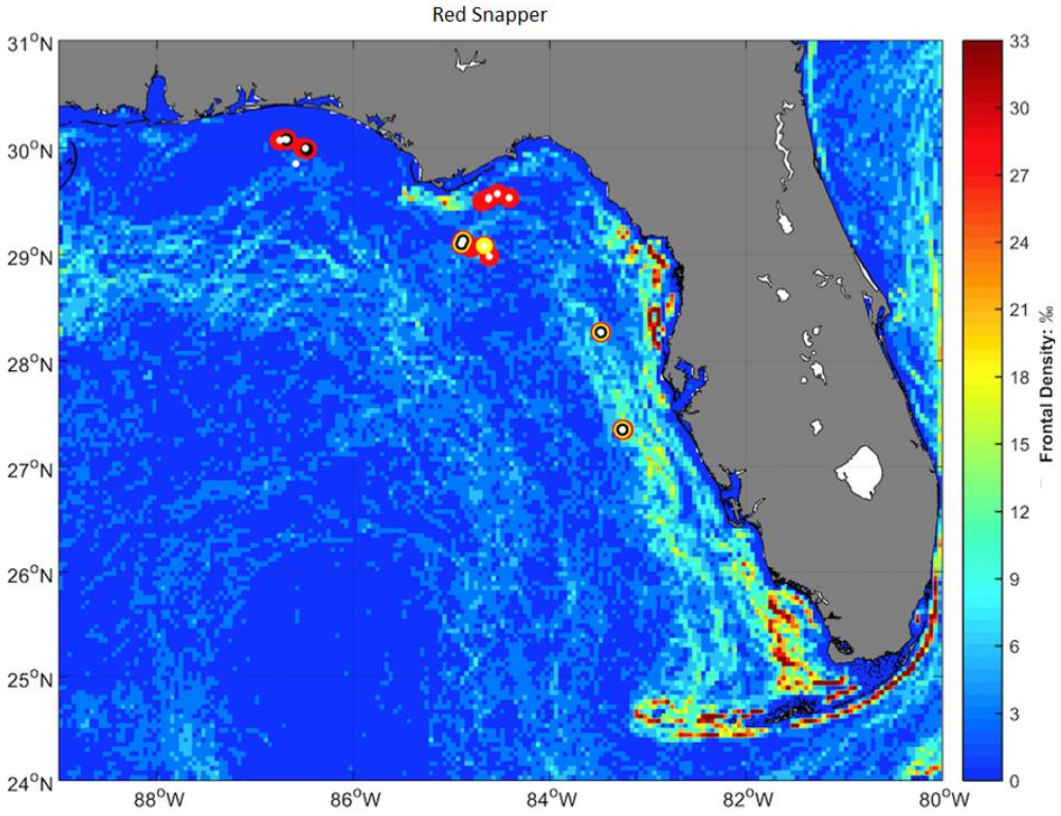

Figure B2. Ocean fronts from color-indexed images of monthly average frontal density for June-July 2017 with Red Snapper represented. Sites where adult fish were caught are indicated with colored circles. Red circles: spawning females, yellow circles: non-spawning females, black circles: spawning males, white circles: non-spawning males.

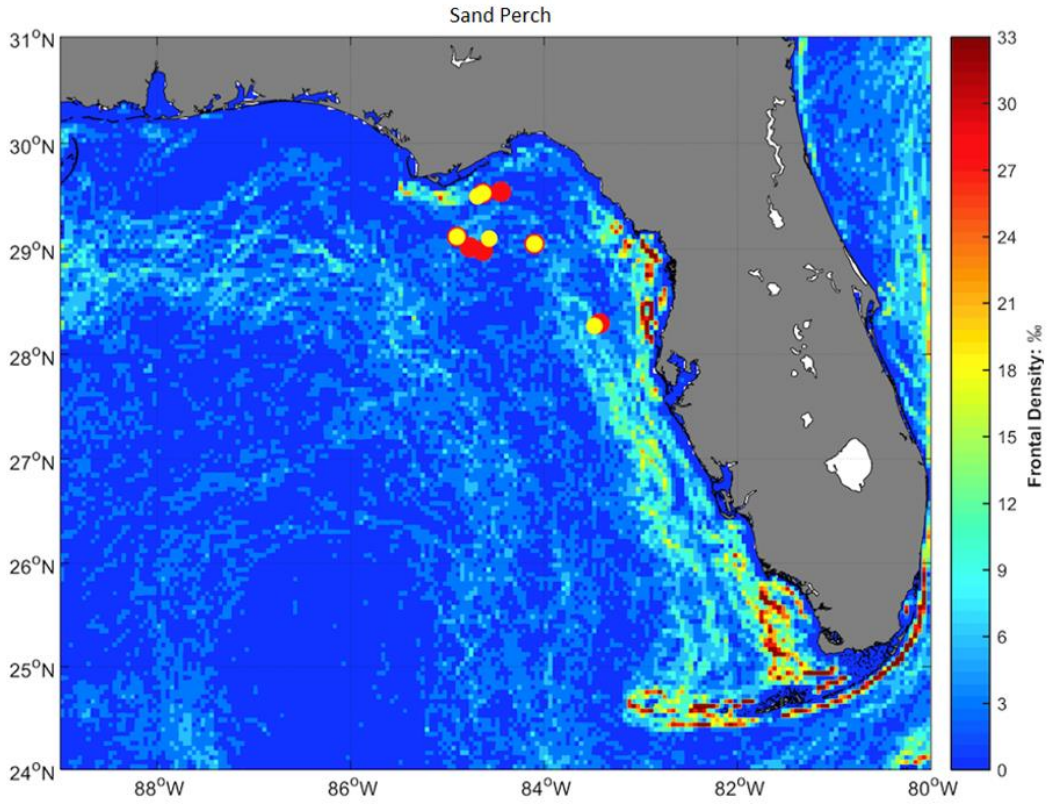

Figure B3. Ocean fronts from color-indexed images of monthly average frontal density for June-July 2017 with Sand Perch represented. Sites where adult fish were caught are indicated with colored circles. Red circles: spawning females, yellow circles: non-spawning females, black circles: spawning males, white circles: non-spawning males.

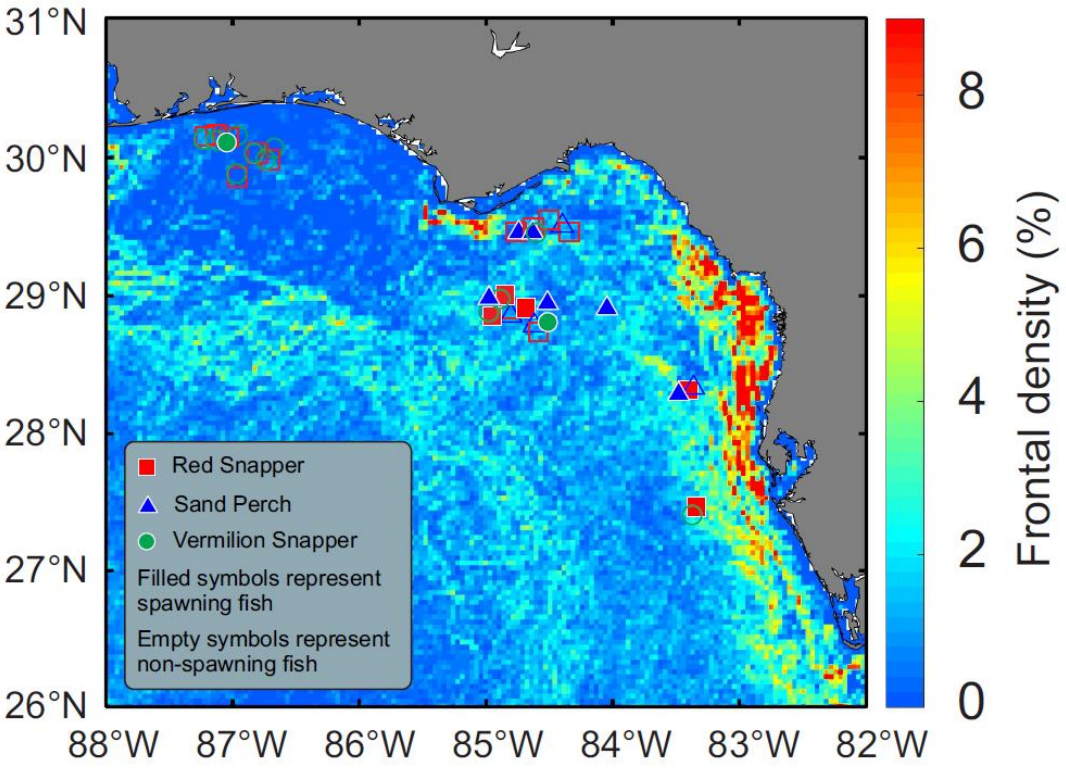

Figure B4. Ocean fronts from color-indexed images of monthly average frontal density for June-July 2017 with adult fish represented and categorized between spawning and non-spawning.

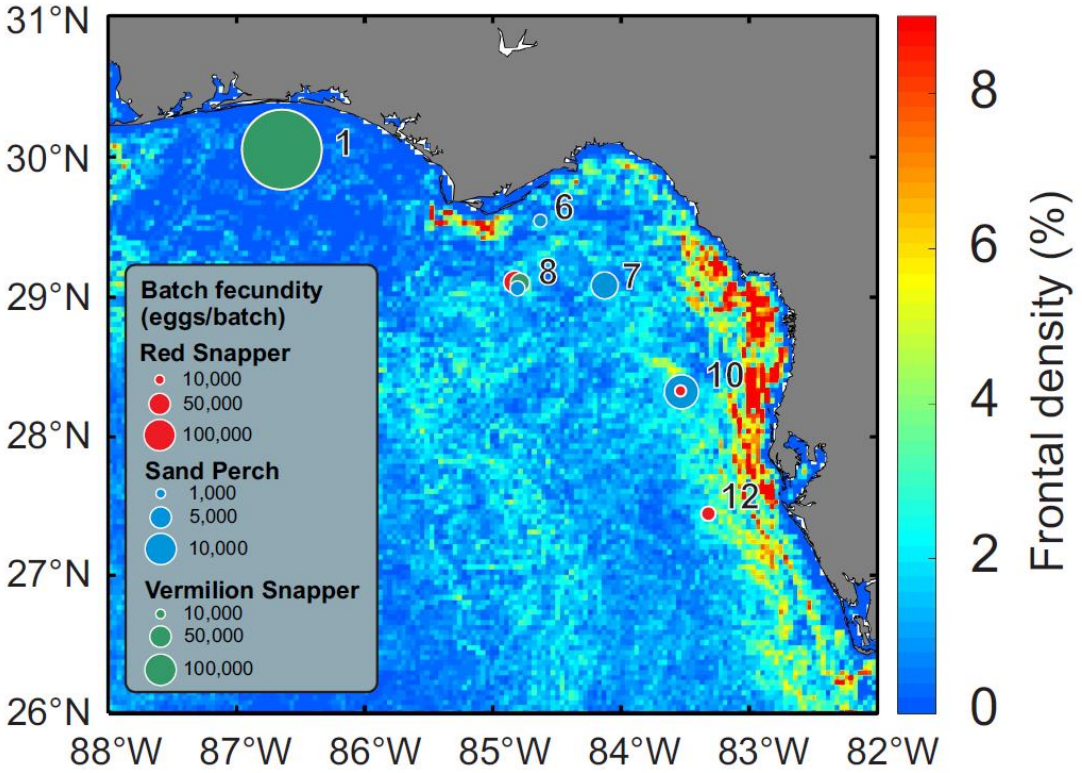

Figure B5. Ocean fronts from color-indexed images of monthly average frontal density for June-July 2017 with average batch fecundity per species represented at each site.

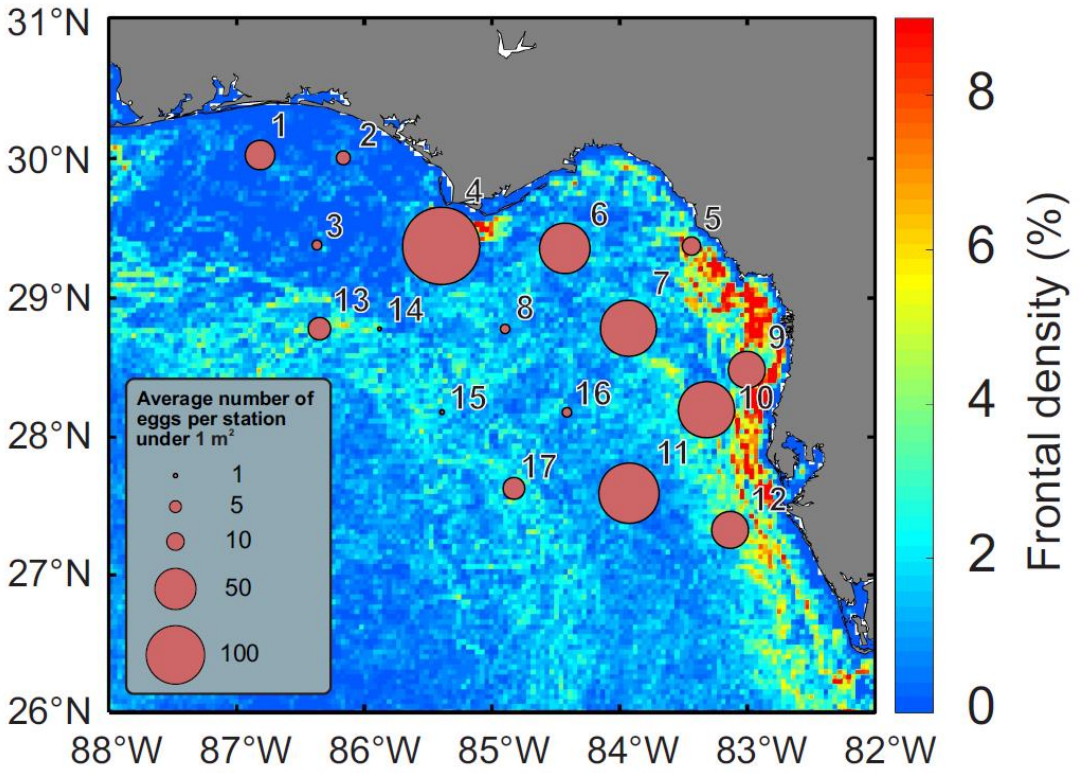

Figure B6. Ocean fronts from color-indexed images of monthly average frontal density for June-July 2017 with fish egg abundance represented at each station.