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FIO: Monty Graham, Nicole Raineault



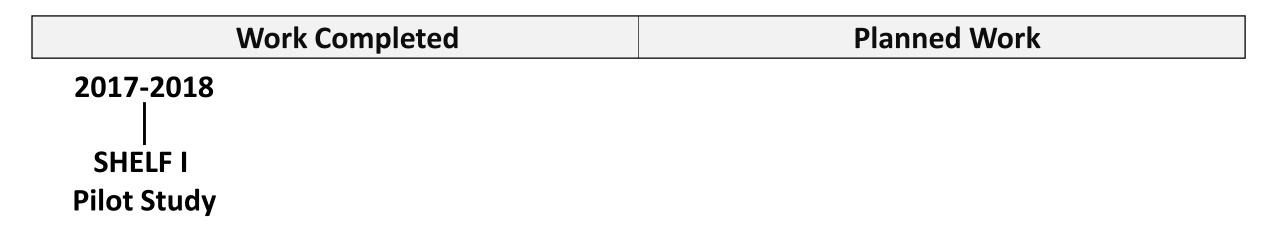
CoPIs: Mya Breitbart, Steve Murawski, Ernst Peebles



Work Completed

Planned Work







Work Completed		Planned Work
2017-2018	2019-2023 SHELF II	
SHELF I Pilot Study	Egg Monitoring Begins; Barcoding Methods	



Work Completed		Planned Work
2017-2018 	2019-2023 	2023-2026
 SHELF I	I SHELF II	I SHELF III
Pilot Study	Egg Monitoring Begins; Barcoding Methods	Egg Monitoring Cont.; Targeted Studies



V	Vork Completed	Planned Work							
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SHELF I Pilot Study	SHELF II Egg Monitoring Begins; Barcoding Methods	SHELF III Egg Monitoring Cont.; Targeted Studies	I SHELF IV-VI LT Egg Monitoring TS; Targeted Studies; Respond to Needs						



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SHELF I Pilot Study	SHELF II Egg Monitoring Begins; Barcoding Methods	SHELF III Egg Monitoring Cont.; Targeted Studies	SHELF IV-VI LT Egg Monitoring TS; Targeted Studies; Respond to Needs				

SHELF I (2017-2018): followed on success of prior FLRACEP funding to DNA barcode fish eggs

Received: 13 April 2018 Revised: 28 June 2018 Accepted: 6 July 2018 DOI: 10.1111/fog.12404

ORIGINAL ARTICLE

WILEY

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

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Funding information Florida RESTORE Act Centers of Excellence Program (FLRACEP)

Abstract We combined research-vessel cruises of opportunity with DNA barcoding to survey planktonic, percomorph fish eggs at 40 stations distributed across and around the Gulf of Mexico (GoM). The objectives were (a) to determine whether eggs of fishes that are potential candidates for the daily egg production method (DEPM) can be readily barcoded, (b) to identify taxa that are spawning in the GoM, (c) to determine encounter rates for eggs of economically valuable taxa, and (d) to characterize individual egg taxa as being primarily neritic, primarily oceanic, or primarily mixed (i.e., both neritic and oceanic). Of the 1.144 eggs that were individually barcoded, 709 (62%) were definitively identified to species (62 species from 42 families), with an additional 20 taxa identified to genus or subfamily level. The eggs of 15 economically important species were identified, most of which had intermediate encounter rates and moderately dispersed spatial distributions, as indicated by an index of aggregation. SIMPROF analysis of stationwise cluster analysis identified eight significant groups within the 35 stations that yielded percomorph eggs; a corresponding specieswise analysis identified six groups of stations as having a neritic egg community and two groups as having an oceanic community, with a community transition located at the shelf break. Although the neritic and oceanic stations did not share important species, it remains possible that coastal pelagic species have mixed neritic-oceanic distributions. Together, these results indicate DEPM fish-egg surveys based on DNA barcoding are feasible at the large marine ecosystem scale.

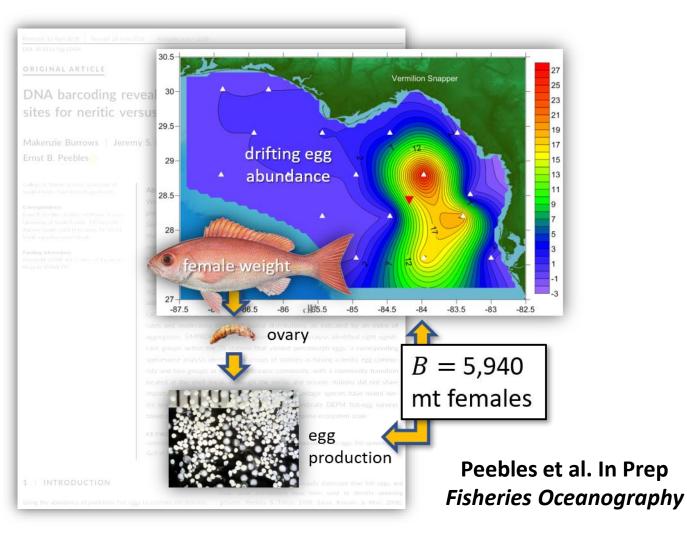
KEYWORDS

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

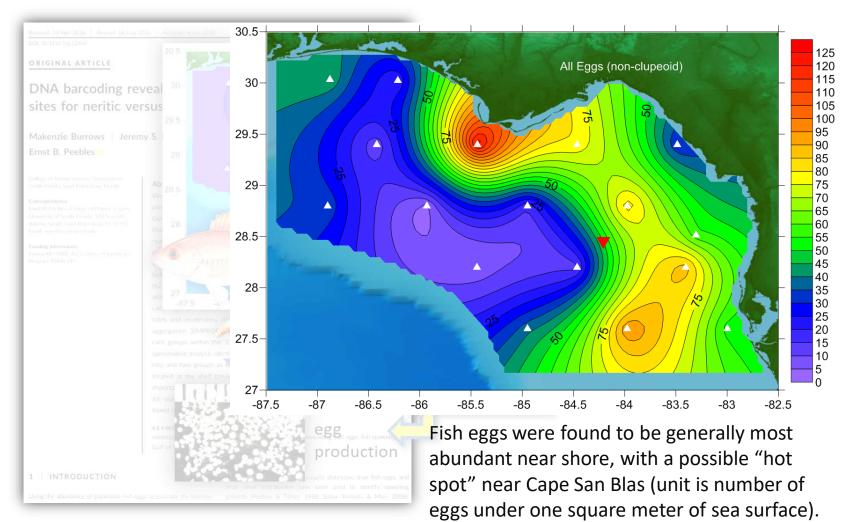
1 | INTRODUCTION

Fish larvae are usually more visually distinctive than fish eggs, and thus, larval distributions have been used to identify snawning Using the abundance of planktonic fish eggs to estimate the biomass grounds (Peebles & Tolley, 1988; Sassa, Konishi, & Mori, 2006).

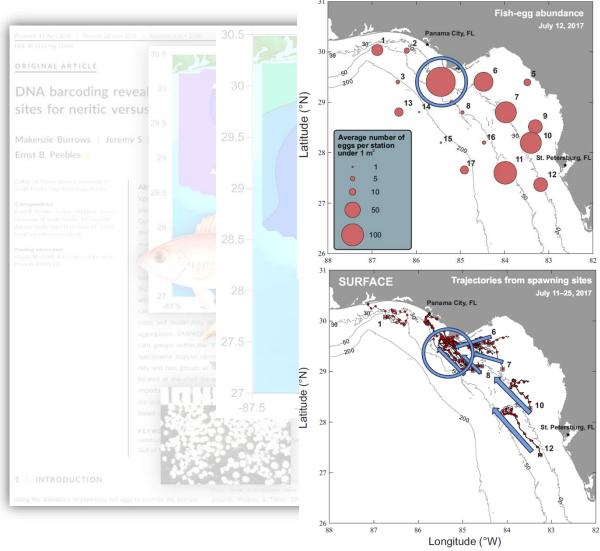
SHELF I (2017-2018): focused on the Daily Egg Production Method to estimate SSB



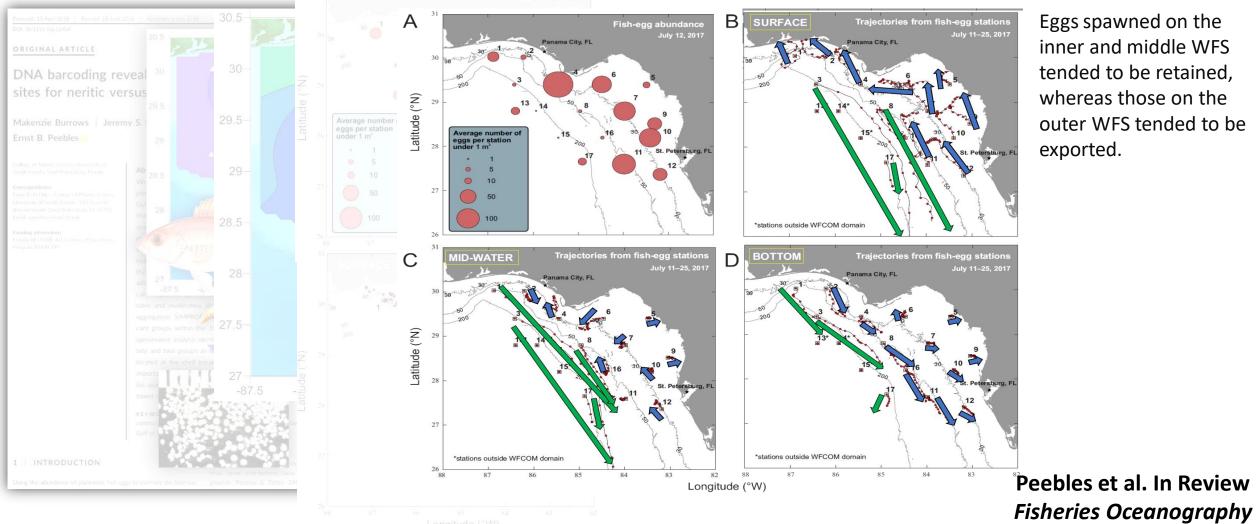
SHELF I (2017-2018): also identified spatial distributions of fish eggs on the WFS



SHELF I (2017-2018): we were able to explain the distribution of eggs based on PO models

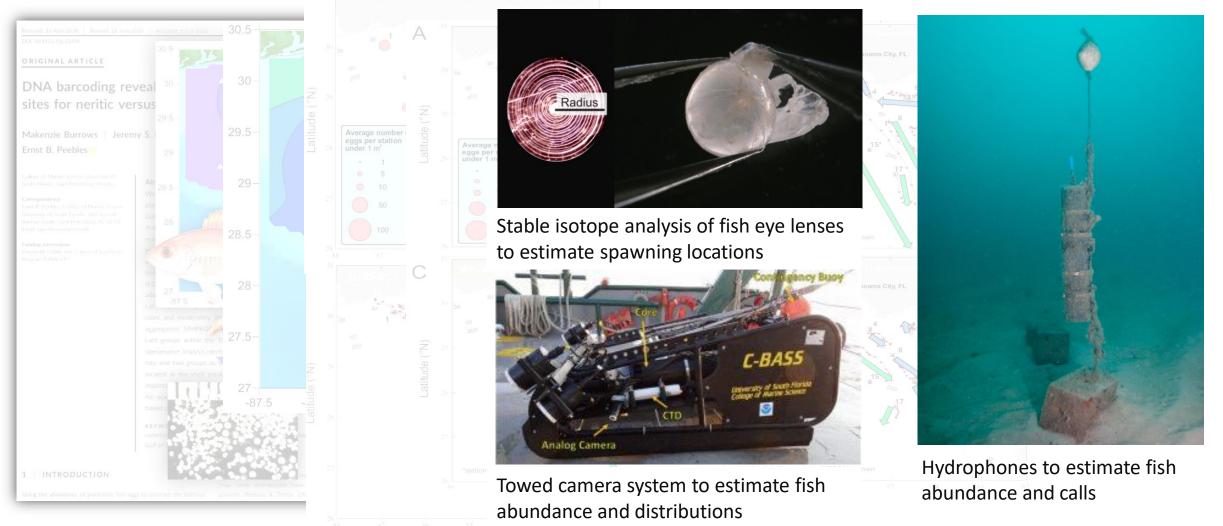


SHELF I (2017-2018): we also used PO models to estimate egg retention and export dynamics



Eggs spawned on the inner and middle WES tended to be retained, whereas those on the outer WFS tended to be exported.

SHELF I (2017-2018): and we explored the use of various complementary field and lab tools



Longitude (



• A final report was submitted, and results of the pilot SHELF project were reviewed and revised by FLRACEP's program management team (PMT)



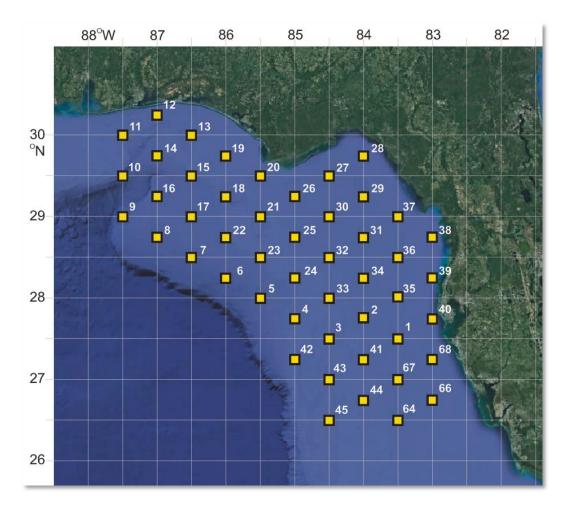
- A final report was submitted, and results of the pilot SHELF project were reviewed and revised by FLRACEP's program management team (PMT)
- After reviewing the diverse array of methods and their results, the PMT prioritized:
 - continued funding for the egg-monitoring component of the program
 - with flexibility and funding for targeted studies



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2017-2018 	2019-2023	2023-2026	2026-2035			
SHELF I Pilot Study	SHELF II Egg Monitoring Begins; Barcoding Methods	SHELF III Egg Monitoring Cont.; Targeted Studies	SHELF IV-VI LT Egg Monitoring TS; Targeted Studies; Respond to Needs			

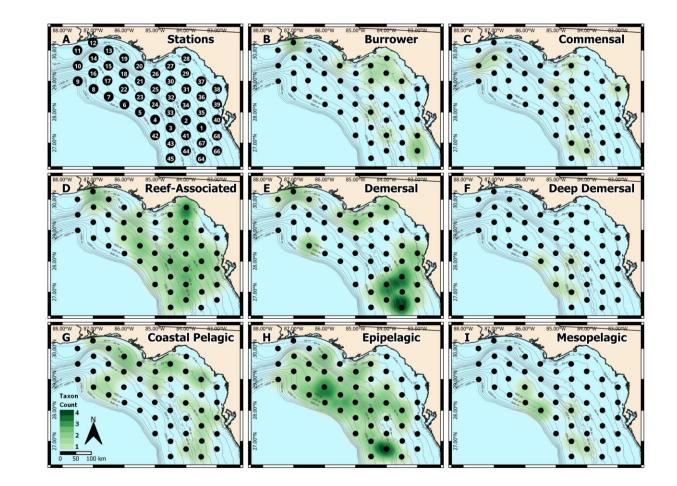
SHELF II (2019-2023): initiation of egg monitoring survey across the WFS

- 49 stations in 10 days at sea (August-September 2019)
- Sample processing was completed Fall 2019
- DNA barcoding was complete by early 2020
- We used DNA metabarcoding...



SHELF II (2019-2023): DNA metabarcoding

- We recovered 37 taxa from 4,719 fish eggs
- Identified taxa corresponded with known habitat types of these taxa



Breitbart et al. 2023 PeerJ

SHELF II (2019-2023): Pros and cons of DNA metabarcoding for long-term monitoring

Parameter	Individual eggs	Metabarcoding
Cost	\$5.15 per egg	\$0.78 per egg ^a
	\$494.40 per site ^a	\$64.82 per site

^a Cost calculated based on 96 eggs per site.

Breitbart et al. 2023 PeerJ

SHELF II (2019-2023): Pros and cons of DNA metabarcoding for long-term monitoring

Parameter	Individual eggs	Metabarcoding
Cost	\$5.15 per egg	\$0.78 per egg ^a
	\$494.40 per site ^a	\$64.82 per site
Quantitative	Yes	No
Ability to return to individual	Yes	No
eggs with additional primers		
Prevalence of false	Low/None	Frequent; dependent on the
positives/negatives		application of a threshold

^a Cost calculated based on 96 eggs per site.

SHELF II (2019-2023): Pros and cons of DNA metabarcoding for long-term monitoring

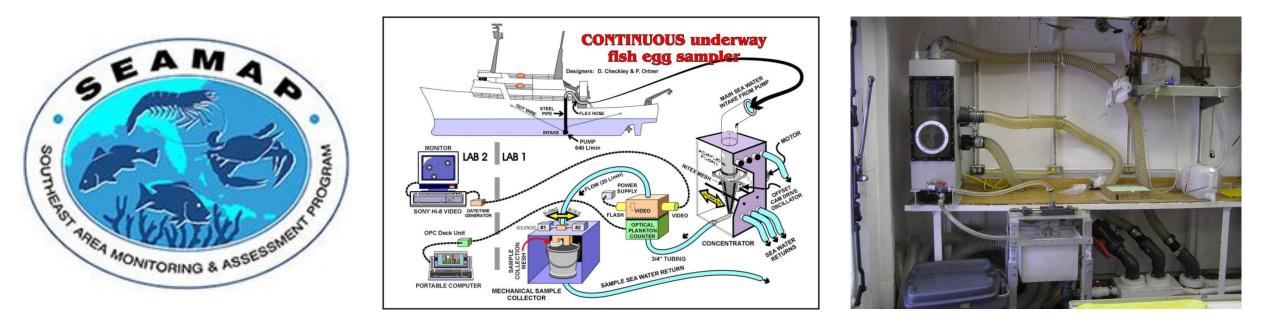
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^a Cost calculated based on 96 eggs per site.

We therefore decided to use individual egg barcoding for the SHELF program

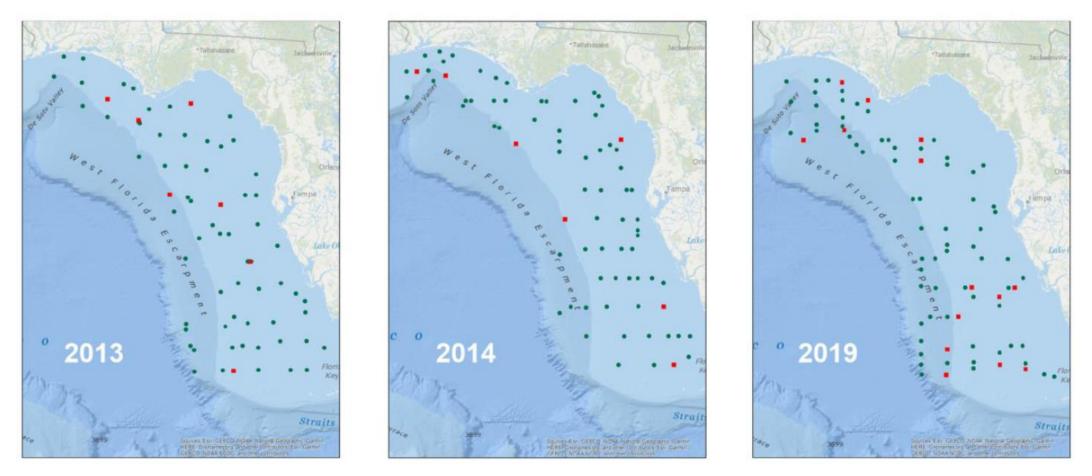
Breitbart et al. 2023 PeerJ SHELF II (2019-2023): COVID created novel challenges to conduct field work / cruises

SHELF II (2019-2023): we were able to overcome these challenges with a new collaboration



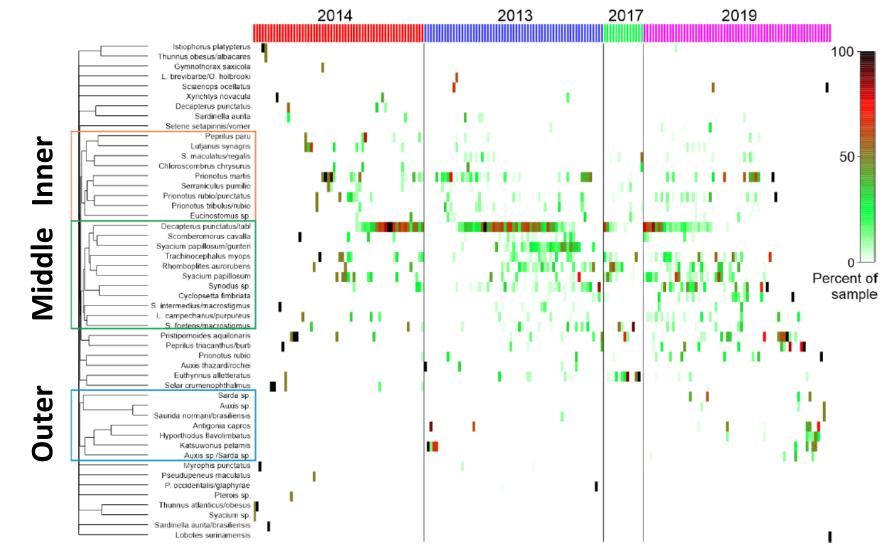
NOAA's Continuous Underway Fish Egg Sampler (CUFES), with the entire system diagrammed in the middle panel and a photo of the concentrator and mechanical sample collector inside the vessel's lab in the right panel (courtesy NOAA NMFS).

SHELF II (2019-2023): we barcoded three years of archived fish eggs collected by NOAA SEAMAP

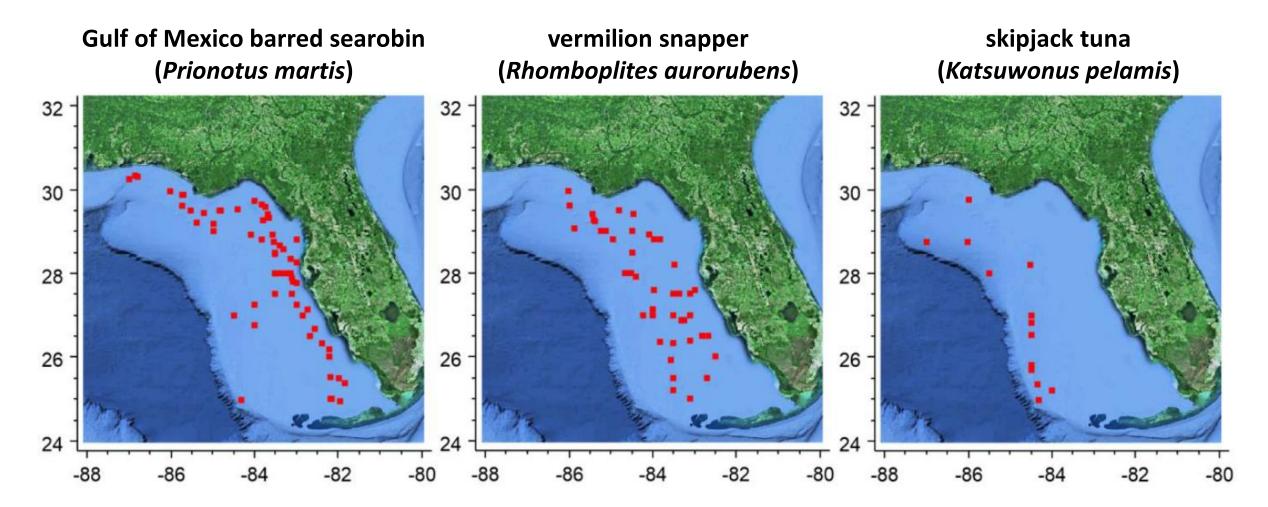


We have barcoded 251 stations, had 80% success rate, and have found 163 taxa.

SHELF II (2019-2023): community structure of fish eggs along an inner-outer shelf gradient

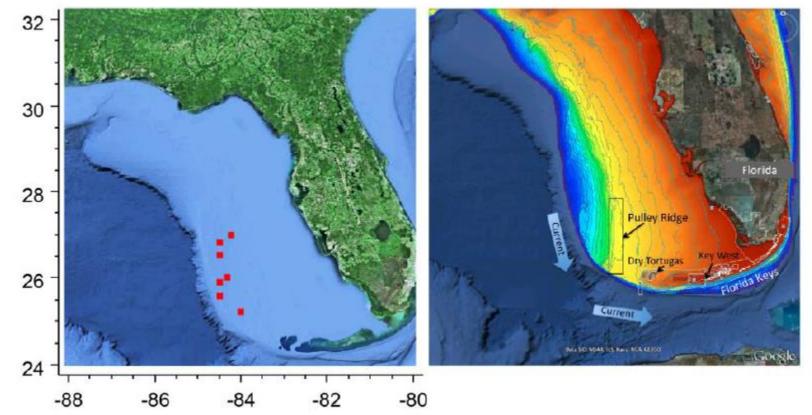


SHELF II (2019-2023): example taxa identified for inner, middle, and outer shelf communities



SHELF II (2019-2023): distribution of yellowedge grouper eggs relative to Pulley Ridge HAPC

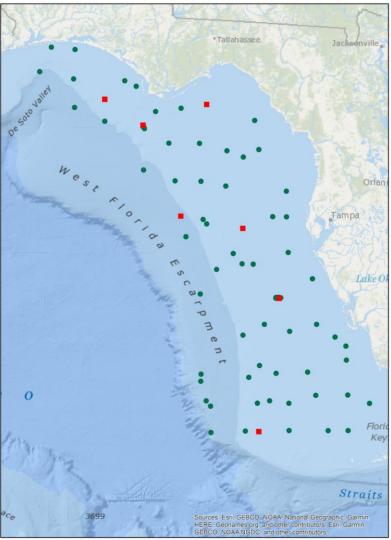
yellowedge grouper (Hyporthodus flavolimbatus)





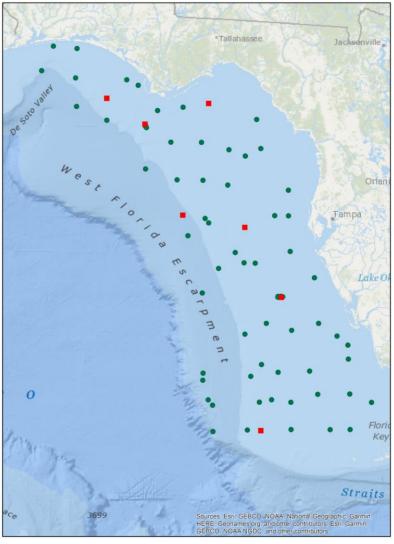
Work Completed		Planne	ed Work
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 SHELF I	 SHELF II	 SHELF III	SHELF IV-VI
Pilot Study Egg Monitoring Begins; Barcoding Methods		Egg Monitoring Cont.; Targeted Studies	LT Egg Monitoring TS; Targeted Studies;
			Respond to Needs

SHELF III (2023-2026): continue long-term egg monitoring times series and targeted studies



Year eggs collected	SHELF Phase	Status
2013	SHELF II	Completed
2014	SHELF II	Completed
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2022	SHELF III	To do
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Targeted Studies

- **1.** Better understand spawning dynamics on the WFS
- 2. Examine key assumptions of our methods

SHELF III (2023-2026): examine eggs collected across seasons on the WFS

FMP category	Common name	Family	Scientific name	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Coastal Migratory Pelagics	King Mackerel	Scombridae	Scomberomorus cavalla												
Coastal Migratory Pelagics	Spanish Mackerel	Scombridae	Scomberomorus maculatus												
Red Drum	Red Drum	Sciaenidae	Sciaenops ocellatus												
Reef Fish	Gray Triggerfish	Balistidae	Balistes capriscus												
Reef Fish	Greater Amberjack	Carangidae	Seriola dumerili												
Reef Fish	Almaco Jack	Carangidae	Seriola rivoliana												
Reef Fish	Speckled Hind	Epinephelidae	Epinephelus drummondhayi												
Reef Fish	Goliath Grouper	Epinephelidae	Epinephelus itajara												
Reef Fish	Red Grouper	Epinephelidae	Epinephelus morio												
Reef Fish	Nassau Grouper	Epinephelidae	Epinephelus striatus												
Reef Fish	Yellowedge Grouper	Epinephelidae	Hyporthodus flavolimbatus												
Reef Fish	Warsaw Grouper	Epinephelidae	Hyporthodus nigritus												
Reef Fish	Snowy Grouper	Epinephelidae	Hyporthodus niveatus												
Reef Fish	Black Grouper	Epinephelidae	Mycteroperca bonaci												
Reef Fish	Yellowmouth Grouper	Epinephelidae	Mycteroperca interstitialis												
Reef Fish	Gag Grouper	Epinephelidae	Mycteroperca microlepis												
Reef Fish	Scamp	Epinephelidae	Mycteroperca phenax												
Reef Fish	Yellowfin Grouper	Epinephelidae	Mycteroperca venenosa												
Reef Fish	Hogfish	Labridae	Lachnolaimus maximus												
Reef Fish	Mutton Snapper	Lutjanidae	Lutjanus analis												
Reef Fish	Red Snapper	Lutjanidae	Lutjanus campechanus												
Reef Fish	Cubera Snapper	Lutjanidae	Lutjanus cyanopterus												
Reef Fish	Vermilion Snapper	Lutjanidae	Rhomboplites aurorubens												
Reef Fish	Tilefish	Malacanthidae	Lopholatilus chamaeleonticeps												
Not Federally Managed	Southern Flounder	Paralichthyidae	Paralichthys lethostigma												
Not Federally Managed	Spotted Seatrout	Sciaenidae	Cynoscion nebulosus												
Not Federally Managed	Black Drum	Sciaenidae	Pogonias cromis												
Not Federally Managed	Sheepshead	Sparidae	Archosargus probatocephalus												
			Number species (all)	11	13	13	19	19	20	16	18	15	9	7	5
			Number species (peak)	3	5	10	9	8	8	8	7	4	2	1	3
					Q1			Q2			Q3			Q4	

SHELF III (2023-2026): examine eggs collected across seasons on the WFS

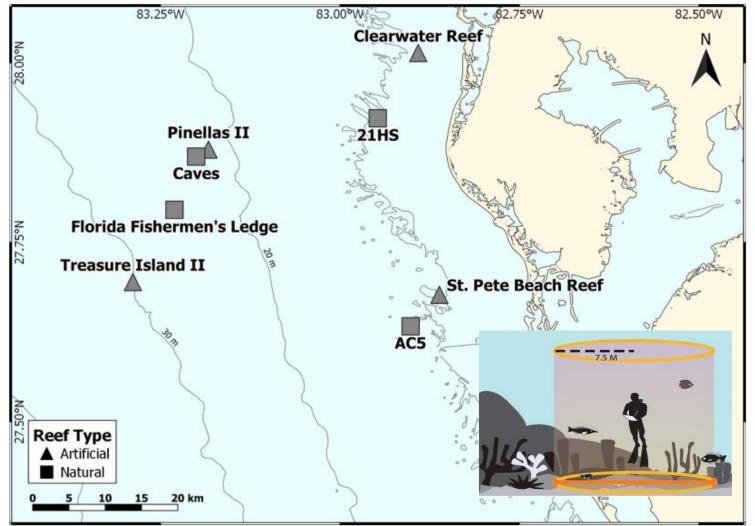
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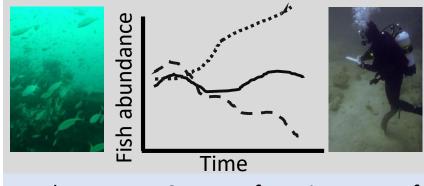


We will build a CUFES to be placed on FIO vessels



SHELF III (2023-2026): test whether we can link adult fish abundances to egg production



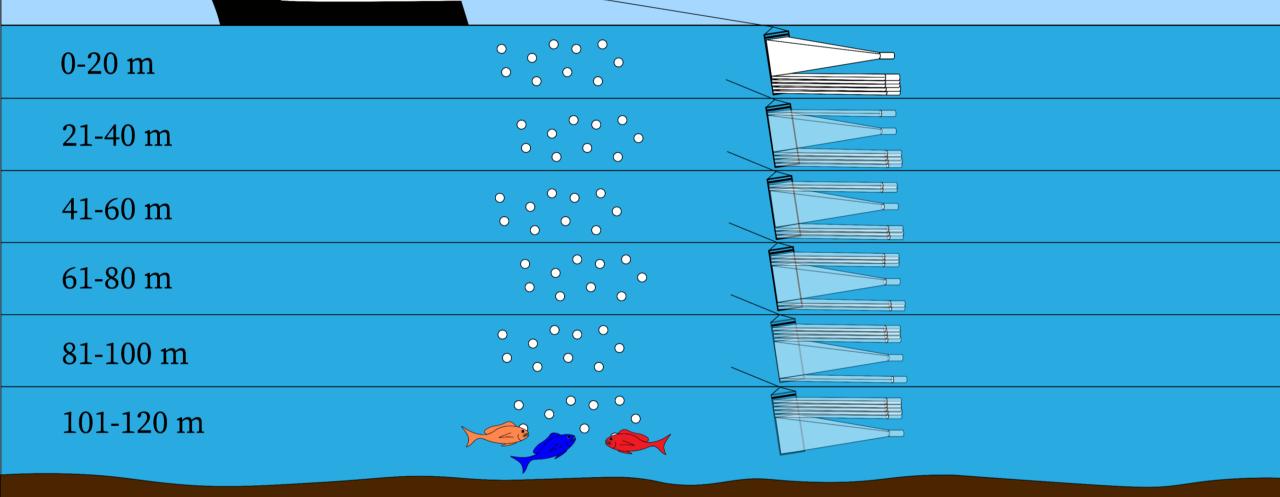


We have over 10 years of continuous reef fish survey data with ~1300 surveys and over 200 taxa

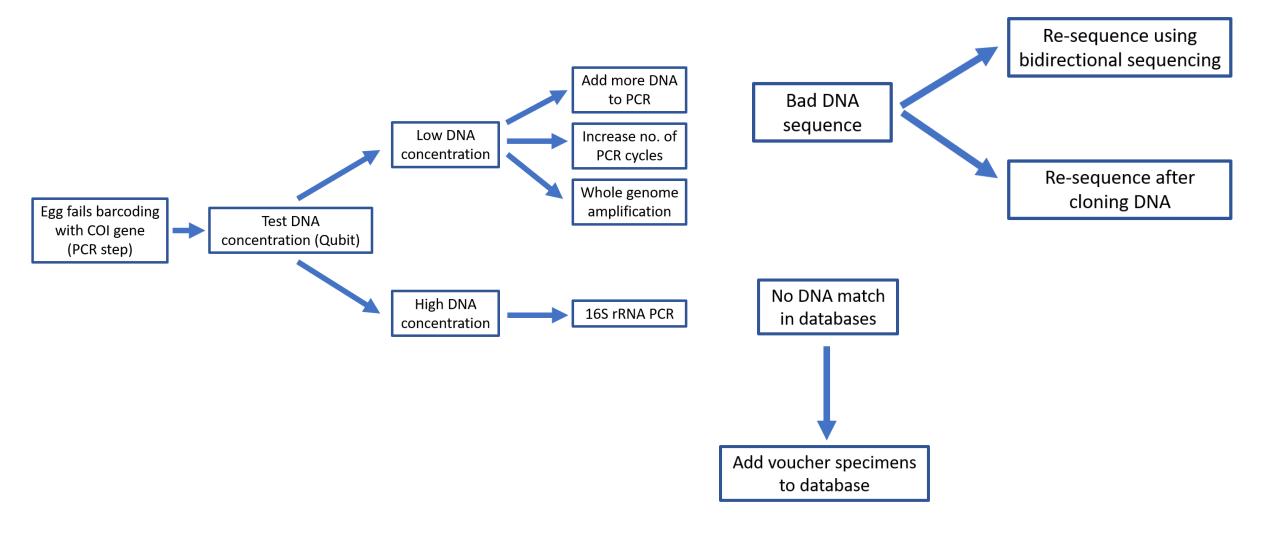


We will build a second CUFES that can be used on our small research vessel

SHELF III (2023-2026): test assumption that surface sampling fully characterizes spawning



SHELF III (2023-2026): determine causes of barcoding failure and improve success rate



Continue existing and develop new collaborations with fisheries scientists







Continue existing and develop new collaborations with fisheries scientists







Disseminate SHELF goals and products to stakeholders



Continue existing and develop new collaborations with fisheries scientists







Disseminate SHELF goals and products to stakeholders



Ensure the SHELF program is fully operationalized







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2029	SHELF IV	Pending continued funding
2030	SHELF V	Pending continued funding
2031	SHELF V	Pending continued funding
2032	SHELF V	Pending continued funding
2033	SHELF VI	Pending continued funding
2034	SHELF VI	Pending continued funding
2035	SHELF VI	Pending continued funding

Long-term time series

- 17 years of identifications across a
 23-year span
- 2. Begin to understand long-term dynamics, response to disturbances, recovery of species
- 3. Potential to leverage funding from other sources to continue time series beyond 2036

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2030	SHELF V	Pending continued funding
2031	SHELF V	Pending continued funding
2032	SHELF V	Pending continued funding
2033	SHELF VI	Pending continued funding
2034	SHELF VI	Pending continued funding
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Flexible to address data needs

- Can respond to issues as they emerge to address data needs (e.g., "Great Counts")
- 2. Potential to incorporate complementary tools and approaches (e.g., PO modeling)

THANK YOU!