# Recreational Effort, Catch and Biological Sampling in Florida During the 2021 South Atlantic Red Snapper Season

Julie Vecchio, Dominique Lazarre, Beverly Sauls, and Ellie Corbett

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# Acknowledgments

We would like to thank Florida east coast recreational anglers and charter vessel operators for their assistance in the collection of catch and effort data as well as biological samples from their catch. Without their cooperation, these sampling efforts could not have been a success. We would also like to acknowledge the marina and boat ramp operators who so graciously allowed us to operate from their facilities. We would like to acknowledge several groups from FWC's Fish and Wildlife Research Institute: including staff from Fisheries Dependent Monitoring, who assisted with local coordination, data collection, sample processing, database management, data entry, purchases and travel reimbursements; Fisheries Independent Monitoring, for use of vehicles and research vessels; Jessica Carroll and staff of the Age and Growth Lab for processing and ageing otoliths. We also thank FWC's Division of Marine Fisheries Laboratory at the University of Florida, who provided valuable assistance in the field. This work was supported by state of Florida funds used to expand the State Reef Fish Survey and from the Southeast Regional Office of the National Marine Fisheries Service.

# **Executive Summary**

Recreational fishery landings for Red Snapper *Lutjanus campechanus* in the southeastern U.S. Atlantic have historically been monitored through a general survey of all saltwater fishing called the Marine Recreational Information Program (MRIP). A majority of landings estimated through the MRIP survey were attributed to the Atlantic coast of Florida. However, the recreational fishery has been managed with an annual harvest season ranging from 0 to 9 days since 2010. In order to improve precision of fishing effort and harvest estimates over such short seasons, the state of Florida has developed specialized survey methods (Sauls et al. 2017).

This report summarizes methods and final results for specialized surveys of the private boat and charter segments of the recreational fishery operating from the east coast of Florida during the 2021 recreational season for Red Snapper in the South Atlantic. Sampling activities were conducted over a single weekend in July (Friday through Sunday, July 9-11, 2021) when Red Snapper recreational harvest was open. Prior to the season opening, a paper log-sheet was mailed to charter vessel operators based in Florida that possess a federal permit for South Atlantic Snapper-Grouper, which was followed up the week after the season closed with telephone contacts to collect information specifically on Red Snapper fishing effort and catch. Final estimates are provided for both the private boat and charter segments of the recreational fishery.

During the 2021 season, weather conditions were favorable for offshore fishing, despite the passage of Tropical Storm Elsa through the northeast Florida region on July 7. High levels of boating activity were observed throughout the study region and an estimated 44,930  $\pm$  4,396 (SE) angler trips targeted Red Snapper over the three-day season A total of 990 private boat parties were interviewed upon returning from trips in the ocean, and 81.6% reported fishing for Red Snapper. An estimated 30,206  $\pm$  3,159 Red Snapper were harvested over the three days. The mean length was  $562 \pm 3.08$  mm midline length and mean weight was  $3.69 \pm 0.06$  kg for fish sampled from private boat trips.

For the federally permitted charter fleet, a total of 472 charter vessels in the MRIP For-Hire Survey Frame with a South Atlantic Snapper-Grouper permit were included in the sample frame, and 426 were selected for this survey. Of those selected, 77.9% responded to the dual mail / telephone survey. More than a quarter of the total responses (26.8%) came from log sheets that were returned via mail. An estimated  $4,196 \pm 172$  Red Snapper were harvested during 3,785  $\pm 152$  angler trips from charter vessels over the three-day season. The majority (98.3%) of charter landings were from northeast Florida (Martin County north to the Georgia border). Captain and crew on for-hire vessels may retain the recreational bag limit, and the mean CPUE was  $1.13 \pm 0.03$  fish harvested per angler trip in northeast Florida. Red Snapper sampled from charter boats averaged  $594 \pm 6.4$  mm and  $4.16 \pm 0.13$  kg.

The Red Snapper harvest season also provided an opportunity to collect fishery dependent biological samples, including length, weight, and otoliths for aging. During 2021, biological data was collected from 1,741 Red Snapper sampled from private boats and 330 sampled from federally permitted charter boats. A new addition during bio-sampling in 2021 was the collection of fin clips for use in a genetic close-kin mark recapture (CKMR) analysis. This work is part of an ongoing two-year grant (Sea Grant College Program NA20OAR4170471) awarded to the University of Florida, in collaboration with FWC and other institutions, to estimate the absolute abundance of age-2+ red snapper in the South Atlantic region (Appendix 1 and <a href="https://www.scseagrant.org/great-red-snapper-count-award/">https://www.scseagrant.org/great-red-snapper-count-award/</a>).

# Section 1. Private Mode

# Methods

The survey design and estimation methods for private boat mode described below were developed over three prior Red Snapper seasons. Details for how methods were tested and validated, as well as results from the first three years, are described by Sauls et al. (2017).

Sample Design — Off the Atlantic coast of peninsular Florida, recreational boaters must pass through one of nine navigable inlets to access Red Snapper fishing grounds in the Exclusive Economic Zone (Figure 1.1). Recreational boat traffic through each of these egress points was monitored during the season. Each day that an inlet was sampled, boat traffic was observed during one of three time periods. The morning period began at local sunrise (6:30 a.m.) and ended at 11:29 a.m., the midday period began at 11:30 am and ended at 3:29 p.m., and the evening period began at 3:30 pm and ended at local sunset (8:30 pm). Each inlet was randomly selected to be sampled during one of the three periods each day of the season. This sample design ensured that recreational boat activity across the region was observed throughout each day, and that variable fishing effort in response to localized weather and offshore conditions was measured and accounted for. Matanzas Inlet is a minor egress point and was not monitored during the Red Snapper season. A ratio adjustment calculated from monitoring in prior seasons was applied to St. Augustine to account for the small amount of additional effort through Matanzas Inlet.

Launch sites for private recreational boats were randomly selected for a complementary access point intercept survey each day of the season. The purpose of the intercept survey was to interview parties as they returned from boating trips to determine whether they were fishing for Red Snapper, measure catch rates, and collect biological samples from harvested fish. The intercept survey also provided data that were necessary for accurately estimating fishing effort. During an assignment, each party that returned from a recreational boat trip was interviewed to determine the proportion that exited through inlets for the purpose of targeting Red Snapper and the proportion that departed before sunrise and were not accounted for in inlet boat count survey. Field procedures for conducting trip interviews with intercepted vessels are described in reports for previous years (Sauls et al. 2013, 2014).

# Estimation.—

The following steps were used to estimate total fishing effort:

1) The numbers of recreational boats observed exiting through each inlet during daylight hours was expanded to generate an unadjusted seasonal estimate of boat trips in the Atlantic Ocean across all inlets;

2) The estimated number of boat trips taken by federally permitted charter vessels (see next section) was subtracted;

3) The remainder was multiplied by the proportion of private recreational boat parties and non-federally permitted charter parties that reported targeting Red Snapper during intercept survey interviews; 4) The estimated boat trips that targeted Red Snapper were adjusted to account for additional boat parties that reported exiting through inlets before sunrise to target Red Snapper; and

5) The adjusted boat trips that targeted Red Snapper were multiplied by the mean number of anglers per intercepted boat party to get the total estimated number of angler trips targeting Red Snapper.

Landings are estimated by multiplying total effort by the mean CPUE (catch per angler trip) estimated from intercept data. Intercept data are weighted proportional to fishing effort across each inlet. A description of calculations is provided in prior years' reports and in Sauls et. al 2017.

## Results

Total estimated fishing effort in 2021 was comparable to recent years, and the impact of shortening the season to three days, compared to four days in 2020, was offset by an increase in the numbers of boat parties that participated each day (Figure 1.2). Overall, weather was favorable for offshore fishing across the three days that the season was open in 2021. NOAA National Data Buoy Center wind speed, wave height and period data from offshore buoys ranging from Fernandina Beach to Fort Pierce indicated wind speeds of no more than 10 mph, wave heights of 0.5-1 m, and wave periods of 6-8 seconds (NOAA, 2021). A total estimated  $30,206 \pm 3,159$  (SE) Red Snapper were harvested by private boat anglers (Table 1.2), and a total estimate of 54,685±5,541 (SE) Red Snapper were discarded (Table 1.3). Overall catch per unit effort (CPUE) for landed fish was 0.673±0.019 (Table 1.2) and has not varied significantly across the past several years, whereas discard rates have continued to increase over the timeseries (Figure 1.3). The current estimate for numbers of discarded fish per angler is  $1.221 \pm$ 0.079 (Table 1.3). Red Snapper landed by recreational anglers, which includes any off-frame charter vessels not included in the for-hire survey described in the next section, averaged  $562 \pm$ 3.08 mm fork length and  $3.64 \pm 0.06$  kg (Table 1.2). Estimated total numbers of fish retained and discarded have remain consistent since 2018 (Table 1.4). At the primary Red Snapper fishing inlets (those with >10 anglers indicating releasing some Red Snapper), approximately 1/3 of anglers reported descending released fish (Figure 1.4). There has been a notable increase in reported use of a descender device to recompress fish during release. Possessing a descender device on board while fishing for reef fishes was required by the S. Atlantic Fishery Management Council in 2020 and went into effect during the Red Snapper harvest season. Overall, during 2021, 33% of boat parties that released Red Snapper reported using a descender device to release fish, compared to approximately 1-3% in prior years (Figure 1.5).

#### References

Sauls, B. J., R.P. Cody, and A.J. Strelcheck. 2017. Survey methods for estimating Red Snapper landings in a high-effort recreational fishery managed with a small annual catch limit. North American Journal of Fisheries Management 37:302-313.

NOAA. 2021. National Data Buoy Center. https://www.ndbc.noaa.gov/

Table 1.1 Effort estimates for private boat mode by nearest inlet. Parameters include total numbers of boat parties intercepted, mean numbers of anglers per party, proportion of trips targeting Red Snapper, proportion of trips departing after sunrise, an estimate of total numbers of targeted boat trips, and an estimate of total numbers of targeted angler trips. All uncertainty estimates are  $\pm$  SE.

Inlet	Number of boat parties intercepted	Mean anglers per party	Proportion of trips targeting Red Snapper	Proportion of trips departing after sunrise	Targeted boat trips	Targeted angler trips
Cumberland	134	3.81±0.126	0.944±0.024	0.836±0.032	458±266	$1,745\pm1,017$
Mayport	168	3.84±0.118	0.821±0.043	0.533±0.041	2,444±1,625	9,384±6,244
St Augustine	186	$4.14 \pm 0.170$	0.816±0.042	$0.611 \pm 0.041$	1,162±671	4,813±2,786
Ponce Inlet	111	4.88±0.216	$0.777 {\pm} 0.056$	$0.541 \pm 0.050$	2,092±1,360	10,200±6,642
Port Canaveral	219	4.16±0.122	0.940±0.024	0.535±0.034	2,277±1,629	9,468±6,781
Sebastian Inlet	117	3.52±0.129	0.805±0.062	0.433±0.048	1,872±1,081	6,589±3,811
Fort Pierce	15	3.22±0.343	$0.500{\pm}0.158$	0.667±0.157	674±439	2,173±1,424
St. Lucie	40	2.88±0.276	0.143±0.066	0.750±0.154	194±126	558±364
Overall	990	4.09±0.057	0.816±0.018	0.600±0.016	11,174±1,064	44,930±4,396

Inlet	CPUE	Landings (# fish)	Mean weight (kg)	Landings (kg)
Cumberland	$0.742 \pm 0.037$	1,295±757	3.06±0.15	3,969±218
Mayport	$0.672 {\pm} 0.038$	6,306±4,204	3.95±0.19	24,938±1,508
St Augustine	$0.826 \pm 0.027$	3,976±2,304	3.64±0.11	14,463±539
Ponce	$0.801 \pm 0.032$	8,258±5,383	4.17±0.14	34,469±1,604
Port Canaveral	$0.729 \pm 0.026$	6,899±4,945	3.99±0.10	27,503±1,060
Sebastian	$0.432 \pm 0.045$	2,848±1,664	$2.71 \pm 0.17$	7,709±549
Fort Pierce	$0.103 \pm 0.074$	225±191	$1.75\pm0.12$	394±194
St. Lucie	0.714±0.161	398±269	2.32±0.71	924±302
Overall	0.673±0.019	30,206±3,159	$3.69\pm0.055$	114,372±14,954
c.v.	0.028	0.296	0.380	0.381

Table 1.2. Mean CPUE (landings per angler trip), estimated total landings, mean weight (kg), and estimated total landings (kg). All uncertainty is expressed as  $\pm$  SE.

Table 1.3. Mean releases per angler trip and estimated total landings  $\pm$ SE.

Inlet	Mean Release per angler trip	Estimated Releases (numbers of fish)
Cumberland	2.280±0.292	3,979±2,356
Mayport	$1.437 \pm 0.188$	13,488±9,071
St Augustine	2.156±0.273	10,379±6,101
Ponce	1.366±0.211	13,927±9,215
Port Canaveral	$0.738 {\pm} 0.081$	6,989±5,034
Sebastian	$0.800 {\pm} 0.168$	5,264±3,176
Fort Pierce	0.241±0.128	524±403
St. Lucie	0.238±0.152	133±108
Overall	1.221±0.079	54,685±5,541
c.v.	0.065	0.286

Year	Month(s)	Number of days	Estimated harvest	Estimated discards $\hat{C}_{disc}(\pm s. e.)$
			$\hat{C}_{harv}(\pm s.e.)$	
2021	July	3	30,206 (±3,159)	54,685 (±5,541)
2020	July	4	30,921 (±5,820)	Not available
2019	July	5	37,750 (±6,292)	56,648 (±10,163)
2018	August	6	30,050 (±6,256)	41,660 (±10,057)
2017	NovDec.	9	5,390 (±475)	4,331 (±561)
2014	July	8	22,013 (±2,782)	9,755 (±1,741)
2013	August	3	6,999 (±1,321)	5,033 (±1,512)
2012	Sept.	6	11,136 (±1,734)	17,587 (±9,031)

Table 1.4 Season length and total catch estimates for private boat mode expressed in numbers of Red Snapper during 2021 as compared to previous monitored seasons.

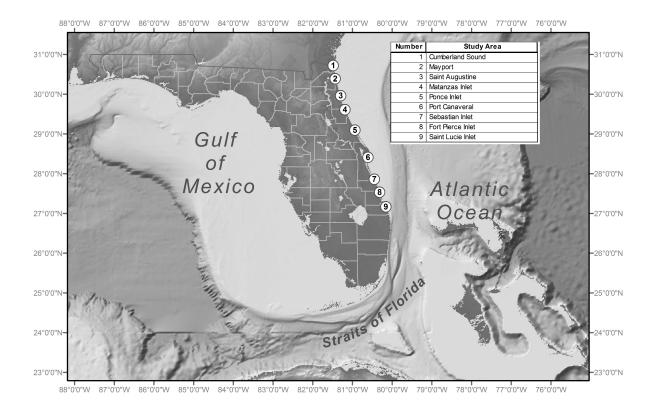


Figure 1.1. Geographic area of study and inlets included in study area.

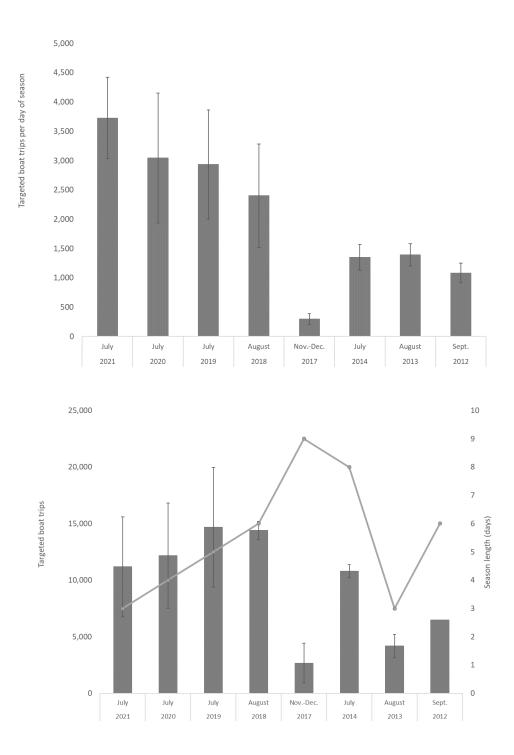


Figure 1.2 Mean boat parties per day that targeted Red Snapper during the harvest season (top panel), and total estimated boat trips with season length as a second axis (bottom panel). Effort has increased in the most recent years that the survey was conducted (2018-2021). Low effort in 2017 was attributed to poor weather conditions for offshore fishing in November and December.

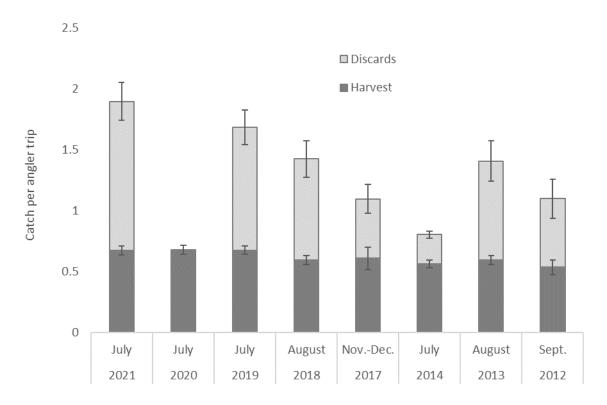


Figure 1.3. Mean catch per unit effort across years. Numbers of fish harvested per angler has remained steady and is constrained by the one fish per person bag limit. Discard rates were not estimated in 2020.

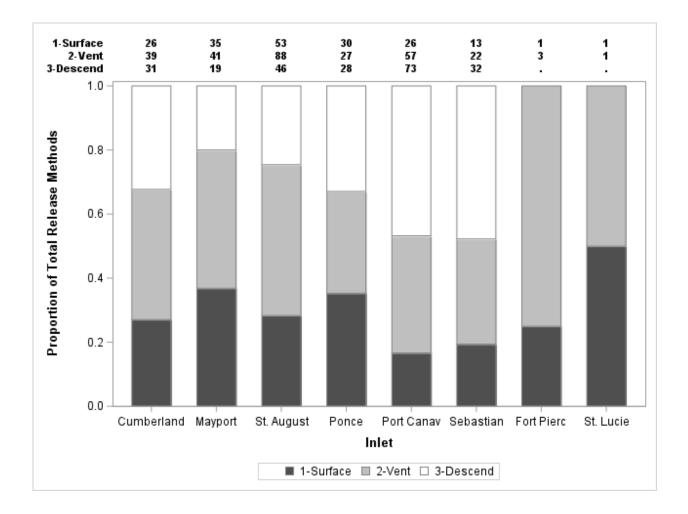


Figure 1.4 Proportion of released fish by mitigation method and inlet 2021. Numbers at the top of each column are total numbers of anglers indicating that they used that mitigation method when releasing discarded Red Snapper. <u>Surface</u> – no barotrauma mitigation. <u>Vent</u> – air was released from an inflated swim bladder with a sharp object before returning the fish to the surface. <u>Descend</u> – discarded fish were descended to depth using a weighted device.

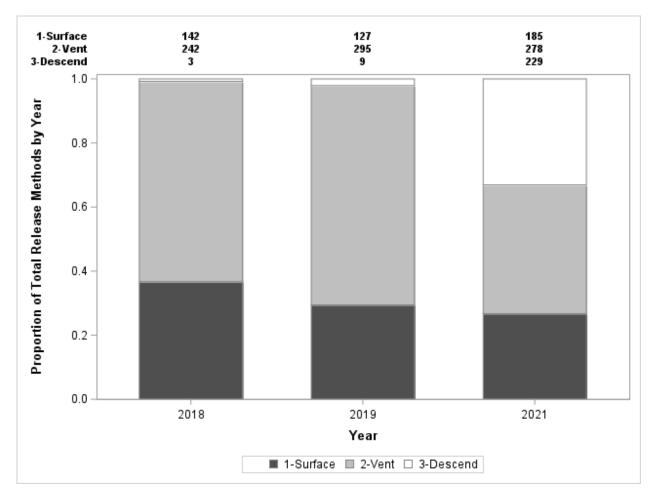


Figure 1.5. Proportion of boat trips that reported releasing Red Snapper, by mitigation method and year. Questions regarding barotrauma mitigation methods were initiated in 2018. The question was asked in 2018 and 2019, was eliminated in 2020, and returned in 2021. Numbers at the tops of each column indicate total numbers of boat trips indicating that they used that mitigation method when releasing Red Snapper. <u>Surface</u> – no barotrauma mitigation. <u>Vent</u> – air was released from an inflated swim bladder with a sharp object before returning the fish to the surface. <u>Descend</u> – discarded fish were descended to depth using a weighted device.

### Section 2: Charter Mode

#### Methods

*Mail / Telephone Survey* — The FWC maintains a list of active charter vessels that is used as the sample frame from which the MRIP For-Hire Telephone Survey (FHTS) weekly draw (10% of active vessels) is selected. For this survey, charter vessels in the wave 4, 2021, of the FHTS sample frame were matched to a list of charter vessels with a valid federal permit to harvest Snapper-Grouper species in the South Atlantic. This permit is required to harvest Red Snapper from the EEZ adjacent to the east coast of Florida. Charter vessels that do not possess a federal permit are more effectively monitored through the MRIP survey, since they may harvest legal sized Red Snapper year-round in state waters. However, this is rare due to the 20" size limit and distribution of larger fish farther offshore, outside state jurisdiction (particularly in northeast Florida). All vessels in the FHTS sample frame with a South Atlantic Snapper-Grouper permit were selected for this survey, with the exception of vessels that were randomly selected by MRIP to participate in the FHTS during the week the South Atlantic Red Snapper season was open.

The week before the July fishing season opened, each selected vessel was sent a letter describing the intent of FWC staff to collect catch and effort data for charter trips targeting or harvesting Red Snapper (Appendix 1). The letter explained that captains could participate in the survey by completing and returning the enclosed log sheet or, if no log sheet was received, FWC staff would attempt to contact them by telephone at the end of the Red Snapper season. The log sheets were printed on waterproof paper to encourage captains to bring the log sheet underway to improve the accuracy of responses, and a pre-paid postage envelope was also provided to encourage prompt return of the log sheet. The logs provided space to record trip and catch level data for up to three trips that targeted Red Snapper on each day of the South Atlantic season, including: number of anglers, number of passengers, trip origin (state and county), distance from shore and depth fished, dock to dock hours, hours fished, and numbers of Red Snapper harvested and released (Appendix 2). Each vessel representative was called up to five times, or until a successful contact was made or their mailed log sheet was received. Vessels that did not return the log sheet or that could not be contacted by the fifth call attempt were marked as non-contacts for the fishing season.

*Catch and Effort Estimation* – Survey responses were used to estimate the total number of charter boat trips that targeted Red Snapper, charter angler trips that targeted Red Snapper, and numbers of fish harvested and discarded by all active federally permitted charter vessels during the July 2021 South Atlantic Red Snapper fishing season. The formula used to calculate the total boat trips, angler trips, and numbers of fish harvested and released for each region and month is:

$$\widehat{Y} = \sum_{i=1}^{n} w_h y_{h,i} \tag{2.1}$$

Where  $y_{h,i}$  corresponds with the total number of boat trips, anglers, or fish reported by respondent *i* in region *h* during the two weekends when Red Snapper harvest was open, and  $w_h$  is a sample weight, calculated as:

$$W_h = \frac{N_h}{n_h} \tag{2.2}$$

Where  $N_h$  is the total number of federally permitted active charter vessels in region *h*, and  $n_h$  is the total number of vessels in region *h* that responded to the survey. The SAS procedure, PROC SURVEYMEANS, was used for this estimation (Appendix 3), and the variance is calculated using the Taylor Series method (SAS Institute Inc., 2008).

The northeast region included counties on the Atlantic coast of Florida north of Palm Beach County, where Red Snapper are most likely to be targeted, the southeast region included southern counties where the species is rarely encountered, and Monroe County was a separate region (Table 2.1). Charter vessels on the Gulf coast of Florida that carry the S. Atlantic Snapper – Grouper permit were also surveyed as a separate region during the July fishery opening to determine if any participate in the short seasonal opening (Table 2.1).

Estimated catch and effort were not adjusted for permitted vessels that are not included in the survey because they were not identified as active charter vessels in the FHTS frame. However, any such vessels also would have been counted as private boats during inlet boat counts (described in Section 1 above). Thus, it would be inappropriate to also account for undercoverage in the charter survey. Charter fishing effort and catch reported by respondents in this survey were not independently validated in the field.

Undercoverage Adjustment – Off-frame charter vessels were encountered during surveys described in section 1, and data collected from these vessels was included in expansions for total effort and catch in the private boat fishery. Thus, no adjustments for under-coverage were necessary in the mail and phone survey of federally permitted charter vessels in the NE or SE region. In the Keys, where private anglers rarely target Red Snapper in the EEZ, no special field surveys are conducted and no information on off-frame charter vessels is available. However, the charter fishery in the Keys is a minor portion of total recreational landings for Red Snapper on the east coast of Florida, and any under-coverage is expected to be small.

Charter vessels without federal permits were not included in this survey; however, when fishing in state waters they must abide by the 20" size limit. Legal sized fish are rare in state waters off the northeast coast of Florida, where Red Snapper are most abundant, although legal sized fish could be targeted in state waters off Dade and Monroe Counties during the South Atlantic season. However, given that state waters are open year-round there is no incentive for state vessels to target Red Snapper during the short South Atlantic season. For this reason, it is unlikely that charter landings were missed by not including state vessels in this survey.

## **Results and Discussion: Charter Mode**

The 2021 South Atlantic Red Snapper season marked the fifth year that a dual mail / phone survey was used to collect trip level data from the federal for-hire fleet. The survey was distributed to over three-quarters of the known, active charter vessels with a valid federal South Atlantic Snapper-Grouper permit (Table 2.2). The overall response rate for the survey remains high at 77.9%. As in years past, the lowest response rate was in southeast Florida at 52% (Table 2.3). However, this value represents an increase in total response as compared to 2020. The southeast region contains only 12% of the population surveyed and landings of Red Snapper are also relatively low, but a higher response rate will guarantee that the trends seen from the respondents are representative of fishing activity in the region. Outreach efforts made to verify contact information for captains before the start of the season have helped to improve the final

status of call attempts, both by reducing the number of attempts made and the number of captains we are unable to contact (Figure 2.1). All other regions of Florida have response rates at or above 70% (Table 2.3). The use of the dual mail survey continues to contribute to the high overall response rates associated with the survey, with 26.8% of the responses coming from returned mail survey logs (Table 2.2).

Before generating catch and effort estimates, the length frequency distribution of vessel lengths of the full charter vessel population was compared to the vessel lengths of the respondents and participants to determine if the latter groups are representative of the full charter population. The vessel length distributions of the full charter population and respondents appear to have similar shape and are likely representative (Figure 2.2).

Estimates of boat trips, angler trips, harvest, and discards were generated for northeast Florida (Nassau to Martin Counties), southeast Florida, and the Florida Keys (Monroe County). No Red Snapper trips were reported by respondents from west Florida (Escambia to Collier Counties). During the 2021 season, an estimated 4,196 ( $\pm$  SE 172) Red Snapper were harvested during 372 ( $\pm$  14) boat trips. Charter fishing effort and landings in northeast Florida continue to account for the majority of charter Red Snapper trips and harvest, 96.9% of angler trips and 98.3% of fish harvested (Table 2.4).

Each vessel provided trip level information about the depth and distance from shore where fishing occurred during charter trips (Table 2.5). Trip details from charter vessels operating in northeast Florida reported an average fishing depth of  $31.65 (\pm 10.51)$  meters and distance from shore of 25.42 ( $\pm 13.57$ ) miles. The 2021 survey yielded 5 trip-level reports from the Florida Keys. These trip reports indicate that charter trips occurred closer to shore ( $6.33 \pm 0.58$  miles from shore), but deeper depths ( $48.77 \pm 3.79$  meters) than northeast Florida Trips. A single trip in southeast Florida reported trip-level information with a depth of 30.48 m and a distance from shore of 12 miles.

Region	Coastal Counties
Northeast	Nassau, Duval, Clay, St Johns, Flagler, Volusia, Brevard, St. Lucie, Martin
Southeast	Palm Beach, Broward, Miami-Dade
Keys	Monroe
West Florida	Escambia, Santa Rosa, Okaloosa, Walton, Bay, Gulf, Franklin, Wakulla, Taylor, Dixie, Levy, Citrus, Hernando, Pasco, Pinellas, Hillsborough, Manatee, Sarasota, Charlotte, Lee, Collier

Table 2.1 Regional groupings of coastal counties used for generating catch and effort estimates.

Table 2.2 Survey frame, summary of response via mail and phone, and response rates by region.

Region	Charter Vessels	Total Selected	Mail Responses	Phone Responses	Response Rate
Northeast	157	143	37	83	0.846
Southeast	53	46	6	18	0.522
Keys	155	136	23	81	0.772
West Florida	107	101	23	61	0.831
Overall	472	426	89	243	0.779

Table 2.3 Table of response rates from 2017-2021, the time frame that the combined mail-telephone survey has been conducted.

Year	Northeast	Southeast	Keys	West Florida	Overall
2021	0.846	0.522	0.772	0.831	0.779
2020	0.731	0.447	0.709	0.886	0.724
2019	0.688	0.683	0.691	0.907	0.740
2018	0.703	0.816	0.777	0.902	0.792
2017	0.846	0.763	0.768	0.875	0.803

Variable	Region	Estimate	S.E.	Var	C.V.
	Northeast	357	13	171	0.037
Poot Tring	Southeast	2	2	3	0.740
Boat Trips	FL Keys	10	3	9	0.289
	Overall	372	14	184	0.037
	Northeast	3667	149	22234	0.040
Anglan Tring	Southeast	18	13	171	0.739
Angler Trips	FL Keys	100	28	775	0.278
	Overall	3785	152	23179	0.040
	Northeast	4124	171	29280	0.041
Harvest	Southeast	13	10	96	0.739
	FL Keys	59	15	152	0.259
	Overall	4196	172	29610	0.041
	Northeast	1.125	0.026	0.00065	0.044
CDUE	Southeast	0.750	0	0	0
CPUE	FL Keys	0.588	0.105	0.0109	0.350
	Overall	1.110	0.026	0.0007	0.046
	Northeast	8683	931	867495	0.107
Discards	Southeast	0	0	0	0
	FL Keys	30	17	281	0.568
	Overall	8713	931	86777	0.107

Table 2.4 Summary of effort and catch estimates for the federally permitted charter fleet from Northeast Florida, Southeast Florida, and the Florida Keys, for the 2021 South Atlantic Red Snapper season.

Table 2.5 Summary of reported depth and distance from shore for charter fishing trips taken during the 2021 South Atlantic Red Snapper fishing season.

Region	Boat Trips	Depth (m)		Distance Fr (m	
		Mean	S.E.	Mean	S.E.
Northeast	82	31.65	10.51	25.42	13.57
Southeast	1	30.48	-	12.00	-
FL Keys	5	48.77	3.73	6.33	0.58
Overall	88	32.57	10.84	24.33	13.86

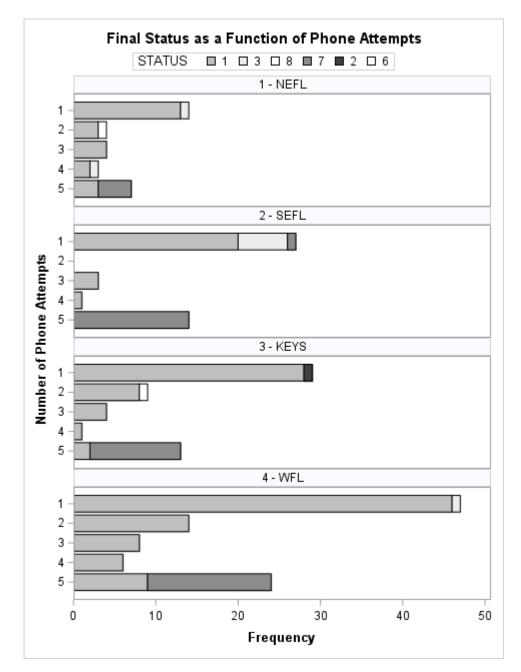


Figure 2.1 Frequency of attempted phone calls to federally permitted charter representatives, as a function of the status after the final call. Status Codes: 1=Complete interview, 2=Incomplete, but all key questions answered, 3=Refusal, 4=Language barrier, 5=Mid-Interview refusal, 6=Ineligible, 7=Unable to Contact, 8=Inactive.

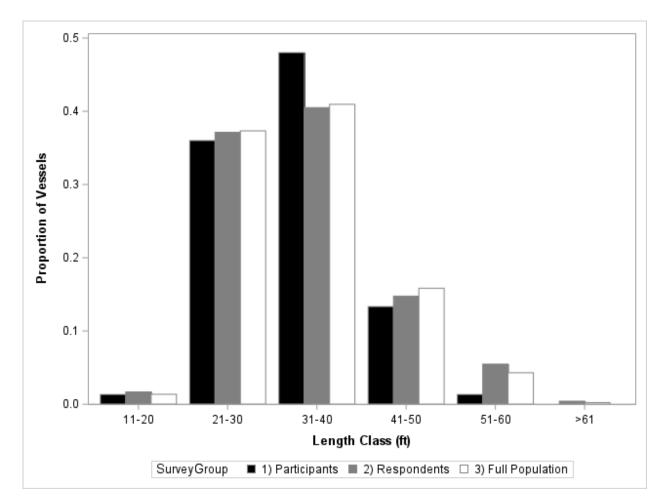


Figure 2.2 Proportion of vessels in 10 ft. length bins for three charter vessel groups: <u>Full</u> <u>Population</u> – Vessels with active federal South Atlantic Snapper -Grouper permits, <u>Respondents</u> – Vessels that responded to the survey, and <u>Participants</u> – Vessels that responded to the survey and conducted Red Snapper trips during the 2021 South Atlantic Red Snapper fishing season.

# Section 3. Biological Sampling

#### Methods

The Red Snapper harvest season provides an opportunity to collect fishery dependent biological samples from a species with a very short open season. When biological samples were obtained, field staff used and buckets to pass harvested fish between parties. These buckets were placed at a safe distance between parties, to maintain a physical distance while during the biosampling process. Biological samples were collected from both the private boat and charter boat fisheries (described above in Section 1). Each fish was measured (at midline in mm), weighed (kg), one otolith was extracted, and a small section of fin was cut for genetic analyses. Priority was given to collecting the left otolith, and this was done to quickly process fish so they could be returned to anglers.

To account for varied sampling rates across inlets in the study area, sample weights were calculated. For private boat catch, sample weights were calculated for each inlet as:

$$W_h = \frac{\hat{C}_h}{n_h} \tag{3.1}$$

where  $\hat{C}_h$  is the estimated landings for inlet *h* (reported in Table 1.3), and *n* is the number of fish sampled in inlet *h* (reported in results section below). Sample weights for each inlet were used to calculate an overall weighted mean for fork length (in mm) and kilograms for landed fish (using the survey means procedure in SAS). The sample weights for fish in each 1 cm length bin were also summed and divided by the sum of all sample weights (equal to total estimated landings) to calculate the weighted proportion of fish in each size category.

Red Snapper otoliths were assigned a unique sample number and associated data entered into the central database for fishery dependent biological samples housed at FWRI. Data are stored on a secure network that is routinely backed up. Otoliths collected during the 2021 season will be sectioned and aged in house at FWRI's Age and Growth Lab. Otoliths from fish sampled by the state of Georgia were also shipped to FWRI for processing. Fin clips taken for genetic analysis were shipped to the Marine Genomics Lab at Texas A&M University – Corpus Christi as a part of the South Atlantic Great Red Snapper Count (GRSC) project. All resulting biological data will be shared with analysts from the NMFS Southeast Fisheries Science Center for the next SEDAR stock assessment update. Age data will also be shared with University of Florida and Texas A&M for inclusion in their analyses for the South Atlantic GRSC.

#### Results

### Biological Samples collected during 2021

Measurements, weights, age structures and fin clips were collected during intercept assignments from both the private boat and charter fisheries. Sample sizes for numbers of Red Snapper measured, weighed, sampled for age and growth, and genetics during 2021 are provided in Table 3.1. In addition to those listed, six Red Snapper were sampled as part of the State Reef Fish Survey in St. Lucie and the Florida Keys. In contrast to previous years, Red Snapper were intercepted near the southern-most inlets of Fort Pierce and St. Lucie, suggesting a potential southward expansion of the population. The length frequency of fish harvested by private boat anglers and charter boats is shown in Figure 3.1. Red Snapper sampled from the private boat fishery (including off-frame charter trips) had a mean length of 562.43 mm (SE=3.08) and mean weight of 3.64 kg (SE=0.06). Red Snapper sampled from charter boats that were included in the charter survey averaged 593.99 mm (SE=6.41 mm) and 4.17 kg (SE=0.13).

Table 3.1. Numbers of fish sampled for length, weight and otoliths from private boat trips and charter boat trips. Numbers in parenthesis indicate additional fish sampled during intercept surveys from vessels that were not included in charter survey and were thus included in the catch estimate for private boats.

PRIVATE BOAT							
Inlet	Number of	Number of	Number of	Number of			
	Length	Weight	Otolith	Genetic			
	Samples	Samples	Samples	Samples			
Cumberland	222	221	222	171			
Mayport	193	188	190	176			
St. Augustine	435	413	428	274			
Ponce Inlet	307	273	306	305			
Port Canaveral	438	434	426	426			
Sebastian Inlet	135	131	135	135			
Fort Pierce Inlet	3	3	0	3			
St. Lucie Inlet	8	8	8	8			
Total	1741	1671	1715	1498			
CHARTER BOA	Γ						
Cumberland	17	17	17	1			
Mayport	21	21	21	21			
St. Augustine	136	127	133	39			
Ponce Inlet	23	23	22	23			
Port Canaveral	115	101	109	100			
Sebastian Inlet	14	14	14	14			
Fort Pierce Inlet	0	0	0	0			
St. Lucie Inlet	4	4	4	4			
Total	330	307	320	202			

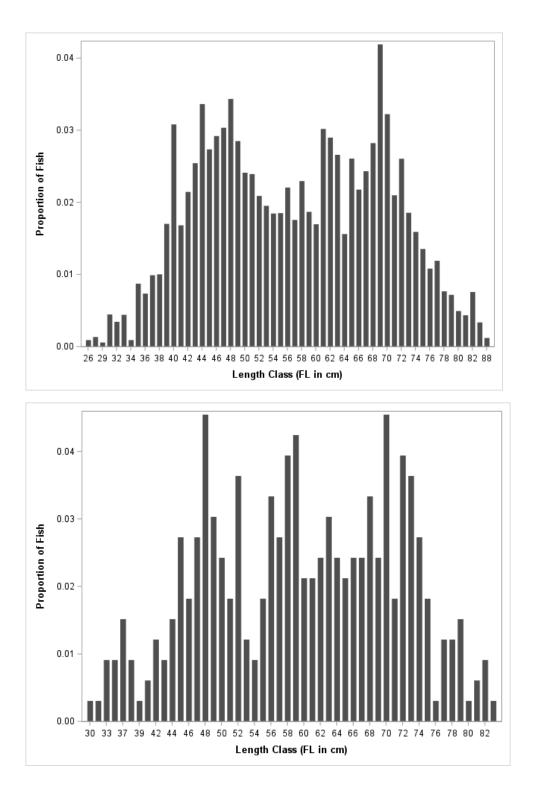


Figure 3.1. Size distribution of harvested Red Snapper sampled from private boat (top panel) and charter boat trips (bottom panel) during 2021. Samples from private boats are weighted proportional to total estimated landings for each inlet.

Appendix 1. Project Description, Estimation of US Atlantic Red Snapper Abundance.

# A. Study Title: Estimation of US Atlantic Red Snapper Abundance

**B. Investigators:** Will Patterson, University of Florida; Jeff Buckel, Nathan Hostetter, Krishna Pacifici, and Paul Ruderhausen, North Carolina State University; Dave Portnoy and Chris Hollenbeck, Texas A&M Corpus Christi; Nate Bacheler, Eric Anderson, and Kyle Shertzer, NOAA Fisheries; Beverly Sauls, Florida Fish and Wildlife Conservation Commission; and, Dan Gwinn, Biometric Research.

# **C. Background and Rationale:**

Red snapper, *Lutjanus campechanus*, is an ecologically and economically significant reef fish in US Atlantic waters between North Carolina and south Florida, where it has been estimated to be overfished [i.e., SSB <  $0.5(SSB_{F30\%SPR})$ ] since the early 1970s (SEDAR 2017). Commercial and recreational regulations put in place to rebuild the red snapper stock failed to accomplish that goal, but it was restrictive management following the passage of Reauthorized Magnuson-Stevens Fishery Conservation Act in 2006 that has caused vocal dissent among various user groups. The current requests for proposals offers the opportunity to estimate the population size of Atlantic red snapper independent of the stock assessment, which should prove to be beneficial to future assessments and fisheries management in the region. Here, we propose a study to produce two independent estimates of age-2+ Atlantic red snapper population size from North Carolina to Florida, one of which will be based on genetic (close-kin) mark recapture and the other which will take advantage of annual Southeast Reef Fish Survey (SERFS) camera-trap sampling along with remotely operated vehicle (ROV) sampling we will conduct in the region.

# **D. Study Objectives:**

Study objectives include 1) estimating the distribution and density of red snapper with ROVs in unknown or unconsolidated habitats within the SERFS sampling frame that are not sampled by SERFS, as well as sites outside the SERFS sampling frame; 2) conducting genetic close-kin mark recapture (CKMR) analysis to produce an estimate of age-2+ red snapper in the study region; and, 3) developing a hierarchical Bayesian integrated abundance model to produce a second estimate of age-2+ red snapper population size in the study region based on SERFS trapcamera and ROV survey data. Below, we describe the rationale and sampling details of each of these approaches. We start with a description of the SERFS sampling frame, site selection, and samples taken as that survey will provide the bulk of tissue samples required to perform CKMR analysis, as well as the data to fit the integrated abundance model.

# E. Methods:

<u>Southeast Reef Fish Survey Design and Sampling:</u> The SERFS collaborative survey, which is represented by co-PI Bacheler, was established in 2010 to estimate fishery-independent abundance trends for reef fishes off the southeast US (SEUS), with red snapper being a species of high interest. The survey annually deploys chevron fish traps with attached video cameras along the southeast

United States Atlantic continental shelf (Bacheler and Shertzer 2020), with ~1,500 trap-video samples are planned in spring and summer 2021 and again in 2022. CTD casts are also made at each site to measure hydrographic parameters of the water column, which will also be done at all ROV sampling sites as well. Annual sampling locations are chosen using a simple random sampling design based a sampling frame of approximately 4,000 sites that are on or adjacent to known hardbottom (Fig. 1) from Cape Hatteras, NC to St. Lucie Inlet, FL. Chevron traps are 0.91 m<sup>3</sup> in volume, baited with 24 *Brevoortia* spp., and have two GoPro Hero 4 video cameras (one mounted over the trap mouth and one over the nose) facing outward. All fish caught in traps are identified, counted, and measured. Fish in video samples are identified and counted. All red snapper captured in SERFS traps are sacrificed and their otoliths are extracted and aged. In 2021 and 2022, replicate fin clips also will be sampled from those fish for CKMR analysis (see below).

Fin clips will be placed in 1.5-ml plastic vials, immersed in saltsaturated DMSO buffer, and stored at room temperature (Seutine et al. 1991). In recent years, ~2,000 red snapper have been captured annually in SERFS trap sampling, with a mean annual increase in catches of ~14% per year since 2015. Therefore, we anticipate sampling >2,000 red snapper samples from SERFS in 2021 and 2022 for CKMR analysis.

Red Snapper Tissue Sampling from Fishery Landings: We will supplement SERFS-collected red snapper tissue samples with samples from commercial and recreational fishery landings off northeast Florida and North Carolina. Red snapper landings are sampled along the Atlantic coast of Florida by the Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute's (FWRI) Fishery-Dependent Monitoring Program (FDSP), which is led by co-PI Beverly Sauls. FWRI-FDMP samples fish lengths and otoliths from landings during the short Atlantic red snapper recreational fishing season. Otolith samples are aged at FWRI. No sampling occurred in 2020 due to Covid restrictions, but sampling is set to resume in summer 2021. In 2018 and 2019, FWRI personnel randomly sampled >2,500 fish per year from St. Lucie Inlet in the south to Cumberland Sound on the Florida-Georgia line. Replicate fin clip samples will be taken from all red snapper samples during summer 2021 and 2022 for the CKMR analysis described below.

Study personnel will sample red snapper landings off



Fig. 1. Map of US Atlantic indicating study sampling frame (pink), 2021 SERFS sites (green circles), seabed mapping locations (multicolored shapes), and 625 km<sup>2</sup> study grid cells.

NC in summer and fall 2021 and 2022. Sampling will occur at recreational docks during the limited recreational season and at fish houses during the commercial season. Fish will be measured and weighed, otoliths will be extracted, and replicate fin clips will be sampled as indicted above. Otoliths will be processed for ageing at UF. All fin clip samples will be shipped to the Marine Genomics Laboratory of co-PIs Portnoy and Hollenbeck at Texas A&M University-Corpus Christi where DNA will be extracted and sequenced as described below.

<u>Remotely Operated Vehicle Sampling:</u> We will conduct ROV video sampling of reef fish communities and shelf habitats in summer 2021 and 2022. We will utilize VideoRay Pro4 ROVs owned and operated by PI Patterson's Marine Fisheries Laboratory at the University of Florida (UF). These mini-class ROVs are small (<6 kg) yet their flight is stable in currents up to 2.6 m/s (~5 knots) due to gyroscopic stabilization and a high thrust:mass ratio. The ROVs are rated to 305 m and are connected to a surface control box by a tether that can range in length from 100 to 200 m. Multiple tethers can be daisy-chained together to achieve required length for deep sites. We also attach a 5-10 kg clump weight to the tether to fly transects or prevent unnecessary sailing of the tether in current behind the vessel. In high (>2 m/s) current conditions, like are often encountered on the outer continental shelf in the study region, the ROV is simply flown near the bottom along with the ship and the direction of the prevailing current to conduct transect surveys.

Having multiple (n = 3) ROV systems at our disposal translates to having backup systems at sea thus no downtime in the case of infrequent technical issues. The ROVs are each equipped with a 170-line color camera and the control panel has a 15-inch high-resolution monitor to view the ROV flight path. The ROVs are also equipped with red laser scalers (75 mm beam width) and stereocameras to measure observed fish. Stereocamera systems are constructed of GoPro Hero7 cameras in deepwater housings mounted to an aluminum bar that is screwed into the ROV frame (Garner et al. in revision). This system allows for easy stereocamera calibrations and the cameras shoot 2.7K resolution at 120 fps and a linear field of view (FOV) of 118°. A third GoPro camera is mounted to the center front of the ROV's float block and angled 45° downward. Based on this angle, the camera's FOV angle, and the ROV's altitude, the width of transects being flown can be estimated precisely (Patterson et al. 2009, 2014). Personnel of UF's Marine Fisheries Laboratory have conducted over 2,500 dives with these ROV systems (e.g., Dahl and Patterson 2014; Lewis et al. 2020), including 908 video samples conducted during sampling of the Florida Gulf of Mexico (GOM) shelf as part of the recently completed Great Red Snapper Count Study. Furthermore, calibration experiments conducted as part of that study with stationary stereocameras, multibeam hydroacoustics, and fine-scale high-resolution 3D acoustic telemetry to track red snapper responses to ROVs and other mobile gear demonstrated red snapper behavioral response to the Pro4 mini ROV was neutral (i.e., no net attraction or repulsion; Garner et al. in review).

There are two objectives to ROV sampling in the current study. The first is to conduct paired sampling to calibrate red snapper density estimates derived from video samples taken during SERFS trap-camera deployments. This will occur over 3 days in summer 2021 off North Carolina, and 3 days off northeast Florida. Our goal will be to conduct ROV transect sampling at a minimum of 10 SERFS sites each day of sampling for a total of 60 paired sampling sites. At each site, the ROV will be deployed following the methods of Patterson et al. (2014) in which the ROV tether is deployed to the bottom with a 5-kg clump weight and then 4 25-m orthogonal transects are flown away from that center point at a height of 2 m. This will produce an area surveyed of ~1,000 m<sup>2</sup> depending on the ROV's altitude throughout the survey. The ROV's position over the seabed also will be tracked and recorded with an ultrashort baseline system to accurately estimate the distance covered on each transect.

ROV sampling also will be conducted cooperatively with for-hire recreational or commercial fishermen in summer 2021 to estimate the density of red snapper in areas of unknown or unconsolidated habitat, as well as habitats outside the sampling frame of the SERFS survey (Fig. 1). There are 226 partial or whole 625 km<sup>2</sup> cells within the ~100K km<sup>2</sup> sampling frame specified in the RFP. Among those, 123 cells contain 2021 SERFS sampling stations (Fig. 1). We

will utilize stratified random sampling to select 44 additional cells to conduct ROV transect video sampling among 26 sampling days in summer 2021, with at least 5 sampling sites located within each cell. Strata will include unsampled cells within the SERFS sampling frame (n = 26 cells over 13 days), cells to the north of the SERFS sampling frame from Cape Hatteras to latitude 36.5N (n = 6 cells over 3 days), cells from St. Lucie Inlet to the Dry Tortugas (n = 6 cells over 3 days), and cells on the outer shelf between St. Lucie Inlet and Cape Hatteras between 120 m (the deepest SERFS trap-camera sets) and 150 m (n = 6 cells over 6 days).

Video from ROV transect sampling will be analyzed at UF. Fish will be identified and counted in each video samples, and then fish density will be computed by dividing counts by total area surveyed. Red snapper fork length will be estimated with the StereoMorph package (Olsen and Westneat 2015; Olsen and Haber 2017) in R (R core team 2020) from stereocamera still images based on synced video and pre-deployment calibrations. Fork length will be converted to total length with a linear regression fit to red snapper data.

<u>Close-Kin Mark Recapture:</u> Genetic mark-recapture methodologies are similar to traditional mark recapture methodologies but differ in several important ways that make the genetic approach ideal for dispersive marine organisms like red snapper. A genetic mark is the composite genotype of an individual across some number of characterized loci. Therefore, it is natural and permanent and can be recovered from a fully processed carcass, fin clips from live animals, or other tissue samples. Genetic marks are transmitted from parent to offspring, thus are partially shared between related individuals. This considerably expands the scope of what may be considered a "recapture" to closely related individuals (i.e., kin). Combining such kin recaptures with models informed by life history information to relate the number of kin to the census population size, is a recent technique known as close-kin mark-recapture (CKMR, Bravington et al. 2016a). While CKMR has not yet been widely applied in marine systems, it has recently been successfully applied to species of conservation concern, including southern bluefin tuna (Bravington et al. 2016b) and great white sharks (Hillary et al. 2018).

The probability is 1.0, or 100%, that an individual in a sample has one mother and one father. In an idealized population model where all adult reproductive contribution is equal, the probability that one individual has the same mother as another is  $1/N_{\text{adult females}}$ . The probability those same two individuals have the same father is  $1/N_{\text{adult males}}$ . Ignoring full siblings, the probability that two individuals are half siblings is  $1/N_{adult females} + 1/N_{adult males}$ , or  $1/N_{adult}$  if the sex ratio is equal. If m pairwise comparisons are made from individuals sampled from the population, then the expected number of half sibling pairs (P) is  $m/(N_{adult})$ . Solving for  $N_{adult}$  yields the equation as  $N_{\text{adult}} = m/P$ . However, in an actual versus idealized population, adult contribution is not equal because it is dependent on viable egg production. Therefore, probabilities for each comparison must be adjusted to account for effects arising from age, year, and sex, through the use of a population dynamics model accounting for adult abundance, size-specific mortality, and size-specific fecundity (Bravington et al. 2016a). The effects of annual random variation in reproductive success must also be controlled by only making comparisons between samples from different cohorts (Bravington et al. 2016a). Therefore, a properly executed CKMR study requires: 1) parameterized life history data, 2) accurately aged samples, 3) a sampling design tailored to the specific kin relationships targeted for estimating  $N_{\text{adult}}$ , and 4) sufficient resolution in genetic data to accurately characterize kin relationships. This method provides great promise for providing an independent estimate of Atlantic red snapper abundance that is both accurate and precise because the required life history data (age- and sex-specific mortality, age- and sex-specific fecundity, age

at maturity) are currently available (Lowerre-Barbieri et al. 2015; SEDAR 2017), and all samples collected in this study will be aged via otolith thin sections. Furthermore, the cutting-edge genomic resources required to accurately define red snapper kin relationships have already been designed by co-PIs Portnoy and Hollenbeck, and co-PIs Portnoy and Anderson, an active developer of modern CKMR techniques, have begun CKMR design and model parameterization for Atlantic red snapper (see below).

*CKMR Experimental Design*: The spatial scale of sampling must be representative of the total population, and the age of individuals sampled, as well as the timing of samples, must be accounted for in the experimental design. Tissue samples collected from red snapper during SERFS chevron trap sampling, as well as from fishery landings between Cape Hatteras, North Carolina and St. Lucie Inlet, Florida, will be extracted and sequenced (described below). Only half-sibling relationships across cohorts will be used to estimate population size to account for recruitment variability (Bravington et al. 2016b). To determine the number of samples required to obtain an estimate of census size with the required CV, forward simulations with CKMRpop (Anderson 2021a) were conducted with Atlantic red snapper life history data to estimate sample sizes required for half-sibling CKMR. Simulations were run twenty times each for age-2+ population sizes expected to be  $5 \times 10^5$ ,  $1 \times 10^6$ , and  $1.5 \times 10^6$ . This range in population size was chosen because there were approximately  $5 \times 10^5$  age-2+ red snapper estimated to be in the US Atlantic population in the most recent assessment (SEDAR 2017), and preliminary estimates from the Great Red Snapper Count study in the GOM produced an estimate that was approximately 3 times the assessment-derived estimate of  $N_{age-2+}$ , which we designate as  $N_{adult}$ .

Population size was estimated with an annual sample size of 2,500 individuals per year for two sampling years, which would be the approximate sample size if only SERFS samples were available, and also with 5,000 samples per year, which is a conservative estimate of the total fin clip samples we actually will collect in this study. Only half-siblings sampled across cohorts were used to estimate  $N_{adult}$ . Estimation of  $N_{adult}$  followed the pseudo-likelihood approach of Bravington et al. (2016a) which will be implemented in the current study. Briefly, for each pair of samples the probability of being half-siblings for a given N<sub>adult</sub> was calculated, accounting for age at sampling, year of sampling, the expected age structure of the population, and age-specific variation in reproductive success. All probabilities were then combined into a likelihood of observing the number of recorded half-siblings for a given population size and the suite of red snapper life history parameters. Likelihoods were evaluated for population sizes between 5 x  $10^5$  and 1.5 x  $10^6$ , normalized, and used to construct posterior probability distributions. The estimated Nadult and CV was then calculated for each posterior distribution and averaged across all 20 runs for each combination of  $N_{adult}$  and sample size. Results indicate the target annual sample size of >5,000 fish is estimated to be more than sufficient to achieve required levels of precision (Table 1), and that would likley remain true even if actual Nadult is several times greater than stock assessment-derived estimate of  $\sim 5 \times 10^5$  age-2+ fish.

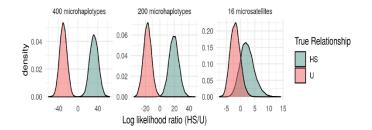
Table 1: Results of CMKR sample size simulations: C is the actual population size, S is the annual number of individuals sampled in each of two years,  $N_{adult}$  is the mean estimated population size among 20 simulations. The CV also was computed among 20 simulations.

С	S	Nadult	CV
500,000	2,500	496,054	16.0%
	5,000	500,428	7.7%
1,000,000	2,500	998,591	23.2%
	5,000	1,023,518	11.1%
1,500,000	2,500	1,568,213	30.0%
	5,000	1,566,668	13.8%

*Molecular Techniques*: Single nucleotide polymorphism (SNP) containing loci suitable for use in Genotyping in Thousands by sequencing (GT-seq; Campbell et al. 2015) panels have been identified using existing double digest restriction-site associated DNA sequencing (ddRAD) data from Atlantic red snapper by co-PIs Portnoy and Hollenbeck. A draft assembly of the red snapper genome also has been constructed by co-PI Portnoy and collaborators via previous funding (Portnoy unpublished data). The SNP-containing loci, also known as microhaplotypes, to be utilized in the current study were selected based on observed heterozygosity and relative position in the genome. Also considered was whether multiple alleles are present at a give locus.

The application of microhaplotypes (SNP-containing loci) in this study provides clear advantages over other markers often utilized in CKMR studies, such as nuclear DNA microsatellites and SNPs. In addition to the increased efficiency and reproducibility of sequencingbased genotyping, hundreds of microhaplotypes, which are themselves multiallelic markers, will be used to estimate kin relationships in this study, as compared to a typical microsatellite data set that might consist of tens of markers, or a SNP data set, which would use only biallelic loci. Therefore, the microhaplotype approach will provide superior confidence in kin relationship designations (Baetscher et al. 2017). To illustrate the power of this approach, siblings and unrelated individuals were simulated using 200 and 400 microhaplotypes, as well as 16 microsatellites, with allele frequencies estimated in previous Atlantic red snapper genetic studies (Hollenbeck et al. 2015, Portnoy et al. unpublished data). The false positive rate (FPR), or the rate at which unrelated individuals would be characterized as half siblings, was then estimated for each data set using CKMRsim, a Monte Carlo CKMR sample size simulator written by co-PI Anderson in R (Anderson 2021b). Estimated FPRs for the 200 data set was 0.0005%, and was 0.0000001% for the 400 microhaplotype data set. These results means that if 400 microhaplotypes were used to estimate kin relationships among 1 x  $10^4$  sampled red snapper (~36 x  $10^6$  pairwise comparisons), then zero unrealted indviduals would be misidentified as half-siblings. The FPR for the 16 microsatellites was ~25%, which indicates CKMR-derived estimates of  $N_{adult}$  based on microsatellites would be biased considerably.

Fig. 2. Relative probability of pairs of red snapper being half-siblings (HS) vs. unrelated (U) based on simulated genotypes for three marker datasets: 400 microhaplotypes, 200 microhaplotypes, and 16 microsatellites. HS and U overlap indicates probability of misassigning unrelated pairs as half-siblings (FPR).



Two GT-seq panels (200 loci each) will be optimized to allow for simultaneous sequencing and genotyping of thousands of individuals required for this project. Mass sequencing will follow a modified version of the GT-seq method of Campbell et al. (2015). Briefly, DNA will be extracted into 96-well plates. Polymerase chain reactions (PCR) will be run with pooled primer mixes that contain ~200 locus-specific primers. Locus-specific primers will be modified by attaching Illumina sequencing adapters to the ends, which will function as priming sites for indexed Illumina PCR primers. A second round of PCR will then be run using a portion of the first PCR as a template to add Illumina flow cell adaptors, which include index sequences that allow for the identification of individual samples bioinformatically after sequencing. Following the second PCR, all 96 amplified samples will be pooled into a single library, cleaned, quantified via qPCR and diluted to a standardized concentration. Equal volumes of many libraries can then be pooled and sequenced on a single lane of an Illumina HiSeq platform, allowing for simultaneous genotyping of thousands of individuals. Sequences retrieved from a sequencing run will be separated by individual fish, using index sequences, and homologous sequences for each individual aligned to a reference sequence to genotype SNPs. Finally, SNPs on the same contig will be collapsed into microhaplotypes (Willis et al. 2017), producing a data set consisting of microhaplotypes.

To ensure accurate identification of half-siblings, a subset of individuals (including all identified putative half-siblings) will also be genotyped using ddRAD sequencing. This approach enables the generation of massive amounts of genome sequence data from a single experiment, and a random sample of thousands of informative (polymorphic) genetic markers spanning an entire genome can be identified and genotyped simultaneously for multiple individuals on an Illumina HiSeq platform (Davey and Blaxter 2010). Based on ongoing research, only ~200 individual red snapper can be genotyped with ddRAD on a single HiSeq lane, making it an inefficient technology for genotyping thousands of individuals, as is required for CKMR. However, it can be deployed efficiently to verify half-sibling designations for hundreds of individuals. For example, in the simulations run for this proposal an average of 171 half-siblings were sampled for a population of 5 x 10<sup>5</sup> individuals and 5 x 10<sup>3</sup> total samples, which would result in ~400 fish being included in ddRAD sequencing (i.e., two sequencing runs) to ensure all putative half siblings were sequenced along with randomly selected putatively unrelated pairs.

Briefly, DNA extraction and purification will follow methods used routinely in Portnoy and Hollenbeck's laboratory at TAMU-CC (Portnoy et al 2015, Hollenebeck et al. 2019), and libraries consisting of  $\sim 50 \times 10^3$  sequence fragments will be generated following an improved modification of ddRAD-tag procedures (Peterson et al. 2012). Genomic DNA will be digested with two restriction endonucleases and the resulting fragments ligated to two adapter oligonucleotides (P1 and P2) that serve as barcodes, enabling DNA from multiple individuals to be sequenced and each individual identified unequivocally on a single Illumina sequencing lane.

Following adapter ligation, barcoded and indexed sequences are pooled and size-selected using a PippinPrep size-selection system (Sage Science), where fragments in a specific size range (375-425 base pairs, bp) are used in the remainder of the library-generation process. PCR amplification of fragments is then used to incorporate adapters necessary for annealing to an Illumina flow cell during a sequencing run. RAD sequences retrieved from a sequencing run will be separated by individual fish, using barcode-index sequences, and homologous sequences for each individual aligned to a reference genome to identify individual SNPs. Quality filtering, *de novo* assembly of RAD loci (SNPs), read mapping, alignment, and SNP calling will be performed with dDocent (Purtiz et al. 2014) and genotyping (identifying homozygotes and heterozygotes) at individual SNP loci will be accomplished using a Bayesian approach (Garrison and Marth 2012). As above, SNPs on the same contig will be collapsed into microhaplotypes to produce a final data set of SNP-containing loci.

*CKMR Statistical Analysis*: Putative half-sibling relationships between pairs of red snapper samples will be identified initially using GT-seq data and CKMRsim. For all identified putative half-siblings, relationships will be revaluated using the full ddRAD sequencing data and CKMRsim, producing a final count of supported half-sibling relationships. Population size ( $N_{adult}$ ) and its CV then will be estimated from the number of half siblings captured across cohorts in the pseudo-likelihood framework using the custom script described above to account for age- and sexspecific mortality and reproductive output. While this will address the primary goal of this study, the broad genome coverage and large number of markers present in the panel, as well as the large spatially complete sampling, will also allow for fine-scale resolution of within and between group genetic diversity (i.e., genetic stock structure) in Atlantic red snapper. Furthermore, the data will be used to estimate the effective number of breeders ( $N_b$ ) using the linkage disequilibrium approach (Do et al. 2014) for the entire US Atlantic red snapper population, but also for any proposed population segments separately, such as apparent population clusters off northeast Florida and North Carolina.

### Estimating Red Snapper Population Size from Trap-Camera and ROV Data:

The second estimate of Atlantic red snapper age-2+ abundance we aim to produce will integrate SERFS trap-camera and ROV data to estimate abundance across the study area. Our integrated abundance model will maximize use of available data by jointly modeling SERFS video-trap and ROV data and directly addresses numerous challenges with estimating red snapper abundance (Table 2). In brief, our integrated abundance model 1) fits a single abundance model where abundance is estimated from multiple observation processes (e.g., trap-camera, ROV), 2) provides a framework to integrate existing as well as newly collected data (e.g.,), and 3) improves precision of abundance estimates by reducing deficiencies and biases relative to separate analyses of each data set (Table 2; Pacifici et al. 2017, 2019; Hostetter et al. 2019; Gwinn et al. 2019;). The Bayesian framework also allows for the natural inclusion of published information about environmental influences on the abundance and detection process through the use of informative priors to increase model resolution (e.g., Coggins et al. 2014; Bacheler et al. 2014; Shertzer et al. 2016; Gwinn et al. 2019).

The primary data supporting our integrated abundance model will be the SERFS trapcamera data, along with ROV survey data. The overlap of ROV and trap-camera sampling at a subset of sites will provide contrast in the data from those two data sources. However, we will also consider data available from tagging-based or fishery-dependent data, such as ongoing conventional tagging of recreational fishery discards by FWC-FWRI personnel off northeast Florida or NOAA observer data.

Challenge	Data	Approach	Citations
Convert counts to density	Video (counts), Chevron (counts), ROV (density)	Integrated Abundance Model, estimate effective sampling area from newly-collected data and prior information	Pacifici et al. (2017), Hostetter et al. (2019), Bacheler et al. (in review), Garner et al. (in review)
Spatial variation in abundance	Spatial covariates, two levels of spatial resolution, ROV surveys of uncharacterized habitat	Abundance modeled as a function of spatial covariates and random effects	Pacifici et al. (2019)
Detection varies by survey method	Spatially and temporally replicated video counts, overlapping ROV and video surveys	ROV and trap data jointly analyzed, N-mixture type detection process for video counts, informative priors	Shertzer et al. (2016), Hostetter et al. (2019), Kazyak et al. (2020)
Spatial sampling	SERFS and ROV	Study design simulation, account for effort and preferential sampling	Pacifici et al. (2016), Coggins et al. 2014

Table 2. List of primary challenges and approaches to confront those challenges.

Our modeling approach will integrate multiple survey methods described above to jointly estimate red snapper abundance at three spatial scales: i) survey site, ii) grid cell, and iii) study area (Fig. 1). To accomplish this, we first discretized the ~100 km<sup>2</sup> sampling frame (Fig. 1) into j = 1, 2, ... J grid cells (25×25 km), reflecting a spatial resolution appropriate for snapper ecology, covariate resolution, and management needs (Fig. 1). Each grid cell is further gridded into i = 1, 2, ... I non-overlapping survey sites (100×100 m), which are larger than the effective sampling area of any single survey method but small enough to assume uniform snapper density within a site. Each survey site may be sampled by s = 1, 2, ... S survey methods (e.g., camera, trap, ROV) or remain unsampled during the study period. As detailed below, nested spatial scales address the importance of local environmental characteristics at survey sites, while balancing data collection at different spatial resolutions, assumptions of population closure, and computational demands.

For the underlying abundance process, we assume  $n_{ij} \sim \text{Pois}(\lambda_{ij}A_{ij}w_j)$ , where  $n_{ij}$  is the latent red snapper abundance at survey site *i* within grid cell *j*,  $\lambda_{ij}$  is the expected abundance, of individuals per unit area,  $A_{ij}$  is the known area of survey site *i* within grid cell *j* and  $w_j$  is an indicator variable denoting if grid cell *j* contains suitable habitat (1) or not (0). Red snapper abundance varies among sites as a function of site-specific covariates ( $C_{ij}$ ; e.g., sea floor habitat, depth, latitude). Specifically,  $\log(\lambda_{ij}) = \beta_0 + \beta C_{ij} + \delta_{ij} + \theta_j$ ,  $\delta_{ij} \sim \text{Normal}(0, \sigma^2)$ , and  $\theta_j \sim \text{MVN}(\mu, \Sigma)$ , where  $\beta_0$  is the log-scale intercept, representing the expected abundance across all survey sites, and  $\beta$  is a vector of estimated parameters describing the relationships between snapper abundance and site-specific covariates,  $C_{ij}$ . Unstructured random effects,  $\delta_{ij}$ , account for heterogeneity at the site level that cannot be accounted for by environmental covariates (we assume variance  $\sigma^2$  is shared across all sites and grid cells for estimation) and  $\theta_j$  are random effects drawn from a multivariate normal distribution that is used to describe spatial correlation among grid cells due to

unmeasured environmental influences. The parameter  $\mu$  represents the mean and  $\Sigma$  represents the variance-covariance matrix. This component of the model allows us to draw stronger inference for grid cells that are unsampled by capitalizing on spatial correlations in abundance.

Suitability  $(w_j)$  will be explored as part of the modeling and can be informed from study data, separate fishery-dependent data (e.g., presence/absence, effort), and informed priors from previous studies (Coggins et al. 2014; Bacheler et al. in review; Garner et al. in review). Suitability of grid cell *j* is modeled as a Bernoulli random variable,  $w_j \sim \text{Bern}(\phi_j)$  and  $\text{logit}(\phi_j) = \gamma_0 + \gamma X_j$ , where  $\phi_j$  is the probability grid cell *j* contains suitable habitat and  $\gamma$  is a vector of estimated parameters describing the relationships between snapper suitability and grid cell covariates.

Each sampling method employed will be formulated with an appropriate observation model to account for idiosyncrasies of each data type. The basic observation model will assume count data  $(y_{ijks})$  result from a binomial process, with sample size  $n_{ij}$  and conditional probability of detection  $p_{ijks}^*$ . The conditional capture probability will then be derived as  $p_{ijks}^* = p_{ijks} \times p_{ijs}^{eff}$ , where  $p_{ijs}^{eff}$  is the effective sampling area of survey method *s*, defined as  $\tilde{A}_{ijs}/A_{ij}$  to represent that each sampling method surveys a portion of the total area within cell *ij*.  $A_{ij}$  is fixed by design and  $\tilde{A}_{ijs}$  will be estimated. For example,  $\tilde{A}_{ijs}$  is known for ROV sampling, but must be estimated for the chevron trap/video combination gear based on Bacheler et al. (in review) and influential covariates such as water clarity and current direction.  $p_{ijks}$  is a site-specific detection probability that can be a function of important site- and survey-specific covariates affecting detection ( $C_{ijs}$ ; Bacheler et al. 2014, Shertzer et al. 2016, Gwinn et al. 2019),  $logit(p_{ijks}) = \alpha_{0s} + \alpha_s C_{ijs}$ .

Total red snapper abundance across the study area ( $N_{total}$ ) will be estimated as the sum of site-specific abundance:  $N_{total} = \sum_{i=1}^{l} \sum_{j=1}^{J} n_{ij}$ . We will fit the full model in a Bayesian framework using Markov Chain Monte Carlo methods, which allows propagation of uncertainty from sampling units to frame-wide abundance estimates and across all estimated and derived quantities. The flexibility of this approach will allow us to derive abundance (and uncertainty) at multiple regional levels by summing across subsets of grid cells, a feature that may be of importance to regional management considerations. Furthermore, we will be able to estimate red snapper population size based on SERFS data alone, which will allow comparison to the multiple survey derived estimate from the integrated abundance model, as well as to the CKMR estimate.

#### F. Study Deliverables

Study deliverables will include two estimates of red snapper population size (with associated CVs) that are independent from one another and from the stock assessment. We will also assess the occurrence and estimate the density of red snapper on unknown and unconsolidated habitats in the region, including areas outside the current SERFS sampling frame. Genetics sampling and analysis will allow further refinement of estimates of Atlantic red snapper population structure and connectivity with the GOM. Beyond the final report produced based on study results, multiple peer-reviewed manuscripts will results from this study. Data and results will also be posted, along with study progress during the course of the research, on a website hosted at the University of Florida for a non-technical audience, which will be widely advertised in the region. Lastly, we will present results to the South Atlantic Fishery Management Council and work closely with Sea Grant agents among states in the study region to produce materials to keep constitutes apprised of study goals, progress, and findings.

## G. Expected Outcomes and Anticipated Benefits

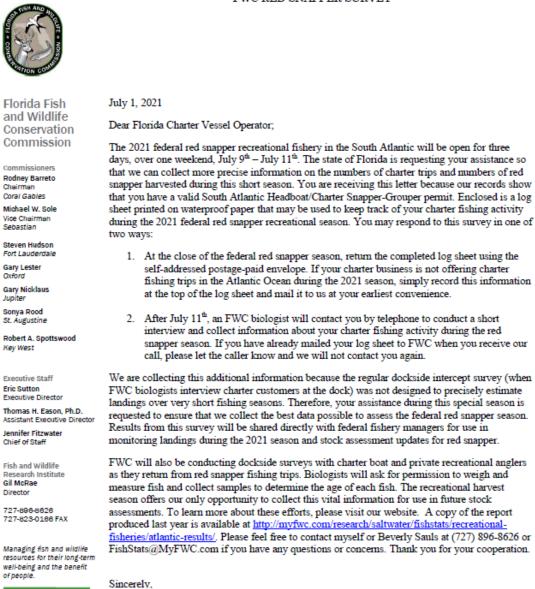
Beyond the Atlantic red snapper population estimates that will be produced during this study, we will also gain invaluable information on the distribution of habitats and reef fish communities across the shelf in the study region. We will actively engage for-hire recreational and commercial fishermen who we will charter to conduct ROV surveys for this project. Cooperative research fosters buy-in from user groups and PIs Patterson, Buckel, Portnoy, and Sauls have long track records of working cooperatively with fishermen throughout the region. In-person meetings with fishermen from northeastern Florida through North Carolina by Paul Ruderhausen will further facilitate buy-in among user groups, as well as further develop the database of reef habitat in the study region. To date, <15% of the study region has been mapped with multibeam or side scan sonar, thus working with the fishermen to inventory hardbottom habitat distribution will greatly benefit reef fish assessment and management into the future. Lastly, the integrated red snapper abundance model can be recomputed with SERFS data in the out years to estimate and track red snapper population abundance on the shelf. The SERFS-only integrated abundance model may provide similar results to the model that also includes ROV data, as well as results from the CKMR approach, but even if that is not the case the SERFS-only model could still be utilized to track relative red snapper abundance among years. Furthermore, subsequent funding could be utilized to conduct ROV or other surveys to produce additional red snapper density information to facilitate direct population abundance estimates in future years.

## **H. Related Work**

Drs. Patterson and Portnoy are members of the scientific team conducting the Great Red Snapper Count in the Gulf of Mexico, with Dr. Patterson's team having conducted 908 ROV surveys to estimate the red snapper population in GOM waters off western Florida. His team also has conducted extensive experimentation to validate ROV methods to be utilized herein and to test the behavioral reaction of red snapper to mini ROVs and other mobile sampling gears. Dr. Buckel's research team has extensive research conducting life history and fisheries ecology research on reef fishes in the study region, with ongoing research related to estimating effects of barotrauma and its mitigation, as well as conducting tagging experiments to estimate natural mortality. Dr. Portnoy and his team have recently completed the mapping of the red snapper genome and the most extensive study to date on red snapper population structure in the US Atlantic and GOM. They have developed many of the SNP-related sequencing and bioinformatics processes that we will utilized in the current study. Dr. Anderson is a quantitative geneticist with ongoing projects ranging from mapping migratory bird population structure to estimating salmon hybridization with genetics techniques. He wrote the modeling program we will utilize to estimate Atlantic red snapper population size with CKMR. Drs. Hostetter, Gwinn, and Pacifici are quantitative ecologists with a range of modeling experience, including development of methods to estimate detection probability in fish and wildlife studies, as well as occupancy and N-mixture modeling. They are currently using integrated modeling approaches to address a range of fisheries and wildlife questions on several continents. Dr. Bacheler leads the annual SEFIS component of the SERFS survey and has conducted extensive research on fish reaction to trap-camera gear, the effective sample area of the SERFS chevron traps, and modeling to estimate red snapper occupancy and habitat utilization on the US Atlantic shelf. Dr. Shertzer is the lead analyst for the Atlantic red snapper assessment and also has conducted extensive modeling to estimate red snapper distribution and population trends in the study region. His membership on our team will be instrumental in seamlessly incorporating study results into the stock assessment model to scale

estimates of population size and productivity. Ms. Beverly Sauls leads the Fisheries-Dependent Monitoring Program at FWC-FWRI and oversees large-scale surveys of fishing effort, total removals, and the biological composition of catch, as well as mark-recapture studies for reef-fishes along the Atlantic and Gulf coasts of Florida.

**Appendix 2.** Letter sent to federally permitted charter representatives the week prior to the South Atlantic Red Snapper Season opening.



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Appendix 3. Log sheet send to federally permitted charter representatives the week prior to the South Atlantic Red Snapper season opening.

#### Florida - Red Snapper Survey Log Vessel Name:

Vessel Number:

Did you participate in the 2021 South Atlantic Red Snapper Season (Trips where you kept, released or tried to catch Atlantic Red Snapper)? YES NO If you circled yes above, please complete the log sheet below. Only report trips where Atlantic Red Snapper were targeted, harvested, or released at sea. Please return all completed log sheets with the self-addressed postage-paid envelope provided. Thank you for your participation.

Date	Day of Week			No. of	No. in Party	Origin of Trip		Miles from	M iles from	Depth Fished	Time Trip	Time Trip	Time Spent Fishing	No. of Atlantic Red	No. of Atlantic Red
						State	County	Shore (range)	Shore (majority of trip)	(majority of trip)	Started (24hr)	E nded (24hr)	r isning (nearest half-hr)	Ked Snapper Kept	Snapper Released
7/9/2021	FRI	1													
7/9/2021	FRI	2													
7/9/2021	FRI	3													
7/10/2021	SAT	1													
7/10/2021	SAT	2													
7/10/2021	SAT	3													
7/11/2021	SUN	1													
7/11/2021	SUN	2													
7/11/2021	SUN	3													
			Pleas	e write a	ny additio	onal con	nments about the	season or you	ur trips belo	w or on the	back of	this sheet	:		
1															

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**Appendix 4.** The PROC SURVEYMEANS code used in SAS to generate the estimated number of charter boat trips that targeted Red Snapper, charter angler trips that targeted Red Snapper, and numbers of fish harvested and discarded by all active federally permitted charter vessels during the 2021 South Atlantic Red Snapper season.

```
*COMPLETE ESTIMATE USING PROC SURVEYMEANS;

□proc surveymeans data=charter total=pop sum sumwgt cvsum std varsum clsum missing ;

strata region season;

weight w;

domain region season region*season;

var rf_trips Anglers Harv Rel;

ods output statistics=charter_estimate domain=charter_strat;

run;
```