

THE GREAT RED SNAPPER COUNT

Estimating the Absolute Abundance of Age-2+
Red Snapper in the U.S. Gulf of Mexico.



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Project Title: Estimating the Absolute Abundance of Age-2+ Red Snapper (*Lutjanus campechanus*) in the U.S. Gulf of Mexico.

August 16, 2021

NOAA Contract Number: NA16OAR4170181

How to cite this report:

Stunz, G. W., W. F. Patterson III, S. P. Powers, J. H. Cowan, Jr., J. R. Rooker, R. A. Ahrens, K. Boswell, L. Carleton, M. Catalano, J. M. Drymon, J. Hoenig, R. Leaf, V. Lecours, S. Murawski, D. Portnoy, E. Saillant, L. S. Stokes., and R. J. D. Wells. 2021. Estimating the Absolute Abundance of Age-2+ Red Snapper (*Lutjanus campechanus*) in the U.S. Gulf of Mexico. Mississippi-Alabama Sea Grant Consortium, NOAA Sea Grant. 408 pages.

Acknowledgements

A project of this magnitude was unprecedented in scope and coverage and required intense preparation, planning, and implementation. It was the result of many meaningful contributions from a variety of legislators, agencies, scientists, steering committees, and review/planning teams. Appendix A names all the primary investigators involved in helping bring this project to completion along with their affiliations, roles, and contributions to the project. The scope of additional personnel who contributed is vast and cannot be encompassed within Appendix A. This included a team of 80 + scientists that were directly involved with this work and was composed of students (undergraduates through Ph.D.) as well as various others that played essential roles. Here we recognize those individuals not previously acknowledged.

Principally, we would like to thank the United States Congress for appropriating funds for this study and recognizing the resources needed for a project of this enormity. The NOAA Sea Grant was the ideal agency for leadership as well NOAA Fisheries facilitating multiple aspects of the study. Sea Grant generously provided their outstanding administration, oversight, and facilitation. In particular, we would like to thank the Mississippi-Alabama Sea Grant Consortium for administering this award. Dr. LaDon Swann and his team including Loretta Leist and Devaney Cheramie, were instrumental in facilitating much of the ‘behind-the-scenes’ work that the immense administrative workload required. This was not a small undertaking, and largely went unnoticed, as they flawlessly executed the administrative tasks letting the research teams remain focused on the science. Their guidance and insight were essential, as they shepherded this process from inception to completion. We are appreciative of the many hours spent dealing with complex budgets, subcontracts, reports, constituent outreach, and many other tasks across 21 individual PIs, 12 Institutions, and over 100 individuals directly involved with the study. We thank the administrative staff at the Harte Research Institute for Gulf of Mexico Studies (HRI) for their assistance with administrative support, promotion of this project through media, as well as managing a variety of logistical needs that enabled us to carry out the goals of the project. We also thank the Steering Committee that had diverse membership with wide-ranging expertise. This team helped guide this project through a multi-phased approach from planning workshops, design, review, and ultimately to a final completed report and constituent engagement. We heavily relied upon several members of this committee as we faced many expected and unexpected challenges throughout the study period. We would also like to thank the external review team of Drs. Steve Cadrin, Mary Christman, and David Eggleston. Their insight on the design, analyses, and interpretation greatly improved this report. Additionally, input from the Gulf of Mexico Fishery Management Council’s Scientific and Statistical Committee on earlier versions helped to refine various aspects of the study. This project could not have been successful without this thoughtful foresight, planning, guidance, and intensive review process.

A few of those challenges included Hurricane Harvey during the initial contractual and research phases, several major hurricanes during the interim, a global pandemic, and ending the project with Uri, a historic polar vortex event at the 11th hour of report completion resulting in no

electricity, running water, gas, or communication for many on the project team. Yet, despite these obstacles, the group of experts was able to manage and meet pressing deadlines. The foresight provided by the steering committee and expert science and review team gave us the adaptability to face these difficulties and provide a robust estimate of Red Snapper abundance in the U.S. Gulf of Mexico.

For a project of this scale, the contributions of various charter captains, commercial fishermen, and individual anglers cannot be overlooked as they worked with us during every aspect of this project. They provided not only their vessels but also their expertise, allowing us to collect visual and acoustic data, as well as tag thousands of Red Snapper throughout the Gulf. Their participation was critical to the successful completion of our research tasks. In Alabama/Mississippi waters, all field work was conducted aboard the F/V *Escape* under the direction of Captain Skipper Thierry. In Florida waters, we thank charter boat captains Johnny Greene, Gary Jarvis, Josh Livingston, and Brad Gorst, as well as their crews, for aid in ROV and sonar sampling. Captains Johnny Greene and Sean Kelley and their crews also provided assistance during tagging trips. In Texas waters, we thank the owner of the Fishermen's Wharf, Will Cocks, and the captains and deckhands of the *Scat Cat* and the *Wharf Cat* who helped conduct ROV and hydroacoustic surveys and tagging operations. Captains Scott Hickman, Mike Jennings, Chad Kinney, Dan Green, and their crews also assisted with scientific tagging. Individual anglers throughout the Gulf were critical in completing the high reward tagging portion of our study, as the unexpectedly high return rate was directly due to their participation.

And finally, the contribution from various research institutes/agencies, oceanographic vessels, and associated research staff were critical to the success of this project. Without the dedication of numerous research staff throughout the Gulf region, little would have been accomplished with the scale of this undertaking. In Alabama/Mississippi, we gratefully acknowledge the assistance of the Dauphin Island Sea Lab. Ed Kim oversaw the endless analysis of video footage. We thank the Florida Institute of Oceanography (FIO) for facilitating Florida outer shelf sampling, and the captains and crews of the R/V *W.T. Hogarth* for making our work possible. In Florida, Jordan Bajema, Jessica Van Vaerenbergh, Miaya Glabach, Allison White, Nick Tucker, Savannah Labua, and Gabriel Diaz provided essential technical support in the field. In the northern and western Gulf, the captain and crew of the FIO R/V *Weatherbird II*, and field scientific parties consisting of Sarah Grasty, Matt Hommeyer, Alex Ilich, Steve Butcher, Chad Lembke, Heather Broadbent, Abigail Vivlamore, Alex Silverman, Ed Hughes, John Gray, Chih-Wei Huang, and Gerardo Toro-Farmer conducted towed camera studies over oil and gas pipelines and unconsolidated sediments. Additionally, Jill Thompson-Grimm, Marie Meranda and Brittany Combs participated as guest scientists. In the Western Gulf, the Louisiana Universities Marine Consortium (LUMCON) and the captain and crew of the R/V *Pelican* were essential to sampling operations for visual and acoustic data collection. We also thank the Louisiana Department of Wildlife and Fisheries (LDWF) for collecting and providing genetic samples through their own tagging operations. In Texas, we also acknowledge the assistance from staff at Texas A&M Galveston particularly graduate students, Jason Mostowy and Phillip Sanchez. We thank the Harte Research Institute for Gulf of Mexico Studies and the tremendous crew of research specialists and post-docs at the Center for Sportfish Science and Conservation

for conducting field operations and data collection for abundance estimations and tag returns in Texas: Jason Williams, Quentin Hall, Kesley Banks, Jeff Kaiser, Ashley Ferguson, David Norris, Jasmine Rodriguez, Daniel Coffey, and Megan Robillard. We cannot overstate the contributions of HRI graduate students Kelsey Martin and Jill Thompson-Grim, who were integral in collecting, processing and analyzing Texas ROV and acoustic data as part of their graduate degrees. We would also like to thank Juan Canchola for creating the graphic figures for this report. Texas Parks and Wildlife Department staff members Jim Tolan and Darin Topping provided insight in initial project development. NOAA's Matthew Laretta, an uncompensated contributor, also offered insightful advice in the early stages of project design.

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I. Executive Summary

Rationale

The primary goal of this initiative was to estimate the absolute abundance of age-2+ Red Snapper (*Lutjanus campechanus*) in the U.S. waters of the Gulf of Mexico (Gulf). The fishery supported by this species is of iconic stature and supports one of the more economically valuable finfish fisheries in the region. Funding was made available by the U.S. Congress and administered through the Mississippi-Alabama Sea Grant Consortium to produce a Red Snapper population estimate independent of the federal stock assessment via a systematic Gulf-wide sampling plan at an unprecedented regional scope and level of funding. Management decisions by the Gulf of Mexico Fishery Management Council based on previous SEDAR (Southeast, Data, Assessment, and Review) stock assessments for Red Snapper have been contentious, and there is concern that the current assessment framework, given the nature of the data involved, is not providing an accurate estimate of abundance. Results of this study provide an independent assessment of Red Snapper stock size via fishery-independent surveys conducted throughout the U.S. Gulf. This is an exceptionally rare opportunity in fisheries science allowing for studies of absolute abundance of this scope and geographic coverage. Moreover, study findings offer a unique opportunity for other approaches to be integrated into the assessment framework. Thus, the **primary rationale** for funding was that a robust estimate of absolute abundance will increase our scientific understanding of the population dynamics for Red Snapper across its range and distribution. Science is a building process, and the independent estimate of abundance derived from this research is not a replacement or in contention with the official SEDAR Red Snapper Stock Assessment. It will supplement and enhance ongoing analyses by allowing for validation, calibration, and further refinement of those models, given absolute abundance has now been estimated independently from the assessment model.

The product reported here is the result of many planning meetings led by a formal Steering Committee. This committee facilitated execution of the study over two separate Requests for Proposals (RFPs). This document represents a report and abundance estimation from an in-depth multiphase design, including project design, implementation, analyses, and interpretation. Briefly, the steering committee convened numerous meetings and hosted several workshops for leadership teams, scientific investigators, review scientists, statisticians, and other constituents. These meetings resulted in an RFP for Phase I (see Appendix E). This phase dedicated \$600,000 to fund six research teams to develop and present independent designs that would accomplish the specified goals and objectives to ultimately generate an absolute abundance estimate for Red Snapper. In a unique and valuable approach, the steering committee then coalesced the most appropriate and desirable aspects from these proposals into an overall prescriptive design to generate an independent abundance estimate in a second RFP. The Phase II RFP (Appendix E) explicitly detailed the scope, goals, and objectives of the study including general methodologies of how a successful team should carry out the abundance assessment including: general statistical analyses, target coefficient of variation (CV), geographic scope, habitat types to assess, depth ranges, and a comprehensive stakeholder engagement component.

Also required was an incorporation of an extensive fish tagging component. Recognizing that a single sampling method was not capable of providing Red Snapper abundance estimates in each habitat type across the entirety of the Gulf region, the RFP recommended and encouraged the use of multiple sampling methodologies that would most likely succeed. In addition, teams were charged with developing new advanced technological methodologies appropriate to meet the goals of the study that would otherwise have not been available. Studies that involved genetic-based methodologies were prohibited; however, collection and archiving of samples for future analyses were encouraged and accomplished. Additionally, the Steering Committee recognized that current bottom habitat mapping was not sufficiently comprehensive to represent the coverage of all Red Snapper habitat in the U.S. Gulf. While teams were encouraged to synthesize existing imagery on habitat distribution, the RFP specifically excluded any additional direct mapping activities. Thus, teams were to use what habitat characterization was available to generate the abundance estimate. For areas that were unmapped/unclassified, yet hypothesized to support a large abundance of Red Snapper, the RFP specified these areas fall into a ‘catch-all’ category of Uncharacterized Bottom (UCB). This process culminated into an intensive written and live three-day peer-review administered by the Gulf of Mexico Fishery Management Council. The process of driven by a team of expert independent external reviews, Drs. Steve Cadrin, Mary Christman, and David Eggleston, including review by members of the Council’s Scientific and Statistical Committee. This report integrates their comments, suggestions, and analytical recommendations.

Sampling Techniques and Design

A major challenge facing this scientific assessment was developing a robust design and relatively unbiased sampling methods that could be applied among the many habitat types and regions across the U.S. Gulf. Both the Steering Committee and our team concluded there was no single method that could efficiently and accurately sample the diversity of habitats given the heterogeneity in geology, habitat types, and water clarity found across the Gulf shelf. Thus, different sampling methods were developed and used in each region to estimate Red Snapper abundance. A stratified random sampling design was developed to generate abundance by region, habitat type, and depth. In this design, the Gulf was separated into eco-regions that closely mirrored state jurisdictional boundaries. Within each region, zones were defined by depth (approximately 10-40m, 40-100m, 100-160m) and habitat type: artificial reefs, natural hard bottom, and uncharacterized bottom (UCB). A suite of methods was deployed to obtain local abundance estimates to accommodate the heterogeneity on the U.S. Gulf shelf, and to fulfill the project mandate of developing and advancing sampling technologies. A preferred method of determining species abundance is through visual means; however, the primary constraint on this technique was water clarity and fish detectability. The visibility limitations resulted in visual methods being primarily used in the east, where visibility was high. In contrast, hydroacoustic methods were primarily used in the west, where water clarity was poor. Remotely operated vehicle (ROV) visual count surveys were used to evaluate densities on artificial and natural substrates in Florida waters. A series of ROV surveys were also used to generate species composition in other regions when visibility allowed. Within Mississippi and Alabama waters, depletion surveys were the primary method used, and in the western Gulf, ROV/Towed camera

arrays (TCA) and hydroacoustics were the methods used to generate estimates. Along pipelines and the vast expanses of UCB, a combination of acoustic and visual approaches was used to efficiently and extensively cover these habitat types. A series of behavioral experiments were conducted in Florida to test whether Red Snapper were attracted to or repelled by any of the mobile sampling gears used among Gulf regions. Finally, a mark-and-recapture study was conducted with high reward tags to provide regional estimates of exploitation and fishing effort. This tagging initiative used fisher participation and engagement in the scientific data collection process and relied exclusively on these stakeholders for the recapture of tagged fish.

Abundance Estimates

Estimates of age-2+ Red Snapper abundance were produced by region, habitat type, and depth. Where appropriate, population estimates for artificial reefs were made for various categories representing the diversity of artificial structures. Overall, we estimated an absolute abundance of 118 million age-2+ (CV 15%) Red Snapper across the continental shelf of the U.S. Gulf (Figure 1, Table 1) during late 2019. In general (see detailed analytical methods), population estimates were derived by expanded mean densities, with means and variances calculated assuming simple random sampling at the lowest strata. Where density estimates were derived from acoustic counts of total fish corrected by a region-specific proportion of Red Snapper, the uncertainty in this average proportion was incorporated into the estimated variance. Means and variances at higher levels of aggregation (region, total) were calculated following stratified sampling methods. Estimates were performed by two independent groups on the same data set to provide validation from different estimation approaches. While the approaches, post-stratification, and application of statistical models differed and were not stipulated *a priori*, these independent analyses produced similar estimates (i.e., within 6.0%; 7.2 million Red Snapper difference from each estimate). While large numbers of fish occurred over well-known habitat features such as artificial reefs and natural hard bottom, a major finding of this study showed that UCB habitat harbored the majority of Red Snapper.

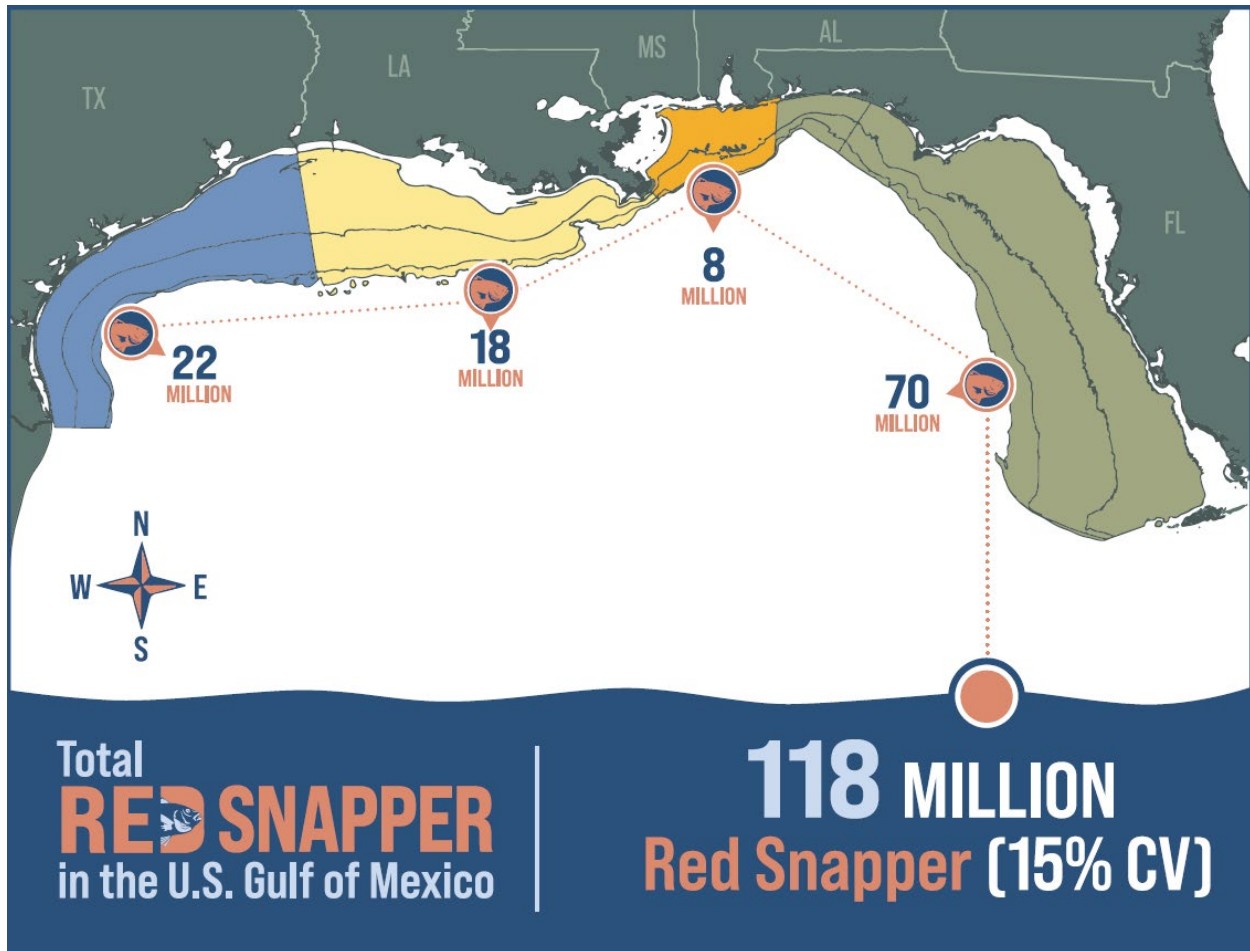










Figure 1. Overall estimate of the absolute abundance of age-2+ Red Snapper by each eco-region/state across the U.S. Gulf of Mexico.

Table 1. Estimate of age-2+ Red Snapper absolute abundance rounded to millions of fish for each state and each of the three main habitat types: Natural hard bottom, Artificial Reefs, and Uncharacterized

State /Region	Habitat Type	Estimated Abundance
 Texas	Natural	7,000,000
	Artificial	<1,000,000
	Uncharacterized Bottom	14,000,000
	 State Total	22,000,000
 Louisiana	Natural	4,000,000
	Artificial	4,000,000
	Uncharacterized Bottom	10,000,000
	 State Total	18,000,000
 Mississippi & Alabama	Natural	4,000,000
	Artificial	1,000,000
	Uncharacterized Bottom	3,000,000
	 State Total	8,000,000
 Florida	Natural & Uncharacterized Bottom	70,000,000
	Artificial	<1,000,000
	 State Total	70,000,000
Total RED SNAPPER in the U.S. Gulf of Mexico		118 MILLION (15% CV)

Project Impacts

The primary outcome of this study was the independent abundance estimate of age-2+ Red Snapper in the Gulf by habitat type including artificial reefs, natural hard bottom, and UCB. This was a large-scale survey using well-established and novel sampling approaches that have been integrated into a larger modeling framework and applied over an unprecedented geographic area, both in size, complexity, and in new habitat types (e.g., UCB) that were previously unassessed. This study provides a robust population estimate and can be further refined as additional spatially explicit habitat mapping becomes available. The scientific approaches to surveying a widespread species occurring in diverse habitats, such as Red Snapper, were advanced by the development, implementation, and evaluation of the gear developed for this study, and that knowledge can be carried to future similar studies. The potential management implications of a higher abundance estimate of Red Snapper need to be carefully considered by policymakers/managers and are beyond the scope of this report. Already, there has been much discussion of how to appropriately integrate the sampling methodologies used in this study with the traditional fishery-independent methods used for stock assessments. This study may help refine population parameters estimated during the Southeast Data Assessment and Review (SEDAR) process and suggest potential strategies for addressing some of the data gaps inherent in the assessment by constructively challenging assumptions made in the current Red Snapper Stock Synthesis assessment model. Thus, the stock synthesis model, and perhaps others, can be calibrated to provide more accurate estimates of stock status.

Stakeholder Engagement

Stakeholder engagement was a major element of this study. The partnerships built throughout this project have been valuable for informing the general public regarding ongoing research in their community, and in many cases, creating a vested interest in the scientific understanding and conservation of our natural resources. Several design components from this project naturally facilitated an RFP-requirement for meaningful participation from recreational anglers, commercial fishermen, and other stakeholders. This component included a high-reward tagging study that was performed regionally throughout the Gulf. While scientific tagging during the initial fishing effort was necessary, recapture of the fish occurred broadly across the entire Gulf by anglers from all fishing sectors. The heavily incentivized reporting (\$250 - \$500 reward) of recaptured fish proved highly successful and was very popular among anglers. The return rate of over 30% eclipsed expectations. While not specifically tested in this study, these data gave key insights to high fishery exploitation over artificial reefs. The documented return rate also shows promise for use of descender devices to reduce discard mortality that should be further investigated. Captains associated with this project have expressed high satisfaction with the partnerships built during this project and conveyed their desire to remain involved in future research endeavors. Comprehensive awareness campaigns developed for the tagging study and other aspects in the abundance estimation also offered the opportunity to engage the general and angling public about this study, and this involvement allowed citizens and regional consortia to provide key support for this project. Certainly, a major benefit from this involvement was the

fishing community remained engaged in the study, recognized the value of and need for advancing science, and remained vested in a sustainable fishery.

Key Takeaways

- This study produced an estimate of 118 million (CV 15%) age-2+ Red Snapper residing in the U.S. Gulf of Mexico through 2019.
- A large percentage of Red Snapper occurred over the uncharacterized bottom habitat type, which may represent a pool of cryptic biomass not previously accounted for in Red Snapper stock assessments. A high abundance of Red Snapper occurring over these areas that are largely unexploited by the fishery may also explain the weak stock-recruit relationship consistently observed in this fishery.
- The tagging results indicate:
 - an astonishing 30% return rate of tagged fish.
 - high fishing exploitation generally occurs over habitat with the highest densities of Red Snapper (i.e., artificial reefs).
 - high angler ‘buy-in’ and engagement with this type of study.
 - that use of descending devices was an effective release strategy.
- This study builds on our scientific knowledge base and improves our understanding of Red Snapper abundance in a non-contentious and constructive approach to federal assessments. This absolute abundance estimate will bolster future assessments and afford other stock evaluation and management options.
- Given new effort recalibrations are underway for Red Snapper, incorporation of these newly discovered fish occurring over UCB, and understanding exploitation patterns of anglers may lead the Red Snapper stock assessment to converge with similar abundance estimates. Moreover, had this information been available for previous stock assessments, those abundance estimates likely would have been higher.
- Stakeholder engagement efforts were successful; approximately 60% of anglers surveyed were familiar with the Great Red Snapper Count. Notably, awareness of the GRSC was associated with up to three times higher satisfaction with fisheries management (Scyphers et al. *In Press*).
- While the survey methods used in the study represent a rigorous application of the best technology available, the specific results of these surveys needed to be extrapolated, since it would be impossible to directly survey all areas. The uncertainty surrounding those extrapolations are linked to the resolution of our habitat maps. We encourage, further mapping, especially of the UCB, to decrease uncertainty in future studies.

- This report is just the beginning of future assessment meetings and activities with managing agencies, Scientific and Statistical Committees, the NOAA Southeast Fisheries Science Center, and the Gulf of Mexico Fishery Management Council. These activities will facilitate direct incorporation of these data into the management process.

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Note: See Appendix A for detailed list of management structure and descriptions of roles and responsibilities for each investigator .

II. Project Description

A. Scientific and Professional Merit

1. Rationale for Project, Background, and Funding Need

Red Snapper, *Lutjanus campechanus*, is a nearly ubiquitous species occurring on natural and artificial reefs across the northern Gulf of Mexico (Gulf) shelf (Patterson et al. 2014; Karnauskas et al. 2016; Streich et al. 2017; Garner et al. 2019), where it supports robust commercial and recreational fisheries. Red Snapper is perhaps the most ecologically, economically, and culturally significant reef fish species in the region, given its widespread distribution, and the fact that its exploitation dates to the 19th century (Stearns 1883; Collins 1885; Jarvis 1935; Camber 1955). The diversity of contemporary Gulf fisheries that target this iconic species directly or, like the shrimp trawl fleet that merely catch it as bycatch, translates to myriad stakeholder groups that have a vested interest in Red Snapper assessment and management. As is often the case in multi-sector fisheries management, balancing the management objectives of numerous stakeholder groups, who often have competing interests or desires, has proven to be exceptionally challenging for Red Snapper.

The first formal stock assessment of Gulf Red Snapper was in 1988, which estimated the stock to have been overfished since the 1960s (Goodyear 1988; SEDAR 2015). In the early 2000s, the stock assessment enterprise for fisheries in federal waters transitioned from a National Marine Fisheries Service driven process to a more open and inclusive process known as Southeast, Data, Assessment, and Review (SEDAR). Stakeholders and a diversity of academic and state agency scientists have participated in multiple SEDAR assessments of Red Snapper since 2005, with the goal of each being to peer-review and challenge data inputs, parameter assumptions, and model structure in a series of transparent workshops aimed at producing the best scientific information available to estimate Red Snapper stock status and project future yields that avoid overfishing while rebuilding spawning stock biomass. What has been consistent across the many Red Snapper stock assessments since 1988 are estimates that the stock has been overfished since the 1960s, although overfishing has mostly been eliminated since 2010 given regulatory requirements of the Magnuson-Stevens Reauthorization Act, which was signed into law by President Bush in early 2007.

An important change in how the Red Snapper stock is assessed occurred during its 2005 SEDAR assessment when, based on population demographics, post-settlement movement, and population genetics data, the stock was divided into eastern and western sub-units for assessment, with the dividing line being the mouth of the Mississippi River (Fischer 2007; Gold and Saillant 2007; Patterson 2007; Porch 2007). The Gulf of Mexico Fishery Management Council (GMFMC) has continued to estimate Gulf-wide annual catch limits (ACL) for the stock despite numerous motions passed by its Scientific and Statistical Committee (SSC) that management as well as assessment should occur separately for eastern and western Gulf Red Snapper populations sub-units of the stock. One reason that particular scientific advice has not been heeded by the GMFMC is because the spawning potential ratio (SPR) of the eastern sub-

unit of the Gulf Red Snapper stock consistently has been estimated to be much lower than that of the western sub-unit (SEDAR 2005, 2009, 2015, 2018); thus, pursuing separate management between the east and the west would mean more restrictive management in the east. The Gulf-wide approach to setting ACLs benefits eastern Gulf fishers by providing greater access, but that approach is estimated to have delayed Red Snapper recovery in the eastern versus western sub-units of the stock (SEDAR 2015, 2018). This has resulted in estimated exploitation rates (landed fish relative to population size) to be much higher in the eastern versus western Gulf (SEDAR 2015, 2018), which has also exacerbated stakeholder conflicts as higher landings would be permitted in the west, given its much higher SPR, if the western population was managed as well as assessed as a separate sub-unit of the overall stock. Thus, many of the controversial issues surrounding the best approaches for Red Snapper Assessment could be allayed by better regional abundance data.

Access issues in the recreational fishery have been caused by other factors as well. For example, as the stock has recovered since 2010, the mean size of landed fish has increased substantially. If larger fish are landed, that translates to annual catch targets (ACT) being reached with fewer fish, which has a negative effect on recreational season length. This created a conundrum for the GMFMC as they had to decrease recreational season lengths in federal waters as catch rates and mean fish size increased faster than spawning stock biomass (SSB). Shortened federal seasons also resulted in recreational effort compression, which put further downward pressure on federal season length to avoid overfishing. States reacted to decreasing recreational season lengths in federal waters by leaving recreational seasons open in states' waters longer than the federal season. This created even more downward pressure on federal season length because landings in states' waters was subtracted from the Gulf private recreational ACT to project season length in federal waters. The federal recreational fishing surveys, the Marine Recreational Information Program, and its predecessor the Marine Recreational Fishery Statistics Survey, were not designed to estimate catch and effort for intense, short (<20 days) seasons, so uncertainty in federal landings estimates increased. This spiral of shorter and shorter federal seasons, with greater and greater uncertainty in recreational landings estimates, during a period of increasing Red Snapper SSB, created increasing stakeholder distrust in Gulf Red Snapper assessment and management. This was particularly the case in the private recreational sector. The issues in part could perhaps be allayed with a better estimate of absolute abundance.

In 2016, the U.S. Congress intervened in the process of Gulf Red Snapper assessment and management in three important ways via the Fiscal Year 2017 Commerce, Justice, Science and Related Agencies appropriations bill. Among the Gulf Red Snapper aspects of that legislation were changing the state-water fishery boundary for all Gulf states to 9 nautical miles from their coastlines, establishment of an exempted fishing permit (EFP) program under which Gulf states could manage their share of the Gulf-wide recreational ACT, and the creation of a \$10 million program (\$12 million total with institutional match) to estimate Gulf Red Snapper population size and exploitation rates independent of the SEDAR assessment process. A study of this complexity, magnitude, and geographic coverage certainly requires extensive resources and in particular planning. Those funds were appropriated to be administered through NOAA's Mississippi/Alabama Sea Grant (MSAL-SG) program. The product reported here is the result of

many planning meetings lead by a Steering Committee. This committee facilitated execution of the study over two separate Requests for Proposals (RFP). A series of workshops were hosted by MSAL-SG in 2016 and 2017, and MSAL-SG awarded funds to six teams around the Gulf (Phase I; see Appendix E) to propose methods and approaches to estimating Red Snapper abundance and exploitation rates. Through that process, a consensus was developed that a combination of advanced technologies, such as remotely operated vehicles (ROV), towed camera arrays (TCA), and splitbeam sonar, should be used to estimate Red Snapper abundance, and a conventional tagging study should be conducted to estimate Red Snapper exploitation rates. The steering committee coalesced the best aspects of these designs into an abundance estimate design that formed the basis for Phase II. The second grant competition was then held for teams of researchers to propose specific studies to meet program objectives, with the primary objective being to estimate U.S. Gulf age-2+ Red Snapper population abundance with a CV <0.3. This RFP (Appendix E) explicitly detailed the scope and goals and objectives of the study including general methodologies of how a successful team should carry out the abundance assessment including: general statistical analyses, expected CV, geographic scope, habitat types to assess, depth ranges, and constituent engagement – specifically, incorporation of an extensive fish tagging component. Recognizing that a single sampling method was not capable of providing reliable absolute abundance measurements in each habitat type, the RFP recommended and encouraged multiple sampling methodologies that were most likely to succeed. In addition, the teams were charged with developing advanced technological methodologies appropriate to meet the goals of the study. Studies that involved genetic-based methodologies were prohibited; although, collection and archiving of samples for future analyses were encouraged and accomplished. Additionally, the Steering Committee recognized the current state of bottom surface mapping was not sufficiently comprehensive to represent all Red Snapper habitat in the Gulf of Mexico. Teams were encouraged to seek out available high-resolution imagery and were precluded from expending funds to do direct mapping of habitats.

This report details the sample design, survey methods, data post-stratification, and Red Snapper abundance estimation that were conducted to fulfill the primary study goal to estimate age-2+ Red Snapper abundance in U.S. waters of the Gulf. We also report tagging methods, the mark-recapture statistical model, and estimates of Red Snapper exploitation rates produced during the tagging component. Red Snapper abundance estimation was performed on an eco-region basis, and these regions fortuitously conformed to state jurisdictional boundaries. The sampling design and methods reflected regional considerations due to habitat distributions, spatial trends in water clarity, and other important but nuanced regional variability that required different approaches. Thus, Red Snapper abundance estimation approaches and procedures are presented on a state-by-state basis.

Sampling Design with Imperfect Knowledge about the Sampling Frame

An undertaking of this magnitude presented major challenges, most notably, the lack of desired detailed mapping for the Gulf's nearly 1.55×10^6 km² continental shelf. The diversity and coverage of habitats that hold Red Snapper is immense and differs dramatically within and

especially among regions, including poor to zero visibility in some regions known to harbor high abundance of Red Snapper. Within the U.S. Gulf's exclusive economic zone (EEZ), there are a variety of unknown natural and artificial reef habitats that support a high abundance as well. Unknowns such as these complicate and constrain a Gulf-wide study to estimate population size and dynamics. For example, in addition to an estimated ~25,000 km² of rock dominant or subdominant natural surficial substrate (i.e., natural hard bottom), there are a myriad manmade reef structures in the northern Gulf, such as oil and gas platforms, state permitted artificial reefs, pipelines, shipwrecks and other obstructions both known and unknown. Since Red Snapper are known to occur at the unknown features in UCB, and based on the recommendation of the RFP, we defined this stratum to include all habitats that fall outside the domains of these 'known' artificial and natural reefs. This stratum recognized and addressed the fact that the bottom in many of these areas is made up of unconsolidated sediments of various types that hold both low and high densities of Red Snapper. However, these areas are vast in extent and likely support high numbers of Red Snapper when areal coverage is expanded to these zones of ephemeral structured bottom, uncharted artificial and natural reefs, and other features. Thus, given the spatial scale and heterogeneity of habitat within and among the regions, and the need to efficiently cover the vast amount of 'open' UCB and a range of visibility constraints, no single sampling technique was appropriate for enumerating the population across such a wide heterogeneous landscape. Thus, specialized gear was developed specifically to survey UCB and pipeline habitat types occurring in UCB (see below for details).

When addressing the challenge facing this scientific assessment and need for common methodology that could be uniformly applied across the many habitat types and regions, many techniques were developed, thoroughly described, and vetted by our teams and rigorous peer-review during the Phase I proposal submission process (Ahrens et al. 2016, Leaf et al. 2016, Powers et al. 2016, and Stunz et al. 2016; see Appendix E), and a subset of these methodologies were selected and used for the Phase II sampling (see design methods). Recognizing that there was not a "one-size-fits-all" sampling approach for all habitats and depth strata across the region, our team believed strongly that in specific areas, similar sampling methodologies may not be appropriate. To account for these discrepancies, we developed appropriate methodologies for Gulf-wide comparisons prior to rigorous sampling of these divergent habitats. The other major challenge was to ensure that abundance estimates were bound by relatively low confidence intervals (coefficient of variation of 30%). Preliminary estimates of confidence intervals around the mean population abundance for many areas known to harbor Red Snapper are very difficult to estimate and frequently unknown. Thus, sampling in these areas required initial surveys designed to estimate variability and refine the most appropriate sampling methodologies tailored to each habitat type.

We addressed these key challenges for estimating absolute abundance by developing a rigorous two-phased approach. First, we evaluated methodologies where necessary, refined techniques, and estimated habitat-specific variance, where they were available. Together these allowed us to determine the most effective methods suitable for making accurate and precise abundance estimates. Second, we proceeded with a more broadly scaled-up sampling approach using these calibrated techniques and refinement of our design simulations. A key feature of this

design was its malleability and adaptability to account for the knowns, unknowns, and in particular habitat nuances (e.g., visibility) in each Gulf region or sub-regions. The teams were particularly sensitive to account for sampling costs and effort required for these regions and habitat types, as ship time is a major driving factor to costs and resulting sample size and effort.

2. Overall Goals and Objectives

The overarching goal of this research initiative was to provide an independent estimate of age-2+ Red Snapper absolute abundance in the northern Gulf of Mexico. This work sought to build confidence in our understanding of population abundance for this species across its range and distribution. The independent estimate derived from this research will enhance stock assessment models used by NOAA Fisheries, allowing for validation, calibration, and further refinement. The detailed design, proposed methodology, and resulting estimate of absolute abundance will enable managers to make the most informed decisions regarding Red Snapper.

Specific study objectives included:

Primary Objectives:

1. To estimate abundance and distribution of age-2+ Red Snapper on artificial, natural, and uncharacterized bottom habitat across the northern Gulf;
2. To estimate Red Snapper exploitation rates in the recreational fishery via a mark-recapture study in specified areas of the Gulf; and
3. To archive biological samples for future life history studies of age and growth, fecundity, trophic ecology, and genetic population structure.

Ancillary Objectives:

1. To develop, optimize, and implement a large-scale survey design that can be used for Red Snapper population estimation and other Gulf-wide population surveys;
2. To ensure the design will result in estimates that can be used for comparison and integration into the NOAA Red Snapper stock assessment;
3. To work directly with the Gulf fishing community and engage stakeholders.

3. PIs and Institutions: Roles and Responsibilities

To accomplish this ambitious task, we assembled a well-integrated multidisciplinary team that included the leading fisheries experts from across the entire Gulf region and beyond. Members of our team are some of the most experienced scientists in characterizing fish abundance among Gulf habitats, depth strata, and regions using a variety of methodologies. The team also included fisheries statisticians to produce the sampling design, including simulating sample sizes required to meet the criterion of producing an age-2+ population estimate with a $CV \leq 30\%$ and conduct abundance estimate analyses. Moreover, these researchers have generated

and relied on the most robust datasets, ongoing research programs/teams, sampling techniques, and possessed the most appropriate analytical skills set available to meet the goals of the project. See Appendix A for list of all PIs, detailed expertise, and affiliated institutions.

B. Regional Sampling Framework

The primary goal of this study was to provide an independent absolute abundance estimate of age-2+ Red Snapper along the northern Gulf of Mexico by habitat type including artificial reefs, natural hard bottom, and UCB. Our design separated the Gulf into eco-regions that fortuitously closely mirrored state boundaries. Within each region, zones were defined by approximate depth bins (10-40m, 40-100m, 100-160m) and habitat type (artificial reefs, natural hard bottom, and UCB). A suite of methods was developed and used to obtain local abundance estimates to accommodate the heterogeneity and geography of the Gulf of Mexico shelf. The primary constraint on the techniques applied within a region was visibility, resulting in a west to east decreasing dependency on hydroacoustic methods. Remotely operated vehicle (ROV) visual count surveys were used to evaluate densities on artificial and natural substrates in Florida waters and natural hard bottoms in Alabama and Mississippi. In areas where local abundances were extremely high (artificial reefs in Mississippi and Alabama waters), depletion surveys were coupled to ROV surveys because of concerns of double counting fish with video surveys. In the western Gulf, ROV/Towed camera arrays and hydroacoustics were used to generate estimates. For deeper waters throughout the Gulf and along pipelines and across vast expanses of UCB, a combination of acoustic and visual approaches was used. Finally, a tagging-based study using mark-and-recapture techniques and high rewards for tag returns was conducted to provide regional estimates of exploitation and fishing effort.

1. Strata Definition and Enumeration

The Gulf contains extensive geographic variability in physicochemical and geological (substrate) conditions, which creates substantial differences in habitat types and associated Red Snapper density across the basin. For example, the continental shelf in the western Gulf compared to the east contains relatively little natural hard bottom and consists predominately of silt and mud (i.e., UCB) requiring different sampling and analytical approaches. Moreover, anecdotal fishery information suggest interspersed within this vast area are large numbers of unmapped structured habitat features (unknown natural bottom, ship wrecks, unknown artificial reefs, etc.) known to harbor Red Snapper; however, the full extent of areal habitat coverage and abundance over the UCB habitat was unknown. Thus, we categorized these areas of unconsolidated sediment and unclassified habitat features as UCB. In contrast, the continental shelf in the eastern Gulf contains extensive hard bottom substrate features interspersed within the UCB habitat. Thus, a major focus of this project was to characterize habitat features in these key areas across the Gulf as extensively as possible as prescribed using existing datasets and cartographic information, as this funding opportunity explicitly restricted novel habitat mapping during the project. Nevertheless, Red Snapper abundance was thought to be disproportionately

related to structured habitat, and it was essential that the geographic extent and composition of habitat types were accurately quantified within the constraints of the RFP. The other habitat types assessed such as known artificial reefs and natural hardbottom (e.g., ‘snapper banks’) were generally well-known and mapped affording detailed areal coverage or enumeration in the case of smaller artificial reefs. Although, natural hardbottom and artificial features that were unknown/mapped occurred over and were considered part of the UCB habitat.

Initial habitat characterization eco-region delineation exercises were conducted during the initial Phase I study. The shelf waters of the Gulf were divided into strata to assign sampling effort using a hierarchical structure based on ecological and management boundaries. Fortuitously, the ecological boundaries generally conformed well with regional boundaries. Thus, due to the great regional/management needs for assigning Red Snapper abundance among the five Gulf states, Eastern and Western regions were split at the Mississippi River, and then further divided along state lines to create four regional groups: Texas, Louisiana, Alabama/Mississippi (the area of the shelf between the Mississippi River and the Alabama/Florida state line), and Florida (Figure 2A). As defined in the RFP, each of these four geographic regions were then classified into 3 depth zones (approximately 10-40 m, 40-100 m, and 100-160 m) creating twelve unique strata (Figure 2A). Within each stratum, habitat was further classified by type: the areal coverage of known hard bottom and UCB habitat, as well as artificial structures (e.g., artificial reefs, oil and gas platforms, shipwrecks and other obstructions; Figure 2, Figure 3, Figure 4).

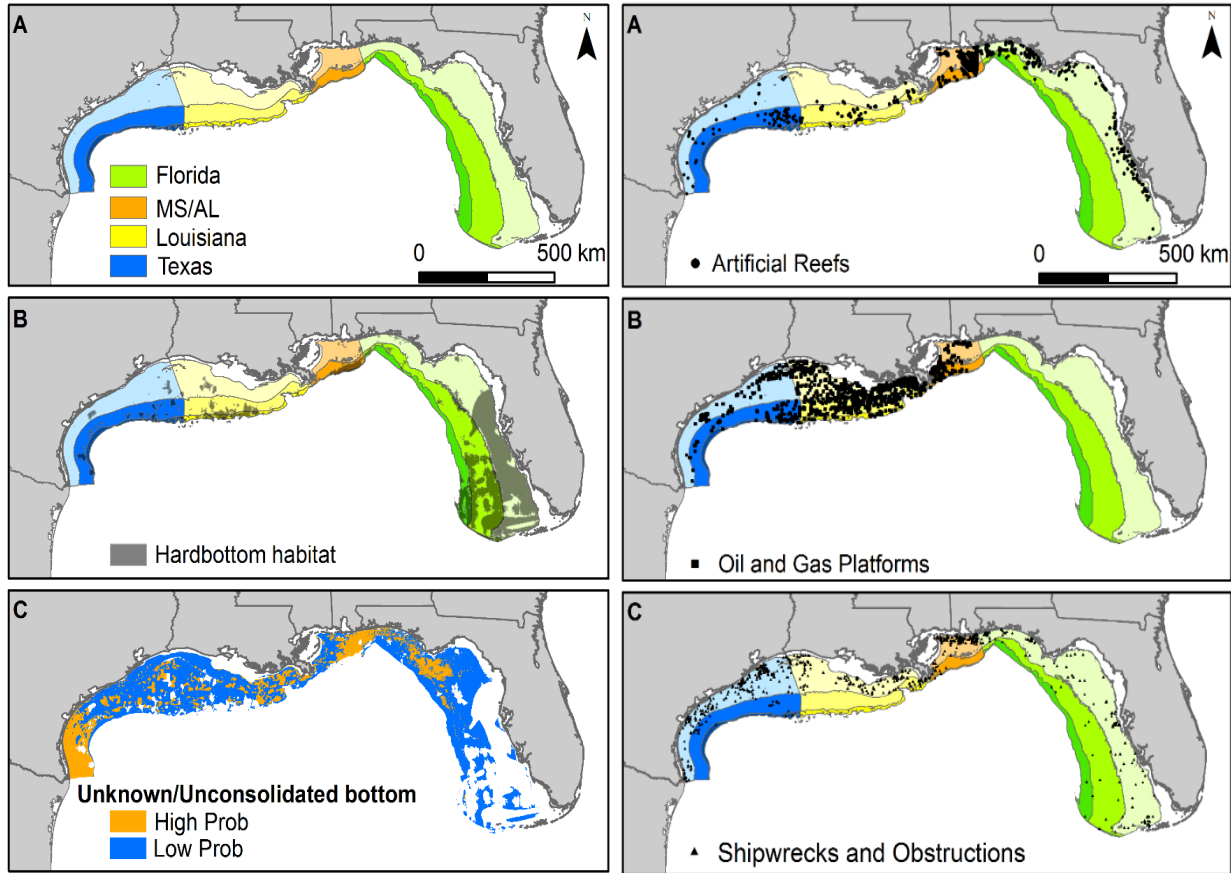


Figure 2. (A) Sampling strata used to estimate the absolute abundance of Red Snapper in the northern Gulf. (B) Natural hard-bottom habitat distribution across strata. (C) Uncharacterized bottom habitat with predicted areas of high and low probability of occupancy for Red Snapper conducted during Phase I of this study.

Figure 3. General distribution across the four geographic boundaries and sampling strata of known: (A) artificial reefs; (B) Standing oil and gas platforms; and, (C) Shipwrecks and other obstructions.

Habitat and boundary data used in defining the sampling strata were obtained from several sources. State boundaries in federal waters were defined by the Offshore Administrative Boundaries developed by the Bureau of Ocean Energy Management (BOEM 2020a) using the National Baseline (Florida) or Supreme Court fixed baselines (Alabama, Louisiana, Mississippi, and Texas). The distribution and number of known artificial reefs in the northern Gulf were acquired from datasets compiled by NOAA’s Office of Coastal Management (updated Dec. 2015; NOAA 2020a), Louisiana Department of Wildlife and Fisheries (LDWF 2020a), Texas Parks and Wildlife Department (TWPD 2020), RGV Reef (2020), Horner (2013), and Stunz, unpublished data. In addition, datasets compiled by NOAA’s Office of Coast Survey (NOAA 2020c) and Horner (2013) were used to identify the locations of shipwrecks and obstructions. The State of Alabama has supported their own surveys of the vast network of artificial reefs off the coast of Alabama (described in Section 4 below). Current locations of standing oil and gas

platforms were obtained from BOEM (2020b) for federal waters and the Texas General Land Office (TGLO 2018) and Louisiana Department of Natural Resources (LDNR 2020b) for Texas and Louisiana state waters, respectively. After aggregating these data, post-processing was completed to ensure that structures were not duplicated and still present at the location. Active oil and gas pipeline habitat was calculated from BOEM (2020c) (described in section 7 below). For the purposes of this study, we defined natural hard bottom habitat as substrates containing at least 1% rock (dbSEABED; Buczkowski et al. 2006, Jenkins 2011) and/or areas identified by NOAA's Coral Essential Habitat (NOAA 2020b), BOEM's confirmed relic patch reefs (BOEM 2020d), Shirley (2012), and Horner (2013). Depth zones were derived from bathymetric data obtained from NOAA National Centers for Environmental Information at a surface area spatial resolution of 90 m. All data were filtered to include only shelf habitats within the 10-160 m depth range, following the Gulf-wide design.

The sampling universe of known artificial reef, natural reef, and uncharacterized habitats for the four Gulf regions was compiled from available data. Artificial structures were categorized by size and/or type as well as active or unremoved unburied oil and gas pipeline greater than 8 inches. Known hard bottom structure in TX, LA, AL/MS were taken from existing habitat maps. Artificial and natural bottom habitats were further divided into depth categories and regions (Texas, Louisiana, Alabama/Mississippi, and Florida (northwest, mid, and south)). Sample sites for natural bottom and artificial structures were selected randomly within each strata (region & depth). In areas outside of Florida, certain modifications and departures from this design were necessary due to high regional variability and logistical constraints. These differences in design and sample collection are detailed in each regional sampling framework below.

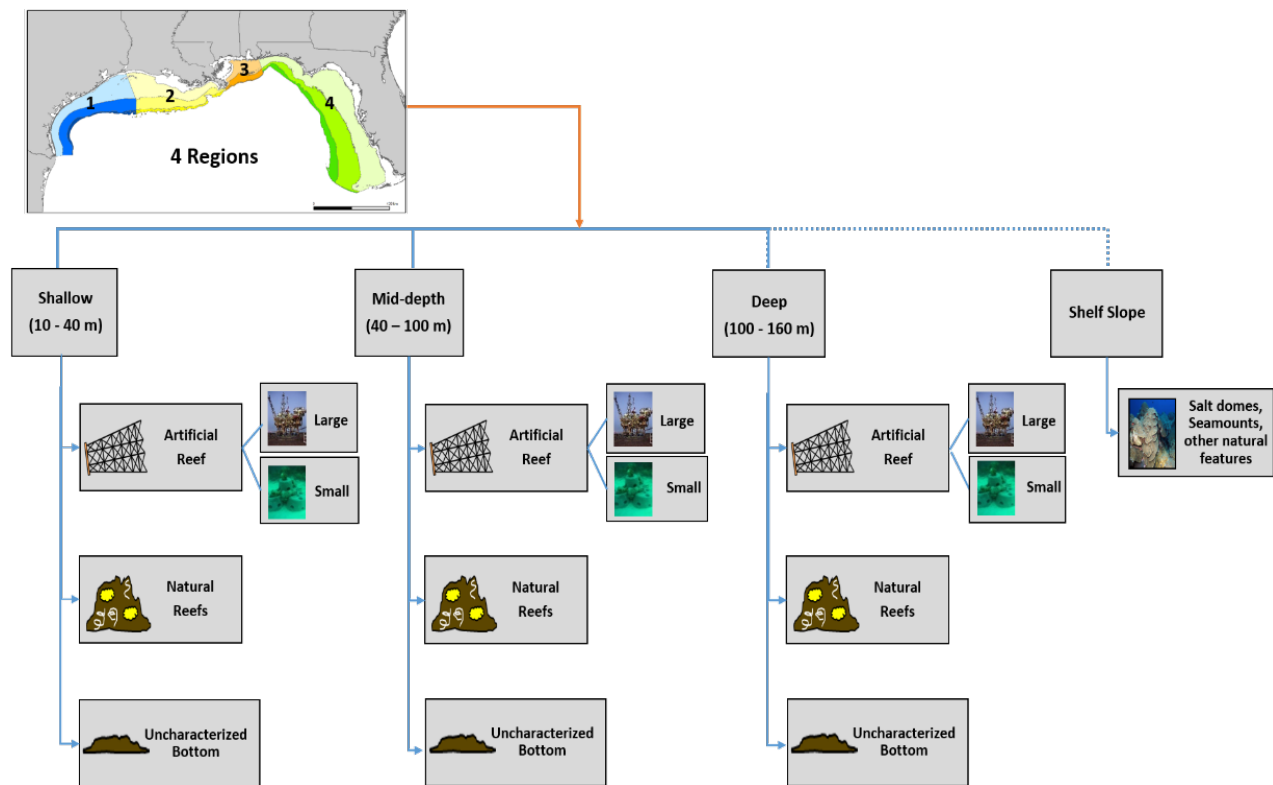


Figure 4. Schematic flow chart representation of the general stratified random design. Each of the 4 regions across the Gulf is broken down into 3 depth strata (shallow, mid-depth, and deep). Habitat types are broken down into artificial reef (large and small), natural hard bottom banks, and uncharacterized bottom for each depth strata. Associated with each region, are other natural features in deeper waters on the shelf slope. These may include salt domes and seamounts that hold substantial biomass and were opportunistically sampled but not included in these abundance calculations (i.e., outside prescribed depth zone requested by the RFP).

2. Regional Survey Introduction

A major challenge facing this scientific assessment was developing a robust design and relatively unbiased sampling methods that could be applied among the many habitat types and regions in the U.S. Gulf. Each study region has an array of habitats and water clarity nuances as well as large differences that vary substantially among regions; thus, multiple sampling approaches were required. A suite of methods was developed and used to obtain local abundance estimates to accommodate seabed and water clarity heterogeneity of the Gulf shelf, and to fulfill the project mandate of using advanced technologies to estimate Red Snapper abundance. A primary constraint on the techniques applied within a given region was visibility, resulting in a dependency on hydroacoustic methods in the western Gulf. Remotely operated vehicle surveys provided visual counts of Red Snapper to estimate their density and abundance on artificial and natural substrates in Florida waters. A series of ROV surveys were also used to determine a species composition in other regions. Within Mississippi and Alabama waters, depletion methods were the primary approach used, and in the western Gulf, ROV, towed camera arrays (TCA), and hydroacoustics were all utilized to generate estimates. For deeper waters throughout the Gulf, and along pipelines and vast expanses of UCB, a combination of acoustic and visual approaches was used. Each of these sampling methods utilized to estimate numbers and density of Red Snapper present at sampling sites carried with them assumptions about detectability, selectivity, or catchability. In the remainder of this report section, we detail sample design, sampling methods, and associated assumptions, including tests of those assumptions, for each region.

3. Florida Region

Red Snapper abundance was estimated on the Florida shelf via ROV surveys. Sample site selection in this region explicitly followed the stratified random design described above. Sampling methods used to conduct ROV surveys differed between artificial and natural reefs due to habitat nuances, but the general approach of using ROV video to estimate Red Snapper density per sample cell, scaling density estimates to cell area, and expanding those estimates via the stratified design were followed throughout. We also conducted a series of experiments to estimate Red Snapper movement dynamics during ROV, TCA, and hydroacoustic sampling to evaluate whether Red Snapper behavior (i.e., attraction to or avoidance of different mobile sampling gears) might be a source of bias when using these gears to estimate Red Snapper abundance. Lastly, we conducted hydroacoustic sampling at over half of 749 natural habitat sampling sites on the Florida shelf, which facilitated comparisons between Red Snapper density estimates produced with ROV versus splitbeam sonar.

a. ROV Visual Surveys

Sample site selection in Gulf waters off western Florida was performed using the stratified random design described in the strata definition with three depth strata (described above) and three regions (northwest, mid, and south). In total, 749 natural habitat sites and 84

artificial reefs were sampled on the Florida shelf between March 4, 2018 and November 21, 2019 (Figure 5). Sampling occurred on chartered fishing vessels, typically on 4-day trips, and on 10-day trips aboard the Florida Institute of Oceanography's *R/V Hogarth*. A SeaBird 19plus CTD was deployed at each sampling site to measure dissolved oxygen, salinity, depth, pH, turbidity, and fluorescence throughout the water column. Video sampling was conducted at each site with an ROV to estimate Red Snapper abundance and density. Splitbeam sonar sampling also was conducted at 410 (54.7%) of natural habitat sites and at 14 (16.7%) artificial reef sites (Figure 5).

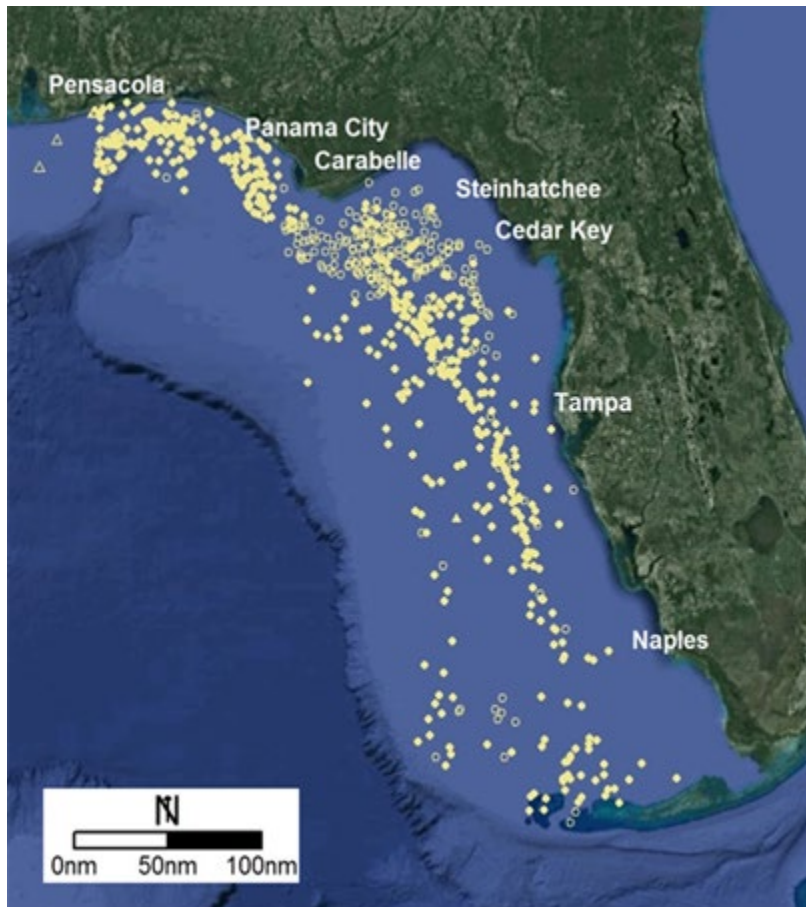


Figure 5. Sample locations among three regions on the west Florida shelf. Triangles are artificial reefs and circles are natural habitat sites. Filled shapes indicate sites at which both ROV and sonar sampling was performed. Open shapes indicate sites where only ROV sampling was performed.

Habitat-specific Red Snapper density has previously been estimated on northern Gulf reefs using modified point-counts or transects completed with a ROV (Patterson et al. 2009;

Dance et al. 2011; Patterson et al. 2014, Ajemian et al. 2015a, Ajemian et al. 2015b, Dahl et al. 2016, Streich et al. 2016), and ROV video surveys were utilized to estimate Red Snapper density and abundance on the Florida shelf in the current study. The ROVs used were VideoRay Pro4 mini-class ROVs. The submersibles have dimensions of 37.5 x 28.9 x 22.3 cm and a mass of 6.1 kg. They have a depth rating of 170 m, a 570-line color camera with wide-angle (116°) lens, a 20-watt halogen light on either side of the forward-facing camera and are tethered to an integrated control box on the surface ship where the video feed from the ROV's camera is used to pilot the ROV. To estimate the length of fish observed during video sampling, the ROVs were equipped with either a red laser scaler or a stereo camera rig, which was developed for this study (Garner et al. In Press; see Appendix D). The laser scaler consisted of 2 parallel 5 mw 635 nm Class IIIa red lasers fixed 75 mm apart. The stereo camera system consisted of two GoPro Hero5 mounted to an aluminum bar at a distance of 610 mm between cameras with the anterior-posterior axis of the ROV bisecting the camera pair. Each camera case was mounted 10° (toe-in angle) inward toward the center line of the ROV, and each camera was set to its narrow field-of-view (FOV; 49.1° vertical and 64.6° horizontal, 28 mm focal length equivalent) at 1080p definition with a 120-fps frame rate. Calibration details for the stereo camera system, as well as methods applied to extract fish estimates are found in Garner et al. (In Press), which is provided in Appendix D. Pool experiments conducted as part of this study indicated both the laser and stereo camera system had a mean error of <3% for fish within 5 m of the ROV that were struck by either the lasers or viewed with the stereo camera system at an angle <20° from perpendicular to the central line of the ROV (Garner et al. In Press; Appendix D). Moreover, system-specific mean and standard deviation of length estimate bias were used to bias-correct fish length estimates obtained under normal conditions (i.e., fish observed within 5 m of the ROV and <20° from perpendicular to the center line of the ROV system) during ROV surveys.

A third GoPro Hero5 camera was mounted to the center front of the float block of the ROV to record high-definition video of fish communities being video sampled. This camera was set to the wide field of view (94.4° vertical and 122.6° horizontal FOV, 14 mm focal length) at 2.7k resolution and 120-fps frame rate. At artificial reef sites, the camera was aimed straight ahead, parallel to the seabed, and the point-count method described by Patterson et al. (2009) was used to survey artificial reefs in a 15-m wide cylinder (area = 176.7 m²) and count Red Snapper and all other reef fishes present. At natural habitat sites, four orthogonal 25-m transects were flown from a center point marked by a 5-kg weight attached to the tether. Transect altitude was typically 2 m with the center GoPro camera angled downward at 45°. Given the known altitude, camera FOV, and camera angle relative to the seabed, the width of transects could be estimated via the method of Patterson et al. (2014). At an altitude of 2 m, the width of video transects flown with the ROV was 8.64 m and the total area surveyed among the 4 orthogonal transects was 1461 m². Video samples were analyzed in the laboratory on a high-resolution monitor and the number of Red Snapper observed during each survey was counted. The Red Snapper count at each site then was converted to a density estimate by dividing total fish counted over estimated area surveyed. One natural site was considered to be an outlier and was omitted from estimate calculations (n=748).

b. Hydroacoustics

Splitbeam transducers were deployed from a stabilized towfish with the exception of the R/V Hogarth which had hull-mounted transducers. Surveys were configured in radial patterns (i.e., flower pattern) with sample site GPS coordinates at the intersection of the transect pattern, ensuring several passes ($n \geq 3$) over each site while also providing spatial coverage of far-field effects. This design has been successfully used in habitat-specific surveys in the Gulf, focusing on standing and toppled oil and gas platform habitats (Simonsen 2013). Acoustic scattering intensity of fishes associated with each station was measured using a calibrated (Demer et al. 2015) multi-frequency split-beam fisheries scientific echosounder system (Simrad EK80). While all regions used a 70 kHz echosounder (Simrad EK80), in Florida, additional frequencies (38, 120 and 200 kHz) were implemented in an attempt to improve classification accuracy of backscatter. Raw data files collected from the echosounder were loaded into the standard hydroacoustic processing software Echoview v. 10. Prior to processing a particular dataset, data were calibrated for sound speed, transducer gain and calibration correction to account for any potential variance in performance of the echo sounder and variation in water column structure on sound speed and sound absorption through the water column. By applying the calibration information, backscatter data collected across surveys can be quantitatively compared (Simmonds and MacLennan 2005). Mean calibration correction and transducer gain values were derived from each specific vessel and frequency, using the standard method of the sphere (Foote et al. 1989; Demer et al. 2005), and then input into the internal Echoview calibration file (.ecs). Water quality parameters used to calculate sound speed and absorption coefficients were derived by calculating the mean of both the water column temperature and salinity, based on processed CTD data collected during each survey.

Several steps were taken to assess the quality of the data and remove unwanted signal (e.g., boat wakes, waves, bottom echo, electrical interference) and echoes generated by sources other than fish. A surface and a bottom line were used to bound the data used for the analysis. The surface line excluded data from the upper part of the echogram affected by vessel noise, bubbles, turbulence and near field conditions (area close to the transducer where the beam pattern is not predictable). For this study, the surface line was estimated at depth that is twice the near field distance (Medwin and Clay 1998). A surface line of 5.5 m was estimated for the 38 kHz and was adopted for all frequencies as this was the largest near field interference calculated.

The seabed line was detected in an automated way using the best bottom candidate algorithm. This line was visually checked for gaps before applying a 0.5 m offset. Any data below this offset were excluded from the analysis to avoid the integration of the bottom echo. Due to presence of electrical interference, data were cleaned for impulse noise using the default settings of the standard algorithm in Echoview. This sequence of steps was applied to both the Sv and the TS echograms of each frequency.

Two approaches were used to distinguish echotraces from fish depending on the number of frequencies available. When only the 70 kHz frequency was available, a threshold of -60 dB re 1 m^{-1} was applied to the Sv echogram. This threshold helps retain fish schools or aggregations in the acoustic data while removing a portion of the signal generated by small organisms such as

plankton or small size fishes. The remaining unwanted signal was manually removed by using the bad region features in Echoview. When more than one frequency was present, all the potential fish schools or aggregations were isolated based on a frequency's summation threshold (Fernandes 2009). A threshold of $-120 \text{ re } 1 \text{ m}^{-1}$ was applied to the echogram resultant from the summation of two frequencies (38 kHz and 70 kHz) and a threshold of $-230 \text{ re } 1 \text{ m}^{-1}$ was applied to the echogram resulting from the summation of four frequencies (38, 70, 120, and 200 kHz). These thresholds were selected to produce the cleanest Sv echogram (Fernandes 2009). A median filter (3x3) was then applied to remove individual samples and a dilation filter (5x5) to augment the fish schools. Finally, the resulting mask was applied to the 70 kHz echograms and subjected to the threshold of $-60 \text{ re } 1 \text{ m}^{-1}$. Summing and thresholding the echogram allowed for the removal of organisms with Rayleigh properties (e.g., zooplankton) and retained only organisms for which the backscatter is persistent across frequencies (e.g., fish schools or aggregations) (Lavery et al. 2007; Fernandes 2009).

Since Red Snapper can occur in both single targets and in aggregations, the total abundance for each sampled site was estimated by using both echo-integration and echo-counting methods. The isolated aggregations on the 70 kHz echogram were detected using the SHAPES algorithm in Echoview (Barange 1994). The detection parameters used were a minimum total aggregation length of 7 m, a minimum aggregation height of 2 m, a minimum candidate length of 2.50 m, a minimum candidate height of 1.75 m, a vertical-linking distance of 0.25 m, and a maximum horizontal-linking distance of 0.80 m. The echograms were visually inspected to ensure the algorithm delineated fish aggregations and not structure on the substrate. Aggregation regions were partitioned into 5 x 5-m cells and their volume backscattering coefficient (Sv, RBC; $\text{m}^2 \text{ m}^{-3}$) was exported.

To obtain the density of individuals within each cell of the aggregation, the Sv is divided by the mean backscattering cross section (linear form of target strength; TS [dB re 1 m^2]) of a single individual, yielding to an estimate of total number of fish per volume of water (fish/ m^3). Measurements of TS were made close to the aggregation border where fish density was lower, and fish echoes were less likely to overlap. It was assumed that the single targets outside the aggregation border and within 10 m of distance from the aggregation depth were representative of the targets inside the aggregation (Scouling et al. 2015).

A combination of Echoview's Mask and XxY statistic operand was used to isolate the region surrounding each aggregation (hereafter called the border region). The border region was subsequently partitioned into cells with horizontal spacing of 25 pings and vertical bin size of 5 m. The fish density (number of fish in the effective reverberation volume for one ping, Nv) within each cell was determined using the method described by Sawada (1993). Only cells with low density, specifically with $Nv < 0.01$ individual per m^3 were used for TS estimation. A single target detection algorithm was then applied to these low-density cells. The following parameters were set for the single target detection: a minimum threshold of $-50 \text{ dB re } 1 \text{ m}^2$, a pulse length determination level of 4 dB, a minimum and maximum normalized pulse length of 0.70 and 1.50 respectively, a maximum beam compensation of 4 dB re 1 m^2 and a maximum standard deviation of minor and major axis angle of 0.6 degrees.

Single targets of the border region were subsequently exported in Echoview and associated to the correspondent school in R for further analysis. All TS value were converted to backscattering cross section (σ_{bs} , m^2), according to $\sigma_{bs} = 10(TS/10)$. A mean target strength ($\sigma_{bs,mean}$) was calculated by averaging together the backscattering cross section of all the single targets associated to the school. Finally, the volume density (ρ_v) estimate of the echo integration (EI) was calculated according to:

$$(1) \quad \rho_{v,cell-EI} = S_{v,RBC} / \sigma_{bs,mean}$$

where RBC is region by cell and $\sigma_{bs,mean}$ is the mean of the targets in the border.

Fish present as individual targets outside of the border region were identified and extracted using the single target detection algorithm in Echoview. A minimum threshold of -50 dB re 1 m^2 was set for the single target detection to exclude small scatterers that do not likely represent fish of interest (Boswell et al. 2020). The pulse length determination level was set at 4 dB and the minimum and maximum normalized pulse length were set at 0.70 and 1.50, respectively. A maximum beam compensation of 6 dB re 1 m^2 and a maximum standard deviation of minor and major axis angle of 0.6 degrees were used. The echogram with the identified targets was binned using a 5x5 m cell grid and exported. The volume density, ρ_v (fish m^{-3}) per cell of the echo-counting (EC) was estimated by simply dividing the number of targets for the beam volume sum (Kieser and Mulligan 1984):

$$(2) \quad \rho_{v,cell-EC} = \#single-targets_{cell} / beam-volume-sum_{cell}$$

The volume density of the echo-integration and the echo-counting were converted to areal density (fish m^{-2} re. cell height) by multiplication with the mean thickness of the cell. The two density estimators were then summed to obtain the total areal density per cell (fish m^{-2}):

$$(3) \quad \rho_{a,cell-EC} = \rho_{v,cell-EC} \times Thickness_meancell$$

$$(4) \quad \rho_{a,cell-EI} = \rho_{v,cell-EI} \times Thickness_meancell$$

$$(5) \quad \rho_{a,cell} = \rho_{a,cell-EC} + \rho_{a,cell-EI}.$$

To be comparable to the area surveyed by video, only cells within 5 m of the bottom were retained for further analysis. Hydroacoustic fish density estimates were summed throughout the bottom 5 m at each survey point. A geostatistical approach was applied to account for any spatial autocorrelation inherent in mobile continuous data collection. First, a variogram was used to model the spatial continuity of the data collected at each site. To automate this process, initial values for the variogram model were calculated from the data. We then applied ordinary kriging to interpolate spatially weighted estimates of area density over a projected survey grid from the predicted variogram. This resulted in hydroacoustic density estimates for 5 x 5-m cells arranged north to south and east to west over the area bounded by the ends of the transects performed at each site. The abundance of fish in each cell was then calculated by multiplying the density estimated by 25 (the area of the cell). To estimate site-specific Red Snapper abundance, the proportion of Red Snapper occurrence relative to all fish species recorded in video survey data was multiplied by the sum of fish abundance calculated for all cells at the site. To estimate site-specific Red Snapper density, total Red Snapper abundance counted in given survey was divided by the areal coverage of the sonar survey conducted at that sampling site.

Assumptions and Assumption Testing Summary

Estimating Red Snapper abundance off Florida with ROV required assumptions that fish of the targeted (age-2+) age classes were fully vulnerable to the gear and that Red Snapper detectability was 100% (i.e., fish were neither avoided nor were attracted to the ROV). By sampling across the shelf from 10 to 160 m, and by following the stratified random design we assume we covered all of the potential habitats they occupied on the Gulf shelf. Furthermore, we have no evidence that some size or age classes were more vulnerable to the gear than others. The size distribution of fish scaled with lasers or stereo cameras in Florida waters was skewed toward fish smaller than 600 mm TL (Figure 6), but fish up to 989 mm TL were observed in Florida samples. Furthermore, the Red Snapper population off Florida is likely to have smaller, younger fish than other regions given the historical overfishing that occurred in this region.

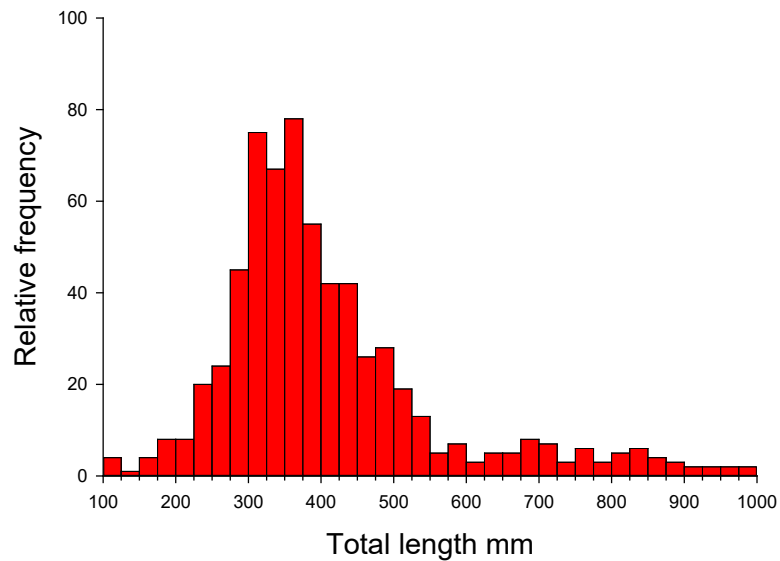


Figure 6. Bias-corrected Red Snapper (n = 637) size composition estimated at Florida sampling sites with a laser scaler or stereo camera system integrated with a VideoRay Pro4 ROV.

We estimated visibility exceeded 5 m on all and 10 m on most (>90%) reef sites sampled off Florida with ROV. Given the fish needed to be visible to a range of <3 m when flying ROV transects, and the fact that adult Red Snapper are conspicuous, we assume that all Red Snapper present were visible with ROV video. However, that does not speak to the potential issue of Red Snapper behavior affecting detectability. To estimate that potential effect, we conducted series of experiments off Florida to estimate the behavioral reaction of Red Snapper to mobile sample gears, including a VideoRay Pro4 ROV and the towed sonar sled used to sample reef fish communities on the Florida shelf, and the Deep Ocean TARAS (Towed Aquatic Resource Assessment System) camera sled used in the western Gulf of Mexico. Experiments examined Red Snapper swimming behavior with high-resolution three-dimensional telemetry of fish externally tagged with acoustic transmitters, as well as using paired stationary stereo camera rigs and passive multibeam sonar deployed on the seabed to estimate the behavioral reaction of Red Snapper to the mobile gears used in the Red Snapper population estimation study. Study details, including methods, results, and inferences, are provided in Garner et al. (In Review) in Appendix D. Briefly, experiment results suggest minimal positive or negative behavioral reaction was displayed by Red Snapper to any of the three mobile sampling gears utilized among Gulf regions, including the ROV approach used in Florida waters (Garner et al. In Review; Appendix D). Therefore, we conclude there was no substantial bias in Red Snapper counts due to their behavioral reaction to the ROV, which was more or less neutral, as well as to the other mobile sampling gears deployed in other regions.

It is important to note that detectability was not tested for any of the mobile gears examined in the Red Snapper behavioral experiments. As stated above, we assumed Red Snapper

detectability was 100% for ROV sampling off Florida, which was based on the 2-m altitude the ROV was flown above the seabed and the fact water visibility was >5 m at all sampling sites, and >10 m at most sites. Our inference of 100% detectability for Red Snapper is also informed by results of previous detectability experiments we have conducted with less conspicuous reef fishes that can be more challenging to survey (Harris et al. 2019). Detectability/visibility issues were precisely why hydroacoustics or depletion-based survey were the principal methods used to estimate Red Snapper abundance in the other regions.

Comparisons Between ROV-derived and Sonar-based Red Snapper Density Estimates:

The estimate of Red Snapper population size in Gulf waters off Florida was derived solely from ROV video sampling. However, we performed a series of studies, where sonar sampling was conducted at a subset of sites sampled with both ROV and hydroacoustics. These paired sampling methodologies provided an opportunity for comparisons between the two methods. In total, 410 of 749 natural bottom sampling sites were surveyed with splitbeam sonar as described above. Red Snapper were observed in ROV video at 25 (6.1%) of those sites. Among those sites, there was a significant ($p = 0.049$) but weak positive correlation (Pearson's $r = 0.40$) between Red Snapper density estimates derived from sonar versus ROV sampling (Figure 7). However, Red Snapper density estimates derived from ROV surveys were on average more than 9X greater than density estimates produced from sonar surveys (Figure 7). It is unclear what the source of this difference might be, or the implications for western Gulf sonar-derived Red Snapper density estimates. One possibility may be that the natural bottom habitat was so patchy on the Florida shelf that $\sim 1,500 \text{ m}^2$ ROV surveys conducted at site coordinates where Red Snapper were observed just happened to be located within a habitat patch with high occurrence of Red Snapper, but sonar surveys, which were also centered on those coordinates but sampled a much broader (i.e., $\sim 100,000 \text{ m}^2$) area, integrated Red Snapper estimates over high as well as low occurrence habitat types. This may lead one to infer that Red Snapper density estimates from ROV samples, therefore, may have been biased high. However, site coordinates were randomly selected based on the random forest model's estimation of high, medium, and low probability of encountering Red Snapper and ROV samples occurred right on selected coordinates. If habitat patchiness did drive the observed difference between sonar and ROV-derived estimates of Red Snapper density, hence abundance, the randomization in the sampling design should have ensured there was no bias in sample location selection.

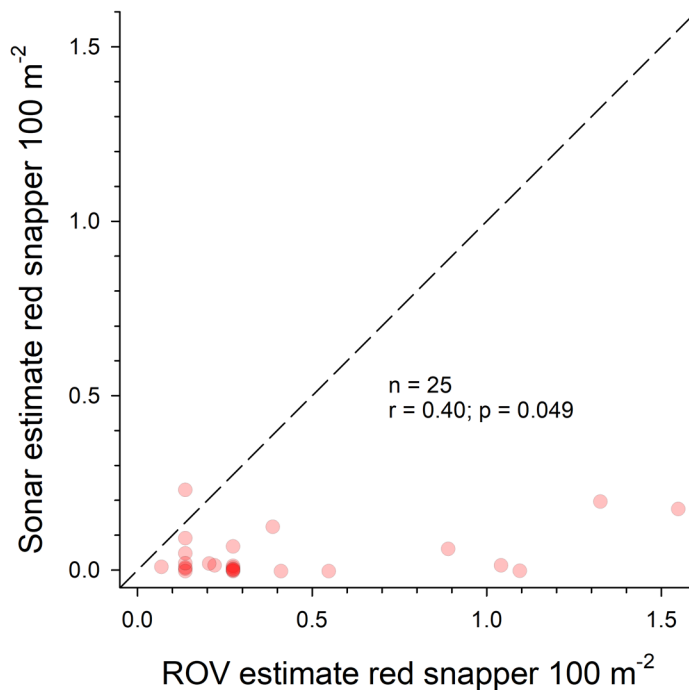


Figure 7. Scatterplot of Red Snapper density (fish/100 m²) estimated with splitbeam sonar versus ROV video samples at natural bottom sites on Florida's Gulf of Mexico continental shelf. Red snapper were observed at 25 of 410 natural bottom sites at which sonar sampling was conducted. Correlation analysis between density estimates from sonar versus ROV methods indicated a significant ($p = 0.049$) but weak (Pearson's $r = 0.40$) linear relationship. However, on average the density estimates produced from ROV video samples were 9.1 times greater than sonar-derived estimates.

It was not possible to estimate Red Snapper detectability with splitbeam sonar sampling, or other potential biases in sonar-derived estimates of Red Snapper density. Results from behavioral experiments suggest Red Snapper do not display much of a positive or negative reaction to VideoRay Pro4 ROVs. Therefore, it does not appear likely that attraction to ROVs explains the difference in Red Snapper density estimates between sonar versus ROV sampling. It is also not clear to what extent Red Snapper detectability with sonar, or post-processing methods employed to estimate Red Snapper counts and density, may have affected sonar-derived Red Snapper density estimates. Given ROV surveys were the primary sampling approach taken to estimate Red Snapper abundance and density in waters off Florida, and the fact that Red Snapper behavioral experiments suggested a more or less neutral reaction of Red Snapper to the ROVs used in this study, stratum- and Florida-specific Red Snapper population estimates were derived from ROV-derived density estimates. Initially, the team developed these studies with the

assumption that the gear with the greatest detectability would be hydroacoustics, but comparisons between ROV and sonar results suggest otherwise. These differences may be due to a variety of factors such as acoustic shadowing of structure and the inability to perceive echoes of fish distributed close to the bottom or within the structure (e.g., oil and gas platforms). Based on these results and given that the direction of any biases was toward an underestimate produced using hydroacoustics, the team recommend proceeding with caution and err on the side of underestimation in other regions. Thus, we did not adjust for these differences in the other regions, where sonar-derived estimates were the primary method used.

4. Alabama/Mississippi Region

For the Alabama/Mississippi region, we used separate habitat-specific approaches to estimate absolute abundances in the three habitat types that were the focal effort of the overall projects (artificial reefs, natural hard bottom, and UCB) due to the habitat-abundance patterns observed in this region. Each habitat type off coastal Alabama and Mississippi posed unique challenges that necessitated these different sampling approaches. For decades, artificial reefs have been placed within the Alabama Artificial Reef Zone (AARZ) by the state and public with few of the locations published. From 2011-2019, our group has completed extensive detailed mapping of this area with acoustic side-scan (approximately 22% mapped) to identify artificial reefs to sample and estimate the overall number of artificial reefs in the area. Our previous data has shown dense aggregations of Red Snapper (100s) surrounding many of the artificial reefs. Because of the high number of Red Snapper, visual surveys cannot use simple count procedures because of the possibility of double counting many fish as the camera sweeps the area. Thus, we chose to use depletion-based approaches to quantify Red Snapper on artificial reefs and combine those with our estimates of the number of structures to extrapolate the count to an absolute abundance estimate. We attempted a similar approach on natural hard bottom areas; however, the density of Red Snapper was relatively low (5-10 individuals per feature surveyed) and depletion efforts did not meet the assumption criteria for depletion. Because densities were low, double counting of Red Snapper was not a major concern. Hence, we used the estimated density per unit area swept by the ROV and multiplied that number by the amount of natural bottom habitat in the deeper areas of our study area (based on USGS mapping of the area) – an approach similar to other methods used in this study. Finally, to sample UCB (n=3) a combination of hydroacoustic and visual approaches were implemented using Camera-Based Assessment Survey System (C-BASS) methodologies described in Section 7 below. In addition, a total of 128 artificial reefs and 32 natural sites were sampled in this region.

Artificial Reefs

Estimated numbers of Artificial Reefs

Because of the vast number of undocumented artificial reefs in Alabama coastal waters, we used the results of our side-scan surveys to estimate the number of artificial reefs and select targets for sampling. The area off coastal Alabama was divided into 2 x 2 km grids. A subset of

these grids (~40 per year from 2011-2019) was randomly selected each year and surveyed with side-scan sonar prior to sampling using both vertical longlines and ROV equipped with video recorders. Grid selection was proportionally allocated to three depth strata based on the bottom area included by each depth. Specifically, 50% of grids selected were in the shallow stratum (18 – 37 m), 33% in the mid-depth stratum (37 – 55 m) and 17% in the deep stratum (55 – 91 m). From 2011-2015, each grid was surveyed using an EdgeTech 4200 dual frequency sidescan sonar (300/600 kHz) and a Biosonic echosounder with a 200 kHz single beam transducer. The sidescan towfish was deployed using a data conducting winch equipped with a digital metering block from the A-frame of a survey vessel and towed at an altitude of approximately 15m above the seafloor. From 2016-2019, similar surveys were conducted using an EdgeTech 4200 DF dual frequency (300/600 kHz) digital sides-can sonar tow fish. Side scan surveys were normally conducted during the spring prior to the vertical longline and ROV surveys to provide time for data processing. All data (position, sonar, and cable-out) were recorded and integrated using Chesapeake Technology Inc. SonarWiz.MAP 4 software running on a ruggedized laptop computer. This software produced a real-time, fully geo-referenced mosaic of the sonar data and served as a navigational aid for the vessel during the course of the survey. Bottom targets visualized by the SonarWiz.MAP 4 program were captured and displayed on the chart plotter of the program. Based on the side-scan generated map of structures, a contact report was generated in which the length, width, height, description, latitude, and longitude of each contact within each grid was produced. Bottom contacts were categorized as either qualifying structure ($> 4 \text{ m}^2$ area and $> 0.5\text{m}$ vertical relief) or non-qualifying structure ($< 4 \text{ m}^2$ area or $< 0.5\text{m}$ vertical relief). To derive an estimate of the number of artificial reefs in Alabama, we used the information in the contact reports of each grid to determine the number of reefs in each sampling grid (Powers et al. 2018).

A total of 432 (out of a possible 1,399) grids were mapped during 2011-2019 and used as the basis for the estimate of the number artificial reefs off coastal Alabama. The contact report summaries could then be stratified by depth (shallow, mid, and deep) and zone (inside or outside of the AARZ) to gain finer spatial resolution on our population estimates. Approximately half of the waters off coastal Alabama are permitted by the U.S. Corps of Engineers for deployment of artificial reefs (Figure 8). However, unreported deployments of artificial reefs have occurred in non-permit waters because the deployment pre-dated the permit, individuals ignored the boundaries, or material was unintentionally planted (i.e., accidental ship sinking, loss of cargo carriers, etc.). Hence the assumption that artificial reefs only occur in the pre-permitted areas is not supported.

For Mississippi, there is a state-maintained list of artificial reefs which, although undoubtedly incomplete, is thought to contain most of the artificial reefs and is the most complete sampling frame available. Reefs providing habitat for Red Snapper are believed to be restricted to 5 grid cells (FH 1, 2, 6, 12, 13), comprising 229 reefs. No systematic survey of grids outside these permitted areas exists. However, based on the number of “snags” reported by the NOAA SEAMAP bottom trawl surveys, it is reasonable to assume a higher number of artificial reefs exist off coastal Mississippi. We used our surveys of western Alabama waters (i.e., Alabama waters not inside the AARZ) to estimate the number of unpublished reefs off coastal

Mississippi. Alabama and Mississippi share similar socio-economic demographics and recreational fishermen communities. The total number of Alabama reefs outside the AARZ is estimated at 2,369. The extent of Alabama waters that is between 10 and 160 m (the extent of our study) and not in the AARZ is similar to that of the total Mississippi shelf area (5,900 km²). Thus, we assumed the same number (n = 2,369) of undocumented reefs for Mississippi coastal waters. Uncertainty in the number of reefs was estimated for AL/MS (see below). Since variance was assumed negligible, any uncertainty in the artificial reef estimate was not carried forward to final variability estimates for AL or any region.

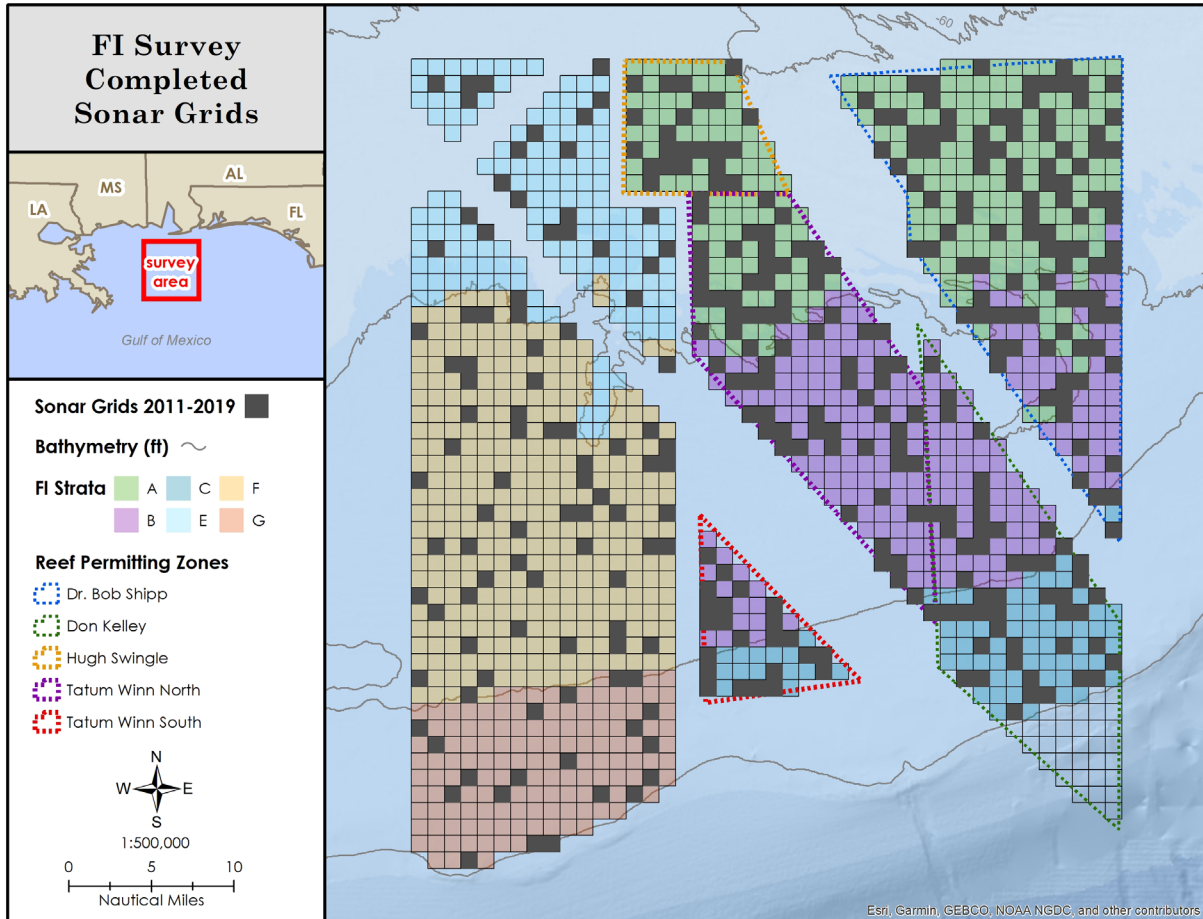


Figure 8. Map of sampling grids off coastal Alabama. The extent of the reef pre-permitted area is shown within the dashed lines as well as the location of grids that have been side-scanned (filled squares).

To derive an estimate of the number of artificial reefs in the AARZ, we computed the average number of artificial reefs per sampled grid, \bar{a} , in the n grid cells surveyed by

$$(6) \quad \bar{a} = \sum_{i=1}^n a_i/n$$

where a_i is the number of reefs in the i th grid cell, and we computed the estimated variance of the number of artificial reefs per grid, $\hat{V}(\bar{a})$, as

$$(7) \quad \hat{V}(\bar{a}) = \frac{\sum_{i=1}^n (a_i - \bar{a})^2}{n(n-1)} \left(1 - \frac{n}{N}\right)$$

The term $\left(1 - \frac{n}{N}\right)$ is the finite population correction which causes the variance of the estimated total to go to zero as one approaches a census of all grid cells. Next, we estimated the number of artificial reefs (A) by multiplying this average by the total number of grid cells in the stratum (N). Thus,

$$(8) \quad \hat{A} = N\bar{a}$$

where the $\hat{}$ symbol denotes an estimate. Estimated variance ($\hat{V}(\hat{A})$) of the number of artificial reefs was calculated by

$$(9) \quad \hat{V}(\hat{A}) = N^2 \hat{V}(\bar{a})$$

with standard error of (\hat{A}) equal to the square root of $\hat{V}(\hat{A})$.

ROV video survey methodology

ROV video-based surveys were conducted at two randomly selected artificial reefs from the total number of artificial reefs found within each sampling grid chosen for side scan surveys. Hence, our design used a two-stage randomization procedure. Video images of the fish community at each site selected for ROV surveys were recorded using a 5-thruster Outland 2000 ROV. The ROV was equipped with a high definition, 1080-line, color camera. The ROV was also equipped with a sonar with a 75-m detection range and 360° viewing capabilities allowing the operator to approach large structures. The ROV was maneuvered at approximately 0.25 ms⁻¹ and 3-4 meters from the bottom. The ROV umbilical (250 m) was attached to a 10-kg depression weight, used to reduce the umbilical's catenary. The terminus of the depression weight was maintained 5-10 meters from the seafloor, followed by 50 meters of unweighted umbilical cord suspended with low buoyancy floats. For each site, the ROV was positioned 5-m away from the structure and the cameras pointed at the structure. The survey area consisted of a 5-m wide halo around the structure. Consequently, we calculated the surface area of a torus shape ($2\pi R * 2\pi r$) where r is the radius of the small circle (2.5 m), R is the radius of the bigger circle (2.5 + 1.72), and π is constant (3.14159) to standardize (#/416 m²) the average observation in each of the two

habitats (artificial reef, natural bottom) assuming an average contact size that covered 17 m² (based on our side scan surveys). Five minutes of video was taken on separate sides of the structure.

Video imagery from the ROV was recorded in HD and analyzed in the laboratory. Fish recorded by the ROV were identified to the lowest possible taxa, enumerated, and measured (see below). For highly mobile fish, abundance was estimated using the MaxN method (Gledhill and Ingram 2004, Ellis and DeMartini 1995). Measurements (total length, TL) of all fish were possible because the ROVs were equipped with a pair of Digi-Key 2.5 milliWatt red lasers aligned in parallel, separated by a distance of 3 cm as a frame of reference (Caimi and Tusting 1987). Fish must be near perpendicular to the camera and have both lasers illuminate their body to be measured (Patterson et al. 2009; Garner et al. In Press).

General description of the index-removal and removal processes

Previous survey work to identify natural and artificial reefs on the Alabama shelf has provided a census of reefs in a randomly selected sample of grid cells and an estimate of the total number of reefs. For each of four spatially defined strata (shallow, mid-depth and deep regions in Alabama plus all of Mississippi waters), a random sample of n_h sites was drawn for stratum h from the master list of all inventoried reefs in the stratum.

At sampled reefs, the following procedures were prescribed. At 9 out of 10 sites, a count of Red Snapper was made on the reef using the ROV and then three sets of vertical longlines were deployed. Each set of vertical longlines consisted of 3 longlines, each with 10 hooks; each line had a different hook size. Hence, there were 3 sets of 30 hooks set at each site (Powers et al. 2018). At every 10th site, an expanded procedure was used consisting of the ROV count, the 3 sets of vertical longlines, and a follow-up ROV count.

The logic behind index-removal estimation is as follows. The ROV count before the removal at a particular site is assumed to be proportional to the abundance, i.e.,

$$E(C_1) = q N \tag{eq 0a}$$

and the expected count after a known removal of R animals is

$$E(C_2) = q (N - R) \tag{eq 0b}$$

where N is the abundance before the removal, q is the “catchability” or calibration factor, and $E(\cdot)$ represents the expected value of the quantity in parentheses. For example, the count before the removal of $R = 100$ animals may be 40 and the count after the removal might be 20.

Logically, the removal of 100 animals caused the population to decline to half of what it was originally. Thus, 100 was 1/2 of the population and the initial population was 200. In symbols,

$$N = R C_1 / (C_1 - C_2) = 100 \times 40 / (40 - 20) = 200. \tag{eq 0c}$$

In theory, the 3 longline sets could also be used to derive a population estimate using a removal estimator, where the decline in catch per set as the population is depleted reflects the initial population size and the efficiency of the vertical longline sampling gear. It is possible to combine the index-removal and removal estimation procedures in an integrated model (Chen et al. 1998). However, it should be noted that the vertical longline gear captures a smaller size range of fish than is seen by the ROV. Consequently, the vertical longlines are sampling a subset of the population seen by the ROV; thus, it follows that the index-removal and removal methods are estimating separate populations. However, it can be shown that the index-removal estimator is robust to heterogeneity in removal probabilities (i.e., size selectivity of harvest) if the indices are not size selective. Thus, the fact that the vertical longlines capture larger animals on average than are seen by the ROV does not invalidate the index-removal method for estimating the size of the populations seeable by the camera.

The general strategy of estimating the size of the Red Snapper population in Alabama/Mississippi was to use the index-removal method to estimate a calibration coefficient that converts an ROV count into an absolute number of fish (a population estimate) and use the calibration coefficient to convert the large number of ROV counts into an estimate of the average population size per reef. This is then multiplied by the number of reefs in Alabama-Mississippi to obtain a total population of Red Snapper in that area. The index-removal method was tested in Alabama in 2014 and 2015 and these data provide additional information on the calibration coefficient. Consequently, these data were included in the integrated analysis of all data on the assumption that the calibration coefficient did not change over time though the estimated population size was free to vary between the two time periods in the model. An additional consideration was that the reefs were stratified into three depth strata with the intensity of sampling varying among the strata. This is addressed in the following Specification of the index-removal and removal processes section.

Specification of the index-removal and removal processes

For the index-removal method, the indices of Red Snapper abundance are generally specified as Poisson random variables. There are a couple of problems with this. First, multiple sites are visited so the total number of fish seen is the sum of independent observations. It is true that the sum of Poisson random variables is itself Poisson distributed. But, the availability of replicate, independent, random observations allows the variance to be estimated from the data rather than relying on the theoretical properties of the Poisson distribution to determine the variance. Second, under the efficient design of Chen et al. (1998) used in the current study, the post-removal index of abundance is obtained by making observations at the same sites used for the pre-removal index, (i.e., the observations are paired). This induces a positive correlation in the counts which reduces the variance of the resulting population estimate. The bivariate distribution of the indices should be specified in the statistical model. We therefore modeled the joint distribution of the *means* of the indices as bivariate normal. This requires a bit of justification.

For each sampling site i , there is an initial population size N_i . When a site is sampled for the first time, the expected count of fish is $E(C_{i1}) = q N_i$ where q is constant across sites. When a site is sampled after the vertical longline removal, the expected count is $E(C_{i2}) = q (N_i - R_i)$ where R_i is the total removal at site i . Since the sites are sampled independently, the expected value of the average pre-removal count over the n sampled reefs is:

$$(10) \quad E(\bar{C}_1) = \frac{\sum_{i=1}^n q N_i}{n} = \frac{q}{n} \sum N_i$$

and, assuming the removals are treated as fixed quantities, the expected value of the average post-removal count is:

$$(11) \quad E(\bar{C}_2) = \frac{\sum_{i=1}^n q (N_i - R_i)}{n} = \frac{q}{n} \sum N_i - \frac{q}{n} \sum R_i$$

It follows that, because the mean (or total) abundance is modeled the same way as the abundance at each site (c.f. equations (10) and (11) with equations (0-a) and (0-b)), the index removal method can be applied to the average (or aggregate) counts even if the abundance varies among sites. The calibration factor q is assumed to be constant over sites. Working with the mean counts has the advantage over working with individual site abundances in that the mean count is almost sure to go down after the removals when the data are treated as an aggregate; in contrast, when estimating abundance separately for each site one encounters situations where the count did not decline after the removal which causes problems (see equation 0-c).

We treated the mean counts in a stratum before and after the removals as bivariate normal with means given by the expectations above in equations (10) and (11) but with an added subscript designating stratum. Note that q was held constant within and among strata. Estimation of the covariance matrix for a stratum requires some thought. We have m_h paired observations on C_{h1} and C_{h2} , and an additional n_h observations on just C_{h1} . We can estimate the variance of \bar{C}_{h1} from the $m_h + n_h$ observations as $S_{\bar{C}_{h1}}^2 = S_{C_{h1}}^2 / (m_h + n_h)$ and, similarly, $S_{\bar{C}_{h2}}^2 = S_{C_{h2}}^2 / m_h$. (Here, $S_{C_{h1}}^2$ and $S_{C_{h2}}^2$ refer to the sample variances of C_{h1} and C_{h2} , respectively.) The covariance of \bar{C}_{h1} and \bar{C}_{h2} is estimated from $\widehat{Cov}(\bar{C}_{h1}, \bar{C}_{h2}) = \frac{\hat{\rho}_h S_{C_{h1}} S_{C_{h2}}}{m_h + n_h}$ where $\hat{\rho}_h$ is the estimated correlation between C_{h1} and C_{h2} in stratum h (based on the m_h paired observations) and $S_{C_{h1}}$ and $S_{C_{h2}}$ are the standard deviations of C_{h1} and C_{h2} based on $m_h + n_h$ and m_h observations, respectively.

We treated the removals as fixed quantities rather than random variables. This means we ignored the information about population size inherent in the three removals at each station (that is, in theory, the three removals could be used to inform a removal estimator of population size). There are two reasons for not using the decline in rate of removal to estimate population size. First, the trend in catches (removals) at the sites is weak, thus indicating that estimates of

population size would be imprecise (but the sum of the three removals is sufficiently large to enable pre- and post-removal camera counts to differ significantly). Second, the removal method estimates a different population than the index-removal method (the removal method estimates a subset of the population seen by the camera). The camera population more closely approximates the target population of the study (Red Snapper age-2+).

The study design also incorporates stratification in Alabama by three depth zones (shallow, mid, and deep) with the addition of (all of) Mississippi as a fourth stratum. The deepest stratum received the least sampling effort due to cost constraints. Thus, the mean counts before and after the removals were calculated as for stratified random sampling as the weighted average of the counts in each stratum; the weights are proportional to the size of the strata (number of reefs). Thus,

$$(12) \quad \bar{C}_{st,i} = \sum_{h=1}^4 W_h \bar{C}_{hi}$$

where $\bar{C}_{st,i}$ is the stratified estimate of the mean count (for sampling event i – either the pre-removal ($i=1$) or post-removal ($i=2$) counts), \bar{C}_{hi} is the mean for stratum h and event i , $W_h = A_h/A$ is the weighting factor for the proportion of the total number of reefs that occurs in stratum h . (We substitute estimates of A_h and A for the true values, noting that it is only the proportion of reefs and not the absolute numbers of reefs that is needed to weight the strata.)

The variances are estimated by:

$$(13) \quad \hat{V}(\bar{C}_{st,1}) = \sum_{h=1}^4 W_h^2 S_{C_{h1}}^2 / (m_h + n_h)$$

$$(14) \quad \hat{V}(\bar{C}_{st,2}) = \sum_{h=1}^4 W_h^2 S_{C_{h2}}^2 / (m_h)$$

where $S_{C_{hi}}^2$ is the sample variance of the catches per station in stratum h for event i , and W_h is as defined above. The simple weighted sum of variances arises by virtue of the fact that the strata are sampled independently. In (13) and (14), the divisors $m_h + n_h$ and m_h are to convert the variances to variances of the means.

The covariances are estimated as:

$$(15) \quad C\hat{o}v(\bar{C}_{st,1}, \bar{C}_{st,2}) = \sum_{h=1}^4 W_h^2 \frac{\hat{\rho}_h S_{h,C_1} S_{h,C_2}}{m_h + n_h}$$

where S_{h,C_1} is the sample standard deviation of observations in stratum h for pre-removal counts, S_{h,C_2} is the sample standard deviation of observations in stratum h for post-removal counts, $\hat{\rho}_h$ is the estimated correlation between C_{h1} and C_{h2} in stratum h , and W_h is the weight for stratum h . In (15), the divisor $m_h + n_h$ is to convert the covariance of C_{h1}, C_{h2} to the covariance of the means $\bar{C}_{st,1}, \bar{C}_{st,2}$.

The likelihood of the data can be expressed as the product of five bivariate normal probability density functions, one for each of the three depth strata in Alabama, one for the data from Mississippi, and one for the data previously collected in 2014-2015; each bivariate normal density is parameterized in terms of the mean number of red snapper per reef (varying within a stratum by site and among strata) and the catchability or calibration coefficient q assumed constant within and among strata. Thus, the likelihood, Λ , is given by

$$\Lambda = \Lambda_{previous} \prod_{h=1}^4 \Lambda_h$$

where $\Lambda_{previous}$ is the likelihood for the previous data (from 2014-2015) and Λ_h is the likelihood for stratum h . Each likelihood for the four strata is bivariate normal with mean vector $\boldsymbol{\mu}_h$ representing the mean ROV counts before and after the removals (given by (10) and (11))

$$(16) \quad \boldsymbol{\mu}_h = \begin{bmatrix} E(\bar{C}_{h1}) \\ E(\bar{C}_{h2}) \end{bmatrix} = \begin{bmatrix} \frac{q}{m_h+n_h} \sum_{i=1}^{m_h+n_h} N_{hi} \\ \frac{q}{m_h} \sum_{i=1}^{m_h} N_{hi} - \frac{q}{m_h} \sum_{i=1}^{m_h} R_{hi} \end{bmatrix}$$

and with covariance matrix $\boldsymbol{\Sigma}_h$ given by

$$(17) \quad \boldsymbol{\Sigma}_h = \begin{bmatrix} \sigma_{\bar{C}_{h1}}^2 & \sigma_{\bar{C}_{h1}, \bar{C}_{h2}} \\ \sigma_{\bar{C}_{h1}, \bar{C}_{h2}} & \sigma_{\bar{C}_{h2}}^2 \end{bmatrix}$$

where $\sigma_{\bar{C}_{h1}}^2$ is determined from $m_h + n_h$ observations as $\sigma_{\bar{C}_{h1}}^2 = \sigma_{C_{h1}}^2 / (m_h + n_h)$ and $\sigma_{\bar{C}_{h2}}^2$ is determined from m_h observations as $\sigma_{\bar{C}_{h2}}^2 = \sigma_{C_{h2}}^2 / m_h$; the covariance is specified as $\sigma_{\bar{C}_{h1}, \bar{C}_{h2}} = \frac{\rho \sigma_{C_{h1}} \sigma_{C_{h2}}}{m_h + n_h}$. The likelihood from the previously collected data from 2014 – 2015 is of the same form as above, with the same q as for the four strata but with a different mean abundance per reef.

The above described likelihood gives estimates of a single q and separate estimates of mean abundance per reef N for each component of the likelihood (four geographic strata plus the previous time period). The population size in each stratum is then obtained as the product of the estimated N and the estimated number of reefs in the stratum, A . The variance of the estimated population size is found using Goodman's (1960) exact formula for the variance of a product.

Unfortunately, the product bivariate normal likelihood described above did not perform well in practice (it was difficult to achieve convergence and the results were unstable). Therefore, we modified the likelihood as follows. We replaced the product of the four likelihoods for the separate strata with one likelihood for the weighted (over strata) mean abundance per reef. Thus,

$$\Lambda = \Lambda_{previous} \Lambda_{stratified}$$

where $\Lambda_{stratified}$ is bivariate normal with mean vector given by

$$\boldsymbol{\mu}_{stratified} = \begin{bmatrix} E(\bar{C}_{st,1}) \\ E(\bar{C}_{st,2}) \end{bmatrix} = \begin{bmatrix} \sum_{h=1}^4 W_h E[\bar{C}_{h1}] \\ \sum_{h=1}^4 W_h E[\bar{C}_{h2}] \end{bmatrix}$$

Here, $E[\bar{C}_{h1}]$ is given by equation (16), and W_h is the stratum weight A_h/A . We treat W_h as a known constant although its value is estimated externally. The variance-covariance matrix for the stratified mean is

$$\boldsymbol{\Sigma}_{stratified} = \begin{bmatrix} \sigma_{\bar{C}_{st,1}}^2 & \sigma_{\bar{C}_{st,1}, \bar{C}_{st,2}} \\ \sigma_{\bar{C}_{st,1}, \bar{C}_{st,2}} & \sigma_{\bar{C}_{st,2}}^2 \end{bmatrix}$$

with estimates given by (13), (14) and (15).

The alternative likelihood based on the stratified mean provides estimates of q , the mean population size per reef for the four geographic strata combined, and the mean population size per reef in 2014-2015. The variance-covariance matrix comes from taking the inverse of the Hessian. Stratum-specific estimates of abundance are obtained by dividing the stratum-specific mean count per reef before the removals by the estimated catchability q and then multiplying by the estimated number of reefs in the stratum. In symbols,

$$(18) \quad \hat{N}_h = \frac{\bar{C}_{h1} \hat{A}_h}{\hat{q}} = \bar{N}_h \hat{A}_h$$

where the $\hat{}$ symbol denotes an estimate. The variance of \hat{N}_h can be approximated by expressing (18) as the product of $\frac{\bar{C}_{h1}}{\hat{q}}$ and \hat{A}_h . The estimated variance of the quotient can be approximated by the Taylor's series approach (or delta method) as

$$\hat{V} \left[\frac{\bar{C}_{h1}}{\hat{q}} \right] = \hat{V}[\bar{N}_h] = \frac{\hat{V}[\bar{C}_{h1}]}{\hat{q}^2} + \frac{\bar{C}_{h1}^2 \hat{V}[\hat{q}]}{\hat{q}^4}$$

where \hat{V} denotes estimated variance of the expression in brackets. Note that the estimates of q and \bar{C}_{h1} are not independent but, because \hat{q} depends on the five \bar{C}_{h1} , the dependence on any one mean count is likely to be small and is ignored here. The estimated variance of the product $\bar{N}_h \hat{A}_h$ can be found using Goodman's (1960) exact estimator

$$(19) \quad \hat{V}[\hat{N}_h] = \hat{A}_h^2 \hat{V}[\bar{N}_h] + \bar{N}_h^2 \hat{V}[\hat{A}_h] - \hat{V}[\bar{N}_h] \hat{V}[\hat{A}_h].$$

Abundance of Red Snapper on artificial reefs in Alabama/Mississippi

We estimated a calibration factor for the ROV camera system of 0.122 ± 0.051 (SE). In Alabama, the number of Red Snapper per reef on artificial reefs is highest in shallow water and lowest in deep water. Red Snapper per artificial reef in Mississippi (considering only the 229

reefs that are believed to harbor Red Snapper) is low, being less than half that seen on artificial reefs in Alabama (Table 2). Almost 90% of the artificial reefs with Red Snapper occur in the shallow and mid-depth strata of Alabama.

The mean number of Red Snapper per artificial reef over all strata can be computed as the weighted mean of the stratum estimates (Table 3). This results in an estimate of 160 Red Snapper/reef. Multiplying by the total number of reefs (9,410) gives an estimated population size of 1.51 million (± 0.461 million standard error of the mean) Red Snapper on known and estimated artificial reefs in Mississippi and Alabama combined. If our assumption of the number of unpublished artificial reefs off Mississippi (2,369) is included, the estimate increases to 1.79 million Red Snapper. However, due to the inherent uncertainty of this unpublished reef estimate, we chose to adopt the more conservative and only use the known reefs for the abundance estimate, and that estimates and associated variability are reported in Tables 4, 5, and 7.

Table 2. Estimated number of artificial reefs in Alabama and Mississippi coastal waters (10 – 150 m depths).

State	Stratum	Number of grid cells (<i>N</i>)	Number of reefs per grid cell		Total number of artificial reefs	
			Mean (\bar{a})	Estimate (\hat{A})	Standard Error	
AL	Shallow (AARZ)	340	11.7	3984	182	
AL	Mid (AARZ)	297	8.45	2510	109	
AL	Deep (AARZ)	111	2.86	317	43	
AL	Shallow (Outside)	165	4.19	691	103	
AL	Mid (Outside)	338	3.38	1144	113	
AL	Deep (Outside)	148	3.62	535	104	
MS	Known Reefs			229		
MS	Unpublished			2369		

Table 3. Estimates of mean population size (number of Red Snapper per artificial reef) for four strata: Mississippi artificial reefs believed to constitute Red Snapper habitat, shallow-water artificial reefs in Alabama, mid-depth artificial reefs in Alabama, and deep-water artificial reefs in Alabama. Also given is the standard error of the estimated mean, the number of reefs in the sampling frame, the stratum weights used in the likelihood (= number of artificial reefs believed to have snapper in the stratum divided by the total number of artificial reefs believed to have Red Snapper), and the number of artificial reefs sampled in each stratum.

Stratum	Mean density (fish/artificial reef)	Standard error (mean density)	Total artificial reefs in sampling frame (<i>N_h</i>)	Stratified weights	Number of artificial reefs sampled
MS	73.761	33.271	229	0.0243	11
Shallow AL	187.416	80.585	4675	0.497	68
Mid AL	163.003	72.049	3654	0.388	45
Deep AL	24.587	15.899	852	0.0906	4

Natural hard bottom habitat

The majority of natural hard bottom is found in the deeper areas of our study region. While bottom habitat in shallow and mid-depth areas can be characterized as mud/sand with almost no natural emergent structure, deep areas of the AARZ include an extensive area of natural hard bottom (rocks, boulders and pinnacles (Figure 9) centered around the 70 m isobath. In contrast, natural reefs are extremely rare in shallow and mid-depth areas, where artificial reefs are common (2 to 3 emergent structures per km²). The most comprehensive survey of the natural bottom areas off coastal Alabama and Mississippi was conducted by the USGS (Figure 9). The total area is estimated to be 1,625 km². Approximately 75% of this area is off Alabama and 25% is off Mississippi. The area includes numerous rock fields, banks and pinnacles scattered throughout a sand bottom. The USGS survey did not have suitable resolution to differentiate the spatial extent of hard bottom areas from sand. To estimate the actual extent of hard bottom, we used our side-scan imagery of randomly selected grids in the area (see description above) to calculate a percent cover estimate of natural hard bottom (Figure 9). Applying this 13% cover estimate, the total bottom area of natural reef was calculated as 211 km².

To estimate the density of Red Snapper on natural reefs, we used our MaxN count of Red Snapper from the ROV and standardize this to the average area surveyed by the ROV (417 m²). The use of MaxN is a conservative estimate since it assumes all Red Snapper were in the image. This is clearly an underestimate. The average MaxN for the 32 natural features surveyed in 2018 was 7.58 (± 1.84 standard error of the mean). Excluding Red Snapper less than 250 mm in length (i.e., only including Red Snapper 2+ years), the average MaxN decreased to 7.41 (± 1.8 SE). The small adjustment reflects the observation that most Red Snapper were above the 250 mm size threshold (Figure 10). In addition, one site was removed from the dataset due to a MaxN that was 5x higher than the average MaxN. The resulting average was 0.017 Red Snapper per m². Multiplying that density by 211 km², yields an estimate of 3.75 million Red Snapper in this habitat strata.

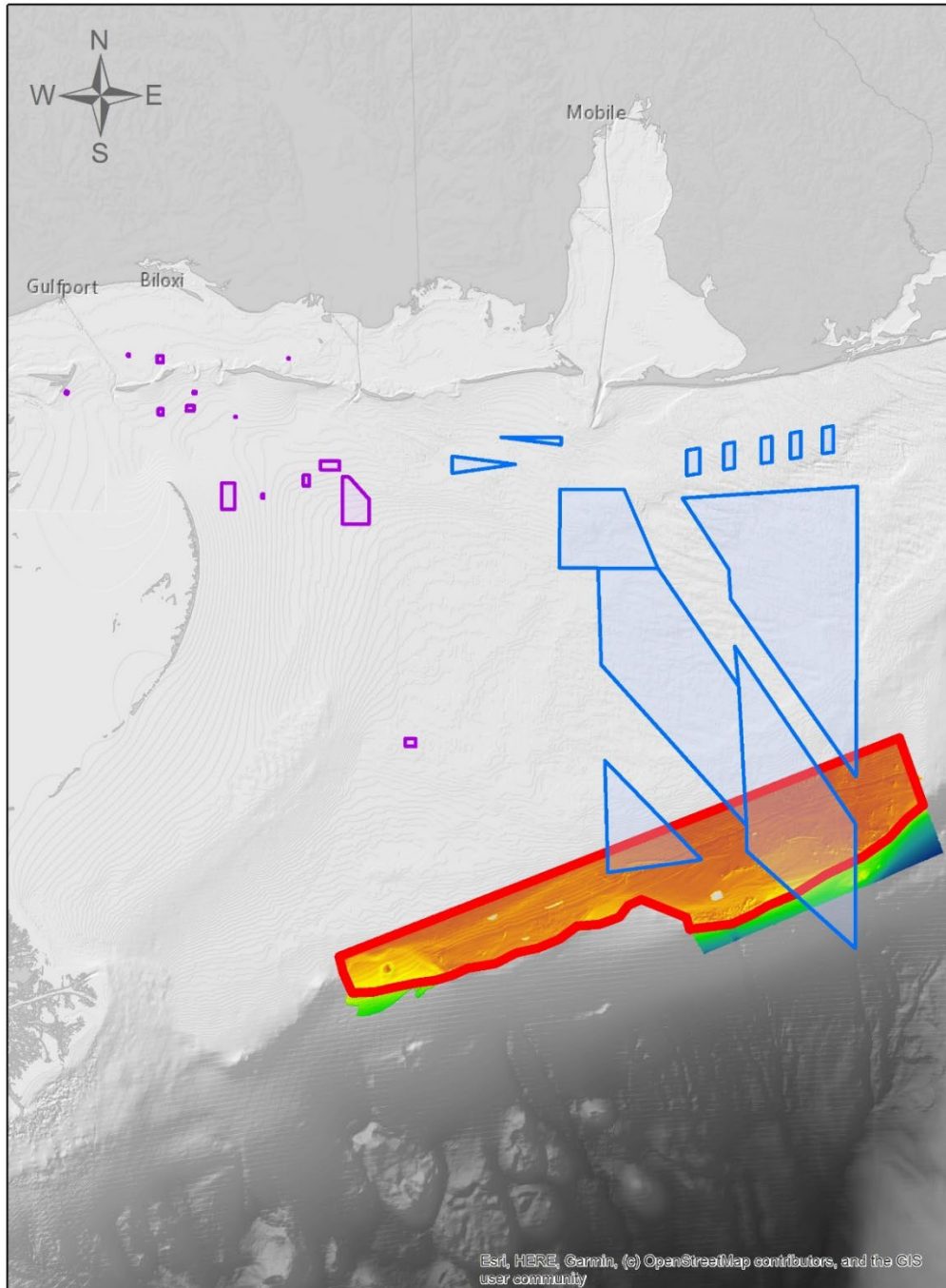


Figure 9. Mississippi and Alabama coastal waters showing the extent of the natural hard bottom banks (Alabama pinnacle and Alps; red polygon) centered around the 70 m isobath surveyed by the USGS multi-beam study. The blue and purple polygons represent the Alabama and Mississippi artificial reef zones, respectively.

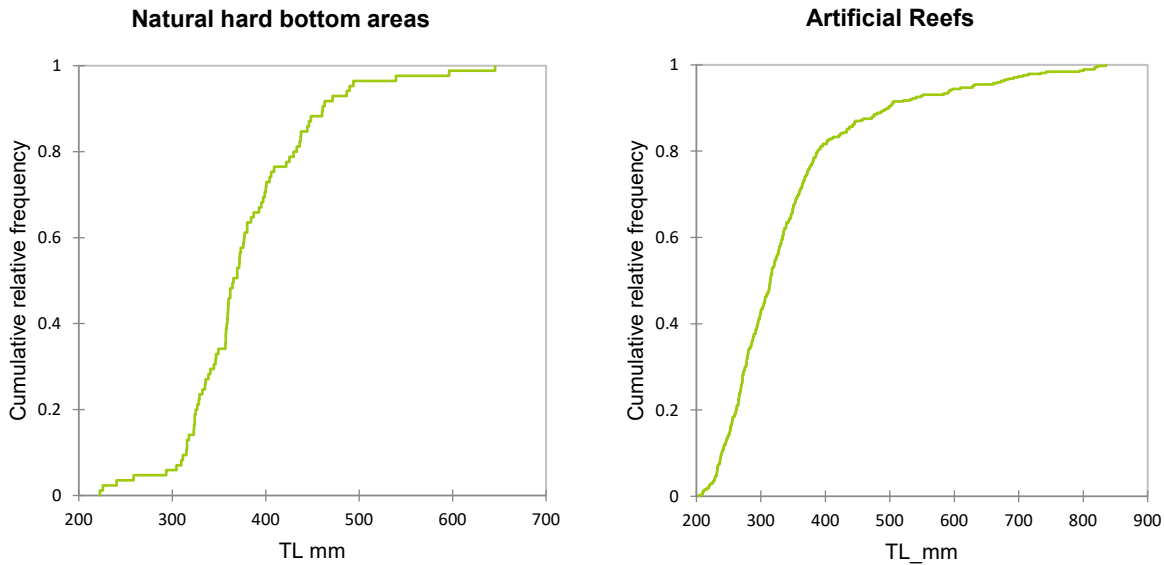


Figure 10. Cumulative histograms of Red Snapper TL (mm) observed on artificial reefs (N = 377) and natural hard bottoms (N = 85) during our survey.

We used two different video-based approaches to estimate the number of Red Snapper in the AL/MS region. The approach for natural hard bottom is similar to that used by other regions in that we derived a density from video (or acoustic) observations and multiplied that density by the extent of the habitat. Our density estimate, which used the MaxN derived number was undoubtedly conservative and could have been improved if we paired the count with an acoustic-based methodology. At the beginning of the sampling program, we felt a depletion-based approach could have worked to calibrate our video count observation (similar to our approach on artificial reefs). However, our trials of this approach over the natural reef areas failed to consistently measure decreases in before and after MaxN counts may be a result of rapid movement of Red Snapper into our natural reef areas in between cycles of video observations. Unlike most artificial reefs which occur in isolation, natural hard bottom areas can be very extensive areas of low relief bottom in which Red Snapper are spread.

As with other regions, improved habitat mapping (i.e., higher resolution to refine our coarse classification of natural hard bottom areas and greater spatial coverage) would decrease our uncertainty substantially. For artificial reefs, we were fortunate to have an extensive side-scan data base to estimate the number of structures currently in Alabama waters both within and outside the pre-permitted AARZ. Unfortunately, no such database exists for Mississippi. We believe if such a database did exist, it would have identified numerous artificial reefs that were deployed but never permitted in Mississippi waters. Likely, the number of such structures is similar to that in Alabama waters. However, the absence of documentation of these habitats (beyond numerous reports of bottom trawl snags) prevented our inclusion of ~320,000 Red Snapper. For natural reefs, our side-scan survey in coastal Alabama only covered a small percentage of the vast expanse of deep water natural hard bottom. We feel that our estimate of

the spatial extent of this area was adequate, but greater stratification of natural hard bottom types (e.g., rock fields, plateaus, ridges, etc.) would have improved our estimates by decreasing variability that is likely explained by habitat type. Unfortunately, we lacked the high-resolution mapping necessary for such an approach. Even if that imagery was available, the additional cost of further deep-water sampling would have been prohibitive for the budget allocated to our region. Although the improvements mentioned would have been helpful, we believe our estimate is quite robust for our region and represents the best systematic survey of natural hard bottom areas off coastal Alabama and Mississippi. More broadly, the sampling in the Alabama/Mississippi region introduced several new aspects of index-removal estimation that we feel advance this technique. The introduction of the multivariate normal distribution for ROV camera count means in the likelihood function (instead of independent Poisson distributions) allows for empirical variances instead of theoretical ones and allows for correlation among the counts.

Key assumptions and implications summary:

Estimating abundance off the AL/MS region required several assumptions depending on the habitat of interest. For natural reefs in AL/MS, we used MaxN counts to estimate abundance. This procedure has a negative bias because some fish can be missed. However, when abundance is low the problem of missed fish (and thus the bias) is lower than when abundance is high. For artificial reefs, we used a variation of the index-removal method. This method is based on the idea that a known removal from the population will cause an index of abundance to decline in proportion to the fraction of the population that is removed. The principal assumption is that the index of abundance is proportional to the size of the population. This implies that 1) the index (not the removal) is not selective (for size, sex, or other factors) and 2) the index represents a constant proportion of the population (over time and location). The second aspect of this assumption implies further that the proportionality of the index does not vary with the size of the population, the type of reef, or any other factor. Although the constant proportionality of the index cannot be easily proved, it can be tested by comparing estimates for aggregates of stations that are, for example, large reefs versus small reefs to see if the estimated calibration coefficients differ. Attempts to subset the data led to imprecise estimates due to small sample sizes.

5. Texas Region

For the Texas region estimate, we used a combination of ROV surveys to generate a species composition coupled with hydroacoustic surveys to determine abundance estimates for artificial reefs and natural hard bottom. We used towed camera arrays coupled with hydroacoustics to generate estimates for the vast expanses of UCB. For pipelines and the deepest UCB sites, a combination of hydroacoustic and visual approaches were implemented using C-BASS methodologies and are described in Section 7 below. Unlike in AL/MS, the location, number and/or areal coverage of artificial reefs and natural bottom was known, and uncertainty was assumed negligible. Since variance was unable to be estimated and assumed negligible, the uncertainty was not carried forward to final variability estimates for any region. Areas where

unclassified/unknown structured habitat occurred (i.e., unknown artificial reefs or natural bottom) that were not part of the ‘known’ universe of habitat types were included in the UCB habitat type. The UCB habitat area was calculated by excluding a 100-m buffer (‘area of influence’; sensu Karnauskas et al. 2017 and Reynolds et al. 2018) around all known artificial reefs and natural hard-bottom habitats within the Texas region. For this area, a total of 183 sites (36 natural and 147 UCB, respectively) were sampled. All of the UCB samples in the deep strata (n=4) were done using the CBASS system, with the remaining 143 done using the towed sled (camera and hydroacoustic equipment, see Section c. with the number of artificial reefs sampled dependent on the analysis used (pyramid and non-pyramid versus large and small designations).

a. ROV Visual Surveys

In Texas, ROV visual surveys were completed with a Mission Specialist Defender ROV (VideoRay, LLC) equipped with a compass, parallel lasers, depth and temperature sensors, LED lighting array, forward-facing Blueprint Oculus 750 kHz/1.2 MHz Dual Frequency sonar, a fixed forward-facing HD camera (160° horizontal viewing angle, and 91° vertical viewing angle), and DVL (Doppler Velocity Logging) Navigation system with GreenSea Software. The DVL system allowed operators to estimate depth and altitude and incorporated auto-depth holding and waypoint following capabilities. The ROV was piloted through an integrated control box, and visual data was transmitted in real-time to the surface and recorded. Time of day, depth (m), altitude (m), heading, and temperature (°C) were embedded in the video. A rear-facing GoPro Hero7 Black (122.6° horizontal viewing angle, 94.4° vertical viewing angle) was mounted externally to the ROV to increase visual area surveyed and account for observed Red Snapper behavior. Both the ROV camera and the GoPro camera were set to an angle of 45° for natural bank surveys and 0° for all artificial reef surveys. The LED array remained off during surveys to reduce potential fish attraction to the ROV (Bowmaker 1990). Longitude and latitude were recorded at the ROV deployment location, and visibility at each site was estimated using paired sonar in conjunction with real-time video. Visibility measurements were collected every 20-m change in depth at artificial reefs and at depth at natural banks. Video was recorded for the entire deployment at each site.

The primary goal of the visual survey for this western region was to generate a species composition rather than an absolute abundance estimate. Visibility was constrained using this method in much of this region; thus, the estimate was derived from ROV-based species composition paired with hydroacoustic surveys (see below). Visual surveys for the western Gulf incorporated transect-based methods as described previously, but also included the use of a rear-facing camera and a comprehensive water-column survey. These addressed concerns about fish behavior and regional visibility, as the artificial reef sites surveyed in the western Gulf were typically oil and gas platforms. These structures are extremely large compared to eastern Gulf artificial reefs, and they are characterized by very high relief from the bottom making sampling throughout the water column and along the entire span of the structure necessary. Unlike in the eastern Gulf, where the entirety of the artificial reef typically can be seen in one frame, only a small fraction of the entire structure can be seen and surveyed. Following previous studies by our

research team (Ajemian et al. 2015a, 2015b), we developed methods to accurately characterize the communities and relative abundance on these structures, even when it is not possible to survey the entire structure due to the enormous size. The entirety of each structure was surveyed with active hydroacoustics for abundance estimation (see b. Hydroacoustics Methods section below), and when conditions allowed, a ROV-based survey was performed which was necessary to generate an accurate habitat-specific community composition that allowed for assignment of a species-specific proportion to the hydroacoustic-derived abundance estimates.

At artificial reefs, the ROV was deployed with a free tether and manually piloted near the structure for the remainder of the survey. Following previously published methods (Ajemian et al. 2015a, 2015b), a five-minute rove was completed at the top of the structure. The ROV then completed a vertical transect toward the bottom at constant speed and distance from the structure. The vertical transect ended at the maximum ambient depth or when visibility was too poor to continue (i.e., ≤ 1 m). Upon completion of the vertical transect, the ROV began ascent and stationary 1-min observations were completed in 10-m depth intervals following previously established methods for surveying artificial structures in the Texas region. During ascent and for oil and gas platforms specifically, a minimum of three horizontal transects were completed at crossbeams (Streich et al. 2017a) with the ROV maintaining an approximately straight path, constant speed, and consistent distance from structure. Due to sampling logistics and ship position top-side, these transects varied in length; however, they generally spanned one entire side of the structure. Varying transect length was accounted for during sample processing.

At natural bottom sites, the ROV was deployed from the vessel using a tethered drop weight to approach the selected deployment site more accurately. Once the ROV was at depth, visibility was estimated with forward-facing sonar, and visual observation of the drop weight, and the navigation system allowed waypoint selection and following to be initiated. Starting and ending waypoints for a minimum of three 40-m transects were randomly selected surrounding the central deployment point. During transects, a constant speed (0.5 m/s) and altitude (1.5 m) were maintained. Transect length, start and end times, speed, altitude, and visibility were recorded for each transect. After completion of the transects, the ROV returned to the surface.

A total of 66 ROV surveys were completed on natural and artificial sites between July 2018 and October 2019. Forty-eight surveys were completed at natural banks and 18 at artificial reefs. These visual surveys were used specifically to determine the species composition of Red Snapper for each site. For all sites, the video was processed by two independent readers. Local time of day, temperature, depth, altitude, visibility, and heading were recorded each time a fish was documented. All fish were identified to the lowest possible taxon, enumerated, and an average count was generated from the two readers. Counts were jointly reviewed if readers differed by more than 5%. Rear-facing video was processed in the same way, and detailed information (i.e., depth, altitude, time of day, etc.) could be aligned with forward-facing data as camera start times were synchronized during deployment. Due to the broader geographic scale of the corresponding hydroacoustic surveys, we used the entire visual survey incorporating both forward and rear-facing camera counts, while avoiding any double counting in generating the

species composition. The inclusion of this large amount of video, allowed us to generate the most robust species composition data for scaling hydroacoustic densities at these habitats.

To allow for integration of the species composition with these hydroacoustics data, further post-processing of the visual dataset was required. First, species that would not be detected acoustically due to size, morphology (e.g., damselfishes, sharks, etc.), cryptic behavior, close-association within the benthic fouling, or on the structure were removed from the dataset prior to further analysis (Simmonds and MacLennan 2005; Wilson et al. 2006). This modified dataset was then used to create robust regional depth and habitat specific species compositions based on project-defined strata (depth: shallow [0-40m], mid [41-100 m], deep [100-160 m]; habitat: natural reef, artificial reef; e.g., mid-natural, deep-artificial, etc.). Species composition data from each survey site occurring within each depth-habitat strata were used to estimate species composition for all sites within the strata. The shallow depth stratum was further divided into shallow I (10-20 m) and shallow II (20-40 m) sub-strata because species composition between these depth strata differs due to ambient habitat characteristics (Bohnsack and Sutherland 1985; Streich et al. 2017a; Streich et al. 2017b; Rosemond et al. 2018; Plumlee et al. 2020).

Hydroacoustic surveys resulted in acoustic abundance binned into 10-m depth layers (see methods below for more detail), and the species composition was partitioned into the same depth/region/habitat data bins for analysis. As sites included within each depth-habitat stratum (e.g., mid-natural) occurred at various depths, a method was developed to account for those depth differences. For each recorded fish occurrence, the proportional depth (or percent distance from the seafloor) was calculated using the site depth and depth of the observed fish occurrence.

$$\text{Proportional Depth (\%)} = \frac{\text{Site Depth} - \text{Fish Depth}}{\text{Site Depth}} \times 100$$

Next, the maximum depth recorded during the hydroacoustic survey at each site was used to determine the number of depth bins for each site surveyed. Bins were created in 10-m intervals starting from the seafloor (i.e., maximum echogram depth), where depth bin 0 equaled the deepest site depth (Table 15, see appendix B). Subsequent depth bins were created in 10-m increments until the shallowest depth bin (i.e., the surface layer) was encompassed. As a result, this shallowest depth bin typically comprised less than 10-m of the water column. To properly allocate fish species composition to bins that mirror the hydroacoustic data, the percent of the water column each depth bin accounted for was calculated for each site. For instance, a 10-m bin in a water depth of 71.2 m (depth bin 0, 71.2-61.2 m) would account for approximately 14% of the water column, (or 14% of the distance from the seafloor). The following 10-m depth bin (depth bin 1, 61.2-51.2 m) could account for an additional 14% of the water column (or 14-28% of the distance from the seafloor). However, at a site with a 53-m water depth, the deepest 10-m bin (depth bin 0, 53-43 m) would account for 19% of the water column. Once calculated for each site surveyed, these percentages were then paired with all fish occurrences within the same depth-habitat stratum that correlated with the proportional depth of the occurrence. For example, fish counts from multiple sites in the Mid-Natural strata occurring at a proportional depth of 14-

28% of the seafloor would be placed into depth bin 1 in the above example site. After allocation of fish occurrences to hydroacoustic depth bins, the MaxN (Ellis and DeMartini 1995; Campbell et al. 2015; the greatest count of a particular species that is on the screen at one time) for each species at each site within the bin was extracted. These MaxN counts were then summed within the depth bin by species, and a species-specific relative abundance based on proportional MaxN counts was generated and applied to hydroacoustic surveys for species-specific scaling to total abundance (Reynolds et al. 2018) based on hydroacoustic data (see below).

b. Hydroacoustics

Hydroacoustic transects were performed along with ROV surveys at each site using a calibrated Simrad EK-80 split-beam echosounder (70-18CD transducer; 70 kHz). These transects resulted in 36 usable natural hardbottom samples (3 additional surveys were attempted but were omitted from the final dataset due to gear malfunction). In addition, 45 large and 4 small artificial reefs were sampled (Table 5), which when separated into pyramid and non-pyramid samples resulted in 31 surveys (13 and 18, respectively Table 7). During hydroacoustic surveys, the transducer was either mounted to a towed body and towed 40 m behind the boat or pole-mounted approximately 1.8 m below the surface depending on vessel requirements. At natural bank and small artificial reef sites, surveys consisted of four 500-m transects in a radial pattern (Reynolds et al. 2018) centered on the geographic station position (Figure 11). At large artificial reef sites, a back-and-forth “*mow-the-lawn*” survey pattern was used to survey the entire artificial reef site (Figure 12). All transects were conducted at 0.256 μ s pulse duration with continuous wave.

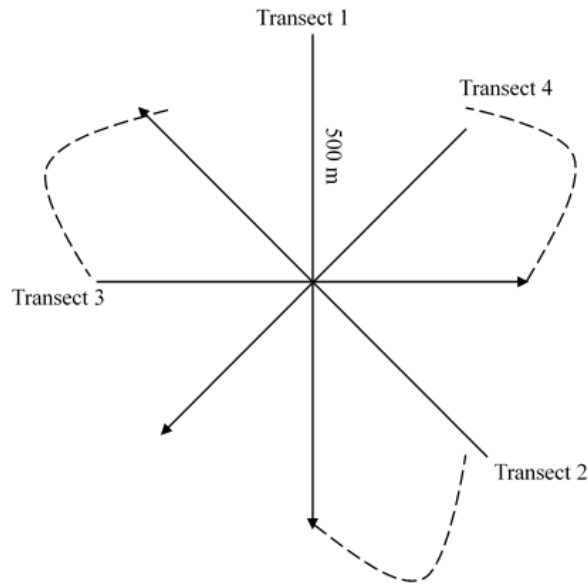


Figure 11. Diagram representing the tow pattern for the four, 500-m echosounder transects centered over the geographic station position for each sampling location.

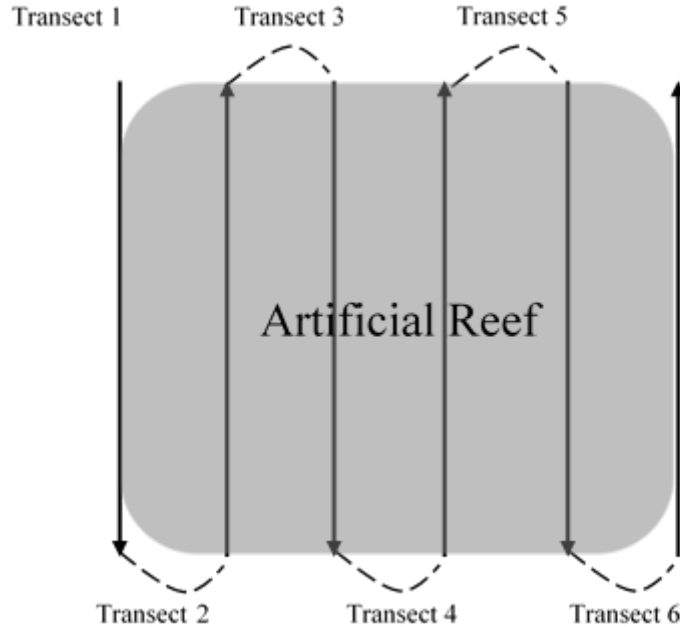


Figure 12. Example of a back-and-forth sweeping (mow-the-lawn) survey used at large artificial reef sites.

Raw acoustic data were processed in the lab using Echoview 10.0 (SonarData Pty Ltd., Hobart, Australia; Boswell et al. 2010). As described above, prior to analyzing, data were edited to exclude unwanted noise and reverberation (Simmonds and MacLennan 2005, Boswell et al. 2007). Echograms were visually inspected for bad data regions (i.e., bubble injection, towed body abnormalities) and corruptions in data integrity (i.e., sudden changes in speed, loss of GPS signal). The sea floor and reef structure were excluded from the analysis by applying a bottom detection algorithm with a 0.5 m backstep (Boswell et al. 2010). Zooplankton layers suspended in the water column were also excluded (Figure 13).

Echo integration approaches were used when individual fish were too closely distributed for echo counting to be successful (Scouling et al. 2015; Gastauer et al. 2017). A 10 x 5-m (10-m depth and 5-m distance) grid was applied over the hydroacoustic data (Figure 13). Both the video and hydroacoustic data were binned by 10-m depth intervals to distinguish pelagic from benthic fish species. Processed hydroacoustic data resulted in two standard outputs: volume backscattering strength (S_v) and target strength (TS). S_v is the sum of discrete targets per unit volume of water (MacLennan et al. 2002) and is often used as a proxy for fish abundance (Simmonds and MacLennan 2005, Boswell et al. 2007, MacLennan and Simmonds 2013). Target strength (in dB re 1 m^{-1}) estimates, used to approximate fish length, were generated with the split-beam single target detection algorithm where targets fulfilling single target criteria with TS greater than -55 dB (3 cm standard length (SL); McCartney and Stubbs, 1971) were accepted into the analysis. When S_v is scaled by TS (Equation 1), a volumetric estimate of fish density can be derived (Simmonds and MacLennan 2005).

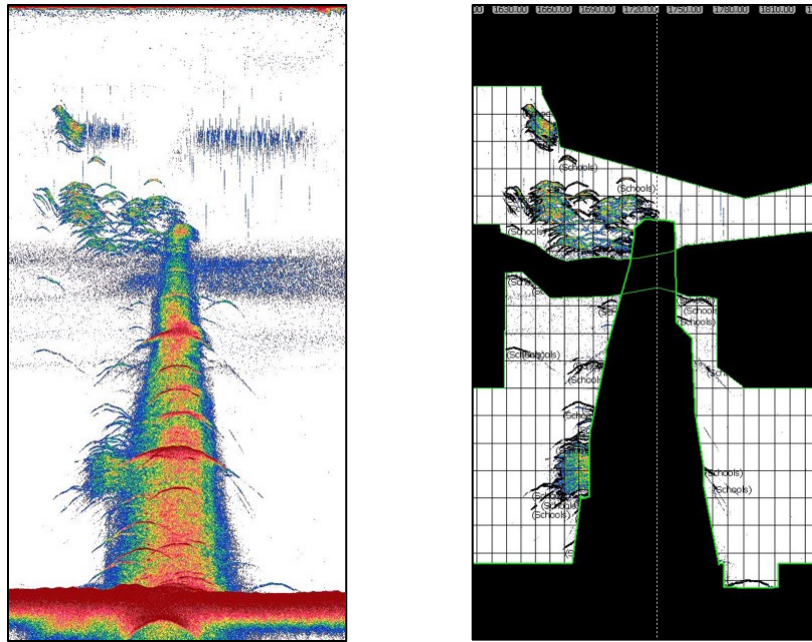


Figure 13. Examples of acoustic echograms from artificial reef MU-A-103 before (left) and after (right) the appropriate zones were excluded, and the grid was applied.

To scale the S_v data, the Sawada index (Sawada et al. 1993) was applied to single targets that were within 7 m of a designated school. These targets, which will be referred to as border targets henceforth, were used to scale the schools based on their target strength to estimate the number of fish within that school. Schools were scaled, and then an abundance estimate was calculated using RStudio (R Core Team version 3.5.1; RStudio Team, 2018). Schools were paired with border targets within 7 m by estimating the minimum distance between border targets and each school. The paired TS and S_v values were converted to linear densities and then to volumetric fish density using the formulae described above. Abundances by layer were produced by averaging the kriged areal densities and multiplying by the total area of the layer. We then took the abundance value and applied the proportion of age-2+ Red Snapper, as indicated by the video data, to produce a Red Snapper abundance by layer. Red Snapper abundances were summed across all layers to produce a Red Snapper abundance estimate for the site.

c. Towed Sled – Camera and Hydroacoustics

Characterizing the distribution and abundance of demersal fishes over UCB is complicated by the methodological restrictions imposed by typical conditions associated with this habitat. Commonly used sampling methodologies such as visual surveys, trawl surveys, and

hydroacoustics each possess their own limitations that may bias abundance estimates because the performance and efficiency of these methods vary as functions of seabed complexity and water column visibility. Visual surveys conducted with SCUBA or ROV-based cameras are only occasionally useful on soft-bottom habitats, and in some regions natural and artificial habitat type, in the Gulf because visibility is often limited by a persistent nepheloid layer (Gallaway et al. 1981, Rezak et al. 1990). Bottom trawls can be used to quantify and identify demersal fishes, but the capture efficiency of this gear is affected by size and species (Wells et al. 2009), complicating calculating an absolute abundance estimate. Moreover, trawled gear does not perform well when bottom complexity or relief anomalies are encountered (Zimmerman 2003). Hydroacoustic surveys are capable of providing high-resolution information on fish abundance across a variety of benthic habitat types, and echo sounders have been successfully used to quantify the relative abundance of fishes on natural (Wilson et al. 2003) and artificial (Boswell et al. 2010) reefs in the Gulf. However, definitive species identification is generally unattainable from narrow-band acoustic backscatter alone (Horne 2000, Parker-Stetter et al. 2009), and thus assemblage composition obtained from visual surveys or bottom trawls is often used in conjunction with echo sounder data to apportion taxon-specific estimates of abundance (reviewed by McClatchie et al. 2000), similar to our approaches for artificial and natural reefs in the western Gulf. New approaches such as imaging sonars which produce near-video quality images of acoustic targets are increasingly used to overcome identification problems in no or low visibility conditions, and integrated surveys that couple these methods show considerable promise for estimating fish abundance across multiple habitats and/or environmental conditions (Holmes et al. 2006, Mueller et al. 2010, Langkau et al. 2012, Able et al. 2014).

Data Collection

The abundance and distribution of demersal fishes on UCB across the continental shelf off Texas was sampled using a randomly stratified approach using integrated hydroacoustic/video. Standard echosounder surveys were used to estimate demersal fish occurrence and density within the survey areas. Concurrent imaging sonar and standard camera videos were collected with the acoustic data. Integrated echosounder, imaging sonar, and standard camera video surveys were conducted in 2018 and 2019 across the continental shelf off Texas. The survey area was bounded between 26.0-29.3°N and 97.3-93.5°E, and surveys targeted shelf areas with no known bathymetric features or relief. The majority of surveys were performed in the summer and fall, during daylight hours or immediately before dawn/after dusk.

Echosounder transects were conducted at 143 sites off Texas (Figure 14) using a SIMRAD EK80 WBT transceiver operating a single split-beam 70 kHz ES70-18CD transducer. During echosounder transects, the transducer was either mounted directly to the side of the vessel or deployed on a custom tow body (Figure 15). The system was calibrated prior to deployment using a tungsten carbide sphere with a nominal target strength (TS) of -40.56 dB. The transceiver was operated at the narrow-band 70 kHz setting, at the maximum pulse rate permitted by the depth with a pulse duration of 0.256 ms. Echo sounder transects were conducted at a vessel speed of 2-4 kts for 20-30 minutes. Headings were selected randomly when possible,

but conditions often necessitated maintaining a heading into the direction of the prevailing swell. Hydroacoustic data were georeferenced at collection time using NMEA GPS string feeds from either a handheld GPS (small vessel) or the shipboard NMEA feed (large vessel). Linear distance of echosounder transects combined was 308.8 km.

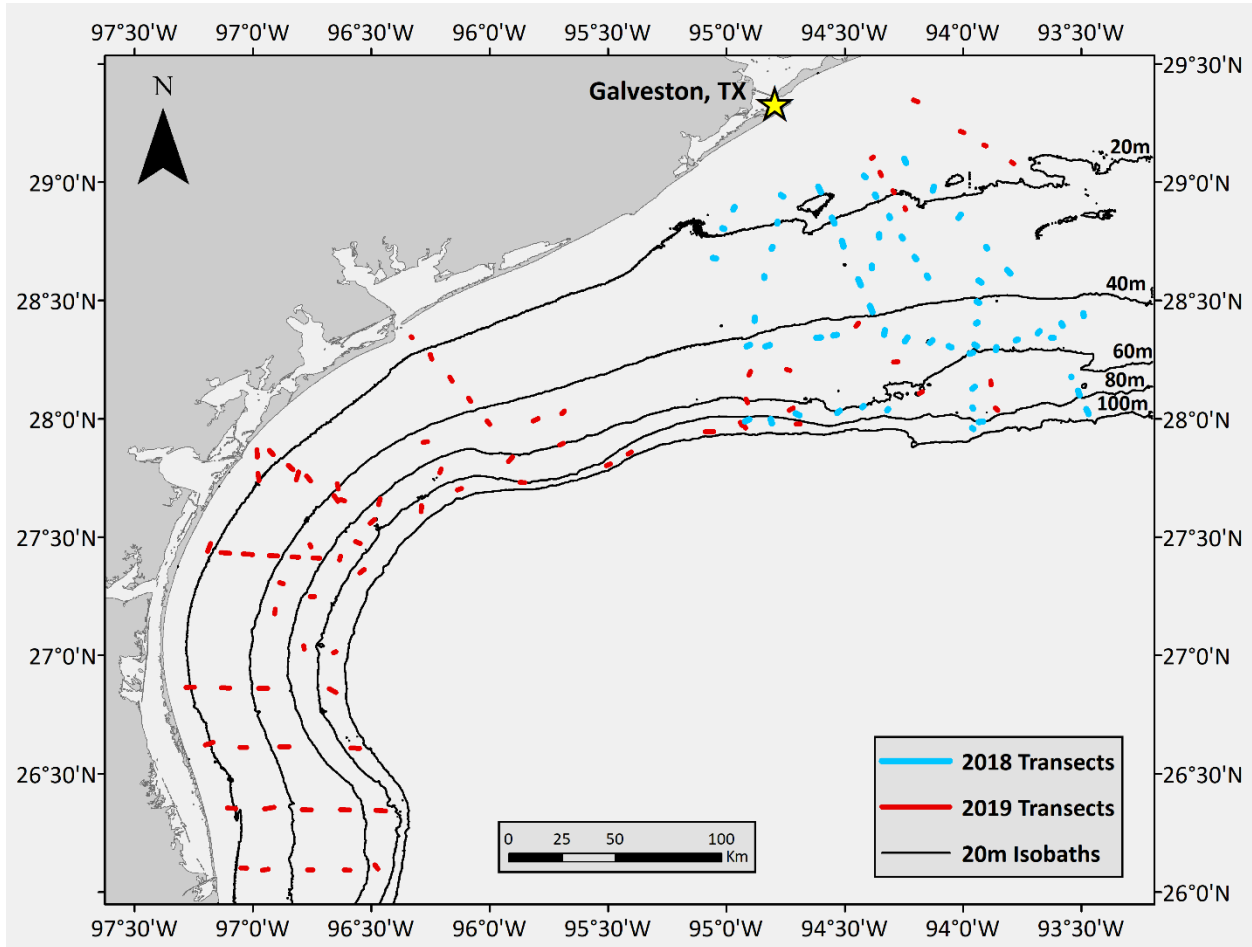


Figure 14. Location, year, and survey extent of the 147 echo sounder transects conducted in this study over uncharacterized bottom habitat along the Texas and LA continental shelf.

ARIS (Adaptive Resolution Imaging Sonar) imaging sonar and standard camera video surveys were conducted simultaneously during echo sounder surveys at transect stations where conditions and vessel capabilities permitted the deployment of video collection gear ($n = 44$). Both gears were mounted on a second tow body (Figure 15). The imaging sonar unit (ARIS Explorer 1800 model) was mounted in the center of this tow body, engaged to a motorized rotator to orient the sonar perpendicular to the plane of the seafloor. One GoPro camera (Hero 6 Black model) was mounted directly on top of the imaging sonar unit, facing downward in the same direction as the sonar swath. A second GoPro camera was mounted at the front of the tow body, facing forward (i.e., in the direction of motion). The imaging sonar was not deployed at

some sites in summer 2018 – during this year, two forward-facing GoPro cameras were mounted on a third tow body. The video collection tow body was towed at a range of 4-15m from the bottom. The imaging sonar was permitted to automatically adjust its operating frequency based on the range to the bottom, switching between the 1.8 MHz identification frequency at close range and the 1.1 MHz detection frequency at longer range.

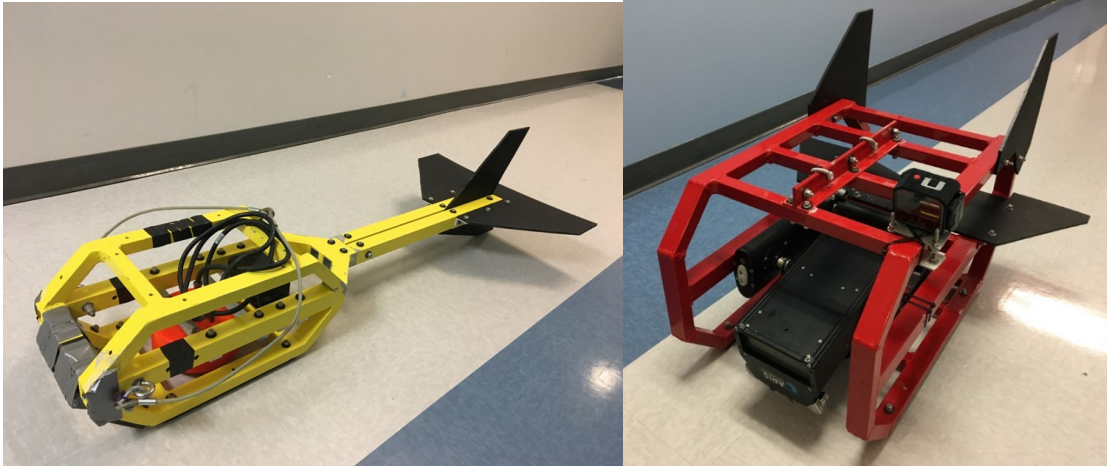


Figure 15. Tow body for the echosounder transducer (orange cylinder at frame center), and video collection tow body for the imaging sonar unit and the standard cameras.

Hydroacoustic Data Processing

Hydroacoustic data from the echosounder were processed using standard echo counting and echo integration methods (Rudstam et al. 2012). All hydroacoustic data processing was performed in Echoview 10 (Echoview, Pty. Ltd.). Echograms were first visually inspected for quality to remove echograms showing excessive transducer movement or ping dropout due to surface noise (Parker-Stetter et al. 2009). Remaining echograms were first preprocessed to remove noise and identify a bottom line. The bottom (seabed) appears on echograms as a conspicuously strong echo; the bottom line delineates the uppermost boundary of this echo and serves as a lower bound for subsequent analyses. The bottom line was identified from the S_v (volume backscatter) data using Echoview's best bottom candidate algorithm. The bottom line was manually edited to span gaps and then raised by 0.25 m to ensure that subsequent analyses were not contaminated by strong seabed echoes. Electrical impulse noise was filtered from the echogram and surface noise was eliminated by excluding data above 5 m range. Echograms were then visually inspected and large regions of non-fish backscatter were removed manually.

The total number of targets within an echogram was determined by combining the abundance attributable to individual single targets (echo counting) with that attributable to school targets (echo integration). First, schools were detected from the cleaned S_v echograms using the SHAPES algorithm (Coetzee 2000) with a minimum data threshold of -60 dB (Parker-Stetter et al. 2009). Schools were manually edited to remove areas where the algorithm had incorporated single fish tracks into a school body.

Non-school fish density was determined through a cone model echo counting method (Kieser and Mulligan 1984). School regions were excluded from the echogram, and single targets outside of schools were detected using the split-beam single target detection algorithm of Soule (1997). As most of the echograms were empty save for a small number of fish tracks, single target detection parameters were widened from their defaults to ensure that marginal targets were included in the analysis. The TS (target strength) threshold was set to -75 dB, the normalized pulse length bounds were 0.5 - 2 lengths, the maximum beam compensation was 9 dB, and the maximum major and minor axis deviations were 0.6 standard deviations. A higher maximum beam compensation can introduce upward bias into the final counts, but the work of Parker-Stetter et al. (2009) suggests that increasing the maximum beam compensation to 9 dB likely introduces only a small bias. After target detection, single target echograms were divided by a 90m horizontal x 20m vertical cell grid. Single target echo density within a cell was calculated by dividing the cell single target count by the cell beam volume sum.

School fish density was determined by scaling the in-school S_v by the TS (target strength) of single targets in close proximity to the school. This was performed under the simplifying assumption that the fish closely associated with a school would have a comparable TS distribution to the fish within the school body and thus could serve as an approximate in situ estimate of mean school TS (MacLennan and Menz 1996). First, a buffer zone of approximately 5 m was drawn around each school to encompass TS measurements from nearby single targets. The single target detection algorithm was applied to the TS within this school buffer. This pass of the single target detection algorithm employed narrower parameters than the algorithm for the single targets described above. Maximum beam compensation was set to 6 dB, normalized pulse length bounds were 0.75 - 1.5 lengths, and the maximum major and minor axis deviation remained at 0.6 standard deviations; hereafter these single targets will be referred to as narrow-scope single targets (NSST).

NSST identified within the school buffer region were filtered using the Sawada index (N_v , (16)) and the ratio of multiple echoes ($M_{\%}$, (17)) to ensure that single targets used to scale the in-school S_v values were not contaminated by echoes from multiple targets (Sawada et al. 1993). NSST were first binned into small cells (5m horizontal x 5m vertical) and N_v and $M_{\%}$ were calculated for each cell. Cells where $N_v < 0.1$ and $M_{\%} < 100$ were considered to contain NSST which were sufficiently isolated to be used to scale the in-school S_v values of nearby schools.

$$(13) \quad N_v = \frac{c \cdot \tau \cdot \psi \cdot r^2 \cdot n}{2}$$

where c is the speed of sound in water (m/s), τ is the pulse duration (s), ψ is the equivalent beam angle (steradians), r is the range (m), and n is the density of targets as determined by S_v scaling.

$$(14) \quad M_{\%} = \frac{n - n_s}{n}$$

where n is the density of targets as determined by S_v scaling and n_s is the density of targets as determined by echo counting.

To scale in-school S_v values, a school mean TS value was first calculated by averaging the backscattering cross-sections ($\sigma_{bs} = 10^{TS/10}$) of all NSST within 5m of the school border. The school S_v echogram was then divided into a 90m horizontal x 20m vertical grid, and the density of single fish targets within the school was calculated according to (18).

$$(15) \quad \rho = \frac{S_v}{\sigma_{bs,\mu}}$$

where ρ is the volume density of fish targets ($\text{ind.}\cdot\text{m}^{-3}$), S_v is the linear volume backscattering coefficient and $\sigma_{bs,\mu}$ is the average backscattering cross-section of the NSST associated with that school. The total density of fish within a cell was calculated by a cell-wise summation of the density of school-associated targets with the density of single targets. A cell fish abundance was also calculated by multiplying the cell fish density by the cell volume. Cells at depths >20m from the bottom were omitted from further analyses, as the focus of this study was demersal fishes.

Relief anomalies (patches of habitat where relief height diverged from the surrounding flat bottom) were identified from echograms by visual inspection using two criteria: 1) a visible height divergence from the surrounding bottom in the echogram; and 2) a discontinuity in bottom echo strength. Once located, the length and height of each relief anomaly feature was approximated by drawing a bounding box around the feature.

Imaging Sonar Video Processing

Imaging sonar recordings from the ARIS were examined for the presence of fish and relief anomalies within a transect. Fish targets were enumerated and their size along their largest presented aspect was estimated using the measurement tool in Echoview. Fish targets were classified into one of four size targets (Figure 16): micro (too small to measure individual targets); small (5-20cm total length [TL]); medium (20-50cm TL); and large (>50cm TL). Distinguishing individual fish targets in compact schools was often difficult and, in these cases, the number of fish in the school was estimated based on the dimensions of the school and the size of targets within the school was estimated from targets at the school margins. Relief anomalies were identified by disruptions in the shape of the bottom echo. As the dimensions of these disruptions were difficult to measure accurately, relief anomaly size was classified by the duration a feature remained in the sonar swath and by a qualitative estimate of its size (Small, Medium, Large) compared with other relief anomalies.

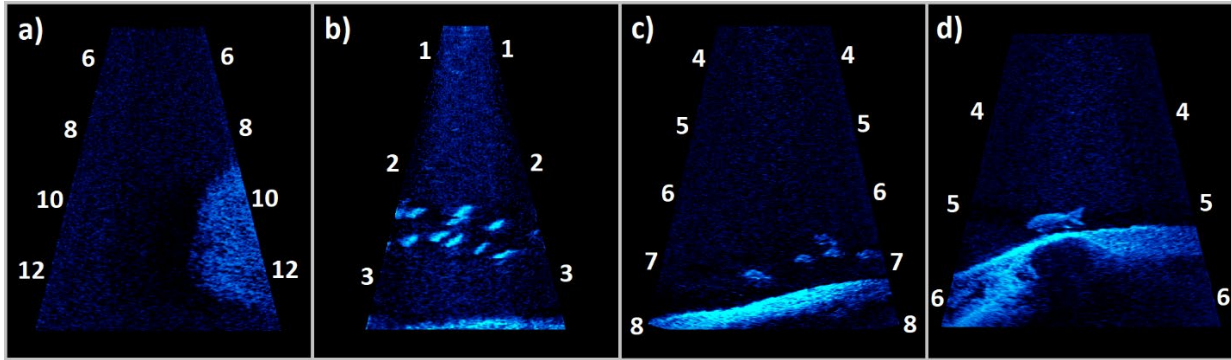


Figure 16. Examples of fish size categories as determined from the imaging sonar video: a) a school of micro fish; b) small fish; c) medium fish; d) a large fish. Numbering shows range from the sonar unit in m.

Standard Camera Data Processing

Fish appearing in the standard camera videos were counted and identified to the lowest possible taxonomic level. Species or genus designations were only assigned if a positive identification could be made. Fish were dispersed enough within videos for each individual to be counted. Fish which were seen to follow or track the tow body were only counted on their first appearance in the frame.

Echosounder transects were conducted at 143 stations on UCB (Figure 17) but the quality of hydroacoustic data collected during 7 transects was too poor for analysis, and these were removed from the dataset. The remaining 136 transects were further divided into 3,323 90m x 20m cells for estimating occurrence/density at a smaller spatial scale. Demersal fish were detected in 929 cells (28.0%), and in cells where fish were present, acoustically derived densities ranged from < 0.1 to $139.3 \text{ fish} \cdot 1000\text{m}^{-3}$; mean of $1.0 \text{ fish} \cdot 1000\text{m}^{-3}$ (Figure 18). Mean target strength (TS, correlates positively with fish target size) of fish targets increased linearly with depth ($R^2 = 0.27$, $p < 0.0001$). Bottom relief anomalies were detected in 412 echogram cells (12.4%). A mean of 1.2 relief anomalies were detected in cells where relief was present and ranged from 1 to 6. Mean Relief Anomaly Linear Proportion (RALP) was 12.0% for cells with bottom relief detected and ranged from 0.3% to 87.2%.

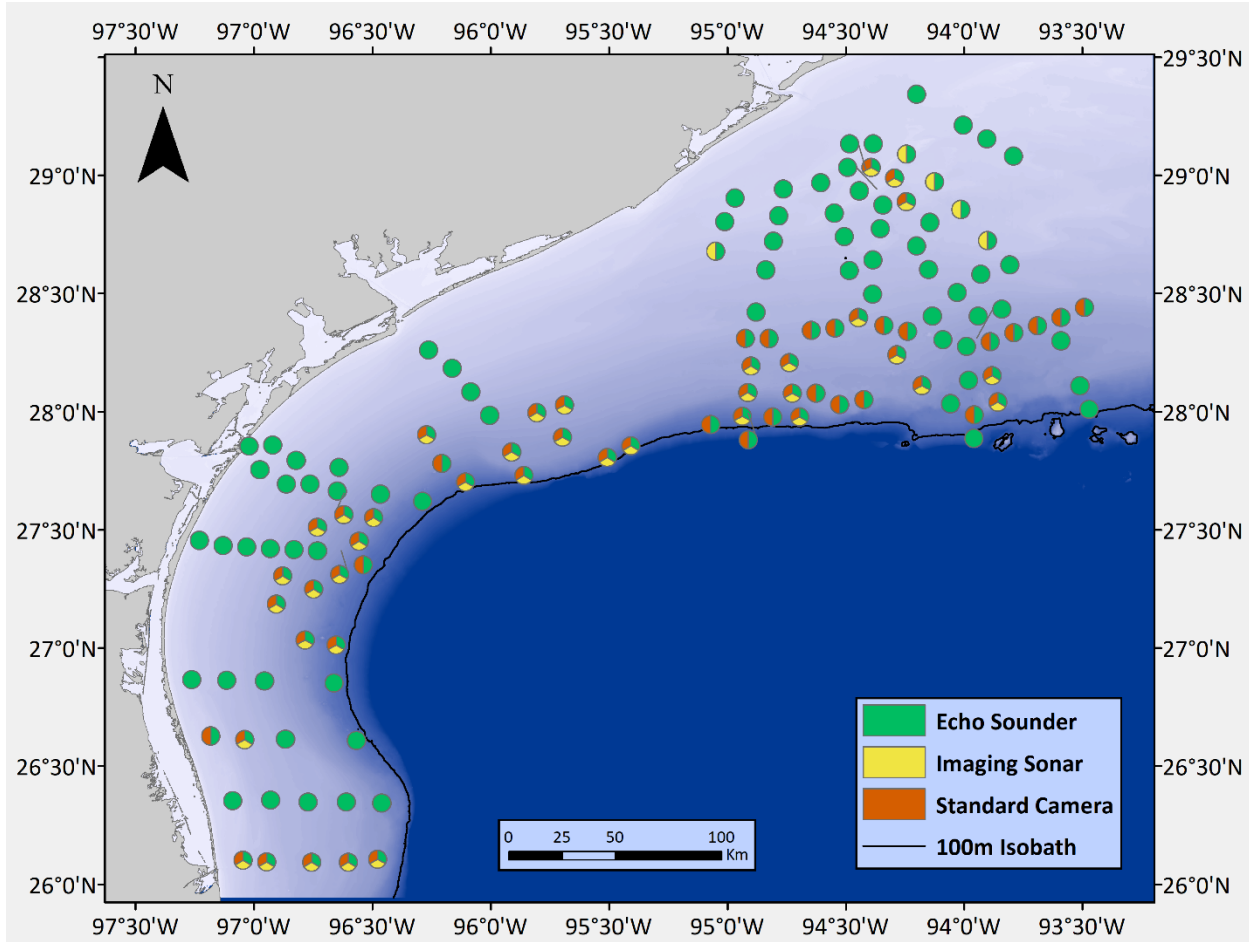


Figure 17. Locations of the 140 usable transects on UCB on the continental shelf off Texas and LA in the nGulf used for data analysis. Symbol colors denote which gear types were deployed at each station. The solid black line marks the 100m isobath.

Imaging Sonar and Standard Camera Video Surveys

Concurrent imaging sonar data were collected at 44 (30.8%) of the stations on UCB, and fish targets were detected in 41 (93.2%) imaging sonar transects. Small (5-20 cm TL), medium (20-50 cm) and large (> 50 cm) fish targets were detected in 86.3%, 68.1% and 9.0% of transects, respectively. Mean per-transect counts of small, medium and large fish targets were 208.3, 21.5 and 0.2, respectively. Bottom relief anomalies were detected in 32 (78.0%) of imaging sonar transects in which fish targets were also detected. Small (mean duration 2.2 ± 0.05 seconds), medium (10.8 ± 0.24 s) and large (16.0 ± 0.45 s) structures were detected in 59.0%, 38.6% and 29.5% of the imaging sonar transects, respectively.

Standard camera video data were collected at 61 (42.7%) of the stations on UCB. A nepheloid layer near the bottom was present during all standard camera transects, and nearly all successful detections of fishes occurred when the cameras mounted on the tow body were several

meters above the bottom and above the nepheloid layer. Fish were detected on 28 (45.9%) of the camera transects, with a total of 524 individual fishes observed. Of these, 82.6% of the individual fish were identified to family and 55.0% to species level. Six families of bony fishes (Balistidae, Carangidae, Echeneidae, Lutjanidae, Rachycentridae, and Scombridae) and one family of sharks (Carcharhinidae) were positively identified in camera transects. The majority of fishes identified to species were Red Snapper, with this species accounting for 46.0% of all fishes identified to at least the family level. Red Snapper were also the most frequently detected species and observed in 18.0% of the camera transects. Other frequently observed taxa included scombrids (11.5%) and carangids (11.5%). Bottom relief anomalies of any kind were never detected in camera transects.

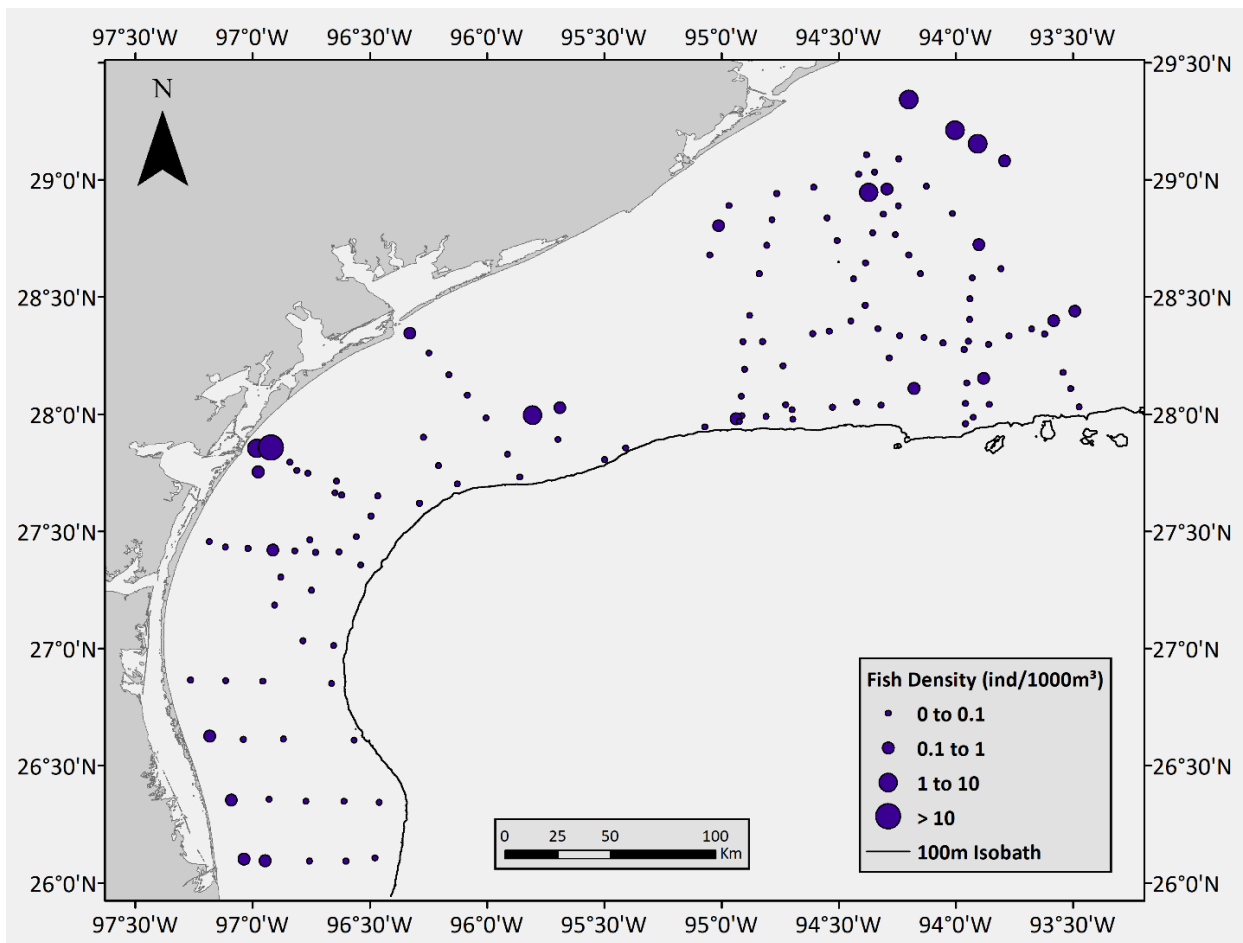


Figure 18. Density of demersal fishes (ind./1000m³) from echosounder transects of UCB on the continental shelf off Texas in the nGulf.

Key assumptions and implications summary:

Estimating Red Snapper abundance off Texas using a paired hydroacoustics-visual species composition approach required two primary assumptions including: 1) all age 2+ Red Snapper were detected by the hydroacoustic survey and 2) species composition estimated using visual methods (ROV, towed camera) accurately reflected actual community composition (and Red Snapper proportion) over all times and locations surveyed. Estimates of total fish abundance using hydroacoustic surveys are negatively biased because of ‘dead zone’ exclusion near the bottom and around and inside of artificial structures. This bias would be more pronounced for demersal fish like Red Snapper that often occur close to the bottom or inside artificial structures but are not counted (see Section 3 for more detail). Species composition estimates were undoubtedly influenced by visibility with more negative bias on Red Snapper proportion in the deeper depth bins where the nepheloid layer was more prevalent. Detection likely changes as a function of distance and visibility, and we were unable to calibrate our gear to account for any of these differences. Thus, detectability was not tested for any of the mobile gears, and the reason hydroacoustics was the principal method used to estimate Red Snapper abundance in the western Gulf regions. Because we were not able to minimize this bias, we estimated depth-habitat strata-specific species compositions using MaxN counts from all sites surveyed within that strata. In doing so, we reduced the influence of sites with poor visibility. Species composition estimates could also be influenced by Red Snapper behavior, but studies of Red Snapper behavioral reaction to mobile gears (see FL above) have estimated a neutral response (neither attracted to nor avoided gear).

6. Louisiana Region

Sampling efforts over the Louisiana shelf used C-BASS and TARAS towed gear methodologies (described in Section 7 below) over natural banks and UCB, including oil and gas infrastructure (e.g., pipelines). In addition, split-beam echo sounder surveys were paired with accompanying video from imaging sonar and standard cameras over uncharacterized bottom habitat at the Louisiana-Texas border (described in Section 5c above). Unexpected complications prevented the initial scope of sampling in Louisiana to be completed. Thus, to augment sampling efforts off Louisiana (particularly for artificial reefs and shallow-depth strata), a subset of surveys conducted over the nearby and similar Texas shelf were integrated and imputed into the Red Snapper abundance estimation for this region. In total, 22 natural hardbottom and 42 artificial reef samples were used for the estimate, with the number of UCB samples dependent on imputation method (n=87 for the primary analysis versus 65 for the secondary analysis; Table 5 and Table 7). By supplementing with nearby TX data in similar ecoregions, the sample size was increased for Louisiana estimates, with the goal to provide small area estimates. Specifically, all surveys conducted over the north and central Texas shelf (between the Louisiana-Texas border and Upper Laguna Madre, ~27.2°N) were deemed most appropriate for integration into the Louisiana Red Snapper abundance estimate for several reasons. First, Red Snapper stocks are assessed separately for the eastern and western Gulf due to demographic differences, with the central stem of the Mississippi River Delta (~89.1°W) dividing the two regions (Cowan 2011;

SEDAR 2018). Second, the western Gulf, relative to the eastern Gulf, contains less natural hard-bottom habitat and consists predominately of silt and mud. During the initial experimental design phase of this project, we used multivariate models based on environmental (sea surface temperature, salinity, dissolved oxygen) and geological conditions (substrate type, % gravel, sand, silt, clay, mud), as well as the presence of artificial reefs (density), to identify major geographic boundaries and regions within the northern Gulf. These models identified four major ecological regions, with extensive overlap across the Louisiana and Texas shelf. Third, a large-scale survey conducted by Gledhill (2001) investigated differences in fish assemblages along the Gulf shelf-edge natural banks from Florida to Texas and identified the Louisiana-Texas shelf banks as spatially and geomorphologically similar based upon fish species composition. The Louisiana-Texas shelf-edge banks result from diapiric salt intrusions uplifting bedrock that is subsequently capped by carbonate reef structure (Rezak et al. 1985). These shelf-edge banks are geologically distinct from the drowned corallgal reefs off south Texas (Rezak et al. 1985; Nash et al. 2013). Hence, the southernmost natural bank (Southern Bank: 27.44°N, 96.53°W) integrated into the Louisiana Red Snapper abundance estimate is located ~55 km offshore of Corpus Christi (central Texas) on the edge of the outer continental shelf and is similar to natural hard-bottom habitat in the salt diapir bank area in the northwestern Gulf (Nash et al. 2013).

Oil and gas structures are predominantly used for artificial reef development in the nearshore (coastline to 30 m water depth contour) and offshore (30 m water depth contour to the U.S. exclusive economic zone boundary) programs of the Louisiana Artificial Reef Program (Kaiser et al. 2020). Outside of Louisiana, the Texas Artificial Reef Program is the second-largest Rigs-to-Reefs program in the Gulf (Kaiser et al. 2020); thus, providing the most appropriate sampling environment for estimating Red Snapper abundances on large artificial reefs over the Louisiana shelf. Because oil and gas structures comprise a large number of artificial reefs off Louisiana, only surveys conducted at oil and gas structures across the north and central Texas shelf were used for estimating Red Snapper abundances at artificial reefs for this region.

Compared to other Gulf regions, the Louisiana shelf has the highest density of standing and reefed oil and gas platforms (Figure 3). Because natural hard-bottom habitat is limited along the nearshore Louisiana shelf (Figure 2), these artificial reefs provide the majority of structured habitat and likely harbor a substantial abundance of Red Snapper (Karnauskas et al. 2017). Moreover, Red Snapper species composition differs among Texas and Louisiana oil and gas structures. To account for potential differences, we determined the percent composition of Red Snapper observed during previous video surveys conducted at standing and reefed oil and gas platforms off Louisiana. The percent composition of Red Snapper (Cowan unpublished data; Reynolds et al. 2018; ~ 15.5%) was used to apportion Red Snapper abundance from the total fish abundance estimated during hydroacoustic surveys conducted at oil and gas structures over the north and central Texas shelf. Combining the Texas hydroacoustic and Louisiana video survey data allowed us to estimate Red Snapper abundances at oil and gas structures for the Louisiana region.

The areal coverage or number of all habitat types within each stratum in the Louisiana region was determined from various databases. Locations and numbers of standing oil and gas platforms, including presumed oil and gas or similar remnant structures (e.g., exposed well-heads, known oil and gas discarded materials, pipeline crossing, etc.) were obtained from the Bureau of Ocean Energy Management (BOEM 2020b), Bureau of Safety and Environmental Enforcement (BSEE 2020), while the locations of known artificial reefs were obtained from data sets compiled by the National Oceanic and Atmospheric Administration (NOAA 2020a), Louisiana Artificial Reef Program (LDWF 2020), GMFMC (2013), K. Rose, unpublished data, and Stunz, unpublished data. The areal extent of natural hard-bottom habitat was calculated using Geographic Information Systems (GIS) data compiled by NOAA's Coral Essential Fish Habitat (NOAA 2020b) and BOEM's confirmed relic patch reefs (BOEM 2020d). Uncharacterized bottom habitat area was calculated by excluding a 100 m buffer ('area of influence'; sensu Karnauskas et al. 2017 and Reynolds et al. 2018) around all artificial structures and natural hard-bottom habitats within the Louisiana region.

7. Uncharacterized Bottom and Pipelines Habitats

A considerable but unquantified proportion of the U.S. Gulf of Mexico Red Snapper stock (east and west components) exists over uncharacterized natural hard bottom (reefs and rock outcroppings), soft bottom (sand and mud) and on oil and gas pipelines. Led by the University of South Florida, College of Marine Science, we used a towed video system to quantify the density of Red Snapper living at or near the bottom along pipeline infrastructure and uncharacterized bottom (UCB) across the majority of the northern Gulf shelf from Alabama to Texas for UCB and pipeline habitat throughout the entirety of the regions including deeper areas not accessible due to other regional gear limitations. Due to the sampling frame of this habitat (see p. 76), pipelines were treated as a unique habitat and therefore population estimates were made not by region, but for the entire Gulf.

The towed camera system, C-BASS, was originally developed at the University of South Florida, under grants from the National Marine Fisheries Service and the National Fish and Wildlife Foundation, to map benthic habitat and estimate associated fish and sea turtle densities (Lembke et al. 2017; Ilich 2018; Grasty et al. 2019; Broadbent et al. 2020) and modified for use here. The C-BASS system (Figure 19) consists of an aluminum tow body frame, an array of digital and analog cameras, a controller pod with on-board data storage and communications instruments, a variety of environmental sensors (e.g., temperature, pressure, salinity, altitude, etc.) and an LED lighting system allowing the system to be used 24 hours per day (Grasty et al. 2014; Lembke et al. 2017).

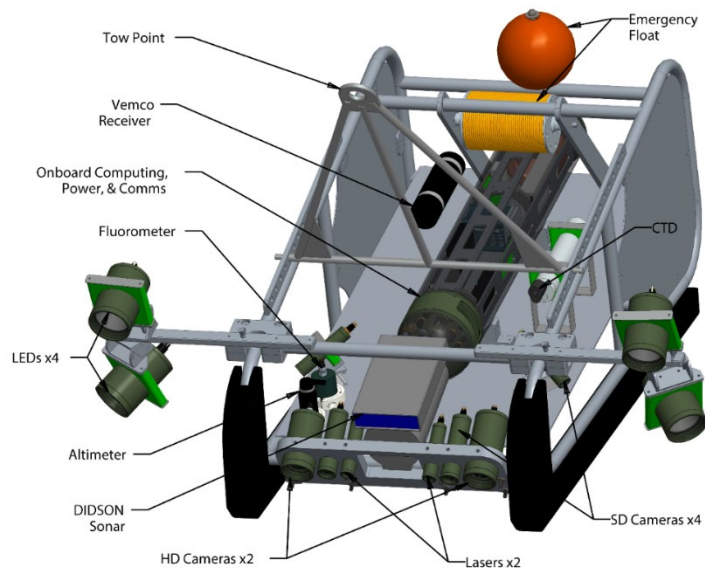


Figure 19. Schematic of the Camera-Based Assessment Survey System (C-BASS) tow body.

The goal for this aspect of the study was to generate density estimates along randomly selected transects on pipelines, over UCB, and on hard bottom banks off of FL, AL, MS, LA, and TX in 30-200 m water depths to estimate densities of Red Snapper. An additional objective was to use the Reson SeaBat 7125 to conduct overnight seafloor mapping surveys of the selected transects to confirm pipeline presence (for applicable transects) and identify any potential obstructions to C-BASS.

To match hydroacoustic observations with camera observations of the identical habitats, the position of the camera sled relative to the ship and its hydroacoustic transducers were computed by calculating the “layback” of the camera system aft of the ship while towing. The layback from the ship was calculated as:

$$Layback = \sqrt{(k * L)^2 - (I + z)^2}$$

Where:

L= Cable Out (m)

k= Catenary Factor

I= C-BASS Depth (m)

z= A-Frame Offset (m)

To estimate fish density from the towed camera data, the total area viewed during each transect was calculated which required knowing the average width and length of the transect for each minute of the survey (Grasty 2014). The value of the angle of the camera to the bottom was first adjusted to account for the pitch of the system (Eqn. 19). The pitch also affects the actual

altitude (A_A) of the system over the bottom so this was corrected for as well (Eqn. 20). The camera center line distance (C_C), or the extent to which the camera view reaches in front of the system, was then computed (Eqn. 21). After adjusting for the differences in refraction between air and seawater for the camera's field of view (horizontal extent of the camera's coverage), the view width of the transect could be estimated (Eqns. 22, 23).

$$(19) \quad \alpha_A = \alpha_{FC} - \alpha_P$$

α_A = Adjusted camera angle to ground (degrees)
 α_{FC} = Measured camera angle to ground (degrees)
 α_P = Pitch of system (degrees)

$$(20) \quad A_A = A_M * \cos(\alpha_P)$$

A_A = Adjusted altitude (meters)
 A_M = Altimeter reading (meters)

$$(21) \quad C = A_A / \sin(\alpha_A)$$

C = Camera center line distance (meters)
 A_A = Altitude of system above bottom (meters)
 α_A = Adjusted camera angle (radians)

$$(22) \quad FOV_A = 2 \sin^{-1} \left(\sin \left(\frac{FOV_C}{2} \right) * \left(\frac{R_A}{R_S} \right) \right)$$

FOV_A = Adjusted Field of View (radians)
 FOV_C = Manufacturer specified camera FOV*
 R_A = Index of refraction for air
 R_S = Index of refraction for seawater
 *Specific to each camera

$$(23) \quad W = 2C * \tan \left(\frac{FOV_A}{2} \right)$$

W = Width of transect

The “area swept” during each camera tow and 15-second bin was then computed as the average transect width surveyed (as above) multiplied by the length of the transect.

Red Snapper density per 15-second bin was calculated as:

$$\gamma_{bs} = c_s / A_b$$

where:

γ_{bs} = Density of species s per 15-sec bin

s = Species

- c_s = Species count for 15-sec bin, b
- A_b = Area covered during 15-sec bin, m (km²)
- b = 15-sec bin

Population size per stratum (e.g., pipelines, unconsolidated sediments) was calculated as:

$$\bar{\gamma}_{hs} = \sum_{1..n}^h \gamma_{bs} * \frac{1}{n_h}$$

where:

- $\bar{\gamma}_{hs}$ = Average density of species (s) per habitat, h (# km⁻²)
- n_h = Number of 15-sec bins sampled in habitat, h
- h = habitat classification

Oil and gas pipeline infrastructure footprints were downloaded from the Bureau of Ocean and Energy Management (BOEM) when fieldwork planning began in late-2017. To limit the PL universe so that only footprints which were likely to contain structure were displayed, the shapefile was loaded into ArcGIS and the Status Code field was filtered as:

Included in PL universe

- A/C- ABANDONED AND COMBINED.
- ABN- ABANDONED.
- ACT- ACTIVE.
- O/C- OUT AND COMBINED.
- OUT- OUT OF SERVICE.
- PABN- PROPOSE ABANDONMENT.
- PREM- PROPOSE REMOVAL.
- R/A- RELINQUISHED AND ABANDONED.

Rejected from PL universe

- CNCL- CANCELLED.
- COMB- COMBINED.
- PROP- PROPOSED.
- R/C- RELINQUISHED AND COMBINED.
- R/R- RELINQUISHED AND REMOVED.
- RELQ- RELINQUISHED.
- REM- REMOVED.

The ‘Generate Random Points’ tool was then used to create several points along the PL Universe vectors which would serve as the midpoints of a transect. The transects were created by extending approximately 7.5 km along the pipeline vectors from the midpoints. If a point was placed close to the end of a pipeline or oil platform, it was simply extended in one direction to achieve a total length of 15 km (which equates to an approximately 2-hour tow with C-BASS). Additional UCB transects locations were chosen by generally offsetting a pipeline transect by 1 nm and making a parallel vector. Some UCB did not follow this protocol, and they were chosen

based on logistical constraints but away from any interactions from the structured pipeline habitats.

Data were collected over three separate cruises between 2018 and 2020: April 2018, July 2018, and January 2020 (see Supplementary figures, tables, etc.). All C-BASS operations occurred during daylight hours (between 11:00 to 01:00+1day UTC) and mapping was completed overnight in preparation for the next day's survey area. It was common to discover numerous unmapped and uncharted obstructions (Figure 20). For each pipeline, or UCB transect that was to be surveyed, we fully mapped the length of the transect during night before to ensure safety and ease of operations. Except for the West Florida Shelf, visibility was relatively occluded for all of the areas that were surveyed with the C-BASS in the northern Gulf and adequate in the western Gulf of Mexico (Figure 21). The combination of using the maps collected overnight (2x2 meter grid size) and the EK-60 for real-time information during C-BASS tows was sufficient to collect quality video and prevent collision with the bottom or other obstructions. No unexpected obstructions were encountered except for discarded fishing gear which was too small to be detected by either echosounder. Federal regulations mandate that in most of the United States' waters, underwater pipelines must be buried if in water depths of less than 60 m (CFR 49 192.327). However, in the Gulf of Mexico the regulations are more complicated, in which offshore pipe in water at least 4.6 m deep (15 ft, 12 feet in other waters) but not more than 61m (200 ft) deep is required to be installed so that the top of the pipe is below the natural bottom unless it is supported by stanchions, held in place by anchors or heavy concrete coating, or protected by an equivalent means (Sections 192.319 and 195.246). This could allow for some structure to be unburied <60m depth. Because of relatively poor visibility during the first leg of the 2018 cruise, we were unable to visually determine if there were any patches of structure along the buried pipelines in the 30-60 m depth range during C-BASS tows. Of note: During the midpoint of the cruise when the vessel was refueling at Port Fourchon, LA several pipelines were bisected by the ship track, and it was observed at a larger scale (via the multibeam echosounder) that there appeared to be no exposed pipelines shallower than 60 meters. Visibility was expected to be less than ideal around the Mississippi River delta due to sediment discharge and potential phytoplankton blooms. Generally, the seafloor could not be viewed on the C-BASS's cameras at typical towing altitude (1.24-2.4 m) in water depths less than 60 m. This improved notably during the second leg of the cruise which surveyed west of the Mississippi River's outflow.

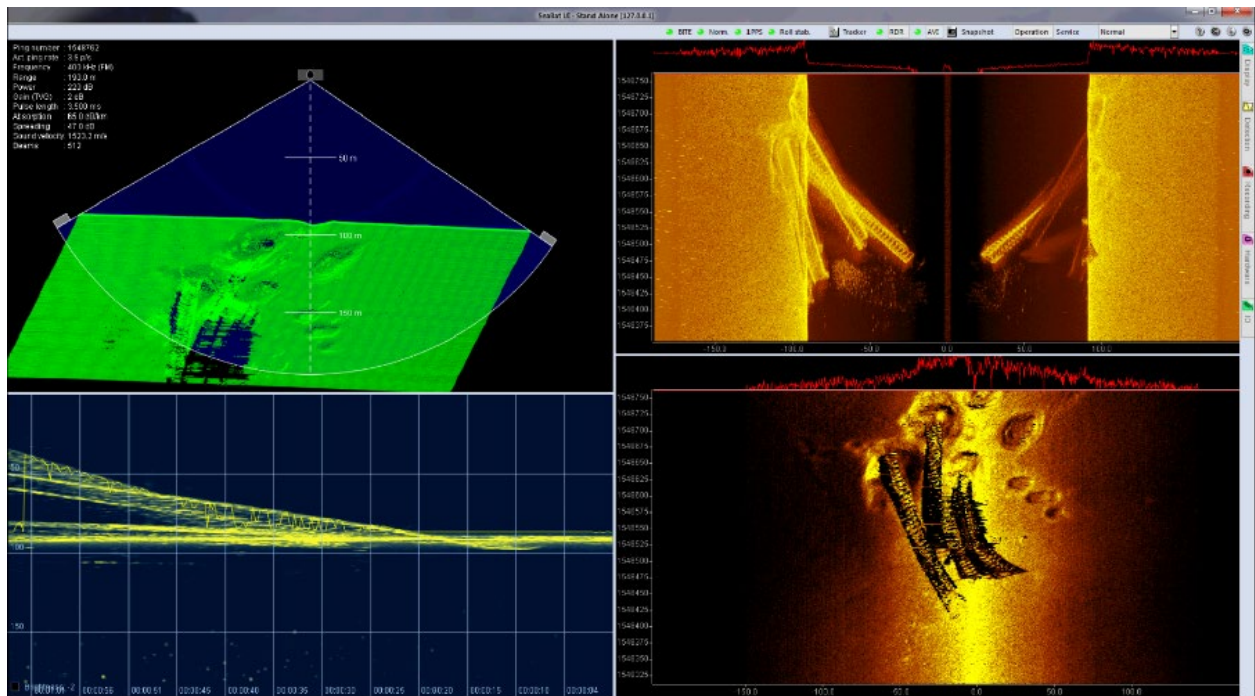


Figure 20. Example of a significant obstruction to towing offshore of Alabama; the above is a screenshot from a RESON Seabat 7125 multibeam echosounder which depicts a toppled oil platform.

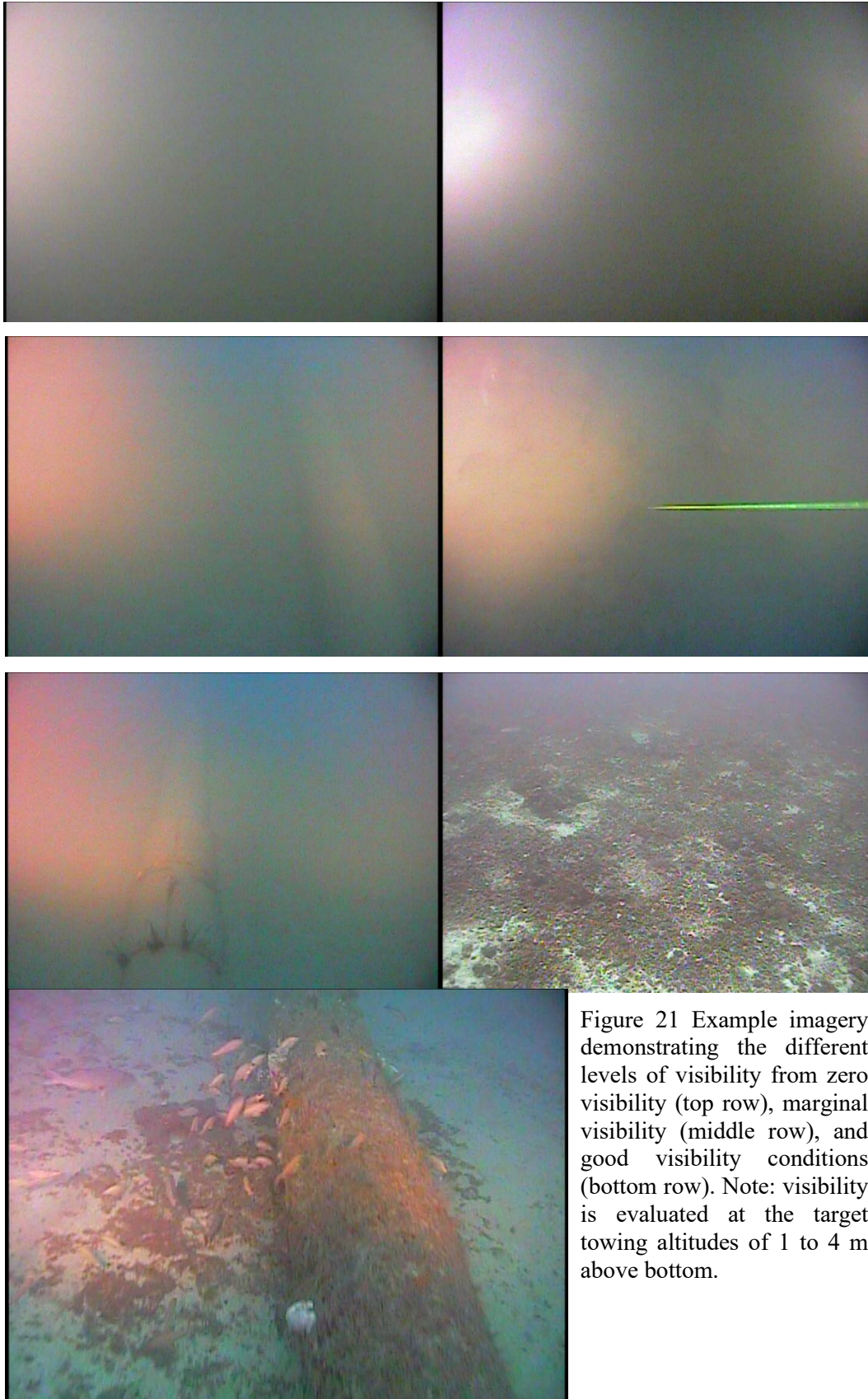


Figure 21 Example imagery demonstrating the different levels of visibility from zero visibility (top row), marginal visibility (middle row), and good visibility conditions (bottom row). Note: visibility is evaluated at the target towing altitudes of 1 to 4 m above bottom.

C-BASS Data Processing

The C-BASS data were collected by analyzing fish presence using the highest quality footage from one of the two forward-facing high definition (HD) cameras. In extreme situations when visibility and/or lighting was very poor, a standard definition (SD), forward-facing camera feed was instead selected for analysis. Transect analysis began by loading one-minute segments of video into CVision's annotation software, Tator (<http://cvisionai.com/project/tator-the-video-and-image-annotator/>). All observed fish were enumerated and identified to the lowest taxonomic level possible. Red Snapper identifications made from C-BASS footage were kept conservative – unless an individual was observed with some combination of the obvious reddish iridescent color, the pointed anal fin, and/or clear profile view of body shape, the fish was classified as a large NoID (no identification). After each one-minute segment was analyzed, a .json file was saved which could be reloaded into Tator and all annotations reviewed. A .csv file was also exported for each segment which compiled all of the counts and identifications. A custom R-script was then used to compile all of the exported, 1-min .csv data and divide the counts into 15 s bins by species. These data were then loaded into an Excel template. Ancillary data were also loaded into the template to estimate the area viewed per 15 s bin which required average towing altitude, system pitch, and ship speed-over-ground. The area viewed was then used to convert the counts into density estimates (number of fish m⁻²). Density estimates were calculated for each of the three transect types (pipeline, unconsolidated sediment, hard bottom) but number of Red Snapper per linear meter of pipeline viewed was also calculated. After calculating density estimates for each 15 s bin (sampling unit) within a transect, each sampling unit was geolocated. This was done by estimating the layback (distance of the system in time and/or space behind the vessel) of the C-BASS via the amount of towing line out, C-BASS depth, and estimated catenary factor of the line.

Key assumptions and implications summary:

Estimating Red Snapper abundance over the UCB habitat type using towed camera sleds relied on several assumptions including: 1) all age 2+ Red Snapper were detected and identified, 2) Red Snapper counts were not significantly biased by gear related behaviors (avoidance or attraction), and 3) available pipeline habitat was accurately estimated. Reduced visibility likely led to reduced detection and hence counts. When visibility at a survey site hindered accurate species composition, the mean proportion of Red Snapper from regional surveys with adequate visibility were often used to derive abundance estimates of Red Snapper (backed out from total fish counts), which may have influenced abundance estimates and associated CVs. Similarly, Red Snapper abundance estimates relied on insignificant effects of gear avoidance or attraction. Both attractive and detractive behaviors are well-documented for various fish survey techniques. In 2019, we began estimating the C-BASS's capture efficiency (analogous to a catchability coefficient) for various target reef fish species, including Red Snapper, by comparing reef fish assemblages observed between the towed camera and Florida Fish and Wildlife's stationary cameras. This work is ongoing, and no final quantitative measures of Red Snapper capture efficiency for the C-BASS, or other towed gear used in this study, are currently available. Our

behavioral observations of Red Snapper (and other snapper and grouper species), however, suggest they react to the presence of the gear once they are very near (and imaged) the towed camera (Grasty 2014). These observations coupled with the gear efficiency experiment from the west Florida shelf suggest that Red Snapper are faithfully imaged via towed camera systems, with little negative bias due to behavioral reactions (Keenan, S. et al., *unpublished data*). Finally, without substantial mapping of available pipeline habitat, expanded Red Snapper abundance estimates are subject to the quantified pipeline lengths. For example, in some areas, pipelines may be buried or exposed, resulting in over- or under-estimating Red Snapper abundance if the actual pipeline status is not in agreement with database records.

C. Final Abundance Estimates

Estimates of age-2+ Red Snapper abundance were produced by region, habitat type, and depth. Where appropriate, population estimates for artificial reefs were made for various categories representing the diversity of artificial structures. In all cases, population estimates were derived by expanded mean densities, with means and variances calculated assuming simple random sampling at the lowest strata level and assuming no error in the individual sample site estimates. Means and variances at higher levels of aggregation (region, total) were calculated following stratified sampling methods. Where appropriate, population estimates for artificial reefs were made for various categories representing the diversity of artificial structures. Estimates were performed by two independent groups on the same data to provide cross validation. While the approaches, post-stratification, and application of statistical models were differed and not stipulated *a priori*, these separate analyses converged with very similar estimates. Overall, we estimated an absolute abundance of 118 million age-2+ (CV 15%) during late 2019. While large numbers of fish occurred over well-known habitat features such as artificial reefs and natural hard bottom, we found that the previously uncharacterized bottom habitat (UCB) harbored the majority of Red Snapper. We observed a lower CV for the overall estimate compared to the subcomponents. The precision of the estimate of the total population is generally lower than that of the component parts. This is because the uncertainty of an aggregated quantity, as measured by CV (or standard deviation) decreases as the number of independent parts in that aggregation grows larger.

What follows is a detailed description of how the team arrived at our final estimate of absolute abundance (Table 4) of Red Snapper by region and habitat type.

1. Abundance Estimates by Region and Habitat Type

Due to the paucity of classified bottom habitat in the Gulf, the largest areal coverage of habitat fell into the UCB category which was stratified by region and depth. The UCB was stratified by state (TX, LA, AL/MS, FL) and depth (10-40 m, 41-100 m, 101-160 m). Given the differences in eco-regions along the FL shelf, it was further subdivided into 3 regions (northwest, mid, south). The resulting 18 strata were used to determine the weights for the stratified

estimates of mean density. For some locations (TX, LA, AL/MS) the areas of well-known large features of hardbottom were removed as separate habitats from the UCB estimates. Given the extensive natural bottom features that occur over wide-spread much less discrete areas in FL, these areas were collectively considered ‘natural bottom’ habitat. Where hardbottom habitat was mapped in detail, population estimates were made for these discrete mapped area by region not including FL. Population estimates were also made for artificial structures and the subcategory of artificial structure pipelines. The overall population estimate was derived by summing over the individual categories. Estimated densities by region, habitat type, and strata specific sampling information are presented in Table 5.

Uncharacterized bottom

To estimate total population size and uncertainty for each stratum, observed numbers of Red Snapper per 100m² were treated as simple random samples and population estimates were calculated as the mean density times the number of 100m² sampling units in each stratum. Mean density estimates were treated differently depended on the region and sampling method.

In Florida, because density was estimated from point counts where 100% detection was observed at the most basic strata level (state, depth) and samples were randomly selected, strata specific mean (\bar{x}_h) and variance (s_h^2) could be calculated following equations 1 and 2. The number of sampling units in a stratum (N_h) relative to the total number of sampling units (N) are used in the estimation of the stratified mean (\bar{x}) following equation 3 where K is the number of stratum and $\frac{N_h}{N}$ is the stratum weight. The variance of the random stratified mean ($s_{\bar{x}}^2$) is a function of the stratum weight, the number of observations in a stratum (n_h), the stratum variance, and the finite population correction and was calculated using equation 4. To estimate total population size (T), the random stratified mean is expanded by the total number of sampling units (N).

$$(1) \bar{x}_h = \sum_{i=1}^n \frac{1}{n} x_i$$

$$(2) s_{x_h}^2 = \frac{\sum_{i=1}^n (x_i - \bar{x}_h)^2}{n-1}$$

$$(3) \bar{x} = \sum_{h=1}^K \frac{N_h}{N} \bar{x}_h$$

$$(4) s_{\bar{x}}^2 = \sum_{h=1}^K \left[\left(\frac{N_h}{N} \right)^2 \left(1 - \frac{n_h}{N_h} \right) \frac{s_h^2}{n_h} \right]$$

In Texas, for the 2 shallowest stratum, where acoustic counts were taken, the total number of fish estimated to have been encountered on a transect over the total area covered by the acoustic gear was used as the total fish density estimate. Total fish numbers were then converted to an estimate of Red Snapper numbers assuming region specific estimates of the proportion of Red Snapper observed in visual observations applied over the region. Transects were post hoc stratified by region (South, Central, and North) to accommodate region specific

estimates of the proportion of Red Snapper. Transects were assumed selected randomly within each strata, with mean and variance calculated following equations 1 and 2. To account for region specific estimates of the proportion of Red Snapper in a sample and the uncertainty associated with this estimate (Table 6) the standard equation for the variance of the product of two independent variables was used. For each region the mean density of fish (\bar{x}_h) was multiplied by the mean proportion of Red Snapper (\bar{y}_h). The resulting variance was calculated using equation 5 where $s_{x_h}^2$ and $s_{y_h}^2$ are the respective variances.

$$(5) \quad s_{xy}^2 = s_{x_h}^2 * s_{y_h}^2 + s_{x_h}^2 * \bar{x}_h + s_{y_h}^2 * \bar{y}_h$$

For the deepest TX stratum the total number of snappers along a transect over the area surveyed from CBASS camera tows were used for density estimate. These estimates assumed 100% detection of Red Snapper within the area surveyed. Each transect was randomly selected and equations 1 and 2 were used to estimate the mean density and variance within a region.

For the deepest stratum in Louisiana, estimates of the total number of snappers along a transect over the area surveyed from CBASS camera tows were used for density estimate. These estimates assumed 100% detection of Red Snapper within the area surveyed. Each transect was randomly selected and equations 1 and 2 were used to estimate the mean density and variance within a region. For the shallow and mid-depth stratum acoustic data from the northern region of Texas as well as a few acoustic transects in the western most area of Louisiana were used to estimate Red Snapper density. The total number of fish estimated along a transect over the total area covered by the acoustic gear was used as the total fish density estimate. Total fish numbers were then converted to an estimate of Red Snapper numbers using the Texas North region estimate (Table 6) of the proportion of Red Snapper observed in visual observations. The additional variance in the estimate due to variability in the observed proportion was incorporated as described above for Texas.

For Mississippi and Alabama the total number of Red Snapper along a transect over the area surveyed from CBASS camera tows were used for density estimate. These estimates assumed 100% detection of Red Snapper within the area surveyed. Each transect was randomly selected and equations 1 and 2 were used to estimate the mean density and variance within a region. Due to the low number of transects, density estimates were not made for each depth stratum.

To estimate stratum specific population size (T_h) mean density per 100m² (\bar{x}_h) was multiplied by the number of 100m² units within a given stratum (equation 6) with the associated variance calculated using equation 7. To estimate regional total population sizes as well as the overall U.S. Gulf population over UCB, strata specific mean density and associated variances were combined using equations 3 and 4 with the stratum weight based on the area of each stratum (note for MS/AL no depth stratification was used).

$$(6) \quad T_h = N_h * \bar{x}_h$$

$$(7) \quad s_{T_h}^2 = N_h^2 * s_{\bar{x}_h}^2$$

The resulting estimates of mean Red Snapper density and variance for each region and depth stratum were combined into a single depth specific mean following equations 3 and 4 for stratified sampling.

Natural Hard bottom

Population estimates for natural hard bottom were calculated as expanded mean densities assuming the data were collected from a simple random sample. Mean and variances were calculated using equations 1 and 2 and expanded to the mapped area of hard bottom for TX, LA, and AL/MS using equations 6 and 7. For LA, samples from TX were substituted.

Artificial structures

Artificial structures in Texas were categorized as small and extra-large. Reef pyramid fields represented a unique habitat type, and 3.25 pyramids were assumed to comprise a small structure given the nature of the sampling conducted by overlaying grid across an entire field of these structures. Structures were also categorized by depth strata. Within each category simple random sampling was conducted and mean numbers and associated variance per structure were estimated using equations 1 and 2 from total fish counts converted to Red Snapper numbers from site specific estimates of the proportion of Red Snapper. For each site, the proportion of Red Snapper was assumed known without error. Total population estimates were calculated from mean numbers per structure expanded by the assumed known number of structures (equation 6). To estimate total number per artificial structure category, mean density per structure was calculated using depth strata following equations 3 and 4.

Population estimates for Louisiana were estimated from data for Texas. All structures in Louisiana were assumed to be extra-large and the number of structures was assumed known without error. Depth specific Texas data was substituted to estimate depth specific mean densities and calculations for each stratum and the combined estimate were calculated similar to the Texas data.

For Alabama the number of artificial structures per depth strata was estimated (see detail in the methods section). As a result, for each depth strata the total variance in the estimate was calculated by combining the variance in the estimated mean numbers per structure multiplied by the estimate of the variance in the number of structures (equation 5). Within each category (depth and authorization zone) simple random sampling was conducted and the mean and variance in numbers per structure were calculated using equations 1 and 2. Samples were stratified by authorization zone category to obtain estimated numbers in a given depth category. Means and variances were calculated using equations 3 and 4. Total numbers were estimated from expanded mean numbers per structure and the estimated number of structures (see methods for greater detail). For Mississippi, the estimated number per structure was calculated from simple random samples and expanded assuming the number of structures was known without error.

No difference in mean densities and variance were apparent for structures in FL and all samples were combined to get a single mean number per structure assuming simple random sampling (equations 1 and 2). Five sites had the same name over the sampling area, so the mean of the duplicated sites was calculated and used as the count. The number of artificial structures by depth was assumed known without error and total population estimated by depth and region were estimated as expanded mean numbers (equation 6 and 7).

Pipelines

The population estimate of Red Snapper on pipelines was estimated from an expanded mean densities per meter of pipeline times the total linear extent of pipeline in the Gulf. The total extent of pipeline was calculated from georeferenced polyline data from the BOEM (Bureau of Safety and Environmental Enforcement, Office of Technical Data Management, Data Administration Unit 2020-12-01, Pipelines vector digital data). Pipeline data was filtered to remove pipeline classified in the "REM", "RR", and "CNCL" categories, as these categories are associated with removed and assumed not present. Pipelines less than 8 inches were excluded, and pipeline arcs were clipped so that only pipe between 5 and 200 m depths were included. 200-m was used as the deepest depth due to the limited bathymetric contours available. Given the rate of depth increase in this zone, the error in the estimate of pipeline length was assumed to be small. Red Snapper density per meter of pipeline was estimated from total video counts per transect, assuming 100% detection of Red Snapper, over the length of pipeline surveyed. Mean density and the associated variance were calculated assuming transects were selected at random out of the available pipeline units in the BOEM database.

As previously outlined, it is possible that some pipeline structure does exist in the 5-60 m depth zone (see pg. 77). However, without extensive acoustic mapping surveys or verification from each pipeline operator, determining whether structure is present was not feasible within the time and resource constraints of this study. The decision was made to retain all pipeline structure within this shallow zone as part of the extrapolated universe, but it is worth noting that removing all of the pipeline structure in 5-60 m does not significantly change the total population of Red Snapper estimated for this habitat type. Another important caveat to clarify is that all data collection for the pipeline stratum occurred between 60 and 200 m. The assumption has therefore been made that the Red Snapper observations at these depths are comparable to the 5 to 60 m zone. Further study is necessary to elucidate this assumption; however, visual surveys would be a challenging sampling approach due to significant visibility issues encountered at these shallower depths.

Estimates of age-2+ Red Snapper abundance were produced by region (FL, AL/MS, LA, and TX) and habitat type (Natural, Artificial, UCB, and Gulf-wide pipelines). Over the entire Gulf, we estimated an absolute abundance of ~118 million age-2+ (± 17.1 million SE; PSE 15%) Red Snapper across the continental shelf of the U.S. Gulf of Mexico during late 2019 (Table 4). Mean density and the areal coverage (natural, UCB) or number of structures (artificial) for each of the regions and habitat types were also calculated and estimates of abundance generated (Table 5). Artificial reefs in TX were sub-divided into large and small categories due to the substantial difference in scale between oil/gas platforms and concrete pyramids. In other regions,

artificial habitat was not sub-divided. In all cases, population estimates were derived by expanded mean densities, with means and variances calculated assuming simple random sampling at the lowest strata level and assuming no error in the individual sample site estimates. Means and variances at higher levels of aggregation (region, total) were calculated following stratified sampling methods. While large numbers of fish occurred over well-known habitat features such as artificial reefs and natural hard bottom, we estimated that uncharacterized bottom habitat (UCB) harbored the majority of Red Snapper.

Table 4. Absolute abundance estimates for each state/region broken into the three habitat strata: Natural hard bottom, Artificial Reef, and Uncharacterized bottom, and pipeline estimates for the entire Gulf. SE = standard error; CV = coefficient of variation.

State/Region	Habitat Type	Number	SE	CV (%)
TX	Natural	7,037,443	2,537,014	36%
	Artificial	417,761	88,469	21%
	Uncharacterized Bottom	14,569,830	6,663,776	46%
	Total	22,025,035	7,130,931	32%
LA	Natural	3,852,652	1,671,470	43%
	Artificial	3,849,325	576,234	15%
	Uncharacterized Bottom	9,729,387	5,699,448	59%
	Total	17,431,364	5,967,375	34%
AL&MS	Natural	3,751,988	752,467	20%
	Artificial	1,509,625	167,506	11%
	Uncharacterized Bottom	3,199,472	1,625,263	51%
	Total	8,461,085	1,798,817	21%
FL	Natural & UCB	69,918,949	14,349,384	21%
	Artificial	127,560	21,088	17%
	Total	70,046,509	16,789,232	24%
Pipelines (Gulf-wide)		507,661	218,961	43%
Gulf of Mexico		118,471,654	17,194,438	15%

Table 5. Absolute abundance estimates for each state/region broken into the three habitat strata: Natural hard bottom, Artificial Reef, and Uncharacterized bottom, and pipeline estimates for the entire Gulf. Estimates of area coverage for natural and uncharacterized bottom, and number of structures for artificial reefs plus mean density per area or structure. SE = standard error; CV = coefficient of variation.

State/Region	Habitat Type	Total Area (km ²) or Structures	Number of Samples (<i>n</i>)	Area Sampled (km ²)	Mean Density (100m ²) or by Structure	Number	SE	CV (%)
TX	Natural	1,570	36	6.13	0.45	7,037,443	2,537,014	36
	Artificial	4,348	49			417,761	88,469	21
	<i>Large</i>	941	45		362	340,905	79,287	23
	<i>Small</i>	3,460	4		22	76,855	39,246	51
	Uncharacterized Bottom	57,535	140	6.26	0.03	14,569,830	6,663,776	46
	Total		225			22,025,035	7,130,931	32
LA	Natural	821	22	<i>n/a</i>	0.47	3,852,652	1,671,470	43
	Artificial	1,771	42		2174	3,849,325	576,234	15
	Uncharacterized Bottom	53,052	87	3.61	0.02	9,729,387	5,699,448	59
	Total		151			17,431,364	5,967,375	34
AL&MS	Natural	211	32	0.013	1.78	3,751,988	752,467	20
	Artificial	9,410	128		160	1,509,625	167,506	11
	Uncharacterized Bottom	18,500	3	0.74	0.02	3,199,472	1,625,263	51
	Total		163			8,461,085	1,798,817	21
FL	Natural & Uncharacterized	143,538	748	0.61	0.05	69,918,949	14,349,384	21
	Artificial	7,763	79		16	127,560	21,088	17
	Total		832			70,046,509	16,789,232	24
Pipelines (Gulf-wide)		26,686 linear km	27	0.49	0.02	507,661	218,961	43
Gulf of Mexico						118,471,654	17,194,438	15

Table 6. Proportion of Red Snapper in acoustic samples for Texas.

Region	Proportion	Variance
North	0.28	0.187
Central	0.475	0.157
South	0.5	0.333

2. Validation Analysis for Abundance Estimate

We performed a separate independent analysis to validate our primary estimate of absolute abundance on the same data set to provide validation. The results of this secondary analysis are shown in Tables 7 and 8. While the approaches, post-stratification, and application of statistical methods differed somewhat and were not stipulated *a priori*, these independent analyses produced similar estimates (i.e., within 6.0%; 7.2 million Red Snapper difference from each estimate).

While these two analyses were performed independently using the same data, guidance was not given in terms of a preferred statistical approach, post-stratification, and various other small nuances regarding how these data were treated. Total abundance estimates were made for 4 regions: Texas, Louisiana, Alabama/Mississippi, and Florida. The primary abundance estimation method for artificial reefs and pipelines is based on a model in which expected abundance in each site is assumed proportional to its area for all sites in the stratum (i.e., it used the average of ratio estimator). The validation method presented here used the standard ratio estimator for abundance, which does not require adherence to a model for consistency. Only small differences in the estimates from the two methods were observed, so the implicit model assumption for the primary estimation method was deemed adequate. Within each region, total abundance was estimated by habitat: artificial reefs (ART), natural banks (NAT), and uncharacterized bottoms (UCB). This section details the different methods used for estimating Red Snapper abundance, data pre-processing, and the mathematical expressions for the different estimators used for estimating total abundance in the various Red Snapper habitats. The rest of the analytical description is organized as follows: the different estimators used to estimate Red Snapper abundance in the different regions/habitats are defined, and the resulting total abundance estimates for each of the regions and habitats as well as their associated estimators are summarized.

Estimators

Within each stratum and post-stratum, a separate estimate of total Red Snapper Abundance was made. Then the estimates of total abundance were summed to achieve a Gulf-wide estimate of abundance. In each stratum or post-stratum, either a Mean-per-unit estimator (if sampling units were the same size or there was no size measure beyond a classification, as for artificial reefs) or a Ratio estimator (if sampling units varied in size, such as varying size transects) was used.

Mean per-unit ($\hat{t}_{y,mpu}$)

In strata in which the sampling unit was artificial structure or grid with fixed size, total abundance was estimated by multiplying the number of artificial structures or grids in the population by the average Red Snapper count per structure or grid (mean per-unit). Let N_h denote the number of units in the stratum h universe (e.g., number of large structures in a region) and n_h denote the number sampled, and let y_{hi} denote the abundance of Red Snapper observed

(or estimated) in the i^{th} sampled unit of the h^{th} stratum. Then, the total abundance estimate for the stratum is given by:

$$(24) \quad \hat{t}_{hy,mpu} = N_h \times \bar{y}_h,$$

where $\bar{y}_h = \text{average abundance observed (or estimated) per structure or grid}$ ($\bar{y}_h = \frac{\sum_{i=1}^n y_{hi}}{n}$).

The variance of the MPU estimator for the h^{th} stratum (Eqn. 24) was estimated as

$$(25) \quad v(\hat{t}_{hy,mpu}) = N_h^2 \times (s_h^2/n_h) \left(1 - \frac{n_h}{N_h}\right).$$

Ratio Estimator ($\hat{t}_{y,r}$)

In strata in which the sampling units were areal and varied in size (e.g., transects), total abundance was estimated with a standard ratio estimator. Let x_{hi} denote the area of the i^{th} sampled unit of the h^{th} stratum and let t_{hx} denote the total area of the stratum. Then, the total abundance estimate for the stratum is given by:

$$(26) \quad \hat{t}_{hy,r} = t_{hx} \times \frac{\sum_{i=1}^{n_h} y_{hi}}{\sum_{i=1}^{n_h} x_{hi}} = t_{hx} \times \hat{d}.$$

The variance of the ratio estimator for the h^{th} stratum (Eqn. 26) was estimated using the Taylor Series approximate variance:

$$(27) \quad v(\hat{t}_{hy,r}) = t_{hx}^2 \times (s_d^2/n_h) \left(1 - \frac{n_h}{\hat{N}_h}\right),$$

where s_d^2 is the sample variance of the residuals $d_{hi} = y_{hi} - \hat{d}x_{hi}$ and the estimated number of transects in the population is $\hat{N}_h = t_{hx}/\bar{x}_h$, where \bar{x}_h is the average area of the sampled units.

Pyramid Structure strata ($\hat{t}_{y,r(pyr)}$)

Artificial structures in TX were classified into two strata, pyramid-like and non-pyramid, because structures are typically large artificial reef (e.g., oil and gas platforms) or smaller artificial reef pyramids (e.g., small discrete structures). These two types required different approaches for estimating abundance. Though abundance of Red Snapper on large structures in Texas were estimated with the mean-per-unit estimator shown in (24), the total abundance on the pyramid structures was estimated by a ratio estimator, as shown in (26). The regions where the pyramids appear was gridded into equal size grid cells. Then a sample of n_h grids cell was selected. However, rather than using the area as the auxiliary variable, the number of pyramids in each grid cell was used. That is, $x_{hi} =$ the number of pyramids in grid unit i in the stratum and the total number of pyramids in the stratum is denoted by t_{hx} . Then total abundance was estimated using the ratio estimator as in (26). Note though that the density estimate \hat{d}_h , is now

the density of Red Snapper per pyramid in the sampled grids. The variance of this estimator is as shown in (27).

Substitution ($\hat{t}_{y,sub}$)

In regions in which samples were not available or missing, total abundance was estimated by substituting the missing samples with samples from similar/nearby areas. The total abundance estimate is:

$$\hat{t}_{hy,sub} = t_{hx} \times \hat{d}_{h,sub}$$

where $\hat{d}_{h,sub}$ is the abundance density for the area where sample is available (the substitute area).

Alabama/Mississippi Estimates

The one exception to the method just described was for Alabama/Mississippi estimates. The AL/MS team produced estimates and their standard errors directly, which are reported in Section B.4 (Alabama/Mississippi Region). The validation estimation team incorporated their estimates into the Gulf-wide total Red Snapper estimate and its variance, using the method we describe subsequently.

Adjustment for calibration variance

The estimated variance expressions in (25) and (27) do not account for uncertainty in the measurement of RS abundance y_{hi} . The so-called “observed” values of Red Snapper count in expressions (24) – (26) are in some cases approximated rather than directly observed. One method for approximating Red Snapper was as a fraction of total fish abundance, which was directly observable by using visual sampling methods (e.g., ROV or TCA). This fraction, called a calibration factor, was itself estimated from experimental data in which fish and Red Snapper abundance could both be measured accurately in a sample of transects. From these data, a proportion of Red Snapper was noted for each of a sample of transects. Then the proportions were averaged to obtain the calibration factor for a specific region. This calibration factor was then multiplied by total fish observed in transects in which the counting technology does not allow species identification, thereby producing an approximate value of Red Snapper counted for the transect. This is the method that was used for the Mid and Shallow depths of the UCB stratum in Texas. A separate calibration factor was estimated by region (Central, North, and South), defining post-strata.

Calibration adds variability to the final estimate beyond what is shown in (25) and (27). To see how much, we must examine the expression for the estimator and calculate an estimate of its variance. Let u_{hi} denote the fish abundance in post-stratum h and transect i and \hat{p}_h denote the calibration factor for post-stratum h , and $\hat{y}_{hi} = \hat{p}_h u_{hi}$ denote the calibrated measure of Red Snapper abundance in transect i . Then the calibrated ratio estimator of Red Snapper in the UCB post-strata, is

$$(28) \quad \hat{t}_{hy,r} = t_{hx} \times \frac{\sum_{i=1}^{n_h} \hat{p}_h u_{hi}}{\sum_{i=1}^n x_{hi}} = t_{hx} \frac{\sum_{i=1}^{n_h} u_{hi}}{\sum_{i=1}^n x_{hi}} \times \hat{p}_h = \hat{t}_{hu,r} \times \hat{p}_h.$$

From (28) we see that the calibrated estimator can be written as a product of two random variables, one in the form of the original estimator (except it is an estimate of total fish abundance rather than Red Snapper abundance) and the calibration factor. Since the calibration data was independently collected from the fish abundance data, the two terms of the product are independent. The variance for a product of two independent estimators that are both approximately unbiased (so that $E(\hat{t}_{hu,r}) \approx t_{hu}$ and $E(\hat{p}_h) \approx p_h$, the true calibration factor, if it could be observed) can be estimated (Goodman 1962) as

$$(29) \quad v(\hat{t}_{hy,r}) = V(\hat{t}_{hu,r} \hat{p}_h) = \hat{p}_h^2 V(\hat{t}_{hu,r}) + V(\hat{p}_h)[\hat{t}_{hu,r}^2 - V(\hat{t}_{hu,r})].$$

Since $\hat{t}_{hy} = \hat{p}_h \hat{t}_{hu,r}$, the first term of (29) can be thought of as an estimate of the variance of the uncalibrated estimator in (28). The second term of (29) is therefore an estimate of the increase in variance due to calibration. When the calibration factor is a sample mean (of proportions) as it is in this case, then $v(\hat{p}_h) = s_{hp}^2/m$, where s_{hp}^2 is the sample variance of the calibration proportions and m is their sample size. This is the method we used to determine the SE's for the estimates of total Red Snapper in Table 7 for the Mid and Shallow UCB strata. (This method was not used for the Deep UCB stratum because calibration was not used, but rather direct counts of Red Snapper were used for estimation where the CBASS gear was used).

Note that the AL/MS estimation team incorporated an adjustment for the uncertainty in the number of artificial reefs in their state, as was described in Section B.4 (Alabama/Mississippi Region). Since the number of artificial reefs was unknown, the expression in (24) also required a product of two random variables for their estimator. As a result, they also used the variance estimate shown in (29), as shown in Section B.4.

Besides the Texas UCB, approximation of Red Snapper count in transects of the natural habitats and artificial reefs in Texas also used calibration methods. The analytical methods needed for this calibration are most likely not possible with the current data and analytical methods available. Thus, no additional variance estimate for this calibration factor was calculated. As a result, we cannot directly assess the effect on the standard error and CV of the estimates of Red Snapper abundance in these strata. Nevertheless, to understand the impact that this calibration might have on the uncertainty of Red Snapper abundance in Texas and the Gulf as a whole, we undertook a conservative “worst case scenario” approach to examine this issue. We estimated the multiplicative increase in variance of Red Snapper abundance due to calibration for each of the post-strata of the UCB in Texas. This quantity, known as design effect or efficiency when comparing sample designs or estimators, ranged from a low of 1.01 (Central region, mid depth of Texas UCB) to a high of 2.77 (South region, mid depth of Texas UCB). The latter value means that the variance of the estimator of Red Snapper abundance in that post-stratum is 2.77 times larger than it would have been if Red Snapper count could have been observed directly, or without uncertainty due to calibration. To examine the impact that calibration might have in the other strata of Texas that used it, we multiplied each variance estimate by 2.77, to determine a “worst-case scenario” for the effect of calibration on variance. Then these conservative estimates

of variance were used to determine a CV for Texas, and for its impact on the estimate of total for the Gulf. Our findings, as shown in the last column of Table 7, are that the estimated CV of Red Snapper abundance for Texas increased from 22% to 25% by applying this factor to all the additional strata of Texas that used calibration. Since LA also used Texas data, we carried out this exercise for LA Red Snapper abundance estimate as well. The CV of Red Snapper abundance in Louisiana increased from 23% to 39% by applying the factor to all its strata, also shown in Table 7.

Total Abundance Estimates

To obtain estimates of total abundance for state areas and Gulf-wide, the estimates in the strata and post-strata (which we refer to collectively as sub-areas) making up those areas were added. The estimated variance of the aggregated estimate was calculated as the sum of the variances for the component sub-areas, and its standard error was estimated as the square root of the aggregate. That is, if we denote the set of sub-areas using MPU estimators as H_{mpu} and the set of sub-areas using ratio estimators as H_r , then we can represent the estimator of abundance for any aggregated area A made of entire sub-areas and its standard error as

$$\hat{t}_A = \sum_{h \in (A \cap H_{mpu})} \hat{t}_{h,mpu} + \sum_{h \in (A \cap H_r)} \hat{t}_{h,r}$$

and

$$SE(\hat{t}_A) = \sqrt{\sum_{h \in (A \cap H_{mpu})} v(\hat{t}_{h,mpu}) + \sum_{h \in (A \cap H_r)} v(\hat{t}_{h,r})}.$$

The results of this validation estimation process are shown in Tables 7 and 8 for various aggregation levels, from individual strata to regions to Gulf-wide estimates. Table 7 includes sample sizes and estimates for individual strata, standard errors of the estimates, their coefficient of variation (standard error divided by the estimated abundance), and a conservative (worst-case) CV, based on assuming a large value (2.77) for the design effect for Red Snapper abundance estimates based on calibration. Table 8 shows estimates, their standard errors, CV's and conservative CV's for aggregations of strata to habitat level for LA and the rest of the Gulf. (We do not combine LA and the rest of the Gulf since LA re-uses data from Texas in its estimate. Thus, combining variances as shown in (30) misrepresents the combined uncertainty.)

Table 7. A detailed breakdown of Red Snapper as calculated with the mean per unit (artificial reef) or ratio estimator (natural and uncharacterized bottom) including total area or number of structures and mean density by state and habitat type. Calculations included stratifying some samples by depth and structure type, depending on state or region. Values in italics are the subcategories within the habitat type and when summed equal the total for that habitat. TX: Natural and uncharacterized bottom samples were stratified into three depths (shallow, mid, deep), with mean density by area used to calculate the abundance by habitat. Artificial reefs were separated to pyramids and non-pyramids due to the vast differences between the two structure types and mean densities for each were calculated and used for the total abundance. LA: The natural and uncharacterized bottom habitat types were grouped into two depth strata- deep and mid & shallow due to the relatively small area in shallow depths off the coast. Artificial reefs were stratified into the three depth categories due to the use of TX artificial reef data to supplement the LA data. AL/MS: The depth and artificial reef types are relatively uniform for this region; therefore, no stratification was required. FL: Due to the size of FL, the natural and uncharacterized bottom habitats were stratified into regions (NW, Mid, South) as well as three depth zones (shallow, mid, deep). All artificial reefs were treated as separate samples, despite site name duplication resulting in 84 total sites sampled.

State/Region	Habitat Type	Area (km ²) or Structures	Number of Samples (n)	Mean Density (100m ²) or by Structure	Number	SE	CV (%)	Conservative CV(%)	Estimator
TX	Natural	1,570	36		5,218,915	1,390,733	27	44	
	Deep	209	11	0.09	178,682	70,111	39	65	$\hat{\epsilon}_{y,r}$
	Mid	953	22	0.35	3,381,753	955,545	28	47	$\hat{\epsilon}_{y,r}$
	Shallow	409	3	0.41	1,658,480	1,008,046	61	101	$\hat{\epsilon}_{y,r}$
	Artificial	12,010	31		706,327	191,728	27	45	
	Pyramids	10,902	13	11	125,300	80,777	64	107	$\hat{\epsilon}_{y,r(2yr)}$
	Non-Pyramids	1,108	18	524	581,027	173,881	30	50	$\hat{\epsilon}_{y,mpu}$
	Uncharacterized Bottom	57,535	140		10,332,018	3,449,733	33	33	
	Deep	4,034	4	0.002	71,460	38,584	54	90	$\hat{\epsilon}_{y,r}$
	Mid-North	8,765	39	0.015	747,705	512,361	69	93	$\hat{\epsilon}_{y,r}$
	Mid-Central	6,450	22	0.033	2,159,374	2,014,526	93	69	$\hat{\epsilon}_{y,r}$
	Mid-South	6,503	16	0.005	340,824	205,910	60	60	$\hat{\epsilon}_{y,r}$
	Shallow- North	17,036	36	0.014	2,335,968	1,426,726	61	65	$\hat{\epsilon}_{y,r}$
	Shallow- Central	8,951	15	0.038	3,367,881	2,183,282	65	61	$\hat{\epsilon}_{y,r}$
	Shallow- South	5,797	8	0.023	1,308,806	856,547	65	65	$\hat{\epsilon}_{y,r}$
Total			198		16,257,260	3,724,454	23	26	
LA	Natural	821	22		3,683,745	958,570	26	43	
	Deep	105	6	0.14	151,361	51,731	34	57	$\hat{\epsilon}_{y,sub}$
	Mid & Shallow	716	16	0.49	3,532,384	957,173	27	45	$\hat{\epsilon}_{y,sub}$
	Artificial	1,771	42		3,849,325	1,341,617	35	58	
	Deep	93	7	710	66,046	38,272	58	96	$\hat{\epsilon}_{y,mpu}$
	Mid	602	29	1,399	842,219	363,261	43	72	$\hat{\epsilon}_{y,mpu}$
	Shallow	1,076	6	2,733	2,941,060	1,290,935	44	73	$\hat{\epsilon}_{y,mpu}$
	Uncharacterized Bottom	53,052	65		11,043,973	4,024,820	36	45	
	Deep	5,348	3	0.01	406,320	387,513	95	159	$\hat{\epsilon}_{y,sub}$
	Mid	19,077	11	0.02	3,756,598	2,715,533	72	120	$\hat{\epsilon}_{y,sub}$
Shallow	28,627	51	0.02	6,881,055	2,945,317	43	71	$\hat{\epsilon}_{y,sub}$	
Total			129		18,577,043	4,349,479	23	39	
AL/MS	Natural	211	32	1.78	3,751,988	752,467	20	20	
	Artificial	9,410	128	160	1,509,625	167,506	11	11	
	Uncharacterized Bottom	18,500	3	0.02	4,425,687	1,730,961	39	39	$\hat{\epsilon}_{y,r}$
	Total		163		9,687,300	1,894,859	20	20	
FL	Natural & Uncharacterized	143,538	748		66,121,747	13,296,205	20	20	
	NW Region- Deep	1,662	26	0.01	92,360	92,214	100	100	$\hat{\epsilon}_{y,r}$
	NW Region- Mid	2,060	29	0.004	85,274	85,757	101	101	$\hat{\epsilon}_{y,r}$
	NW Region- Shallow	3,553	52	0.05	1,859,201	1,298,879	70	70	$\hat{\epsilon}_{y,r}$
	Mid Region- Deep	3,759	4	-	-	-	-	N/A	$\hat{\epsilon}_{y,r}$
	Mid Region- Mid	12,113	20	0.12	15,114,169	8,521,792	56	56	$\hat{\epsilon}_{y,r}$
	Mid Region- Shallow	33,977	425	0.11	37,891,216	9,203,445	24	24	$\hat{\epsilon}_{y,r}$
	Southern Region- Deep	12,189	25	-	-	-	-	N/A	$\hat{\epsilon}_{y,r}$
	Southern Region- Mid	37,043	37	0.01	3,510,529	2,532,121	72	72	$\hat{\epsilon}_{y,r}$
	Southern Region- Shallow	37,180	130	0.02	7,568,998	3,368,997	45	45	$\hat{\epsilon}_{y,r}$
	Artificial	7,763	84	16	123,377	20,016	16	16	$\hat{\epsilon}_{y,mpu}$
Total			832		66,245,124	13,296,220	20	20	
Pipelines (Gulf-wide)			27	0.02	546,988	358,761	64	64	$\hat{\epsilon}_{y,r}$
Gulf of Mexico					111,313,716				
TX, MS, AL, FL					92,736,673	13,942,031	15	14	
Louisiana*					18,577,043	4,349,479	23	39	

Table 8. Red Snapper abundance estimates by habitat.

	Habitat	Estimate	SE	CV	Conservative CV (%)
Gulfwide (excluding LA)	Artificial	2,339,329	255,379	11	15
	Natural	75,092,650	13,389,899	18	18
	Uncharacterized Bottom	14,757,705	3,482,480	24	26
	Pipelines	546,988	358,761	66	64
Louisiana*	Artificial	3,849,325	1,341,617	35	58
	Natural	3,683,745	958,570	26	43
	Uncharacterized Bottom	3,849,325	1,341,617	35	58

*LA estimates were made using TX data for ART TX and LA data for NAT and UCB

Determining Age-2+ Fish Length

Generally, Red Snapper do not recruit or are excluded from reef structure until ~ age-2 or older (Patterson 2007). At this point, these fish have joined the exploitable population for recreational and commercial fishers, and these fish are the population of concern for this study. However, in some regions, particularly in the eastern Gulf where overfishing was intense but now characterized by rapid re-colonization, Red Snapper may use small reef habitat at younger sizes/ages (Bailey et al. 2001; Workman et al. 2002). Based on our size data, we observed a slight portion of fish slightly smaller than what would be considered size age-2. This was not the case in Texas and Louisiana regions, where ontogenetic shifts, behaviors, sampling methods, and post-processing excluded these smaller fish from our analyses. While these fish are clearly part of the population and experience fishing, discard mortality, and exploitation, we adopted a conservative approach, despite some fish not reaching this somewhat arbitrary benchmark (> age-2+) set forth in the Phase II RFP. Thus, the size distribution of Red Snapper was derived from laser scaler (Figure 24a) or stereo camera (Figure 24b) samples to reduce from the estimate the proportion of fish that may have been < age-2 in ROV video and depletion samples collected from both Florida and Alabama. Red Snapper size-at-age data (n = 1,755) was estimated using a von Bertalanffy growth function (VBGF) from Patterson et al. (2001), as well as more recent otolith-derived age data (n = 388) from northwest Florida and Alabama (Patterson et al. 2010). We first examined the standardized residuals from the VBGF $\{L_t = 978.6[1 - e^{-0.178(t+0.22)}]; P < 0.001, R^2 = 0.85\}$ for Red Snapper with biological (fractional) ages of 0.92 to 2.43 years old to evaluate the fit of the function to fish <2.5 years old. The residuals plot indicated a slight but not substantial positive bias in the fit (Figure 25). Based on cumulative probability distributions computed from predicted size-at-age from the VBGF fit and its CV for fish 1.0 to 2.25 years old (Figure 26), a threshold of 250 mm TL was established for considering fish to be age-2 or older. The rationale for this was the fact that Red Snapper median hatch date is July 1, and they begin forming their first opaque zone in January (Patterson et al. 2001). Therefore, fish with a biological age of 1.5 years on January 1 would have an integer age of 2 years based on opaque zone counts. Fish that are 1.5 years old, or have an integer age of 2 years, would have a relatively

high (~60%) probability of being >250 mm TL, while fish estimated to be 1.25 years old would have a relatively low (~20%) probability of being >250 mm TL (Figure 26).

Among length estimates in our data, 10.8% of samples were estimated to be <250 TL. However, only 15.9% (n = 613 of 3,856) of the Red Snapper observed in video samples were able to be measured with either the red laser scaler or the stereo camera system, so we had to expand sample-specific proportions of Red Snapper <250 mm TL to the total number of individuals observed at a given site. As a result, the total number of Red Snapper <250 mm TL (i.e., estimated to be age-2 or older) was 3,510, which represented an 8.9% reduction from our total Red Snapper count of 3,856. Site-specific Red Snapper density estimates were computed from samples estimated to be >250 m TL for expansion from sample-specific Red Snapper density estimates to stratum- and Florida-specific population estimates. Thus, these estimates presented here only include fish that are > 250 mm TL (age-2+), and this model and size distribution was also applied to fish collected from the Alabama/Mississippi region.

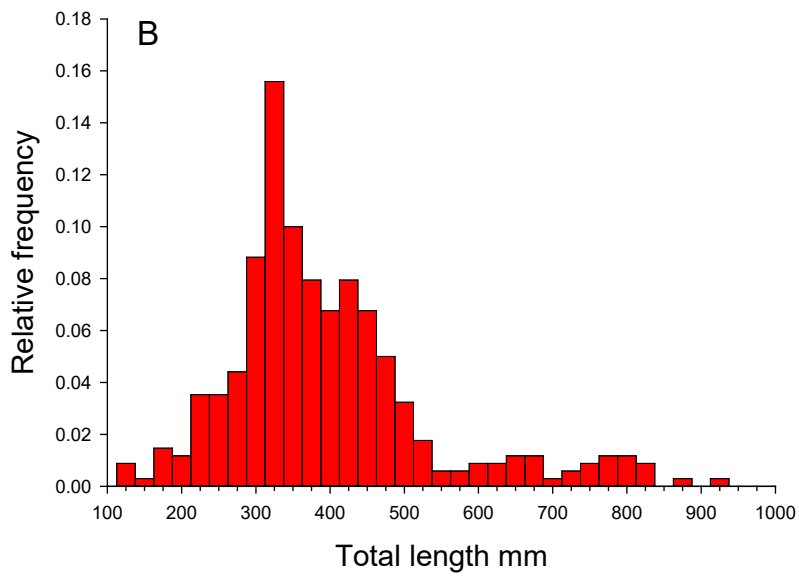
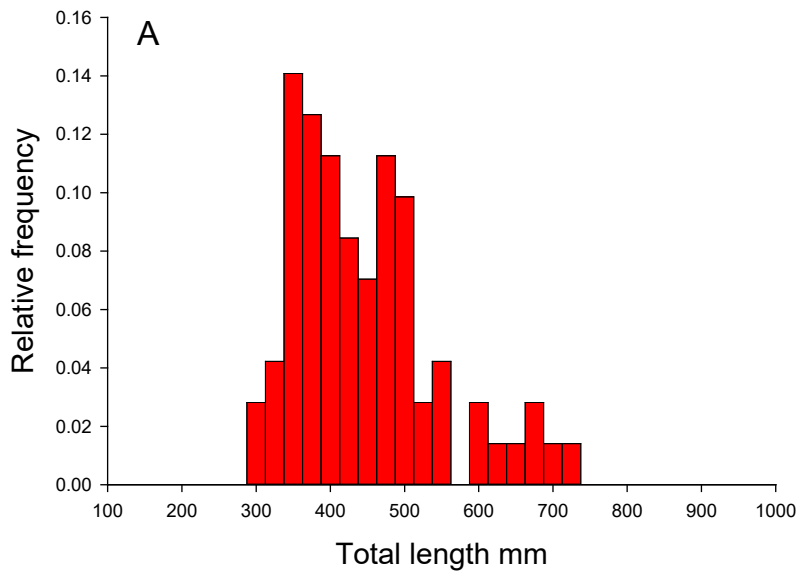


Figure 22. Size distribution of Red Snapper total lengths estimated with A) a red laser scaler (n = 215) or B) a stereo camera system (n = 398) at sites sampled in Gulf of Mexico waters off Florida.

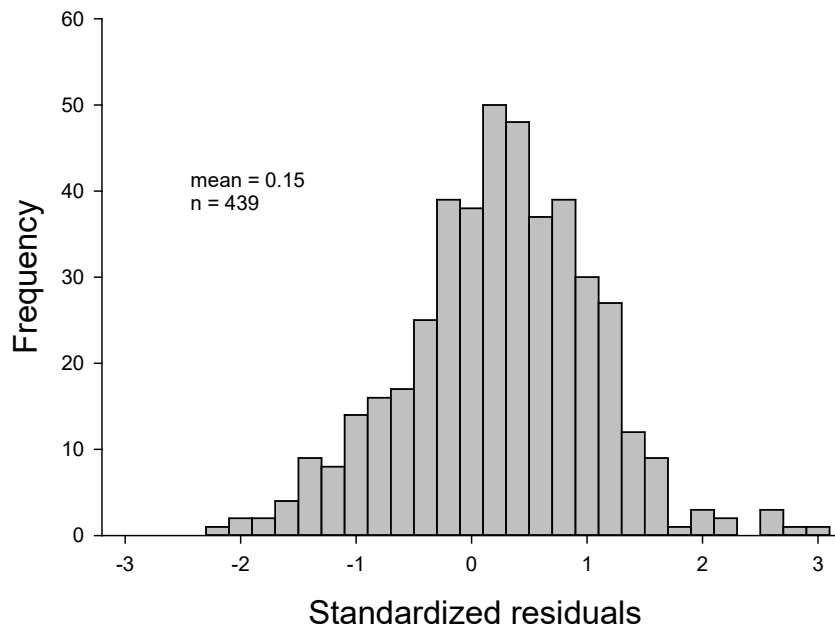


Figure 23. Standardized residuals for Red Snapper aged to be 2.5 years old from a von Bertalanffy growth function fit to otolith-aged fish (n = 2,143) sampled off northwest Florida and Alabama.

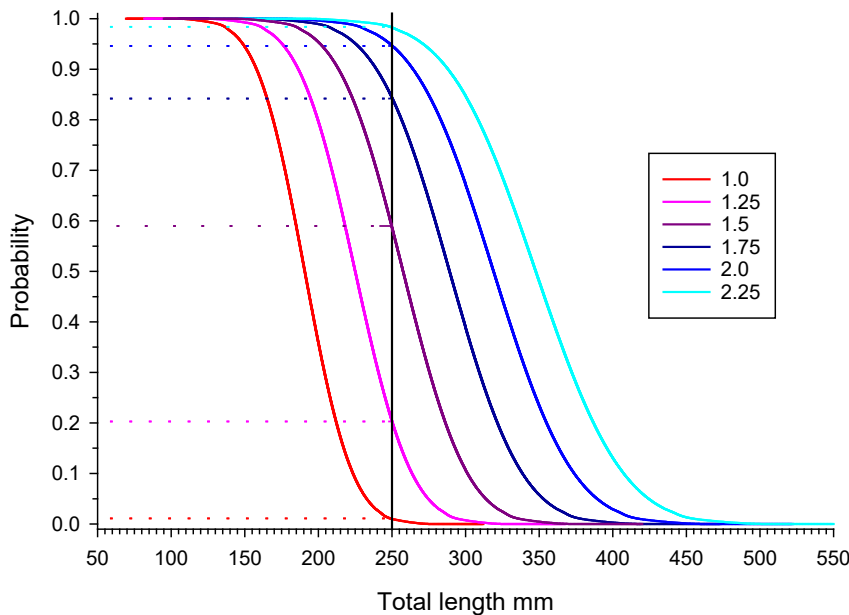


Figure 24. Estimated cumulative size distributions of age-1 to age-2.25 fish given the von Bertalanffy growth function fit to Red Snapper otolith-aged samples and the CV from that fit. Vertical line indicates a total length (TL) of 250 mm. Dashed horizontal lines indicate the cumulative probability where 250 mm TL intersects the age-specific distributions.

Because many sampling methodologies (e.g., visual surveys, hydroacoustics) used in this project did not provide length data, ancillary length data was compiled by project PIs from past Red Snapper studies occurring between the years 2010-2020 using a variety of gear types (e.g., ROV laser and stereo cameras, vertical longline, bottom longline; Table 9), where accurate length measurements had been obtained. Since the focal point of the abundance calculations were for age-2+ Red Snapper, data were truncated at 250 mm TL based upon the analysis described above (Figure 27, Figure 28, Table 9). Raw data used to compile the following table and figures have been provided to the NOAA Southeast Fishery Science Center to supplement existing length composition from the SEDAR process, and are available upon request as needed in compliance with our data management plan.

Table 9. Compilation of ancillary length data from projects outside the scope of the Great Red Snapper Count. Data sources span multiple years (2010-2020) and were collected with various gear types (ROV laser and stereo cameras, vertical longlines, and bottom longlines). No data was available for Uncharacterized Bottom for the Western Gulf (n/a).

Region	Habitat Type	n	Mean TL (mm)	SE	Min TL (mm)	Max TL (mm)
Gulf of Mexico	All	17,969	481	1.141	251	990
	Natural	2,002	499	2.52	256	976
	Artificial	14,635	453	1.111	251	990
	Uncharacterized Bottom	1,332	761	2.803	371	977
Eastern Gulf	All	12,932	482	1.469	251	990
	Natural	676	471	4.932	256	976
	Artificial	10,924	449	1.358	251	990
	Uncharacterized Bottom	1,332	761	2.803	371	977
Western Gulf	All	5,037	478	1.527	251	871
	Natural	1,326	513	2.778	257	855
	Artificial	3,711	465	1.775	251	871
	Uncharacterized Bottom	n/a	n/a	n/a	n/a	n/a

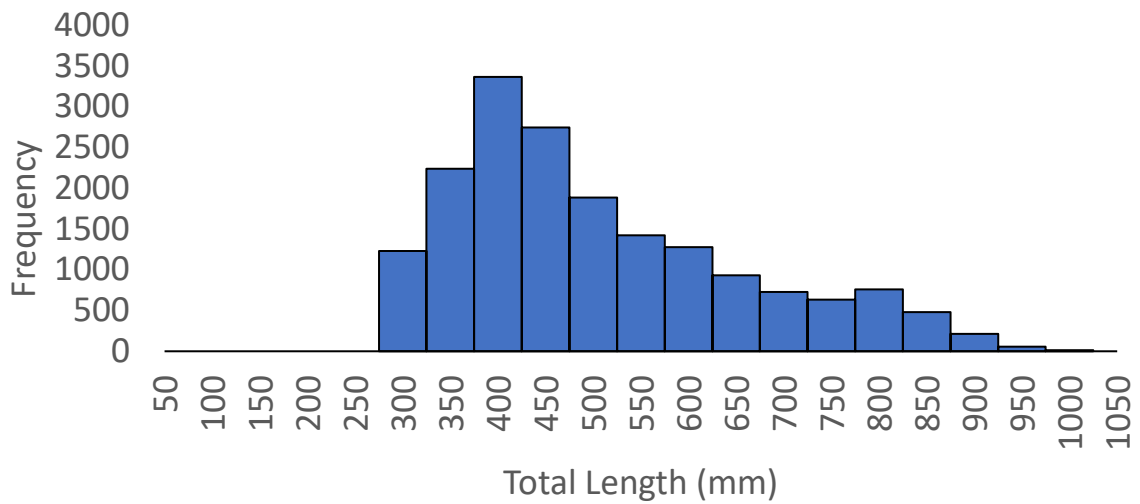


Figure 25. Histogram of Red Snapper total length (mm) for all habitat types and all geographic regions across the Gulf of Mexico. Data sources span multiple years (2010-2020) and were collected with various gear types (ROV laser and stereo cameras, vertical longlines, and bottom longlines).

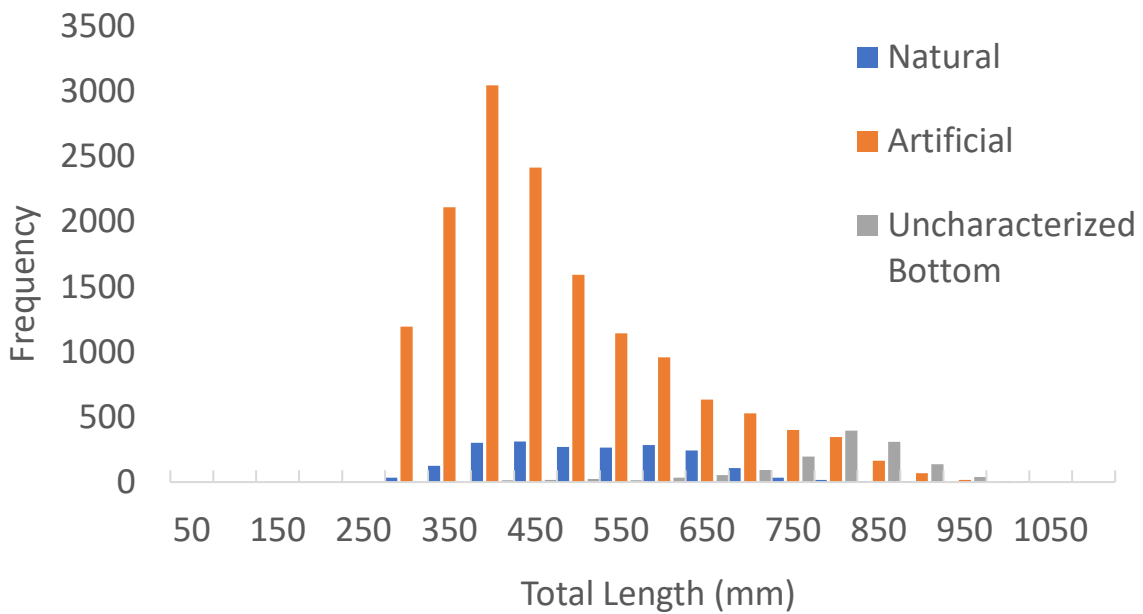


Figure 26. Histogram of Red Snapper total length (mm) separated out by the three habitat types (natural hard bottom, artificial reef, uncharacterized bottom) sampled in this project across the Gulf of Mexico. Data sources span multiple years (2010-2020) and were collected with various gear types (ROV laser and stereo cameras, vertical longlines, and bottom longlines).

3. Sampling Biases: Direction, Uncertainty, and Validity of the Abundance Estimate

The team strived to minimize and account for bias and uncertainty that would affect the accuracy of our Red Snapper abundance estimates. Nevertheless, uncertainty does exist with any study, and we recommend it is important to identify these biases, accounting for them where possible, as well as indicating the magnitude and direction (e.g., increasing/decreasing the ultimate population size estimate). This will provide for the most robust estimate of absolute abundance and ultimately provide the most effective integration into the management process. Potential sources of bias include necessary departures from the original core stratified random sample design to deal with regional differences, issues affecting Red Snapper detectability with video and sonar, the potential to double count Red Snapper with video-based methods, and meeting assumptions of depletion methods employed on select habitats off AL/MS. This section identifies potential sources of bias or uncertainty that managers should take into consideration when interpreting and integrating these data into management. Additionally, we offer future research recommendations based on ‘lessons-learned’ given we now have the benefit of hindsight post-completion of this study (see Section F below).

Direction of Bias – Generally, we suggest the direction of the overall estimate is conservative and thus, likely underestimates the absolute abundance of Red Snapper in the Gulf. The research team applied decisions and assumptions at all phases of this study that are conservative in nature. This was principally due to the fact that an over-estimation has far greater implications for the sustainability of the fishery. Some key examples of the directionality and likely significance of sampling biases are captured within the body of the main document, while some of the key aspects are highlighted below:

- For the western Gulf of Mexico, we were often challenged by a persistent nepheloid layer (i.e., near zero visibility). These constraints required the use of hydroacoustics paired with species composition from visual methods (e.g., ROV, towed camera sled) to estimate abundance. This was particularly problematic for surveys of UCB because a large fraction of the fish abundance (fish targets from echosounder surveys) were within the nepheloid layer, limiting our ability to directly estimate the proportion of Red Snapper in the fish assemblage. In response, the mean proportion of Red Snapper from regional surveys conducted during periods of adequate visibility were used to derive abundance estimates of Red Snapper.
- Additional visibility constraints may have resulted in underestimates of Red Snapper when in the presence of congeners (e.g., gray snapper). For example, fish that resembled Red Snapper but could not be identified with certainty were classified as Lutjanidae or unknown and were not included in our estimates of Red Snapper abundance. This reduced the calculated percent composition of Red Snapper resulting in a downward estimate bias.
- Hydroacoustic surveys may have missed additional Red Snapper close to the bottom and inside structure (e.g., large oil and gas platforms), potentially underestimating the number

of Red Snapper. For example, video surveys of artificial reefs commonly observe Red Snapper within the structure matrix (See Ajemian et al. 2015), yet the gear limitation of hydroacoustics fail to account for these fish due to acoustic returns hindered by the structure, thereby reducing the total count.

- We relied on hydroacoustics for many regions due to visibility constraints working from the initial assumption that this method would be the most efficient gear. However, recent calibration studies have shown that hydroacoustics may actually underestimate Red Snapper abundance (see section II.B.3). We recommend future experiments to fully describe the magnitude of the difference. We did not calibrate for these differences in our abundance estimate.
- We observed a lower CV for the overall estimate compared to the subcomponents. The precision of the estimate of the total population is generally lower than that of the component parts. This is because the uncertainty of an aggregated quantity, as measured by CV (or standard deviation), decreases as the number of independent parts in that aggregation grows larger. As such, overestimates in some of the component subareas are compensated for with underestimates in others, making the total aggregated abundance estimate less variable. Moreover, the metric of variability associated with the stratified estimate of mean density, and ultimately the population estimates, is the standard error, a metric that is an estimate of the variability in the mean should similar samples be taken from the population. The standard error is used to calculate confidence intervals and is approximated as the square root of the variance divided by the sample size.
- We accounted for the influence of extraordinarily high sampling abundance measurements on overall population size estimates. The research team spent considerable time discussing and ultimately removing some outlier data points that had large effects on population estimates (higher if included). While team members confirmed Red Snapper counts at those sites were accurate, they were nonetheless outliers given they were at least an order of magnitude higher than other sites within the same habitat and depth stratum. The consensus reached by the team was those sites should be censored from the data set given they were not characteristic of Red Snapper densities typically observed and due to the effect of single data points increasing regional population estimates by 10-20% through the stratification process.
- Unlike other regions, where the universe of artificial reef (and natural bottom) was known, we used surveys in AL/MS to estimate the number of unpublished reefs. For other regions, the location, number and/or areal coverage of artificial reefs, natural bottom, and pipeline are required to be reported to county, state, or federal agencies, thus were assumed to be known with negligible uncertainty. Areas where unclassified/unknown structured habitat (i.e., unknown artificial reefs or natural hard bottom) may have occurred are included in the UCB habitat type.
- We have evidence using C-BASS video surveys (mentioned above) of Red Snapper occurring in areas outside of the nominal study area/depths (e.g., deep salt dome and

pinnacles) prescribed for this study. These fish were not included in the abundance estimate but occur off the U.S. continental shelf in some regions.

- This study specifically tasked the researchers to quantify the abundance of age-2+ fish. The size distribution was region-specific (see above). Thus, the proportion of these fish that were < 250 mm TL were removed. While there is general agreement in the literature that age-2 fish do occur at lengths < 250 mm TL, and despite these individuals being a component of the exploitable population, we adjusted the estimate downward to account for any large age-1 individuals that may have been part of the sample population. The result was a deflation of the total abundance estimate.
- The number of Red Snapper on pipeline habitat can be difficult to estimate. In some areas, pipelines may be covered by sediment overwash or scoured. Estimating the amount of available pipeline habitat is very difficult. In these cases, multiplying fish density by quantified pipeline lengths may under- or over-estimate population size. Similar to visual surveys discussed above, low visibility (especially near the Mississippi River outflow) may have resulted in population counts of fish actually appearing within the field of view of cameras to be biased low, thus resulting in underestimation of the abundance.
- Habitat mapping was beyond the scope of the project. Consequently, for Mississippi, the mean abundance per artificial reef was expanded to total abundance by multiplying the mean by the number of artificial reefs in the public list of registered artificial reefs. This list is an underestimate of the total number of reefs because artificial reefs are frequently created without seeking a permit. To overcome this negative bias, the number of artificial reefs per unit area in western Alabama waters (determined through a designed survey) can be applied to Mississippi to get an approximate value for the total number of artificial reefs in Mississippi. For natural reefs, depletion estimates did not prove feasible or appropriate. Consequently, visual counts were used to determine the number of fish per natural feature on the assumption that the maximum number of fish seen at a site represented the total. This is a minimal estimate but is believed to represent a small bias because the density of fish on natural hard bottom is generally quite low so most or all fish can be seen. The amount of natural reef habitat (hard bottom) is poorly known so that the application of an estimated mean fish per unit area to the total area of hard bottom is sensitive to error in the amount of hard bottom.
- Several factors could contribute to the towed sled gears in this study leading to an underestimate of actual population size. First, due to visibility concerns (low light levels, turbidity), some Red Snapper within the estimated field of view of towed systems may have been undetected by analysts. This is especially possible in the central and western Gulf, where Mississippi River inputs were high when these areas were surveyed. We do not currently have a siting function estimate for correction (proportion of fish within the viewing window that were actually observed, as a function of water clarity). Thus, this resulted in an underestimate due to these detectability issues. Secondly, if fish sense the presence of the towed camera before they are imaged, such behavioral avoidance will

underestimate abundance (Ryer et al. 2009, Stoner et al. 2008, Trenkel et al. 2004). Both attractive and detractive behaviors are well-documented for various fish survey techniques. In 2019, we began estimating the C-BASS's capture efficiency (analogous to a catchability coefficient) for various target reef fish species, including Red Snapper, by comparing reef fish assemblages observed between the towed camera and Florida Fish and Wildlife's stationary cameras. This work is ongoing, and no final quantitative measures of Red Snapper capture efficiency for the C-BASS, or other towed gear used in this study, are currently available. Our behavioral observations of Red Snapper (and other snapper and grouper species), however, suggest they react to the presence of the gear once they are very near (and imaged) the towed camera (Grasty 2014). These observations coupled with the gear efficiency experiment from the west Florida shelf suggest that Red Snapper are faithfully imaged via the towed camera system, with little negative bias due to behavioral reactions (Keenan, S. et al., *unpublished data*). A third potential bias of towed systems is that some fish (particularly over high-relief natural or artificial structures) are located above the viewing height above bottom imaged by the camera systems. Based on previous research using combined hydroacoustics and simultaneous camera observations, these fish would not have been accounted and lead to and underestimate.

- Towed gear can be influenced by spatial autocorrelation. The tendency of fish to spatially aggregate into clusters may invalidate mean density estimates based on the assumption of stratified random sampling. For some towed estimates we estimated the degree of autocorrelation and sub-sampled at scales to minimize this effect. Specifically, transect data were divided into a series of separate clips and how we included breaks (1 min or more) in data to minimize autocorrelation.
- Local environmental/stochastic biases that we are unable to quantify may lead to uncertainty. For example, nearshore artificial reefs along the Louisiana and northeastern Texas shelf are exposed to a seasonally ephemeral hypoxic zone (dissolved oxygen $<2 \text{ mg l}^{-1}$) associated with high freshwater and nutrient inputs from the Mississippi-Atchafalaya River System (MARS; Rabalais et al. 2002). While hypoxia reduces the availability of suitable habitat for fishes, the high relief of oil and gas platform structures provide Red Snapper and other reef-associated fishes refugia in the well-oxygenated waters overlaying hypoxic bottom waters (Stanley and Wilson 2004; Hazen et al. 2009; Reeves et al. 2018; Munnely et al. 2019, 2020). Therefore, the presence of hypoxic zones may temporarily shift the vertical and/or horizontal distribution of Red Snapper rather than drive changes in overall abundances; however, the magnitude and direction is unknown.

While the team feels it is important to point out these sources of uncertainty to better understand the estimate, provide caution for interpretation, and guide future studies, we suggest this study provides a robust estimate of absolute abundance of age 2+ Red Snapper.

D. High-Reward Tagging Study

Alternative methods for estimating exploitation for Red Snapper in the northern Gulf of Mexico would be valuable as independent sources of information to assist with management decision-making. This is especially true for a stock such as Red Snapper when considering its economic and social importance to the region. Recent regulatory changes have led to more state-level control of Red Snapper management; however, the current catch-at-age model used in the SEDAR process does not provide exploitation rate estimates at these smaller spatial scales. Exploitation rates likely vary across the northern Gulf of Mexico due to spatial gradients in fishing effort and fish size/age structure (e.g., Alabama Artificial Reef Zone, AARZ; Szedlmayer and Shipp 1994, Minton and Heath 1998, Patterson et al. 2001).

Tagging studies have the potential to provide exploitation rate estimates at smaller spatial scales depending on study design considerations. More localized estimates could be valuable for the management of this site-attached species with low adult movement rates (Patterson 2007, Saillant et al. 2010, Karnauskas et al. 2017). Many tagging studies have been conducted for Red Snapper in the northern Gulf of Mexico, but these efforts have typically not been methodologically sufficient to estimate exploitation rates.

Reward tagging studies are one type of mark-recapture approach used to estimate exploitation rates (Cowan et al. 2011, Sackett et al. 2017). This approach usually involves tagging by teams of biologists using standardized methods but then relies on anglers to report the recapture of tagged fish. Anglers are then provided with a reward for reporting these recaptures. This type of study has the potential to increase stakeholder engagement because it provides an opportunity for anglers to become directly involved in the stock assessment process while receiving a monetary reward for their participation (Jentoft and McCay 1995, Coffey 2005, Pita et al. 2010). In addition, the direct contact between biologists and anglers during the tag reporting process provides an opportunity to obtain detailed information on fishing patterns such as depth and habitat type (Hood et al. 2007, Cowan et al. 2011).

The estimation of exploitation from these models is relatively simple within a single fishing season and is obtained as the fraction of fish released with a tag that are reported as recaptured by anglers after adjusting for tag loss, tagging mortality, and angler non-reporting.

We sought to apply this approach to Red Snapper across the northern Gulf from Texas to the Florida Panhandle. The objectives of this study were to (1) estimate regional (state-level) open-season recreational exploitation rates, vulnerability to capture, and tag shedding rates; (2) examine the association between exploitation rates and distance to angler access points such as ports; and (3) evaluate movement patterns of recaptured Red Snapper throughout these regions.

Methods

We used angler tag returns from a high-reward tagging program to estimate Red Snapper recreational exploitation rates, length-based vulnerability to capture, and tag shedding rates for the 2019 fishing season in the northern Gulf of Mexico. We also estimated movement patterns

from a subset of tag returns in which anglers reported the latitude and longitude of the recapture location. Red Snapper were tagged using standardized methods by regional science crews led by the Project PIs aboard cooperator-owned or hired vessels. Captains of hired vessels were vetted to ensure that tagging locations were kept confidential. We then relied on anglers to report the recapture of tagged Red Snapper.

Site Selection

We divided the northern Gulf of Mexico into five regions and attempted to tag 300 fish distributed across a minimum of 30 sites in each region. The regions were: Florida Panhandle, Alabama, East Texas, and West Texas. In each region, sites were selected from lists of known waypoints that contained artificial bottom structures that typically exceeded 0.5 m in vertical relief and were located at a depth of less than 40 m. This maximum depth was chosen because it has been associated with the high survival of released fish (Curtis et al. 2015). In Texas and Florida, these sites were primarily public artificial reef zones and known oil and gas platforms. We also included in the sample set a list of waypoints obtained from cooperating charter boat operators. In Florida, the site list was developed from well-known artificial reefs assembled by the Florida Region PI. In Alabama, sites were selected from targets identified in side-scan sonar surveys of randomly selected 1.0-km square grids inside and to the west of the Alabama Artificial Reef Zone. The Alabama surveys were conducted by the Alabama Region PI since 2011. From these lists, sites were randomly selected. This selection was random in Florida and Alabama but stratified by distance to the nearest port in Texas due to the much longer distances in that region. Our goal was to ensure that our data set contained ample variation in distance to the nearest port so that we could estimate the relationship between that covariate and Red Snapper exploitation rates. We had originally planned to tag Red Snapper in Louisiana, but we had difficulty generating these data. Moreover, rough seas and associated high turbidity pushes much of the Red Snapper fishing effort beyond 40 m. Thus, we did not include release and recapture data from Louisiana hereafter.

Field Methods

Tagging occurred in spring 2019, just prior to the opening of the federal recreational season. At each site, tagging crews attempted to tag a wide size range of Red Snapper by simultaneously employing different gear types and hook sizes. We used hook-and-line with a single hook, and double drop rigs, “sow” rigs, with size 7/0 and 11/0 hooks baited with live or cut bait. Crews generally fished a particular site for 15-30 minutes before moving if catch rates were not sufficient to achieve target numbers of tagged Red Snapper. Crews tagged federally legal-sized (>406 mm TL) Red Snapper with yellow Hallprint PDAT dart tags. Each tag was inserted just below the dorsal fin with a 4 mm diameter needle. The tags were uniquely numbered and included a telephone number for anglers to call to report recaptured fish. The tags also included the words “\$250 REWARD” to ensure that anglers were aware of the reward program if they inspected the tag. One third of the Red Snapper received two tags to enable

estimation of tag shedding rates. Tagging crews limited the number of Red Snapper tagged at each site to 10 to reduce spatial autocorrelation in tag returns. Each tagged Red Snapper was measured prior to release. Fish with prolonged handling time, signs of barotrauma, and hooking injuries were not tagged. After tagging, all fish were released unvented using the SeaQualizer fish descending device. Field data that was recorded included the tagging date, latitude/longitude, depth, and structure description (if known).

Tag Returns

Tag returns were reported by anglers by calling the toll-free number printed on the tags and publicized in print and online media. Voicemails left by anglers were monitored regularly and anglers were called back within 24-48 hours. Once the angler was contacted, a brief questionnaire was administered (see Appendix F). Anglers were asked to report the following information: tag number(s), general location and lat/long coordinates, date, fishery sector (private recreational, charter, commercial), and whether the fish was harvested or released alive. Anglers were also asked whether they were aware of the tagging program prior to capturing the tagged fish. The purpose of this question was to better understand the potential for non-reporting of recaptures due to lack of awareness about the program. Anglers were paid a cash reward of \$250 for reporting recaptures after the physical tag was submitted. This reward amount was set such that it was expected to elicit a high reporting rate as close to 1.0 (i.e., 100%) as possible. We based this assumption on previous studies (Nichols et al. 1991; Denson et al. 2002; Taylor et al. 2006). Nichols et al. (1991) showed that a reward of \$100 resulted in a 100% reporting rate by duck hunters. Denson et al. (2002) also found this amount was sufficient to produce 100% reporting in a tagging study of red drum (*Sciaenops ocellatus*). Adjusting for inflation via the consumer price index resulted in a reward amount of approximately \$200 that was adjusted upward to \$250 to further guard against non-reporting by accounting for unanticipated demographic or socio-economic differences between Red Snapper anglers and anglers/hunters in these previous studies.

We included in the analysis tag returns from recreationally caught Red Snapper that were captured between the opening date of the 2019 fishing season in each state and the end of October of that year. We excluded fish that were captured before the opening date in each state. Tag returns from the commercial fishery were excluded from the analysis because reporting rates of recaptures from the commercial fishery are unknown and are assumed to be much less than 100%. However, we included release data from fish that were ultimately recaptured in the commercial fishery to ensure recreational exploitation rates would be unbiased. Reporting rates of tagged Red Snapper captured in the recreational fishery were assumed to be 100%.

Tag Return Model

We used a Bayesian approach to model the tag return data. The model was fitted to two observed response variables. The first was a categorical variable representing observed fates of each individual fish with respect to capture sector (private vs. charter recreational) and tag retention. The second variable described whether each fish was reported as released alive or harvested after capture to enable an estimate of discard rates. The model estimated regional (Florida, Alabama, East Texas, West Texas) and sector-specific (private vs. charter) recreational exploitation rates, the tag shedding rate, length-based vulnerability parameters, the live release rate, site-specific fishing mortality anomalies and their variance, and the tagging mortality rate.

The model assumed that the population was closed to natural mortality, commercial fishing mortality, and movement. By excluding movement, tag returns were modeled as a function of the release location. Clearly these factors have the potential to affect the fates of tagged Red Snapper and thus estimates of instantaneous fishing mortality rates but finite exploitation rate estimates for the recreational fishery should be unbiased.

The sector-specific (s) instantaneous capture rate (F'_{ijrs}) for an individual fish i released at site j in region r was generated as the product of a site- and region-specific fully-vulnerable capture rate \bar{F}'_{jrs} and region-specific length-based vulnerability to capture for fish i (v_{ir}):

$$(28) F'_{ijrs} = \bar{F}'_{jrs} v_{ir}$$

The \bar{F}'_{jrs} was modeled as a log-linear function of the straight-line distance (d_{jr} ; km) between each site and the nearest port to account for systematic spatial variation in fishing effort due to accessibility and cost factors:

$$(29) \bar{F}'_{jrs} = e^{B0_{rs} + B1d_{jr} + \omega_{jr}},$$

where $B0_{rs}$ is the regional and sector-specific intercept, $B1$ is the slope of the relationship that is assumed constant across regions and between sectors, and ω_{jr} is a site-specific capture rate anomaly. The capture rate anomalies were incorporated to account for non-independence of the fates of fish released at the same site due to site-specific variation in fishing effort and/or catchability. The anomalies were assumed to be drawn from a normal distribution with mean = 0.0 and an estimated precision (τ) that was drawn from an uninformative Gamma prior distribution (shape=0.001, rate=0.001). An important assumption is that the capture rate anomalies were shared between the two fishery sectors. This approach presumes that the spatial distribution of effort and/or catchability of the two sectors was identical. The $B0_{rs}$ and $B1$ were drawn from uninformative normal prior distributions ($\mu = 0.0$ and precision = $1e-12$).

Vulnerability was modeled as an exponential-logistic function (Thompson 1994) of the total length (l_{ir}) of each fish upon release in region r . The function can accommodate a wide range of sigmoidal and dome-shaped vulnerability curves:

$$(30) v_{ir} = \left(\frac{1}{1-\gamma_r} \right) \left(\frac{1-\gamma_r}{\gamma_r} \right)^{\gamma_r} \left(\frac{e^{\alpha_r \gamma_r (\beta_r - l_{ir})}}{1 + e^{\alpha_r (\beta_r - l_{ir})}} \right),$$

where γ_r describes the degree to which vulnerability is dome-shaped, α_r determines the rate of change in vulnerability as a function of length, and β_r represents the length at 50% vulnerability. The β_r was modeled as a function of the length at peak vulnerability (X_r):

$$(31) \beta_r = X_r - \left(\frac{1}{\alpha_r}\right) \ln\left(\frac{1-\gamma_r}{\gamma_r}\right).$$

The prior distribution for γ_r was a weakly informative Beta (2,2) distribution. This distribution provided a slightly higher probability mass near 0.5 than at the boundaries to prevent the γ_r parameter from becoming entrenched at the boundaries. The prior distribution of α_r was an uninformative log-Normal ($\mu = 0.0$, $\text{precision} = 1e-12$). The X_r were given uniform prior distributions with bounds set at the minimum and maximum length of fish observed in the study to ensure that vulnerability achieved a maximum of 1.0 across the observed size range. An important assumption was that vulnerability was assumed to be identical between the private and charter sectors.

Sector-specific finite capture rates of individual fish (i.e., probability of capture; U_{ijrs}) were obtained by assuming that private and charter sector fishing mortality occurred simultaneously:

$$(32) U'_{ijrs} = \frac{F'_{ijrs}}{\sum_s F'_{ijrs}} (1 - e^{-\sum_s F'_{ijrs}})$$

The region-specific tag shedding rate (T_r) was estimated as the probability that an individual tag would be shed immediately after fish release. We did not include a term for long-term tag shedding because we found no relationship between the proportion of recaptures with a shed tag and the number of days at large (logistic regression: $\chi^2 = 3.39$, $df = 1$, $P = 0.07$). The prior distribution of the tag shedding rate was an uninformative Beta (1,1). Thus, the probability of a fish tagged with t tags retaining z tags was obtained via:

$$(33) Q_{rtz} = \begin{cases} 1 - T_r & \text{for } t = 1, z = 1 \\ 2T_r(1 - T_r) & \text{for } t = 2, z = 1 \\ (1 - T_r)^2 & \text{for } t = 2, z = 2 \end{cases}$$

We collected no data to inform tagging mortality rates (m) in this study. Therefore, informative Beta priors were specified to result in a mean tagging mortality of 0.1 (CV of 0.15) to roughly approximate predictions from a meta-analysis by Campbell et al. (2010).

There were five possible recapture fates (f) for double tagged ($t = 2$) Red Snapper i released at site j in region r , and three fates for single tagged fish ($t = 1$). The five possible fates were: fate 1, not reported as captured; fate 2, reported as captured in the private sector ($s = 1$) with both tags retained ($z = 2$); fate 3, reported as captured in private sector with one tag retained ($z = 1$); fate 4, reported as captured in the charter sector ($s = 2$) with both tags retained ($z = 2$); fate 5, reported as captured in the charter sector with one tag retained ($z = 1$). For single-tagged fish, fates 2 and 4 were undefined, and any fish with shed tags would have been unobservable. The probability of each of the observable fates (fates 2-5) was modeled as a function of spatially

invariant tagging mortality (tm), regional tag retention, finite capture rates, and spatially invariant angler reporting rate (λ) via:

$$(34) \pi_{ijrf} = (1 - tm)Q_{rt(ijr)z(f)}U'_{ijrs(f)}\lambda$$

The probability of not being reported as captured ($f = 1$) was modeled as:

$$(35) \pi_{ijrf(1)} = 1 - \sum_{f=2}^5 \pi_{ijrf}$$

The prior distribution for the tagging mortality rate was constructed from three previous studies (Campbell et al. 2010, Bohaboy et al. 2019, Curtis et al. *unpublished*) by taking a weighted average of the individual estimates, weighted by their precision. The prior was Beta distributed with shape parameters set to match the weighted mean of 0.19 and CV of 0.21. The observed fate data for each released Red Snapper was assumed sampled from a categorical distribution with probability π_{ijrf} .

Anglers were asked to report whether or not each captured fish was released (d_{ijr}). The observed d_{ijr} data were sampled from a Bernoulli distribution with a regional discard rate D_r . The prior distributions for the D_r were assumed an uninformative Beta (1,1). Discard rates were used to estimate fully vulnerable fishing mortality rates that accounted for the mortality of harvested fish and the death of a proportion (dm) of the discards:

$$(36) \bar{F}_{jrs} = \bar{F}'_{jrs}D_rdm + \bar{F}'_{jrs}(1 - D_r),$$

where dm is the discard mortality rate (i.e., proportion of released fish that die). The discard mortality rate was not estimated in this study. Instead, we constructed an informative Beta prior distribution from a meta-analysis by Campbell et al. (2010) for recreational surface releases which had a mean of 0.1 and CV = 0.15. Estimates of the exploitation rates that included discard deaths were made by substituting \bar{F}_{jrs} for the F'_{ijrs} in equation 5, above.

Eight different candidate models were constructed to evaluate the degree of empirical support for regional variation in vulnerability, tag shedding rates, and release rates. For each of these three factors, two models were constructed, one that allowed for regional variation and one that did not. All possible combinations of models were considered which resulted in eight candidate models. The posterior distributions of each model were simulated via Markov Chain Monte Carlo simulation. Two chains were initiated at different starting values and run for 10,000 iterations after a burn in period of 2,000 iterations. Convergence was confirmed by inspection of trace-plots, and posterior distributions, and computing the Gelman-Rubin statistic for all model parameters. Model comparison was carried out by estimating the Watanabe-Akaike Information Criterion (WAIC; Watanabe 2010, Gelman et al. 2014). Model uncertainty was considered by calculating model weights and weighted-average posterior distributions.

Movement Analysis

Movements of recaptured Red Snapper were assessed by comparing release locations with angler-reported recapture locations. First, we evaluated broad-scale movements among the four regions by tallying the number of Red Snapper that were recaptured within their release region and those that were recaptured in a different region. Next, movement distance and direction were estimated for recaptured Red Snapper in which anglers provided latitude/longitude coordinates of the recapture location. For these recaptures, we measured the straight line-distance between the release and recapture locations and the direction in degrees. The average movement distance and direction was then compared among release regions.

Results

Tag releases and returns

Field crews tagged 1,208 Red Snapper greater than 406 mm total length in spring 2019. There were 187 fish tagged at 33 sites in Florida, 335 tagged at 64 sites in Alabama, 386 tagged at 31 sites in the Eastern Texas Region, and 300 tagged at 35 sites in the Western Texas Region (Figure 27). Anglers captured and reported 326 of these tagged fish through October 2019 and thus these were included in the Bayesian tag return model (Figure 28). Returns continued into 2020 with an additional 51 tags reported through October (Figure 29). At the time of the release of this report (March 2021), a total of 425 fish had been recaptured across all Gulf states for a total recapture rate of 32%, with Florida reporting the highest recapture rate at 43% and AL/MS and Texas both reporting 28% (Table 10). Returns from the private recreational sector were highest across regions, with returns from the charter sector coming in at approximately half of private returns. Tag returns from the commercial sector were lowest with only 11 returns, and as previously mentioned, tagged fish that were ultimately recaptured in the commercial sector were included in the number of fish tagged but were excluded as a recapture in the analysis of recreational exploitation rates.

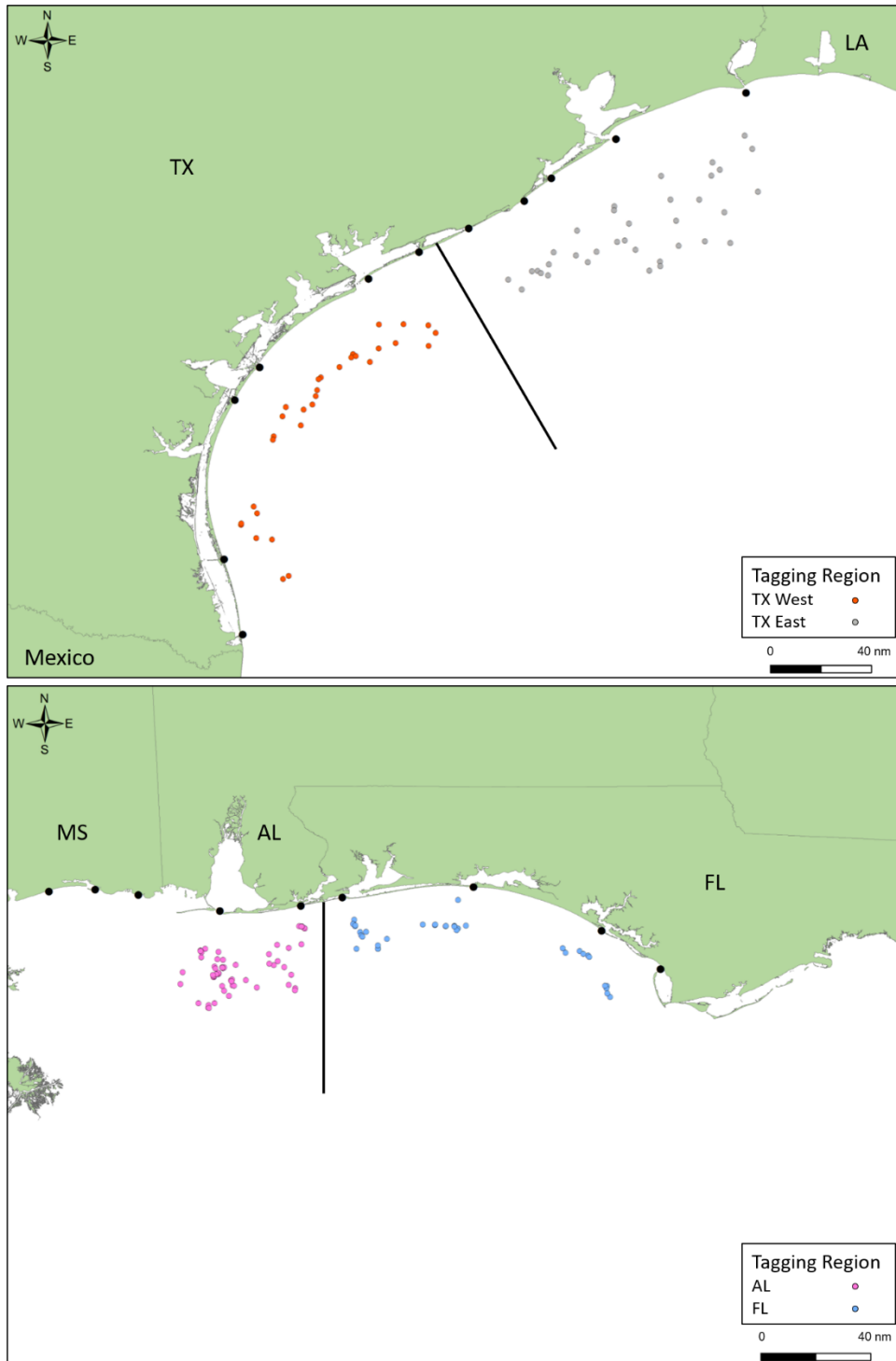


Figure 27. Release locations (open circles) of tagged Red Snapper in TX (upper panel) and AL/FL (lower panel). Port locations are indicated by the closed circles. The solid lines represent the dividing line between the West and East Texas regions and the Alabama and Florida regions.

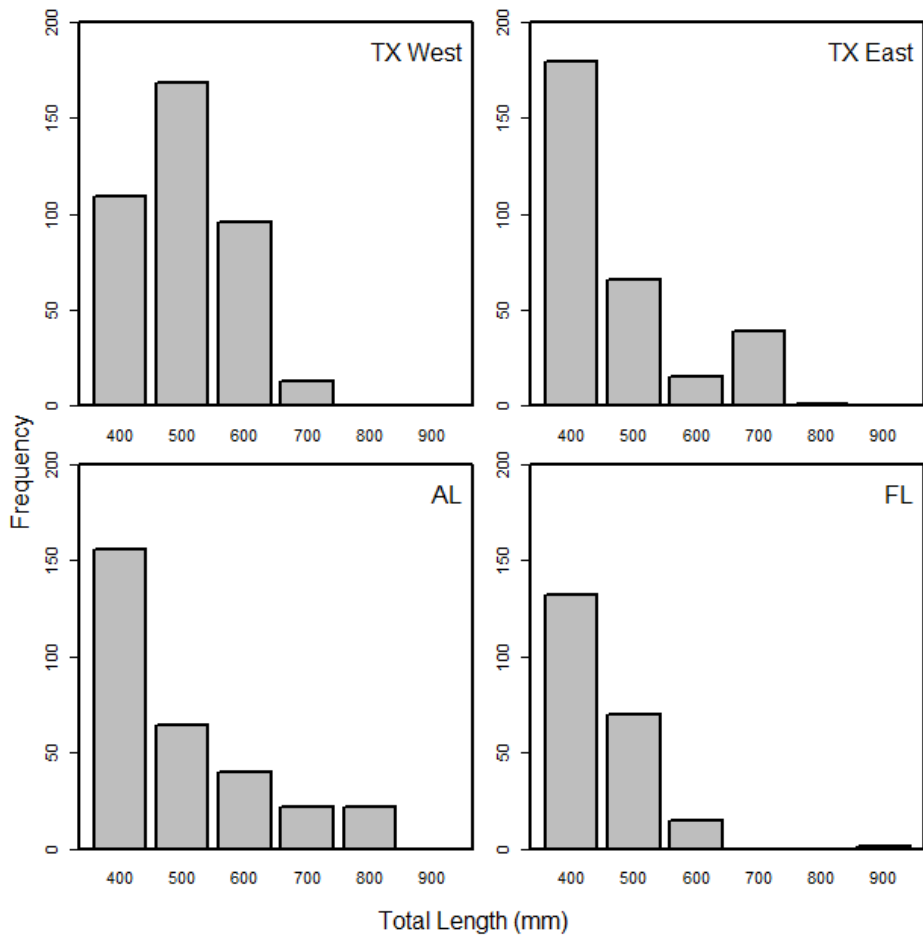


Figure 28. The number of >406-mm Red Snapper tagged in each total length interval (x-axis) and region.

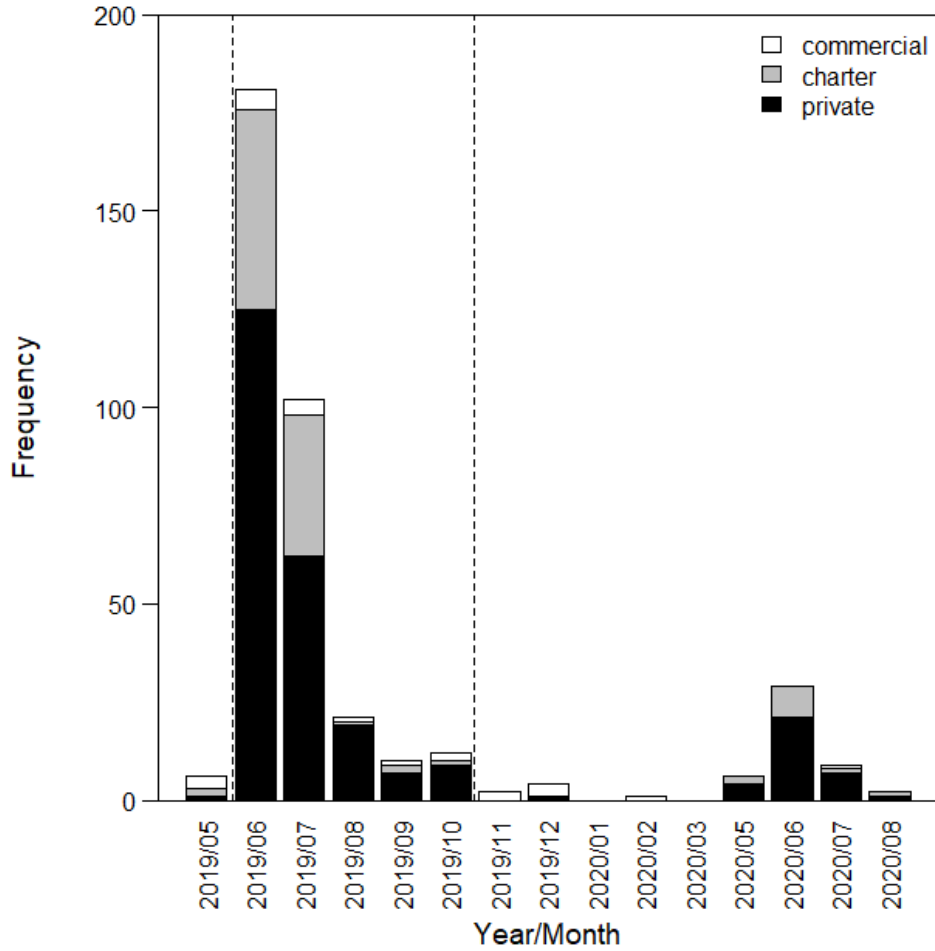


Figure 29. The monthly number of tagged Red Snapper reported by anglers as captured in the commercial (white), charter (grey), or private (black) sector. The vertical dashed lines enclose the period of months to which the Bayesian model was fitted.

Table 10. Total number of recaptures by state through the end of 2020 with percentage (%) calculated from the number scientifically tagged.

State	Tagged	Recaptured	Percentage
MS/AL	342	97	28%
FL	310	133	43%
TX	697	195	28%
Total	1349	425	32%

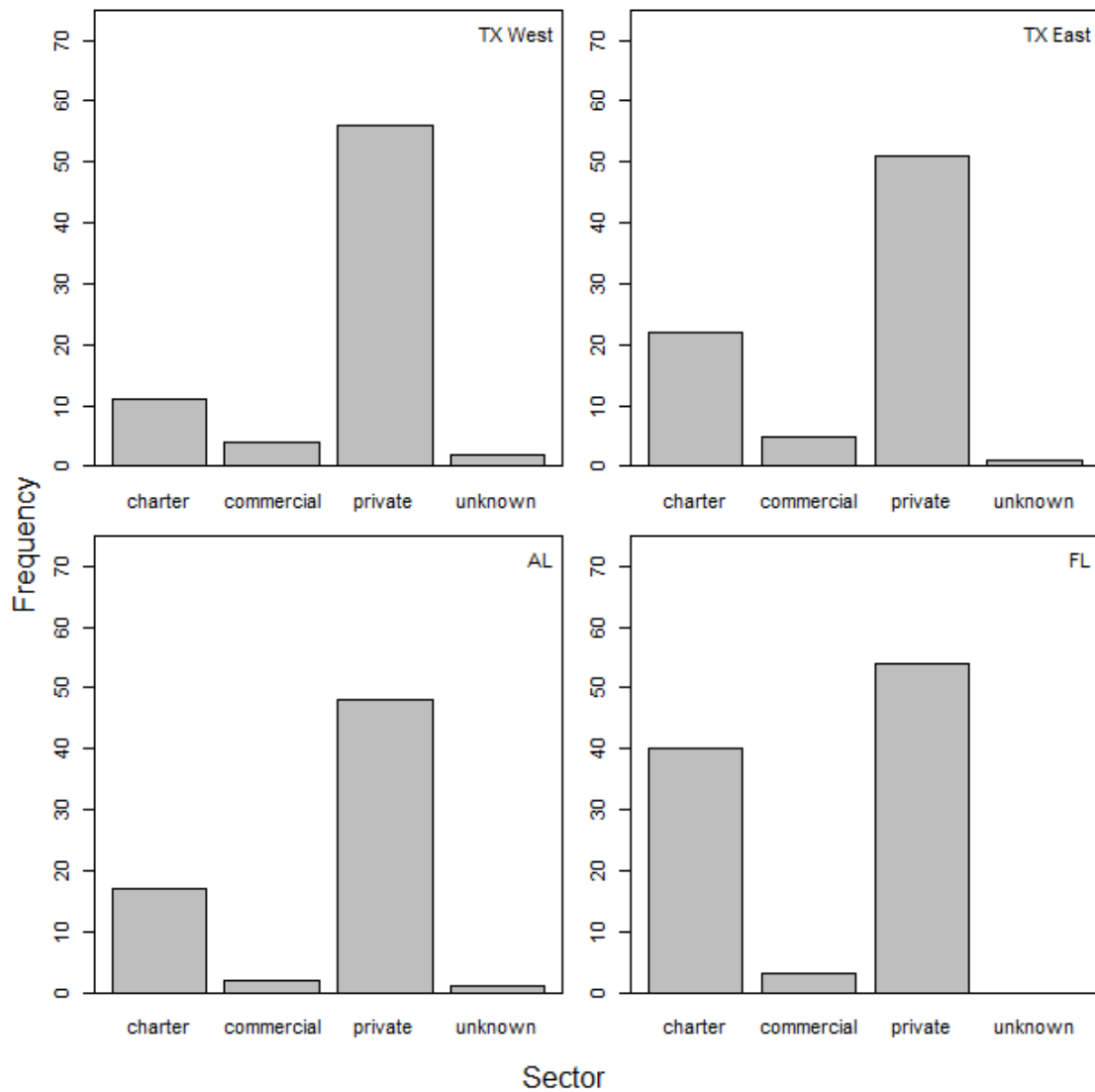


Figure 30. The number of Red Snapper recaptured through August 2020 in each fishery sector and region.

A majority of anglers (60%) that reported a tag were not aware of the tagging study prior to capturing a tagged fish (Figure 31a) and became aware of the study by inspecting the tag (Figure 31b). The next most prevalent mode of awareness was word of mouth (20%). Less than 10% of anglers reported becoming aware of the study by viewing social media and websites (Figure 31b).

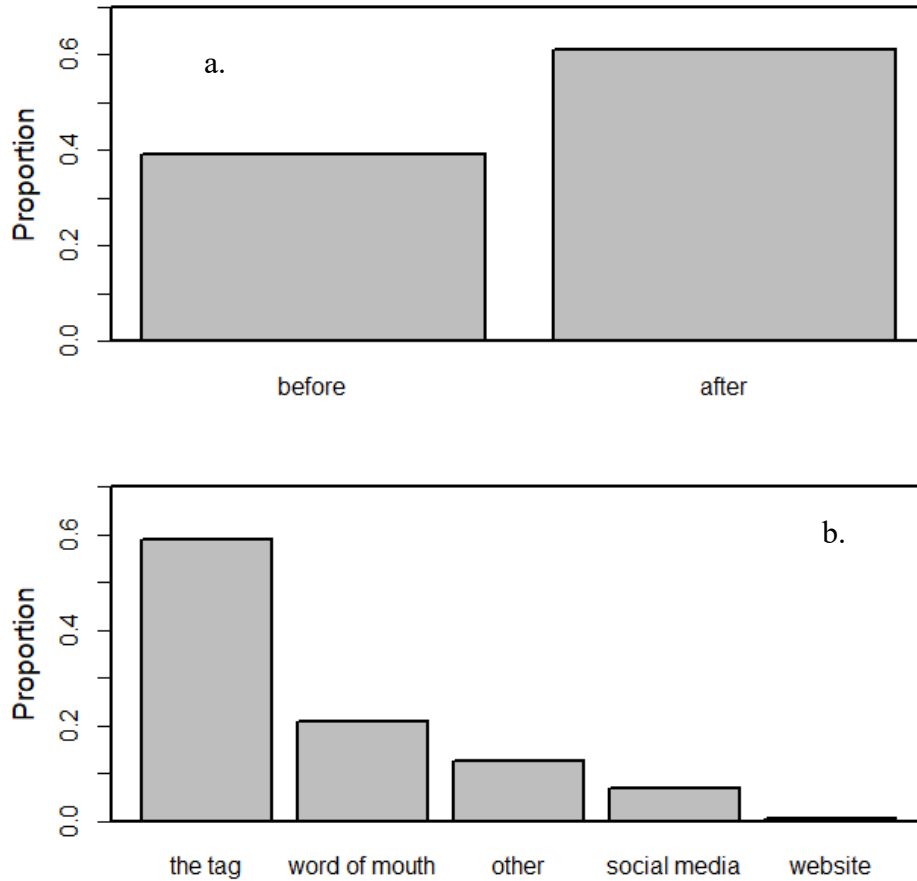


Figure 31. (a) Proportion of anglers reporting a tag that became aware of the Great Red Snapper Count tagging program either before or after they captured a tagged fish. (b) Proportion of anglers reporting a tag that became aware of the Great Red Snapper Count via each of five different methods. Anglers were queried verbally over the telephone by tag return clerks when the anglers called to report their capture of a tagged Red Snapper.

Movement

Movement of Red Snapper among regions was rare, and movement distances within regions were generally small. Two Red Snapper that were tagged in Alabama were subsequently recaptured in Florida (Table 11). All other Red Snapper were recaptured in the same region in which they were tagged. The average absolute distance moved by individual Red Snapper between release and recapture locations ranged from 5 to 15 km among regions (Figure 32a). An analysis of movement distance and direction indicated that the average movement vector ranged from 1 to 3 km from the release location across regions (Figure 32b), and the average direction (i.e., degrees from due N) was a small negative value in West Texas and Alabama indicating a general NNW movement (Figure 32c). Average movement direction of Red Snapper released in East Texas and Florida indicated a general westward movement ($\sim -90^\circ$; Figure 32c).

Table 11. The number of Red Snapper released in each region (rows) that were subsequently recaptured by anglers through August 2020 in each region (columns).

Release Region	Recapture Region			
	FL	AL	TX East	TX West
FL	95	0	0	0
AL	2	86	0	0
TX_east	0	0	105	0
TX_west	0	0	0	83

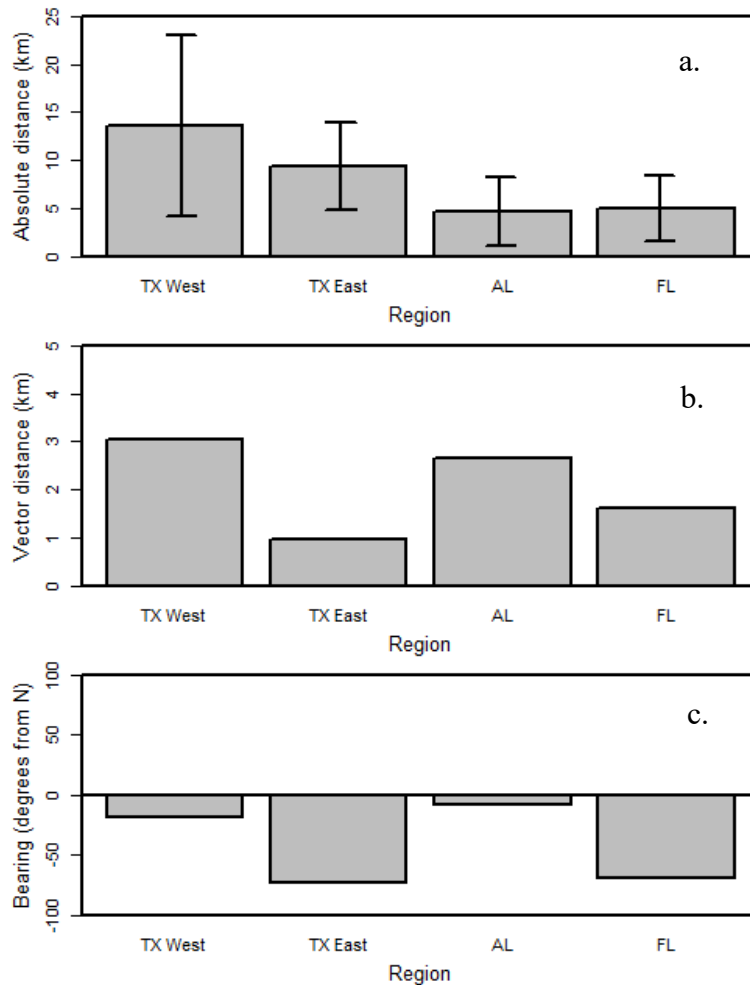


Figure 32. Regional average (a) absolute distance between release sites for tagged Red Snapper and angler-reported recapture locations, (b) length of the mean movement vector, and (c) bearing (degrees from due North) of the mean movement vector. A bearing of 0° indicates movement to the north, 90° indicates east, -180° or 180° south, and -90° west. Error bars represent 95% confidence intervals.

Tag return model

The tag return model with the minimum WAIC allowed for regional variation in vulnerability but assumed a spatially-invariant rate of tag shedding and probability that a legal Red Snapper would be caught and released by anglers (Table 12). The second-ranked model assumed spatially invariant vulnerability, tag shedding, and release rates. The entire set of eight models was separated by no more than 2.5 WAIC units. Thus, we computed model weights and reported model averaged posterior distributions for each of the parameters that incorporated results from each of the eight models.

Table 12. Watanabe-Akaike Information Criterion (WAIC), effective number of parameters (pD), and model weight for eight models representing different combinations of regional or spatially-invariant tag retention, vulnerability to capture, and/or the proportion of fish released by anglers after capture.

Model	pD	WAIC	Weight
tag retention(\cdot), vulnerability(\cdot), release(\cdot)	24.09	586.8	0.16
tag retention(\cdot), vulnerability(\cdot), release (r)	25.14	587.8	0.1
tag retention(\cdot), vulnerability(r), release(\cdot)	25.3	586.03	0.24
tag retention(\cdot), vulnerability(r), release(r)	26.17	587.33	0.12
tag retention(r), vulnerability(\cdot), release(\cdot)	24.3	588.54	0.07
tag retention(r), vulnerability(\cdot), release(r)	24.99	588.54	0.07
tag retention(r), vulnerability(r), release(\cdot)	24.85	587.7	0.1
tag retention(r), vulnerability(r), release(r)	25.87	587.09	0.14

Posterior median estimates of fully-vulnerable private sector exploitation rates across sites ranged from 0.18 in the West Texas region to 0.44 in the Florida Panhandle region (Figure 34). Fully vulnerable charter exploitation rates ranged from 0.06 in West Texas to 0.22 in East Texas (Figure 34). Coefficients of variation on these exploitation rate estimates were less than 0.25 for the private sector estimates and less than 0.35 for the charter sector. After accounting for regional patterns in vulnerability and the size distribution of the tagged population, estimates of the realized exploitation rate of the tagged population of Red Snapper in each region ranged from 0.15 in West Texas to 0.28 in Florida for the private sector (Figure 33), and 0.05 in West Texas to 0.16 in East Texas for charters (Figure 33). Coefficients of variation on these exploitation rate estimates were less than 0.15 for the private sector and less than 0.3 for the charter sector.

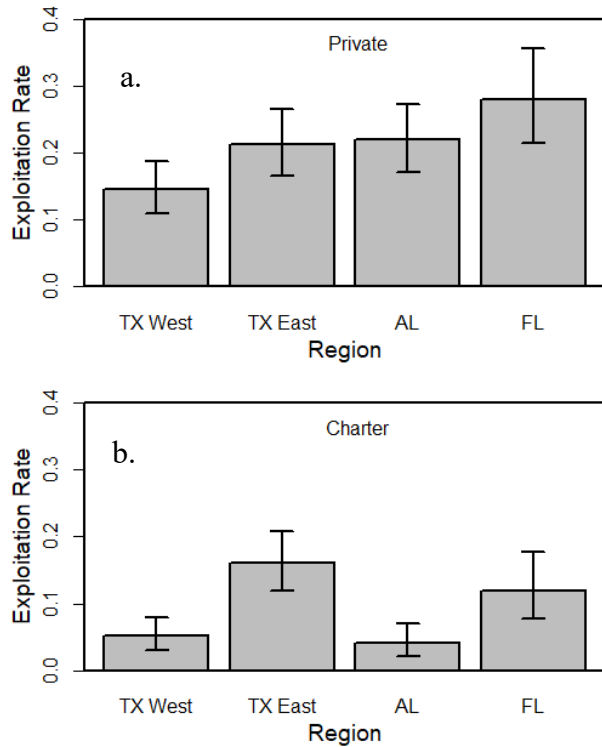


Figure 33. The posterior distributions of the model-averaged regional exploitation rates (grey bars) of the tagged population of Red Snapper by the private (a) and charter (b) sectors. The exploitation rates are obtained by averaging over the predicted exploitation rates of the individual Red Snapper released in each region. The bars represent the posterior medians and error bars depict the 95% credible intervals.

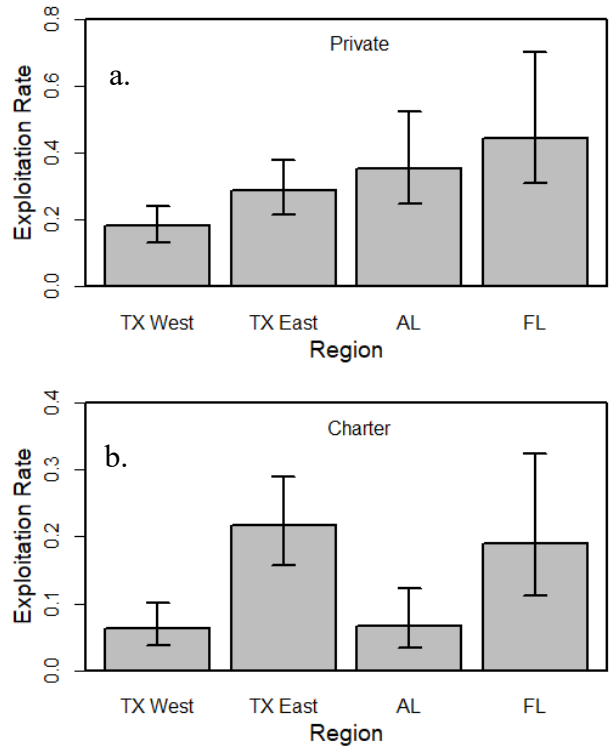


Figure 34. The model-averaged posterior medians of regional fully vulnerable exploitation rates for the private (a) and charter (b) sectors. The error bars depict the 95% credible intervals. The exploitation rates were obtained by averaging the site-specific fully-vulnerable exploitation rates in each region.

Site-specific fully-vulnerable exploitation rates were negatively related to the distance of the site to the nearest port (Figure 35). The posterior median of the slope estimate for this relationship was -0.018 (95% CI: -0.031, -0.005). The 95% credible interval on the slope did not include zero for each of the eight models. This slope estimate is consistent with an 1.8% decline in the instantaneous fishing mortality rate per additional km from the nearest port. For example, under this slope estimate, fishing mortality would be expected to be 49% lower at a site 60 km from the nearest port when compared with another site that is 20 km from the nearest port.

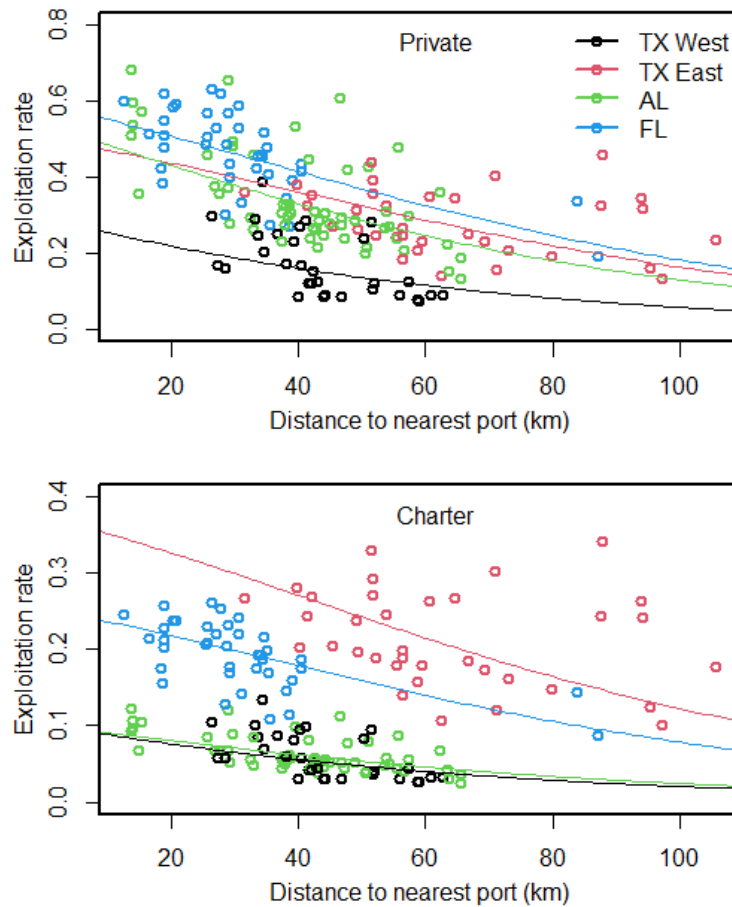


Figure 35. Site-specific exploitation rates as a function of distance to the nearest port for the private (upper) and charter (lower) sectors. The open circles are the model-averaged median exploitation rates for each site at which at least one Red Snapper was tagged and released. The lines show the model averaged posterior median relationship. The colors indicate the four regions.

Vulnerability was dome-shaped in each of the regions (Figure 36). There was a weak west-east gradient in peak vulnerability with the peak occurring between 500 and 600 mm total length interval in Texas, but at or above 600 mm in Alabama and Florida. Credible intervals on the posterior vulnerability curves indicated a substantial amount of uncertainty in these patterns. Some of this uncertainty was likely due to variation in the observed fraction of recaptured Red Snapper as a function of length and small sample sizes. For example, in the East Texas region, returns of 700-800 mm Red Snapper were much higher than predicted by the model. In Florida, few Red Snapper greater than 600 mm were tagged and thus the dome-shaped pattern in that region was based entirely on the lack of recaptures of a single Red Snapper in the 650-700 mm length interval.

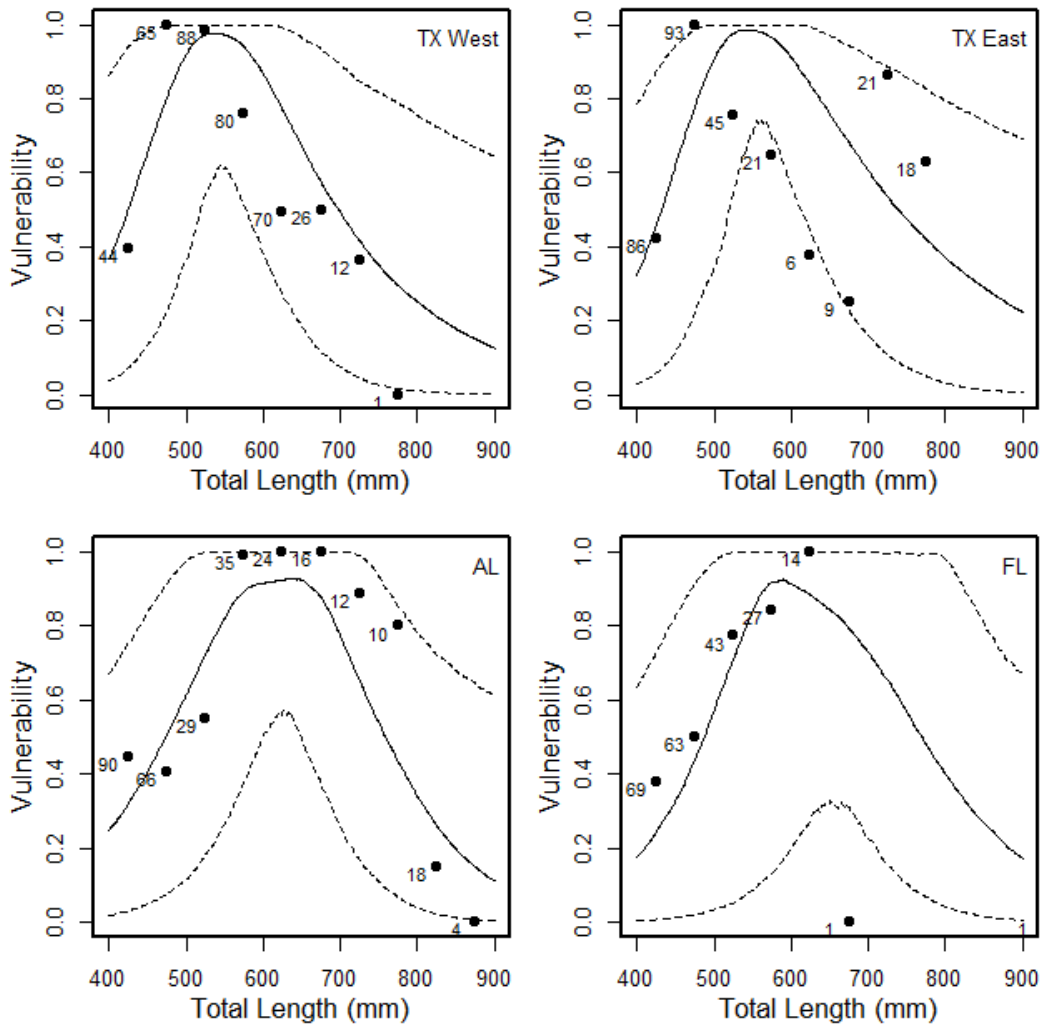


Figure 36. The posterior distribution of vulnerability to capture as a function of total length (mm) in each region. The solid line represents the model-averaged posterior median vulnerability and the dashed lines depict the 95% credible intervals. The filled circles depict the proportion of tagged Red Snapper in 50-mm length bins (scaled to the maximum proportion across bins) that were reported captured by anglers. The numbers indicate sample sizes.

The posterior median tag shedding rate ranged from 0.081 in East Texas to 0.091 in Florida. Coefficients of variation in the tag shedding rates ranged from 0.34 in East Texas to 0.41 in Florida. Under these tag shedding rates, approximately 15% of double-tagged fish were predicted to be returned with only one tag remaining attached, which generally matched observed tag shedding observations from returns of double-tagged fish (Figure 37).

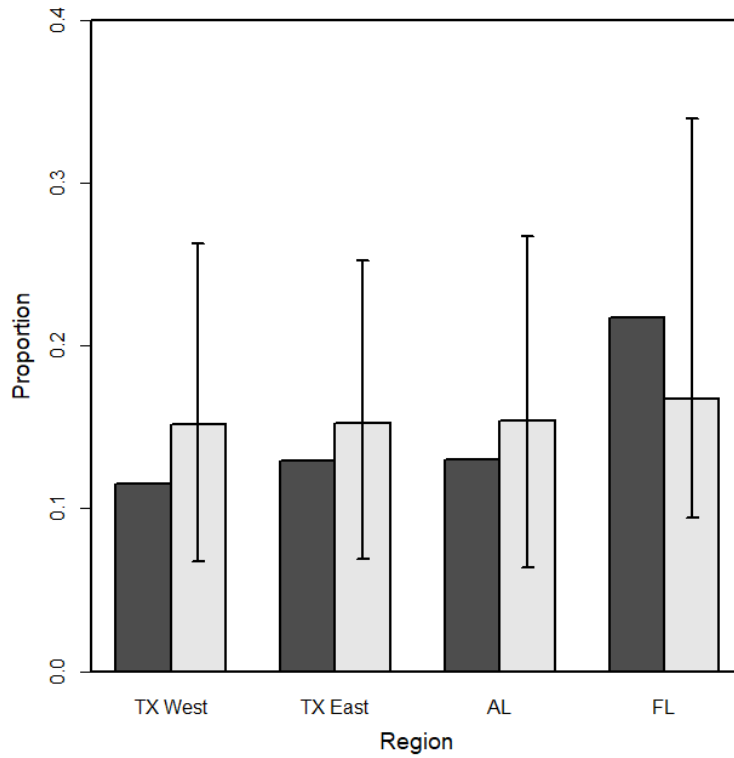


Figure 37. Observed (black bars) and model-averaged posterior median (grey bars) of the regional predicted proportion of double-tagged Red Snapper recaptured with one shed tag. The error bars represent 95% credible intervals.

The posterior median rate at which captured Red Snapper were released by anglers ranged from 0.14 in Alabama to 0.16 in Florida (Figure 38). Coefficients of variation in the release rates ranged from 0.20 in East Texas to 0.27 in Alabama.

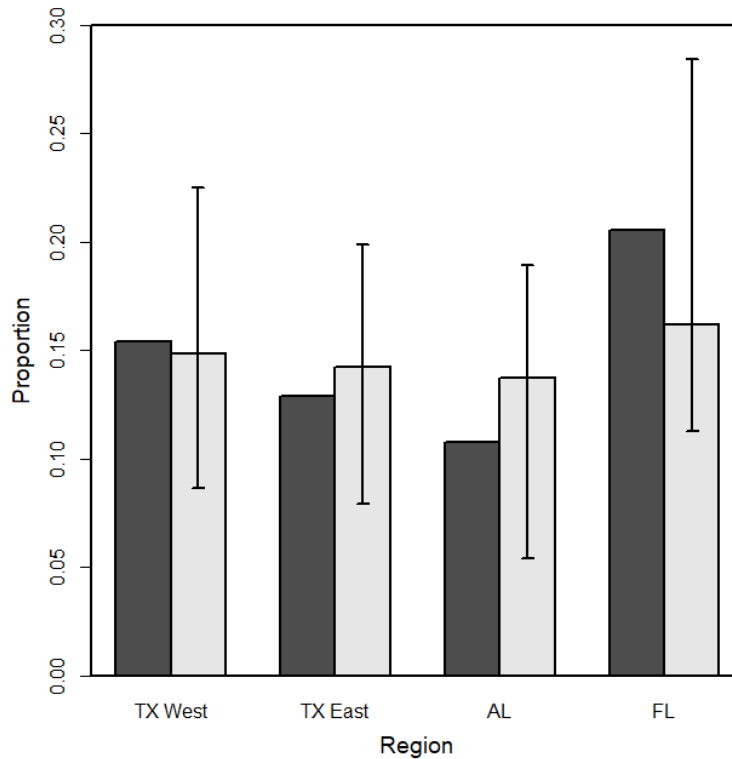


Figure 38. Observed (black bars) and model-averaged posterior mean (grey bars) proportion of recaptured Red Snapper that were released alive by anglers in each region. The error bars depict the 95% credible intervals.

The model-predicted tag returns of Red Snapper by region, sector, number of tags, and fish length generally captured patterns of variation in the observed tag returns (Figure 39, Figure 40). Uncertainty in the predicted tag returns was quite high for low frequency observations such as the returns of double-tagged fish, particularly those with a shed tag (Figure 40).

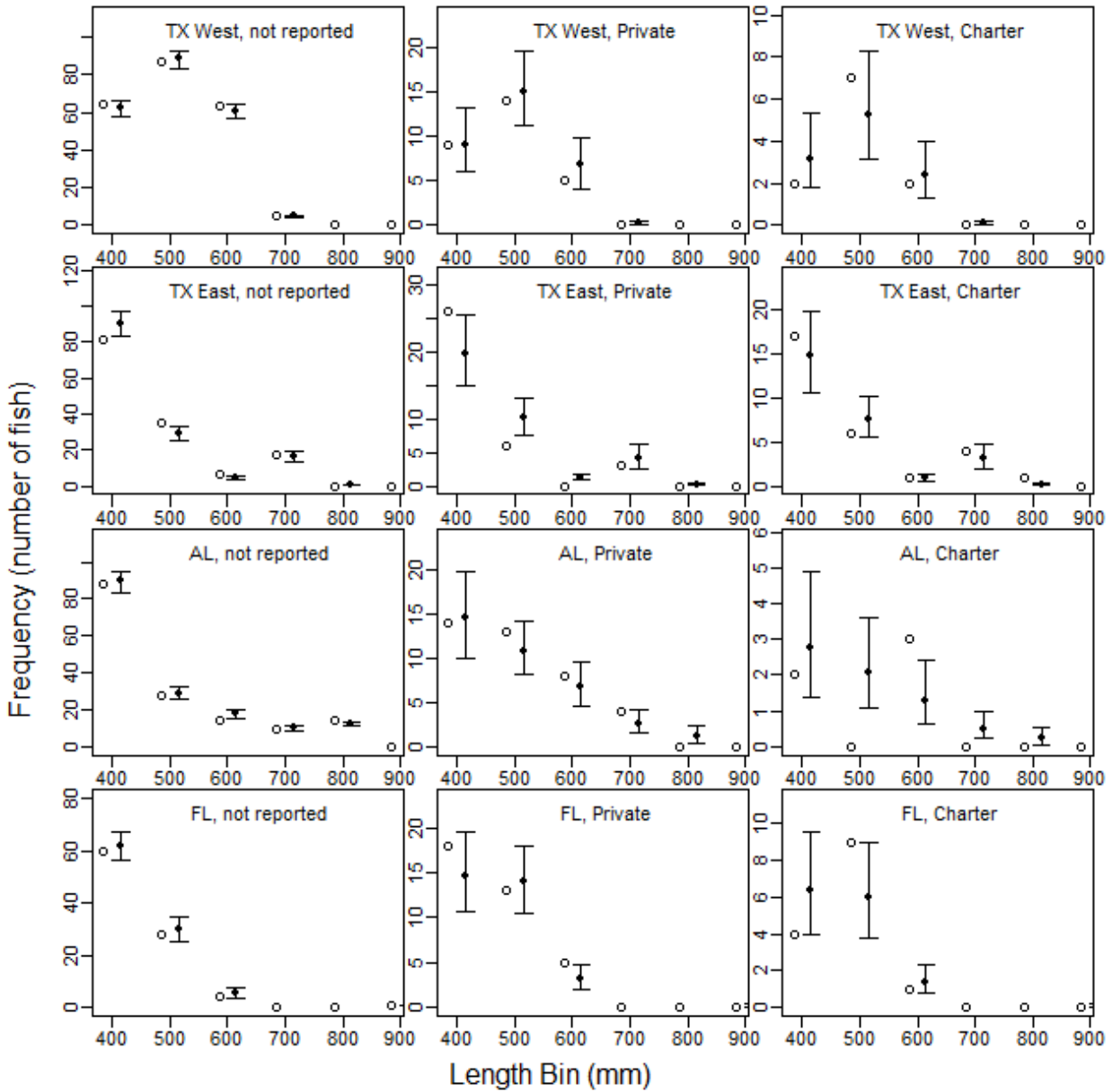


Figure 39. Observed (open circles) and predicted (closed circles and error bars) number of single tagged Red Snapper that were not reported as captured (left column), captured in the private sector (middle column), or captured in the charter sector (right column) as a function of fish length (mm; x-axis) in each of the regions (rows). Closed circles represent the model-averaged posterior medians and error bars depict the 95% credible intervals.

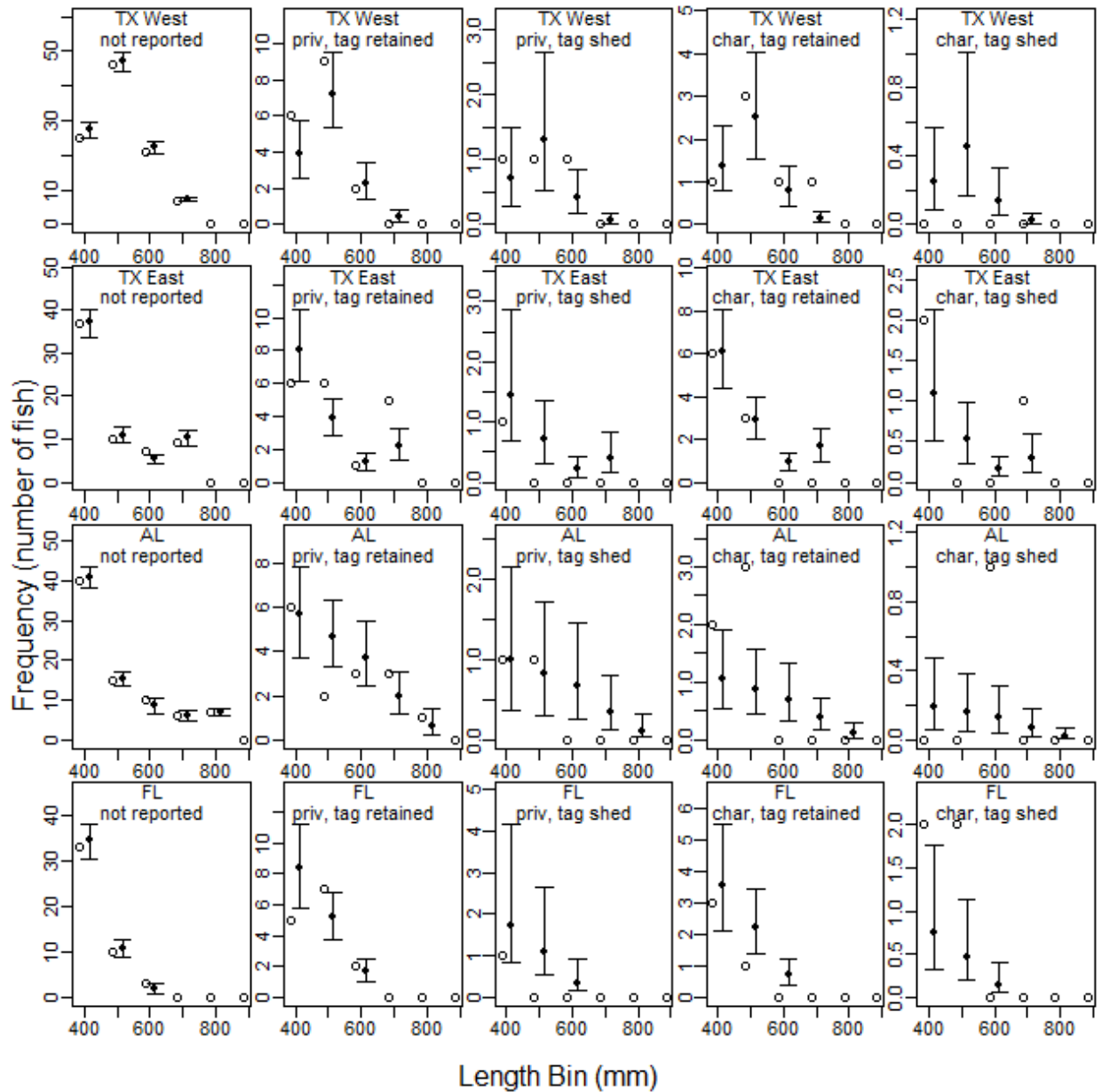


Figure 40. Observed (open circles) and predicted (closed circles and error bars) number of double-tagged Red Snapper per length bin (mm; x-axis) that were not reported as captured (left column), captured in the private sector with tags retained (center left) or with one shed tag (middle), or captured in the charter sector with tags retained (center right) or one shed tag (right) in each region (rows). Closed circles represent the model-averaged posterior medians and error bars depict the 95% credible intervals.

Tagging Implications

The tagging-based total (i.e., private + charter) recreational exploitation rates estimated for the 2019 fishing season likely exceeded 0.2 in each of the regions. These estimates are substantially higher than the most recent estimate of 0.052 from the SEDAR model (SEDAR 2018). However, our estimates were similar to estimates from a previous Red Snapper tagging

study conducted in Alabama from 2016-2018 which reported a recreational exploitation rate of 0.2 at sites less than 36.5 m deep (Sackett et al. 2018). Previous telemetry-based estimates of Red Snapper fishing mortality on artificial reefs in Alabama were also high, averaging 0.44 yr^{-1} from 2012-2014 (Williams-Grove and Szedlmayer 2016). Differences between these tagging studies and the SEDAR assessment likely resulted from the restriction of tagging to high-relief artificial reefs in relatively shallow water (<40 m in our study). These tagging sites harbor a subset of the Red Snapper population that likely experiences higher fishing effort than fish in deeper water or associated with lower-relief structure that is more difficult for anglers to locate with sonar. Our analysis indicated that sites closer to ports, even within our relatively restricted sampling frame, experienced higher exploitation than those sites located farther out. This finding suggests that the fraction of the stock located farther offshore will likely be protected from high recreational exploitation. This spatial gradient in exploitation could drive down the overall exploitation rate on the stock so long as the spatial distribution of the stock does not match the distribution of exploitation. Clearly, broad conclusions regarding the exploitation status of the entire Red Snapper stock should not be based exclusively on exploitation rate estimates from tagging at these shallow sites that are especially accessible to angling.

An important contribution of our work is the broad spatial scale of the project and the estimation of the magnitude of decline in exploitation with increasing distance from port. Our study has a much larger spatial extent than any previous study to estimate Red Snapper exploitation. Previous studies have been conducted at the state (Sackett et al 2018) or artificial reef complex (Williams-Grove and Szedlmayer 2016) scale. Our study indicated that exploitation was quite high at these sites in all regions but generally increased from west to east. Our estimates of declining exploitation with distance from port could be used to construct heat maps of exploitation as a function of distance from ports. Spatially-explicit exploitation estimates could be combined with spatial estimates of Red Snapper abundance (i.e., the Great Red Snapper Count) to attempt to quantify population-level recreational exploitation rates. Another avenue for application of our tagging-based exploitation estimates would be to estimate abundance by dividing landings by exploitation. This approach would require landings data from an identical sampling frame as the tagging study, which would require current creel survey designs to be altered.

We found that exploitation by the charter sector was less than that of private anglers, which is qualitatively consistent with the quota allocation between these two sectors. Two important assumptions that may affect the estimation of the relative magnitude of the two sectors was that our model assumed that length-based vulnerability and the site-to-site variation in exploitation was identical between the two sectors. These assumptions would be violated if the spatial distribution of the two sectors differed. This assumption was likely violated to some degree, but the magnitude of the potential bias is unknown. Estimation of sector-specific vulnerability and spatial exploitation patterns would require a much larger number of fish to be tagged, which may be cost-prohibitive. We also cannot rule out that angler reporting rates were similar for both sectors.

Another important contribution of this study is the direct estimation of length-based vulnerability to capture. Our findings agree with previous estimates from the Sackett et al. (2018) tagging study in Alabama and the SEDAR assessment that vulnerability is dome-shaped with respect to length/age. Our finding of peak vulnerability of between 600 and 700 mm agreed with Sackett et al. (2018). Although this is an important finding, it remains uncertain whether these patterns could be at least partially explained by size-dependent angler reporting of tags. For example, if anglers are more likely to harvest the largest Red Snapper within the legal range, and if anglers are more likely to notice and/or inspect tags on harvested fish, then relatively small legal Red Snapper would be under-reported. This reporting bias could strengthen a dome-shaped vulnerability curve, and/or shift the peak toward larger size classes. Understanding the potential for this source of bias in vulnerability estimates (and exploitation estimates) would require independent estimates of length-based reporting, which would be challenging to estimate.

An important assumption of our study was that anglers reported 100% of tagged Red Snapper captured in the recreational fishery. Anglers may fail to report tags for a variety of reasons including (1) the reward is not large enough, (2) failure to notice tags in the field, or (3) animosity toward the science and/or management process. We attempted to address the first possibility by employing a \$250 reward, but we cannot confirm that this amount was sufficient to eliminate nonreporting. Surprisingly, few studies have estimated the relationship between the reward amount and angler reporting rate. We set the reward amount in excess of amounts that have been associated with full reporting in previous studies, after adjusting for inflation (Nichols et al. 1991, Denson et al. 2002, Taylor et al. 2006, Meyer et al. 2012), but certainly no studies have been conducted on this topic for Red Snapper. Failure of anglers to notice the tag or inspect it closely could relate to their prior awareness of the existence of the tagging program. Presumably a high level of awareness about the tagging program within the angling community would lead to higher reporting rates because anglers would be looking for tags and taking the time to clean off and read the reward amount and phone number printed on the tag. We attempted to address this possibility with substantial outreach and advertising effort aimed at recreational anglers. These efforts were generally well-received and generated a large amount of enthusiasm within the angling community. Despite this perception, only 40% of anglers that reported a tag indicated that they were aware of the tagging program prior to capturing a tagged Red Snapper. This finding brings into question whether we were able to reach a large enough fraction of the angler population to eliminate nonreporting due to lack of awareness.

If the angler reporting rate was less than 100%, then our model would underestimate the exploitation rate. The degree to which exploitation would be underestimated is generally proportional to the non-reporting rate. For this reason, one could view the estimates from our model as a minimum bound for the exploitation rate, all else being equal. This is an important consideration because our estimates are substantially higher than the SEDAR model, and would only go higher if the angler reporting rate was less than 100%. If tagging will become an important component of the stock assessment in the future, then a study to estimate the relationship between the reward amount and the reporting rate would be valuable. This could be accomplished by releasing tags with a range of reward amounts to estimate the reward amount at which the reporting rate reached an asymptote. Alternatively, acoustic telemetry arrays can be

used to estimate reporting rate if angler captures can be reliably identified from the time series of detections for individual fish (Williams-Grove and Szedlmayer 2016).

We excluded commercial recaptures from our analysis because the reporting rate of tagged fish from that sector is unknown and presumably lower than the recreational sector (Vandergoot et al. 2012). Exclusion of these recaptures should not introduce bias into recreational exploitation rate estimates but would bias recreational instantaneous fishing mortality rates. Thus, we have emphasized in our results the exploitation rates and not the underlying fishing mortality rates. Inclusion of commercial recaptures in future studies will necessitate the development of a program to identify commercial recaptures from the landings rather than relying on commercial fishers to report the tags. Such an approach would likely require a large fraction of the landings to be inspected for tags, which may be cost-prohibitive.

Another limitation of our study design was that post-release mortality (tagging and angler discard mortality) was not directly estimated in our study. Instead, we constructed informative prior distributions for these variables. Using prior distributions allowed uncertainty in these variables to be propagated into the resulting estimates of exploitation, but any bias in the literature-based priors would lead to bias in exploitation rates. Downward-bias in the prior for tagging mortality would lead to an underestimate of the exploitation rate. We recommend that future studies include concurrent telemetry-based estimation of tagging mortality from within the spatial stratum in which tagging was conducted.

Although not a primary focus of our analysis, we assessed movements of tagged Red Snapper between tagging and angler-reported recapture locations. There are several potential limitations to using these data for an analysis of movement. For example, movement estimates depend on the spatial distribution of fishing effort. Moreover, the accuracy of angler-reported recapture locations is unknown. Nevertheless, our findings generally confirmed previous studies that indicated Red Snapper exhibit low rates of movement and are often captured within 30 km of their release location within a few months of tagging (Szedlmayer and Shipp 1994, Patterson et al. 2001). High site fidelity has also been demonstrated more directly from telemetry studies (Szedlmayer and Schroeffer 2004, Topping and Szedlmayer 2011). We also examined directional movements which indicated westward or northward movements, but the magnitude of these movements was small (<10 km), thus the directionality should be interpreted with caution.

E. Stakeholder Engagement

Involving stakeholders in the research process has been shown to increase buy-in of science and management while fostering improved trust between the public and scientists (Johnson and van Densen 2007). Conversely, failure to adequately transfer scientific findings to stakeholders exacerbates their inherent suspicion of science and the management process (Dedual et al. 2013). For the Great Red Snapper Count, we developed a comprehensive, digitally-driven stakeholder engagement plan designed to A) inform the fishing community, resource managers, and all other interested stakeholders about the project and B) assess how

awareness of the project influenced angler satisfaction with Red Snapper populations and management. Much of the work that has been implemented throughout various stages of the project have proven tremendously successful (Scyphers et al. 2021). Our strategy was accomplished through a 3-phase approach:

Phase 1: Introduction to the research approach

During Phase 1, we created a series of 5 brief whiteboard videos (approximately 2 minutes each) and accompanying 2-page fact sheets. These materials described the goals and methods of the project in an easily digestible format. The 5 topics were as follows: project overview (i.e., who/what/when/where/why), habitat classification, direct visual counts, depletion studies, and the tagging study. The videos were uploaded to the project's [YouTube channel](#). The videos and fact sheets were then disseminated to the public via the project's [website](#) and various social media platforms. Additional information about the project was shared through several Extension/outreach publications. The tagging study materials received the most attention. As a result of these materials, an article was published by the Associated Press, resulting in a reach of 81 newspapers with 135.6 million unique views.

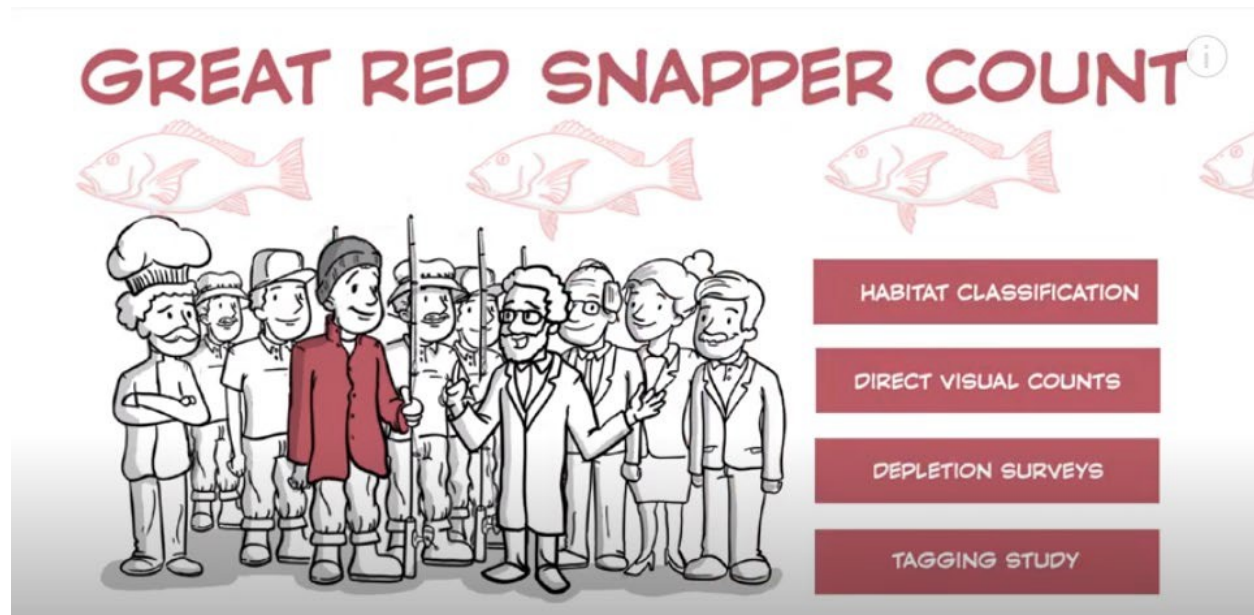


Figure 41. Screenshot of the opening to one of the whiteboard videos. See Appendix C for all whiteboard videos and fact sheets.

Phase 2: Electronic survey

During Phase 2, we designed and implemented a Gulf-wide electronic survey. To accomplish this, we enlisted the expertise of a researcher who specializes in coupled social-ecological systems (Dr. Steven Scyphers, standing member of the GMFMC SSC). The objectives of the survey were to characterize the social dimensions of Red Snapper anglers, measure

satisfaction with current Red Snapper populations and regulations, assess overall patterns of awareness of the Great Red Snapper Count, and evaluate the potential benefits of the Great Red Snapper Count stakeholder engagement videos. The electronic survey was distributed to 1,000 anglers (200 per Gulf state) using “Qualtrics Panels,” a highly robust survey method. Based on the survey results, awareness of the Great Red Snapper Count was high, with 60% of participants familiar with the Great Red Snapper Count. Also, awareness of the project was associated with higher satisfaction with Red Snapper populations. Lastly, participants presented with a stakeholder engagement video showed higher satisfaction with management compared to those in the control treatment (no video). A manuscript detailing this Phase 2 work is in press at North American Journal of Fisheries Management (see Appendix D).

Phase 3: Summary of findings

Phase 3 is in progress as of March 2021. As noted above, the whiteboard videos and fact sheets from Phase 1 detailed the goals and methods of the project. Presently, we have created a final whiteboard video (approximately 1 minute) and accompanying fact sheet, which summarize the results and implications of the project. Once the Red Snapper abundance estimates are finalized, the video and fact sheet will quickly be disseminated.

F. Next Steps

1. End-users, Partners, and Co-sponsors

a. SEFSC, SEDAR74 stock assessment for Gulf Red Snapper

Primary end users of these estimates will be stock assessment scientists at the NOAA Southeast Fisheries Science Center (SEFSC), the Southeast Regional Office (SERO), the Gulf of Mexico Fishery Management Council (GMFMC), and the Scientific and Statistical Committee (SSC) of the GMFMC. The final implementation for these estimates will fully involve representatives from the SEFSC assessment team and the SERO office and integration into management through the GMFMC. Throughout the process we have maintained open lines of communication with these groups to ensure the outcome of the study will generate parameters suitable for integration into current interim stock assessments and future research track assessments for Gulf Red Snapper. For example, co-PI Drymon is leading the Life History Working Group for the SEDAR 74 Red Snapper research track assessment, a group that includes 6 co-PIs from the Great Red Snapper Count, where these results will be most relevant and have a direct avenue for integration.

b. Stakeholder Partnerships

The partnerships built throughout this project with the stakeholders have been extremely important in not only informing the general public about ongoing research in their community but, in many cases, creating a vested interest by the public in understanding and conserving our natural resources. Several design components from this project easily facilitated participation for recreational and commercial anglers. The primary component included the high-reward tagging

study that was performed regionally throughout the Gulf. While scientific tagging during the initial fishing effort was imperative, recapture of the fish occurred broadly across the entire Gulf from anglers from all sectors. The heavily incentivized reporting (\$250 reward per tag) of these captured fish proved extremely successful and eclipsed our highest expectations. Captains associated with this project have expressed high satisfaction with the partnerships built during this project and have expressed desire to stay involved in future research endeavors by project PIs. Certainly, a major benefit from this involvement is the fishing community remains engaged in the study, and thus the fishery. Comprehensive awareness campaigns developed for the high-reward tagging study also offered the opportunity to engage the general and angling public about this study. As the tagged fish were recaptured at a high rate, this study helped to develop grass-roots angler buy-in for the use of descending devices primarily through extensive social media coverage. While not the focus of the study, nor specifically tested here, these results do indicate to anglers that descending fish has merit. This involvement allowed citizens and regional consortia to provide key support in obtaining accurate and precise Gulf-wide abundance estimates.

2. Next Steps and Future Components

a. Archived Genetic Samples

A total of 3,753 tissue samples (fin clips) were collected from individual Red Snapper caught across the northern Gulf of Mexico during the project (Figure 42). Table 13 provides the breakdowns of the number of tissues collected in state waters of the eastern Gulf (Florida, Alabama and Mississippi) and western Gulf (Louisiana and Texas). Along with each fin clip collected in the field the following data was recorded: total length (mm), tag number, location of tagging (latitude and longitude), and environmental variables (e.g., water temperature, salinity, etc.) when possible. Once obtained, fin clips were immediately immersed in thermally stable, salt saturated, 20% DMSO buffer and tubes were sent either to the Marine Genomics Laboratory at Texas A&M University -Corpus Christi (TAMUCC) or to the Laboratory of Eric Saillant at University of Southern Mississippi (USM). Tissues at TAMUCC were inventoried on arrival and each assigned a unique barcode which was linked to an entry in a relational database. This allows the user to pull up the metadata for a specific archived tissue by scanning the barcode or identify all tissue sample that share characteristics of metadata (e.g., location, depth, size, etc.). The database is maintained on a local workstation and backed-up locally and on a remote secure server at TAMUCC. Upon reception at USM, samples were logged in a database that records unique sample IDs and all field data provided by samplers, including the field ID used during tagging and/or field data recording. The database is an Excel workbook which is stored on one local computer in E. Saillant's laboratory and backed-up through the University secured server using the Sync SharePoint system of MS Office 365. Copies of files uploaded to the server are stored at the USM's Technology Data Center in Hattiesburg. Samples are physically stored in the collections maintained by D. Portnoy and E. Saillant at their respective home institutions in climate-controlled locations with restricted access. The large number of samples and their fairly even spread across the northern Gulf of Mexico makes them ideal for a landscape genetics

approach to understanding population structure and connectivity in the region. Such a project would involve a population genomics approach and would take advantage of two independently generated drafts of the Red Snapper genome (Portnoy unpublished data, Saillant et al. 2020).

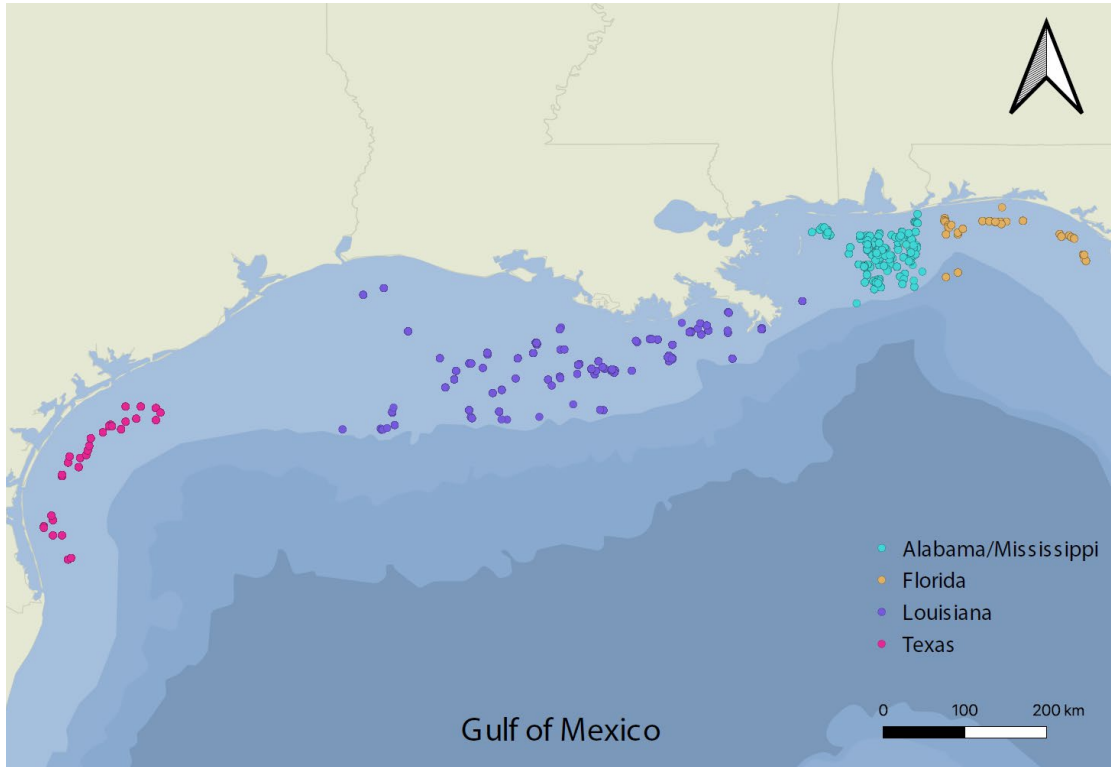


Figure 42. Distribution of sampling locations where tissue samples were collected.

Table 13. Breakdown of number of tissues from individual fish (N) in waters of the eastern Gulf (eGulf); including Florida (FL), Alabama (AL) and Mississippi (MS) and the western Gulf (wGulf); including Louisiana (LA) and Texas (TX). The location (Loc) at which samples are archived, either University of Southern Mississippi (USM) or Texas A&M University -Corpus Christi (TAMUCC), is also indicated.

State	Region	N	Location
FL	eGulf	310	USM
AL/MS	eGulf	2347	USM
LA	wGulf	689	TAMUCC
TX	wGulf	407	TAMUCC
Total		3753	

b. Management Integration and Future Research Recommendations

As is the case with virtually all studies, and especially at the scale, magnitude, and novelty of this research investigation, they open the door for many other questions, future studies, and research recommendations. We are now armed with the value of hindsight and many lessons learned. While not comprehensive, below are a few key areas of research that the teams suggest for future studies and analyses:

- More access to improved bathymetric and habitat mapping. Certainly, our estimate is only as good as the known current maps of the areal coverage of the structured features that harbor Red Snapper. Increasing high-resolution mapping efforts to elucidate the spatial distribution and areal coverage of bottom types in the Gulf of Mexico will lead to more spatially resolved and refined abundance estimates.
- Uncharacterized Bottom (UCB) should be better characterized by conducting fine-scale habitat mapping studies over these regions. Additional sampling effort in this habitat is considered a priority given its influence on the overall Red Snapper population estimate including age/size structure using this area.
- Geographic locations of Red Snapper aggregations over UCB are available from our surveys, and the nature of these features should be further investigated.
- Studies examining large- and fine-scale movement of Red Snapper are needed. For example, how much exchange occurs among UCB, natural banks, and heavily exploited habitat types such as artificial reefs. This is especially the case given the exploitation pattern discovered through the tagging component of this study.
- Improved size-at-age data in general for all habitat types, but given the large abundance of fish using UCB, detailed age-and-growth studies should be performed over UCB for each region.
- Technology and gear improvements occur at a rapid pace. Rigorous gear calibration and validation experiments should be conducted.
- Our success with the tagging and exploitation study showed these studies have high value. Tagging studies should also be conducted over natural bottom and UCB habitat types.
- We recommend collaborative discussions in the near-term with the NOAA Southeast Fisheries Science Center to begin to develop a mechanism to incorporate these data into the assessment process.

While we have made some recommendations regarding the integration of these data into the management process, it can be much more nuanced than what can be presented here in written format. Or perhaps, there are data or other aspects of the study valuable to assessment and management that are unknown at this point. Our research team welcomes others such as the Southeast Regional Office and NOAA Southeast Fisheries Science Center to use these data to the fullest extent possible, and we are ready and willing to facilitate future collaborations to explore

these data and findings as needed. Finally, this study is very complex with many facets. While many other peer-reviewed papers, ancillary studies and additional lessons learned will be forthcoming, the knowledge gained here goes well-beyond what can be codified in a report such as this. Our research team is available to share these insights with others, and especially for future abundance estimation projects that are on the horizon.

c. Estimate Reconciliation through Simulation Modeling

The reconciliation of the estimates derived in this report are substantially higher than the estimates presented in the recent Red Snapper stock assessment. We propose that a variety of analytical approaches primarily centered on evaluating, through sensitivity analysis, the impacts of parameter estimates, data inclusion, and model structure in the stock assessment model be used to address these alternative conclusions about the stock. In this section we discuss how the results of this work can be used both within the stock assessment and as a complementary approach for understanding the Gulf Red Snapper stock.

Sensitivity analyses in stock assessment is a standard and widely used approach for understanding how the variation in assumed (often fixed) values of parameter estimates impact metrics of stock and fishery status. Experiments to systematically change the impactful parameters that have influence on the abundance of age-2+ Red Snapper are recommended as a promising future approach. Our efforts to do this have been limited and primarily focused on evaluating the $\ln(R_0)$ parameter as a way to understand if variation in this parameter could result in greater estimates of age-2+ fish. Other parameters that could be examined include those that describe the life-history of Red Snapper, primarily instantaneous age-specific natural mortality and reproductive biological parameters and their associated confidence intervals. Although these aspects of Red Snapper biology are relatively well studied, there are substantial variations that could be further explored in the stock assessment (given the results of the estimates presented in this work).

In terms of the data included in the model, our finding of substantial biomass in small patch reefs that had been previously overlooked as productive Red Snapper habitat is certainly worth inclusion in future studies. This indicates that survey effort on those areas should be expanded – it is likely that given the estimates of age-2+ abundance developed in this work, that the information derived in the fishery-independent survey effort may need to be expanded in terms of habitat strata to better understand the dynamics of the stock in these areas.

A relevant source of data developed in this work that could be expanded and formalized is the data that describes the capture and recapture of Red Snapper. Data from tagging experiments can be included into the Stock Synthesis assessment model. To our knowledge, this methodology is currently being developed and expanded in the software. Given the ongoing and historical tagging effort of Red Snapper by participants in this study (that have developed information from tagging that includes habitat affinity, natural mortality rates, participant characteristics of cooperative tagging, and movement) it may be informative to incorporate these data into the stock assessment. The inclusion and exclusion of these data, even simulated data

based on the characteristics of the recapture histories of previous tagging studies, in the SS model will indicate the utility of these data.

The differences between SEDAR stock assessment model estimates and those derived in this work are influenced by aspects of model specification, as well as process, and measurement errors discussed in this section. The employment of management strategy evaluation, a widely used simulation approach to evaluate alternative management regimes, data, and biological processes and their feedback, may be promising to reconcile alternative states of nature of the Red Snapper. In particular, differences between SEDAR estimates of Red Snapper stock sizes and those herein are driven to a great extent by the numbers of fish encountered over UCB. More detailed spatially-disaggregated data on exploitation rates, population densities from fishery-independent surveys, and detailed fishing effort information may be extremely useful in reconciling SEDAR and GRSC estimates.

3. Data Management Plan

a. Data repositories and storage

We understand our role in satisfying the directives for sharing environmental data and peer-reviewed publications expressed in version 3.0 of the NOAA document *Data and Publication Sharing Directive for NOAA Grants, Cooperative Agreements and Contracts* and will adhere with guidance, definitions, directives, and requirements contained therein. All data collected from this award will be made available to the MS-AL Sea Grant Program. We also have the full intention of sharing and making these data readily available to end-users such as the Gulf of Mexico Fishery Management Council, Science and Statistical Committee, and the NMFS Southeast Regional Office and Southeast Fishery Science Center

The Harte Research Institute for Gulf of Mexico Studies (HRI) at Texas A&M University-Corpus Christi has an outstanding history with data management and access. Project PIs are assisted with data archiving by the Gulf of Mexico Research Initiative Information and Data Cooperative (GRIIDC, <http://data.gulfresearchinitiative.org/>) housed at HRI. This in-house data management team and system allows for safe archiving and serving of these data to end users. GRIIDC is both a data management system and a human network of scientific data experts compiling and documenting the vast and varied datasets acquired through the Gulf of Mexico Research Initiative (GoMRI), a \$500-million dollar, 10-year research program investigating the impacts of oil spills on the ecosystems and human wellbeing in the Gulf of Mexico region. GRIIDC is working with more than 500 scientists from more than 100 academic institutions employing a variety of scientific methods. The mission of GRIIDC is to ensure a data and information legacy that promotes continual scientific discovery and public awareness of the Gulf of Mexico ecosystem.

The GRIIDC program was designed to receive and process data from a variety of sources and from various scientific disciplines. These include structured and unstructured data from remote sensing instruments, oceanographic and atmospheric observing stations, autonomous

vehicles, research vessels, on-the-ground field surveys, socioeconomic studies, laboratory analyses, and numerical modeling. Scientists submit their data to GRIIDC for long-term archiving and public discovery. GRIIDC ensures efficient data transfers for data providers and recipients, proper dataset documentation, and provides data discovery capabilities. A data repository is installed in a hardened Network Operations Center on the Texas A&M University-Corpus Christi campus and a duplicate site is being installed on the Texas A&M University campus in College Station.

At the conclusion of this project PIs will have contributed data via ftp, email, websites, or web services. GRIIDC will provide the data via direct download or Globus/GridFTP. Scientific publications often include small datasets; larger datasets can be referenced via a Digital Object Identifier (DOI). GRIIDC and NCEI will use EZID to mint and assign DOIs to their datasets.

b. Data products/expected publications

A substantial number of direct and ancillary documents along with a variety of data products will arise from the work completed for this project and reported in this document. These will include scientific publications, data workshop papers, ancillary figures and tables, and numerous other data products. A preliminary list of these anticipated data products, though not exhaustive, is provided here:

Current:

- Garner et al. 2021. Estimating reef fish size distributions with a mini remotely operated vehicle-integrated stereo camera system. *PloS ONE* 16 (3), e0247985.
- Garner et al. *in review*. A multidisciplinary approach to estimating Red Snapper, *Lutjanus campechanus*, behavioral reaction to mobile camera and sonar sampling gears. *Fisheries Research*.
- Scyphers et al. 2021. Understanding and Enhancing Angler Satisfaction with Fisheries Management: Insights from the “Great Red Snapper Count”. *North American Journal of Fisheries Management* 41(3):559-569.
- Dance MA., Rooker JR. 2019. Cross shelf habitat shifts by Red Snapper (*Lutjanus campechanus*) in the Gulf of Mexico. *PLoS ONE* 14 (3), e0213506

Future:

- Stunz et al. Absolute abundance of Red Snapper in the U.S. Gulf of Mexico: The Great Red Snapper Count.
- Patterson et al. Integration of studies of absolute abundance into fisheries management: A case study for the Great Red Snapper Count.
- Catalano et al. Exploitation patterns of Red Snapper over a variety of habitat types in the U.S. Gulf of Mexico

G. Discussion, Conclusions, and Key Takeaways

The primary goal of this initiative was to estimate the absolute abundance of age-2+ Red Snapper in the U.S. waters of the Gulf by habitat type, including artificial reefs, natural hard bottom, and UCB. This study produced an estimate of 118 million (CV 15%) age-2+ Red Snapper residing in this region. This team of fisheries scientists developed an independent estimate of abundance derived from surveys throughout the stock's range which can be integrated into the current stock assessment through a reconciliation process that will likely involve challenging assumptions about parameters such as natural mortality and discard mortality, while also including revised catch and effort estimates. This was a rare opportunity in fisheries science to compare stock assessment-derived estimates of population size, hence productivity, in such a fundamental way. Moreover, these findings offer a unique opportunity for other approaches and data to be integrated into the assessment framework. To be clear, science is a building process, and the independent estimate of abundance derived from this research is not intended as a replacement or in contention with the SEDAR Red Snapper Stock Assessment. Instead, this research will supplement and bolster ongoing analyses by allowing for validation, calibration, and further refinement of those models, given absolute abundance has now been estimated independently from the assessment model.

This was a large-scale survey using established as well as novel sampling approaches that have been integrated into a modeling framework and applied over an unprecedented area of study and in new habitat types (e.g., UCB) that were previously unassessed. The Gulf contains extensive variability in oceanographic and geologic conditions that create substantial differences in habitat types and associated Red Snapper densities across the basin. We developed a robust design to fully characterize the expansive shelf waters using stratification and sampling effort in a hierarchical structure based on ecological regions that closely aligned with jurisdictional management boundaries. The scientific approaches to surveying a widespread species occurring in diverse habitats, such as Red Snapper, were advanced by the development, implementation, and evaluation of the gear and approaches used in this study, and that knowledge can be applied in new studies. Already, there has been much discussion of how to appropriately integrate these sampling methodologies with the traditional fishery-independent methods used for stock assessments. With a robust estimate, a major benefit is a better understanding of the population dynamics leading to improved assessment procedures for Red Snapper throughout the Gulf. This study will help refine population parameters estimated during the SEDAR process, and it will provide potential strategies for addressing some of the data gaps inherent in the assessment while also evaluating assumptions made in the current Red Snapper assessment models. Thus, the stock synthesis (SS) model, and perhaps others, can be calibrated to provide the most accurate estimates of stock status.

It is well-known that Red Snapper use habitat types such as artificial reefs and natural bottom, and we estimated large abundances of Red Snapper using these habitat types in each region. Though, we also observed a large percentage of Red Snapper abundances over UCB habitat throughout the Gulf. Red Snapper have been previously observed over UCB, and some have speculated that a large cryptic biomass of fish may occur over this habitat (Mitchell et al.

2004; Porch 2007; Gallaway et al., in review); however, these populations have never been thoroughly studied on large scales as was done in the current study. These results are important because the large numbers of Red Snapper inhabiting UCB are not targeted by any fishery and may be poorly indexed by current fishery-independent surveys (e.g., NMFS Bottom Longline Survey); thus, data from the proportion of the Gulf Red Snapper population observed in these habitats is largely missing from the data streams utilized in the SS assessment model (SEDAR 52). The discovery of this biomass may also explain several aspects of the fishery that have puzzled scientists for many years. First, it may help to explain the lack of a stock-recruit relationship consistently observed for the Gulf Red Snapper stock. For example, we can now account for a previously unknown spawning biomass occurring over areas that were not indexed from fishery-dependent or -independent data streams.

We estimated 70 million age-2+ Red Snapper in the Florida region. A relatively small number of fish occurred over artificial reefs, which is unsurprising given the relatively modest numbers of artificial reefs in waters off Florida versus other Gulf states. The vast majority of fish were estimated to inhabit the combined habitat of natural hard bottom and UCB. Again, that is intuitive given the prevalence of those habitat types on the Florida shelf. The Florida region is also much larger in relation to the other study regions given its shelf comprises nearly 50% of the entire shelf area in US waters of the Gulf. There are natural hard bottom features in the Florida portion of the Gulf of Mexico that are similar to other regions; however, both large and small natural hard bottom features are much more prevalent in Florida and decrease moving westward.

The size distribution of fish observed in ROV video samples in Florida waters were skewed toward small (<600 mm TL), young fish (Figure 6). This truncated size distribution likely reflects the high exploitation rates estimated for the recreational fishery in Florida. However, those estimates were produced only from fish tagged in the Panhandle region off northwest Florida. Another potential explanation for the observed size distribution is that Florida waters, while once the center of the Gulf Red Snapper fishery, have been historically overfished for decades. Fishery-independent surveys suggest the Red Snapper population south of Cape San Blas, Florida has been rapidly increasing in recent years (SEDAR 52), and the observed size distribution in that region likely reflects that recovery. Given Red Snapper can live to be nearly 60 years old, it will likely take decades to rebuild the Red Snapper age structure in Florida. Furthermore, the presence of mostly small, young fish in Florida, despite their high abundance, is consistent with the stock assessment result that the spawning potential ratio in the east has lagged behind that of the western sub-unit of stock, given that fecundity increases exponentially with length (SEDAR 52).

For the Alabama/Mississippi region, we used separate habitat-specific approaches to estimate absolute abundances in the three habitat types that were the focal effort of the overall project. We estimated a total of 8.5 million Red Snapper occur in the AL/MS region. Large numbers of Red Snapper occurred over artificial reefs and natural banks, and similar to other regions, 3 million Red Snapper were estimated to occur over UCB. Like other regions, each habitat type off coastal AL/MS posed challenges that necessitated different sampling approaches, including an estimation of the number of artificial reefs. We chose to use depletion-based

approaches to quantify Red Snapper on artificial reefs and combine those with our estimates of the number of structures to extrapolate the count to an absolute abundance estimate. Unlike other regions where the universe of artificial reef (and natural bottom) was generally known, we used previous surveys in AL/MS to estimate the number of unpublished reefs in each spatial stratum along with the variance of the estimate. For other regions, the location, number and/or areal coverage of artificial reefs, natural hard bottom, and pipeline was known, and uncertainty was assumed negligible. Since this variance was unable to be estimated and was assumed negligible, this source of uncertainty was not carried forward to final variance estimates for any region. Areas where unclassified/unknown structured habitat (i.e., unknown artificial reefs or natural bottom) may have occurred are included in the UCB habitat type.

We estimated 22 million Red Snapper occur in the Texas region. The estimate included 14.5 million fish over UCB, and 7 million and less than 1 million occurring over natural hardbottom and artificial reefs, respectively. Artificial reef and natural hard bottom habitats were surveyed using a combination of ROV coupled with hydroacoustics due to the low visibility in waters of the western Gulf. The size of many of the artificial reefs (e.g., oil and gas platforms, large ships, etc.) necessitated a modification of the survey approach used over the smaller artificial reefs seen in FL and AL/MS, where the entire reef is typically visible in a single frame. For TX, a standardized combination of roving and transect-based sampling was developed to survey these large structures. Red Snapper proportion estimated from these surveys was then used to scale hydroacoustic total fish abundance estimates for the entire artificial reef structure to obtain Red Snapper absolute abundance. The universe of artificial reefs was well-documented, which made the total abundance calculations for artificial reefs direct; thus, we did not have to estimate/model the number of artificial reefs as for AL/MS. Natural bottom habitat in the western Gulf region is defined by discrete and very large features (km²). We included an additional habitat stratum of these known features into the design for this region. Estimates were conducted similarly by obtaining abundance estimates, and then scaling up to the areal footprint of the natural bottom, which was also known from the universe of large high-relief natural banks well documented from previous multibeam sonar mapping projects. The remaining unclassified bottom types were grouped into the UCB habitat. These areas were surveyed using a TCA with hydroacoustics. Density estimates were calculated and scaled based on the best estimate of UCB habitat along the continental shelf. Undoubtedly, this region contained some unmarked artificial reefs, ephemeral natural habitats (e.g., ‘mud lumps’, scouring depressions, etc.), and perhaps large unmapped natural bottom features, all of which may harbor Red Snapper. Thus, even at low densities of Red Snapper, when expanded by the large areal coverage of UCB habitat, this habitat type accounted for a significant proportion of the estimated Red Snapper population off TX as well as other regions.

We estimated 17.4 million Red Snapper occur on the Gulf shelf off Louisiana, although for reasons noted above, the Red Snapper abundance estimate is least certain in this region. The Louisiana estimate included approximately 4 million fish on natural hard bottom and artificial structures, respectively, and the vast majority (~10 million) occurring over UCB. This pattern was evident in the TX region of the western Gulf; therefore, we treated sampling of habitat types similar to this region. Sampling efforts over the Louisiana shelf used C-BASS tows over natural

banks and UCB. We also used hydroacoustics surveys, paired with accompanying ROV video to generate species composition, and to sample artificial reefs (exclusively oil and gas infrastructure). Unexpected complications prevented the full scope of sampling in Louisiana from being completed as anticipated for both the Red Snapper abundance estimate as well as the tagging component. To supplement sampling efforts in this region a subset of surveys conducted over the nearby and similar TX shelf were integrated into the Red Snapper abundance estimation for this region. Thus, we recommend caution in interpreting the estimate for Louisiana due to the lack of robust sampling for some areas of the Louisiana shelf. Moreover, ongoing independent Red Snapper abundance estimates conducted by the Louisiana Department of Wildlife and Fisheries should bolster data and analyses presented here in the near future.

A relatively low number (~500,000) of Red Snapper was estimated to occur along pipelines compared to other habitat types investigated. We have suggested this number is likely an underestimate, as fish on pipeline habitat can be difficult to estimate for a variety of reasons. In some areas, pipelines are covered by sediment over wash, and in some instances, pipelines are available as habitat above the sediment or through scouring. Thus, the extent of the availability of this habitat type can be difficult to determine. Depending on the actual status of pipeline segments (i.e., exposed or buried), multiplying fish density by total quantified pipeline lengths may result in under- or over-estimation of population size. However, visibility issues, especially near the Mississippi River outflow and western Gulf, and other areas with persistent nepheloid layers near the seafloor, may have resulted in low detectability of fish within the camera field of view, leading to an underestimation of Red Snapper abundance. Nevertheless, Red Snapper do associate with pipelines, and this habitat should be evaluated further. These pipelines varied in diameter from ~ 15 cm (small) to larger ~ 1-m. Thus, pipelines offer a comparatively low amount of hard bottom, much of which is not high relief as some the more 'desirable' features of complex structure that harbored higher numbers of Red Snapper. Similar to many of the habitats considered here, as our mapping of known habitat and areal coverage improves, the accuracy of population estimates are likely to improve as well.

Comparative studies of Red Snapper abundance are useful for validation and can inform our findings. While studies of absolute abundance of Red Snapper by habitat type are rare, especially for natural bottom and UCB, some efforts have been successful albeit at much smaller geographic scales given more limited resources than were available here. Several previous studies estimating Red Snapper abundance at oil and gas structures in the western Gulf are typically lower but generally align with our estimates of Red Snapper per structure in TX (362) and LA (2174). For example, Gallaway et al. (2020) estimated mean Red Snapper abundance per platform of 359 within 10-17 m depths, 1015 within 18-30 m depths, 2980 within 31-90 m depths, and 133 within 91-300 m depths. Estimates of absolute abundance are lacking from natural habitats in the Gulf; however, results of numerous studies are consistent with the finding that Red Snapper density is significantly lower on natural reefs compared to artificial reef habitat (Patterson et al. 2014; Karnauskas et al. 2017; Streich et al. 2017a; Powers et al. 2018). For example, ROV-based habitat comparisons have suggested Red Snapper density is approximately 7.8 times higher at artificial reefs than natural reefs in Texas (Streich et al. 2017a) and approximately 6 times higher at artificial reefs than natural reefs in Florida (Patterson et al.

2014). Our results are also congruent with the previous Gulf-wide analysis of Karnauskas et al. (2017) that indicated natural hard bottom habitats hold a much greater proportion of the Gulf Red Snapper stock than artificial structures despite higher fish densities on artificial reefs. For instance, our estimate suggests approximately 5.0% of the stock occurs at artificial structures, while Karnauskas et al. (2017) estimated a slightly higher proportion (13.3%) for that habitat type. Perhaps the most important finding of this study is the high proportion of the stock occurring over UCB. Although it has long been known that larger, older fish can occur over this habitat (see Mitchell et al. 2004), only relative abundance estimates were previously available. Estimates of total abundance from this study indicate approximately 82% of the Gulf Red Snapper stock occurs over UCB (including FL's natural and UCB combined area) – a habitat that experiences relatively little effort and subsequently limited landings from commercial or recreational fisheries in the Gulf. It appears these fish represent the “cryptic adult biomass” that Porch (2007) and others have suggested could contribute to the apparent increase in stock spawning potential in recent decades.

The tagging component of this study proved to be informative on a variety of aspects from spatial exploitation patterns, movement, discard mortality, and angler engagement. The tagging-based total (i.e., private + charter) recreational exploitation rates estimated for the 2019 fishing season likely exceeded 0.2 in each of the regions. These estimates are substantially higher than the most recent estimate of 0.052 from the SEDAR model (SEDAR 2018). Tagging sites were shallow (<40 m) artificial reefs that harbor a subset of the Red Snapper population that likely experiences higher fishing effort than fish in deeper water or associated with lower-relief structure. Our analysis indicated that sites closer to ports, even within our relatively restricted sampling frame, experienced higher exploitation. This finding suggests that the fraction of the stock located farther offshore is less vulnerable to recreational exploitation. Our study had a much larger spatial extent than any previous study designed to estimate Red Snapper fishery exploitation and indicates exploitation was quite high at shallow artificial reef sites in all regions but generally increased from west to east. Not only are these results consistent with the stock assessment, but they also are consistent with analyses presented by Karnauskas et al. (2017). Lastly, we estimated that exploitation by the charter sector was less than that of private anglers, which is qualitatively consistent with the quota allocation between these two sectors.

A series of experiments were conducted in the Florida region to estimate potential differences in the behavioral reaction of Red Snapper to mobile sampling gears. Details of this study can be found in Garner et al. (in review; see Appendix D). Briefly, the calibration experiment results suggest minimal positive or negative behavioral reaction displayed by Red Snapper to any of the three mobile sampling gears utilized in this study to estimate Red Snapper density and abundance. Thus, we concluded that there is no substantial bias in Red Snapper counts due to their behavioral reaction to mobile sampling gears used in this study. With ROV sampling in Florida waters, we assumed detectability to be 100%, and that assumption was also informed by results of previous work on other species (Harris et al. 2019). However, for other regions and methods, the detectability may be lower under some conditions; yet, it was not possible to test these assumptions, and we were not able to account for or correct for this uncertainty.

In comparisons of Red Snapper abundance estimates derived from ROV versus hydroacoustic sampling, we reported Red Snapper abundance estimates produced in ROV surveys were much greater than those produced from sonar surveys. It is unclear to the team what the source of this difference might be, or the implications for western Gulf sonar-derived Red Snapper density estimates. Moreover, it was not possible to estimate Red Snapper detectability with split-beam sonar sampling, or other potential biases in sonar-derived estimates of Red Snapper density, based on study data. Given behavior experiments suggest Red Snapper display a more or less neutral reaction to our mobile sampling gears, it does not appear likely that attraction to ROVs is occurring. It is also unclear to what extent Red Snapper detectability with sonar, or post-processing methods employed to estimate Red Snapper counts and density, may have affected sonar-derived Red Snapper density estimates. Initially, the team assumed that the gear with the greatest detectability would be hydroacoustics, but these results may indicate otherwise. However, it is important to note that comparisons between ROV and sonar surveys were conducted at sites where Red Snapper were observed in ROV video. If the habitat was patchy and the GPS coordinates for the comparison sampling site just happened to be in a habitat patch that favored Red Snapper occurrence, but surrounding areas had more open substrates, then spatial heterogeneity of habitat types could have driven the incongruity between ROV and sonar results. If that in fact did occur, this would not necessarily implicate Red Snapper abundance estimates derived from ROV video to be biased high because coordinates for the 749 natural habitat sites sampled on the Florida shelf were random selected based on the stratification described above.

No study is without some level of bias, and the team strived to eliminate or minimize potential biases wherever possible to provide the most robust estimate of Red Snapper age-2+ abundance. Nevertheless, uncertainty exists with any study, and we recommend it is important to identify these biases, mitigate them where possible, and indicate the magnitude and direction of any remaining ones. Generally, the overall direction of bias for this study is likely to be erring on the side of under- versus over-estimation of the Red Snapper population size in U.S. Gulf waters. We believe conservative decisions and assumptions were made in all phases of this study. This was principally due to an over-estimation of population size has far greater implications for the sustainability of this important fishery. In addition to conservative decisions and assumptions, we also have evidence that Red Snapper occur in areas outside of the prescribed sampling frame (10-160 m) of this study. This study provides the first estimate of Red Snapper population size in the U.S. Gulf; though, we also believe we have demonstrated in this report that this estimate is a robust one.

Stakeholder engagement was a major element of this study. The partnerships built throughout this project have been extremely valuable for informing the general public about ongoing research in their community, but in many cases, creating a vested interest in the scientific understanding and conservation of our natural resources. Several design components from this project fulfilled the RFP requirement of meaningful participation from recreational anglers, commercial fishermen, and other stakeholders. This included the high-reward tagging study that was performed regionally throughout the Gulf. While scientific tagging during the initial fishing effort was necessary, recapture of the fish occurred broadly across the entire Gulf

from anglers from all fishing sectors. Heavily incentivized reporting (\$250 - \$500 reward) of recaptured fish, and the return rate of over 30% eclipsed our *a priori* return rate expectations, which not only reflects high exploitation rates, but also high angler participation in the study. The tag return data provided key insights into high fishery exploitation rates over artificial reefs. Charterboat captains associated with this project have expressed high satisfaction with their partnerships built during this study and have conveyed their desire to stay involved in future research endeavors. Comprehensive awareness campaigns developed for the tagging study and other aspects in the abundance estimation also offered the opportunity to engage the general and angling public about this study, and this involvement allowed citizens and regional consortia to provide key support for this project. Certainly, a major benefit from this involvement is the fishing community remained engaged in the study and recognized the value of and need for advancing science.

As is the case with virtually all studies, results and outcomes open the door for many other questions, future studies, and research recommendations. We are now armed with the value of hindsight and many lessons learned. We have provided a detailed listing of recommended future studies (see Research Recommendations section above). However, a few key aspects needing attention are improved high-resolution habitat mapping, better characterization of UCB including demographic parameters of fish occurring over that habitat type, and additional studies to evaluate gear biases and calibrations among gear types. Studies examining large- and fine-scale movement of Red Snapper are needed to elucidate the exchange among the various habitat types. We also highly recommend collaborative discussions in the near-term with the NOAA Southeast Fisheries Science Center team to begin to develop a mechanism to incorporate these data into the assessment process.

This study builds on our scientific knowledge base and improves our understanding of Red Snapper abundance in a non-contentious and constructive approach to enhancing Gulf stock assessments. While we have made recommendations regarding the integration of these data into the assessment process, it can be much more nuanced than what can be presented here. Furthermore, there are likely data or other aspects of this study that have value to assessment and management that are currently unknown. Our research team welcomes other scientists and managers such as the Southeast Regional Office, NOAA Fisheries Southeast Fisheries Science Center, scientists involved in the SEDAR assessment process, and the Gulf of Mexico Fishery Management Council to use these data to the fullest extent possible, and we are ready and willing to facilitate future collaborations to explore these data and findings as needed.

Key Takeaways:

- This study produced an estimate of 118 million (CV 15%) age-2+ Red Snapper residing in the U.S. Gulf of Mexico through 2019.
- A large percentage of Red Snapper occurred over the uncharacterized bottom habitat type, which may represent a pool of cryptic biomass not previously accounted for in Red

Snapper stock assessments. A high abundance of Red Snapper occurring over these areas that are largely unexploited by the fishery may also explain the weak stock-recruit relationship consistently observed in this fishery.

- The tagging results indicate:
 - an astonishing 30% return rate of tagged fish.
 - high fishing exploitation generally occurs over habitat with the highest density of Red Snapper (i.e., artificial reefs).
 - high angler ‘buy-in’ and engagement with this type of study.
 - that use of descending devices was an effective release strategy.
- This study builds on our scientific knowledge base and improves our understanding of Red Snapper abundance in a non-contentious and constructive approach to federal assessments. This absolute abundance estimate will bolster future assessments and afford other stock evaluation and management options.
- Given new effort recalibrations are underway for Red Snapper, incorporation of these newly discovered fish occurring over UCB, and understanding exploitation patterns of anglers may lead the Red Snapper stock assessment to converge with similar abundance estimates. Moreover, had this information been available for previous stock assessments, those abundance estimates likely would have been higher.
- Stakeholder engagement efforts were successful; approximately 60% of anglers surveyed were familiar with the Great Red Snapper Count. Notably, awareness of the GRSC was associated with up to three times higher satisfaction with fisheries management (Scyphers et al. 2021).
- While the survey methods used in the study represent a rigorous application of the best technology available, the specific results of these surveys needed to be extrapolated since it would be impossible to directly survey all areas. The uncertainty surrounding those extrapolations are linked to the resolution of our habitat maps. We encourage, further mapping, especially of the UCB, to decrease uncertainty in future studies.
- This report is just the beginning of future assessment meetings and activities with managing agencies, Scientific and Statistical Committees, the NOAA Southeast Fisheries Science Center, and the Gulf of Mexico Fishery Management Council. These activities will facilitate direct incorporation of these data into the management process.

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IV. Appendices

A. Investigators' Roles and Responsibilities

Gregory W. Stunz, Ph.D.

Texas A&M University-Corpus Christi

Lead principal investigator on the project responsible for assembling and overseeing all investigators on the project. Also responsible for the abundance estimates of natural and artificial habitats in TX. Sampling methods for abundance estimate included ROV visual surveys and hydroacoustics. Also coordinated the tagging effort of Red Snapper in south TX waters.

William F. Patterson III, Ph.D.

University of Florida

Regional lead investigator responsible for the FL abundance estimates. Sampling methods included direct count visual surveys to determine Florida stock using ROV, direct count method calibration, data analysis. Also coordinated the Florida tagging of Red Snapper for the high reward tagging study.

Sean P. Powers, Ph.D.

University of South Alabama

Regional lead investigator responsible for the MS/AL abundance estimates. Sampling methods included ROV visual surveys and depletion. Also coordinated the tagging of Red Snapper in both states for the high reward tagging study.

James H. Cowan, Jr, Ph.D.

Louisiana State University

Regional lead investigator responsible for the LA population estimates. Sampling methods included visual direct counts using ROV, towed camera arrays, and hydroacoustics.

Jay R. Rooker, Ph.D.

Texas A&M University at Galveston

Regional lead investigator responsible for the abundance estimate of uncharacterized bottom for TX. Sampling methods included towed camera arrays (TARAS and ARIS) and hydroacoustic surveys. Also coordinated the tagging efforts for north TX.

Robert A. Ahrens, Ph.D.

University of Florida

Principal Investigator responsible for helping develop the overall project design and population estimation using a random forest model. All data used in the model was provided by the lead investigator for each state.

Kevin Boswell, Ph.D.

Florida International University

Principal Investigator responsible for providing guidance on the use of hydroacoustic equipment including assistance with determining total fish abundance using visual software and data script code.

Liese Carleton, Ph.D.

Virginia Institute of Marine Science

Investigator responsible (with J. Hoenig) for developing the estimation scheme and sampling design, and in charge of calculating the population on artificial and natural reefs using the depletion method for AL/MS.

Matthew Catalano, Ph.D.

Auburn University

Principal Investigator responsible for the high reward tagging study design and statistical analysis. Fishing effort, exploitation, and recapture rate were calculated using these data.

Judson M. Curtis, Ph.D.

Texas A&M University-Corpus Christi

Investigator responsible for coordination and planning of TX sampling, study design and logistics associated with the high reward tagging study (with M. Catalano).

Michael Dance, Ph.D.

Texas A&M University at Galveston

Investigator responsible for habitat characterization and mapping, planning, and assisting with north TX sampling and abundance calculation.

Marcus J. Drymon, Ph.D.

Mississippi State University

Principal Investigator responsible for all outreach materials and stakeholder engagement. Final products included six videos and associated fact sheets describing the project and the methods used to calculate Red Snapper abundance. Also was an integral part of designing, planning, and testing the use of depletion methods on artificial reefs in AL and MS.

Marta D'Elia, Ph.D.

Florida International University

Investigator responsible (with Dr. Boswell) for processing the acoustic data in the Eastern Gulf. Final products included abundance estimates of Red Snapper at 410 sites.

Steve Garner, Ph.D.

University of Florida

Investigator that designed cruise tracks for FL sampling, coordinated and oversaw sampling crews to video sample artificial and natural habitats with ROV, and led stereo camera calibration work and the red snapper behavioral experiments.

Sarah Grasty, M.S.

University of South Florida

Investigator responsible (with S. Murawski) for conducting a Gulf-wide visual survey using a towed camera array (CBASS) with emphasis on pipeline and open uncharacterized bottom habitats.

Crystal L. Hightower, M.S.

University of South Alabama

Investigator responsible for project management and implementation (with Dr. Sean Powers) of Alabama and Mississippi components of study (ROV, VLL depletion studies, and high dollar tagging).

John Hoenig, Ph.D.

Virginia Institute of Marine Studies

Principal Investigator responsible (with L. Carleton) for developing the estimation scheme and sampling design, and in charge of calculating the population on artificial and natural reefs using the depletion method for Alabama/Mississippi.

Amanda Jefferson, M.S.

Mississippi State University

Investigator responsible (with M. Drymon) for producing outreach materials for stakeholder engagement. Final products included six videos and associated fact sheets describing the project and the methods used to calculate Red Snapper abundance.

Danielle Kulaw, M.S.

Texas A&M University-Corpus Christi

Investigator responsible for administrative leadership and overall project team facilitation, assisting with recapture reward distribution and delivery to stakeholders.

Robert Leaf, Ph.D.

University of Southern Mississippi

Principal Investigator responsible for assisting to develop the initial model design and calculating the projected Red Snapper population size at present day using the data from the last SEDAR stock assessment for Red Snapper.

Vincent Lecours, Ph.D.

University of Florida

Principal investigator that assembled habitat classification and bathymetry data from a variety of sources and worked (with R. Ahrens) on sampling design and stratification issues.

Zhaoce (Charlie) Liu, Ph.D.

Southern Methodist University, current affiliation Mathematica, Washington, D.C.

Investigator responsible (with L. Stokes) for producing an independent analysis of population estimates. All data was provided by the lead investigators for each state.

Hui Liu, Ph.D.

Texas A&M University at Galveston

Investigator responsible for planning and assisting with north TX sampling and abundance calculation.

Steven Murawski, Ph.D.

University of South Florida

Principal Investigator responsible for conducting a Gulf-wide visual survey using a towed camera array (CBASS) with emphasis on pipeline and open uncharacterized bottom habitats.

David Portnoy, Ph.D.

Texas A&M University-Corpus Christi

Principal Investigator in charge of archiving DNA tissue for future genetics studies stemming from the tagging component of the project. Physical samples are housed at TAMUCC and data is available through the GRIIDC.

Dana Sackett, Ph.D.

Auburn University

Investigator responsible for tag recapture study design, data collection, database management, data analysis, and presentation of tag-recapture results through written and oral means (with M. Catalano). Also developed outreach materials and e-mail and phone recapture systems.

Eric Saillant, Ph.D.

University of Southern Mississippi

Principal Investigator responsible for archiving DNA tissue for future genetics studies stemming from the tagging component of the project.

Steven Scyphers, Ph.D.

Northeastern University

Investigator responsible (with M. Drymon) for designing stakeholder surveys and interpreting/analyzing survey results relative to angler awareness and satisfaction.

Zach Siders, Ph.D.

University of Florida

Investigator involved in initial project design and performance of the random forest modeling and post-stratification of the data (with R. Ahrens) to produce Red Snapper density estimates.

Lynne Stokes, Ph.D.

Southern Methodist University

Principal Investigator responsible for an independent analysis of population estimate. All data was provided by the lead investigator for each state.

Matthew Streich, Ph.D.

Texas A&M University-Corpus Christi

Investigator responsible for developing and leading ROV and hydroacoustic sampling at natural and artificial reefs off Texas.

Joseph Tarnecki, M.S.

University of Florida

Investigator responsible for overseeing a field crew estimating red snapper density on Florida artificial reefs and natural bottom habitats. He also oversaw red snapper size estimation from stereo camera and laser data, as well as performed red snapper counts from video samples.

Tara Topping, M.S.

Texas A&M University-Corpus Christi

Investigator responsible for assistance in the Texas estimate of Red Snapper using visual abundance. Administrative coordination of meetings regarding the final report culminating with preparation, compilation, and review of the final report.

R. J. David Wells, Ph.D.

Texas A&M University at Galveston

Principal Investigator responsible for coordination and planning of north TX sampling and abundance calculation.

Jennifer Wetz, M.S.

Texas A&M University-Corpus Christi

Investigator responsible for coordinating field work, developing and implementing the visual survey design, and devising/supervising subsequent visual data finalization for TX.

Shalima Zalsha, Ph.D.

Southern Methodist University

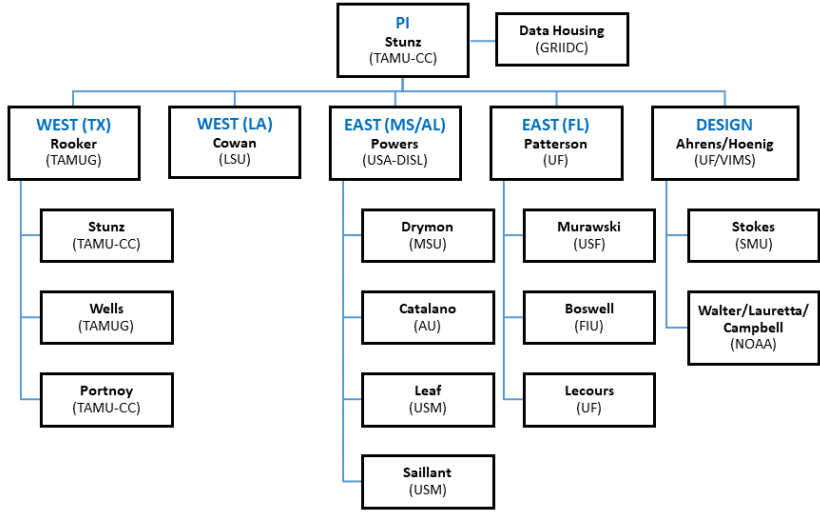
Investigator responsible (with L. Stokes) for producing an independent analysis of population estimates. All data was provided by the lead investigator for each state.

NOAA/Non-Compensated Collaborators:

The following individuals advised the committee regarding the initial design and the desired final outcomes required for this study. Their expertise was utilized to ensure the project goals were adequately reached and the investigators stayed within the scope of the project to calculate the final abundance.

John Walter, Ph.D.

Matt Campbell, Ph.D.



B. Supplementary figures, tables, etc.

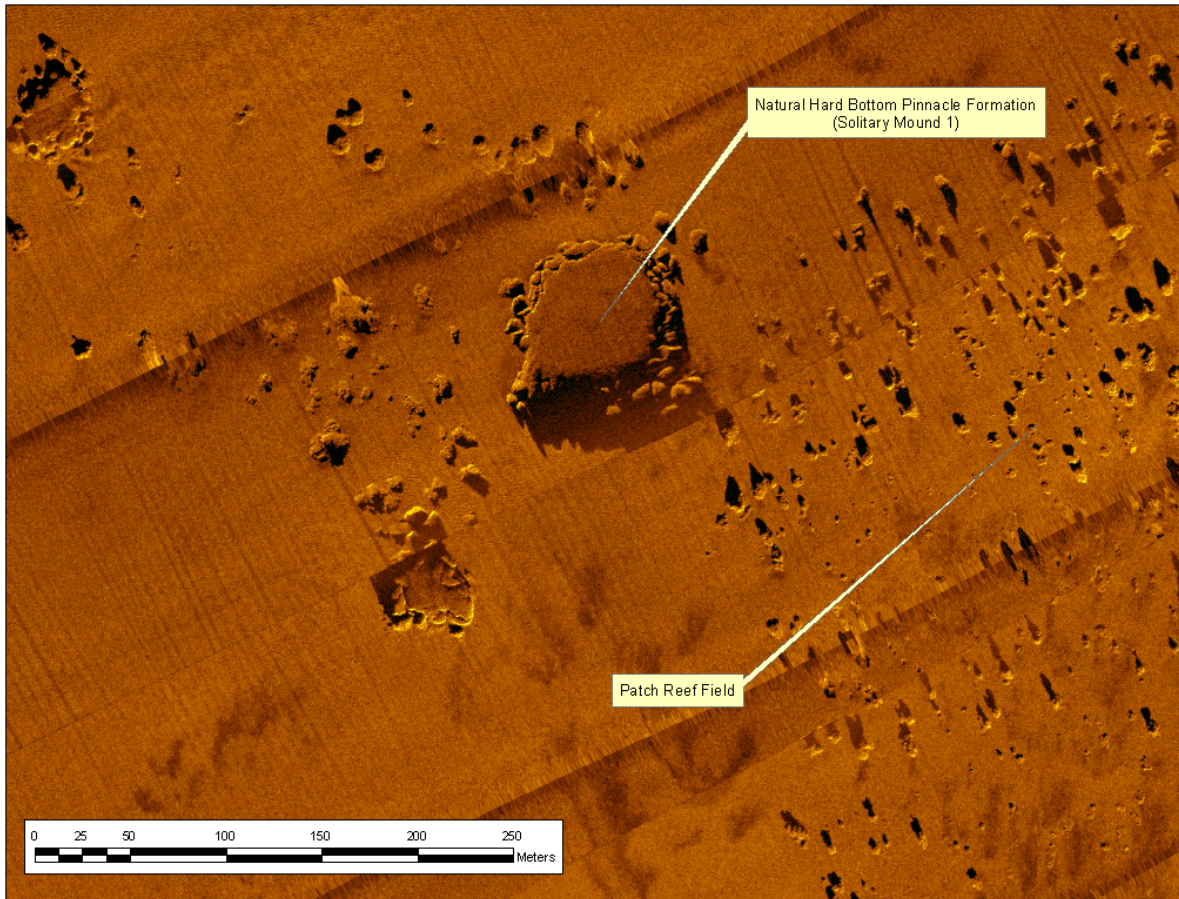


Figure 43. An example side scan image of natural hard bottom in the AARZ.

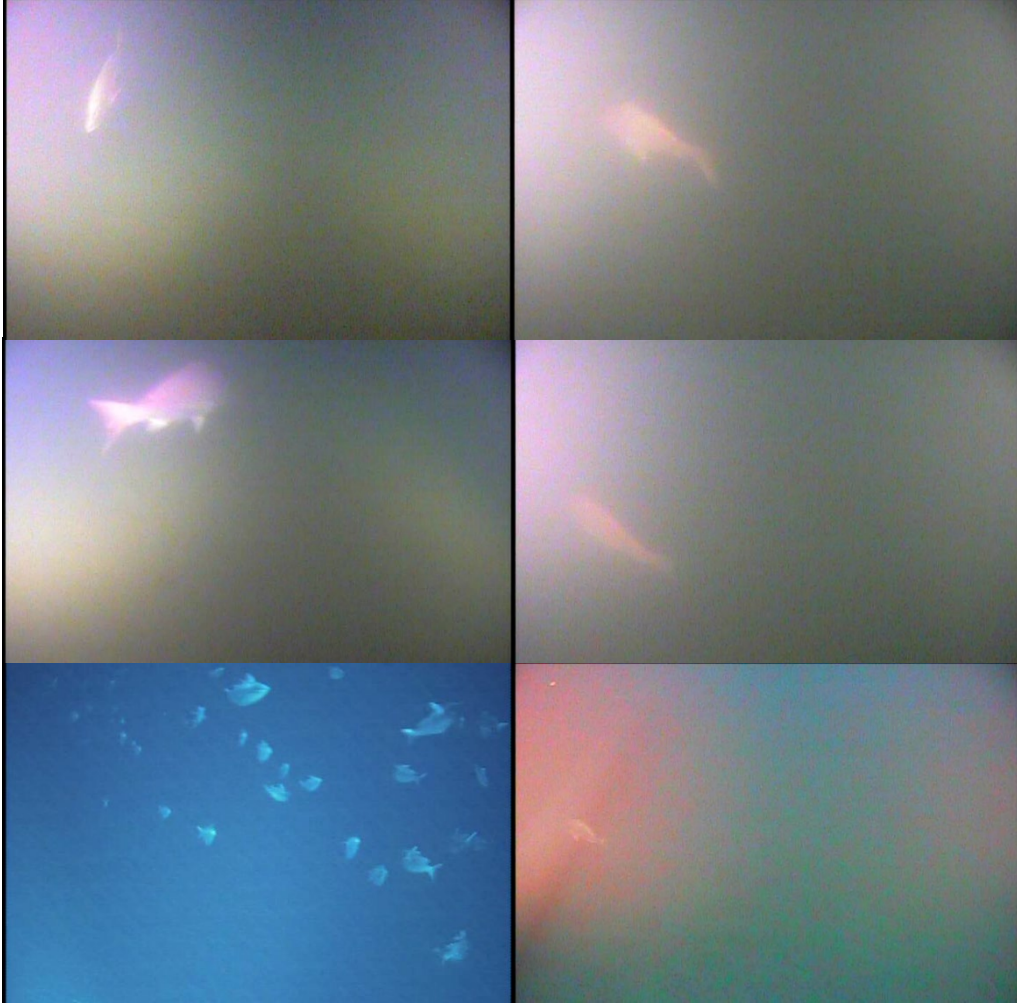


Figure 44. Example C-BASS imagery of Red Snapper (*Lutjanus campechanus*) observed over pipelines and hard bottom during the July 2018 research cruise.

Table 14. Example of hydroacoustic layers with their actual and proportional depths reported for a site where hydroacoustic survey was completed and the maximum depth was 72.1m. Layer numbering starts at the deepest hydroacoustic depth (layer 0) and increases by 10m increments. Proportional layer size corresponds to the percentage of the water column the layer makes up and the cumulative percent is the sum of the water column between the hydroacoustic maximum depth and the top of the layer in question.

Layer	Actual Depths Spanned (m)	Actual Bin Size (m)	Proportional Layer Size	Cumulative Percent	Percent Distance from Bottom
0	71.2-61.2	10	14.114	14.114	0-14.114
1	61.2-51.2	10	14.114	28.228	14.114-28.228
2	51.2-41.2	10	14.114	42.342	28.228-42.342
3	41.2-31.2	10	14.114	56.456	42.342-56.456
4	31.2-21.2	10	14.114	70.57	56.456-70.57
5	21.2-11.2	10	14.114	84.684	70.57-84.684
6	11.2-1.2	10	14.114	98.798	84.684-98.798
7	1.2-0	1.2	1.2	100	98.798-100

Table 15. Summary of C-BASS transects completed during the April 2018 research cruise, July 2018, and January 2020 research cruises

Activity	Date	Time In (UTC)	Time Out (UTC)	Lat (Start/End)	Long (Start/End)	Total Time (hh:mm)	Length (km)
PL-T1 D4	4/27/2018	20:15	22:29	29.8105/29.85297	-87.3833/-87.56848	02:14	17.2
PL-T2 D5	4/28/2018	11:28	12:26	29.586/29.613760	-88.325/-88.389440	00:58	7.3
PL-T3 D6	4/28/2018	16:38	17:35	29.3958/29.34528	-88.04833/-88.09472	00:57	6.8
PL-T4 D7	4/28/2018	18:05	19:27	29.336957/29.256222	-88.069942/-88.09990	01:22	9.2
PL-T5 D8	4/29/2018	11:49	13:52	29.384695/29.3215	-87.920358/-87.8006	02:03	14.2
PL-T5_2 D8	4/29/2018	13:52	14:56	29.3215/29.3465	-87.8006/-87.8652	01:04	7.3
PL-T6 D9	4/29/2018	19:48	23:16	29.80207/29.730363	-87.43891/-87.190208	03:28	24.2
PL-T7 D10	4/30/2018	11:47	17:38	29.14732/28.9348	-85.956235/-85.60335	05:51	28.7
PL-T7_Cont D11	4/30/2018	19:32	22:05	28.8033/28.7041	-85.4398/-85.2799	02:33	17.4
Total						20:30	132.3
GSPL_T1_D1	07/06/2018	12:33	20:57	27.905737/28.1578	-83.924805/-84.5456		
RS_MUD_T1_D2	07/08/2018	12:05	12:36	29.237497/29.257928	-88.124943/-88.111253	00:31	6
RS_MUD_T1_D3	07/08/2018	13:13	14:26	29.232852/29.30715	-88.128733/-88.07486	01:13	12.8
RS_PL_T1_D4	07/08/2018	15:16	16:41	29.338403/29.266603	-88.059048/-88.126137	01:25	13.4
RS_HB_T1_D5	07/08/2018	18:54	19:29	29.25868/29.226927	-88.340525/-88.31988	00:35	4.3
RS_PL_T2_D5	07/08/2018	19:29	21:14	29.226927/29.176450	-88.31988/-88.440975	01:45	11.3
RS_PL_T3_D6	07/08/2018	22:38	00:44*	29.307687/29.338582	-88.531855/-88.362368	02:05	15.7
RS_MUD_T2_D7	07/09/2018	12:12	12:40	29.358409/29.35243	-88.35598/-88.393983	00:28	
RS_MUD_T2_D8	07/09/2018	13:28	14:52	29.350462/29.330003	-88.402128/-88.517038	01:24	12.6
RS_PL_T4_D9	07/09/2018	16:19	17:32	?/29.218725	?/-88.764167	01:13	12.5
RS_PL_T5_D10	07/09/2018	18:32	20:55	29.199852/29.047953	-88.065178/-88.779223	02:23	17.1

RS_PL_T6_D11	07/10/2018	12:56	14:41	28.58329/28.69059	-89.39217/-89.34125	01:45	16.1
RS_MUD_T3_D12	07/10/2018	15:16	15:30	28.75676/28.74756	-89.36285/-89.37569	00:14	2
RS_MUD_T3_D13	07/10/2018	16:24	17:42	?/28.68323	?/89.4432	01:18	10.8
RS_PL_T7_D13	07/10/2018	17:56	20:16	28.68063/28.7878	-89.46083/-89.59042	02:20	18.9
RS_PL_T8_D14	07/10/2018	21:14	22:34	28.7319/28.7356	-89.7039/-89.8242	01:20	13.4
RS_PL_T9_D15	07/11/2018	13:31	15:48	28.36027/28.19967	-90.19396/-90.20052	02:17	18.2
RS_PL_T10_D16	07/11/2018	16:56	18:35	28.12018/28.20031	-90.27087/-90.33797	01:39	9.4
RS_PL_T10_2_D16	07/11/2018	18:47	19:56	28.19709/28.11782	-90.33389/-90.2793	01:09	6.4
RS_HB_T2_D17	07/11/2018	22:35	23:32	28.09638/28.09328	-90.73031/-90.67634	00:57	8.6
RS_PL_T12_D18	07/13/2018	12:12	13:42	28.24189/??	-91.06138/??	01:30	10
RS_PL_T12_2_D19	07/13/2018	14:51	17:23	28.16844/28.01152	-91.07113/-91.02033	02:32	24.9
RS_HB_T3_D19	07/13/2018	18:21	19:23	28.06754/28.13497	-91.01762/-91.01788	01:02	12.1
RS_PL_T13_D20	07/13/2018	20:32	22:39	28.04921/28.07082	-91.09931/-91.25211	02:07	14.8
RS_MUD_T4_D20	07/13/2018	22:50	23:50	28.06165/28.05153	-91.24881/-91.17011	01:00	7.7
RS_PL_T14_D21	07/14/2018	12:19	15:36	28.32145/28.10032	-91.92132/-91.92132	03:17	23.5
RS_PL_T15_D22	07/14/2018	16:34	19:06	28.0755/27.93939	-91.98821/-91.93199	02:32	18.7
RS_HB_T4_D22	07/14/2018	19:22	20:58	27.94117/??	-91.95149/??	01:36	12.4
RS_HB_T5_D22	07/14/2018	21:09	22:00	27.95476/27.95998	-92.06322/-92.99593	00:51	5.9
RS_PL_T16_D23	07/15/2018	12:18	14:22	28.19003/28.1021	-92.67167/-92.5225	02:04	16.9
RS_HB_T6_D25	07/15/2018	16:29	17:24	27.97602/27.94894	-92.61778/-92.56493	00:55	6.1
RS_MUD_T5_D26	07/15/2018	17:47	19:00	27.93953/28.00783	-92.51691/-92.44835	01:13	9.5
RS_PL_T17_D26	07/15/2018	19:20	20:52	28.01736/27.94228	-92.46457/-92.52816	01:32	11.3
RS_PL_T17_2_D27	07/15/2018	21:44	23:17	27.9424/28.0216	-92.5268/-92.4425	01:33	11.3
RS_PL_T20_D28	07/16/2018	12:27	13:54	27.92837/27.85185	-92.88603/-92.93393	01:27	11.3
RS_HB_T7_D29	07/16/2018	15:04	16:23	27.78277/27.86823	-93.04827/-93.07704	01:19	9.3
RS_PL_T18_D30	07/16/2018	19:17	21:04	28.23376/28.1436	-93.02814/-92.92034	01:47	14.8
RS_PL_T19_D31	07/16/2018	21:46	23:38	28.10981/27.97638	-92.97773/-92.97577	01:52	13.4
Total						04:54	210.8
TX_MUD_T2D2	01/12/2020	18:26:11		27.84388/27.939095	-93.441157/-93.526133	01:54	19.1
TX_PL_T1D3	01/12/2020	21:58:57		28.069458/28.263708	-93.717672/-93.751065	03:07	19.1
TX_PL_T4D4	01/13/2020	13:10:13		28.14927/27.837885	-94.244227/-94.2972	05:11	31.7
TX_MUD_T3D4	01/13/2020	18:56:06		27.842857/28.073248	-94.261234/-94.111493	03:19	30.1
TX_MUD_T6D5	01/15/2020	13:03:57		27.895083/27.881688	-95.233527/-94.896808	04:56	34.5
TX_PL_T5D6	01/15/2020	18:58:36		27.95162/28.051183	-94.846422/-95.065642	02:57	22.9
TX_PL_T7D7	01/16/2020	13:18:55		27.781527/27.6982	-95.962732/-95.8855	01:40	11.1
TX_MUD_T8D7	01/16/2020	15:17:39		27.6842/27.608753	-95.914/-96.085845	02:44	21.1
TX_MUD_T9D8	01/16/2020	18:43:29		27.5242127.6414	-96.062852/-95.883415	03:06	21.1
Total						04:54	210.8

C. Stakeholder Engagement and Outreach

The Stakeholder Engagement and Outreach team created a number of materials including six informational videos and corresponding fact sheets regarding the project and the suite of methods used to conduct the research and estimate the total abundance of Red Snapper in the U.S. Gulf of Mexico. These videos and fact sheets are available for public viewing and download at snappercount.org or visit our YouTube channel [here](#).

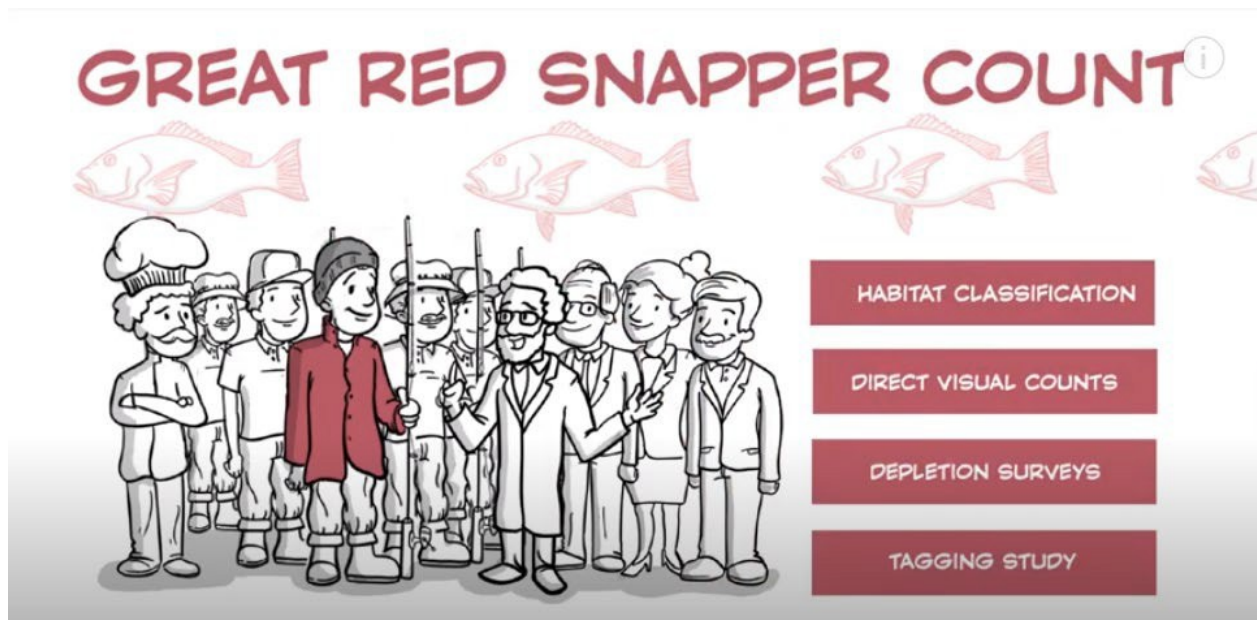


Figure 47. Screenshot from the first informational video created by the Engagement and Outreach team describing the purpose and general sampling plan of the study.



Figure 48. A screenshot from the second video describing how the investigators classified the habitat in the Gulf of Mexico into one of three categories: natural reefs, artificial reefs, or uncharacterized bottom.

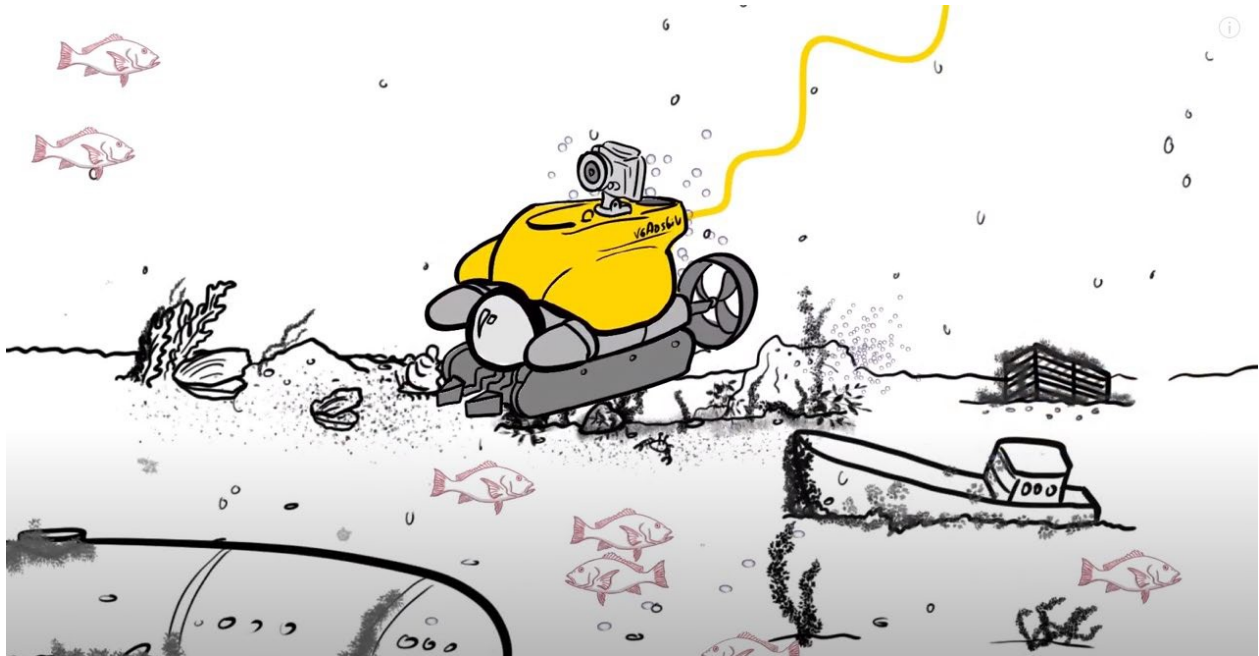


Figure 49. A screenshot from the third video describing the direct visual counts done throughout the Gulf used to help calculate the total abundance of Red Snapper.

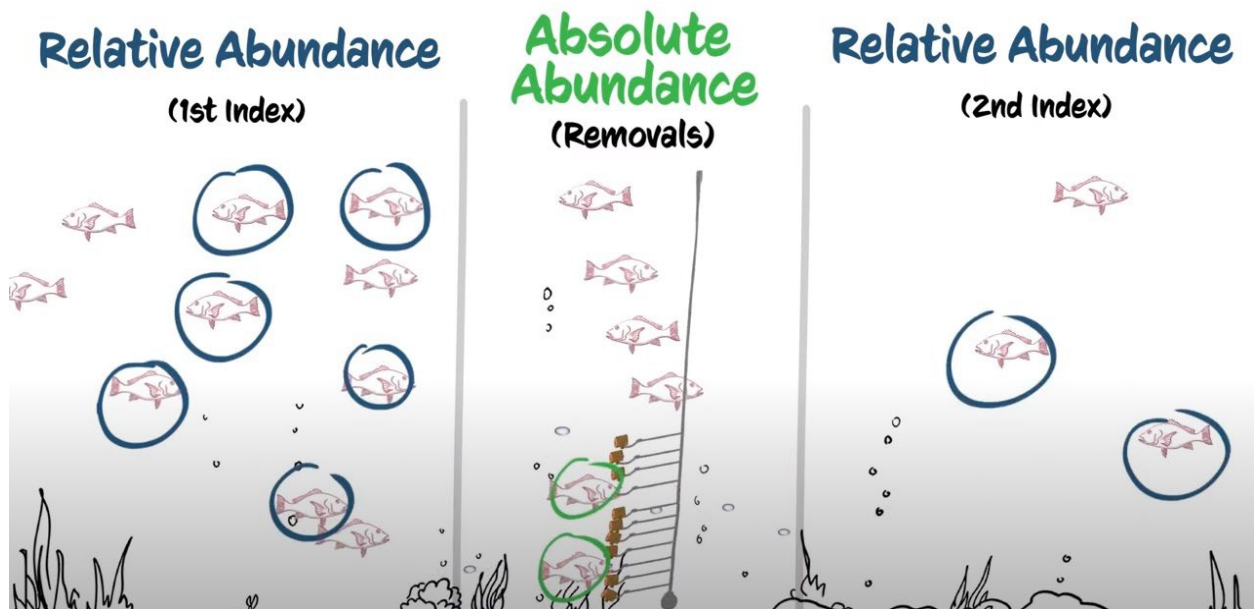


Figure 50. A screenshot from the informational video about the depletion method used to help calculate the number of Red Snapper off Alabama and Mississippi.

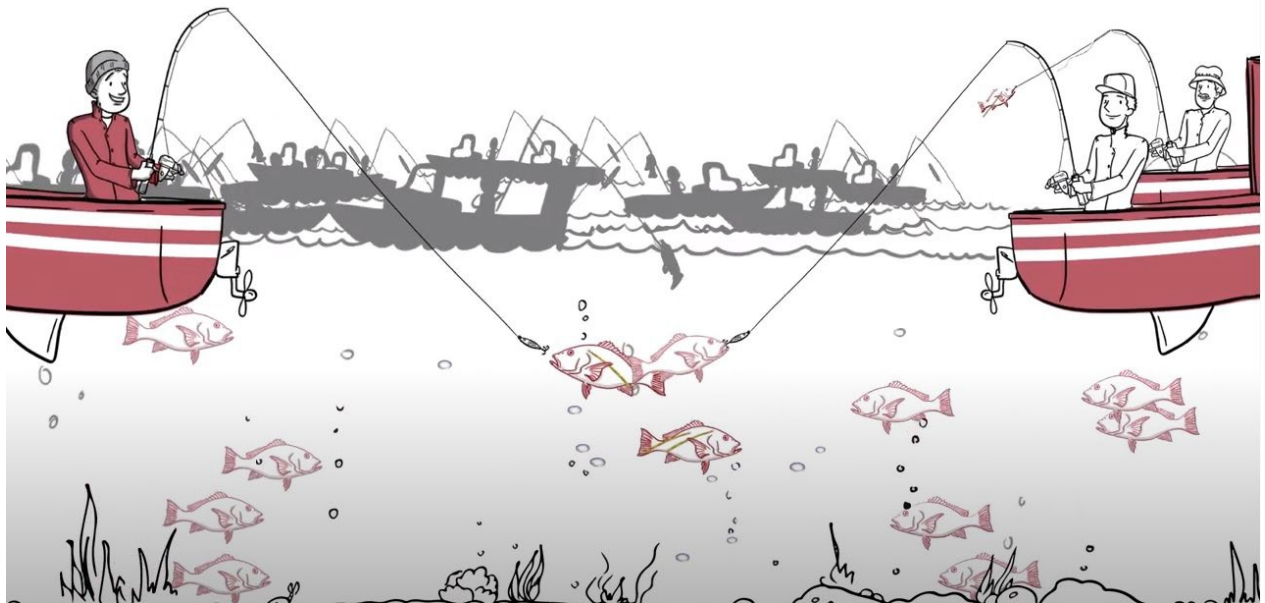


Figure 51. A screenshot describing the Gulf-wide high reward tagging study conducted to help calculate the abundance of Red Snapper. This study also helped scientists collect better data about fishing effort and discard mortality, two very important factors to consider when managing the stock.

Region Estimates:

Texas - 23 million

Louisiana - 28 million

Mississippi/Alabama - 11 million

Florida - 48 million



Figure 52. A screenshot from the final video describing the results of the study, including the final estimate of 110 million Red Snapper.

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The Great Red Snapper Count

PROJECT OVERVIEW



Photo by Quentin Hall

This project overview describes the **Great Red Snapper Count**, which is a two-year research project to estimate the abundance of red snapper in the U. S. Gulf of Mexico.

Why is this study important?

- Red snapper comprise an economically valuable and culturally relevant fishery in the Gulf of Mexico.
- The stock is currently under a rebuilding plan.
- Although the red snapper fishery is showing signs of recovery, anglers are frustrated by restrictions, such as shortened seasons.
- Stakeholders collectively desire a healthy, well-managed red snapper stock.
- A lack of abundance data hinders the best possible stock management.

Who is funding the study?

- Congress made \$10 million in funding available for research projects designed to independently estimate red snapper abundance.
- After a competitive review process, Mississippi-Alabama Sea Grant awarded the \$10 million for a 2-year (2017-2019) project.

What is the goal of the study?

The central objective of this study is to independently (separately from NOAA Fisheries) estimate the abundance of red snapper in the U.S. Gulf of Mexico.

Who is involved in the study?

A well-integrated, multidisciplinary team of investigators, which includes leading fisheries experts from the Gulf region and beyond, is leading the project.



A scientist measures a red snapper that was caught during a scientific research study.

Photo by Dauphin Island Sea Lab/University of South Alabama Fisheries Ecology Lab

Questions or comments? Contact the project team at snappercount@harterresearchinstitute.org
For more information, visit snappercount.org

The Great Red Snapper Count - PROJECT OVERVIEW

How will scientists develop the abundance estimate?

Scientists will use a suite of methods, including habitat classification, direct visual counts, depletion surveys and a tagging study, across the entire U.S. Gulf of Mexico.

What are the expected outcomes of the study?

- Legislators and fishery managers will review the abundance estimate from this project and use it to make more informed management decisions.
- This will lead to:
 - Calibration of the current stock assessment
 - Increased confidence in the status of the stock
 - Maximum fishery access for stakeholders



Scientists use remotely operated vehicle (ROV) footage to conduct direct visual counts and estimate the number of fish inhabiting artificial reefs.

Photo by Dauphin Island Sea Lab/University of South Alabama Fisheries Ecology Lab



In The Great Red Snapper Count, yellow high-reward tags will be placed below the dorsal fin.

Photo by David Hay Jones



Scientists conduct sampling surveys in conjunction with ROV surveys to provide another estimate of fish abundance.

Photo by Dauphin Island Sea Lab/University of South Alabama Fisheries Ecology Lab

This independent study is being conducted by a leading team of red snapper scientists from across the Gulf of Mexico and beyond:



MASGP-18-019-01
 This project and publication are funded by the U.S. Department of Commerce's National Oceanic and Atmospheric Administration under NOAA Award NA16OAR4170181 and the Mississippi-Alabama Sea Grant Consortium. The views expressed herein do not necessarily reflect the views of any of these organizations.

The Great Red Snapper Count

HABITAT CLASSIFICATION



Photo by Trey Spearman, Dauphin Island Sea Lab/
University of South Alabama Fisheries Ecology Lab

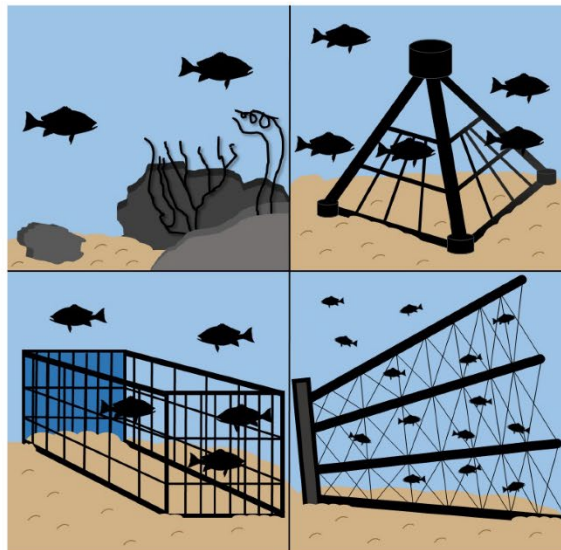
This fact sheet describes the habitat classification phase of the Great Red Snapper Count, which is a two-year research project to estimate the abundance of red snapper in the U.S. Gulf of Mexico.

Where do red snapper live in the U.S. Gulf of Mexico?

- Red snapper are distributed across a variety of habitats.
- The seafloor consists primarily of sand and mud, along with natural reefs; these areas provide habitat for red snapper.
- Concentrated areas of artificial structures also serve as red snapper habitat.
- The coverage of sediments, natural reefs, and artificial structures differs dramatically across the Gulf.

What types of artificial structures exist in the Gulf?

- Large oil and gas platforms are common in the western Gulf.
- Smaller structures (chicken transport cages, pyramids, military tanks, planes, car bodies, and others) are deliberately placed on the seafloor to create fish habitat.



This illustration shows some of the various reef types present in the U.S. Gulf of Mexico (clockwise from top left: natural reef, pyramid, toppled rig, and chicken transport cage).

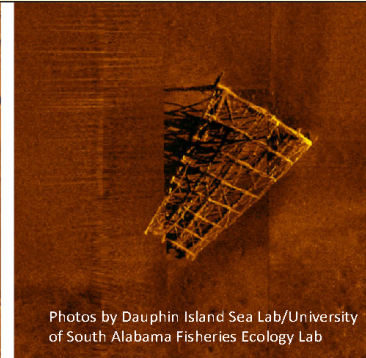
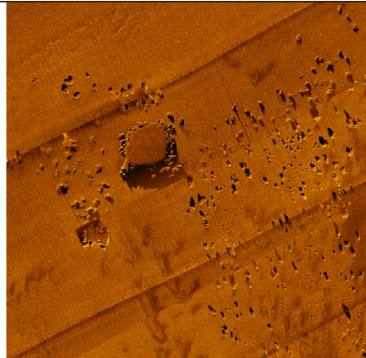
Image by Amanda Jefferson, Mississippi State University/Mississippi-Alabama Sea Grant

Questions or comments? Contact the project team at snappercount@harterresearchinstitute.org
For more information, visit snappercount.org

The Great Red Snapper Count - HABITAT CLASSIFICATION

What is habitat classification?

- Habitat classification is "Phase 1" of the Great Red Snapper Count.
- This phase involves determining where each of the various habitat types exist across the Gulf.



Photos by Dauphin Island Sea Lab/University of South Alabama Fisheries Ecology Lab

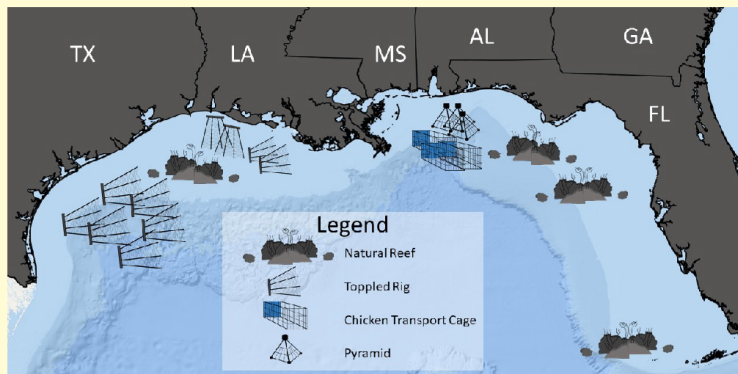
How did scientists approach the habitat classification process?

These rocky outcrops (left) and a toppled rig were discovered during a side-scan sonar survey.

- U.S. Gulf waters were separated into four regions: Texas, Louisiana, Mississippi-Alabama, and Florida.
- Each region was divided into three depth zones, creating 12 unique sections.
- For each section, scientists compiled existing data from various sources to characterize known habitat features.

What did scientists learn from this process?

- Scientists calculated the amount of the U.S. Gulf seafloor that is covered by sand, mud, and natural reefs.
- Scientists also determined the quantity of existing artificial reef structures.



This conceptual map shows the general types of habitat present across the U.S. Gulf of Mexico.

Why is this information useful?

- Based on the distribution and number of different habitat types, scientists decided which sampling approaches, or "gear types," to use in the Great Red Snapper Count.
- This will result in the best possible estimate of red snapper abundance in each section of the U.S. Gulf of Mexico.

Map by Amanda Jefferson, Mississippi State University/Mississippi-Alabama Sea Grant

This independent study is being conducted by a leading team of red snapper scientists from across the Gulf of Mexico and beyond:



MASGP-18-019-02
This publication was supported by the U.S. Department of Commerce's National Oceanic and Atmospheric Administration under NOAA Award NA16OAR4170181, the Mississippi-Alabama Sea Grant Consortium and the Mississippi State University Extension Service. The views expressed herein do not necessarily reflect the views of any of these organizations.

The Great Red Snapper Count

DIRECT VISUAL COUNTS



Image by the Center for Sportfish Science and Conservation

This fact sheet describes the direct visual counts phase of the Great Red Snapper Count, which is a two-year research project to estimate the abundance of red snapper in the U.S. Gulf of Mexico.

Where will scientists count red snapper?

Counts will be performed across the U.S. Gulf of Mexico at various habitat types (for more details, see our “Habitat Classification” video and fact sheet).

What types of equipment will scientists use onboard research vessels?

- Scientists will use two types of camera equipment: remotely operated vehicles (ROVs) and towed cameras.
- An ROV is deployed from a stationary vessel and driven by an operator in a specific pattern, much like the operation of a remote-controlled car.
- A towed camera is pulled behind a research vessel at a constant speed and altitude above the seafloor, along a predetermined path.
- Both camera types will record video footage to be analyzed later.

(A) An ROV, the VideoRay Pro 4, and (B) a towed camera, the Camera-Based Assessment Survey System (C-BASS), are used for direct visual counts.

Photos by (A) the Center for Sportfish Science and Conservation and (B) the Continental Shelf Characterization, Assessment, and Mapping Project (C-SCAMP)



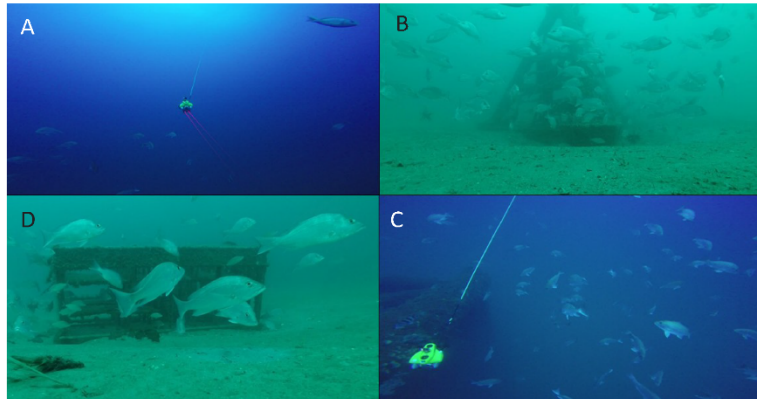
Questions or comments? Contact the project team at snappercount@harterresearchinstitute.org
For more information, visit snappercount.org

The Great Red Snapper Count - DIRECT VISUAL COUNTS

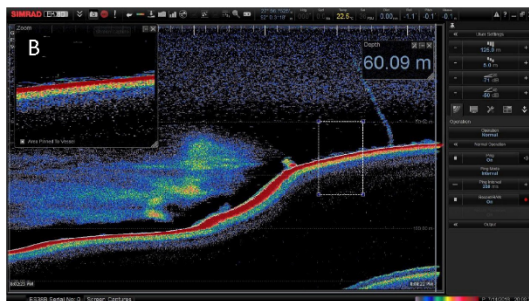
Why do scientists need to use two different types of equipment?

- ROVs are best for surveying discrete artificial and natural habitats.
- Towed cameras are best for surveying large expanses of sand and mud bottom.

Photos by (A, C) the Center for Sportfish Science and Conservation and (B, D) the Dauphin Island Sea Lab/University of South Alabama Fisheries Ecology Lab



(A, C) A VideoRay ROV surveys red snapper along a transect. (B, D) ROV screenshots show red snapper congregating at a pyramid and chicken transport cage, respectively.



How will scientists collect fish count data from the video footage?

- The videos from the two types of cameras will be transferred to laboratory computers and analyzed.
- First, scientists will count the number of red snapper in each ROV and towed camera video.
- Then, these counts will be converted to density estimates, which will yield abundance estimates.
- In areas with poor visibility or very large structures, bioacoustic sonar (imagine a 'fish finder') will be used with ROV surveys to confirm red snapper abundance estimates.

(A) An ROV screenshot shows gray snapper along the Gulfstream pipeline. (B) An EK-80 bioacoustic sonar display depicts an elevated feature with fish near the seafloor, and a prominent gas seep at far right.

Photos by (A) the Continental Shelf Characterization, Assessment, and Mapping Project (C-SCAMP) and (B) Edward Hughes, C-SCAMP

This independent study is being conducted by a leading team of red snapper scientists from across the Gulf of Mexico and beyond:



MASGP-18-019-03
This publication was supported by the U.S. Department of Commerce's National Oceanic and Atmospheric Administration under NOAA Award NA16OAR4170181, the Mississippi-Alabama Sea Grant Consortium and the Mississippi State University Extension Service. The views expressed herein do not necessarily reflect the views of any of these organizations.

The Great Red Snapper Count

DEPLETION STUDIES



This fact sheet describes the depletion studies phase of the Great Red Snapper Count, which is a two-year research project to estimate the abundance of red snapper in the U.S. Gulf of Mexico.

Index-removal depletion gear. (A) A remotely operated vehicle (ROV), used to record video footage; fish counts from this footage yield relative abundance indices. (B, C, D) Hook-and-line gear is used for removals, which provide an absolute abundance estimate.

Photos by (A-C) David Hay Jones and (D) Trey Spearman, Dauphin Island Sea Lab/ University of South Alabama Fisheries Ecology Lab

What is a depletion study?

A depletion study is a scientific survey which collects two types of information: relative abundance (in our case, counts of red snapper *relative* to other species present) and absolute abundance (a known number of fish removed from the population).

Where are scientists conducting depletion studies?

Depletion studies for red snapper are being conducted at both natural and artificial habitat types.

How do scientists determine the abundance of red snapper from the depletion data?

The ratio of relative abundance to absolute abundance yields a population size estimate.

Questions or comments? Contact the project team at snappercount@harterresearchinstitute.org

For more information, visit snappercount.org

The Great Red Snapper Count - DEPLETION STUDIES

What types of depletion studies are being used to estimate abundance?

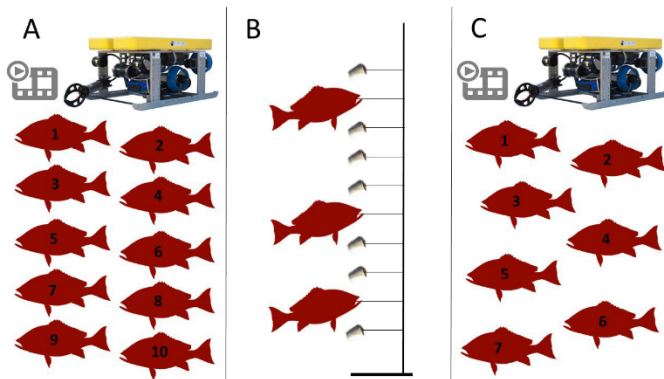
1. Index-Removal:

- This method involves successive cycles of indexing (or counting) the population using video footage collected with a remotely operated vehicle (ROV), then removing individuals from the population using hook-and-line gear, and then indexing again with the ROV.
- After at least one index-remove-index cycle is completed, the second index should be a reduction of the first index, based on the number of individuals that were removed.



The Outland Technology ROV-2500, which is being used to record video footage for relative abundance indices.

Photo by Outland Technology, Inc.



One cycle of the index-removal depletion method involves indexing (A), removing fish (B), and indexing again (C).

Image by Amanda Jefferson, Mississippi State University/Mississippi-Alabama Sea Grant

2. Change-in-Ratio:

- This method applies the same principles as index-removal but collects relative and absolute abundance data in a different way.
- Relative abundance is determined during scientific surveys immediately before and after a recreational fishing season, while absolute abundance is simply the number of fish removed by the recreational fishery.

For the change-in-ratio depletion method, indexing occurs immediately before and after the red snapper recreational fishing season, and recreational landings are used to determine removals.

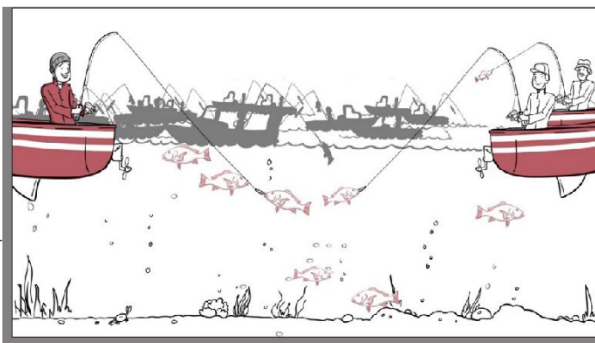


Image by HypnoVid

This independent study is being conducted by a leading team of red snapper scientists from across the Gulf of Mexico and beyond:



MASGP-18-019-04
 This publication was supported by the U.S. Department of Commerce's National Oceanic and Atmospheric Administration under NOAA Award NA16OAR4170181, the Mississippi-Alabama Sea Grant Consortium and the Mississippi State University Extension Service. The views expressed herein do not necessarily reflect the views of any of these organizations.

The Great Red Snapper Count

TAGGING STUDY



This fact sheet describes the tagging component of the Great Red Snapper Count, which is a two-year research project to estimate the abundance of red snapper in the U.S. Gulf of Mexico. This study will include recreational and commercial fishers as a critical component of the scientific process.

A scientist uses a dart tag applicator (above) to tag a red snapper. Photo by David Hay Jones

Who will tag the fish?

To ensure consistency, all red snapper will be tagged by scientists who are working on the Great Red Snapper Count, in collaboration with recreational and commercial fishers.

How many red snapper will be tagged?

Approximately 4,000 legal-sized red snapper will be tagged.

When and where will tagging occur?

Tagging will happen in spring 2019, prior to the federal Gulf of Mexico red snapper recreational fishing season. Red snapper will be tagged and released across the U.S. Gulf of Mexico.

What do the tags look like, and where will they be located on the fish?

The tags are yellow and have text beginning with "RS" followed by a unique 5-digit ID number, along with a phone number to call for reporting the recaptures. Tags will be placed beneath the dorsal fin. Some fish will have two tags so that tag shedding rates can be estimated.

Tags used in this tagging study, shown below, will be bright yellow with a unique 5-digit ID number.

Photo by Judd Curtis, Texas A&M University-Corpus Christi



Questions or comments? Contact the project team at snappercount@harteresearchinstitute.org

For more information, visit snappercount.org

The Great Red Snapper Count - TAGGING STUDY

A tagged red snapper is ready to be released into Gulfwaters.

Photo by the Center for Sport-fish Science and Conservation



What types of information should fishers document before reporting a recapture?

- The fishing port from which they departed
- The date the fish was caught
- The fish's tag ID ("RSXXXXX")
- The fish's length and weight
- The latitude and longitude where the fish was caught

How can recreational and commercial fishers become involved in the study?

Fishers can become involved by recapturing tagged fish and then reporting those recaptures by calling the phone number printed on the tags.

What is the reward for reporting a tagged fish?

Tags from recaptured fish will be worth \$250 per fish. Some double-tagged fish may be worth up to \$500. The physical tag must be mailed in to claim the reward, so fishers should always clip off and save the tag, even if they plan to release the fish. Fishers who report their recapture information AND return the physical tag to the research team will receive a reward. If a fish has two tags, both tags should be reported and returned. Rewards will be issued through Dec. 31, 2019.

How will this tagging study contribute to the overall abundance estimate?

Tag returns will be combined with estimates of catch and effort from participating fishers to estimate red snapper abundance.

This independent study is being conducted by a leading team of red snapper scientists from across the Gulf of Mexico and beyond:



MASGP-18-019-05
This publication was supported by the U.S. Department of Commerce's National Oceanic and Atmospheric Administration under NOAA Award NA16OAR4170181, the Mississippi-Alabama Sea Grant Consortium and the Mississippi State University Extension Service. The views expressed herein do not necessarily reflect the views of any of these organizations.

D. Manuscripts Related to Project

Design of a Multidisciplinary Study to Estimate Red Snapper Population Size, Population Connectivity, and Mortality Rates in the US Gulf of Mexico

Robert Ahrens¹, Kevin Boswell², James Cowan^{3*}, Steve Midway³, William Patterson⁴, David Portnoy⁵, and David Wells⁶

¹University of Florida, ²Florida International University, ³Louisiana State University, ⁴University of South Alabama,

⁵Texas A&M University-Corpus Christi, and ⁶Texas A&M University-Galveston

*Principal investigator

Keywords: red snapper, population estimation, population dynamics, genetic tagging, videosurvey

Introduction

Marine fisheries are exceedingly important to coastal economies of the northern Gulf of Mexico (nGOM). On the shelf, no group is more economically important than reef fishes, even though several marquee species, including red snapper, are estimated to be overfished. Increasingly restrictive fishery regulations, following passage of the Magnuson-Stevens Reauthorization Act of 2006, have had positive impacts on red snapper recovery, but shortened recreational fishing seasons have also brought angst and mistrust, particularly among recreational fishery lobby groups. Among the issues routinely cited by these groups is the perception that artificial reefs have greatly increased the productivity of red snapper in the nGOM yet that is not accounted for in stock assessment models, as well as the perception that red snapper population size is greater than estimates derived from those same models. Part of the persistence of these perceptions owes to the failure of scientists to adequately convey to the fishing public that the catch at age matrix routinely has the greatest influence on estimates of stock biomass and productivity in statistical catch at age assessment models. Therefore, if most of the catch in each region is taken at artificial reefs, then clearly the influence of those reefs is captured in assessment models.

Recent funding made available by Congress to estimate red snapper population size provides a unique opportunity to conduct GOM-wide sampling to challenge data inputs and parameter estimates of the GOM red snapper stock assessment model. This is an awesome challenge given the GOM's nearly 1×10^6 km² shelf. Furthermore, within the US GOM's EEZ is a variety of natural and artificial reef habitats that further complicate designing a GOM-wide study to estimate red snapper population size and dynamics. For example, in addition to an estimated ~ 27 thousand km² of rock dominant or subdominant natural surficial substrate, there are myriad manmade reef structures on the nGOM, such as oil and gas platforms ($\sim 2,000$ and ~ 20 km²), state permitted artificial reefs (0.13 km²), and shipwrecks and obstructions (0.74 km²) (Froesche and Dale 2014). Given the spatial scale and heterogeneity of habitat within the region, evaluation of the scale and methodological composition of sampling programs required to estimate absolute red snapper (*Lutjanus campechanus*) abundance is essential.

Here, we present our evaluation of the efficacy of two general approaches, tagging or video-based counts, for estimating the abundance of age 2+ red snapper throughout U.S. waters of the GOM. The primary objective of this study is to explore sampling design options to estimate the abundance of age-2+ red snapper in U.S. waters of the northern Gulf of Mexico (GOM) with a coefficient of variation of 0.3, which was prescribed by Congress. Potential secondary benefits from this design are estimates of red snapper growth, mortality, site fidelity, and population connectivity. Ultimately, population parameters estimated during the implementation phase of the design will either challenge assumptions of rates utilized within the red snapper stock assessment model (e.g., natural mortality), or will be compared to estimates computed with the model (e.g., population abundance).

The first step in our analysis involved compiling estimates of adult red snapper distribution and density among various artificial and natural habitats that were either produced in dedicated research studies or were derived from fishery catch per unit effort (CPUE) data. Collectively, these somewhat limited data were used to compute a spatial simulation of adult red snapper density in the

nGOM. Next, simulations were constructed for both tagging and video-based estimation of adult red snapper abundance to estimate the sampling effort and costs required to produce GOM-wide estimates of red snapper abundance. Beyond preliminary estimates of the distribution of red snapper biomass, other assumptions had to be made involving the percentage of nGOM habitats occupied by red snapper and shiptime and manpower costs to conduct surveys. Simulation results are presented below along with caveats as to the impact of assumed parameters being incorrect.

Methodology

Physical characteristics

The first stage in estimating the distribution of red snapper biomass in U.S waters of nGOM involved dividing the nGOM into 3 arc-second squared sampling units (~35 million) between 10 and 160 m depths. These sampling units were then partitioned into 15 strata (Figure 1) representing broad geopolitical/biological boundaries from west to east and 3 depth zones (10-40m, 40-100 m, 100-160 m). Bottom physical characteristics for each stratum were derived using the National Center for Environmental Information (NCEI) 3 arc-second U.S. Coastal Relief Model (CRM) (NOAA National Centers for Environmental Information August 2016), the U.S. Geological Survey usSEABED: Gulf of Mexico and Caribbean (Puerto Rico and U.S. Virgin Islands) Offshore Surficial Sediment Data Release 2006, Version 1.0 (Buczowski et al. 2006), the National Center for Environmental Information Gulf of Mexico Hypoxia Watch bottom oxygen database (<http://www.ncddc.noaa.gov/hypoxia/products/>), NOAA Office of Coast Survey Wrecks and Obstructions database (http://www.nauticalcharts.noaa.gov/hsd/wrecks_and_obstructions.html), the Bureau of Ocean Energy Management platform location database (<https://www.boem.gov/GOMR-GIS-Data-and-Maps/>), and the Gulf of Mexico registered artificial reef database (<http://marinecadastre.gov/data/>). Physical characteristics derived for each sampling units are presented in Table 1.

Red snapper visual density estimates

Red snapper density estimates (count per unit area) were compiled from various data sources and converted to numbers per 3 arc-second square area. Most often, empirical estimates were derived from red snapper counts made from video samples collected with remotely operated vehicles (ROV). This was true for natural and artificial reefs off the Florida Panhandle and Alabama, where both point-count and transect methods were utilized (Patterson et al. 2014; Dahl et al. 2016). In Louisiana, red snapper density estimates were computed from available data at standing (Stanley and Wilson 1996, 1997, 2000) and toppled (Boswell et al. 2010) oil and gas platforms, in addition to natural habitats (Wilson et al. 2006). These estimates were derived from active acoustic methods, aimed at examining the fish community structure associated with these habitats. The measured acoustic energy from detected fish was scaled to red snapper based on the proportion of red snapper identified in ROV-based video surveys of each habitat. In Texas, limited information was available to compare

unit-area estimates, however Streich (2016) reported density estimates across both natural and artificial reef habitats based on standardized transect ROV-based video methods.

Estimating fish density on large-volume structures such as standing or toppled petroleum platforms is more difficult than smaller artificial and typical natural. Therefore, red snapper density on those structures was estimated with a combination of acoustics and ROV video. That approach and associated methods were developed by Stanley and Wilson (1996, 1997, 2000), which revolutionized our ability to estimate fish density and community composition on petroleum platforms. More recent work by Wilson et al. (2006) informed red snapper density estimates on western GOM petroleum platforms in the current study, but also were utilized as estimates of platform-associated red snapper density off Alabama/Mississippi and Texas.

Density estimates on artificial structures in each spatial stratum were assumed representative and the arithmetic means and standard deviations were calculated. Density estimates on natural bottom generally came from high relief habitat, thus were assumed to represent the 95th percentile of red snapper density from a log-normal distribution. Arithmetic means for these samples were adjusted accordingly assuming a log-normal distribution and standard deviation in log-space adjusted so that the standard deviation of unlogged values was similar to that indicated by the coefficients of variation. Empirical density estimates were not available for many strata. In these instances, values were calculated based on a region-specific reference strata (Table 2) and a scalar. Scalars were calculated as the relative difference in the summed annually averaged catch per fishing point within 10 km x 10 km areas within the strata determined from an analysis of vertical longline fleet vessel monitoring data (VMS). Catch per point was calculated from a uniform allocation of trip ticket catches to VMS points per trip estimated to be fishing points.

VMS point classification was done using a random forest model trained on the vertical line fishery observer data.

Red snapper population density modeling

A delta log-normal generalized additive model (GAM) was used to estimate expected relative red snapper density based on physical characteristics using georeferenced red snapper catch rate (biomass per hook hour) information from the GOM Reef and Shrimp Observer Program (OBS) for the vertical line fishery (Figure 2). These data classified catch records for 13,283 locations from January 2007 through February 2014. Forward and backward stepwise regression was used to determine the final model. Final variable fits and resulting spline functions are presented in tables 3 and figures 3-4. Spatial location, depth, bottom oxygen, topographic ruggedness, standard deviation in slope, slope, and the relative contribution of mud, sand, and gravel were generally significant in both the binomial and log-normal models. The binomial model has a null deviance of 3,053.106 on 13,282 degrees of freedom, and a residual deviance of 2,514.639 on 13,241 degrees of freedom with an AIC of 15,673.89. The log-normal model had a null deviance of 10,422.77 on 4,755 degrees of freedom, and a residual deviance of 9,665.607 on 4,714 degrees of freedom with an AIC of 16,955.74.

Assuming local biomass caught per hook hour reflects local abundance, the final GAM was then used to predict relative abundance over the full grid of ~35 million spatial areas (Figure 5). Due to low sample sizes in the extreme southwestern and southeastern GOM, binomial GAM predictions were not reliable and reduced to 10% of predicted values. The resulting GAM predicted densities are overdispersed relative to the distribution indicated by commercial fishing. To create a more patchy distribution, 25% of the gulf was assumed to be occupied by redsnapper. To achieve this level of occupancy, binomial model predictions of <77.1% occupancy were excluded from the final population model. Relative biomass was converted to absolute numbers assuming the spatial distribution of relative biomass within each zone (see Figure 1) could be mapped to absolute numbers using observed density data. To achieve this mapping, the probability from a cumulative normal distribution for each grid cell was calculated using the stratum-specific mean predicted catch rate and its standard deviation. These cumulative values were then projected onto a lognormal distribution to determine the quantile values given the mean and standard deviation in log space of the observed or estimated density for each stratum (Table 2). Total density on both artificial and natural bottom are estimated to be noticeably higher than values suggested from stock assessment given this approach. If the population of age-2 and older snapper is approximately 43 million individuals, with 10% of the population on artificial structure, and the count values presented are representative of counts on natural and artificial habitats, the model over predicts density by 2X on artificial structure and by 18X on natural bottom. To align the model simulated population with that estimated in the stock assessment (~43 million), model areas were subsampled. Approximately 5% of natural bottom was randomly sampled and, to preserve the number of artificial structures, densities on structures were reduced by 50% of values allocated given observed counts. This approach preserve the number of artificial structures which are better documented than areas of natural bottom used.

This resulted in a population much less dispersed than predicted by the GAM model, with 3,361 areas designated as having artificial structure and 34 million natural bottom areas. This population model is the base model used to evaluate sampling designs for both the mark recapture and random stratified visual surveys. To simulate the fishery, a fishing mortality rate of 0.1 was assumed. Areas were targeted based on expected catch rate with the threshold target level set to the mean expected catch rate. This approach is a simplistic way of focusing effort on high snapper density.

Table 1. Physical characteristics derived for each 3 arc-second square cell.

Characteristic	Code	Description
Depth	Bathy	Depth in meters
Bottom DissolvedOxygen	O ₂	Summer annually average oxygen concentration at the seafloormeasured on SEAMAP surveys 2001-2015
Topographic Ruggedness Index	TRI	The difference between the value of a cell and the mean of an 8-cell neighborhood of surrounding cells. Created form a 3 cell x 3 cell smoothed DEM
Topographic Position Index	TPI	A terrain ruggedness metric and a local elevation index createdform a 3 cell x 3 cell smoothed DEM
Standard Deviation in slope	SD	Created form a 3 cell x 3 cell smoothed DEM
Slope	Slope	Created form a 3 cell x 3 cell smoothed DEM
Focal Flow	Focfl	The flow of the values in the DEM within each cell's immediateneighborhood.
Presence of Artificial Structure	Arti	Boolean flag indicating the presence of an artificial structure inthe 3 arc-second square area.
Proportion of surficial substrate as mud	Pm	Classification based on grain size
Proportion of surficial substrate as sand	Ps	Classification based on grain size
Proportion of surficial substrate as gravel	Pg	Classification based on grain size
Proportion of surficial substrate as rock	Pr	Classification based on grain size

Table 2. Estimated mean density and coefficient of variation per 90 m x 90 m area, as well as density per 10³ m² for natural bottom and artificial structure. Bold and italicized entries were used as reference values. Values in other strata were scaled relative to these values using a scalar calculated from the sum of catch rates within 10 km x 10 km areas within the strata determined from VMS data. Shading indicates grouping for the use of reference values.

Region	Depth(m)	Natural			Artificial				
		Scalar	Mean	CV(%)	10 ³ m ²	Scalar	Mean	CV(%)	10 ³ m ²
TX	10-40	1.271	116.13	82	14.34	1.271	1740	136	214.81
	<i>40-100</i>	<i>1.000</i>	<i>91.37</i>	<i>82</i>	11.28	<i>1.000</i>	<i>1344</i>	<i>136</i>	165.93
	100-160	0.556	50.80	82	6.27	0.556	761.1	136	93.96
LA	10-40	1.270	44.34	150	5.47	1.270	2110	95	260.49
	<i>40-100</i>	<i>1.000</i>	<i>34.91</i>	<i>150</i>	4.31	<i>1.000</i>	<i>1661</i>	<i>95</i>	205.06
	100-160	0.609	21.26	150	2.62	0.609	1012	95	124.94
MS-AL	10-40		297.21	109	36.69	<i>1</i>	<i>4004</i>	<i>38.6</i>	494.32
	<i>40-100</i>	<i>1</i>	<i>17.68</i>	<i>109</i>	2.18	0.95	3803	38.6	469.51
	100-160	0.450	7.96	109	0.98	0.46	1841	38.6	227.28
N.FL	10-40		186.11	148	22.98	<i>1.00</i>	<i>356.4</i>	<i>221</i>	44.00
	<i>40-100</i>	<i>1.00</i>	<i>10.12</i>	<i>156</i>	1.25	<i>0.971</i>	346	221	42.72
	100-160	0.560	5.67	156	0.70	0.557	198.5	221	24.51
W.FL Shelf	10-40	0.117	4.08	150	0.50	0.117	220	95	27.16
	<i>40-100</i>	0.114	<i>3.98</i>	150	0.49	0.114	215	95	26.54
	100-160	0.070	2.45	150	0.30	0.070	132	95	16.30

Table 3. GAM fit summary for binomial and log-normal models.

Binomial Model						Log-normal Model					
Variable	df	SS	MS	F value	P value	Variable	df	SS	MS	F value	P value
s(long)	268.1	268.	1411.7	<0.001	***	s(long)	206.5	206.5	100.7	<0.001	***
s(lat)	3.77	3.77	19.8	<0.001	***	s(lat)	442.2	442.2	215.7	<0.001	***
s(bathy)	26.4	26.4	138.9	<0.001	***	s(bathy)	72	72.0	35.1	<0.001	***
s(o2)	24.2	24.2	127.2	<0.001	***	s(o2)	1	0.98	0.48	0.489	
s(tri)	56.8	56.8	299.1	<0.001	***	s(tri)	0.8	0.76	0.37	0.543	
s(sd)	0.06	0.06	0.30	0.587		s(sd)	8.7	8.66	4.22	0.040	*
s(slope)	5.58	5.58	29.4	<0.001	***	s(slope)	15.4	15.4	7.52	0.006	**
s(pm)	0.39	0.39	2.07	0.150		s(pm)	47.1	47.1	23.0	<0.001	***
s(ps)	12.9	12.9	68.0	<0.001	***	s(ps)	50.2	50.2	24.5	<0.001	***
s(pg)	17.9	17.9	94.4	<0.001	***	s(pg)	47.6	47.6	23.2	<0.001	***
arti	1.92	1.92	10.1	0.001	**	arti	0.1	0.07	0.03	0.855	
Residuals	13241	2514.6	0.190			Residuals	4714	9665.6	2.05		

Significance codes: p<0.001 '***'; 0.001<p<0.01 '**'; 0.01<p<0.05 '*'

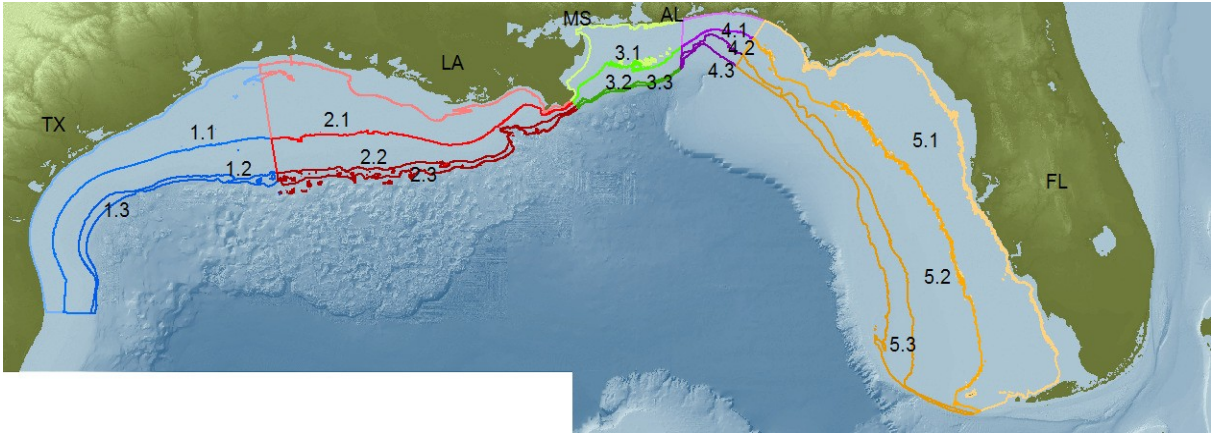


Figure 1. Zonation of the Gulf of Mexico used for data compilation. 5 major zones: 1. TX, 2. LA, 3. MS-AL, 4. N.FL, and 5. W.FL Shelf were subdivided by depth, 10-40 m = 0.1, 40-100 m = 0.2, and 100-160 m = 0.3.

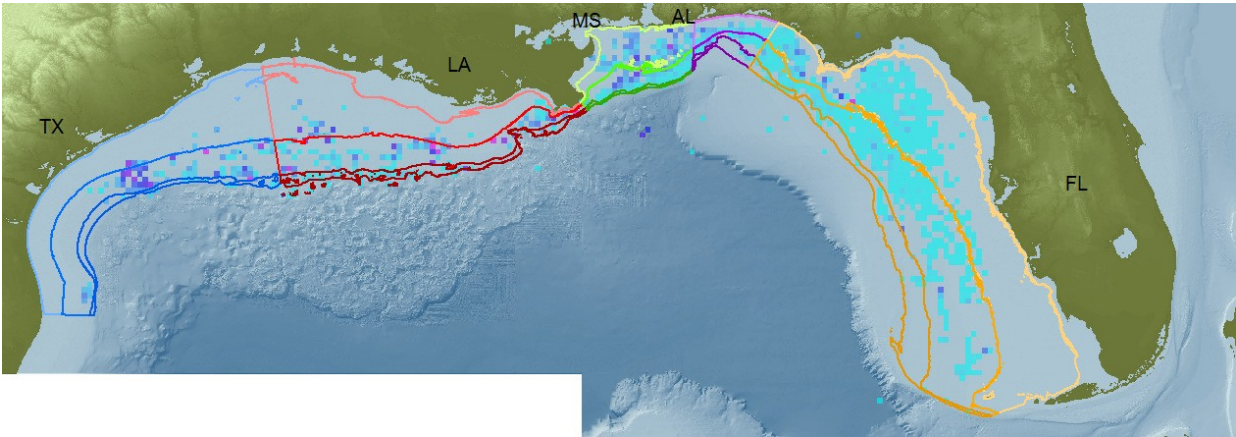


Figure 2. Annually averaged, mean monthly red snapper biomass caught per hook hour (cphh) for 2007-2014 aggregated at a 10 km x 10 km resolution for vertical line trips covered in the NOAA observer program. Light blue indicates low cphh (0.03) and purple indicates high values (587).

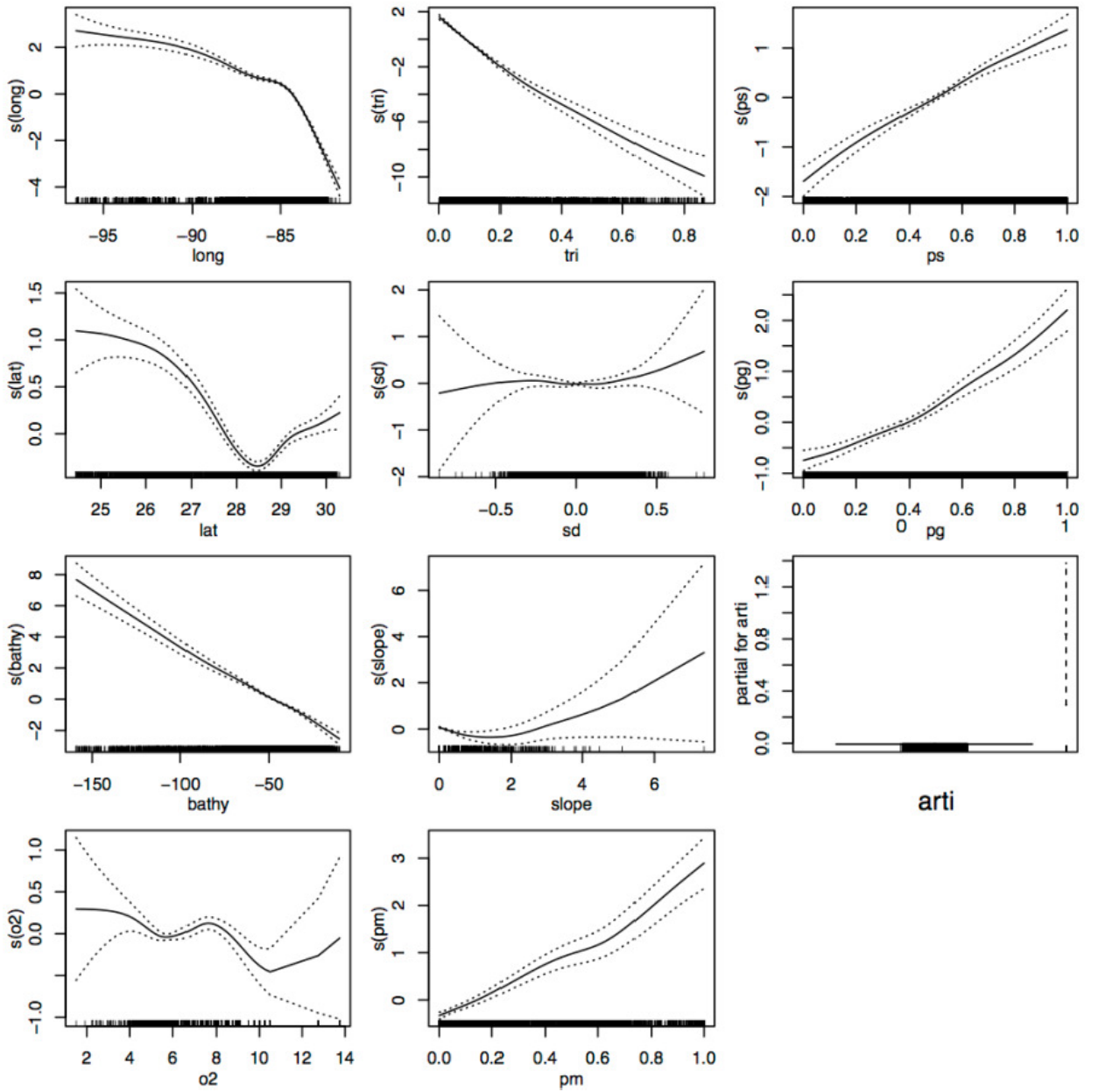


Figure 3. Spline fits with standard error for the final binomial GAM model.

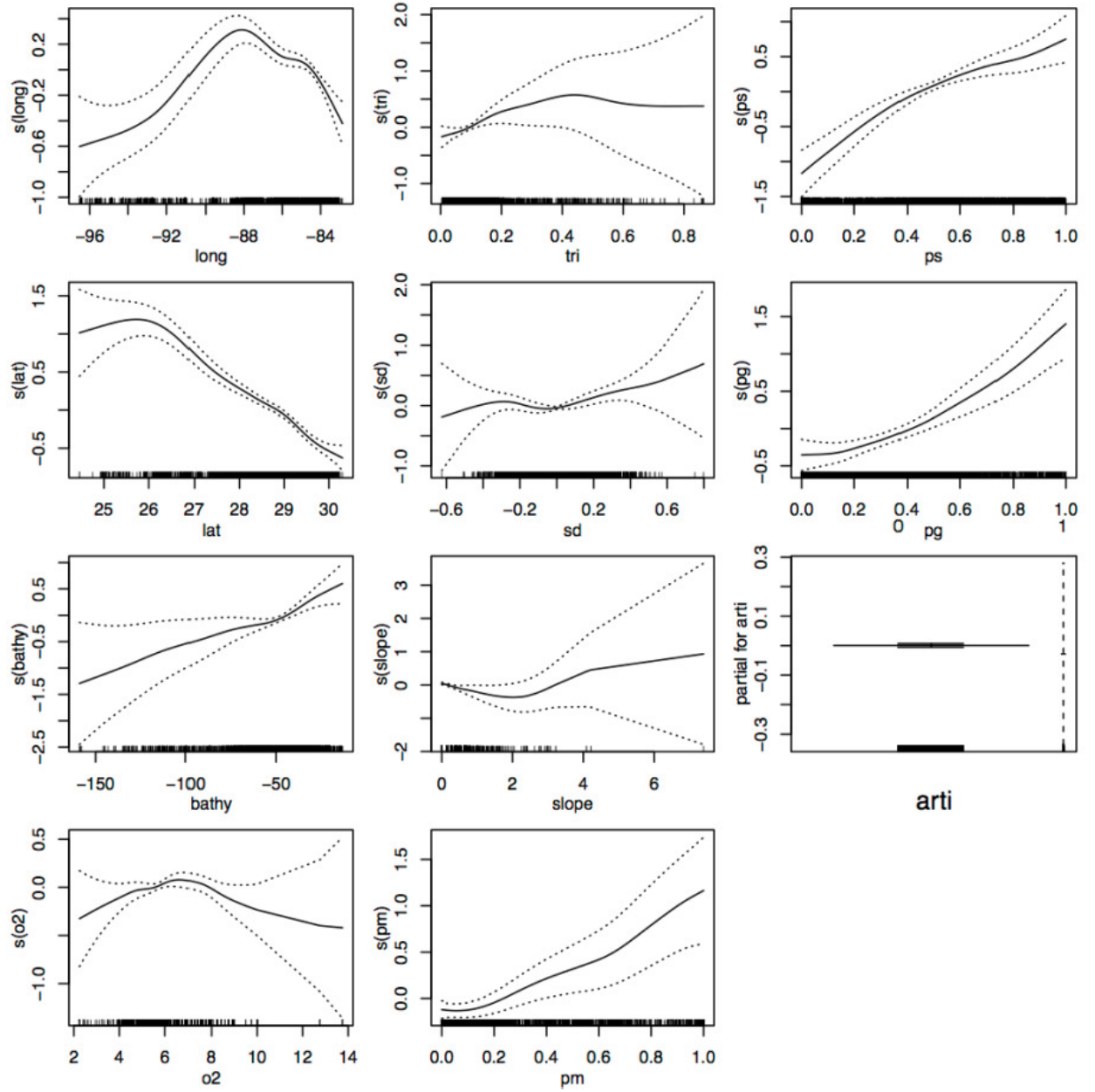


Figure 4. Spline fits with standard error for the final positive value GAM model.

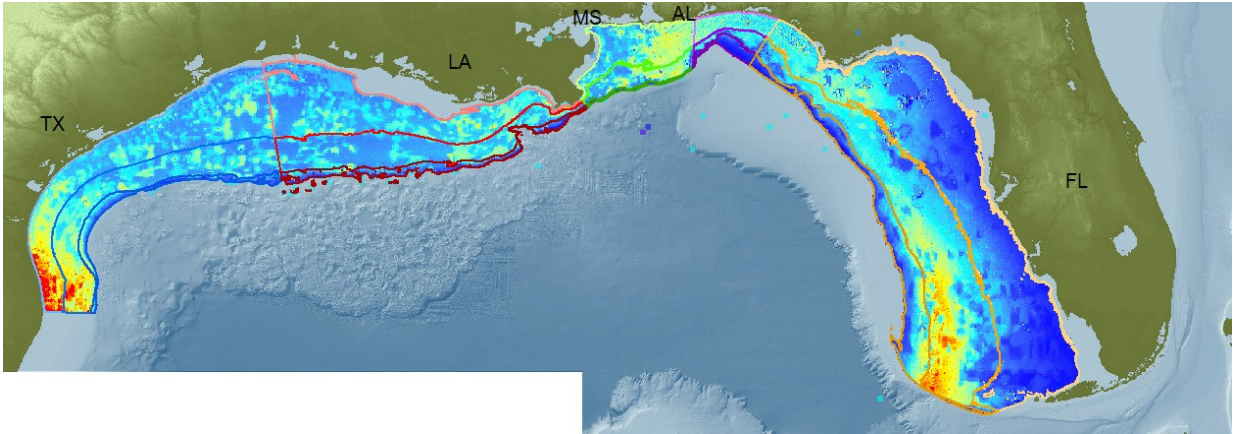


Figure 5. Estimated relative red snapper distribution from GAM-predicted biomass caught per hook hour. Estimated values in the extreme south east and west are a result of data deficiencies as are down scaled in the final model Dark blue indicates low cphh (0.001) and red indicates high values (0.6)

Mark recapture

Rationale

Conventional tagging and genetic mark recapture methodologies would use the same statistical procedures for estimating the total number of marked red snapper required, as well as the sample size for recaptures. The only difference between the two methodologies is that a genetic mark is natural and permanent, thus cannot be shed or disposed of and can be recovered from a fully processed carcass. Genetic marks also are transmitted from parent to offspring, making it possible to estimate the number of breeding individuals (as opposed to the total number of individuals) by marking potential breeders and sampling subsequent groups of recruits. Finally, while both conventional tags and genetic marks can be used to examine contemporary movement, genetic marks may also be used to assess long-term connectivity, effective population size, and aspects of local adaptation. The ability to address this last set of questions would require a higher diversity or greater number of molecular markers than would be necessary for genetic mark recapture alone. However, the benefit of simultaneously collecting additional genomic data useful for fisheries management is high relative to the increase in costs associated with examining a greater number of molecular markers (see Sample Processing and Cost Evaluation). Obtaining tissue for genetic mark recapture involves catching and handling animals and the expense of conventional external tags is relatively small; therefore, it would be beneficial to simultaneously deploy conventional and genetic tags because fishermen may report conventional tags but cannot be expected to take tissue samples. This approach also would allow one to estimate tag loss of conventional tags.

Past studies

Several conventional tagging studies have been conducted in the nGOM to estimate red snapper site fidelity, movement dynamics, and mortality rates (Table 4). Tagging depths among these studies ranged from 10 to 180 m. Overall, the percentage of tagged fish reported as being recaptured

by fishermen ranged from 2.3 to 34.1%. However, the 34.1% recapture rate was produced in a heavily fished area off northwest Florida in the mid to late 1960s; that level of recapture rate may be unlikely today. For example, in that same region from 2013-2016, FloridaFish and Wildlife Research Institute biologists achieved a recapture rate of 3.8% (Beverly Sauls, FWRI, personal communication). Overall, we can account for 56,477 red snapper having been tagged with conventional tags in the nGOM since the late 1970s, with 2,833 of these being reported as recaptures by fisherman. This yields an overall recapture rate of 5.0%. We surmise that a similar rate might be expected in the implementation phase of the current research program. However, higher rates also might be achieved if the program were widely advertised and high reward tags or tag return lotteries were employed, both of which may increase the likelihood of high participation rates among fishing constituencies were achieved.

Release mortality

Numerous studies have been conducted to examine red snapper release mortality in both the commercial and recreational fisheries of the nGOM. Direct comparison of results among these studies is problematic, however, given the various methods utilized to estimate release mortality (e.g., surface observation, caging, conventional tagging, acoustic tagging), differences in sample sizes and time scales of observation (e.g., immediate at release to months post-release), and differences in gear and handling times and conditions in recreational versus commercial fisheries. Campbell et al. (2014) conducted a meta-analysis of known estimates ($n = 75$) of red snapper release mortality in which they estimated the effect of depth (0-100 m) on red snapper release mortality while accounting for the effects of fishing sector, timing of observation (immediate versus delayed), venting, season, and hook type (circle versus J) in a mixed effects model.

The main effects of depth, fishing sector, and season were significant ($p < 0.01$) in Campbell et al.'s (2014) model, but timing, venting, and hook type were not ($p > 0.30$). While venting and timing were not significant, their interaction was ($p = 0.045$). Vented fish had lower estimated release mortality when observed at the surface immediately following release (i.e., a higher percentage could swim down rapidly), but their delayed release mortality estimates were higher than non-vented fish. However, recent results from hyperbaric chamber experiments (Drumhiller et al. 2014), as well as from acoustic tagging of released fish in the wild (Curtis et al. 2015), indicate that red snapper display lower release mortality when vented or released with descender devices. Therefore, while the evidence that venting lowers release mortality may be equivocal (Wilde et al. 2009), it appears that the rapid recompression achieved with return-to-depth strategies, such as descender devices, can lower release mortality in red snapper.

Model results produced in Campbell et al.'s (2014) meta-analysis are informative with respect to the effects of depth and season on red snapper release mortality. It is likely that conventional or genetic tagging approaches that may be employed to estimate red snapper abundance in the GOM would utilize recreational-type fishing techniques (i.e., 1-3 hooks fished with manual rod and reel) to capture fish. Therefore, the recreational fishery estimates are likely to be the most informative with respect to release mortality experienced by fish released after tagging or fin clipping. Campbell et al. (2014) reported overall recreational and commercial release mortality estimates by depth, and then season-specific estimates for combined recreational and commercial releases. Given the implementation phase of this program will most likely occur in summer or early fall when sea state is more amenable to working offshore, we contacted Matt Campbell at NMFS-

Pascagoula and asked if he would be willing to re-run the Campbell et al. (2014) model to predict season-specific release mortality by depth for the recreational fishery alone, which he graciously agreed to do. Results from that analysis indicate that release mortality is predicted to increase non-linearly with depth and to increase with water temperature (Table 5).

This vector of predicted release mortality at depth provided in Table 5 represents the best information available and was utilized as a data input in simulation models described below to estimate sample sizes, costs, and predicted CVs for different approaches to estimating GOM- wide red snapper abundance. However, uncertainty remains in predicted release mortality remains despite the comprehensive meta-analysis conducted by Campbell et al. (2014). For example, while we feel it would be prudent to utilize return-to-depth tools to release study fish, it unclear exactly what level of reduction in release mortality their usage would impart. Ongoing studies in the nGOM may shed further light on this, but currently only the estimates reported by Curtis et al. (2015) exist. Another source of uncertainty is depredation on released fish by marine mammals or upper trophic level fishes, such as sharks. Ongoing research in several laboratories in the nGOM may shed light on the magnitude of this issue and, more importantly, provide estimates of depredation rates that can be incorporated into models computed during the implementation phase of this program. Furthermore, if descender devices are utilized to return fish to depth during tagging operations, cameras mounted above descender devices can be utilized to monitor interactions with predators during descent.

Sample acquisition for conventional and genetic tagging

The sampling effort required to apply conventional tags or clip fins to develop molecular tags could likely be accomplish through cooperative research utilizing commercial, for-hire recreational, or private recreational fishing vessels. However, liability concerns may preclude the widespread use of private recreational fishing vessels as tagging platforms. Other potential sampling platforms include academic or agency research vessels, but their typically large size and high daily rates (>\$10k per day) may render them less than ideal for this work. Therefore, we assumed that either commercial or for-hire recreational vessels with a mean daily usage rate of \$4,500 would be utilized. An additional benefit of this cooperative approach would likely be buy-in by fishing constituencies involved in the research.

We envision recreational-type fishing gear (i.e., 1- to 3-hook rigs fished with manual rod and reel) being utilized to capture fish for tagging or fin clipping, with the idea being that each fish that is handled should have a conventional tag applied and have a fin clip taken. Once on deck, fish will be removed from hooks, measured to FL, and a stainless steel dart tag applied beneath the dorsal fin. Fin clips of approximately 1-2 cm² will be taken from the trailing edge of the soft dorsal fin or from the anal fin using a sterile pair of dissecting scissors. In between sampling, scissors will be cleaned with sterile water to remove remnant tissue in order to avoid cross- contamination. Tissue samples will be immersed in thermally stable fixative, such as 95% non-denatured ethanol or 20% DMSO buffer saturated with NaCl. Costs associated with buffer preparation and storage vials have already been figured into cost-per sample processing.

Resampling marked fish

Resampling events under a conventional mark recapture framework would require additional boat days to recapture fish. Resampling using a genetic mark recapture approach could occur via state-run recreational fisheries intercepts or by sampling at commercial fish houses because genetic marks remain intact even if conventional tags are removed. Given an estimate of 43 million red snapper in the Gulf of Mexico and the required CV of 30%, it is likely that ~250,000 fish would need to be resampled if 15,000 fish are tagged initially (see below). Accomplishing such large-scale resampling will require a large collaborative effort among state, federal, and academic institutions. The biggest difficulty will be coordinating resampling to make sure each region is appropriately sampled and that protocols for tissue preservation and data archiving are consistent across collaborating entities. The logistics of this undertaking will likely require that a single person is hired for the duration of the project with the task of coordinating, overseeing, and documenting all resampling effort.

Sample processing for genetic tags

Several laboratory approaches are applicable to genetic mark recapture, including PCR amplification of panels of microsatellites, generation of automated SNP assays, and sequencing of reduced-representation genomic libraries. Generating enough genotypic data to create a unique identifier (genetic mark) for each individual would be possible with any of the three technologies. The differences in methodologies reside in the price per individual, the time required to genotype ~250,000 individuals, and the applicability of the data acquired to address secondary questions, such as population structure, genetic demography, and local adaptation.

Each methodology is briefly outlined below with an estimate of cost per unit sample.

Microsatellites are short segments of DNA that contain repetitive motifs of 2-6 base pairs (bp). They tend to be highly variable with three or more alleles present at each locus (individual microsatellite marker). Given the high levels of polymorphism seen in microsatellites, a modest number of loci (10-20) would be required for a genetic mark recapture study and loci are currently available for red snapper that amplify reliably. A downside to microsatellites is that they are prone to artefacts caused by PCR amplification and this can lead to genotyping error (see below). Therefore, personnel who are experienced with scoring microsatellites will be required and it may be necessary to rerun as much as 25% of individuals at a given locus to ensure accurate genotyping. Furthermore, while the data generated from ~20 microsatellites may be used to assess long-term connectivity and effective population size, they will not be useful for detecting local adaptation that may be important in metapopulation dynamics.

SNPs (single nucleotide polymorphisms) are single base pair changes and SNP assays involve automated simultaneous genotyping of multiple SNPs. SNPs have two alleles and assay methodologies involve the deployment of probes specific for each allele at a given locus. To accomplish this, specific chips or arrays must be designed prior to deployment, a process that can be quite difficult for less experienced researchers, and specialized equipment is needed for genotyping. Several companies offer assay design and genotyping services but there is an upfront cost involved with production of the assay. Fluidigm assays, for example, are capable of scoring 96 SNPs across 96 individuals but the manufacture of the assay costs \$5,000-7,000. Furthermore, each assay would be viable only for ~20,000 samples, at which time another assay would have to be manufactured. While a 96 by 96 assay would likely be adequate for genetic mark recapture, it would not likely provide

appropriate data for a high-resolution assessment of long-term connectivity and effective population size, nor would it be useful for detecting local adaptation.

Reduced-representation genomic libraries also can be used to genotype SNPs across many individuals simultaneously. Briefly, high molecular weight DNA can be sheared physically and/or digested with restriction endonucleases to create many small fragments of DNA. A subset of fragments can then be selected based on size and/or the sequence of base pairs at the ends and sequenced on a high-throughput next-generation platform. For example, in the MGL Dr.

Portnoy's group at Texas A&M-Corpus Christi uses a double digestion protocol to make reduced representation libraries that are sequenced on the Illumina HiSeq platform. For red snapper, they genotype approximately 200 individuals simultaneously at more than 1,000 SNP-containing loci. By changing aspects of library preparation (e.g., restriction endonucleases used or size selection window) the number of fragments per individual can be altered. It is likely that up to 400 individuals could be genotyped for >500 SNP loci in a single run. In addition, because individual fragments are sequenced and may contain more than one SNP, these loci can have more than two alleles present. The number of loci generated by this technique far exceeds what would be needed for genetic mark recapture but will provide high resolution data for assessment of connectivity and estimation of effective size and would be useful for exploring the potential for local adaptation.

Cost per sample estimates for genetic tagging are based on material costs (lab supplies), costs associated with machine usage, and personnel (see additional costs below for personnel estimates). Of the three methodologies outlined (microsatellite (micros), SNP assays, and reduced-representation library sequencing (SNP seq)), SNP assays will involve the least amount of labor as most steps, other than DNA extraction, would be outsourced. Microsatellites will involve the most labor, though efficiency can be improved by running the microsatellite in panels of multiplexed loci. For estimated costs presented below it was assumed that 20 microsatellites could be run in three multiplex reaction following Renshaw et al. (2006). For reduced-representation library sequencing a major portion of the expense comes from the use of the Illumina HiSeq platform. For estimated costs, we consider (Table 3.2) the cost when sequencing is outsourced (SNP seq A) and the cost when an Illumina HiSeq is available at a researcher's home institution and sequencing can be done at cost (SNP seq B). For both, we consider the cost when 400 samples are run simultaneously and sequenced in a single direction (single-end). Further savings could be realized, by reducing personnel costs, if DNA extraction and PCR are automated using PCR robots. Given the number of samples to be processed several robots would likely have to be purchased, as any given lab is unlikely to have more than one.

Total costs were calculated for 250,000 samples.

Genetic ID protocol and uncertainties

An important consideration for any genetic mark recapture study is minimizing error associated with identification of individuals. Two types of error are possible and can be accounted for in different ways. First, it is possible to sample two individuals with the same multi-locus genotype which would then be interpreted as a recapture. This error results from genotyping too few loci per individual and can be exacerbated if individuals are highly related and/or inbreeding is occurring. To deal with this problem it is standard practice to calculate the probability of identity (probability that two individuals selected at random have the same composite genotype across n -loci). This number can be calculated by considering the number of loci to be deployed and the observed frequency of their alleles and can be corrected for the presence of kin. All the methodologies proposed here involve enough markers that risks associated the first type of error would be minimal (e.g., probability of occurring $\ll 0.001\%$). The second type of error involves resampling the same individual but recording a different multi-locus genotype due to experimenter error or artefactual differences in allele calls at specific loci. This can be accounted for by rerunning a certain number of samples and estimating levels of genotyping error. Of the methodologies proposed, microsatellites will be most problematic because artefacts associated with allele calling can be common within loci and because so few total markers will be employed. Therefore, it is likely that a greater percentage of individuals will need to be rerun at each locus (perhaps as great as 25%).

Additional costs for conventional and genetic tagging

In addition to the cost per sample presented above cost associated with sample acquisition and processing are considered. The following assumptions were made with regards to marking individuals: 50 conventional tags per sampling day were assumed to be deployed, ship time costs of \$4500 per day, and a minimum of 4 technical biologists per region (5 regions) being required for conducting tagging in the field, with each costing \$45,000 per year including fringe. These regional biologists would also be available to engage in resampling port and dockside for recapture. In addition, 6 masters level technicians would be required for 2 years to process the genetic samples as SNP sequencing takes a person approximately two weeks to make 2 libraries. If there are $\sim 250,000$ samples and 400 samples can be processed per library, that equals 625 libraries so about 625 weeks of work, which is 12 years. If 6 full-time personnel were hired for this, it would take two years. Microsats would take approximately the same amount of labor, although possibly more and SNP panels less. This results in a total man-hour requirement of 32 years at \$45,000 per year plus any indirect costs.

Sampling design and evaluation

We assume that population abundance will be assessed using a simple Petersen mark recapture estimator. To evaluate the number of marks and recaptures required to achieve a level of precision of $CV = 30\%$, we developed a simple simulation. Assuming a population size of ~ 43 million red snapper and a fishing mortality rate F of 0.1 y^{-1} , combinations of initial numbers marked

(M) and total numbers resampled (C) can be evaluated for the level of precision achieved and the total cost of the project. The total number of individuals resampled for marks was calculated as a proportion of the catch sampled (ps). Total catch was calculated as the population size (N) times the fishing mortality rate (Equation 1). For this simulation, we assumed that the total number marked is not reduced by release mortality (this will be more fully explored within the evaluation simulation). The number of marks recaptured (R) was calculated as the total catch times the proportion of the population marked (Equation 2). Confidence intervals on the estimated population size were calculated in a maximum likelihood framework and assuming a binomial likelihood. 95% confidence intervals were determined assuming 2 times the likelihood ratios follow a chi-squared distribution. If the number of resamples (i.e., number of fish sampled from the population or fishery once fish have been marked) is some proportion of the total catch in each year from both commercial and recreational fisheries, and the number of marks recaptured is the proportion of the population marked times the number of resamples, sample size requirements and costs can be coarsely approximated from Figure 6.

$$(1) \quad C = ps * F * N$$

$$(2) \quad R = M/N * C$$

In our initial simulation, we have assumed that the initial marks and recaptures are distributed and drawn randomly from the population. The reality is that marking effort will need to be allocated regionally and that recaptures from the fishery will more than likely be sampled in proportion to anticipated catch rates and regional effort. Allocation of marks can be done in proportion to expected population size (Table 7) within each stratum which have been estimated from total mean red snapper catch per fishing point (see Figure 7). Mean catch per fishing point was determined by classifying VMS data from 2007-2014 for the vertical line fleet using a random forest model conditioned on NOAA observer coverage. VMS points with observer coverage were determined to be fishing or not fishing based on the timing of vertical line sets within the observer data. These classified points were used as a training set to fit the random forest models based on a suite of characteristics. The random forest model was then used to classify the remaining VMS points as fishing or not fishing. VMS points deemed fishing were then aligned with trip ticket information and catch from each trip ticket was uniformly allocated over the corresponding VMS fishing points.

An alternative is to estimate required marks per stratum based on assumed stratum weight (proportion of total population in a stratum) and an assumed sampling efficiency per stratum. Relative precision of the total population estimate (r_t) can be expressed as a function of stratum-specific relative precision (r_h) and weighting (w_h) (Equation 3). If r_h is assumed constant across strata, then it can be estimated using Equation 4. Assuming a hypergeometric model (Chapman 1951), r_h is a function of the stratum population (N_h), the total marks (M_h), and capture efficiency (e_h) (Equation 5). Assuming a capture efficiency (Table 7) and a r_h derived from Equation 4, marks required for a given stratum can be estimated as Equation 6, where K is a constant that depends on the desired type I error rate (see Carlson et al. 1998 for a table of values). K and the associated stratum efficiency determine the expected number of marks recaptured, which is recommended to be >10 per stratum (Chapman 1951). Capture efficiency can be estimated as the proportion of fishing mortality (if F is low) that occurs in a stratum times the expected proportion of the catch sampled.

Using an F of 0.1 y^{-1} , assuming effort is allocated in proportion to the stratum weight resulting in a stratum-specific fishing mortality of $F * w_h$, and $\sim 0.5\%$ (200,000) of the catch sampled for marks results in stratum efficiencies presented in Table 5. Changes in fishing mortality and the proportion of catch sampled will alter the stratum efficiencies. Using this method, the marks required are presented in Table 5. Capture efficiencies of 4 times (see Table 6) those in Table 5 are required to achieve the initial mark numbers presented in Figure 6, which has an inherent efficiency of 0.005 due to aggregation over strata. If red snapper are distributed over a narrower distribution that initially assumed, the efficiency is likely to be higher and results will be similar.

$$(3) \quad r_t = \sqrt{\sum r_h^2 w_h^2}$$

$$(4) \quad r_h = r_t / \sum w_h^2$$

$$(5) \quad r_h = \frac{2 \sqrt{N_h^2 [(M_h e_h)^{-1} + 2(M_h e_h)^{-2} + 6(M_h e_h)^{-3}]}}{N_h}$$

$$(6) \quad M_h = \frac{K}{e_h(100)}$$

A wide array of sample designs and cost structures can be explored using the red snapper population model. Here we consider a simple design based on the sample size estimates in Table 6. Each of the 5 main strata are allocated 2,000 tags with 10% of the tags deployed on artificial structure which is assumed to hold $\sim 10\%$ of the population. The goal at each sampling location is to deploy 10 tags that survive for a total of 180 sample locations over natural bottom and 20 artificial structure sample locations. For a given area, the number of fish tagged depends on the depth of the sampling location. The target 10 surviving tags is divided by the depth based survival rate (see release mortality, Table 5) to determine the number of tags to deploy. We assumed tags will be deployed using recreational methods in summer. The actual number of tags deployed depends on the availability of fish for capture, which is assumed to be 20% of the local population. Tags are then subjected to tagging mortality and assumed recaptured a short time after deployment. Note that sample locations are chosen at random from a sampling frame of location that have red snapper. The detail of how this can be accomplished is dependent on local knowledge and flexibility in a radius around sampling points. The red snapper population is then subjected to fishing and recaptures are determined by binomial draws assuming a proportion of the catch is sampled (e.g., 7%). In instances where no recapture occurs in an area, information on the probability of capture is borrowed from other areas. Precision on the estimate of total red snapper population size is estimated using 1000 bootstraps using the binomial distribution. The simulation is run for 1000 iterations to determine the performance of the proposed sampling design. Results for the simple design proposed above are presented in Figure 8. Total cost for this sampling program is estimated around \$4 million. Precision is expected to be greater than the target 30% with 85% of the simulations resulting in precision greater than 30%. In addition to lower than desired precision, 60% of the simulations result in a relative error greater than 30%.

Table 4. Estimates of recapture rates for past mark recapture studies.

Study	Region	Tagged	Recaptures	%Recaptured
Beaumariage (1969)	wFL	1,126	384	34.1
Fable (1980)	TX	299	17	5.7
Szedlmayer and Shipp (1994)	AL	1,155	146	12.6
Burns et al. (2002)	wFL	5,272	386	7.3
Patterson and Cowan (2003)	AL	2,932	364	12.4
Strelcheck et al. (2007)	AL	4,317	217	5.0
Diamond et al. (2007)	TX	5,614	130	2.3
Addis et al. (2016)	wFL	2,141	137	6.4
LDWF unpublished	LA	7,577	414	5.5
FWRI unpublished	wFL	27,170	1,039	3.8

Table 5. Estimated release mortality (proportion) by depth and season for the recreational fishery computed by Matt Campbell with the model reported by Campbell et al. (2014) in their meta-analysis of red snapper studies. Estimates are model-predicted values from their mixed-effects model computed to test the effects of depth, fishing sector, timing of observation, venting, timing*venting, season, and hook type on red snapper release mortality.

Depth	Winter	Spring	Summer	Fall
0	0.05	0.06	0.12	0.09
5	0.05	0.06	0.13	0.09
10	0.06	0.07	0.14	0.10
15	0.06	0.08	0.15	0.11
20	0.07	0.08	0.17	0.12
25	0.08	0.09	0.18	0.14
30	0.08	0.10	0.20	0.15
35	0.09	0.11	0.21	0.16
40	0.10	0.12	0.23	0.18
45	0.11	0.13	0.25	0.19

50	0.12	0.15	0.27	0.21
55	0.13	0.16	0.29	0.23
60	0.14	0.17	0.31	0.25
65	0.16	0.19	0.34	0.27
70	0.17	0.21	0.36	0.29
75	0.19	0.22	0.38	0.31
80	0.20	0.24	0.41	0.33
85	0.22	0.26	0.43	0.35
90	0.24	0.28	0.46	0.38
95	0.26	0.30	0.49	0.40
100	0.28	0.33	0.51	0.43

Table 6. Estimated cost per sample and total cost based on 250,000 samples for alternative methods of genetic identification.

Method	#Loci	Cost per Sample	Total Cost
Micros	20	\$8.60	\$2,150,000
SNP Assay	96	\$9.15	\$2,287,500
SNP seq A	500+	\$14.60	\$3,650,000
SNP seq B	500+	\$9.60	\$2,400,000

Table 7. Strata specific population proportions (stratum weight), estimated capture efficiency and required mark rates depending on the desired rt and resulting rh .

Stratum	wh	eh	$rt=0.3 / rh=0.62$	$rt=0.35 / rh=0.73$	$rt=0.4 / rh=0.84$
TX	0.32	0.0015	5,903	4,582	3,717
LA	0.21	0.0010	8,842	6,863	5,568
MS-AL	0.20	0.0009	9,654	7,493	6,080
N.FL	0.08	0.0004	23,558	18,285	14,835

W.FL Shelf	0.19	0.0009	9,921	7,700	6,248
Total	57,877	44,923	36,448		

Table 8. Stratum-specific population proportions (stratum weight), estimated capture efficiency and required mark rates depending on the desired rt and resulting rh .

Stratum	wh	eh	$rt=0.3 / rh=0.62$	$rt=0.35 / rh=0.73$	$rt=0.4 / rh=0.84$
TX	0.32	0.0089	1,476	1,145	929
LA	0.21	0.0060	2,210	1,716	1,392
MS-AL	0.20	0.0055	2,414	1,873	1,520
N.FL	0.08	0.0022	5,889	4,571	3,709
W.FL Shelf	0.19	0.0053	2,480	1,925	1,562
Total	14,469	11,231	9,112		

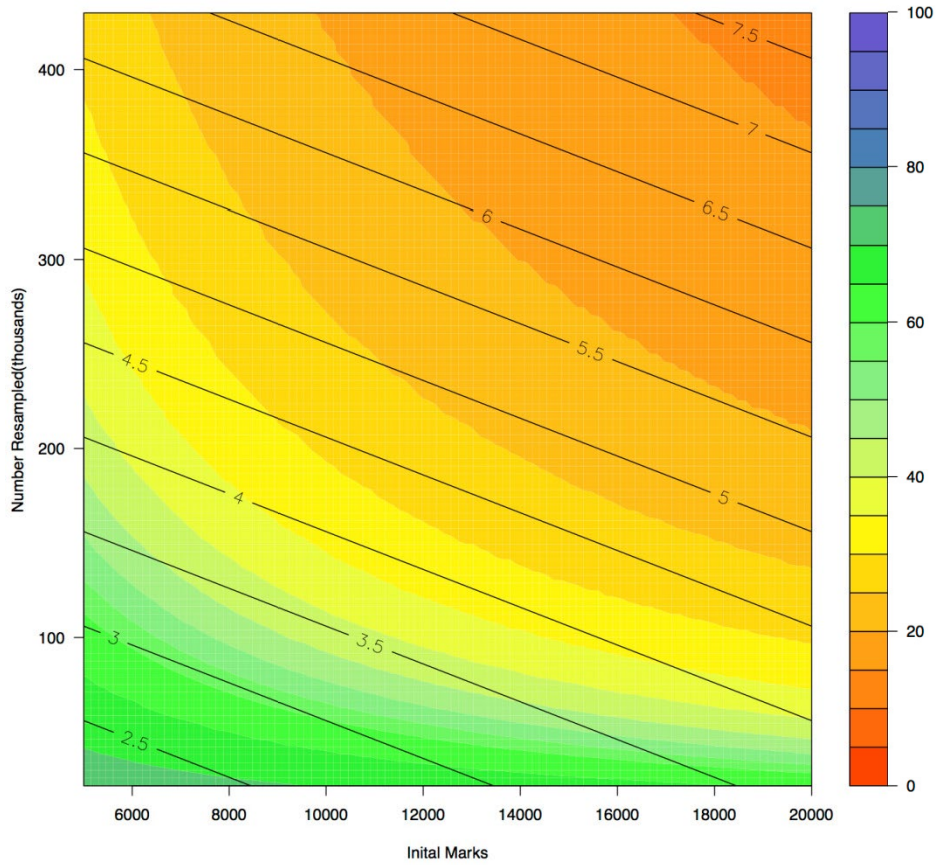


Figure 6. Estimated precision and costs for mark recapture sampling. Colored bands indicate precision (CV) in percentage and black contours indicate cost in millions of dollars.

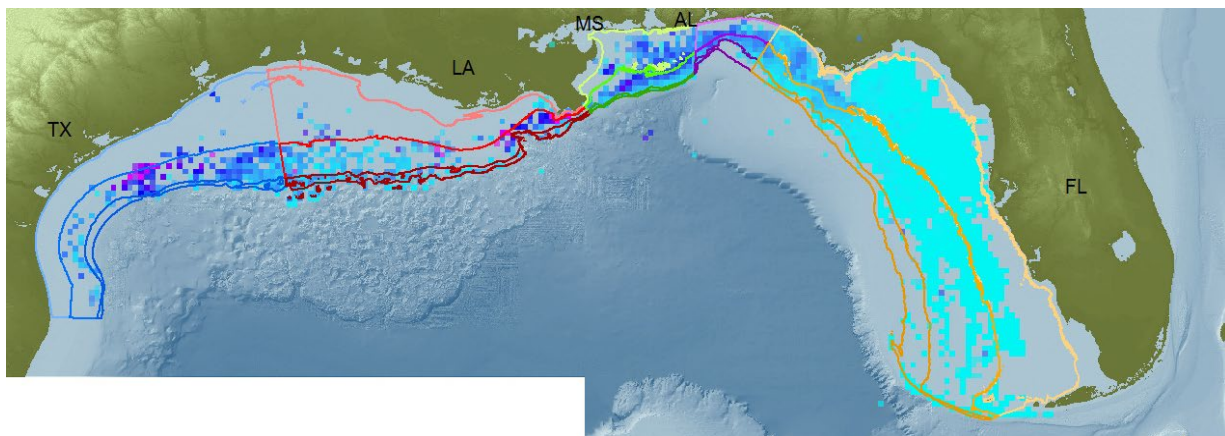


Figure 7. Annually averaged, average monthly red snapper biomass caught per VMS point (cpp) classified as a fishing point for 2007-2014 aggregated a 10 km x 10 km resolution for vertical line vessels with VMS. Light blue indicates low cpp (0.001) and purple indicates high cpp (393).

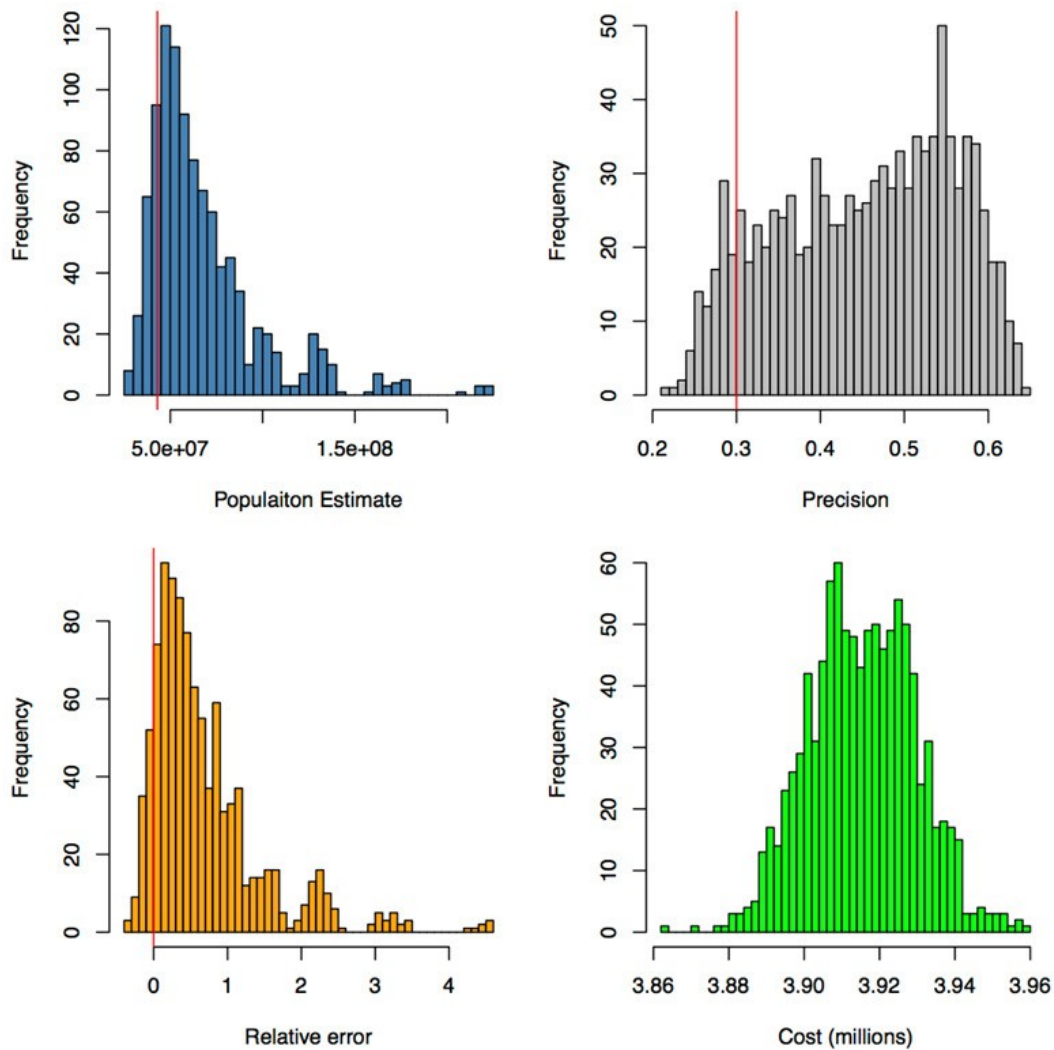


Figure 8. Simulation results from 1000 iterations for tagging study assuming a target mark density of 2000 per strata. Red vertical lines indicate target or true values. Precision represent the level of precision in the 95% confidence interval on the total population estimate. Relative error compares estimates with the true simulated population size.

Video and acoustic sampling

The second general approach to estimating red snapper abundance that we explored was combining ROV-based video methods with fishery acoustics. Geo-referenced ROV video allows one to estimate numbers of fish observed within an area surveyed, thus estimate fish density.

Either point-count (e.g., Patterson et al. 2009; Dance et al. 2011; Dahl et al. 2016) or transect (e.g., Dahl et al. 2014; Patterson et al. 2014) methods can be employed, and both have been utilized in the nGOM to estimate habitat-specific red snapper density. However, it can be difficult to examine fish community structure or density at complex, large-scale habitats, such as petroleum platforms. For that particular habitat, fisheries acoustics have been successfully employed to estimate fish biomass density, which was then groundtruthed with ROV-based video to estimate species-specific distributions (Stanley and Wilson 1996, 1997, 2000; Wilson et al. 2006). Below, we describe the general principles involved with each of these approaches, and then simulate sample effort and associated costs that would be required to utilize these combined approaches to estimate red snapper abundance in the nGOM.

Fishery acoustics

Acoustics have proven a useful tool for quantifying the spatial and temporal distributions of fish communities across a variety of aquatic ecosystems (Simmonds and MacLennan 2005). Acoustic technology has advantages over traditional approaches given its potential as a rapid non-invasive approach capable of acquiring high-resolution spatio-temporal data across large areas (km^2), which can be related back to taxa of interest. However, this approach often requires direct comparison with biological community data to provide inference at relevant taxonomic scales.

Most commonly, acoustically-derived metrics (e.g., fish density) are directly compared with catches from direct biological sampling or visual data (Stanley and Wilson, 1996, 1997, 2000; Wilson et al. 2006), but the extension to inform fishery independent indices has not been examined in this region. This is principally due to the difficulties associated with partitioning acoustic scattering responses attributed to individual taxa and the general lack of robust acoustic models which describe these responses. In the past, acoustic scattering data have been scaled at taxonomic resolution based on the proportional abundance observed via visual methods or catch data (Simmonds and MacLennan 2005).

There are two primary outputs derived from acoustic surveys: backscatter from individual targets (i.e., fish) distributed in the water column and volume backscatter from aggregated targets. In general, fish that are diffusely distributed in the water column can be detected as individual targets and enumerated to yield a density estimate within the ensonified volume (fish m^{-3}).

Echoes from these individuals can be measured to derive the acoustic size (i.e., backscattering cross-section, a_{bs} [m^2]) which is related to an individual's target strength ($\text{TS} = 10 \log_{10}(a_{bs})$ [dB]), often used to approximate fish length through relationships describing the length dependence on acoustic scattering intensity (see Simmonds and MacLennan 2005). Targets that are too tightly packed, or aggregated do not permit target counting and instead echo integration must be used to estimate fish density and abundance, where the echo integral (summation of echo energy within a volume) is taken throughout the ensonified volume (s_v , volume backscatter [$\text{m}^2 \text{m}^3$]).

Volume backscatter is generally linearly proportional to fish density for aggregated targets (Foote 1983, Simmonds and MacLennan 2005), however in some cases pelagic schools have been shown to demonstrate non-linear effects (i.e., mackerel or herring; Furusawa et al. 1992, Zhao and Ona 2003). In many of the reef-related surveys, when applying shorter pulse durations, which mediates target resolution and therefore separation, the potential effects of highly-aggregated targets is not anticipated to be an issue to characterize the fishes in the nGoM.

Recent advances in the development of acoustic technologies afford researchers the ability to examine scattering responses across a spectrum of acoustic frequencies. This new development, commonly termed ‘broadband,’ ‘wideband,’ or ‘chirp’ sonar allows a frequency-modulated sweep (e.g., 25-50 kHz) in comparison to a single discrete frequency typically used (e.g., 38kHz). Benefits of this newly available technology in scientific echo sounders include the opportunity to extract additional information from each echo, which may contain species-specific information (Boswell, unpublished), similar to an acoustic ‘fingerprint’. Additional benefits of this approach include increased target resolution which permits improved target detection and separation from neighboring targets, permitting density estimation within aggregated targets.

Sample acquisition

We estimate that 6 stations could be sampled each day and randomized surveys across the region would require approximately. In each site, acoustic surveys will be conducted following a flower-shaped pattern, with 6 linear transects offset by 30 degrees. Following acoustic transects, an ROV will be deployed to characterize the fish community. Details of each methodology are provided below.

Acoustic methodology

Multifrequency scientific echosounders will be calibrated following the standard sphere method (Demer et al. 2015) and used to derive fishery independent estimates of habitat specific fish density and abundance. Acoustic surveys will be conducted following ROV transects and will be designed to minimize the effects of spatial correlation among sampling points (Petitgas 1993) along the acoustic transect. Acoustic data will be apportioned relative to the ROV derived estimates of composition. Where possible, echo counting will be performed to derive in situ density estimates, in addition to target strength for deriving size distributions of ensonified fish.

Recent empirical work has been undertaken to derive numerical models to compute the acoustic scattering response of many common reef species found in the nGOM, including red snapper (Boswell et al., unpublished). These efforts have demonstrated the potential to extract species-specific acoustic responses which may prove to allow researchers to acoustically distinguish among common reef fish species (Figure 9). These efforts are still in development; however, they provide increased resolution for signal processing conducted to estimate community composition from acoustic data. For now, video-based methods provide proven approaches to estimate community structure, as well as to ascribe biomass estimates from acoustic data to observed reef fishes.

Video methodology

Video sampling will be conducted with a Video Ray Pro4 micro remotely-operated vehicle (ROV), or similar, to estimate fish community structure. In the case of the Pro4, Real-time ROV movement is observed on a high-resolution monitor with a live feed from the ROV's 570-line resolution video camera; the camera is capable of 160° vertical tilt, and has a wide focus range and a wide viewing angle (116°). Depth and heading of the ROV will be electronically overlain on the video image. Lighting, when needed, will be provided by twin 20-watt high efficiency halogen lights mounted on the ROV. Video output from the ROV will be recorded on digital video tape with a Sony GVD1000 digital VCR, as well as on the hard drive of a Panasonic Toughbook laptop computer running the Pro4's navigational software.

Methods for conducting ROV-based sampling will either be the transect method described by Patterson et al. (2014) for widely-distributed natural reef habitats, or the point-count method described by Patterson et al. (2009) for artificial reefs. The transect sampling method involves video sampling a 5-m wide transect as the ROV moves forward at a rate of approximately $0.5 \text{ m}\cdot\text{s}^{-1}$ along a 25 m long transect. The width of the transect is controlled by flying the ROV with a camera angle of 45° approximately 1 m above the seabed given the 116° viewing angle of the camera. Four orthogonal transects are flown over the habitat, thus a total area of approximately 500 m² is surveyed. The distance covered on a given transect is controlled by flying the ROV with a fixed scope of tether away from a 5-kg clump weight attached in-line to the tether. Transect distance is confirmed with a Tritech MicronNav ultrashort baseline acoustic positioning system deployed with the ROV.

In the point-count method, the ROV is positioned 1 m above the seafloor and approximately 5 m away from a given artificial reef and then slowly pivoted 360° and then moved to the opposite side of the reef. Once there, it was again positioned 1 m above the seafloor and approximately 5 m away from the reef and pivoted 360°. The ROV is flown to 1 m directly above the reef and pivoted 360° to video fishes in the water column above the reef. Next, the ROV is flown to 10 m above the reef and pivoted 360°. Once all sample segments were completed, the ROV is flown back down to the reef to observe fishes located on the reef's surface or inside the reef structure.

Cost per sample

Costs associated with the collection and processing of ROV video and acoustic methods were estimated to include the equipment use costs, operational ship costs, and costs for data processing, including personnel. Based on the variety of vessels available throughout each region, we adopted a generalized daily rate (see Table 9), with the understanding that there may be location-specific variation in these daily rates. Personnel costs were estimated as technicians earning \$14/hour, time and half for more than 8 hours worked in a day, 12-hr days in the field, and fringe = 7.6%. We assumed that 6 stations could be sampled each day for a total daily cost, including post processing of \$9,223. Note: these cost estimates only include fish count estimation and post-processing acoustic data. They do not include further analysis of data and abundance estimation.

Sampling design

Stratified random sampling assessing numbers per unit area is an alternative to or can be used in conjunction with mark-recapture (i.e., mark recapture in high density areas with per area sampling in others). Sample size needed to achieve a given relative error (i.e., CV = 30%) is a function of expected total variance (s_h^2), stratum weights, stratum variances, and sampling cost per stratum (Equation 7), which can be allocated to each stratum using Equation 8.

$$(7) \quad n = \frac{(\sum w_h s_h \sqrt{c_h}) \sum w_h s_h / \sqrt{c_h}}{V + (1/N) \sum w_h s_h^2}$$

$$(8) \quad n_h = n \frac{w_h s_h / \sqrt{c_h}}{\sum (w_h s_h / \sqrt{c_h})}$$

As above, sampling units are assumed to be 3 arc-second square areas (~90 m x 90 m). Catch from both commercial and recreational fisheries as well as spatially allocated commercial catch using VMS information (see Figure 5) provide some guidance about relative population densities. In this analysis, stratum weight (w_h) is based on the apparent physical number of sampling units (n_h ; see Table 10) in each stratum after filtering the suitability of sampling unit using the GAM as in the population model. Sampling units were filtered (using a binomial GAM prediction threshold of >77% probability of occupancy) so that 25% of all sampling units designated as red snapper habitat. This filtering excludes sampling units assessed to have artificial structure. All artificial structure units were included. This assumption is not supported by any hard data but seems reasonable given the spatial distribution of observed commercial catch rates, but is recognized as a **fundamental uncertainty**. To address this uncertainty, we present sample size analyses for a range of proportion of habitat utilized from 0.05-0.25 with the explicit assumption that red snapper density within ‘unused habitat’ is consistently very low so that the variance is near 0. This is an assumption that would need to be verified. Within each stratum, population was divided between ‘natural’ and ‘artificial’ (i.e., having artificial structure) based on the proportion of each cell type within the strata and the relative ratio of ‘natural’ to ‘artificial’ density on in each type (see Table 2). Variances for each stratum were calculated using the coefficients of variation from observed counts (see Table 2). The desired variance for the stratified mean (V) is estimated assuming a population of 43 million red snapper 2 years and older and a total number of sampling units dependent on the assumed proportion of area occupied. Given a desired precision of CV=30% on the estimate of the mean, required sample sizes and associated costs are presented in Table 10. A minimum of 30 sampling units were placed in each strata. Evaluation of the random stratified method will be done assuming 10% of the habitat is utilized by red snapper requiring 1,733 units sampled allocated between strata (see Table 11) over 289 field days plus a post processing time for a total cost of ~\$2.66 million US dollars.

Stratified visual surveys were evaluated using the simulation model under the **fundamental assumption** that locations of fish bearing strata could be identified. As in the simulations used to evaluate the mark recapture experiments the true population was set to 43 million individuals by randomly subsampling (5%) the areas deemed to be occupied by red snapper from the GAM

(~25% of the sampling units in the Gulf). As in the mark recapture evaluation, the number of artificial structure was preserved but the densities on each structure was halved so that the percentage of the population on structure was 10% of the simulated population. Two potential sampling protocols were assessed: red snapper occupying 5% and 10%, respectively, of the potential sampling (see Table 11). In general, both sampling approaches produce highly accurate but slight underestimates of the true population (Figures 10 and 11). In each simulation, the precision is estimated to be higher than that expected from required sample size calculations.

This increase in precision is due to the requirement for a minimum of 30 samples to be taken in each stratum, effectively increasing the sample size, and due to the reduction in sample variance that arises given the allocation of population density to each sampling unit. The variance used to determine required samples sizes is taken from the variance in observed counts, while this variance is reduced given the method allocating densities in the model. The slight negative shift in population density is an artifact of the modeling when the spatial distribution of the population is reduced so that total population density equals 43 million individuals. This process slightly alters the stratum weights and caused the negative bias.

Table 9. Cost breakdown for video and acoustic sampling assuming 6 samples collected per day

Cost Category	Collection costs per day	Processing costs per day sampled
Vessel	\$4,500	-
Instrument- ROV	\$1,000	\$586
Instrument-Acoustics	\$1000	\$625
Field Personnel	\$632 (3 people)	\$180
Travel/per diem	\$700	-

Table 10. Stratum characteristics used to estimate required sample sizes for stratified random sampling. The number of sampling units (n_h) for natural habitat must be multiplied by the assumed proportion of 'natural' habitat used by red snapper. Weight (w_h) presented assume 25% of natural habitat is used by redsnapper.

Region	Depth (m)	Natural			Artificial		
		n_h	w_h	s_h^2	n_h	w_h	s_h^2
TX	10-40	4,292,264	0.0220	34,495.63	252	3.974E-05	5.5998E+06
	40-100	2,958,088	0.2701	21,352.22	111	3.057E-04	3.3410E+06
	100-160	546,099	0.0276	6,600.85	19	2.946E-05	1.0714E+06

LA	10-40	3,824,615	0.0244	8,287.37	1,033	9.165E-04	4.0180E+06
	40-100	2,563,186	0.1385	5,138.74	575	4.320E-03	2.4899E+06
	100-160	737,459	0.0448	1,905.32	81	6.849E-04	9.2429E+05
MS-AL	10-40	1,372,955	0.0843	292,291.61	1,171	2.323E-03	2.3887E+06
	40-100	689,849	0.0839	1,033.94	314	1.968E-02	2.1549E+06
	100-160	77,209	0.0052	209.53	8	2.977E-04	5.0499E+05
N.FL	10-40	491,592	0.0325	144,673.73	362	1.329E-04	6.2038E+05
	40-100	254,284	0.0390	443.52	19	2.989E-04	5.8470E+05
	100-160	204,632	0.0082	139.09	1	4.210E-06	1.9244E+05
W.FL	10-40	8,915,942	0.1043	70.31	628	1.156E-03	4.3681E+04
	40-100	6,069,518	0.0793	66.83	7	1.443E-05	4.1718E+04
	100-160	1,964,020	0.0057	25.25	0	0.000E+00	0.0000E+00

Table 11. Required sample size, total number of sampling units, and total cost as a function of the proportion of ‘natural’ habitat assumed occupied by red snapper.

Proportion used	Sample size	Days required	Total number of units	Total cost (\$million)
0.05	455	76	1,756,579	0.7
0.1	1733	289	3,508,579	2.66
0.15	3837	640	5,260,578	5.9
0.2	6765	1127	7,012,578	10.4
0.25	10516	1753	8,764,576	16.16

Table 12. Required sample size by stratum assuming 25% of habitat is used by red snapper. Strata depth ranges are in m.

Region	Natural			Artificial		
	10-40	40-100	100-160	10-40	40-100	100-160
TX	659	382	30	30	30	30
LA	415	158	30	30	30	30
MS-AL	54	30	30	30	30	30
N.FL	30	30	30	30	30	30
W.FL Shelf	30	30	30	30	30	30

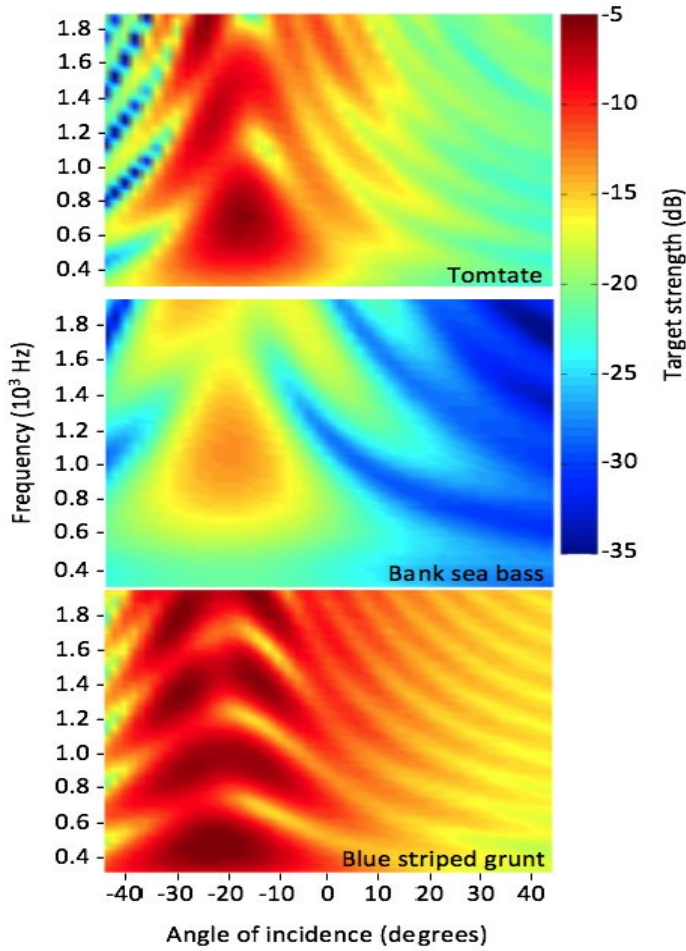


Figure 9. Theoretical acoustic scattering responses of three dominant reef-associated species commonly found in the Gulf of Mexico. The color maps represent the normalized acoustic intensity (target strength) across orientations, relative to normal incidence, and acoustic frequencies spanning commonly used frequencies for fisheries surveys. Responses indicate clear frequency-dependent structure across frequencies among modeled species indicating high-potential for classification.

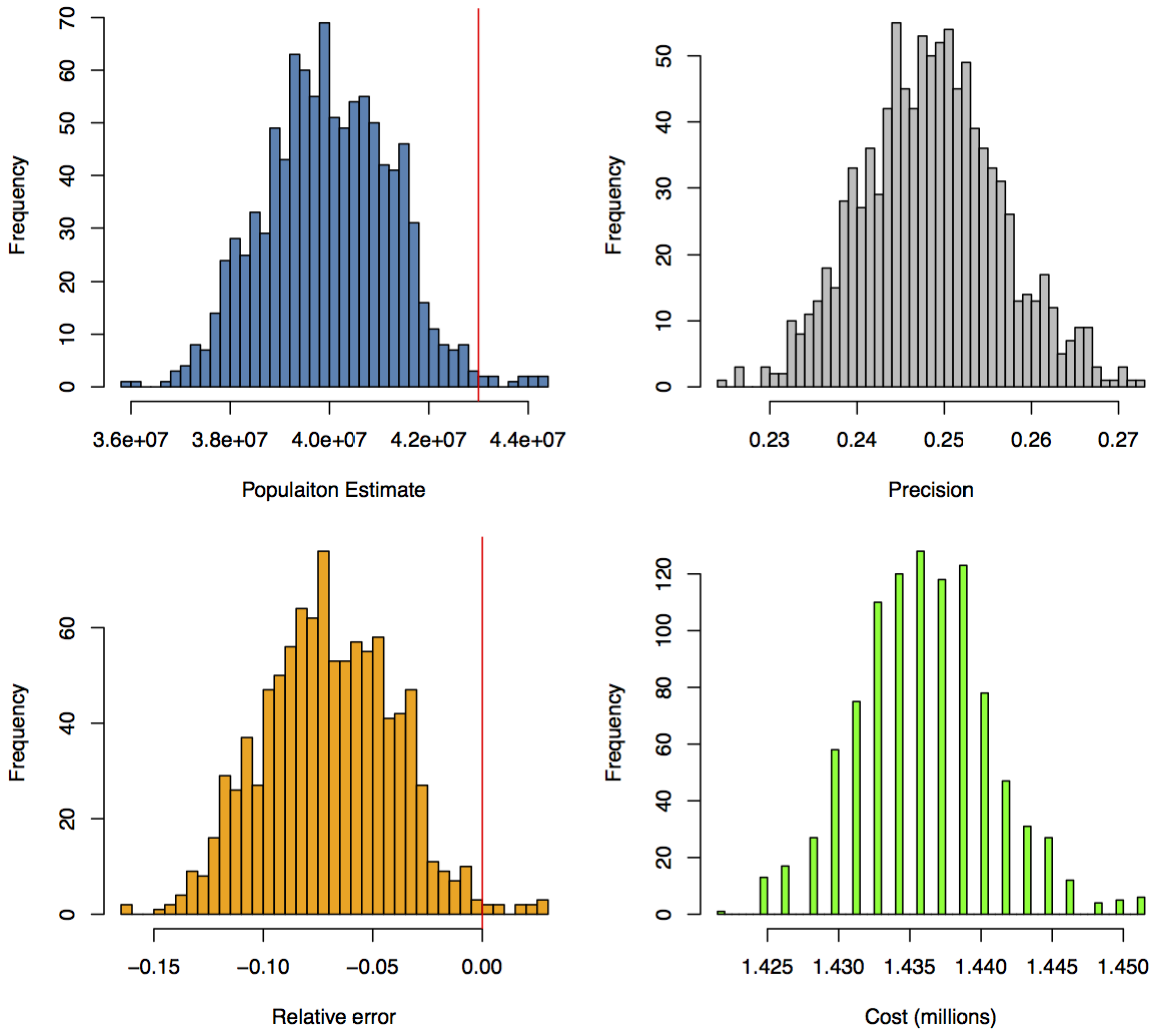


Figure 10. Simulation results from 1000 iterations for stratified video survey assuming snapperoccupy 5% of the natural habitat. Red vertical lines indicate target or true values. Precision represent the level of precision in the 95% confidence interval on the total population estimate. Relative error compares estimates with the true simulated population size.

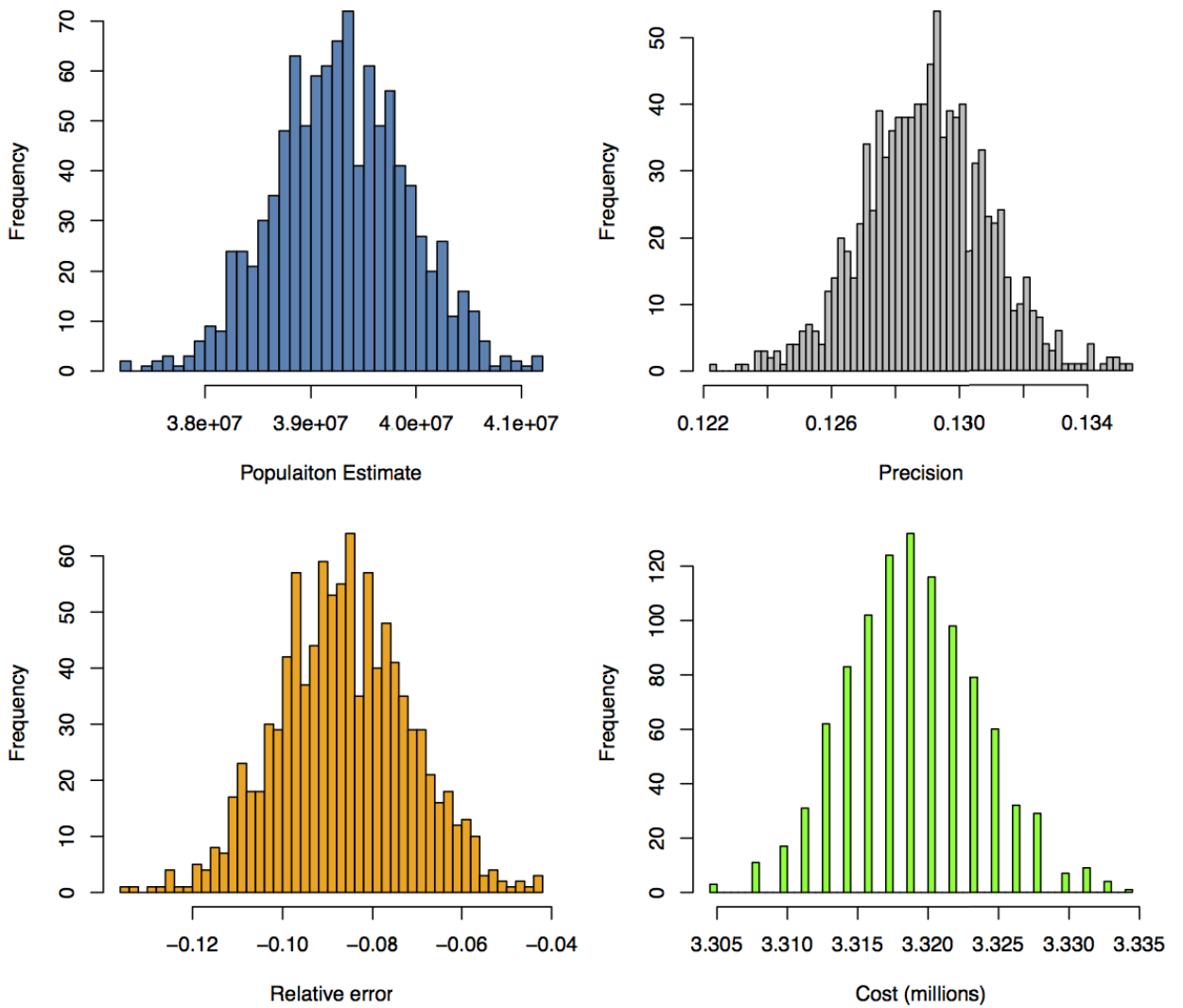


Figure 11. Simulation results from 1000 iterations for stratified video survey assuming snapperoccupy 10% of the natural habitat. Red vertical lines indicate target or true values. Precision represent the level of precision in the 95% confidence interval on the total population estimate. Relative error compares estimates with the true simulated population size.

Additional considerations

One of the first steps in any sampling program is to define the sampling frame. We attempted to accomplish this in an informed way for red snapper, but sparse data in most regions, and the lack of empirical density estimates for some, meant that assumptions had to be made to estimate the distribution of red snapper in the nGOM. Clearly, there is much uncertainty as to how close the spatial distribution estimated in the first component of our study approximates reality. Yet, some estimate of the sampling frame is required to apply models we derived to estimate red snapper abundance in U.S. waters of the GOM with either tagging or video-based methods. A critical component to any approach employed during the implementation phase of this program will be to more fully understand the spatial distribution of red snapper in the nGOM. While some insight can be gained from the spatial distribution of commercial catches and the GAM modeling exercise presented in this document, the reality is that any recommendation from these modeling exercises could result in placing sampling units in inappropriate habitats. There are at least two potential solutions to the current lack of information. The first would be to canvas commercial and recreational fishermen to develop a more explicit map of red snapper occupancy. This process would involve the sharing of GPS locations of known positive catch rate areas at a spatial resolution similar to that presented in this analysis. The second option is to recognize the shortcomings of the information and adapt flexibility into any sampling program such that sampling locations could be adjusted so that they would sample 'suitable' red snapper habitat near predefined locations. This approach still requires 'suitable' habitat to be defined in the Gulf for total population estimates to be made. Despite limitations in the red snapper distribution and density data, the modeling performed herein indicates there is the potential to obtain a GOM-wide population estimate within the prescribed relative error and for the allocated costs with either of the two methods we explored. It should be stressed however, that this inference is contingent upon the estimated sampling frame being somewhat accurate. In the end, we think this exercise should perhaps be viewed as an iterative approach in that information gained in the implementation phase, at the very least, will provide a more robust estimate of the sampling frame (i.e., habitat specific distribution and occupancy of red snapper in the nGOM).

The last additional consideration stems from the issue we encountered when attempting to rectify our original model-derived estimates of red snapper abundance with the estimates derived from the 2015 stock assessment. Essentially, we had to down-weight empirically derived red snapper density estimates, sometimes considerably, to match estimated abundance from the stock assessment. This might owe to initial estimates made about the quality of habitat where empirical density estimates were made, or it might be due to erroneous habitat occupancy estimates.

However, an alternative explanation would be that red snapper abundance in the GOM is in fact higher than current stock assessment-derived estimates. Results of the implementation phase of this program should indicate which of these scenarios is more likely to be correct.

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2016 Red Snapper Phase I Experimental Design

5 December 2016 Sea Grant and NOAA Fisheries

Final Report

Red snapper CIR study design

A study design to estimate the population of age 2+ red snapper in the US waters of the Gulf of Mexico: use of change-in-ratio, index removal and removal estimators

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Summary

To estimate the population size of age 2+ red snapper in the US waters of the Gulf of Mexico, three types of habitat need to be studied: 1) artificial reef structures, platforms and other known areas of high fish density, 2) known, natural, low relief reefs, and 3) areas of featureless bottom or of unknown bottom type. We propose the use of change-in-ratio (cir), index-removal (ir) and removal estimators. The cir and ir methods involve a survey, followed by a partial depletion of the population or a component of the population (i.e., legal-size animals), followed by another survey. The greater the depletion, the better the results. The removal method involves conducting a series of two or more fishing (e.g., longline) sets at each sampling place and noting the progressive decline in catch per set at the sampling place. Again, the greater the depletion, the better the results.

For artificial structures (first habitat type), the cir and ir methods are recommended. The cir estimator is used with the surveys conducted prior to and after the sport fishery to take advantage of the sizeable depletion of legal-size animals due to the fishery. For the ir method, the surveys and depletion are completed by research scientists within a day to ensure that survey catchability does not change appreciably between surveys.

For known natural reefs (second habitat type), the ir method with surveys and depletion completed within a day is recommended because exploitation rate on these reefs is believed to be quite low due to lower abundance compared to artificial reefs; consequently, we cannot rely on sport fishers to deplete the populations in these areas appreciably.

For areas of featureless bottom and unknown bottom type (habitat type 3), it is recommended that a random sample of geographic locations be sampled by repeatedly setting bottom longlines and deploying vertical longline (bandit) gear at the selected locations; population size at these locations will then be estimated using a removal estimator and possibly an index calibration method.

As proof of concept, the cir and ir methods have been tried for 4 years in the Alabama Artificial reef zone (first habitat type) to good effect. The abundance of red snapper on a sample of natural, low relief reefs (second habitat type) was surveyed by this project and abundance was found to be much lower than what has been observed on artificial reefs, in accordance with expectations and general knowledge about red snapper distribution. The proposed structure for field sampling lends itself well to the idea of using multiple, complementary methods (mark recapture, visual surveys, etc.), and integrated models, to strengthen the inferences that can be made about the population. In order to implement this – or any other study design – it is highly advisable to conduct auxiliary and pilot studies to better define habitat maps, evaluate calibration factors, determine allocations of sampling effort to strata, and determine necessary sample sizes.

Background

Gulf of Mexico red snapper are a valuable natural resource and, as such, there is a clear need to independently and directly evaluate the abundance of this stock. The purpose of this project is to design a study that can be used to estimate the size of the population of red snapper age 2 and older in the US waters of the Gulf of Mexico. The study design must be implementable in three types of habitat as follows:

Artificial reef structures, platforms and other known areas of high fish density

Known, natural, low relief reefs

Other areas with featureless bottom and areas of unknown bottom type.

Artificial structures (type 1 habitat) occur at known locations so that it is possible to select a random sample of these structures for the purpose of estimating population sizes associated with the reefs. Artificial structures are important because they have the highest densities of red snapper and account for most of the recreational catch (see pilot study results in Table 1).

For some areas of the Gulf, bottom type has been mapped and the locations of natural, low relief reefs (type 2 habitat) are known so that a sampling frame of these known reefs can be compiled and a random sample can be drawn randomly from the frame.

The remaining areas of the Gulf (type 3 habitat) either have habitats associated with low densities of red snapper (mud or sand) or have not been mapped sufficiently for determining areas of concentration of snapper. It should be noted that featureless bottom still needs to be sampled because, even if snapper are present in low densities (number per unit area), the enormity of the area with featureless bottom may nonetheless give rise to a substantial population of snapper. For example, considerable numbers of large red snapper on featureless bottom near the shelf break. For purposes of sampling to estimate population size, geographic coordinates can be sampled randomly from the area of type 3 habitat.

Diagrammatically, the study area (the US waters of the Gulf) can be divided into regions as in Figure 1. Key points are:

For type 1 habitat, there is a frame of artificial reefs, platforms and other high use sites that can be used to draw a random sample of discrete habitat patches.

For type 2 habitat, maps exist with natural, low-relief reefs delineated, though not all sites are listed and not all listed sites will have red snapper. These low-relief reefs can be small or many kilometers long. Large reefs will have to be divided into smaller reefs for logistical reasons. Thereof study areas should be small enough that they can be completely surveyed and a significant depletion can be made; they should be large enough to minimize boundary problems (i.e., minimize exchange of fish among areas and minimize uncertainty over the size of the area being studied and depleted).

For type 3 habitat (the remaining areas), maps of bottom features are not available or are not adequate for defining red snapper habitat. Consequently, random coordinates within type 3 habitat will be sampled.

In designing a program to estimate the size of the population of red snapper in the US waters of the Gulf of Mexico, we were cognizant of, and tried to take advantage of, two salient features. First, the recreational fishing season in federal waters is extremely short (less than 2 weeks) and is concentrated at high catch rate “hot spots” associated with artificial reefs, platforms and some natural features (type 1 habitat). Second, the exploitation rate at these hot spots is appreciable.

These conditions are ideal for the use of methods that assess population size by noting the effect of harvest on indices of relative abundance – change-in-ratio, index-removal, and removal methods. We

propose to use the change-in-ratio (cir) and index-removal (ir) methods for type 1 habitat and to use the ir method for type 2 habitat. For type 3 habitat, the ir method or a removal estimator is proposed. The cir and ir methods require a survey followed by a harvest and then a follow-up survey (Hoenig and Pollock 1998; Pollock and Hoenig 1998). There are two ways this can be implemented: within-day depletion and seasonal depletion. For the within-day depletion, the scientific team conducts a survey in a localized area, then fishes the population to (partially) deplete it, then conducts a follow-up survey, all within a day. For the seasonal depletion, the scientific team conducts a survey prior to the fishing season, then the population is depleted by the anglers during the regular fishing season, and then the scientific team conducts a follow-up survey. The removal method is similar to the ir method except that the processes of monitoring the population (the surveys) is combined with the process of depleting the population. Thus, for example, a series of successive sets with bottom longline gear should show a decline in catch per set (the monitoring) due to the cumulative removal of longlined fish (the depletion).

The cir and ir approaches have been used to good effect in the University of South Alabama (USA) paired vertical longline (VL) and remotely-operated vehicle (ROV) surveys before and after the recreational fishing season in the Alabama Artificial Reef Zone (AARZ) from 2011 – 2014 (see Gregalis et al. (2012) for a description of the program). We draw upon the results from this program to develop the sampling design proposed here.

It is important to note that this structure to the sampling lends itself well to the idea of using multiple, complementary methods to strengthen the inferences that can be made about the population (see, e.g., Seber (1982), Chen et al. (1998a)). Thus, the ideas in this proposal might be integrated with other study proposals for Gulf red snapper.

Change-in-ratio, index-removal, and removal methods

Change-in-ratio

The change in ratio method is generally attributed to Kelker (1940) though methods using the same mathematical structure go back several centuries. The cir method looks at how the population composition changes due to a known, selective harvest of one component of the population. In the case of red snapper, the population can be considered to be composed of two components, legal-sized animals (hereafter, legal animals), and immediately sub-legal-sized animals (hereafter, sublegal animals). We propose to use the cir method for artificial reefs and other known hot spots of sport fishing activity (type 1 habitat). A survey is conducted just prior to the recreational fishery at n of the N known artificial reefs, platforms and high use sites and the proportion of fish that are legal size is estimated. The sport fishery then depletes the population of legal-sized animals over the very short sport fishing season. The total catch of legal animals (and sublegals, if any) is obtained from the existing surveys of recreational fishing activity. The sport catch is assumed to come from the artificial reefs and high use sites (though this can be investigated if additional questions are added to the existing creel survey programs and, if necessary, the catch can be apportioned to the three habitat types). The follow-up survey is then conducted and the after-season proportion of the survey fish that is legal is noted. The idea is that if a given harvest, say 100,000 legal-sized fish, changes the

proportion seen in the surveys a great deal then 100,000 must be a major component of the population, i.e., the population is small, whereas if the catch of 100,000 hardly changes the proportion legal at all then the population must be quite large. Formally, when only legal-size fish are harvested the exploitation rate, u_L (fraction of legal size fish harvested), can be estimated by

$$\hat{u}_L = \frac{p_1 - p_2}{p_1(1 - p_2)} \quad (1)$$

where p_1 and p_2 represent the proportion of legal-size fish in the pre- and post-depletion survey, respectively, and the $\hat{}$ symbol denotes an estimate. The population of legal-size fish before the depletion, N_L , is estimated as

$$\hat{N}_L = u_L / c_L \quad (2)$$

where c_L is the depletion (amount of legal-size fish caught and removed from the population). The total number of fish in the population (sublegal and legal) before the depletion is N_L / p_1 .

When some sublegal size fish are also harvested, the population size can still be estimated provided the exploitation rate for legal and sublegal fish differs. The estimators for this situation are

$$\hat{N}_L = \frac{p_1(c_L - cp_2)}{p_1 - p_2} \quad (3)$$

$$\hat{N} = \frac{c_L - cp_2}{p_1 - p_2} \quad (4)$$

where c_L is the removal of legal-size animals, c is the total removal (sublegal- and legal-size), and \hat{N} is the estimate of the total population (sublegal and legal). Variance formulae for these estimators are given in Seber (1982), Chen et al. (1998) and Pollock and Hoenig (1998).

Under very general conditions these are maximum likelihood estimates. We have tried this method in the Alabama Artificial Reef Zone (AARZ) and the approach worked well for obtaining exploitation rate estimates (more on this, below and in Table 1). The assumptions of this method are (Pollock and Hoenig 1998):

the population is closed except for the removals (depletion).

the removals are known exactly.

legal and sublegal fish have equal catchability (sightability) in the pre- and post-harvest surveys so that the estimated proportion of fish that are legal size is unbiased.

animals remain in their category over the course of the study, i.e., sublegal animals remain sublegal.

There are some important subtleties to these assumptions that have important implications. First, consider assumption #1. It implies that there is no recruitment, mortality other than the known depletion (removal), immigration, or emigration. Pollock and Hoenig (1998) note that this may be reasonable if the time between the two surveys is short. However, suppose that the first survey is immediately before the depletion but the follow-up survey occurs after a delay such that there is emigration and/or natural mortality between the depletion and the survey. If the animals emigrate or die without regard to their size or age then the post-depletion ratio of legal to sublegal animals will not change. Consequently, the estimate of pre-depletion abundance should not change. (Immigration would likely affect the ratio of legal to sublegal animals and thus cause a bias.)

Also, if the time between surveys is appreciable, some sublegal animals may grow into the legal-size category thus affecting the ratio of legal to sublegal fish without reflecting differential harvest. And, growth of animals smaller than the lower limit of the sublegal size class (i.e., recruitment) could affect the ratio. In these cases, adjustments to the observed ratios must be made (by redefining size boundaries for the categories) to reflect growth so that the two categories are tracked properly over time. This was done in our pilot study in the AARZ.

Finally, it can be shown (e.g., Seber 1982 p. 353) that an unbiased estimate of the abundance of legal-size animals can be obtained if only legal-size animals are removed, even if the catchability of legal and sublegal animals is unequal. It is assumed that the ratio of catchabilities remains constant from survey to survey (but is not necessarily 1:1). The estimate of sublegal abundance is biased to the degree that the catchability between the two groups differs. It has been shown that increased precision can be had if one uses the same stations for the pre- and post-harvest surveys rather than rerandomizing (Chen et al. 1998b).

Index-removal

The index-removal method is generally attributed to Petrides (1949). It looks at how an index of population size changes because of a known removal. For example, we might assume that catch rate (catch per unit effort) is proportional to abundance. Then if a harvest of 100,000 animals causes the catch rate to decline to 1/2 of its pre-harvest level (say, from 40 to 20), then we can infer that 100,000 animals represents 1/2 of the initial population size. Hence, we infer that the initial population was $100,000/0.5 = 200,000$.

Formally, the exploitation rate is estimated by

$$\hat{u} = \frac{i_1 - i_2}{i_1} \quad (5)$$

where i_c and i_* represent the catch rate of legal-size fish in the pre- and post-season survey and the population size is estimated by

$$\hat{N} = c \frac{i_1}{i_1 - i_2} \quad (6)$$

where N is the estimated population size and c is the depletion (removal). Variance formulae are given in Hoenig and Pollock (1998).

The assumptions behind the method are (Hoenig and Pollock 1998):

the population is closed except for the removals

all animals have the same probability of capture in the surveys, and this probability does not vary from survey to survey sampling is with replacement, or the fraction of the population taken in the surveys is negligible.

It has been found that, when the assumptions of the cir and ir methods can be met, the ir method provides more precise results (e.g., Dawe et al. 1993; Chen et al. 1998a,b and see below). However, the assumptions for the ir method are stronger and harder to meet than those for the cir method. Ihde et al. (2008) relaxed the second assumption by allowing the catchability to change from the pre-depletion survey to the post-depletion survey. They did this by assuming the ir method is repeated multiple years with the catchability changing the same way each year between the two surveys. They thus had to estimate an extra parameter, i.e., two catchability coefficients instead of one. The index-removal method can be used in two ways for type 1 habitat – using within-day and seasonal depletion. For within-day depletion, the procedure is as follows:

At each of n sites, a camera survey is conducted to obtain an index of relative abundance.

Bandit (hook and line) fishing gear is deployed at each station to remove fish. This serves several purposes, primarily to:

Deplete (partially) the population

Obtain a sample for determining the age composition of the population being studied

Determine the selectivity of the bandit fishing gear by comparing size composition in the camera survey with the hook and line catches

At each of the same n sites, conduct a post-depletion camera survey to determine the new relative abundance.

Optionally, repeat steps 2 and 3 as many times as needed to get the desired level of depletion and thus the desired precision of the estimated abundance (see Chen et al. 1998a).

For type 1 habitat, the seasonal depletion approach would entail surveying a random sample of reefs just before the recreational fishing season, then determining the catch by recreational fishers during the short fishing season, and then resurveying the reefs. Our experience with this method in the AARZ raises concerns about the use of this method for the seasonal depletion approach (see below). The ir estimates of exploitation were consistently lower than the cir estimates and were sometimes infeasible. This raises a concern that the survey catchability may change between two surveys. If the survey is repeated over multiple years, the assumption of constant catchability can be relaxed and replaced with the assumption that the catchability changes between the two surveys the same amount each year (Ihde et al. 2008). However, if the red snapper program is only conducted for one year then the generalization of Ihde et al. cannot be used.

For type 2 habitat, the within-day ir method can be used as described above. The seasonal method is not recommended because angling pressure in type 2 habitat is believed to low so there won't be sufficient depletion.

Removal method for type 3 habitat

For type 3 habitat, the number of fish anticipated to be seen by the standard camera gear used in the AARZ is expected to be low. And, the catch of red snapper by the bandit gear is also likely to be low due to low density of fish. Consequently, the ir method may not work well for this habitat and more efficient gear may be needed. We propose that bottom longline sampling following Drymon et al. 2010 be used to both monitor the abundance of the population and deplete the population. This gives rise to the well-known removal estimator described by Zippin (1956) and discussed in detail by Seber (1982) and other texts. A series of study areas is defined, each measuring say 1 km x 1 km. The study areas should be large enough that boundary issues are minimal but small enough that the areas can be surveyed completely and depleted. Within each study area, k longline sets are made. For $k = 2$ sets, the removal estimator of abundance in a study area is

$$\hat{N} = \frac{c_1^2}{c_1 - c_2}$$

where c_1 and c_2 are the catches in the first and second bottom longline sets, respectively. Note the close resemblance of this formula to that for the ir method, the difference being that the depletion c in equation (6) is taken to be the catch in the first bottom longline set, c_1 . The procedure is easily extended to handle any number of sets with the bottom longline gear, and with $k > 2$ it is possible to increase the precision and also to test and relax the stringent assumptions. Variance formulae are available in Seber (1982).

Calibrating an index of abundance for type 2 and type 3 habitats

Another possibility for type 3 habitat is to develop a calibration factor such that catch per unit of sampling effort in a research survey can be converted into absolute density. This can be accomplished as follows for the bandit gear deployed in habitat type 2. We model the expected catch, c , in one deployment of the bandit gear as

$$c = a e d$$

where a is the area prospected or fished by the gear (the area of attraction), e is the efficiency of catching fish given that a fish is in the area of attraction, and d is the density of fish, i.e., the number per unit area. The average density of fish estimated by the ir method in the studies of type 2 habitat furnishes the value of d . The average catch by bandit gear in type 2 habitat furnishes the value of c . Consequently, the product ae can be estimated by

$$\widehat{ae} = c/d \quad (7)$$

Given an estimate of ae , we can then conduct survey fishing with bandit gear in other areas, i.e., in habitat type 3, and estimate the average density of fish in habitat type 3 as

$$d_{\text{habitat } 3} = c_{\text{habitat } 3} / \widehat{ae}. \quad (8)$$

The absolute number of fish in habitat type 3 is estimated as the average density estimated from

$$(8) \text{ times the total area of type 3 habitat, } N = dA.$$

This is predicated on the assumption that the product ae is the same in habitat type 3 as in habitat type 2. It also presumes that the same gear will be used in the two habitat types. However, the density of fish in habitat type 3 is believed to be generally low so that use of bandit gear might not be efficient enough to produce adequate sample sizes. In this case, alternative gear such as bottom longlines might be preferred. Developing a calibration factor for bottom longlines could be accomplished in two ways.

First, bottom longlines and bandit gear could be deployed simultaneously to estimate the ratio of catching power, i.e., $ae_{\text{longline}}/ae_{\text{bandit}}$. This ratio is then multiplied by ae_{bandit} to get the catching power for longlines. That is,

$$\widehat{ae}_{\text{longline}} = \widehat{ae}_{\text{bandit}} \frac{c_{\text{longline}}}{c_{\text{bandit}}}. \quad (9)$$

Second, the catching power for bottom longlines in type 3 habitat could be estimated the same way the catching power of bandit gear was estimated for habitat 2: the absolute abundance is estimated in randomly selected patches of known size of type 3 habitat using, say, the removal method, and the abundances are converted to fish density; then the average catch per bottom longline set is related to the absolute estimates of density. That is,

$$\frac{\widehat{N}_r}{a} = d \quad (10)$$

where \widehat{N}_r is the estimate of abundance by the removal method, a is the area for which the estimate is determined, and d is the density of fish. Then, from equation (8), we have

$$\widehat{ae}_{\text{longline}} = \frac{c_{\text{longline}}}{d}.$$

In practice, the estimates are obtained from multiple patches by computing averages, i.e., for k patches, the sum of the k abundances is divided by the sum of the k areas to obtain the average density, and the average catch rate is divided by the average density to get the average value of the product ae .

Double sampling

For both habitat types 2 and 3, it might be advantageous to adopt a double sampling scheme whereby the survey gear (camera or bottom longline set) is deployed once at a large number of stations on the assumption that this can be done quickly. At a subset of the stations, the more time-consuming procedure of doing index-removal or removal estimation is conducted. The results are then combined as a ratio estimator. For example, suppose the n sites studied with the full assessment procedure happened to be above average in abundance, say twice the average true abundance. This would give rise to an overestimate of abundance by a factor of two.

However, if we could establish that the collection of n sites was above average we could make an adjustment. The large number of sites where just the survey gear was used would establish that the n sites had twice the catch rate of the average of all sites. Hence, the estimated abundance should be adjusted downward by the ratio $\frac{1}{2}$. In the AARZ study, this type of double sampling is used.

Implementation of a Gulf-wide program for red snapper based on cir, ir and removal estimation

The first step is to divide the US waters of the Gulf of Mexico into broad study regions based on logistical concerns, and b) project objectives with respect to spatial specificity. For example, estimates might be desired by state, and project personnel may find it convenient to survey the waters of their state. Managers may wish to compare exploitation rates among states and may wish to know if high catches at artificial reefs and platforms are due to heavy exploitation versus high abundance. The information provided by region-specific estimates sets the stage for area-based management. We propose four study regions: Florida, Alabama, Western Louisiana and Texas. Within each study region, three strata are delineated based on the three habitat types described above. Each of the 12 resulting strata is treated as an independent study.

The second step is to review all available information on red snapper distribution. The study of Karnauskas et al. (in press) can be used as a starting point and further refined (see Figs 2 and 3). For example, areas that have high predicted abundance (Fig 2) as well as high numbers of artificial reefs or platforms (Fig 3) can be considered type 1 habitat. These areas could include the Alabama Artificial Reef Zone, waters on the Texas-Louisiana border, etc. Further determination of exact locations of potential Type 1 (and other) habitats would involve additional input from local experts. Type 2 habitat could be determined via Fig 2 as those areas with high percentages of rock or gravel, such as portions of the Florida shelf. Finally, Type 3 habitat would be considered as all other areas, i.e., areas which can be seen in Fig 2 as those with low percentages of rock (and therefore would have a high probability of being mud-dominated) as well as areas for which the bottom type is poorly known. Large portions of the Gulf will likely be classified as type 3 habitat.

Type 1 habitat

For type 1 habitat, we recommend the study protocols of Gregalis et al. (2012) as enhanced in this proposal. These have been used for six years in the AARZ. The field protocol is as follows:

From the list of N known artificial reefs, platforms and high use spots, a sample of n is selected for study.

Just prior to the sport fishery (as close to it as possible), a field survey is conducted consisting of the following elements at each site:

An ROV equipped with two video cameras, a single-beam scanning sonar, and a pair of lasers is deployed to record fish assemblage and measure fish length. On a single artificial reef, an ROV is deployed and positioned on the bottom within 5 meters of the target feature. Examples of target features sampled with this gear include artificial pyramids, tanks, chicken coops, cement drums, etc. The ROV heading, depth, range to target, GPS position (for the boat) and start time of the video are recorded for the feature. Video is recorded for two minutes at the designated heading (in degrees); then the ROV is flown to the opposite side of the feature for two additional minutes for sampling as described above (i.e., on the bottom, within 5 meters of the feature). The second heading and range to feature is recorded (~180 degrees from first heading). Finally, the ROV is positioned ~1 meter above the feature for a slow 360-degree spin and a vertical view of the structure. After recording is complete, the video stop time is recorded. Total time for video recording is usually between 7-10 minutes. Lengths can be obtained in instances where the fish are illuminated by the ROV mounted lasers, spaced 3 cm apart.

An index of abundance of red snapper is taken to be the maximum number of fish observed in a single screenshot. This minimal estimate of abundance is a standard way of generating an index from video data because it represents an absolute minimum number of fish at that station while avoiding the issue of double counting (Bacheler and Shertzer, 2015).

Bandit (vertical longline) fishing gear is deployed in a standard fashion. The catch is retained to deplete the population and to provide otoliths for determining the age composition of the population. The gear and procedures used in the AARZ, which can serve as a model for other areas, is as follows:

The fishing vessel is outfitted with three vertical longline reels, loaded with 167 m of 400 lb test monofilament that terminates in a 6.5 meter monofilament backbone. At the bottom of each line is a 4 kg weight. A mono backbone (300 lb test) is assigned a hook size (8, 11, and 15) and attached to a mainline via a three way (2/0) snap swivel. Each gangion (100 lb. test, twisted) is 45 cm in length with its 10 assigned circle hooks and all three backbones are fished on site to address hook selectivity. Gangions are spaced equally (60 cm apart) along the 6.5 meter length. All hooks are baited with Atlantic mackerel (*Scomber scombrus*). Soak time is 5 minutes. The line remains attached to the vessel for the 5 min soak while the boat is held on station. The entire catch is retained, each fish is measured, and otoliths are removed from the fish. The gear configuration and sampling procedure described above have been adopted by NOAA SEAMAP as a standardized method for vertical longline sampling throughout the Gulf of Mexico.

The camera survey with the ROV is repeated, as in (a).

Optional: repeat steps (b) and (c) as many times as needed to achieve desired precision of the estimates.

The recreational catch of red snapper in the type 1 habitat of the study region is obtained from the existing angler surveys (MRIP and some state surveys). It is assumed that virtually all of the recreational harvest comes from the type 1 habitat because the catch rates are highest in this habitat (see Appendix A – experimental fishing on natural, low-relief reefs). If possible, questions could be added to the MRIP and state surveys to determine the habitat where the snapper were caught. Additionally or alternatively, aerial surveys of recreational fishing activity could be conducted during the short federal recreational fishing season to verify that the vast majority of the fishing is taking place in habitat type 1. The field survey described in (2) above is repeated as soon after the recreational fishery as possible. The data analytic procedures are as follows:

Selectivity of the bandit fishing gear (needed for the seasonal *cir* estimate) is estimated by comparing the size composition of fish caught by the bandit gear with that seen by the camera (see Myers and Hoenig 1997). This has been done using four years of data from the AARZ study program but the results can be made more precise by considering the additional data accruing from the Gulf-wide study.

The within-day IR estimate of abundance is calculated using equation (6) using a range of sizes of approximately constant selectivity in the bandit gear. The estimate can then be extrapolated to the total population at the site by dividing the estimated abundance by the proportion of the population in the included size range. The proportion is obtained from the size composition of the fish in the camera survey. For example, suppose the index in the pre-depletion survey is 20, and 15 of these fish are larger than 30 cm and thus (at least for the purposes of this example are assumed to) have approximately equal selectivity in the bandit gear. Thus, the proportion $p = 15/20 = 0.75$ of the population has approximately equal catchability. The pre-depletion index of population for the selected size range is 15. Suppose 50 fish in the selected size range are caught by the bandit gear, and 5 fish in this size range are caught in the post-depletion survey. The estimate of abundance in the selected size range is (from equation (6)) $N = 50(15)/(15-5) = 75$ and the total estimate is $N/p = 100$. Note that there is a check on the reasonableness of these calculations: we have an estimate of the number of fish less than 30 cm at the start of the experiment (it's 25) and we know how many fish under 30 cm were caught and removed by the bandit gear. Thus, we can estimate what fraction of the fish less than 30 cm were removed by the bandit gear. The index of fish < 30 cm in the post-removal survey should have been reduced by this amount. An alternative way to do the IR calculations is to make separate IR estimates for different size ranges of fish (such that selectivity is approximately constant within a size range) and add the results for the different size ranges.

The procedure in this step can be modified to include several observations with the ROV interspersed with removals using the bandit gear. This will increase the precision of the results (Chen et al. 1998a). Note that the double sampling procedure (whereby all sites are surveyed with the ROV camera and a subset is studied with two ROV one bandit deployments) is not included in this step but it could easily be done.

To obtain the seasonal *cir* estimate of abundance, the index of abundance in the pre-season surveys (prior to deploying the bandit gear) are divided into legal- and sublegal- sized fish and, similarly, for the post-season survey. The indices are then averaged over sites. The *cir* estimate of pre-season abundance is obtained from equation (2) (if only legal-sized fish are harvested) or from equation (4) if some sublegal size fish are harvested (as determined from existing angler surveys).

For the pilot study described here, the bandit data were used to compute the pre- and post-season proportions after correcting for selectivity of the bandit gear; the ROV data were used just to determine theselectivity for correcting the bandit estimates of proportions.

Type 2 habitat

For low-relief, natural reefs, only the within-day ir method is used. A decision must be made about how large a natural reef can be and still obtain a sufficient depletion to afford the needed precision. Any reefs larger than this must be divided into manageable segments. After selecting a random sample of natural reefs, steps (2) and (4) (from the procedure for type 1 habitat) are implemented. Steps (1) and (2) from the data analytic procedures are then performed.

Type 3 habitat

Type 3 habitat constitutes a vast area with generally low abundance of red snapper. It includes regions for which the bottom type has not been surveyed and mapped. It is anticipated that at many sites, test fishing will produce no red snapper. However, it is believed that, even with very low densities of snapper, the habitat may contain a considerable biomass of snapper due to its vast size. In particular, there may be considerable numbers of large red snapper near the shelf break. It is not clear that vertical longline gear will effectively capture snapper is this habitat.

Therefore, test fishing with both vertical longline and bottom longline gear should be done to establish what catch rates might be expected. In addition, sampling with vertical longline gear in type 3 habitat ensures a common gear type is sampled across all habitat types in the Gulf, which allows for the development of calibration factors. Furthermore, the procedure described below should be tried for different sized study sites to ensure that adequate depletion can be accomplished in as large an area as is feasible.

The field procedure is as follows:

select n sites measuring 1 km x 1 km (say) for study.

deploy a 1 km bottom longline with equal numbers of hooks of three sizes (8, 11, and 15) for a short duration set (say 1 hour). The length is recorded and the otolith is removed for every fish caught.

At a subset of m sites, the bottom longline procedure is repeated until a total of k sets ($k \geq 2$) has been made, with the locations of the sets within the study site randomized. Note that the short sets are so that many sets can be made in one day to deplete what's there with minimal movement of fish into and out of the study site. The value of k will need to be determined empirically as the smallest value capable of producing the desired level of precision in a removal estimate.

The above data give rise to two kinds of inference: what fraction of the type 3 habitat contains red snapper, and what is the average density of red snapper (number/km²) at those sites containing snapper.

Proposed Budget for Implementation in Phase II

We have experience in using the within-day and seasonal estimators for type 1 habitat (within the AARZ) and so estimated budgets can be made for type 1 habitats. The cost for the Alabama survey, which includes pre- and post-season surveys, is approximately \$350,000 for a one-year study. This estimate includes all vessel, equipment and personnel costs associated with sampling approximately 50 stations during each survey (100 stations per year). Assuming a) Gulf-wide strata were at the state level, b) we excluded Mississippi because there are no known hotspots there, and c) we sampled two hotspots in Texas and two hotspots in Florida, that would mean that sampling type 1 habitats would cost approximately six times that amount, or \$2.1 million.

For habitat type 2 we assume there are 4 state regions (excluding Mississippi) and for habitat type 3 we assume there are 5 state regions (including Mississippi). Thus, an additional 9 surveys are needed for these habitat types at a cost of around \$3.0 million (assuming a cost of \$350,000 per survey). However, implementation in Phase II would require auxiliary and pilot studies to determine the importance and relative effort required to appropriately sample these areas. These studies include:

Surveys to determine catch rates in type 2 and type 3 habitats obtained with bandit gear and bottom longlines and to determine performance of ROV with camera for sighting red snapper

Estimation of calibration factors for converting an index of abundance into absolute abundance, which enables the catch rates in type 2 and type 3 habitats to be interpreted in absolute rather than relative terms

Experimental depletions to determine feasibility of depleting study areas given various options for sampling effort, sampling gear, and size of study sites

Expert consultation to define working maps of habitat types

The cost of these additional studies would need to be determined and included in the final comprehensive budget. An additional consideration when thinking about the budget is that these surveys might be incorporated into existing monitoring programs (e.g., SEAMAP bottom longline surveys), which could substantially reduce costs.

Evaluation of options and rationale for adoption

Comparison of seasonal cir and ir methods in AARZ

We have examined the potential use of seasonal cir and ir on type 1 habitat in Alabama. The University of South Alabama Fisheries Independent Ecosystem Survey is conducted before and after the federal recreational snapper season, typically about four months apart. The survey uses vertical line (VL) gear with three circle hook sizes (8/0, 11/0, and 15/0) to catch red snapper at artificial reef sites. At a subsample of sites, an ROV equipped with video cameras, single-beam scanning sonar, and a pair of laser scanners is deployed prior to and after fishing with VL gear to record fish assemblage and measure fish length.

Data analysis from 2011-2014 showed that several modifications needed to be made to traditional seasonal cir and ir analysis methods. First, due to the length of time between the surveys,

individual growth between the two survey periods needed to be accounted for to correct the estimated post-season indices. Growth patterns from two sources (Patterson et al. 2001, SEDAR 2013) were tested for sensitivity and yielded nearly identical results in correcting for the four-month period between surveys. Similarly, natural mortality was incorporated for completeness, however changes to the indices after incorporating natural mortality were negligible due to the short time period. Finally, we used the vertical longline gear in preference to the ROV camera data to obtain the indices of abundance and composition because of the larger sample sizes with the vertical longline gear; however, selectivity of the vertical longline gear needed to be accounted for to ensure the composition of the catch was representative of the population. After reviewing the literature on red snapper hook selectivity (Garner et al. 2014, Campbell et al. 2014), different selectivity patterns were tested to examine how changing the selectivity assumption would alter the proportions of legal and sublegal fish. Results were quite sensitive to different selectivity curves. Due to this sensitivity, we recommend a more direct method of determining gear selectivity. In our analysis we relied on the length measurements taken by the ROV, and assumed that these measurements were representative of the local population of red snapper. Selectivity was estimated directly by computing ratios of survey catch-at-size to ROV abundance-at-size and a generalized linear model with binomial error structure was fit to the ratios (Myers and Hoenig 1997).

After processing the data to incorporate growth, natural mortality, and selectivity, traditional seasonal cir and seasonal ir methods were used to estimate exploitation via the equations above. Exploitation estimates using the ir method were systematically lower and had a much larger variance than those estimated via cir (Table 2, Figure 4). The large variances and unrealistic estimates produced by the ir method were likely due to the additional restrictive assumption of the ir method that catchability is constant between survey seasons. The cir method has the more relaxed assumption of constant selectivity as opposed to catchability, and so the cir method was more appropriate for the seasonal depletion approach.

The traditional ir method was deemed inappropriate for this survey, and so we further explored this approach by applying an ir method with changing catchability (see Ihde et al. 2008). This method assumes that the pre- and post-season survey catchabilities are different, but that they are constant among multiple years. While we were successful in applying this method and were able to estimate the pre- and post-depletion catchability coefficients, q_{pre} and q_{post} , the variances around these estimates (and therefore the variance surrounding the estimated abundance) were extremely large, resulting in coefficients of variation on the order of 1000% (Table 3). The high variability implies that either there was not enough contrast in exploitation during the four years that were considered (2011 – 2014), or that catchability is not constant from year to year.

After evaluating three methods using seasonal survey data from the AARZ, the cir method was found to produce the most reliable estimates of exploitation. Because these methods are predicated on estimating exploitation in order to then calculate abundance, the variance of the estimated exploitation will directly affect the variance of the estimated abundance. Therefore, when considering applying *seasonal* methods to other locations in the Gulf of Mexico, we recommend using the cir method due to issues with the ir method's constant catchability assumption.

Based on the exploitation rates calculated via the cir method, local abundance of both legal and sublegal fish can be calculated based on known removals (Table 1). Given that almost all

recreational fishing in Alabama federal waters is done on artificial reef platforms, MRIP data for the whole state were used to calculate abundance. In the future, it would be possible (and indeed, perhaps preferable) to use alternative data sources (e.g., iSnapper, LA Creel, mandatory reporting to Alabama MRD). The high level of variability inherent in MRIP removals data increased the variability around the abundance estimates quite a bit (Table 1). In order to use this seasonal approach to estimate abundance, more precise removals estimates would be desired.

Advantages/disadvantages of within day versus seasonal estimates

The advantage of using the seasonal cir estimator is that one can make use of the considerable depletion of the red snapper population by sport fishers. This is important because the precision of estimates depends on the degree of depletion and it takes effort on the part of research scientists to effect local depletion. The disadvantage of using the seasonal cir estimator is that it relies on an estimate of total removals from angler surveys. The reported cv for MRIP estimates of red snapper harvest are high, thus dissipating some of the benefit of having anglers do the depletion. Also, the use of the seasonal cir estimator requires a post-season field program. The effort allocated to the post-season sampling could be reallocated to pre-season sampling to increase the sample size for within-day ir estimation.

The advantage of using the within-day ir estimator is that it generally produces more precise estimates than the cir method because it makes stronger assumptions about catchability being constant. By restricting the data collection to one day (per site) it is likely that the catchability won't change much during the day.

Of course, comparing the results of two separate methods for assessing population abundance is advisable for validation. Every method relies on a set of assumptions which can be difficult to assess. Agreement among methods thus gives increased confidence while disagreement can point to possible problems. In particular, the NMFS assessment of red snapper relies on the catch at age matrix which is determined in part by recreational harvest. Thus, comparing the seasonal cir estimates with the within-day ir estimates, both in terms of magnitude and variability, may give an indication of the reliability of the estimates of recreational harvest.

Effect of sample size on precision

The precision of the abundance estimates for habitat 1 from the seasonal cir estimator are in large part tied to the imprecise MRIP removal data. We evaluated the effects of changing the sample size using the pilot data in the AARZ. We chose to simulate altering the sample size using data from a single year, 2013, for simplicity. In 2013, 36 stations were sampled on artificial structure for both the pre- and post-season surveys. At each station there were three sets fished simultaneously, with each of the three backbones having a designated hook size, and with each backbone having 10 gangions. In practice what this means is that at a single station, there is a maximum of 30 fish (10 gangions times three hook sizes) that could be caught and measured, and thus during a single survey there was a maximum of 1,080 fish (30 gangions at 36 stations) that could be caught. It's rare that all

hooks are saturated at a station, so there are many instances where there are ‘zero’ fish that were caught on a particular gangion in the data.

We investigated the effects on precision if a fewer or greater number of stations had been fished. In order to simulate the effects of altering the survey effort, first we sampled the stations with replacement in order to obtain the desired target level of effort. Then the fish captured on individual sets were sampled with replacement 10 times (to match the level of effort of 10 gangions per set). These data, which include both fish that were measured as well as the hooks that came back with no fish, now constitute a single sample. These data can be analyzed via the cir method described above (which includes the alterations for growth, natural mortality, and selectivity). Then the sampling procedure was repeated 100 times to generate 100 estimates of exploitation, abundance, and variances of exploitation and abundance. This was fairly computationally intensive, otherwise more repetitions would have been computed. The simulation aspect of this work was to generate estimates of exploitation with different variances. Then, we used the MRIP removals estimate and variance to deterministically calculate the abundance estimate and variance. For this work we did not alter the variance around the removals to simulate what would happen if these data were more or less precise.

As expected, coefficients of variation for exploitation, and therefore for abundance, decreased with increasing effort, and vice versa (Table 4). However, it appears as though the CVs around abundance are still heavily influenced by the high variability in the removals data at simulated higher levels of effort. In other words, even if sampling effort is quite large and therefore exploitation is known precisely, the resulting abundance estimates can still have high variance due to the variance of the removals data. This simulation is not intended to provide guidance on how much effort should be placed at other hotspots or type 1 habitats, but instead showcases the value of obtaining accurate and precise removals data.

Importance of type 1 habitat

One of the major issues in calculating red snapper abundance in the entirety of the Gulf is the sheer magnitude of the area. The current proposal outlines a multi-tiered strategy that will effectively be able to sample all habitat types and generate values of absolute abundance (Fig 5). That said, we have experience with and have tested our methods for type 1 habitat and have found them to be sound, and therefore think they can be applied to other hotspots. Given that these relatively small areas support the highest amounts of recreational fishing, it is perhaps of most use and importance to come up with local abundance estimates for type 1 habitats. We compared the estimates of abundance of legal size red snapper in the AARZ (from Table 1) with the Gulf-wide estimates of abundance from the NMFS stock assessment and estimated the fraction of legal size snapper that is within the AARZ was 16.4%, 17.9% and 15.7% for 2011, 2012 and 2013, respectively. The bulk of the catch comes from type 1 habitat in the Gulf, and much of the fished population is also within this habitat. It is for this reason that type 1 habitat deserves appreciable attention.

Importance of type 3 habitat

Fishing on natural reefs in Alabama (type 2 habitat) produced catch rates that were about 7 timeslower than on artificial reefs (see description of methods in Appendix A). This corroborates the recent predictive model for relative abundance (Karnauskas et al. in press), which estimates that red snapper densities on artificial structures could be 18-24 times as high as on natural habitats. Densities of red snapper on featureless bottom (type 3 habitat) are expected to be much lower than in type 2 habitat. Despite this, type 3 habitat is believed to contribute substantially to the population abundance due to the sheer magnitude of the area of type 3 habitat.

Consequently, we've developed a fourth-tier, "fail-safe" strategy for estimating relative abundance in type 3 habitat using a gear type common to all four strategies: bandit gear. Tier four is predicated on the assumption inherent in every stock assessment (and indeed the recent approach used by Karnauskas et al. in press), which is that catch-per-unit-effort is proportional to population abundance. Ideally, will estimate absolute abundance in type 3 habitat using the removal estimator approach described above; however, given the expanse (and thus relative importance) of type 3 habitats, our fourth-tier approach ensures that if sampling with bottom longlines is insufficient to apply the removal estimator, we will have catch-per-unit-effort generated from the bandit gear, a sampling technique common across all habitat types.

Recommendations and Conclusions

Determination of habitat maps should be done as an expert consultation. Errors in the assignment of sites to habitats does not induce bias but does increase variance. Thus, it makes sense to solicit expert and local opinion about habitat usage to refine maps. Whatever study design is selected, there will be a need for ancillary studies and pilot studies to evaluate options and establish proof of concept. These studies should better define habitat maps, evaluate calibration factors, determine allocations of sampling effort to strata, and determine necessary sample sizes. For cir/ir/removal estimation, the following ancillary and pilot studies are advisable:

Trial of field procedures in habitat type 3, to see what kind of catch rates can be obtained with bottom longlines, ROV cameras and bandit (vertical longline) gear. Trials are needed to determine optimal size of study sites, and number of bottom longline or bandit sets needed to achieve adequate depletion and adequate precision. Trial of field procedures in habitat type 2 to determine a workable patch size and to determine the amount of sampling effort to achieve adequate precision. In particular, the tradeoff between more intensive sampling of a site and visiting more sites should be determined. The possibility of developing calibration factors for habitat types 2 and 3 so that a survey of catch rates can be converted into estimates of absolute abundance should be investigated further. Detailed exploration of the most appropriate sources of recreational effort (e.g., MRIP vs. state-specific surveys).

For artificial structures (first habitat type), the cir and ir methods are recommended. The cir estimator is used with the surveys conducted prior to and after the sport fishery to take advantage of the sizeable depletion of legal-size animals due to the fishery. For the ir method, the surveys and depletion are completed by research scientists within a day to ensure that survey catchability does not change appreciably between surveys.

For known natural reefs (second habitat type), the ir method with surveys and depletion completed within a day is recommended because exploitation rate on these reefs is believed to be quite low due to lower abundance compared to artificial reefs; consequently, we cannot rely on sport fishers to deplete the populations in these areas appreciably.

The abundance of red snapper on a sample of habitat type 2 (i.e., natural, low relief reefs) was surveyed by this project and abundance was found to be much lower than what has been observed on artificial reefs, in accordance with expectations and general knowledge about red snapper distribution. The ir method has not been tested in low density areas so a pilot study is advisable.

For areas of featureless bottom and unknown bottom type (habitat type 3), it is recommended that a random sample of geographic locations be sampled by repeatedly setting bottom longlines in conjunction with deploying vertical longline (bandit) gear at the selected locations; population size at these locations will then be estimated using a removal estimator and possibly an index calibration method. A pilot study is advisable to verify the viability of this approach. As proof of concept, the cir and ir methods have been tried for 4 years in the Alabama Artificial reef zone (first habitat type) to good effect.

Any method for quantifying abundance based on indices (i.e., counts) will need calibration factors. And, all methods are predicated on a set of assumptions being met. Therefore, careful consideration should be given to using multiple, complementary methods such that the methods contribute to the generation of calibration factors and afford opportunities to investigate whether assumptions are being met. The proposed structure to the field sampling lends itself well to the idea of using multiple, complementary methods (mark recapture, visual surveys, etc.), and integrated models.

Tables and Figures

Table 1. Preliminary estimates of exploitation and abundance of legal (A) and sublegal (B) sizefish in the AARZ from 2011 – 2014 using the seasonal CIR method. Sublegal-size fish are defined as between 20 – 40.6 cm total length, and legal-size fish are larger than 40.6 cm total length. Exploitation was calculated using survey data with corrections for gear selectivity, natural mortality, and growth. Abundance estimates calculated using the exploitation rate and MRIP/TPWD removals data (with SEFSC corrections) from 2011 – 2013.

A	Exploitation of Legal-Size Fish within AARZ			Abundance of Legal-Size Fish within AARZ		
	Year	Mean	95% CI	CV	Mean (million lbs)	95% CI (million lbs)
2011	0.56	(0.45, 0.67)	0.10	6.32	(0.12, 11.4)	0.41
2012	0.39	(0.26, 0.52)	0.17	6.83	(0.02, 15.2)	0.56
2013	0.67	(0.60, 0.74)	0.05	6.21	(3.67, 8.75)	0.21
2014	0.47	(0.37, 0.58)	0.11	4.39	(2.25, 6.53)	0.25

B	Abundance of Sublegal-Size Fish within AARZ		
Year	Mean (million lbs)	95% CI (million lbs)	CV
2011	0.71	(0.004, 1.42)	0.51
2012	0.93	(-0.003, 2.24)	0.64
2013	1.10	(0.53, 1.67)	0.26
2014	0.47	(0.03, 0.92)	0.47

Table 2. Exploitation of legal size fish in the Alabama Artificial Reef Zone. Comparison of traditional seasonal cir and ir to calculate exploitation. A negative (nonsensical) estimate of exploitation rate in the ir method arises when the post-season catch rate is higher than the pre-season.

Year	Change in Ratio			Traditional Index Removal		
	Mean	95% CI	CV	Mean	95% CI	CV
2011	0.56	(0.45, 0.67)	0.10	0.16	(-0.27, 0.61)	1.35
2012	0.39	(0.26, 0.52)	0.17	-0.09	(-0.55, 0.37)	2.49
2013	0.67	(0.60, 0.74)	0.05	0.32	(0.003, 0.65)	0.50
2014	0.47	(0.37, 0.58)	0.11	0.34	(0.11, 0.57)	0.33

Table 3. Estimated seasonal catchabilities as evidence of evaluation of the changing catchabilityir model (Ihde et al. 2008); estimated abundances not shown to avoid confusion. CV calculated via SD/mean; in other words, values in the table are not percentages.

	Estimated Mean	95% CI	CV
$K_{\$J8}$	0.028	(-1.09, 1.15)	19.8
$K_{\$5L<}$	0.018	(-0.53, 0.56)	14.8

Table 4. Simulation of how altered sampling effort affects variability of exploitation and abundance estimates for seasonal cir analysis. The formulation of sampling effort is relative to the effort expended in the 2013 survey in the AARZ, so a sampling effort of 0.5 has half the number of stations that was used in 2013. For reference, the effort in 2013 was 34 stations (with 3 sets of 10 gangions per station) in both the pre- and post-season surveys. Note that the sampling effort labeled ‘1’ was not bootstrapped, as it represents the control or standard level of effort.

Sampling Effort (Relative to 2013 Survey)	Exploitation of Legal Fish			Abundance of Legal Fish		
	Mean	95% CI	CV	Mean (million lbs)	95% CI (million lbs)	CV
0.25	0.63	(0.22, 1.04)	0.33	6.52	(1.28, 11.76)	0.41
0.5	0.65	(0.53, 0.77)	0.09	6.42	(3.27, 9.57)	0.25
1	0.67	(0.60, 0.74)	0.05	6.22	(3.67, 8.75)	0.21
1.5	0.66	(0.61, 0.72)	0.04	6.32	(3.84, 8.80)	0.20
2	0.66	(0.62, 0.70)	0.03	6.32	(4.09, 8.55)	0.18

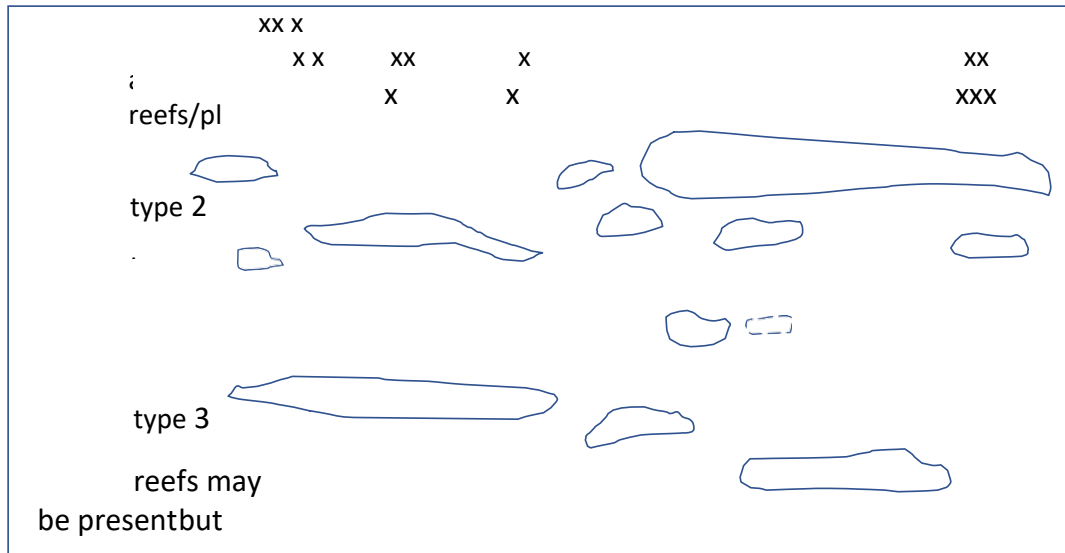


Figure 1. Diagram of a region (e.g., state) composed of three types of habitat. Habitat type 1 consists of a list of reefs, platforms and known hot spots of red snapper abundance, denoted by x's. A random sample can be drawn of these sites. Habitat type 2 consists of known (or suspected) natural, low-relief reefs. For logistical reasons, some of these reefs will need to be divided into sampling segments. A random sample of reefs from this habitat type can be selected. Habitat type 3 consists of the remaining areas in the Gulf. This habitat type contains either featureless bottom or unsurveyed bottom. As there are no discrete units to sample, the habitat type 3 must be sampled by selecting random geographic coordinates.

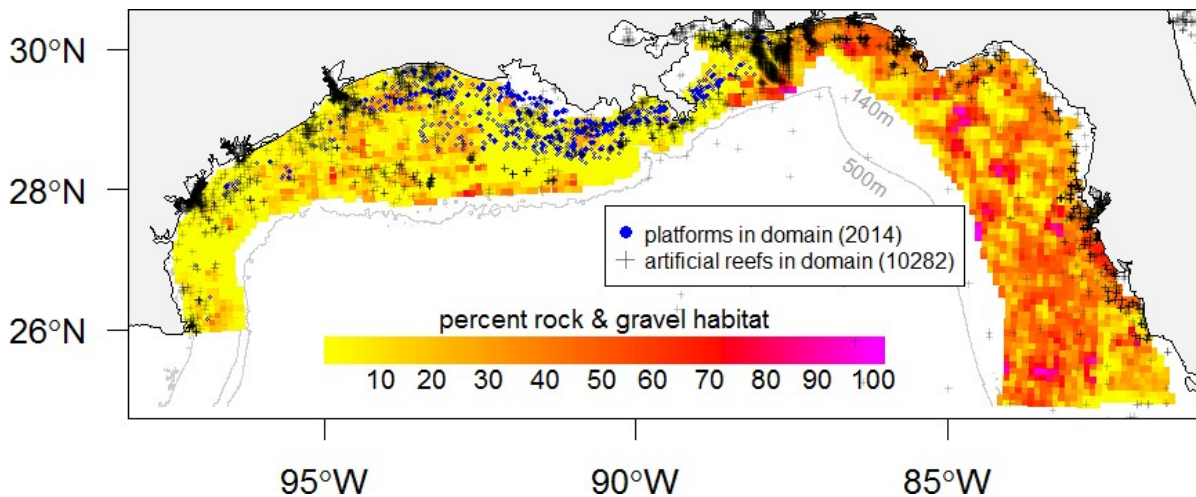


Figure 2. Habitat features map for the Gulf of Mexico from Karnauskas et al. (in press). Warm colors refer to natural habitat, and is classified as a percentage of rock and gravel. Blue dots refer to oil platforms, and black crosses represent artificial reefs. Expert opinion of scientists, stakeholders and other interested parties can be used to define habitat type zones (1 to 3) in the Gulf based on this and the next figure.

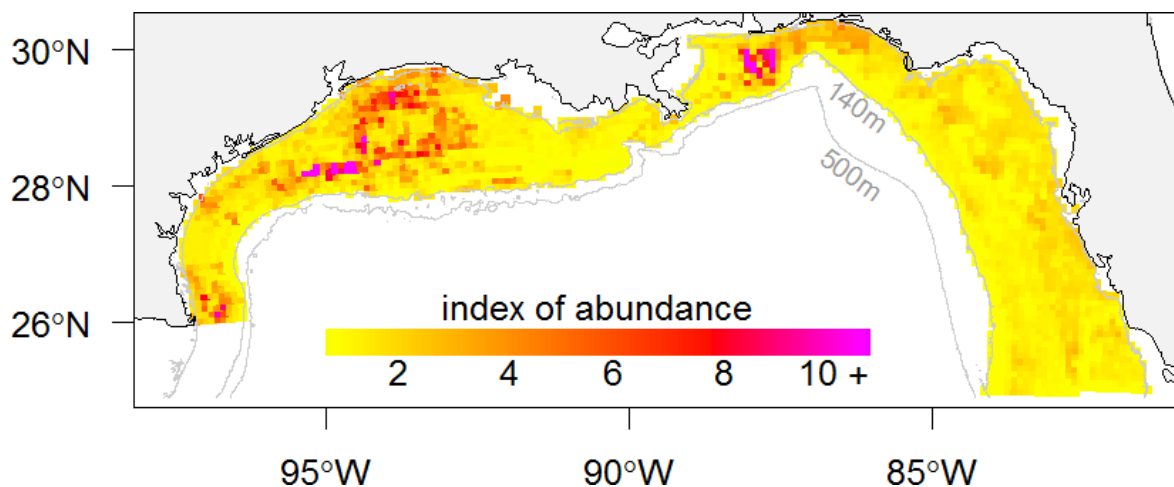


Figure 3. Predicted relative abundance of red snapper from Karnauskas et al. (in press). The predictive model includes the influence of artificial structures.

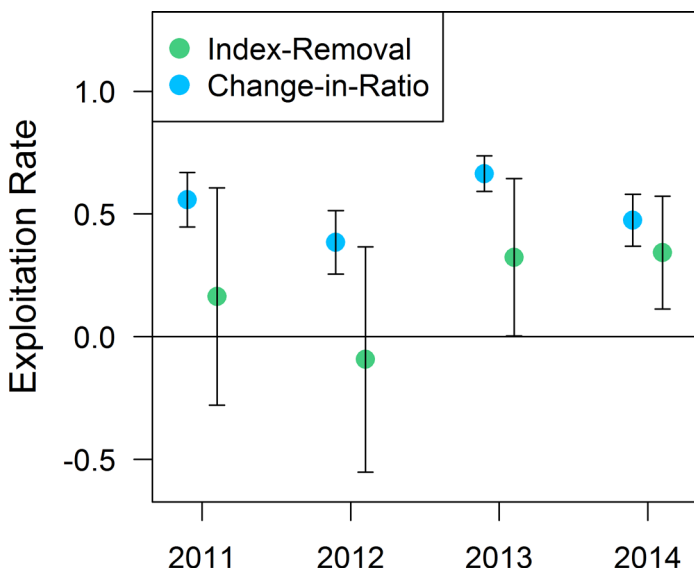


Figure 4. Local exploitation rate within the Alabama Artificial Reef Zone, as calculated via the index removal (green) and change in ratio (blue) methods. The index removal estimates show greater variability from year to year, have much higher estimated variances, and the estimate for 2012 is not feasible.

HABITAT TYPE 1

Artificial reef structures, platforms and other known areas of high fish density

HABITAT TYPE 2

Known, natural, low relief reefs

HABITAT TYPE 3

Featureless bottom and areas of unknown bottom type

TIER 1: Preferred
(absolute abundance)

Change in ratio (cir)
Approach: before/after season bandit gear sampling through scientific surveys
Provides: site-specific abundance, catchability & density estimates

TIER 2: Secondary
(absolute abundance)

Index removal (ir)
Approach: scientific depletion surveys with bandit gear & camera
Provides: site-specific abundance, catchability & density estimates

Index removal (ir)
Approach: scientific depletion surveys with bandit gear & camera
Provides: site-specific abundance, catchability & density estimates

TIER 3: Tertiary
(absolute abundance)

Removal estimator
Approach: scientific depletion with multiple sets of bandit gear
Provides: site-specific abundance, catchability & density estimates

Removal estimator
Approach: multiple bottom longline & bandit surveys
Provides: site-specific abundance, catchability & density estimates

Removal estimator
Approach: multiple bottom longline & bandit surveys
Provides: site-specific abundance, catchability & density estimates

TIER 4: "Fail-safe"
(relative abundance)

Relative abundance
Approach: bandit gear surveys
Provides: catch-per-unit-effort, used to estimate abundance from catchability from Tiers 1, 2 and 3

Relative abundance
Approach: bandit gear surveys
Provides: catch-per-unit-effort, used to estimate abundance from catchability from Tiers 1, 2 and 3

Relative abundance
Approach: bandit gear surveys
Provides: catch-per-unit-effort, used to estimate abundance from catchability from Tiers 1, 2 and 3

Figure 5: Schematic depiction of our multi-tiered approach for estimating absolute abundance of age 2+ red snapper across three habitat types in the US Gulf of Mexico. Alternatives are listed in order of preference, with Tier 1 (preferred) to Tier 4 (“fail-safe”).

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Red Snapper CIR and IR project NATURAL REEF SURVEY RESULTS

September 20, 21, 26, 27, 29, and 30, 2016

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Vertical Longline Survey

F/V Escape

North-Central Gulf of Mexico

SURVEY PERIOD: September 20, 21, 26, 27, 29, and 30, 2016

AREA OF OPERATION: The north-central Gulf of Mexico (Figure A1).

MATERIALS AND METHODS:

A total of 54 natural structure stations were randomly selected within the deep depthstrata (180-360ft) of the Alabama Artificial Reef Permit Zone. The fishing vessel *Escape* was outfitted with three vertical longline reels, loaded with 167 m of 400 lb test monofilament that terminated in a 6.5 meter monofilament backbone. At the bottom of each line was a 4 kg weight. A mono backbone (300 lb test) was assigned a hook size (8, 11, and 15) and attached to a mainline via a three way (2/0) snap swivel. Each gangion (100 lb. test, twisted) was 45 cm in length with its 10 assigned circle hooks and all three backbones were fished on site to address hook selectivity. Gangions were spaced equally (60 cm apart) along the 6.5 meter length. All hooks were baited with Atlantic mackerel (*Scomber scombrus*). Soak time was 5 minutes. The line remained attached to the vessel for the 5 min soak while the boat was held on station. Fish order (1 - 10 = deepest to shallowest hook placement along the backbone), hook size, species identification, condition, size (standard, fork, and stretched total lengths), and weights as well as station information (location, bottom type, depth, time of day) were recorded. All fish were retained and processed for life history studies.

RESULTS:

Fifty-four (n=162 sets) vertical longline stations were completed over 6 sea days. One hundred seventy-six fish were caught, representing eight species; only 40% of total catch were Red Snapper (Table 1). Red Porgy were the second most abundant (28%) and Vermilion Snapper third most abundant fish species (19%). Species composition and station location data are included in the digital copy.

CRUISE PARTICIPANTS:

Name

Skipper Thierry (Vessel Captain) *F/V Escape*

Crystal Hightower (Chief Scientist) USA

Trey Spearman USA

Matt Jargowsky USA

Pearce Cooper USA

Brandy Malbrough USA

Courtney Buckley USA

Kyle Hafstad USA

Sarah White USA

Trish Vosburg USA

Lauren Still USA

Oliver Ho USA

Michelle Louie USA

Steven Stokes USA

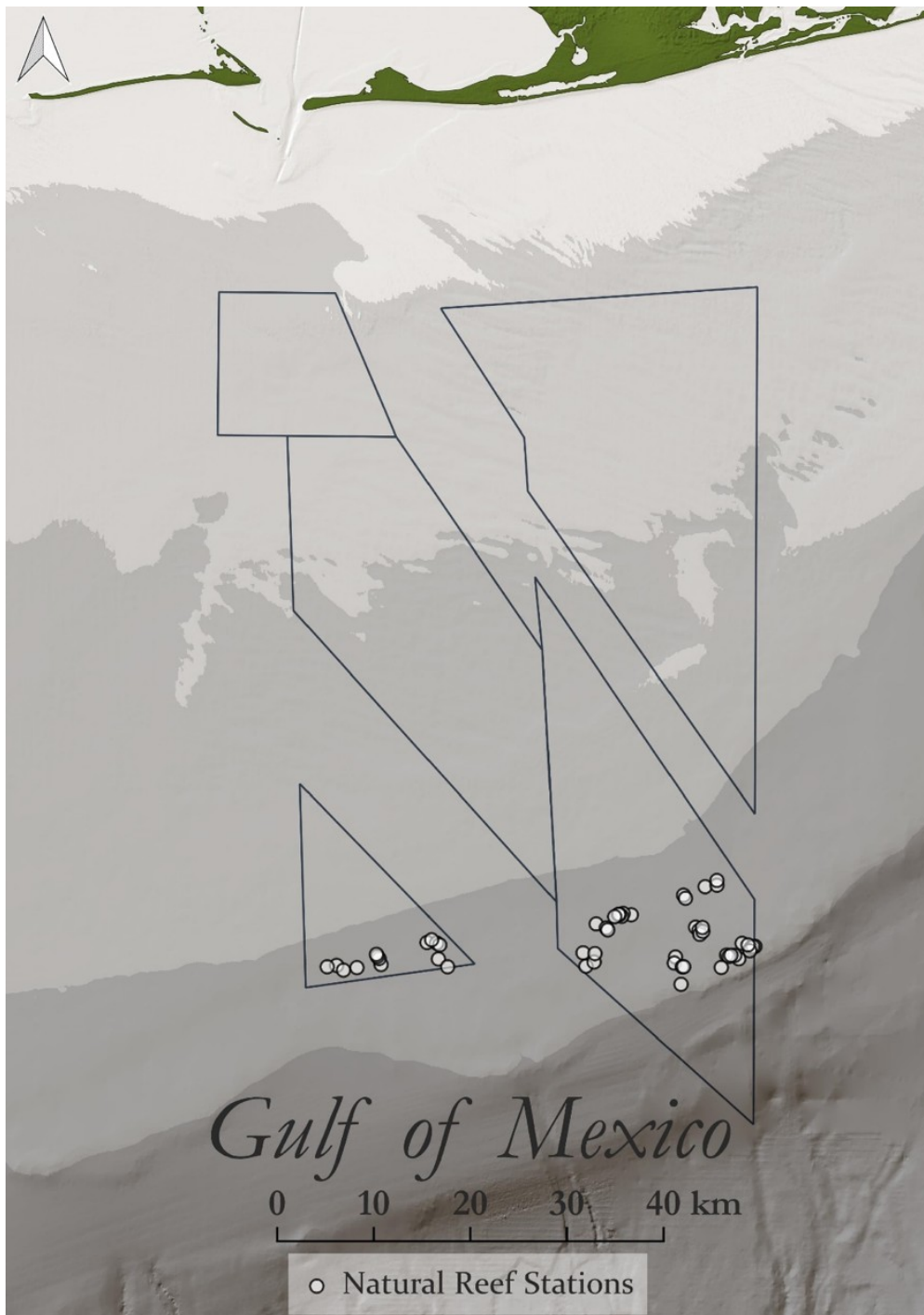


Figure A1: Survey area and locations for natural reef vertical longline surveys ES092016VL, ES092116VL, ES092616VL, ES092716VL, ES092916VL, and ES093016VL.

Table 1: Species composition and catch totals for natural reef vertical longline surveys ES092016VL, ES092116VL, ES092616VL, ES092716VL, ES092916VL, and ES093016VL.

Species	Captured
Red Snapper	70
Red Porgy	50
Vermilion Snapper	34
Silky Shark	11
Scamp	8
Red Grouper	1
Yellowedge Grouper	1
Gray Triggerfish	1
TOTAL	176

Table 2: Station identification and coordinates for natural reef vertical longline surveys ES092016VL, ES092116VL, ES092616VL, ES092716VL, ES092916VL, and ES093016VL.

STATION	STA_LAT	STA_LON
092016VL03	2927.785	-8742.437
092016VL04	2927.562	-8741.793
092016VL05	2927.454	-8741.697
092016VL06	2926.162	-8743.266
092016VL07	2925.416	-8743.082
092016VL08	2925.650	-8742.558
092016VL09	2926.126	-8742.508
092116VL01	2926.744	-8733.080
092116VL02	2926.575	-8732.257
092116VL03	2926.484	-8732.514
092116VL04	2926.562	-8732.385
092116VL05	2926.330	-8732.592
092116VL06	2926.656	-8732.704
092116VL07	2925.873	-8733.291
092116VL08	2926.068	-8733.553
092116VL09	2926.080	-8734.071
092116VL10	2926.003	-8733.924
092116VL11	2925.383	-8734.412
092116VL12	2926.070	-8733.791
092616VL01	2928.308	-8740.195
092616VL02	2928.380	-8740.716
092616VL03	2928.220	-8740.803
092616VL04	2928.323	-8740.952
092616VL05	2928.332	-8741.007
092616VL06	2928.199	-8741.255
092616VL07	2927.633	-8736.070
092616VL08	2927.449	-8735.853

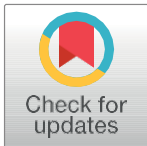
092616VL09	2927.233	-8735.793
092616VL10	2927.402	-8735.602
092616VL11	2927.618	-8735.632
092716VL01	2929.905	-8735.471
092716VL02	2929.992	-8734.711
092716VL03	2930.255	-8734.716
092716VL04	2929.421	-8736.849
092716VL05	2929.250	-8736.726
092716VL06	2925.957	-8737.345
092716VL07	2925.621	-8737.421
092716VL08	2925.412	-8736.913
092716VL09	2925.415	-8736.819
092716VL10	2924.429	-8736.981
092916VL01	2925.423	-8756.202
092916VL02	2925.763	-8756.334
092916VL03	2925.880	-8756.444
092916VL04	2925.995	-8756.503
092916VL05	2925.356	-8759.048
092916VL06	2925.274	-8759.650
092916VL07	2925.241	-8757.745
092916VL08	2925.092	-8758.605
093016VL01	2926.679	-8753.267
093016VL02	2926.654	-8752.650
093016VL03	2925.762	-8752.531
093016VL04	2925.327	-8751.939
093016VL05	2926.548	-8752.454
093016VL06	2926.834	-8752.975

Estimating reef fish size distributions with a mini remotely operated vehicle-integrated stereo camera system

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Abstract

We tested the efficacy of a stereo camera (SC) system adapted for use with a remotely operated vehicle (ROV) to estimate fish length distributions at reef sites in the northern Gulf of Mexico. A pool experiment was conducted to test the effect of distance (1, 2, 3 or 5 m), angle of incidence (AOI; 0° to 40° at 5° increments), and SC baseline distance (BD; BD1 = 406, BD2 = 610, and BD3 = 762 mm camera separation) on the accuracy and precision of fish model length (288, 552, or 890 mm fork length) estimates compared to a red laser scaler (RLS). A field experiment was then conducted at 20 reef sites with SCs positioned at BD1 to compare fish length distribution estimates between the SC and RLS systems under *in situ* conditions. In the pool experiment, mean percent errors were consistently within the *a priori* selected threshold of $\pm 5\%$ at AOIs $\leq 10^\circ$ at all distances with all four systems. However, SCs produced accurate estimates at AOIs up to 30° at all distances tested; 2–3 m was optimal. During reef site surveys, SCs collected 10.4 times as many length estimates from 4.3 times as many species compared to the RLS. Study results demonstrate that, compared to laser scalers, ROV-based SC systems can substantially increase the number of available fish length estimates by producing accurate length estimates at a wider range of target orientations while also enabling measurements from a greater portion of the cameras' field of view.

Introduction

Fish length data are commonly used to examine ecological processes [1–4] or assess the status of populations [5, 6] because length is strongly correlated with various life-history parameters [7]. Thus, size-composition data can provide critical demographic information in a variety of ecological modeling and stock assessment contexts including evaluations of predator-prey relationships [8–10], ontogenetic shifts in habitat use [11–13], sustainable harvest levels [14–16], or ecosystem-level effects [17–19]. Video-based methods for estimating length distributions can reduce sampling bias due to gear selectivity and provide an ethical improvement

OPEN ACCESS

Citation: Garner SB, Olsen AM, Caillouet R, Campbell MD, Patterson WF, III (2021) Estimating reef fish size distributions with a mini remotely operated vehicle-integrated stereo camera system. PLoS ONE 16(3): e0247985. <https://doi.org/10.1371/journal.pone.0247985>

Editor: Hudson Tercio Pinheiro, California Academy of Sciences, UNITED STATES

Received: July 23, 2020

Accepted: February 17, 2021

Published: March 4, 2021

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Data Availability Statement: All relevant data are within the manuscript and its Supporting information files.

Funding: Funding for this work was provided by a grant from Sea Grant and the National Marine Fisheries Service (#NA16OAR4170181) and the Florida Fish and Wildlife Research Institute (#FWC-16188) to WFP.

Competing interests: The authors have declared that no competing interests exist.

over traditional sampling methods when incidental mortality is common [20, 21]. Visually derived length estimates are particularly valuable when even minor handling-induced mortality is a concern (e.g., endangered species) or when fish reside in protected areas and are thus unavailable for collection [22].

Methods for collecting fish length data with stereo cameras (i.e., photogrammetry) developed rapidly in the 1980s and have since been adapted for a wide variety of scientific needs, including use in reef fish community surveys conducted with divers or remotely operated vehicles (ROVs) [23–28]. To collect viable length estimates with stereo cameras, paired cameras are fixed to a survey gear and positioned so that fields-of-view overlap; their respective orientations are then calibrated [23, 29–31]. Length estimates can be collected for a wide variety of objects in the environment, provided objects are viewed simultaneously by both cameras [28–30], which may increase sample size and accuracy compared to traditional methods that utilize visually estimated size classes [32–35] or laser scalers [36–39]. Compared to other common survey gears, ROVs can avoid duration limits, descend to deeper depths, and minimize fish attraction or avoidance behaviors associated with divers while also allowing density estimates unavailable with stationary camera systems.

Stereo-camera methods have recently been adapted to both working-class [27] and mini-class ROVs [28] and length measurements collected via commercially available software. However, Olsen and Westneat [40] recently developed an efficient stereo camera calibration and measurement software (StereoMorph [41]) freely available as a package within the R environment [42]. The goal of this study was to assess the efficacy of collecting robust length estimates for reef fish communities via small, low-cost (<\$500), hand-held action cameras in stereo integrated with a (micro) mini-class ROV (<10 kg; hereafter referred to simply as ROV) using the newly developed freeware. This was accomplished by first conducting a pool experiment to test the effects of distance, angle-of-incidence, and SC baseline distance (i.e., inter-camera distance, BD) on length estimates for fish models of known length compared to a traditional red laser scaler (RLS). Based on the results from the pool experiment, a single SC pair with optimal BD was selected for field trials by integrating it with the ROV that is also equipped with a red laser scaler. Field trials were then conducted to estimate fish length distributions at northern Gulf of Mexico reef sites to compare the amount and quality of length composition data produced by either system.

Materials and methods

No permits were necessary to conduct the study because field sites were not on privately owned land and no animals were collected.

Pool experiment

Underwater video was collected with high-definition GoPro Hero5 digital cameras ($n = 3$ pairs) that were enclosed in standard submersible GoPro camera housings (60 m depth rating) and mounted to a VideoRay Pro4 ROV (375 x 289 x 223 mm; 6.1 kg; 305 m depth rating) equipped with an RLS (2 parallel 5 mw 635 nm Class IIIa red lasers, 75 mm BD). All three camera pairs were mounted to a single aluminum bar (800 x 38 x 6 mm) via an adhesive mounting pad with two stainless-steel through-bolts and lock nuts. The aluminum bar with cameras was attached perpendicularly at the midpoint to a 76 x 6 mm flat aluminum plate via three stainless-steel through-bolts and lock nuts to form a T-shaped bar. The T-bar was then mounted to the ROV via manufacturer-drilled, threaded mounting holes on the underside of the ROV's sled. Camera pairs were mounted to the aluminum bar at BDs of 406 (BD1), 610 (BD2), or 762 mm (BD3), with the anterior-posterior axis of the ROV bisecting each SC pair (Fig 1).

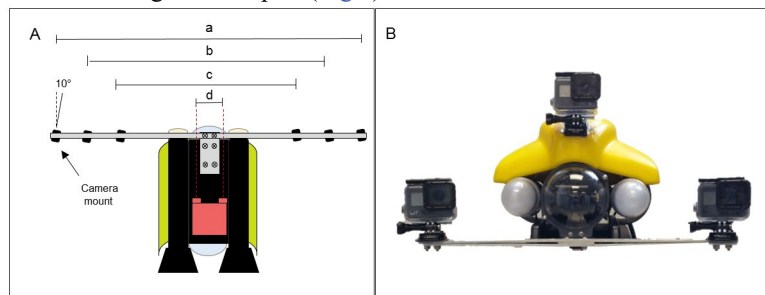


Fig 1. Stereo camera attachment and orientation. Schematic showing A) bottom view of the three stereo camera pairs mounted to the ROV at BDs of a) 762 mm (BD3), b) 610 mm (BD2), or c) 406 mm (BD1) and the d) RLS (75 mm BD) used to estimate model fish lengths in the pool experiment. Panel B indicates the front-view of the digital cameras (GoPro Hero5)

mounted to the ROV in stereo at BD1 for use in the field experiment. Black rectangles in panel A indicate the mounting positions of the six GoPro Hero5 model cameras (3 pairs), each of which had 10° inward rotation.

<https://doi.org/10.1371/journal.pone.0247985.g001>

Each camera case was mounted inward 10° (toe-in angle) toward the center line of the ROV and each camera was set to the narrow field-of-view (FOV; 49.1° vertical and 64.6° horizontal, 28mm focal length equivalent) at 1080p definition with a 60-fps frame rate. A black and white checkerboard printed on vinyl and mounted to a 610 x 457 mm Lexan polycarbonate sheet was used to calibrate all SCs. Each square of the checkerboard measured 63.7 x 63.7 mm, with a total of 7 horizontal and 5 vertical inner corners (Fig 2A and 2B). Immediately after initializing recording on each of the six cameras, a flashlight was triggered to allow post-processing synchronization between video cameras for extracting paired images. Following the methods of Delacy et al. [43], the checkerboard was positioned throughout the FOV by forming expanding concentric circles in a clockwise pattern at AOIs from 0 to 20° from perpendicular at distances of 1, 2, 3, and 5 m from the ROV. Paired images used for calibration were extracted at a representative number (n = 50) of AOI and distance combinations throughout the FOV. Checkerboard image pairs were taken simultaneously from all three SC pairs to minimize the

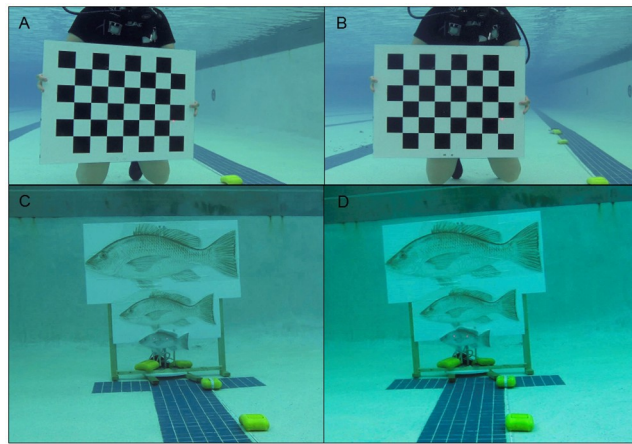


Fig 2. Paired images of calibration checkerboard and fish models. Example paired images from A) left or B) right view of calibration checkerboard (457 x 610 mm; 63.7 mm square size; 2 m distance) and C) left or D) right stereo camera view of fish display board indicating small (288 mm FL), medium (552 mm FL), or large (890 mm FL) paper red snapper models viewed at 3 m distance and perpendicular to the ROV. Laser points are visible on the smallest red snapper model in panels C and D.

<https://doi.org/10.1371/journal.pone.0247985.g002>

potential effect of image pair selection on measurement errors. Three replicate trials were conducted to account for differences in calibration quality.

Following completion of the calibration video, red snapper, *Lutjanus campechanus*, models of known length (288, 552, or 890 mm fork length, FL), were submerged in the pool and simultaneously filmed by each SC pair. Fish models were two-dimensional images printed on water-proof paper and adhered to a polyvinyl chloride (PVC) board affixed to a weighted wooden stand (Fig 2C and 2D). A circular disk was demarcated from 0 to 40° at 5° increments and mounted to a metal rod affixed to the back of the PVC board, which enabled the entire stand to be precisely positioned at each AOI (0, 5, 10, 15, 20, 25, 30, 35, or 40° from perpendicular). A transect tape was used to position the ROV at each designated distance (1, 2, 3, or 5 m) from the PVC board, and tile grids built into the floor of the pool allowed for perpendicular positioning of the ROV throughout the experiment. For each model distance and AOI, the ROV was positioned at a level height above the pool floor such that the RLS was visible along the lateral midline of each fish model (Fig 2C and 2D). Thus, up to 9 measurements were available for each fish model from each SC pair at each model distance and AOI combination from all three trials. However, all three models were not visible in every image at 1 m distance due to either the narrow FOV setting or the AOI.

Calibration and measurement videos were synchronized and still images extracted with

CyberLink's PowerDirector 15 video processing software. Camera calibration parameters and model length estimates were estimated with the StereoMorph package [40, 41] in R [42]. The StereoMorph package identifies common points (i.e., internal corners for checkerboard squares of known dimension) among paired images of checkerboard positions to estimate image distortion parameters and 6 optimal transformation parameters (3 translational and 3 rotational) to sequentially relate each set of checkerboard image pairs in 3-dimensional space based on minimizing calibration error. The transformation parameters are then used to estimate calibration coefficients (by direct linear transformation) to transform 2-dimensional image coordinates into 3-dimensional coordinates for collecting measurements [40, 41]. Each image pair was digitized and two landmarks were identified for each target: the anterior-most point of the premaxilla and the posterior-most point of the caudal fin at the midline (i.e., the tail fork). The distance between these two landmarks comprised the fork length (FL) estimate used for analyses.

Single still images used to estimate model lengths with the RLS were taken from an additional, forward-facing GoPro mounted atop the ROV at 0° tilt and 0° toe-in-angle (Fig 1B). Length estimates were generated for the RLS by dividing model fork length by laser inter-point distance as measured on screen when striking each model. This ratio was then multiplied by the RLS BD (75 mm) to generate each model length estimate. As laser position is fixed in parallel and calibration quality was not a concern with the RLS, length measurements were estimated with the RLS during only one trial producing up to 3 measurements for each model at each distance and AOI combination. As with the SCs, all three models were not always visible in every image at 1 m distance due to either the narrow FOV setting or the AOI.

Bias in each fish FL estimate was calculated as percent error (PE) with the equation:

$$PE_j = \left(\frac{\text{estimated length}_j - \text{actual length}_j}{\text{actual length}_j} \right) * 100 \quad (1)$$

where estimated length is the length estimate derived for each fish model i from each measurement system j . Percent error was used to identify measurement bias, with the threshold accuracy set *a priori* to $\pm 5\%$. However, the absolute value of the percent error estimate (absolute percent error, APE) was calculated as the response variable for comparing differences in bias among SC pairs and the RLS. The effect of each factor, and their interactions, on APE was tested in a generalized linear model (GLM) framework in R [42] by specifying a Gamma distribution with logistic (i.e., log-link) link function between the independent factors and the response variable (i.e., $x+1$ transformed APEs). The dispersion parameter was set equal to 1 (i.e., an exponential distribution) because the APE data were always positive with multiplicative errors. The factor effects modeled were the measurement system (4 levels: SC BD1, BD2, or BD3, or RLS), model FL (3 levels: 288, 552, or 890 mm FL), distance (m), and AOI (degrees) at an *a priori* significance level of $\alpha = 0.05$. The relative position (3 levels: centered or above or below midline) of each model in the camera's view was included as a covariate to test the effect of distortions or reduced calibration accuracy as models were measured farther from the center of view. Centered referred to the model at the center of view in each still image as indicated by laser points. For example, if lasers were visible at the midline of the small fish model, then the medium model (one position above center) would be classified as 1 and the large model would be given a classification of 2; with lasers present at the midline of the middle fish model, both small and large models would be classified as 1.

Stereo camera field trials

Fish communities were surveyed with an ROV integrated with both a SC and RLS system at 20 northern Gulf of Mexico (nGOM) reef sites to test the efficacy of each system for estimating fish lengths *in situ*. The SCs were positioned at BD1 during all reef site surveys because all three BDs provided length estimates that were below the accuracy threshold in the pool experiment, but BD2 and BD3 reduced ROV maneuverability in strong currents. The two GoPro Hero5 cameras mounted in stereo were set to the narrow field of view (49.1° vertical and 64.6° horizontal FOV, 28 mm focal length) with 1080-p resolution and 120-fps frame rate. Increased light intensity and much shorter video durations during field surveys enabled the use of higher frame rates reduces motion blur and enhances species identification during video processing in the laboratory.

The calibration checkerboard used in the pool experiment was attached to an aluminum pole and submerged alongside the research vessel to collect paired videos for calibrating SCs in the field. A small, handheld flashlight was triggered immediately prior to deploying the ROV to allow for video synchronization and image extraction during post-processing of videos in the laboratory. The ROV was then deployed just below the surface and several transects were flown perpendicular to the submerged checkerboard to enable extraction of paired images for calibration. Transects were flown by initially positioning the ROV perpendicular to the checkerboard at a distance of approximately 1 m. The ROV then was slowly flown in reverse until the checkerboard pattern was no longer clearly visible (i.e., >5 m).

The ROV was then flown slowly towards the checkerboard until it filled the camera FOV (<1 m). This process was repeated three times to ensure at least 50 paired checkerboard images were available for the calibration algorithm. The calibration procedure took ≤ 5 minutes to complete.

A single calibration was used for all successive reef site surveys for the duration of each camera pair's battery life ($n = 5-8$ reefs). Calibration videos were not collected prior to every survey because this would greatly reduce the number of sites sampled on a given day, and therigid cases with secure mounting hardware provided reliably fixed camera positions during normal operations. An object of known length (603 or 364 mm PVC pipe, or 275 mm PVC disc) was submerged at each site and filmed, and later measured during video processing, at the end of each survey as a means to validate the SCs calibrated positions throughout each survey and through the duration of each pair's battery life. A new calibration video was immediately collected following removal of cameras from their cases for battery replacement or to exchange memory cards.

Following SC calibration, the ROV was retrieved and the research vessel was positioned over a reef site. The flashlight was triggered onboard the vessel several times in simultaneous view of all three digital cameras immediately prior to each ROV survey. The ROV was then deployed to survey reef fish communities with a transect method at natural reefs, as described in Patterson et al. [44], and a point-count method adapted from Bohnsack and Bannerot [45] at artificial reefs, as described in Patterson et al. [38]. At natural reefs, four orthogonal 25-m long transects were flown from a central stationary point at a height of 1 m above the seafloor at a constant speed of ~ 1 kt. A weight (~ 7 kg) was attached to the ROV's tether via a short (~ 2 m) rope 25 m from the ROV and deployed at the GPS coordinates, which provided the central point for each of four cardinal direction survey transects [44]. The GoPro Hero5 placed atop the ROV (center camera; Fig 1B) was positioned at a 45° downward angle from the horizontal axis to identify and enumerate reef fishes during transects, as well as to estimate fish lengths with the red laser scaler. The center camera was set to the wide FOV (94.4° vertical and 122.6° horizontal FOV, 14 mm focal length) at 2.7k resolution and 120-fps frame rate. At artificial reefs, the GoPro Hero5 was positioned at 0° angle from the horizontal axis to observe reef fishes during 360° spins conducted on opposite sides (1 m above the seafloor), atop (1 m above the top of the reef structure), and above (10 m above the top of the reef structure) each reef structure. During spins on opposite sides of each reef, the ROV was positioned such that the artificial reef module occupied 20% of the ROV's FOV in real-time [38]. Regardless of reef type, one of the PVC objects described above was attached to the ROV's tether such that the object was suspended 1 m above the seabed. At the end of each survey (~ 10 minutes in duration), the deployed PVC object was located along the tether and a single perpendicular transect (with the same methods described above for collecting video of the calibration checkerboard) was flown with the ROV to collect 10 paired images.

Calibration and survey videos were processed in the laboratory. The GoPro Hero5 provided high-resolution video for fish identification and enumeration during processing. In addition to collecting fish community data, the center camera was also used to estimate whether fish were appropriately oriented for collecting length measurements. Results of the pool experiment indicated that targets struck with both lasers simultaneously at an AOI $\leq 10^\circ$ and a distance ≤ 5 m from the ROV could be measured with the RLS with a mean error within $\pm 5\%$. The SCs with BD1 were capable of accurately measuring targets (mean error within $\pm 5\%$) at an AOI $\leq 30^\circ$ and a distance ≤ 5 m from the ROV, but we chose a conservative AOI of $\leq 25^\circ$ to ensure that targets were oriented sufficiently for collecting accurate length measurements. Therefore, length was estimated for all reef fish that met the orientation criteria for either measurement system. Length was also estimated for the PVC object at each reef site and Eq 1 was utilized to compute the percent error in object length estimates as a means to validate the calibration file at each site.

Results

Pool experiment

Mean PE of target length estimates measured with the RLS was within the $\pm 5\%$ error threshold at AOIs $\leq 10^\circ$ at all distances, and up to 30° at 5 m but with greater variability (Fig 3; S1Data). Mean PE was also within the $\pm 5\%$ threshold for SCs at all three BDs for nearly all angles at all distances tested. At BD1 (Fig 3, column B), variability increased for measurements at the 5 m distance for small and medium fish models with increasing AOI. At BD2 (Fig 3, column C), variability in MPE estimates at 5 m increased slightly for small and medium targets at 5 m but was greater at 1 m with some AOIs exceeding $+5\%$. At BD3 (Fig 3, column D), all mean PE estimates were within the error threshold at all distances and AOIs except when the small model was measured at 40° at 5 m. All estimates of the large model were within the $\pm 5\%$ error threshold, regardless of the BD. However, the large target could not be measured at 1 m distance for most AOIs with SCs at either BD1 or BD3 due to the narrow FOV. The 552 mm red snapper model also could not be viewed at several AOIs at 1 m distance with BD3. Bias was

BD-specific, with BD1 having increasingly negative bias (underestimated lengths) with increasing AOI, BD2 having no consistent pattern, and BD3 having increasingly positive bias (overestimated lengths) with increasing AOI. Length estimates produced with the RLS showed a negative bias that increased in magnitude with AOI but not with distance.

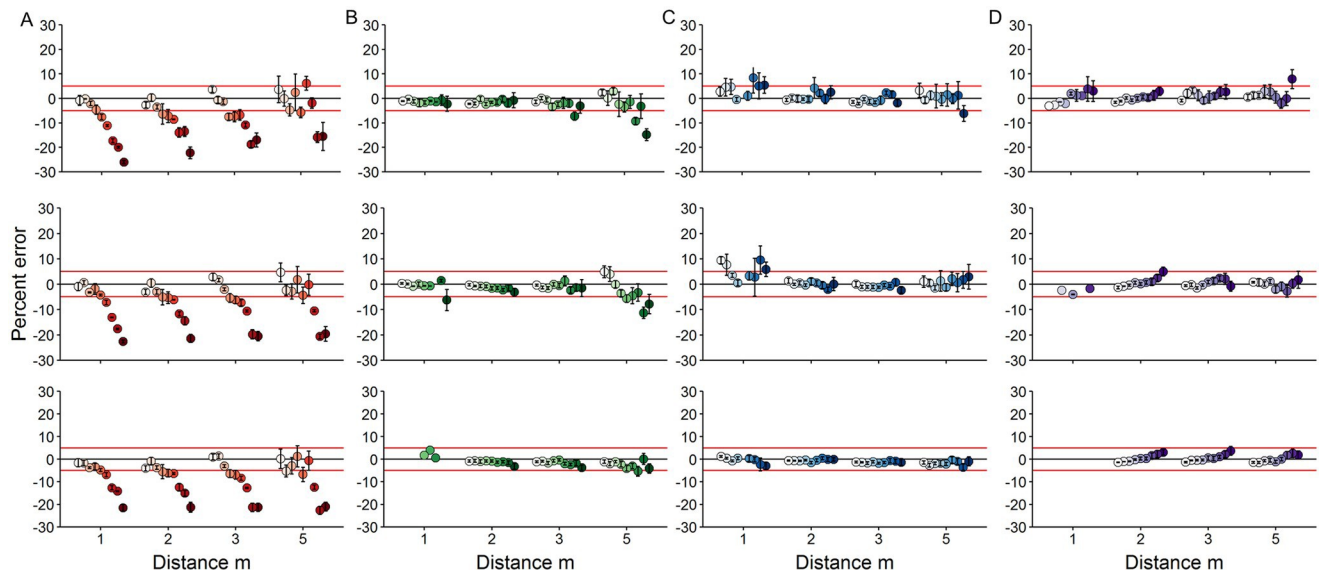


Fig 3. Pool experiment length estimate error plots. Mean percent error ($\pm 95\%$ CIs) in red snapper fork length estimates with increasing distance (m) and angle of incidence (AOI, degrees) as measured with the A) RLS (75 mm baseline, red gradient), or SCs at baseline distances of B) 406 mm (BD1, green gradient), C) 610 mm (BD2, blue gradient), or D) 762 mm (BD3, purple gradient) in the pool experiment. Filled circles ($n = 9$) from left to right in each panel indicate AOIs from 0 to 40° at 5° increments. Horizontal red lines indicate the $\pm 5\%$ error thresholds. Top, middle, and bottom rows in each column indicate measurements for small (288 mm FL), medium (552 mm FL), or large (890 mm FL) paper red snapper models, respectively. Distance and AOIs with length errors below 5% were deemed viable for collecting fish length measurements from ROV survey videos in the field experiment.

<https://doi.org/10.1371/journal.pone.0247985.g003>

Results of the Gamma GLM model for mean APE indicated that AOI was a significant main effect ($p < 0.001$) but that AOI interacted significantly with distance and the measurement system. Specifically, the $BD1 * Distance * AOI$ (coefficient = 0.015; $p = 0.031$) and $BD3 * Distance * AOI$ (coefficient = 0.015; $p = 0.027$) interactions indicated SC systems significantly decreased APE with increasing AOI by distance compared to estimates from the RLS (the base level); the p -value for the interaction term $BD2 * Distance * AOI$ was $p = 0.066$ (coefficient = 0.012). Mean APE for the small model was not significantly different from length estimates for medium ($p = 0.980$) or large ($p = 0.653$) models, nor was error significantly different when a fish model was one ($p = 0.518$) or two ($p = 0.376$) positions above or below the center view of the camera. Regressions of fish model FL versus APE with fitted data from the Gamma GLM for each SC BD indicated the minimum estimable fish model FL was 194 mm for SCs at BD1 and 147 mm at BD2; all model lengths were estimable at BD3 (Fig 4).

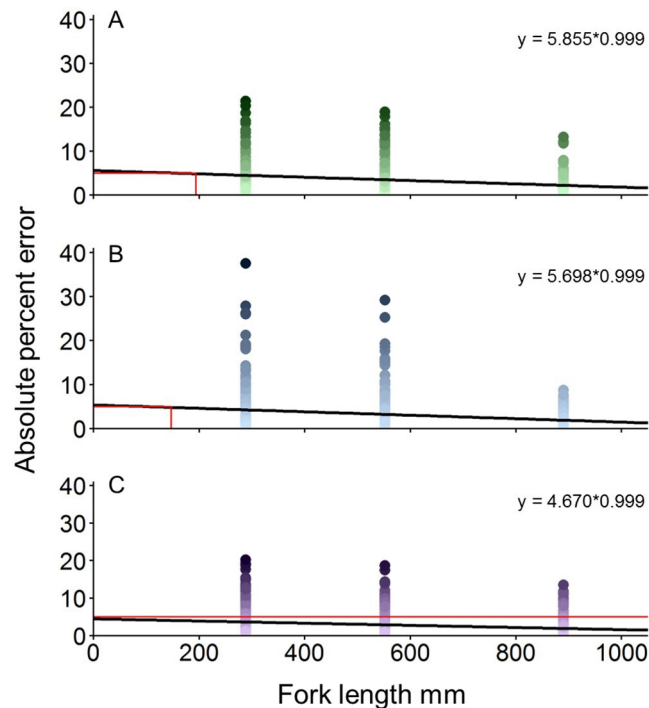


Fig 4. Pool experiment length estimate regression plots. Gamma GLM regressions of absolute percent error estimates versus model fork length for SCs at A) BD1 (406 mm), B) BD2 (610 mm), or C) BD3 (762 mm). Red lines indicate the length at which each regression intersects the *a priori* specified 5% error threshold, which occurs at 194mm in A and 147 mm in B. All length estimates were below the error threshold in C.

<https://doi.org/10.1371/journal.pone.0247985.g004>

In situ target estimates

Twenty reef sites were surveyed during field trials with the ROV integrated with the RLS and SC systems (See [S1 Data](#) for GPS locations). Survey sites consisted of unstructured hard bottom ($n = 8$), low-relief natural reef ($n = 10$), and artificial reefs ($n = 2$). PVC object lengths ($n = 189$) estimated at each of the 20 sites had a mean PE (\pm SE) of 0.76% ($\pm 0.21\%$) among all sites, and at no site did mean PE exceed the $\pm 5\%$ threshold ([Fig 5](#); [S1 Data](#)). In total, 3,249 individuals among 40 species were observed during ROV surveys ([Table 1](#)). An additional 175 individuals were unable to be identified due to small size, distance from camera, or visibility issues. Of the total number of individuals and species observed, 19 individuals among 4 species were scaled with the RLS, while 197 individuals among 17 species were measured with the BD1 SC system ([Fig 6](#); [S1 Data](#)). All individuals scaled with the RLS also were observed and measured with the SCs; red snapper were the most frequently measured with either method.

Red snapper mean fork length estimated with the SC system (393.1 ± 12.8) was similar to the mean estimated with the RLS (394.4 ± 22.0), but the SC system provided 47 additional length measurements compared to the RLS, including eight individuals < 300 mm and four individuals > 600 mm FL that could not be measured with the RLS because they were not struck simultaneously by both laser points at the correct orientation ($\leq 10^\circ$ from perpendicular to the ROV's center axis). No red snapper < 300 mm FL were scaled with the RLS and only one individual scaled exceeded 600 mm FL. The number of individual length measurements for species other than red snapper was 10x greater with the SC system than the RLS. Furthermore, four fishery species (i.e., gray snapper, *Lutjanus griseus*, lane snapper, *Lutjanus synagris*, tomtate, *Haemulon aurolineatum*, and vermilion snapper, *Rhomboplites aurorubens*) were measured with the SC system that were never or rarely scaled with the RLS ([Fig 6](#)). The SC system also produced length estimates for an additional 12 low-abundance species that were never measured with the RLS ([Table 1](#); [Fig 6](#)). These low-abundance species were predominantly comprised of small individuals ≤ 300 mm, which were rarely struck by both laser points simultaneously due to a combination of small body size and orientation

in the horizontal plane (see Fig 7 for example image). Larger individuals also are only infrequently scaled by lasers because the RLS requires they swim directly into the center-of-view.

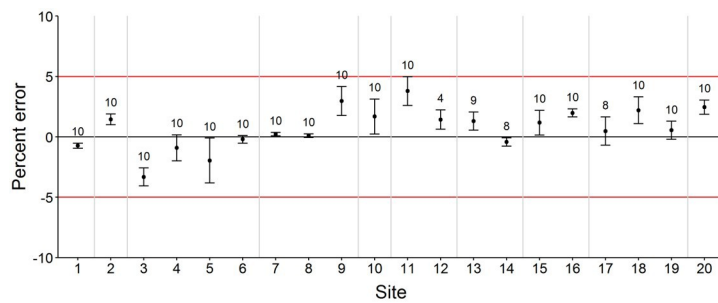


Fig 5. Field experiment length estimate error plot for objects of known length. Mean percent error estimated for objects of known length from paired still images obtained with SCs at BD1 (406 mm) mounted to an ROV deployed at reef sites ($n = 20$) in the northern Gulf of Mexico in 2018. Error bars indicate ± 1 standard error of the mean. The number of observations is shown above each estimate. Horizontal red lines indicate the $\pm 5\%$ error threshold. Object length estimates for sites 1–4 and 20 were for a 603.3 mm PVC pipe, sites 5–10 were for the diameter of a PVC disc (274.6 mm dia), and sites 11–19 were for a 364.0 mm PVC pipe. Vertical gray lines bracket length measurements estimated with each set of calibration parameters.

<https://doi.org/10.1371/journal.pone.0247985.g005>

Discussion

Study results indicate that a SC system integrated with a (micro) mini ROV is a viable means to collect robust length estimates for a wide variety of reef fishes. Compared to an RLS, SCs substantially increased the number of reef fish length estimates by enabling measurements to be taken for fishes at greater AOIs from a much greater portion of the video field-of-view. In addition to reducing length estimation error, SCs also reduced overall bias in reef fish length estimates by allowing measurement of fishes shorter than the RLS baseline (75 mm), which greatly increased the number of estimates taken for relatively small fishes (< 300 mm). Shorter SC BDs produced robust reef fish length estimates up to 30° , but the majority of reef fishes could be viewed at a variety of distances and angles, especially at 2 to 3 m where precision and accuracy were high for SCs at all three BDs tested in the pool experiment. Wider BDs may be necessary when a significant portion of target species occur at great distances (≤ 5 m), such as when studying highly mobile, shy, or rare species [24, 46, 47], species that exhibit avoidance behaviors [28, 47, 48], or relatively small individuals for which AOI is difficult to estimate reliably (e.g., Pomacentrids). The efficacy of stereo cameras for collecting robust length measurements have been demonstrated with a variety of stationary platforms [30], divers [49–52], and working-class ROVs [53], but studies utilizing SCs integrated with (micro) mini-class ROVs [28] to collect *in situ* length estimates via short BDs are limited [52].

Accuracy and precision estimates reported in our study are similar to results from previous works conducted in controlled enclosures with manually positioned cameras, which demonstrated the potential for small action cameras to collect highly accurate ($< 5\%$ error) length measurements at a range of distances and AOIs [43, 49, 54, 55]. Greater BDs provide more contrast between paired images and allow greater accuracy and precision in length estimates at greater angles of incidence [40, 50, 54]. Shortis and Harvey [50] concluded that a camera separation of 1.4 m was ideal for collecting fish measurements up to 5 m away (following a BD to target distance ratio of 3.6) based on frequently observed distances between divers and reef fishes. For BDs > 700 mm, estimates are highly accurate ($< 5\%$ error) at target distances ≤ 5 m and AOIs up to 40° while decreasing the BD decreases the distance and AOI available to collect viable length estimates [49, 54, 55]. Field experiments suggest that a smaller window of opportunity for collecting *in situ* length measurements is not problematic because mobile survey gears do not induce strong behavioral responses in many species and observe most individuals at a variety of distances and angles; baited gears draw in many carnivorous or scavenging species to relatively close distances from cameras [28, 46]. Although we did not include multiple calibration techniques or brands of video recording devices in our pool experiments, Boutros et al. [49] reported similarly high accuracy in target length estimates collected between 2D (checkerboard) and 3D (cube) calibration techniques [56, 57]. Letessier et al. [55] reported similar accuracy and precision to that reported in our study with earlier model GoPro cameras (Hero 2) and found no significant difference in length estimates between Sony and GoPro brand cameras between paired fish length estimates collected *in situ*.

Table 1. List of observed species and their composition.

Scientific name	Common name	Count	%Frequency
<i>Aluterus schoepfii</i>	orange filefish	2	<0.1
<i>Balistes caprisicus</i>	gray triggerfish	4	<0.1
<i>Calamus proridens</i>	littlehead porgy	11	<0.1
<i>Caranx crysos</i>	blue runner	1	<0.1
<i>Carcharhinus obscurus</i>	dusky shark	2	<0.1
<i>Centropristis ocyurus</i>	bank seabass	1	<0.1
<i>Chaetodon aya</i>	bank butterflyfish	1	<0.1
<i>Chaetodon ocellatus</i>	spotfin butterflyfish	12	<0.1
<i>Chromis enchrysur</i>	yellowtail reeffish	33	<0.1
<i>Chromis scotti</i>	purple reeffish	1	<0.1
<i>Diplectrum formosum</i>	sand perch	6	<0.1
<i>Epinephelus itajara</i>	goliath grouper	1	<0.1
<i>Epinephelus morio</i>	red grouper	3	<0.1
<i>Equetus lanceolatus</i>	jackknife fish	1	<0.1
<i>Pareques umbrosus</i>	cubbyu	2	<0.1
<i>Haemulon aurolineatum</i>	tomtate	786	0.2
<i>Halichoeres bivittatus</i>	slippery dick	3	<0.1
<i>Halichoeres poeyi</i>	blackear wrasse	1	<0.1
<i>Holacanthus bermudensis</i>	blue angelfish	9	<0.1
<i>Lactophrys quadricornis</i>	scrawled cowfish	1	<0.1
<i>Lutjanus campechanus</i>	red snapper	261	0.1
<i>Lutjanus griseus</i>	gray snapper	28	<0.1
<i>Lutjanus synagris</i>	lane snapper	116	<0.1
<i>Mycteroperca microlepis</i>	gag	2	<0.1
<i>Mycteroperca phenax</i>	scamp	1	<0.1
<i>Ogocephalus radiatus</i>	polkadot batfish	1	<0.1
<i>Pagrus pagrus</i>	red porgy	9	<0.1
<i>Pristigenys alta</i>	short bigeye	3	<0.1
<i>Ptereleotris calliura</i>	blue dartfish	49	<0.1
<i>Pterois volitans</i>	red lionfish	24	<0.1
<i>Raja texana</i>	roundel skate	1	<0.1
<i>Rhinobatos lentiginosus</i>	Atlantic guitarfish	1	<0.1
<i>Rhomboplites aurorubens</i>	vermillion snapper	1822	0.6
<i>Rypticus maculatus</i>	whitespotted soapfish	3	<0.1
<i>Seriola dumerili</i>	greater amberjack	20	<0.1
<i>Seriola rivoliana</i>	almaco jack	14	<0.1
<i>Sphoeroides spengleri</i>	bandtail puffer	1	<0.1
<i>Stegastes leucostictus</i>	beaugregory	5	<0.1
<i>Synodus intermedius</i>	sand diver	1	<0.1
<i>Xyrichtys novacula</i>	pearly razorfish	6	<0.1

Number of observations and relative frequency of fishes observed during ROV video surveys at 20 reef sites in the northern Gulf of Mexico in 2018.

<https://doi.org/10.1371/journal.pone.0247985.t001>

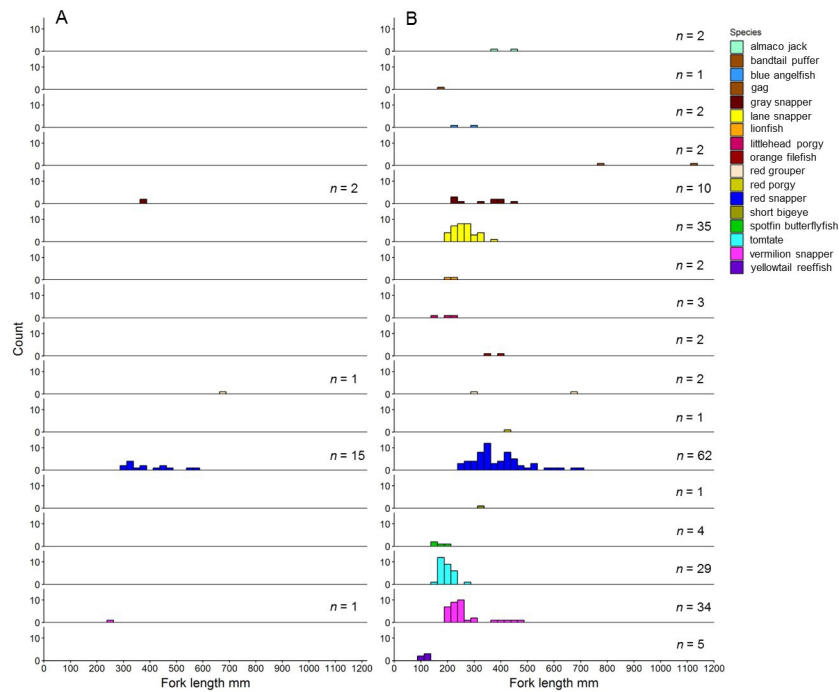


Fig 6. Field experiment frequency histograms. Frequency histograms (counts, 30 mm bins) of fork length estimates for 197 individuals among 17 species measured with a A) red laser scaler or B) stereo cameras with BD1 (406 mm) at reef sites ($n = 20$) surveyed in the northern Gulf of Mexico in 2018. The number of length estimates for each species is indicated on the right side of each panel.

<https://doi.org/10.1371/journal.pone.0247985.g006>

Stereo cameras integrated with mini ROVs provide several advantages compared to other methods for collecting fish length estimates. Unlike larger survey gears, mini ROVs are easily deployed from small vessels by a single person, which facilitates lower operating costs. Compared to divers, ROVs can access greater depths (>300 m) without any cumulative restrictions on dive duration or health risk. Unlike baited stationary camera systems, quantitative survey methods with mini ROVs allow the collection of density estimates by estimating the area sampled [38, 44]. However, Schramm et al. [28] recommends that stationary baited cameras should complement mobile gears when estimates of diversity are desirable because baited systems are likely to observe significantly more species.

Integrating ROV-based methods with stereo cameras and StereoMorph video analysis software provides a means to fully eliminate the need for divers and pool facilities because the 2-dimensional checkerboard needed to calibrate the SC systems can be easily submerged and positioned from the side of a vessel via an extendable aluminum pole. Attaching the checkerboard to an extendable pole provides a rapid (<5 min) and easily deployed field method to obtain new calibration coefficients should any unexpected movement occur in the fixed position of the SC system. In our experience, the careful handling required when deploying expensive ROV equipment along with slow flying speeds underwater virtually eliminate potential collisions that could necessitate recalibration. Instead, recalibration is nearly always associated with camera battery replacement, which typically occurs only once or twice per day due to the relatively short survey times (<10 min) required for each site. Letessier et al. [55] reported that removing GoPro cameras multiple times per day from the standard underwater housing for battery replacement had no meaningful effect on accuracy or precision of *in situ* length estimates. We simply chose to recalibrate after battery replacement as a precautionary measure because the minimal time required to collect additional calibration videos is short (<5 min). We also utilized objects of known length to verify measurement accuracy in successive survey videos for each calibration to maximize our confidence in collecting consistently accurate length estimates.

Small, lightweight, extended-life batteries (up to 24 hrs) with waterproof cases have recently become available for GoPro brand cameras that could reduce the number of daily calibrations to a single event to eliminate this potential source of error.

The checkerboard square size and corner number appropriate for successful SC calibrations depends upon the size of desired target observations and water conditions [40, 49]. Olsen and Westneat [40] recommend a checkerboard image at least 40 pixels wide with as many internal corners as possible to maximize calibration accuracy. More internal corners can increase calibration accuracy by providing more data points, particularly for correcting lens distortion.

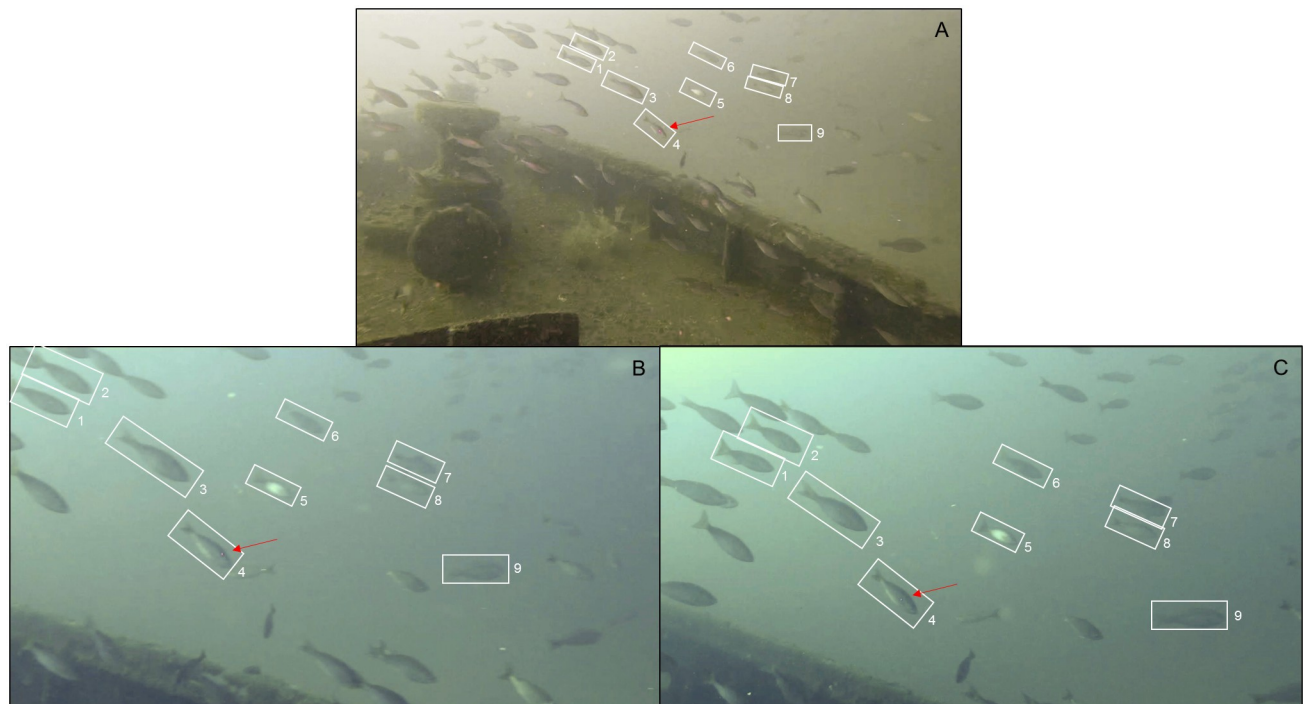


Fig 7. Example stereo camera vs red laser scaler sample availability. School of vermilion snapper passing in front of A) the center GoPro camera mounted atop the ROV, and the B) left and C) right stereo cameras during an artificial reef survey in the nGOM in 2018. Numbered white boxes indicate individuals whose lengths could be estimated with the SC system because they are entirely visible in both views simultaneously and are oriented 25° from perpendicular to the ROV's longitudinal axis based on their orientation in the horizontal plane in panel A. A single laser point is visible on individual 4 in all three images as indicated by the red arrow, which is insufficient to estimate length with the RLS.

<https://doi.org/10.1371/journal.pone.0247985.g007>

However, the checkerboard squares must also be large enough for corners to be detected at the expected target distances. Thus, smaller checkerboards may be better suited to calibrating measurements of smaller individuals at close range, while larger ones are more appropriate for larger individuals viewed at greater distances. Our checkerboard design was successfully detected in Stereomorph less often at distances of 5 m due to decreasing contrast between the black and white squares and interference from bubbles and drifting particles. Regardless, the overwhelming majority of fishes observed during ROV survey videos were between 1 and 5 m from the SC system.

The accuracy of length measurements collected with laser-based methods are ultimately limited by 1) the parallax effect, 2) laser separation distance, 3) size bias towards larger individuals, 4) the frequency of scaling events at appropriate AOIs, or 5) beam contact distortion [36, 38, 53, 58]. In a controlled pool experiment, Patterson et al. [38] estimated that an RLS with 100 mm baseline provided accurate (mean PE within $\pm 5\%$) length estimates of fish models at distances ≤ 2.5 m and AOIs $\leq 15^\circ$. Our estimates of measurement accuracy likely differed from Patterson et al. [38] due to differences in video capture technology and laser spacing (100 ver- sus 75 mm baselines).

Patterson et al. [38] utilized a VideoRay Pro3 mini ROV and captured still images directly from the ROV's internal camera, which had lower resolution than the digital GoPro Hero5 cameras utilized in our study.

With ROVs, especially mini-class ROVs, considerations that compete with image resolution and minimizing bias include payload, hydrodynamics, and maneuverability. The VideoRay Pro4 ROV produces 9.5 kg of thrust at a maximum speed of 2 m/s but can support only a relatively small payload without considerable reduction in hydrodynamic performance, especially when operating against a hydrodynamic current. Thus, the SC system, including waterproof cases and mounting bracket, could not greatly exceed the total ballast weight typically utilized with the ROV (1.5 kg) without necessitating additional specialized flotation. The camera and bracket design for the SC system with BD1 had a mass of only 0.7 kg, which enabled us to simply remove ROV ballast weights to offset that mass. The ROV's flotation block can be adapted or additional flotation can be affixed to the sled to offset the increased weight of heavier cameras or sturdier cases designed to withstand greater pressures at increased depth but will likely reduce ROV maneuverability.

Regarding camera settings, we recommend setting GoPro cameras to the narrow FOV to minimize barrel distortions [43, 52, 54, 59]. When ambient light is sufficient, we recommend maximizing the video resolution and/or frame rates of cameras used in field surveys to increase placement accuracy of landmark points to maximize measurement accuracy during image digitization. In our pool experiment, we noticed pixilation effects at 5 m distance that decreased our ability to accurately place digital landmarks for estimating fish model lengths. The importance of maximizing accuracy and precision is inversely related to fish size because a relatively small absolute errors will result in disproportionately greater percent errors when measuring smaller individuals. Higher frame rates can reduce motion blur as mobile fish swimthrough view, but inadequate lighting or rolling shutters can distort image quality of fast moving objects [60]. Higher resolution or frame rate settings will decrease camera battery life, which will necessitate more frequent recalibration. Ultimately, the capabilities of the ROV and specific research goals of the study will determine the appropriate SC design.

Conclusions

Our results clearly demonstrate the efficacy of SC systems integrated with (micro) mini ROVs for estimating length distributions of reef fish communities. The specific design used (e.g., camera models, video resolution, and BD) must be considered against the size and behavior of the species of interest, the hydrodynamic capabilities of the ROV, and goals of the study. We recommend using the greatest BD possible to maximize measurement accuracy and precision, but all three BDs we tested produced accurate fish length estimates over most distances and target AOIs encountered during field surveys of fish communities commonly observed at northern GOM reef sites. We were successful in calibrating SC systems using a checkerboard deployed from the side of a vessel, but weather conditions, turbidity, and fine-scale visual obstructions can increase the duration of video necessary to extract a sufficient number (~50) of checkerboard image pairs for successful camera calibration. Thus, the checkerboard dimensions and deployment methods should be considered for the expected sea conditions and vessel design used for collecting calibration videos. Regardless of the region or application of interest, integrating SC systems with stationary gear or mobile platforms like ROVs enables much greater length composition data to be collected than with an RLS. Video surveys that collect only relative abundance estimates can be coupled with SC systems to also provide length composition estimates for input into stock assessments as well as hypothesis tests in ecological studies of length-dependent factors.

Supporting information

S1 Data. Complete set of data and metadata underlying all reported findings. (XLSX)

Acknowledgments

We thank Miaya Glabach of the University of Florida Marine Fisheries Laboratory for providing diver support during the pool experiment and research technicians, Jordan Bajema and Jessica VanVaerenbergh

(University of Florida), for field support during reef surveys. We also gratefully acknowledge the captains and crews of participating charterboats and the R/V Hogarth for the invaluable assistance they provided in the field.

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IN REVIEW: Fisheries Research**Do Not Cite Without Author Permission****A multidisciplinary approach to estimating red snapper, *Lutjanus campechanus*, behavioral reaction to mobile camera and sonar sampling gears**

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Keywords: gear bias, acoustic telemetry, ROV, red snapper, Gulf of Mexico

Abstract

We examined the potential for Gulf of Mexico red snapper behavior to bias count estimates collected with a remotely operated vehicle (ROV), towed camera sled (TCS), towed acoustic sled (TAS), or a SCUBA diver at artificial reef sites. Large-scale responses were evaluated by examining depth, acceleration, or distance from reef data derived from high-resolution three-dimensional acoustic telemetry. Small-scale responses were evaluated by examining fish counts and 3-dimensional movements collected with digital video stereo cameras positioned on the seafloor. Responses to gear deployments were compared between 15-minute gear or diver deployment periods and the respective 15-minute acclimation period prior to each gear deployment. Mean red snapper counts estimated from stereo-camera video were higher for ROV(+0.6 fish) deployments, but fish counts were not significantly different than acclimation periods for the TCS or TAS gears or the diver. Results from 3D telemetry indicated red snapper distance to reefs, height off bottom, or acceleration were not significantly affected by any of the sampling gears or the presence of divers after positioning the fixed camera and sonar stands. Overall, results suggest red snapper behavior is relatively unaffected by mobile video or sonar gear, thus alleviating potential concerns of bias when estimating their abundance.

1. Introduction

Abundance estimates provide important information for single-species or ecosystems-based assessments of fish populations (Hutchings et al., 2010; Stuart-Smith et al., 2013; Edgar and Stuart-Smith, 2014; FAO, 2018). Stock assessments typically rely on a time series of catch and effort data to estimate the total population, with indices of abundance included when possible to track population trends with both fishery-dependent and -independent data (Chen et al., 2003; Haddon, 2010; Hutchings et al., 2010; Maunder and Punt, 2013). An absolute estimate of abundance is an alternative approach to assessing stock size when density estimates are available for all habitats occupied by the stock, the sampling window is appropriate for the scale of movement of individuals, and detectability is well estimated (Fréon, et al. 1993; Rivoirard et al., 2008; Keiter et al., 2017). Habitat-specific density estimates are then scaled up to the total areal extent of each strata provided that habitat-specific detectability and gear biases are known and the area surveyed is estimated reliably for each sample (Rivoirard et al., 2008; Marques et al., 2013; Keiter et al., 2017). The precision of the population estimate is then dependent upon the sample size relative to the variance of density estimates (Rivoirard et al., 2008; Ramsey et al., 2015; Keiter et al., 2017).

A species inhabiting multiple habitat types likely requires multiple sampling gears, each with potential biases that must be evaluated in order to provide robust density estimates (Watson et al., 2005; Schramm et al., 2020). Common survey gears include acoustic profilers (Kotwicki et al., 2013; Davison et al., 2015), digital video cameras (Koslow et al., 1995; Shortis and Harvey, 1998; Letessier et al., 2015; Schramm et al., 2020), or visual census techniques with divers (Bohnsack and Bannerot, 1986; Thompson and Mapstone, 1997; Schramm et al., 2020).

Acoustic profilers are best-suited for estimating fish abundance over large areas in simple habitats with low species-diversity (Lawson and Rose, 1999; Kotwicky et al., 2013; Davison et al., 2015); stationary camera systems are effective in sampling relatively small areas of complex habitat (Somerton and Gledhill, 2005; Watson et al., 2005; Schramm et al., 2020); towed cameras or remotely operated vehicles are effective for sampling either large or small areas of simple or complex habitat (Somerton and Gledhill, 2005; Schramm et al., 2020). Acoustic surveys may lose resolution over complex habitats with diverse fish communities (Lawson and Rose, 1999; Zenone et al., 2017); mobile gears (Somerton and Gledhill, 2005; Lorance and Trenkel, 2006; Stoner et al., 2008; Somerton et al., 2017) or divers (Brock, 1982; Cailliet et al., 1999; Edgar et al., 2004; Dickens et al., 2011) may elicit positive or negative behavioral responses; and estimating the area sampled with stationary cameras is problematic (Harvey et al., 2007; Langlois et al., 2010; Schramm et al., 2020).

Reef fish densities are especially difficult to estimate due to a myriad of factors influencing our ability to perceive their presence or number within a surveyed area. Reef fish communities are highly diverse including cryptic and shy species that take cover in crevices while large mobile predators can easily move beyond the range of visual detection. With visual or video methods in clear water, one can confidently assume that relatively large, non-cryptic species are fully detectable within the sampled area (MacNeil et al., 2008; Bozec et al., 2011; Stewart et al., 2017). However, gear deployments may induce behavioral avoidance or attraction effects that alter fish distribution at scales larger than the sampled area that are not detectable without secondary sampling gear (Fréon et al., 1993; Yule et al., 2007; Schramm et al., 2020). For example, carnivorous individuals evenly distributed over a large reef area may contract their distribution around a baited camera stand but may expand their distribution to avoid a rapidly approaching mobile sampling gear.

Here, our objective was to assess potential behavioral responses to mobile video and acoustic sampling gears commonly used to survey reef fishes in the northern Gulf of

Mexico. Our model species was red snapper, *Lutjanus campechanus*, due to its abundance in the system, its ecological and economic importance in the region, and the fact that research efforts were being developed to produce an estimate of absolute abundance of age-2+ in US waters of the Gulf of Mexico. Red snapper behavioral response to a mini remotely operated vehicle (ROV), atowed camera sled (TCS), and a towed acoustic sled (TAS), was assessed via high-resolution three-dimensional acoustic telemetry, as well as with epibenthic stereo cameras and an epibenthic acoustic profiler. The effect of mobile sampling gears on red snapper acceleration, distance from reef, or height off bottom was tested with telemetry data, and their effect on red snapper position relative to reef modules was examined with position data derived from stereo cameras. Lastly, fish counts derived from stationary stereo cameras or the acoustic profiler were utilized to test for differences when mobile gears were deployed versus acclimation periods when no gear was in the water. The cumulative data collected were utilized to produce a comprehensive assessment whether red snapper displayed positive (attraction) or negative (avoidance) behaviors relative to the ROV, TCS, or TAS gears. Results have implications for surveys designed to estimate red snapper absolute abundance in the Gulf of Mexico, as well as for assessing behavioral responses of other fish species to video or sonar sampling gears.

2. Material and methods

This study was conducted at a series of artificial reef sites (depth = 38-39 m) located approximately 35 nm SSE of Destin, FL from September through November 2019 (Fig. 1A). Artificial reefs were deployed by the Florida Fish and Wildlife Conservation Commission in 2003 and have persisted on the shelf since then (Dance et al., 2011; Lewis et al., 2020). Reefs were composed of 1 or 2 prefabricated concrete modules that were 1.83-3.1 m tall with volumes of 4.1-4.9 m³. The coordinates of these reefs were never published, thus

minimizing the likelihood of fishery removals of tagged individuals during the study.

1.1. Acoustic telemetry

Study reefs were surveyed with ROV in September 2019 to ensure red snapper aggregations were present prior to the deployment of the acoustic array. After identifying five reefs with sufficient red snapper abundance (>10 fish per site), a total of 70 Vemco (Bedford, Nova Scotia, Canada) VR2Tx acoustic receivers were deployed on September 25-26, 2019. Receivers were deployed 470 m apart in a 11.9 km² Vemco Positioning System (VPS) array such that all sampling reefs were located within the array and >500 m from the array perimeter (Fig. 1B).

Receiver spacing and overall VPS array design was intended to provide high-resolution red snapper geolocation estimates and to maximize the probability of acoustic tag transmissions being detected by at least three receivers under predominant environmental conditions based on previous studies within this region (Dahl et al., 2020; Bohaboy et al., 2020). All acoustic receivers had their internal synchronization transmitters set to 160 dB and were attached at the top of 2-m PVC support pipes with heavy duty UV stabilized nylon cable ties (250-lb tensile strength), with an additional paracord safety line (550-lb tensile strength) attached between each receiver and the cement base (~0.5 m diameter) which anchored the PVC support pipe. A high-density foam buoy was attached to the top of the PVC pipe with a 2-m long section of paracord to enable the vessel captain to accurately identify the GPS coordinates of each receiver via the vessel's acoustic echosounder (AIRMAR series) and chart plotter (Garmin GPSMAP series) for receiver recovery at the end of the experiments.

Red snapper (n = 50) were captured with hook and line at 5 study reefs (n = 10 fish per reef) on October 28-29, 2019 and tagged externally with Vemco V9AP acoustic tags

following the methods of Bohaboy et al. (2020). Acoustic tags were programmed to emit a 151 dB unique acoustic identification code (ID) at 69 kHz with a 30 second mean transmission interval (range 15 to 45 sec) for 21 days. In addition to unique ID codes, acoustic tags also transmitted acceleration ($m \cdot s^{-2}$) and pressure data, with the later utilized to estimate depth occupied by tagged fish. Tags were attached externally to minimize handling time, avoid surgery required for internal tagging, and facilitate quicker post-tagging acclimation (Bohaboy et al., 2020).

Following tagging, fish were attached to a descender device clamp onto their lower jaw, returned to depth, and released. The descender device was deployed with rod and reel and set to release fish at 4 atm, or at a depth of approximately 33 m. A GoPro (Hero5) digital camera in an underwater housing was attached to the line 1 m above (oriented downward toward the seabed) and another one 1 m below (oriented upward toward the sea surface) the descender device to observe fish behavior (e.g., swimming activity and orientation) and potential depredation events during release of tagged fish (Bohaboy et al., 2020).

1.2. Red Snapper Behavioral Experiments

Behavioral experiments were conducted on November 10 (sites 1 and 2), November 11 (sites 3 and 4) and November 18 (sites 5) at study reefs where red snapper had been acoustically tagged at the end of October 2019. This gave fish at least two weeks to acclimate to external tags. However, 3D movement data indicated tag acclimation was accomplished after 2 days, which is consistent with findings reported by Bohaboy et al. (2020).

The sampling protocol, which began at least a half hour after sunrise and ended at least a half hour before sunset, was similar among all study reefs. Upon locating a given reef with the ship's bottom profiler, a weighted aluminum camera stand equipped with two pairs

of stereo cameras was deployed to the seabed. Stereo-camera pairs were fixed to the camera stand and consisted of two pairs of GoPro (Hero5) digital cameras housed in rigid waterproof cases bolted 75 cm apart to the stand. The upper pair of stereo cameras was positioned 10° upward from horizontal while the lower pair were positioned parallel with the seafloor. Both camera pairs were positioned with a 10° toe-in angle. Cameras were set to 2.7k resolution at a frame rate of 60 frames per second. Cameras were fitted with extended-life batteries (24-hr maximum lifespan) to capture the entire experiment conducted at each reef with a single continuous video. Prior to deployment at each reef site, a small flashlight was triggered in view of all four cameras immediately prior to deployment of the stand to allow for video synchronization during video analyses in the laboratory.

A benthic, stationary multibeam imaging sonar (500 kHz Mesotech M3) secured atop a 1-m tall tripod was deployed 15 m from each reef at a 90° heading to the camera stand to measure the broad-scale distribution of fish. The M3 was powered by an underwater battery system with an embedded computer to operate the sonar and record data. The M3 was angled horizontally to aim the major axis of the beam parallel and the minor axis perpendicular to the seabed. The M3 was configured to transmit a 120° (horizontal) by 30° (vertical) beam at 2 Hz, sampling out to a range of 25 m. In this configuration, the sampled beam volume was approximately 9,300 m³.

A 1-hour acclimation period followed deployment of the stereo-camera stand and M3, after which divers were deployed to the seabed to position the stereo-camera stand 5 m from the reef and the M3 15 m from the reef. GoPro Hero5 cameras have a vertical and horizontal field of view of 49.1° and 64.6°, respectively, which results in a 29.0 m² (4.6 m x 6.3 m) viewing window at a 5-m distance. Thus, the stereo cameras with a 75 cm baseline had a common viewing window of 19.3 m² (4.6 m x 4.2 m) to track red snapper movements and collect length measurements. Stereo cameras were calibrated underwater by the diver positioning a 5 x 7 square (63.47 mm) checkerboard (610 x 457 mm) at a variety of

distances (between 1 and 5 m) and angles of incidence ($<20^\circ$) following the methods of Delacy et al. (2017) and Garner et al. (in revision). The diver initially positioned himself adjacent to the reef holding the checkerboard in front towards the centerline of the camera stand and swam slowly forward while tilting the checkerboard forwards, backwards, to the right, and to the left (20° range from perpendicular in each direction) in decreasingly large circular motions until the diver was 1 m from the camera stand. The diver then repeated the same motions while swimming backwards and away from the camera towards the reef. The circular checkerboard movements allowed the checkerboard to be viewed by all four cameras during each transect.

Red snapper behavioral experiments commenced once the diver completed positioning the M3. Behavioral experiments at each reef site consisted of three 15-minute gear deployment periods, in addition to the diver deployment, consisting of one of three mobile sampling gears (i.e., ROV, TCS, TAS) and three 15-minute acclimation periods (range: 14-21 min depending on haulback times) without mobile gears that occurred in an alternating fashion (i.e., acclimation, gear, acclimation, etc). The 15-minutes between the diver exiting the water and the first mobile gear deployment served as the acclimation period for the diver treatment as well as the first mobile gear deployed at each site. The 15 minutes prior to diver deployment could not be used as the acclimation period for the diver because the stereo cameras and M3 had not yet been positioned. The order of deployment for each mobile sampling gear was randomized among the 5 sampling reefs.

The ROV utilized in this study was a VideoRay Pro4 (375 x 289 x 223 mm; 6.1 kg; 305 m depth rating) equipped with an integrated live-view, forward-facing, internal camera (1080 p) and provided real-time depth and heading information. The TCS was a Towed Aquatic Resource Assessment System designed and built by Deep Ocean Engineering on a modified Phantom ROV frame and equipped with a a Deep Sea Power & Light Multi SeaCam 2060 low-light color video camera, two 500 watt underwater lights (model 710-

0400601), a Tritech PA200/20-PS sonar altimeter, a SeaLaser 100 parallel compass, and a depth (pressure) sensor. The TAS consisted of a 1 m by 0.25 m aluminum frame with ¼” PVC board “fins” attached for stability that carried a downward facing echosounder (70 kHz).

The ROV was deployed as close to each reef as possible and flown in the immediate proximity (<10 m) of the reef for the duration of the 15-minute survey period. The TCS and TAS were each deployed approximately 100 m from each reef site and towed in three transects that crossed immediately above reefs such that each transect had a total distance of approximately 200 m. During TCS transects, the vessel maintained constant forward motion at intermittent speeds <3 kts to maintain a target sled depth of 2-3 m above the seafloor. This was accomplished by monitoring the TCS’s integrated depth sensor and live-feed camera in real time to ensure transects crossed over reefs. During TAS transects, the towing vessel maintained a speed of 3 kts and the sled remained at a depth of 3 m below the sea surface. The stereo camera and M3 stands were retrieved by divers following the last mobile gear deployment at each site to extract digital video and sonar data. After all reef sites were sampled, acoustic telemetry receivers were retrieved from the seabed by divers between November 19 and 22, 2019.

1.3. Data processing

Data stored on acoustic receivers were downloaded onto a laptop in the field. Digital files were transmitted to Innovasea, Inc. in Dalhousie, Nova Scotia, Canada for data processing with proprietary software (Espinoza et al. 2011; Smedbol et al. 2014). Geoposition (latitude and longitude coordinates), depth (m), and acceleration ($m \cdot s^2$) was estimated for each tag-specific acoustic ping heard by array receivers. Fate (e.g., tag loss, depredation, emigration) of acoustically tagged fish was estimated based on movement data

following the approach of Bohaboy et al. (2020).

Video data were processed in the laboratory to estimate fish abundance and fork length to the nearest mm and track fish movements in response to mobile gear deployment. While the ROV, TCS, and TAS were the primary gear treatments of interest, diver presence was also included as a treatment in statistical analyses of video data because diver surveys are a commonly used method to sample reef fish communities. During each 15-min gear deployment and the preceding 15-min acclimation period, red snapper were counted from one camera of the top pair and one from the bottom pair. Camera-specific counts were performed for each minute of the gear deployment and acclimation periods and constituted the maximum number of red snapper viewed among the 60 video frames for each 15-min period.

Red snapper tracking analysis conducted with stereo camera data was performed with the freeware package XMAlab (Knörlein et al., 2016) available in R (R core team, 2019). X-ray motion analysis (XMA) software was developed to study *in vivo* skeletal movements in humans and animals using X-ray videos of surgically implanted radio-opaque markers but can also be applied to standard video files for tracking points identified on moving objects through a series of still images (Knörlein et al., 2016). Video data from stereo cameras were synchronized and stills of the checkerboard ($n = 50$) were extracted for calibration. Calibration files had $<1\%$ error for all but one reef site which had an estimation error of 1.5% due to a missing video segment that required manual synchronization prior to calibration. Each red snapper viewed simultaneously by both cameras of the stereo-camera pair was tracked if it remained in view for at least three seconds with a position estimated for each second the individual was in view.

Tracking consisted of first identifying the anteriormost point of the jaw of an individual when first viewed by both cameras. Successive paired images were taken of that same individual every second (minimum of 3 seconds i.e., 3 still images) throughout the

duration of its occurrence in the viewing window. Tracking concluded when the anteriormost point of the jaw exited the viewing window shared by both cameras or when it could no longer be confidently identified due to distance from the camera (>5 m). Tracking data consisted of a set of x (left to right), y (top to bottom), and z (near to far) coordinates in real units (cm) with the point (0,0) corresponding to the center point of view shared by both cameras. The mean values for all initial and final positions (x , y , and z values) of all individuals tracked within each minute were estimated for each gear deployment treatment.

Following retrieval of the M3, data were downloaded and stored for analysis. Fish were detected and enumerated in Echoview (v10; Hobart, Australia) following methods described by Boswell et al. (2008). A background subtraction algorithm was applied to remove static background objects (i.e., substrate and reef structure), followed by a 3×3 median filter and multibeam single target detection algorithm. Targets that exceeded the minimum criteria (>30 cm TL) were recorded for each ping (2 Hz), which produced a time series of fish abundance associated with each site and used to compare with coincident estimates of abundance from stereo-camera videos. Targets that met the minimum length criteria were enumerated in each ping and summed across each 1-minute interval. The minute-specific count was then multiplied by the corresponding minute-specific proportion of red snapper observed on digital video. Red snapper could not be differentiated to species directly from sonar signatures. However, video data indicate that reef sites had very low diversity (~ 5 species per site), red snapper were the numerically dominant species at >30 cm TL, and other species were viewed infrequently.

1.4. Statistical analyses

A generalized linear model (GLM) was computed in R (R core team, 2019) to test

the effect of FL and handling time on red snapper fate. Distance of red snapper from reef sites was estimated by calculating the distance between red snapper GPS position estimates and study reef center locations. We excluded GPS position estimates with horizontal position error (Smith, 2013) in the upper 5th percentile of the data to filter out estimates that were highly uncertain or likely resulted from false detections (Bohaby et al., 2020). Detections of red snapper >100 m from the study reef being examined also were excluded from statistical analysis of red snapper position for the series of gear deployments at that reef. Depth data recorded from acoustic tags were converted to height off bottom (HOB). Distance, HOB, and acceleration data were analyzed with separate generalized linear mixed models (GLMMs) with the “glmmTMB” package (Brooks et al., 2017) in R (R core team, 2019). Separate models were computed for each gear treatment (i.e., Diver, ROV, TCS, or TAS), all specified with a gamma distribution with log-link function due to the data having all positive values with right skewed distribution. Tag ID and site were included as random effects.

Red snapper count data derived from stereo-camera video and the M3 sonar were also analyzed with GLMMs. The response variable for video samples was the mean number of red snapper observed per minute, which was the average of the per minute counts between top and bottom cameras, and was assumed to be Poisson-distributed with mean λ . Separate GLMMs were estimated for each gear treatment with the mean red snapper count during the deployment period was compared to each gear's pre-deployment period. Minute also was included in each model as an explanatory variable along with the interaction term; site was included as a random effect. The AR1 covariance structure was specified to account for autocorrelations among observations between time intervals but the option to specify zero-inflated data was not necessary. The same approach was used to analyze mean red snapper counts estimated with M3 sonar data, but statistical models could not be estimated for the diver deployments due to interference from bubbles in the water column.

3. Results

Mean FL (\pm 95%CI) of tagged red snapper was 448.1 mm (\pm 27.4 mm). Thirty individuals were tagged at three study reefs on October 28, 2019 and the remaining 20 were tagged at two sites on October 29, 2019 (Fig. 1B). Three of the initially tagged fish returned to the surface in poor condition and had their tags recovered and redeployed on different fish. Of the final 50 tagged individuals, 30 (60.0%) survived and were detected at study reefs throughout the 22-day duration of the behavioral study, 12 (24.0%) were estimated to suffer depredation, 2 (3.3%) shed their tags, and 6 (12%) tags were never detected within the array. Results from GLM analysis indicated neither fish FL ($p = 0.335$) nor handling time ($p = 0.649$), or their interaction ($p = 0.524$), significantly affected the probability of red snapper surviving and being detected throughout the study period.

In total, 1,004 acoustic detections were logged during red snapper behavioral experiments conducted among the five study reefs. Of those, 184 detections occurred within 100 m reefs when mobile gears were actively deployed. Most of the remaining detections were due to individuals being detected at reef sites that were not actively being sampled. One tagged red snapper was detected within 100 m of two different survey reef sites (sites 1 and 5) during gear deployments, but detections occurred 8 days apart.

Analysis of red snapper distance to reef, HOB, and acceleration data before and after the stereo camera and M3 sonar stands were deployed indicated no significant difference in red snapper distance to reef, HOB, or acceleration immediately after (post-deployment minutes 1-15)(Stands treatment), or well after (post-deployment minutes 16-60) deployment of the stereo camera and M3 sonar stands during the acclimation period (Acclimation) (Fig. 2; Table 1). There was a significant effect of Minute on red snapper acceleration ($p = 0.007$) but the magnitude of the coefficient was minimal (1.02). Analysis of red snapper counts per minute during the acclimation period (i.e., prior to divers pointing the camera toward and 5 m

from the reef) indicated fish initially were seen in the view of the camera at elevated numbers that quickly equilibrated at background levels during the initial acclimation period (Fig. 3).

Mean distance of tagged red snapper from study reefs during gear deployments was similar among diver, ROV, and TCS treatments and slightly lower during TAS deployments (Fig. 4A). Statistical models of mean red snapper distance to reefs during behavioral experiments indicated no significant gear effects existed (Table 2). Red snapper HOB was less variable when the diver and ROV were deployed as compared to the other treatments but differed at most by only ~1 m among treatments (Fig. 4B). Height off bottom was not significantly different when divers ($p = 0.372$), ROV ($p = 0.299$), or TAS ($p = 0.458$) were present as compared to acclimation periods, but the interaction between the TCS and minute was significant ($p = 0.002$; Table 3).

Acceleration was lower during the ROV, TCS, and TAS gear deployments and higher during the diver deployment compared to the respective acclimation periods (Fig. 4C), but not of the gear effects were significant in among the acceleration models (Table 4).

Analysis of stereo-camera video data indicated red snapper counts per minute were significantly greater during some gear deployments relative to their respective acclimation periods (Table 5; Fig. 5). The presence of the ROV ($p = 0.001$) or the TCS ($p = 0.002$) significantly increased mean ($\pm 95\%$ CIs) red snapper count, but only by 0.64 (± 0.37) and 0.63 (± 0.41) individuals, respectively. Neither the presence of the diver ($p = 0.834$) nor the TAS ($p = 0.659$) had a significant effect on red snapper count, but the interactions between diver and minute ($p = 0.016$) and TCS and minute ($p < 0.001$) were significant. However, the coefficients for the diver*minute (0.04) or TCS*minute (0.10) interaction terms were quite small, thus indicating only minor effects existed.

Red snapper counts estimated with the M3 sonar (Fig. 6) were similar to count estimates derived from video samples (Fig. 5). Statistical models for sonar-derived red snapper count estimates indicated no significant difference in counts per minute for ROV ($p =$

0.517) or TAS gears ($p = 0.270$) but did indicate a significant effect of the TCS ($p < 0.001$) and the interaction between TCS*Minute ($p < 0.001$; Table 6). However, the coefficients for the TCS effect (1.21) and the TCS*minute interaction (-0.13) were relatively small. Visual inspection of mean red snapper counts per minute show relatively stable red snapper mean (\pm SE) counts per minute across all three gears except for mean counts during minutes 3 and 4 for the TCS where mean redsnapper counts were 6.3 (± 4.2) and 9.1 (± 7.4), respectively (Fig. 6, column A). Inspection of scaled mean counts during these two time points well exceeded the overall mean of 2.4 (± 0.4) red snapper per minute for the TCS gear treatment (Fig. 6, column B).

Tracking data estimated for red snapper from stereo-camera video samples indicate little difference in observed red snapper position among diver, ROV, TCS, and TAS deployments. Overall, observed fish tended to be between 0.5 and 2 m above the seabed and within 3-4 m of reef modules. Red snapper did tend to be closer to reef modules and aggregated above reefs during diver (Fig. 7A) and ROV (Fig. 7B) deployments, while movements were greater and fish were somewhat more diffuse around reefs during TCS (Fig. 7C) and TAS (Fig. 7D) deployments. Lastly, tracking plots indicate red snapper tended to be closer to the bottom during TCS deployments and tended to utilize more of the water column during TAS deployments.

4. Discussion

Study results indicate behavioral effects associated with the survey gears used in this study were minor and mostly non-significant. Therefore, we infer that none of the mobile survey gears examined would be likely to introduce substantial bias into estimates of red snapper density.

Video data reveal that red snapper may infrequently be inquisitive towards and

approach foreign objects, like the stereo camera and M3 sonar stands, which might be interpreted as attraction when viewed only with gears that have small sampling volumes (10s of m³) that are less than the volumes typically occupied by red snapper around reef sites (Piraino and Szedlmayer, 2014; Williams-Grove and Szedlmayer, 2016; Bohaboy et al., 2020). It is also challenging to infer much about red snapper movement behavior from video data alone because visibility is often limited to <10 m in this region, which makes it difficult to continuously track individuals seen on video (Stoner et al., 2008). Nonetheless, stationary stereo cameras can provide important insight regarding red snapper behavior (Somerton et al., 2017), but fine-scale 3D acoustic telemetry provides much greater information on larger-scale movements to examine the potential for fish behavior to cause survey bias. Red snapper were not observed to significantly contract the volume they occupy in response to the mobile survey gears deployed, when tracked at larger spatial scales (i.e., 1000s of m³).

Response behavior by benthic fishes to survey gear (stationary or mobile) can be variable (Lorance and Trenkel, 2006; Stoner et al., 2008) and depend on light levels (Brock, 1982; Thorne et al., 1989; Ryer et al., 2009), habitat characteristics (Brock, 1982; Cailliet et al., 1999; Lawson and Rose, 1999; Edgar et al., 2004), gear characteristics (Koslow et al., 1999; Cailliet et al., 1999; Lorance and Trenkel, 2006; Stoner et al., 2008), and ecology (Norcross and Mueter, 1999; Lorance and Trenkel, 2006). In their synthesis of behavioral studies of fishes surveyed with underwater vehicles, Stoner et al. (2008) reported most of the taxa studied exhibited some type of response behavior to survey vehicles with more than half of the fish taxa examined exhibiting avoidance behavior while a third exhibited some degree of attraction. MacNeil et al. (2008) and Bozec et al. (2011) both reported that larger fishes on coral reefs tended to display stronger avoidance behavior. Somerton et al. (2017) observed negative response behaviors for vermilion snapper, *Rhomboplites aurorubens*, a congener commonly associated with red snapper at nGOM reefs, when approached by a TCS.

Despite being the most studied fishery species in the nGOM, little information exists

in the published literature regarding responses of adult red snapper to fishery-independent survey gears. We saw no evidence of avoidance behavior by red snapper in response to the presence of any of the survey gears. Startle responses were not observed on digital video and mean acceleration data were similar among all gear treatments, as well as between paired gear deployment and acclimation periods. Telemetry-derived GPS position data did not indicate an increase in mean distance from survey reefs, which would have been indicative of large-scale avoidance unobservable on digital video. Regardless, a negative behavioral response can only contribute to survey bias if the response directly or indirectly (e.g., startle response of individuals in view induces startling by others at the edge of or out of view) prevents species identification, increases enumeration error (e.g., blurring of individuals on video during startle response), or individuals avoid survey gear entirely and thus are not observed. Although Stoner et al. (2008) caution against characterizing species-specific responses from a single study, we believe that red snapper are unlikely to demonstrate meaningful negative behavioral responses in subsequent studies because they can be inquisitive, are not benthic or cryptic, do not exhibit schooling behavior, are highly active with low swimming speeds, and have distinct profiles from other taxa and most congeners that allow them to be confidently identified and enumerated.

Based on our video observations, attraction (positive bias) would be the more important potential issue than gear avoidance when conducting red snapper surveys, especially ones designed to estimate absolute abundance or density. Although red snapper, especially small (<600 mm), young fish, are strongly reef-associated (Patterson et al., 2001; Westmeyer et al., 2007; Strelcheck et al., 2007; Bohaboy et al., 2020), they are a mobile species that may meander over areas 10s of meters in radius from reef sites during daylight periods when collecting video data is feasible (Piraino and Szedlmayer, 2014; Williams-Grove and Szedlmayer, 2016; Bohaboy et al., 2020). Therefore, there is considerable potential for red snapper to contract the volume they occupy around reefs during surveys if they

respond positively to survey gear. Despite this potential, and the fact that more red snapper were observed on stereo-camera video immediately following deployment of the camera stand, neither benthic sonar nor telemetry data indicated any large-scale attraction of red snapper to the stereo camera or M3 stands, or to any of the three mobile survey gears examined. Fish did appear to display greater curiosity with respect to divers being present, as demonstrated by changes in movement metrics estimated with 3D telemetry, and fish were estimated to be more clustered near reefs, hence divers, based on stereo camera-derived tracking of individual fish. However, when the various data sources are examined in their entirety, it does not appear that any of the three mobile survey gears substantially affected red snapper behavior, thus their detectability or the potential to double-count fish.

A potential attraction issue was observed during the TCS deployment at reef site 1. Several red snapper were seen oriented toward but swimming behind (i.e., following behavior) the TCS on two of three transects when it passed over the reef in view of the benthic cameras. However, we did not detect directional movements or red snapper following behavior when the TCS was deployed at the other four reef sites. The individuals observed following the TCS at site 1 also were unlikely to meaningfully bias survey-derived density estimates because they were initially observed to exhibit typical swimming behavior and only began orienting towards the TCS as it passed the reef module. During the period the exhibited following behavior, these fish had already been viewed by the TCS's forward-facing camera and were out of view when they began following the sled. Towed camera gears are typically deployed in a single unidirectional linear transect over relatively great distances with forward-facing cameras that would not record following behavior and thus avoid numerical bias when estimating animal density for the area surveyed. Furthermore, GPS positions of acoustically tagged red snapper indicated neither an increase in distance from the reef during TCS surveys nor was there an increase in variance associated with the distance of tagged red snapper from the reef compared to other treatments.

Tagged individuals following the TCS would likely have a large effect on the variance of the distance estimate due to the relatively low number of GPS positions available during each 15-minute period.

Red snapper swimming behavior appeared to be least affected by TAS deployments. Issues with survey bias have been previously reported with TAS-type gear when surveying demersal fishes associated with complex habitats if the fishes seek vertical or structural refuge in response to hydrodynamic (i.e., pressure waves) or auditory (i.e., vessel noise) stimuli (Lawson and Rose, 1999; Kotwicky et al., 2013; Kotwicky et al., 2015). Potential detectability issues are well-known with TAS gears in complex benthic habitats, especially ones with vertical relief, due to acoustic shadows or “dead zones” that reduce fish detectability (Ona and Mitsen, 1996; Hjellvik et al., 2003; Kotwicky et al., 2013). In this study, the TAS was deployed approximately 3 m below the surface at reef sites that were nearly 40 m deep, thus minimizing the possibility of red snapper displaying behavioral interactions with the TAS. No vertical response behaviors (e.g., synchronized downward directional swimming or persistent changes in proximity to the benthic surface) by red snapper were observed on stereo-camera video during TAS tows. Stereo-camera tracking data also indicated red snapper were the most dispersed around reefs during TAS deployments, and 3D telemetry data indicated no effects of the TAS on red snapper movement metrics. Therefore, red snapper movement behavior did not appear to be affected by the TAS as has been reported in other reef fish taxa.

Unlike the mobile survey gears, red snapper did demonstrate persistent attraction to divers when they were present. Red snapper counts also were elevated when the stereo camera and M3sonar stands were first deployed, but that effect quickly dissipated. Furthermore, telemetry data did not indicate red snapper were attracted over even moderate (>5 m) distances to the stands.

The stands were the first gear introduced at each of the survey sites and they

disturbed the sediment when they landed on the sandy seabed, which may have explained the initial attraction of red snapper if the fish perceived the disturbed or suspended sediment as a feeding opportunity. Divers working on the seabed to move the stereo camera and M3 sonar stands into position also disturbed the sediment and thus possibly exposed benthic prey fauna. This could explain the persistent rather than fleeting attraction of fish to the divers.

Overall, study results indicate none of the three mobile survey gears had much of an effect on red snapper swimming behavior, thus the potential to bias abundance or density estimates. However, there are two caveats to this interpretation. First, stereo camera and M3 sonar stands always were deployed first at each reef fish in our multidisciplinary attempt to estimate the effect of mobile survey gears on red snapper behavior. It is unknown, and unknowable from our design, whether red snapper would have displayed attraction to any one of the mobile survey gears if it had been the first gear deployed at a reef. Given the different movement metrics that can be quantified with high-resolution 3D acoustic telemetry, it is yet possible to test potential attraction issues without deploying the stereo camera or M3 sonar stands, which in hindsight perhaps should have been done at additional study reefs.

A second caveat to interpreting study results with respect to mobile survey gear effects on red snapper swimming behavior is that all experimental work was performed at artificial reefs that were distributed on otherwise featureless sand bottom. The reason for conducting the experiment in this habitat was because the probability of locating red snapper on nGOM artificial reefs is much higher than on natural reefs (Dance et al., 2011; Patterson et al., 2014) where their density is typically an order of lower for reefs on the nGOM shelf (Patterson et al., 2014; Karnauskas et al., 2017). There are no published studies on red snapper swimming or foraging behavior on natural reefs, thus no comparisons with results from the numerous published red snapper acoustic telemetry papers is possible. If artificial reefs altered red snapper movement behavior, then study results may not provide an accurate picture of how the mobile survey gears examined affect red snapper behavior, or whether

patterns observed are likely to be applicable to natural reef habitats as well. However, red snapper are known to range 100s of m away from artificial reefs (Piraino and Szedlmayer, 2014; Bohaboy et al., 2020), which was seen in the current study as well, thus are not closely site-attached to the structure of artificial reefs.

Furthermore, adult red snapper trophic position and diet, which ranges from small zooplankton to relatively large fishes, are consistent between natural and artificial reefs (Tarnecki and Patterson, 2015), thus indicating red snapper foraging behavior for mostly non-reef prey is consistent between the habitat types.

In conclusion, from the data collected during this study it does not appear that any of the mobile survey gears deployed, which included an ROV, a towed camera sled, and a towed acoustic sled, had much effect on red snapper swimming behavior, thus minimizing an important potential source of bias in estimating red snapper abundance or density. Fishery-independent surveys utilizing a variety of gears have become an integral part of stock assessments, but abundance data are also important for examining ecological questions, including via ecosystem models. This study was not designed to compare red snapper abundance or density estimates among the gears examined to develop gear-specific correction factors, but obviously the issue of detectability is important to assess whether camera-based or sonar approaches are utilized in a given survey. Quantifying potential gear biases can help reduce variability in density estimates or indices of abundance and thus reduce scientific uncertainty in stock assessments or reduce measurement error in ecosystem models. Understanding the sources and magnitude of gear bias can also increase stakeholder confidence and acceptance of management regulations that in turn can help achieve management objectives.

Acknowledgements

Funding for this work was provided by a grant from Sea Grant and the National Marine Fisheries Service (#NA16OAR4170181) and the Florida Fish and Wildlife Research Institute (#FWC-16188) to WFP. We greatly appreciate the efforts of Erin Bohaboy in providing technical details and guidance for acoustic tagging methodology and data processing. We thank Jessica VanVaerenbergh and Savannah Lebuja for field support during field experiments. We also gratefully acknowledge the captain and crew of the charterboat vessel Dreadknot II for their invaluable assistance they provided in the field.

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Figure List

Fig. 1. Location of array (red dot inside red box) in A) the central northern Gulf of Mexico ~35 nm southeast of Destin, FL, and B) locations of reef sites within the 4.23 x 2.82 km (11.9 km²) acoustic array. Numbered circles indicate acoustic receiver positions while triangles indicate artificial reef sites. Numbered triangles indicate site locations where, and the order in which, redsnapper were tagged with acoustic transmitters and released (10 per site). No fish were tagged at reef sites indicated by numberless triangles. Receivers were deployed 472 m apart in any cardinal direction all with equal spacing.

Fig. 2. Distance to reef (m), height off bottom (m), and acceleration (m•s⁻²) of acoustically tagged red snapper at survey reefs 55 minutes prior to and after the stereo camera and M3 sonar stands were deployed. Individual data points are means ± 95% CIs of 5-min time bins. The vertical grayline indicates timing of stand deployment.

Fig. 3. Exponential decline of red snapper observed on digital video during the initial acclimation period at each site, prior to divers being deployed to position the stereo camera and M3 sonar stands. The acclimation period began when the stereo camera stand contacted the seabed and ended when the diver entered the water to position the stands. Data plotted are mean ±SE red snapper counts per minute at the 5 sites surveyed. The fitted line is a non-linear regression with its equation indicated on the figure.

Fig. 4. Plots of A) mean distance (m), B) depth (m), or C) acceleration (m•s⁻²) of acoustically tagged red snapper that were near the survey site (≤100 m) where the diver, remotely operated vehicle (ROV), towed camera sled (TCS), or towed acoustic sled (TAS) were actively deployed (filled circles) as well as during their respective acclimation periods (filled triangles). Sample sizes are shown above each point. Error bars indicate ±SE.

Fig. 5. Mean (column A) and scaled mean (column B) counts of red snapper observed per minute on digital video during the diver (dark gray), remotely operated vehicle (gold), towed camera sled (dark blue), or towed acoustic sled (dark orange) gear treatments. Scaled mean values were

estimated by subtracting the site-specific mean for the 15-minute period before each gear deployment from the mean count estimate per minute for each gear treatment. Mean values shown at the top right of each panel in the left column indicate the overall mean of the pre-deployment period for each gear treatment. Error bars indicate \pm SE.

Fig. 6. Mean (column A) and scaled mean (column B) counts of red snapper observed per minute with a lateral-viewing, benthic echosounder (M3) during the remotely operated vehicle (gold), towed camera sled (dark blue), or towed acoustic sled (dark orange) gear treatments. Scaled mean values were estimated by subtracting the site-specific mean (shown on panels in column A) for the 15-minute period before each gear deployment from the mean count estimate per minute for each gear treatment. Mean values shown at the top right of each panel in the left column indicate the overall mean of the pre-deployment period for each gear treatment. Error bars indicate \pm SE. The diver treatment could not be included due to acoustic interference.

Fig. 7. Minute-specific mean directional red snapper movement computed from stereo camera tracking of individual fish during the A) diver, B) remotely operated vehicle, C) towed camera sled, or D) towed acoustic sled gear deployments among all study sites. The black triangle indicates the position of the artificial reef module relative to the stereo camera stand (black square) oriented towards the reef. The number of observations contributing to each mean position is indicated by the number at each arrowhead, while the legend indicates the observed minute during the 15-min gear deployment.

TABLES

Table 1. Results from generalized linear mixed-effects models (GLMM, gamma family with loglink) for distances from reef (m), height off bottom (m), and acceleration ($m \cdot s^{-2}$) of acoustically tagged red snapper immediately following stereo camera and M3 sonar stand deployment (Stands = post deployment minutes 1-15) and during the subsequent acclimation period (Acclimation = post-deployment minutes 16-60) relative to the 55-minute period prior to deployment of the stands.

Response		Estimate	SE	t-value	p-value
Distance from reef	Intercept	22.88	1.19	18.19	<0.001
	Stands	0.13	3.24	-1.74	0.083
	Acclimation	1.17	1.46	0.43	0.670
	Minute	0.99	1.00	-1.10	0.273
	Stands*Minute	1.03	1.02	1.74	0.084
	Acclimation*Minute	1.00	1.00	-0.06	0.956
Height off bottom	Intercept	1.73	1.17	3.49	<0.001
	Stands	1.22	6.39	0.11	0.916
	Acclimation	0.60	1.66	-1.00	0.320
	Minute	1.00	1.00	1.37	0.174
	Stands*Minute	0.99	1.03	-0.25	0.803
	Acclimation*Minute	1.00	1.01	0.24	0.812
Acceleration	Intercept	0.50	1.27	-2.89	0.005
	Stands	0.32	27.48	-0.35	0.729
	Acclimation	0.66	3.27	-0.35	0.727
	Minute	1.02	1.01	2.75	0.007
	Stands*Minute	1.01	1.05	0.13	0.894
	Acclimation*Minute	0.99	1.01	-0.69	0.491

Table 2. Results from generalized linear mixed-effects models (GLMM, gamma family with loglink) of acoustically tagged red snapper distances from surveyed reef sites during each gear treatment deployment. Exponentiated coefficients are relative to each gear's acclimation period which represents the 15 minutes prior to each mobile gear deployment. The diver acclimation period was last 15 minutes of the initial 60-minute acclimation period. Distances were calculated as the distance between each tagged red snapper GPS position and the reef site where gears were actively deployed.

Gear		Estimate	SE	z-value	
		p-value			
Intercept		15.54	1.18	16.75	<0.001
Diver		0.93	1.16	-0.46	0.645
Minute		1.00	1.01	0.00	0.998
	Diver*Minute	1.00	1.02	0.27	0.791
Intercept		15.26	1.18	16.15	<0.001
Remotely operated vehicle	ROV	0.98	1.16	-0.14	0.889
	Minute	1.00	1.01	0.30	0.767
	ROV*Minute	1.00	1.01	-0.35	0.728
Intercept		11.88	1.25	11.22	<0.001
Towed camera sled	TCS	1.18	1.18	0.97	0.336
	Minute	1.00	1.02	0.25	0.807
	TCS*Minute	1.00	1.02	-0.20	0.839
Intercept		18.11	1.18	17.33	<0.001
Towed acoustic sled	TAS	0.80	1.16	-1.58	0.120
	Minute	0.98	1.01	-1.99	0.051
	TAS*Minute	1.03	1.02	1.51	0.136

Table 3. Results from generalized linear mixed-effects models (GLMM, gamma family with loglink) of acoustically tagged red snapper height off bottom (m) at surveyed reef sites during each gear treatment deployment. Exponentiated coefficients are relative to each gears' acclimation period which represents the 15 minutes prior to each gear deployment. The diver acclimation period was last 15 minutes of the 60-minute acclimation period, 45 minutes after the camera stand was deployed. Heights off bottom were calculated only for red snapper that were ≤ 100 m from the site where gears were actively deployed when each gear entered the water.

Gear		Estimate	p-value	SE	t-value
Intercept		1.38	1.28	1.29	0.203
Diver		1.35	1.40	0.90	0.372
Minute		1.04	1.02	1.71	0.095
	Diver*Minute	0.97	1.04	-0.72	0.473
Intercept		1.34	1.32	1.03	0.308
Remotely operated vehicle	ROV	1.39	1.37	1.05	0.299
	Minute	1.01	1.03	0.50	0.618
	ROV*Minute	0.99	1.03	-0.16	0.871
Intercept		2.53	1.22	4.69	<0.001
Towed camera sled	TCS	0.79	1.11	-2.38	0.023
	Minute	0.96	1.02	-3.02	0.005
	TCS*Minute	1.05	1.02	3.33	0.002
Intercept		2.38	1.24	3.99	<0.001
Towed acoustic sled	TAS	0.87	1.21	-0.75	0.458
	Minute	0.99	1.01	-0.53	0.603
	TAS*Minute	1.02	1.02	0.91	0.370

Table 4. Results of generalized linear mixed-effects models (GLMM, gamma family with log link) of acoustically tagged red snapper acceleration ($m \cdot s^{-2}$) at surveyed reef sites during each gear treatment deployment. Exponentiated coefficients are relative to each gears' acclimation period which represents the 15 minutes prior to each gear deployment. The diver acclimation period was last 15 minutes of the 60-minute acclimation period, 45 minutes after the camera stand was deployed. Heights off bottom were calculated only for red snapper that were ≤ 100 m from the site where gears were actively deployed when each gear entered the water.

Gear		Estimate	SE	t-value
Intercept		0.74	1.29	-1.19
Diver		1.30	1.28	1.07
Minute		0.98	1.02	-0.79
	Diver*Minute	0.99	1.03	-0.39
Intercept		0.66	1.53	-0.98
Remotely operated vehicle	ROV	1.01	1.58	0.03
	Minute	0.98	1.04	-0.39
	ROV*Minute	1.03	1.05	0.63
Intercept		1.72	1.33	1.90
Towed camera sled	TCS	0.26	1.37	-4.28
	Minute	0.87	1.03	-5.30
	TCS*Minute	1.19	1.03	5.38
Intercept		0.56	1.49	-1.45
Towed acoustic sled	TAS	0.77	1.53	-0.61
	Minute	0.99	1.04	-0.23
	TAS*Minute	1.08	1.06	1.32

Table 5. Results of generalized linear mixed-effects models (GLMM, poisson family with loglink) of red snapper mean counts per minute from digital video for each gear treatment. Coefficients are relative to each gears' acclimation period which corresponds to the 15 minutes prior to each gear deployment. The diver acclimation period corresponds to the 15 minutes after each diver deployment because the camera stand was not oriented towards the reef prior diver deployment.

Gear		Estimate	SE	z-value
Intercept		1.51	0.32	4.74
Diver		0.03	0.14	0.21
Minute		-0.02	0.03	-0.61
	Diver*Minute	0.04	0.02	2.41
Intercept		0.58	0.42	1.37
Remotely operated vehicle	ROV	0.64	0.19	3.39
	Minute	0.01	0.03	0.28
	ROV*Minute	-0.02	0.02	-1.09
Intercept		1.24	0.29	4.26
Towed camera sled	TCS	-0.63	0.21	-3.04
	Minute	-0.04	0.03	-1.39
	TCS*Minute	0.10	0.02	4.29
Intercept		1.02	0.20	4.99
Towed acoustic sled	TAS	-0.10	0.22	-0.44
	Minute	-0.01	0.02	-0.23
	TAS*Minute	-0.01	0.03	-0.24

Table 6. Results of generalized linear mixed-effects models (GLMM, poisson family with loglink) of mean red snapper counts per minute from the M3 epibenthic acoustic profiler for each gear treatment. Coefficients are relative to each gears' acclimation period which represents the 15 minutes prior to each gear deployment.

Gear	Estimate	SE	z-value	p-value	
Remotely operated vehicle	Intercept	0.59	0.62	0.96	0.339
	ROV	0.16	0.24	0.65	0.517
	Minute	-0.02	0.05	-0.47	0.642
	ROV*Minute	0.03	0.03	1.04	0.299
Towed camera sled	Intercept	-0.99	0.87	-1.14	0.256
	TCS	1.21	0.26	4.73	<0.001
	Minute	0.14	0.07	1.96	0.050
	TCS*Minute	-0.13	0.03	-4.71	<0.001
Towed acoustic sled	Intercept	-0.42	0.85	-0.50	0.619
	TAS	0.35	0.31	1.10	0.270
	Minute	0.03	0.03	1.00	0.319
	TAS*Minute	-0.03	0.04	-0.73	0.467

Figures

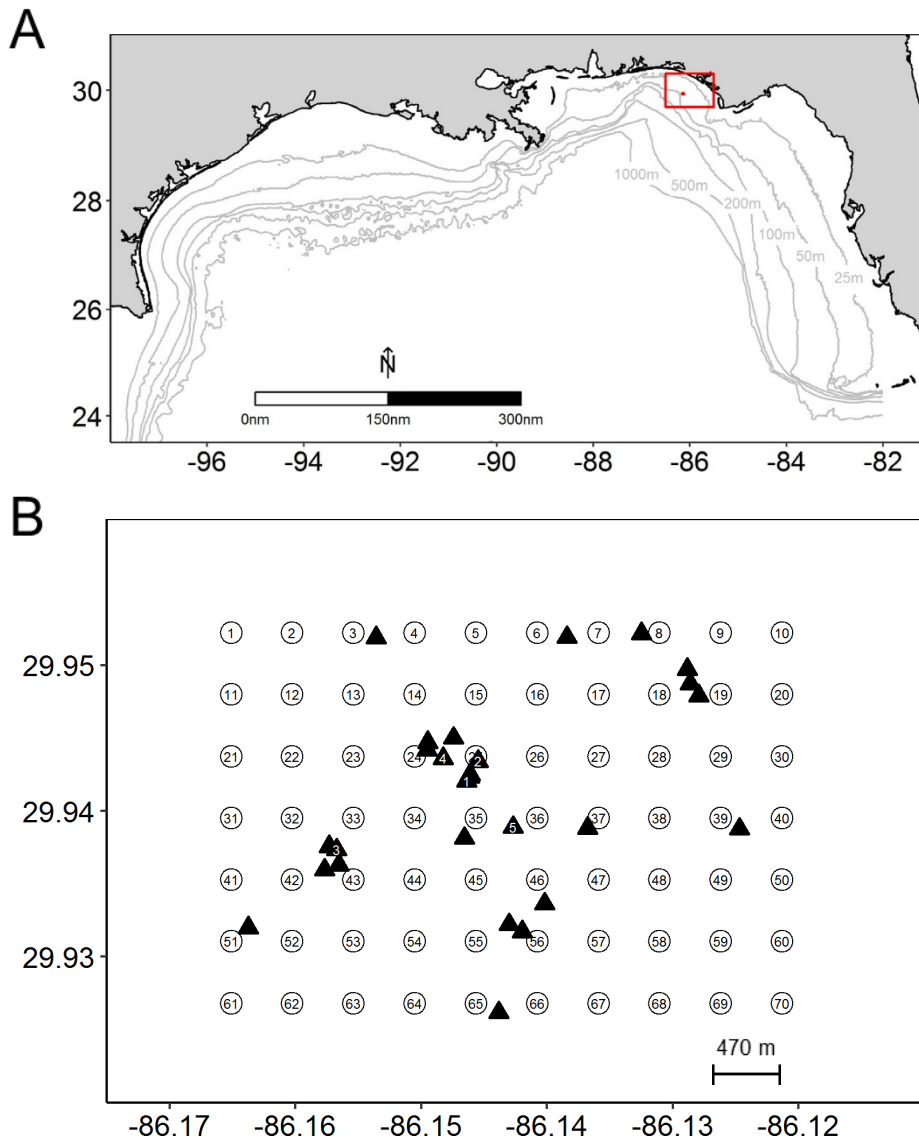


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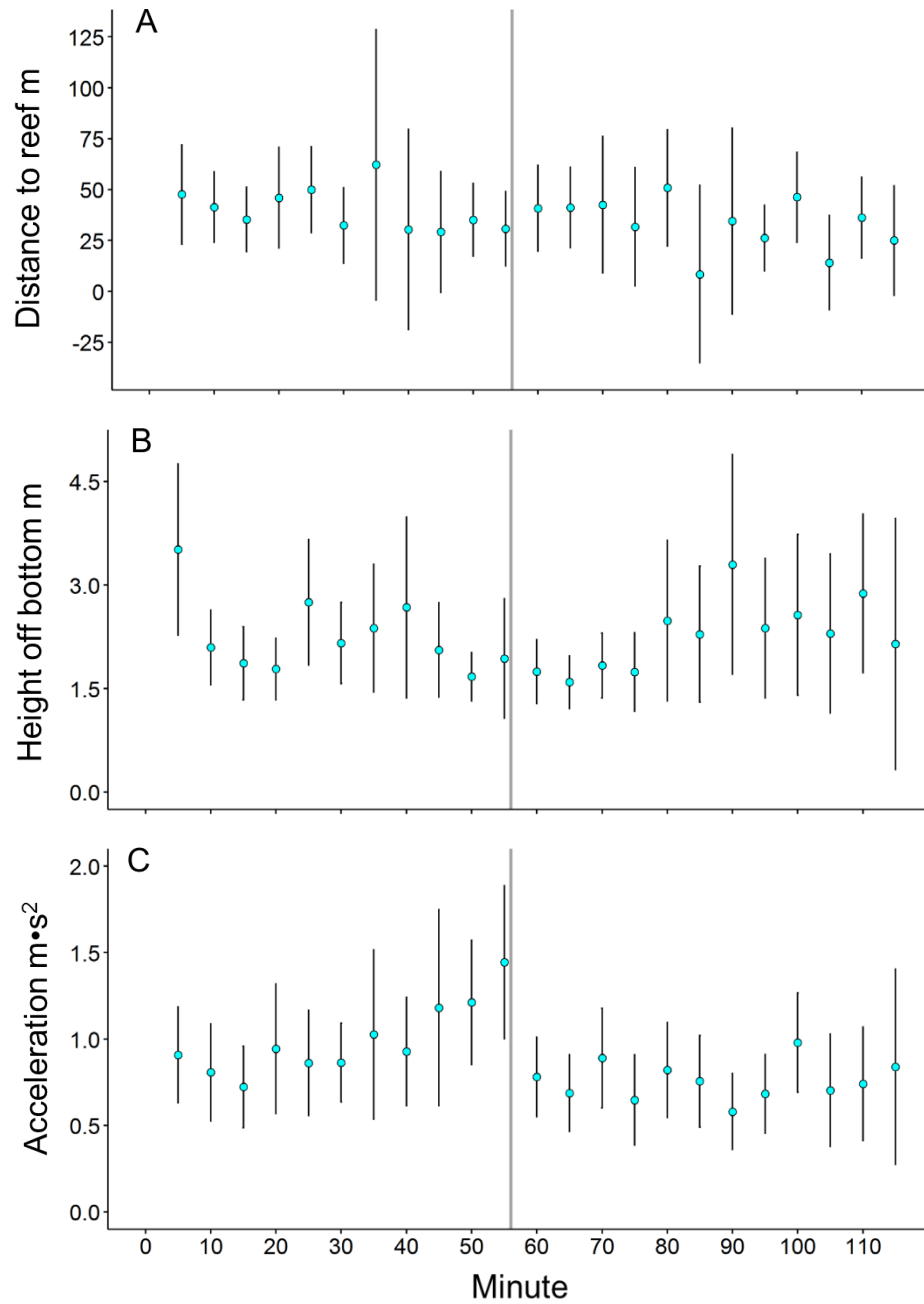


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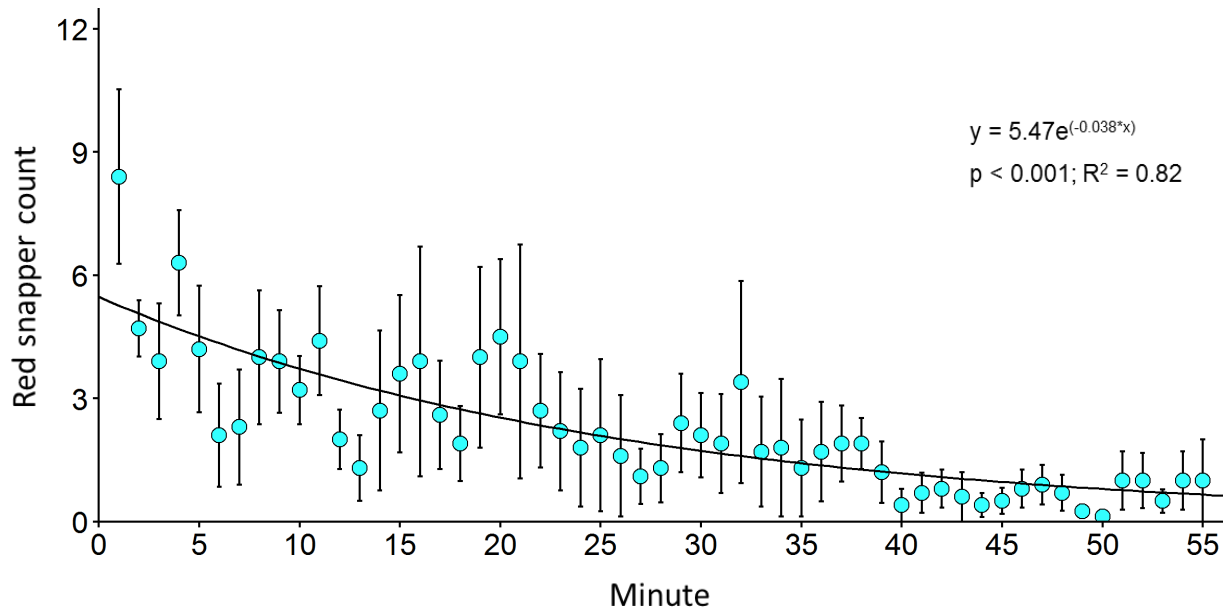


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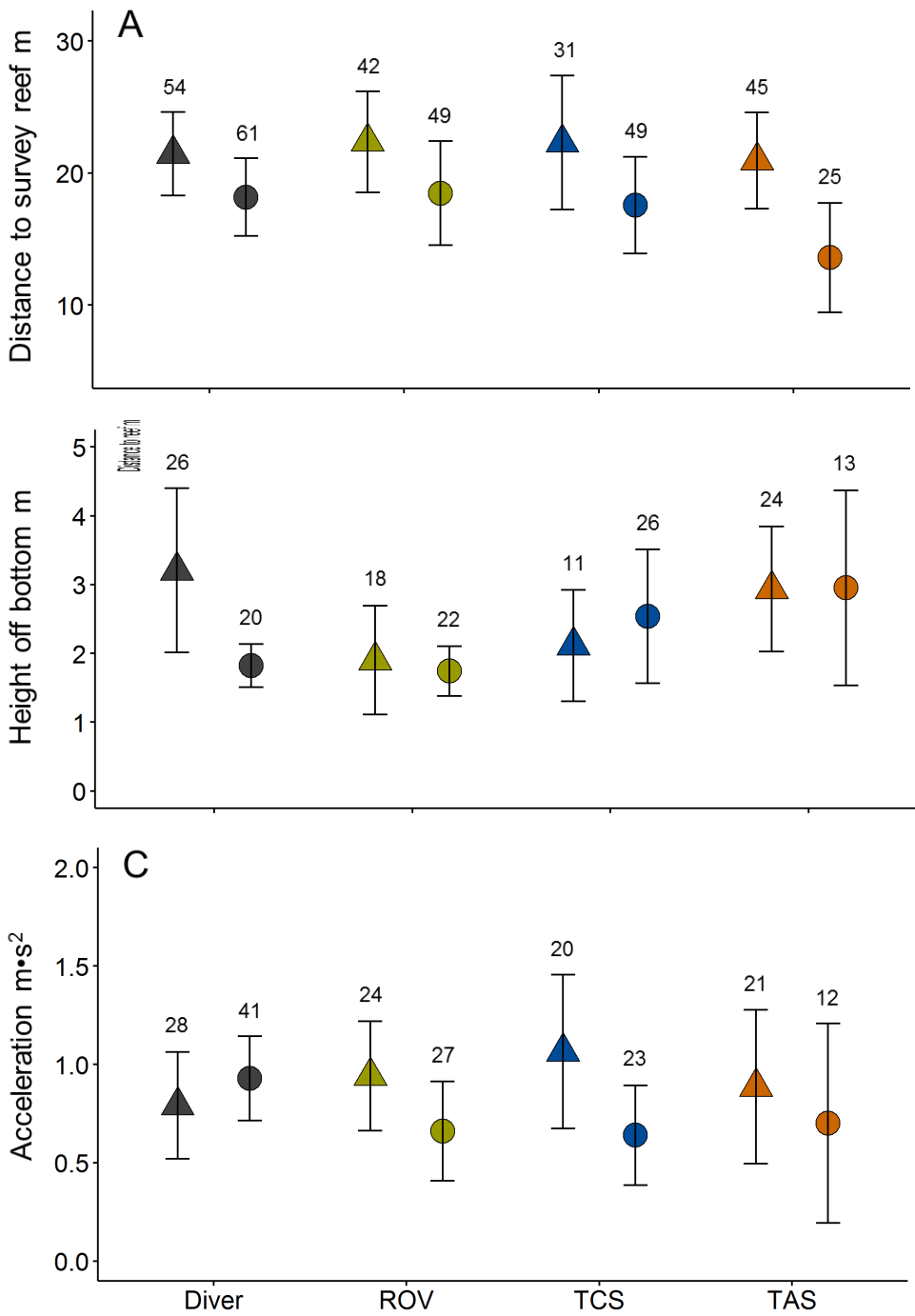


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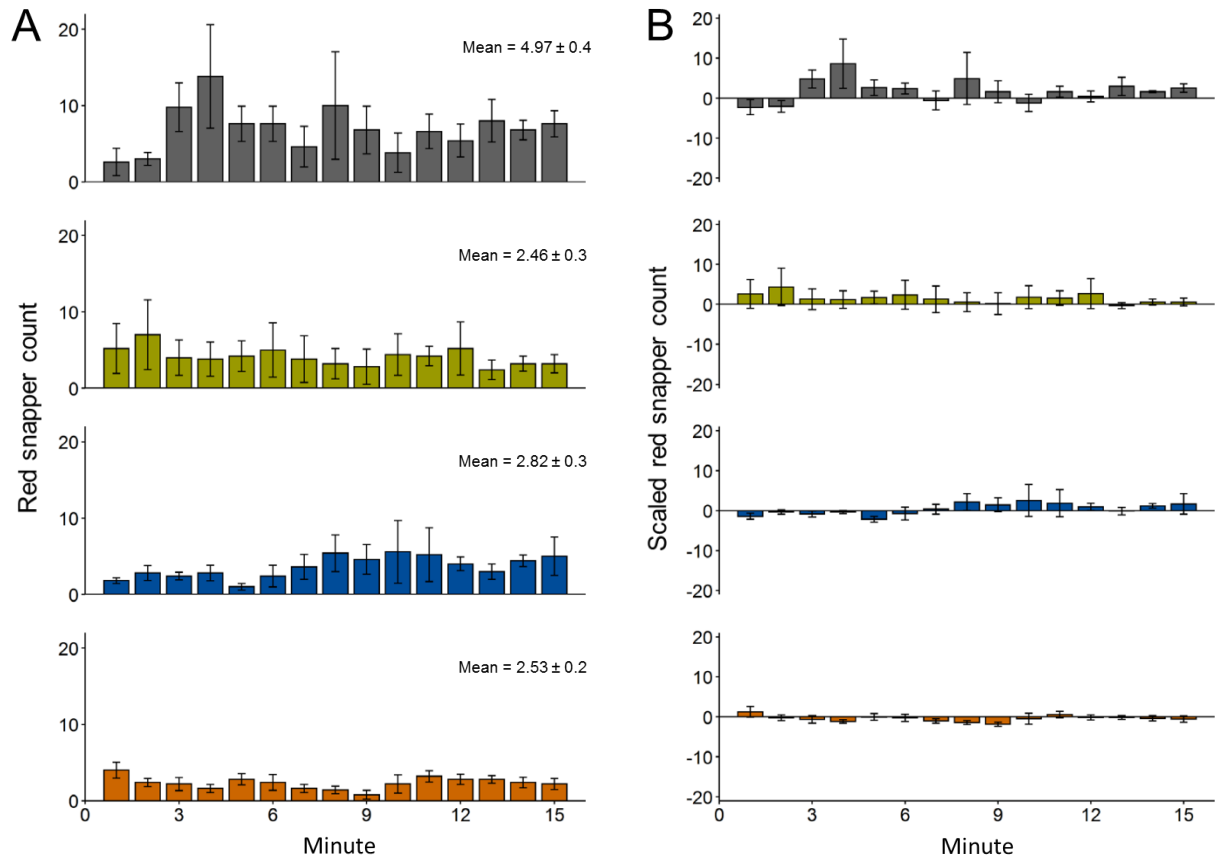


Fig. 5. Mean (column A) and scaled mean (column B) counts of red snapper observed per minute on digital video during the diver (dark gray), remotely operated vehicle (gold), towed camera sled (dark blue), or towed acoustic sled (dark orange) gear treatments. Scaled mean values were estimated by subtracting the site-specific mean for the 15-minute period before each gear deployment from the mean count estimate per minute for each gear treatment. Mean values shown at the top right of each panel in the left column indicate the overall mean of the pre-deployment period for each gear treatment. Error bars indicate \pm SE.

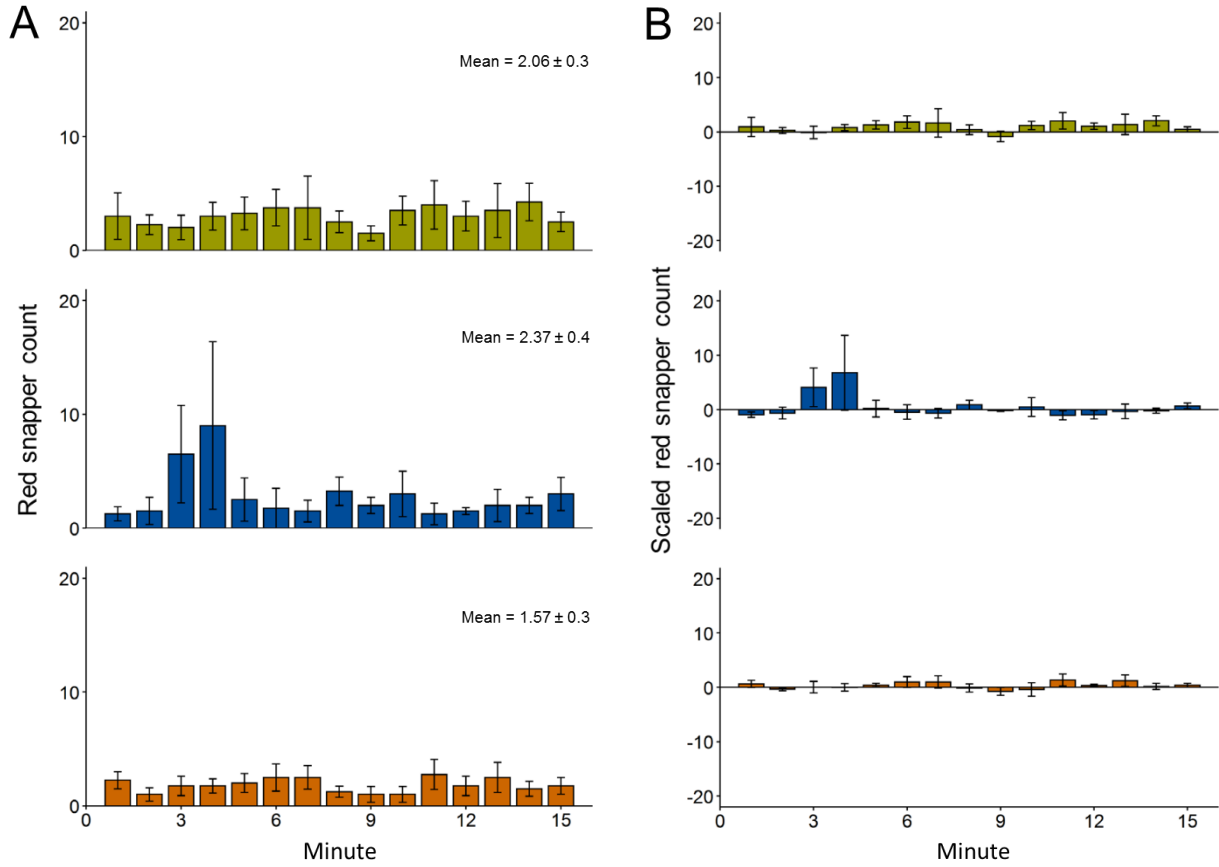


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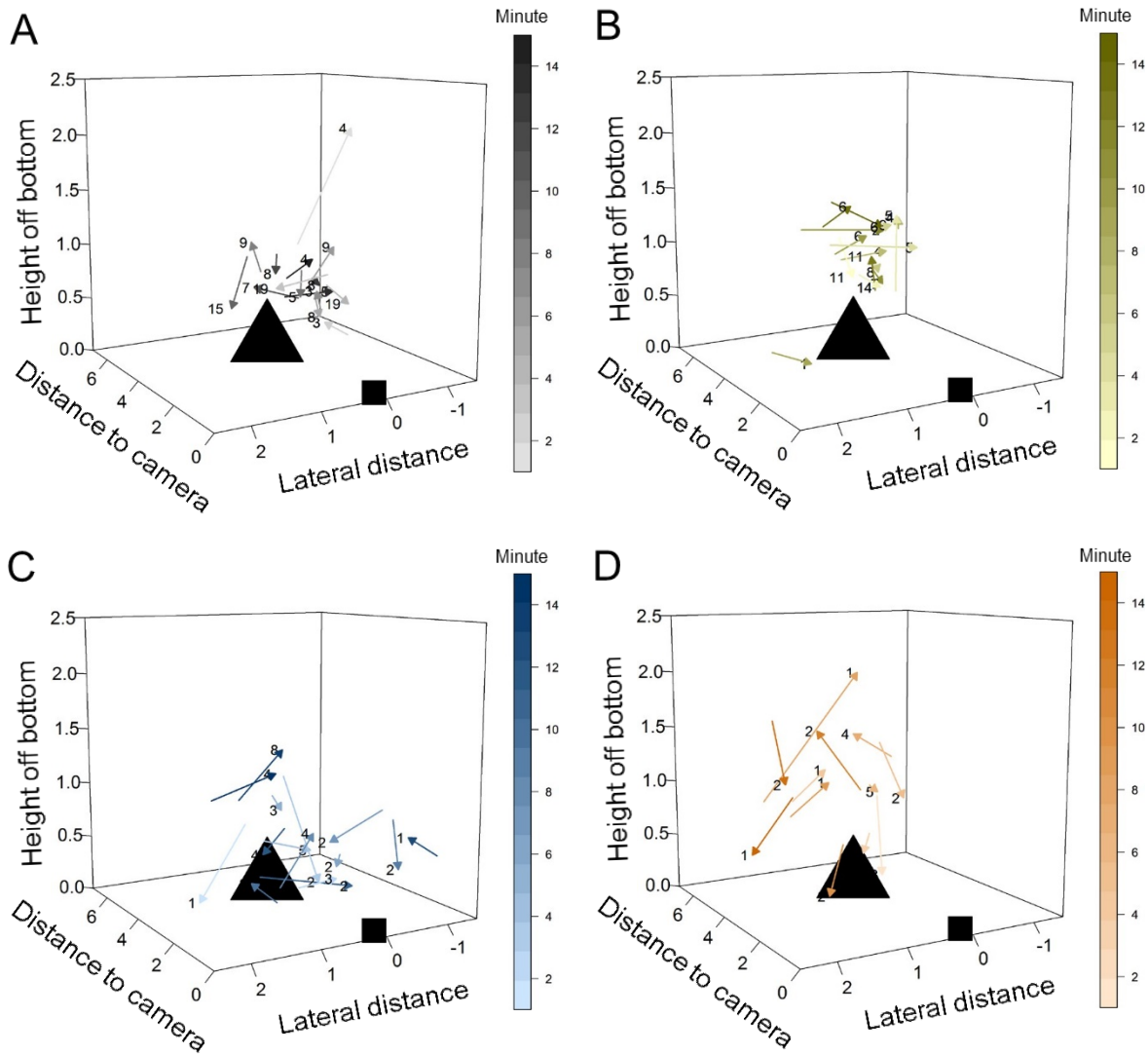


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FEATURED PAPER

Understanding and Enhancing Angler Satisfaction with Fisheries Management: Insights from the “Great Red Snapper Count”

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Abstract


Management of Gulf of Mexico Red Snapper *Lutjanus campechanus* has been a topic of much scientific debate and intensive public scrutiny. In response to political, public, and management desires for more robust data on Red Snapper populations, a gulfwide initiative commonly referred to as the “Great Red Snapper Count” (GRSC) was funded to estimate the absolute abundance of Red Snapper in the U. S. Gulf of Mexico. Here, we describe the results of an online survey designed to (1) characterize the social dimensions of Red Snapper anglers, (2) measure satisfaction with current Red Snapper populations and regulations, (3) assess overall patterns of awareness of the GRSC, and (4) evaluate the potential benefits of GRSC stakeholder engagement videos. A key finding of our survey was that awareness of the GRSC was associated with up to three times higher satisfaction with fisheries management. Through an in-survey experiment, we found that anglers that were presented a video on specific GRSC project components reported slightly higher management satisfaction than those presented an overview video or no video. Collectively, our results indicate that angler awareness, when underpinned by effective engagement and outreach activities, can enhance angler satisfaction.

In the U. S. Gulf of Mexico, populations of Red Snapper *Lutjanus campechanus* and their management have been subject to intense scientific debate and public scrutiny

(Cowan 2011; Cowan et al. 2011). Over the past decade, Gulf of Mexico Red Snapper has undergone multiple formal assessments through the Southeast Data, Assessment,

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Received August 7, 2020; accepted January 5, 2021

	NAFM	10579	WILEY	Dispatch: 8.2.21	CE:
	Journal Code	Manuscript No.		No. of pages: 10	PE:

1 and Review program (SEDAR). The 2013 assessment sug-
2 gested that Gulf of Mexico Red Snapper was overfished but
3 not experiencing overfishing (SEDAR-31 2013). In contrast,
4 the most recent assessment, completed in 2018 with a
5 revised approach for stock status determination, deemed
6 the stock as neither overfished nor experiencing overfishing
7 yet needing to remain on a rebuilding plan (SEDAR-52
8 2018). Much of the controversy and angler disenfranchise-
9 ment in the Red Snapper fishery can be attributed to a rela-
10 tively unique problem of a rebounding fishery with very
11 high catch per unit effort, coupled with decreasing season
12 lengths for recreational sectors (i.e., access).

13 The recreational fishery for Red Snapper has undergone
14 several management changes in recent years. Since 1990,
15 Red Snapper recreational fishing regulations have gener-
16 ally become more restrictive with per-person bag limits
17 decreasing from seven to two fish, minimum size limits
18 increasing from 33.0 to 40.6 cm, and season lengths
19 decreasing from a full calendar year down to as low as
20 several days. Compounding the problem, a 2014 federal
21 court ruling requiring greater accountability measures in
22 the fishery led to the implementation of more conservative
23 annual catch targets. In subsequent years, recreational
24 fishing seasons in federal waters were as short as 3 to 4 d.
25 However, studies of angler behavior revealed that the
26 shorter seasons did not proportionally reduce catches,
27 instead promoting “derby-style fishing” and worsening
28 perceptions of angler dissatisfaction (Powers and Anson
29 2016; Farmer et al. 2019). In recent years, recreational
30 season lengths have been extended and landings for Red
31 Snapper have been at all-time highs, collectively providing
32 a major source of concern and conflict within the fishery.

33 In 2016, the National Oceanic and Atmospheric
34 Administration Sea Grant invested approximately US\$9.5
35 million (plus an additional \$1.5 million in matching funds
36 for a total budget of \$12 million) to provide an indepen-
37 dent estimate of Red Snapper absolute abundance in the
38 U. S. Gulf of Mexico (MASGP-18-019-). Commonly
39 referred to as the “Great Red Snapper Count” (GRSC),
40 this research was largely in response to both scientific
41 uncertainty and public interest in the Red Snapper fishery.
42 The GRSC was implemented by academic research institu-
43 tions in each of the five gulf states and involved four com-
44 mon components aimed at assessing Red Snapper
45 populations: habitat characterization, direct counts using
46 video, fishing depletion experiments, and tag-and-recap-
47 ture studies. Through working directly with legislators and
48 fisheries managers, the desired outcomes of the study
49 included an improved stock assessment, increased public
50 and scientific confidence in the status of the fishery, and
51 maximum access to the fishery for stakeholders.

52 Notably, the GRSC was designed with an angler
53 engagement priority “to work directly with the gulf fishing
54 community and engage stakeholders”. For instance, the

GRSC’s tag-and-recapture study was modeled after long-
standing and widely popular tagging programs throughout
the Gulf of Mexico, where anglers report data on the tagged
fish they catch. An overarching goal of the GRSC was to
increase public understanding of the scientific tools and
processes involved in estimating fish populations, such as
Gulf of Mexico Red Snapper. One specific effort towards
this goal involved the development of a series of whiteboard
videos describing the GRSC and its various scientific
components. The series of five videos included a project
overview and four more focused videos detailing each of the
GRSC’s scientific methodologies: habitat char-
acterization, video counts, depletion experiments, and tag-
and-recapture studies. While other studies have previously demonstrated
that short educational videos can be effec-
tive tools for promoting stakeholder understanding and management
support (Giglio et al. 2018; Jacobson et al. 2019), these
strategies have not been explicitly tested or evaluated for
diverse and contentious fisheries like Gulf of Mexico Red
Snapper.

In this paper, we describe the results of a gulfwide sur-
vey focusing on four objectives: (1) characterizing the social
dimensions of Red Snapper anglers, such as avidity and
specialization, (2) measuring satisfaction with current Red
Snapper populations and fishing regulations, (3) assessing
overall patterns of awareness of the GRSC, and
(4) evaluating the potential benefits of stakeholder engage-
ment videos using an in-survey experiment.

METHODS

The human subjects research in our study was approved
by Northeastern University’s Institutional Review Board
(IRB # 13-07-16), and informed consent was acquired from
all participants.

Survey instrument and experimental design.— The gen-
eral structure of our survey instrument and experimental
design is shown in Figure 1. After screening for qualified
participants and obtaining informed consent, the first three
sections of the survey were presented identically to all par-
ticipants. The questions in these sections spanned three
general themes: (1) general fishing characteristics (e.g.,
location, specialization, etc.), including the importance of
Red Snapper and other reef fishes as target species, (2)
general attitudes and beliefs towards reef fishes, and (3)
specific attitudes and beliefs regarding Red Snapper,
including awareness of the GRSC.

Next, the fourth section of the survey involved a video
experiment that was designed to evaluate the GRSC stake-
holder engagement videos on the overall GRSC program and
specific research components. For this part of the sur-
vey, we used a split sample design with randomization. First,
each survey participant was randomly assigned to one of
three top-tier treatments, where they were presented

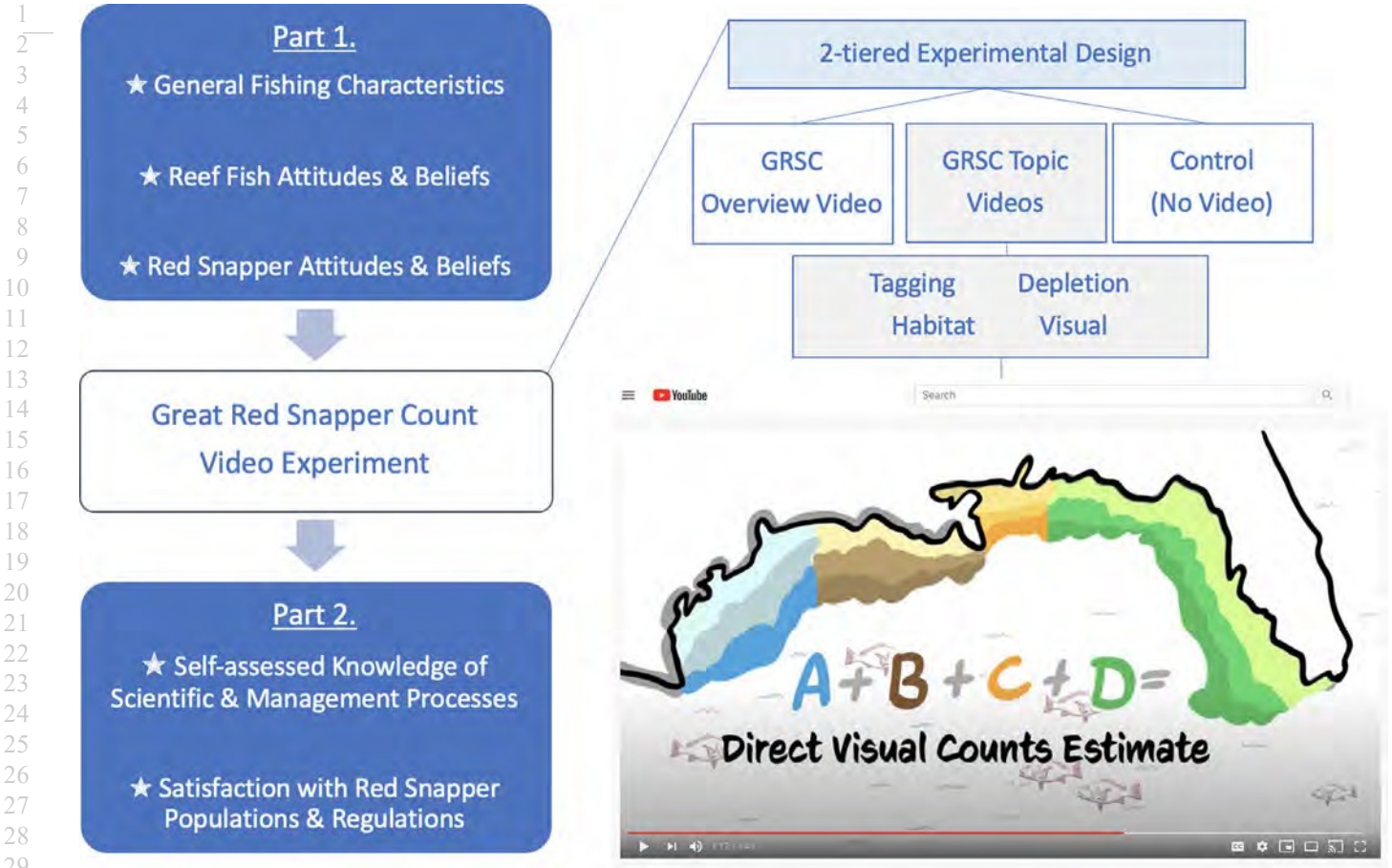


FIGURE 1. Schematic showing the core sections, survey flow, and experimental design of our study. The example video image shows one of the videos developed as part of the stakeholder engagement activities of the Great Red Snapper Count (GRSC).

either a GRSC overview video, a video about a specific research topic, or no video as a control. Within the research topic video treatment, participants were randomly shown one video describing one of the four core project components: habitat characterization, direct counts using video, fishing depletion experiments, and tag-and-recapture studies. All videos are available at <https://www.youtube.com/channel/UCEjpASgofRSoaFvul-N-Kmw>.

Finally, following the video experiment, the survey included two additional sections of questions that were identical for all respondents. The fifth section measured self-assessed knowledge and satisfaction with Red Snapper populations and regulations (Table 1). The sixth block of questions collected demographic information, including age, gender, education, and income. The survey instrument with all the questions described in the paper is provided in the supplement (available in the online version of this article).

Data collection, quality assurance, and quality control.— We used Qualtrics Research Panels to recruit a

sample of 1,000 individuals (200 per gulf state) who salt-water fish in the Gulf of Mexico. Panel samples have rapidly gained popularity over the past decade as a quick and cost-effective approach to online surveys, and Qualtrics Research Panels has been described as among the most robust tools (Zack et al. 2019). As with all nonprobability sampling methodologies, it is important to consider and minimize potential issues of data quality. The panel sample was proportioned to the general public and randomized before the survey was deployed. To evaluate and assure data quality, we applied a multistep process during and after survey implementation. First, we used a self-affirmation screening question, where only participants who committed “to providing their best answers” were allowed to proceed with the survey. Additionally, we included two “attention check” questions to detect “straight-lining” (i.e., respondents who repeatedly selected the same answer), and we set a completion time threshold of 50% of the mean completion time to identify “speeders” (i.e., respondents who rapidly answer questions without closely

TABLE 1. Key concepts and associated questions included in the survey. The survey instrument with all the questions described in the paper is provided in the supplement (available in the online version of this article).

Concept	Question	Responses
Satisfaction with Red Snapper populations	How would you describe your overall level of satisfaction with Red Snapper <i>population levels</i> ?	Very dissatisfied (1) to very satisfied (5)
Satisfaction with Red Snapper regulations	How would you describe your overall level of satisfaction with <i>current fishing regulations</i> for Red Snapper?	Very dissatisfied (1) to very satisfied (5)
Self-assessed scientific knowledge	How would you describe your overall level of knowledge on the <i>scientific processes</i> involved in assessing Red Snapper populations?	Not knowledgeable (1) to extremely knowledgeable (5)
Self-assessed management knowledge	How would you describe your overall level of knowledge on the <i>management processes</i> involved with setting regulations for Red Snapper fisheries?	Not knowledgeable (1) to extremely knowledgeable (5)

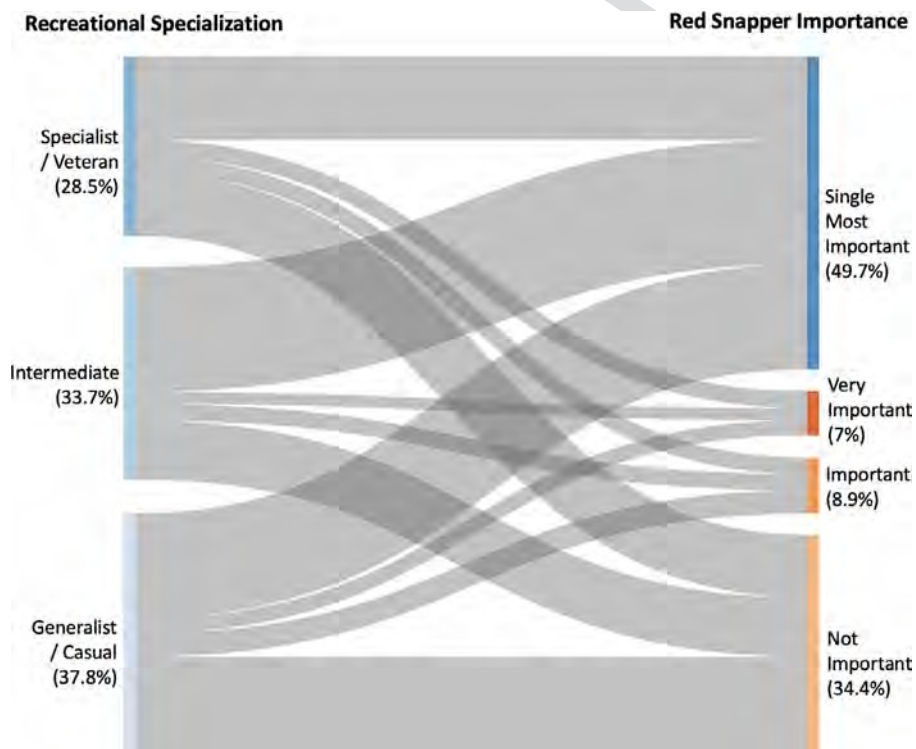


FIGURE 2. Sankey diagram showing relationships between recreational fishing specialization (left) and the importance of Red Snapper as a target species (right). Line width represents the numerical crosstabs between these two survey questions.

reading them) (Zhang and Conrad 2014). After the survey closed, we reviewed all open-ended responses using a three-category system: *definitely bad*, *possibly bad*, or *not suspicious*. All cases of duplicate entry were coded as *definitely bad*. As a second step, we reviewed all *possibly bad* and *not suspicious* responses for duplicate entry, such as a respondent pasting the same answer into multiple questions. From this process, we flagged 16% of responses as *definitely bad* and 11% as *possibly bad*, leaving 73% as *not suspicious*. Following this review, all bad responses were

replaced by Qualtrics and new responses were subsequently reviewed.

Analysis.— We used Fisher's exact tests to assess potential relationships among recreational fishing specialization and Red Snapper importance. We used nonparametric Kruskal–Wallis tests to evaluate whether awareness of the GRSC was associated with differing levels of satisfaction. Among respondents not previously aware of the GRSC, we also used Kruskal–Wallis tests to explore potential influences of the video treatments on self-assessed

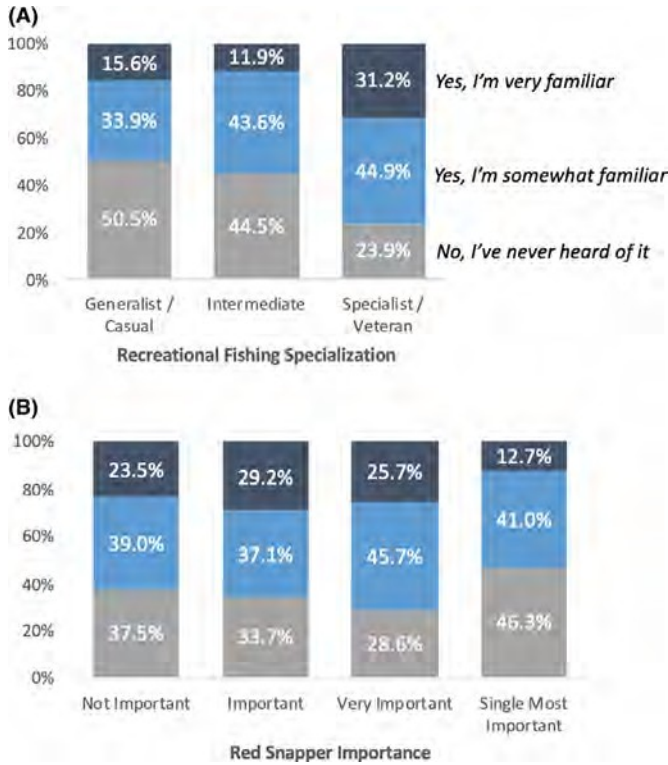


FIGURE 3. Awareness of the Great Red Snapper Count by (A) recreational fishing specialization and (B) the importance of Red Snapper as a target species

knowledge and satisfaction. All data were analyzed using the Statistical Package for the Social Sciences (SPSS version 26), and results were considered statistically significant at $P \leq 0.05$.

RESULTS

Panel Sample Demographics and Fishing Characteristics

All 1,000 anglers in our study had completed at least one saltwater fishing trip within the past 2 years. Compared to the general population of each state, the survey panel sample was generally similar for household income, education, and race. However, as is common in panel surveys, our dataset was overrepresented by female participants. Using a self-classification measure for “recreational fishing specialization” (Needham et al. 2009), 37.8% of anglers were generalist/casual, 33.7% intermediate, and 28.5% specialist/veteran. In the context of all saltwater fishing, offshore fishing for reef fishes was considered extremely important by 25.5%, very important by 25.3%, moderately important by 27.7%, slightly important by 11.2%, and not at all important by 10.3%. Among a list of 32 reef fishes, Red Snapper was considered the most important reef fish species, with 65.6% of anglers considering it at least “important” for their fishing and among these 49.7% considering it the single most important species.

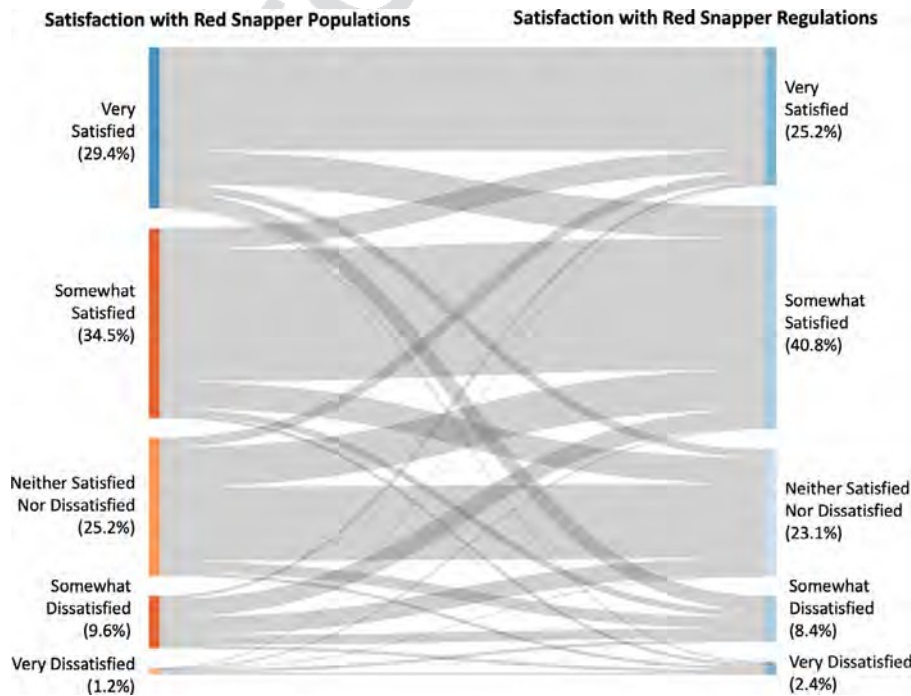


FIGURE 4. Sankey diagram showing relationships between satisfaction with current Red Snapper populations (left) and satisfaction with current Red Snapper regulations (right). Line width represents the numerical crosstabs between these two survey questions.

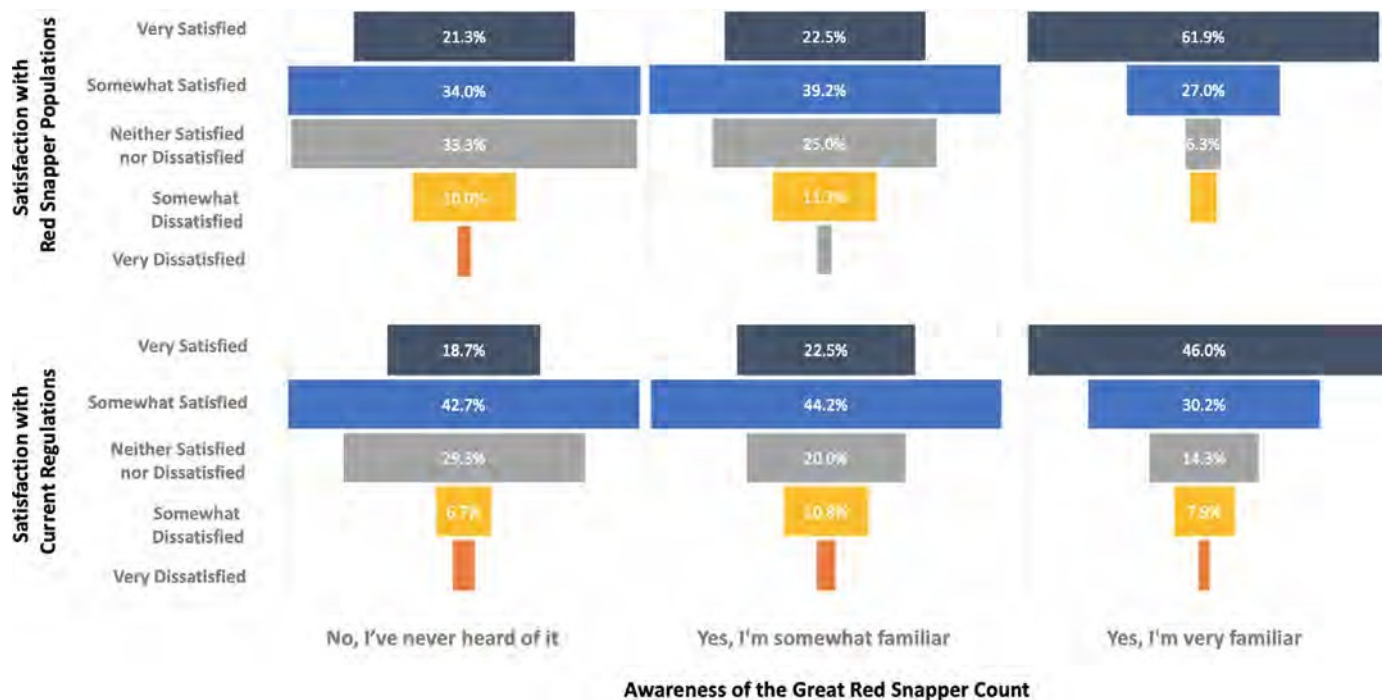


FIGURE 5. Funnel plots showing the categorical response to survey questions measuring angler satisfaction with Red Snapper populations (top row) and current regulations (bottom row) across categories of awareness of the Great Red Snapper Count.

We calculated crosstabs and created a Sankey plot to visualize the relationship between recreational fishing specialization and Red Snapper importance (Figure 2). Among anglers who considered Red Snapper as their single most important target species, 26.6% self-classified as specialist/veteran, 39.8% as intermediate, and 33.6% as generalist/casual anglers. From the sorting direction of recreational fishing specialization, Red Snapper was considered the single most important target species among 46.3% of specialist/veteran, 58.8% of intermediate, and 44.2% of generalist/casual anglers.

Awareness of GRSC and Satisfaction

Overall, our results indicate that roughly 60% of anglers were aware of the GRSC prior to taking the survey, with 18.8% stating they were very familiar (Figure 3A). Among the four core GRSC components, overall awareness of the tagging and rewards program was the highest at 35.2%, followed by habitat characterization (21.5%), visual and camera fish counts (21.2%), and fish depletion experiments (17.7%). Overall GRSC awareness generally increased with recreational fishing specialization, with 76.1% of specialist/veteran anglers at least somewhat familiar with the program and 31.2% very familiar. However, awareness across categories of Red Snapper importance was more complex, with the lowest familiarity existing among the group of anglers that considers Red Snapper as their most important target species.

Two other core questions in our survey measured angler satisfaction with current Red Snapper populations and current regulations. To assess overall patterns of satisfaction, we looked at responses among anglers within the control treatment (i.e., respondents who did not view any videos during the survey). We found that most of these anglers were satisfied with both current populations and regulations; moreover, these factors were significantly related ($\chi^2 = 202.991$, $df = 16$, $P < 0.001$; Figure 4).

Overall, our results show that angler awareness of the GRSC was positively associated with higher satisfaction with both Red Snapper populations (Figure 5; $n = 333$, $H = 36.751$, $df = 2$, $P < 0.001$) and current management (Figure 5; $n = 333$, $H = 11.535$, $df = 2$, $P = 0.03$). For satisfaction with Red Snapper populations, there were large differences across categories, with 62% of individuals very familiar with the GRSC reporting that they were very satisfied with Red Snapper populations compared with only 21% of individuals who had never heard of the GRSC. Likewise, for satisfaction with current regulations, there were also substantial differences across awareness levels, with 46% of individuals very familiar with the GRSC also very satisfied with current regulations compared with only 19% of individuals who had never heard of the program.

Stakeholder Engagement Video Experiment

Our survey design involved an experiment to assess potential influences of the GRSC angler engagement

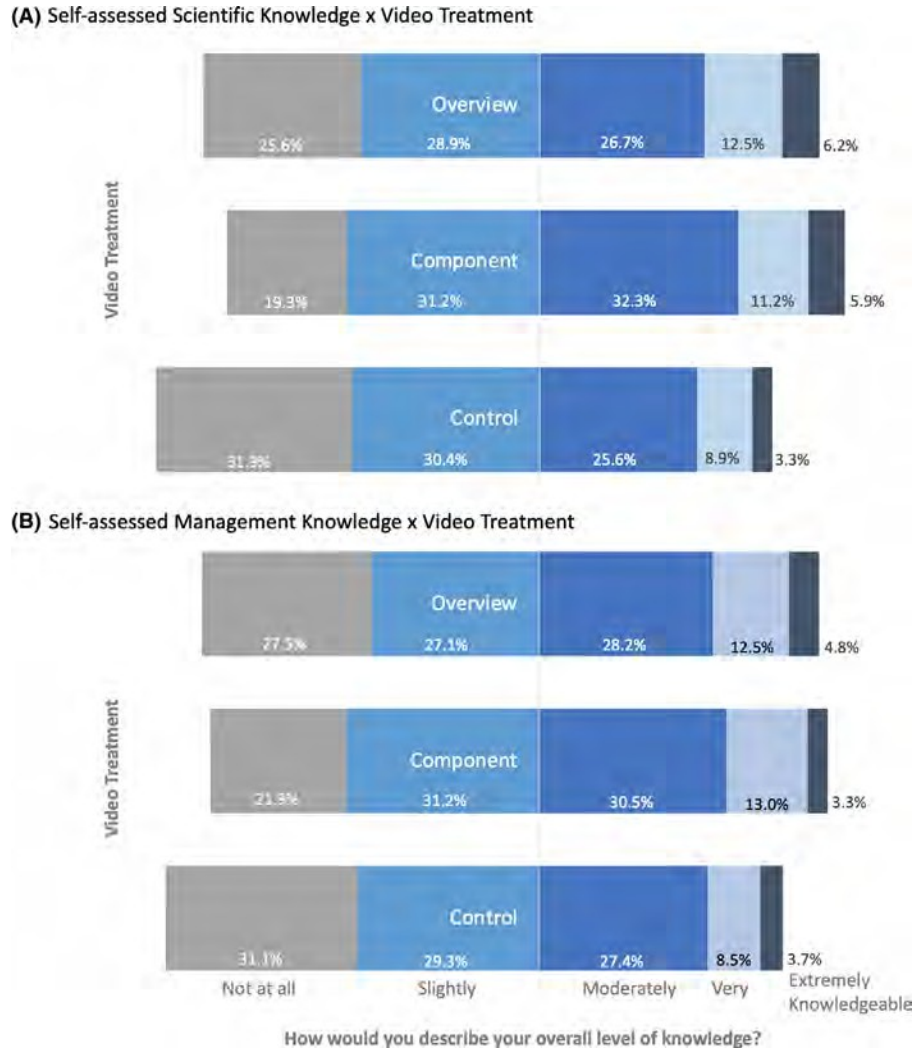


FIGURE 6. Categorical response to survey questions measuring self-assessed angler knowledge of (A) scientific processes and (B) management processes across video treatments.

videos on angler knowledge and satisfaction. Among survey participants who were somewhat or not at all familiar with the GRSC prior to taking the survey, our analyses found that respondents in video treatments self-rated their knowledge of scientific processes significantly higher ($n = 812$, $H = 11.734$, $df = 2$, $P = 0.003$) and their knowledge of management processes marginally higher ($n = 812$, $H = 5.428$, $df = 2$, $P = 0.066$) than respondents in control treatments (Figure 6).

We also compared satisfaction levels across treatments in our video experiment. In this context, video experiment treatment was associated with satisfaction with current regulations ($n = 812$, $H = 7.362$, $df = 2$, $P = 0.025$) but not satisfaction with population levels ($n = 812$, $H = 0.293$, $df = 2$, $P = 0.864$) (Figure 7). When comparing patterns across the specific component videos, some additional trends were

visible. For instance, satisfaction with Red Snapper populations was qualitatively highest among the group of individuals presented a short video about the habitat characterization component of the GRSC at 74.6% compared with 58.2% among those not shown a video as part of the control treatment. Similarly, the four component video treatments qualitatively aligned as having the highest levels of satisfaction with current regulations.

DISCUSSION

As one of the most socially important and economically valuable fisheries in the Gulf of Mexico, Red Snapper poses many challenges for scientists and managers (Cowan et al. 2011; Powers and Anson 2016; SEDAR-52 2018). Consequently, the overarching goal of the GRSC

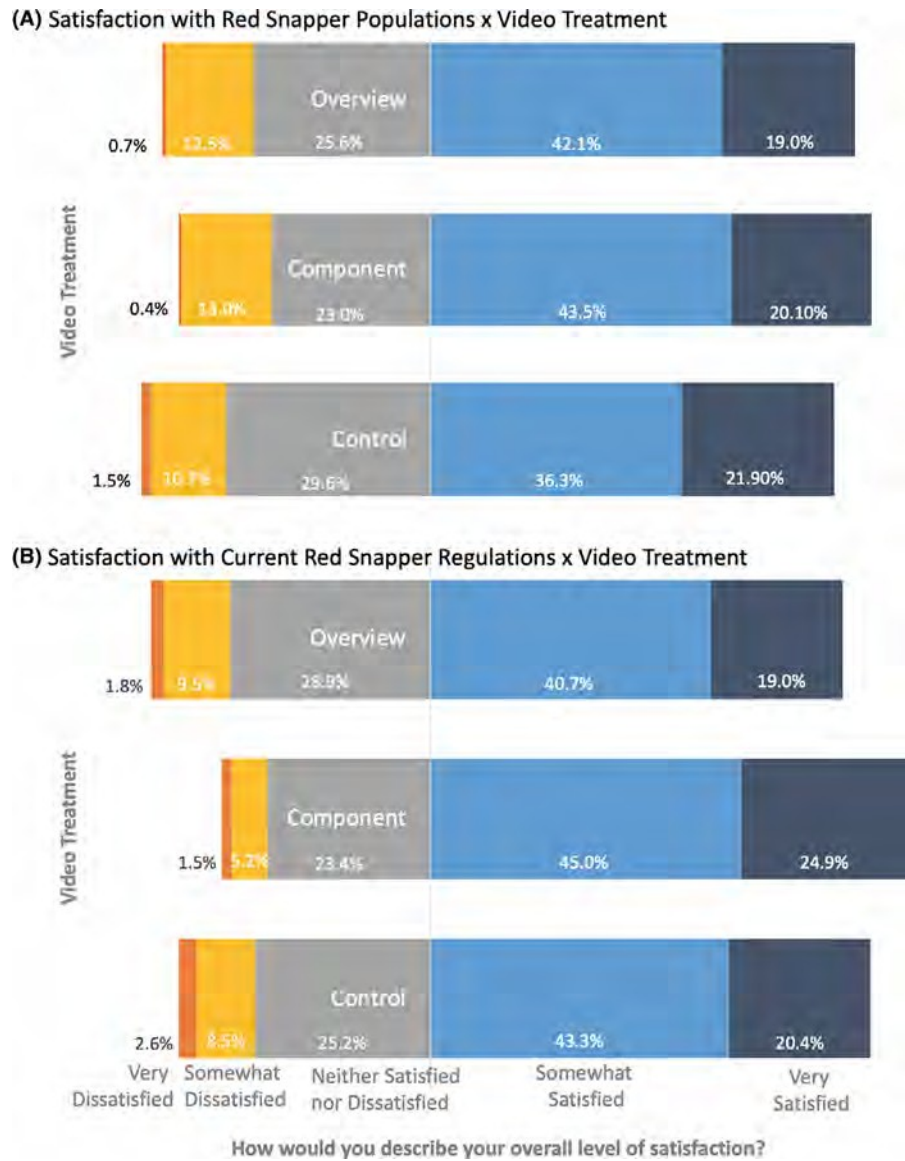


FIGURE 7. Categorical response to survey questions measuring angler satisfaction with Red Snapper (A) populations and (B) current regulations across video treatments.

was to reduce public uncertainty on the status of Gulf of Mexico Red Snapper populations. A top priority of our study focused on understanding how these issues, and the GRSC initiative, were perceived by Gulf of Mexico anglers. In particular, the video experiment component of our study presented a unique opportunity to test how specific stakeholder engagement materials influenced self-assessed angler knowledge and satisfaction. From our survey results, we identified a series of key findings relevant to the current management of Gulf of Mexico Red Snapper.

Awareness of the GRSC was generally associated with higher satisfaction with Red Snapper fisheries. As

expected, GRSC awareness was highest among the most avid and specialized anglers who consider fishing to be their primary outdoor activity. Given the widespread use of social media among this subset of the fishing community (e.g., fishing forums), high awareness among this group was not particularly surprising. Conversely, however, the lowest awareness of the GRSC was among anglers who considered Red Snapper to be their most important target species. One plausible explanation for this pattern is that many casual anglers only saltwater fish a few times per year, for example during summer vacations to coastal areas, yet many of these individuals consider Red Snapper as very important for their fishing

satisfaction. Given the diverse constituency of the Red Snapper fishery, adequately engaging all of these stakeholders presents a substantial challenge. However, our results highlight the need to understand and connect with these individuals.

Another key finding of our study emerged from the video experiment. We found that anglers that were presented a video on specific GRSC project components reported higher scientific knowledge and higher management satisfaction than individuals presented an overview video or no video. While the project overview video provided the most comprehensive project description, one potential explanation for this pattern is that anglers may desire both in-depth yet understandable insight on the scientific methodologies for assessing fish populations. For instance, while modern stock assessments are generally transparent (e.g., the SEDAR process), the assessments themselves are incredibly complex and focus on data analyses.

A number of other studies have also shown that educational videos can be effective tools for promoting management support and conservation objectives (Giglio et al. 2018; Jacobson et al. 2019). For instance, Giglio et al. (2018) conducted a video experiment with recreational scuba divers and found that divers who were shown an educational video were more likely to implement conservation-oriented diving behaviors than a control group. In another study, Jacobsen et al. (2019) used short 1–2-min videos in a large experiment of college students and found that positively framed messages were more effective at motivating willingness to donate money to conservation organizations than negatively framed videos. In our study, it is worth noting that the overview video was more negatively framed than the component videos as it highlighted the general landscape of angler dissatisfaction.

Angler engagement and participation have been widely described as key components of satisfaction (Arlinghaus 2006; Hutt and Bettoli 2007; Beardmore et al. 2015; Crandall et al. 2019). Considering that the tagging and rewards component of the GRSC had the highest awareness, it is important to recognize that the GRSC is a short-term program built upon many previous and ongoing fisheries-independent research studies (Scott et al. 1990; Sackett and Catalano 2017; Grüss et al. 2018). For instance, the fishery for reef fish in the Gulf of Mexico has a long history of engaging and relying on anglers for the success of tagging programs (Szedlmayer and Shipp 1994; Patterson et al. 2001) and other management strategies that provide relatively high buy-in through angler participatory opportunities (Scyphers et al. 2013; Crandall et al. 2017). However, satisfaction is most common when angler engagement or input in management processes is followed by meaningful action (Crandall et al. 2019), as well as when the expected benefits of proposed management adjustments are clear and realistic (Seeteram et al. 2019).

One important consideration for interpreting our study is understanding our survey methodology using Qualtrics Panels, which has several key strengths but also a few known limitations. For instance, the overarching strength of our approach was the ability to rapidly and cost-effectively survey diverse anglers engaged in Red Snapper fisheries in the Gulf of Mexico across multiple states. For instance, when compared with email-based surveys, our study was not limited to anglers who met licensing criteria, which vary across states and many saltwater anglers are not required to purchase licenses. Moreover, when compared with address-based mail sampling, our approach targeted a similarly broad population of coastal anglers yet was significantly faster and more cost-effective. Some criticism of nonprobability survey panels, such as Qualtrics Panels, focus on their representativeness (Zack et al. 2019); however, recent studies have increasingly shown that effective panel design and sampling can lead to robust and representative samples, with many of these studies involving Qualtrics Panels (Harlan et al. 2019; Boas et al. 2020; Miller et al. 2020).

In summary, recreational fishing satisfaction is complex, multidimensional, and generally defined as “the difference between the outcomes an angler desires or thinks should be received and the perceived fulfillment of the desired outcomes” (Fedler and Ditton 1986; Graefe and Fedler 1986). The GRSC was designed and implemented to reduce public consternation on the population size and sustainability of Red Snapper. Our survey results indicate that the program may have had ancillary benefits for fisheries management by increasing satisfaction among anglers, at least initially, independent of those biological outcomes. However, it is also important to consider that recently increased season lengths and high catch rates are likely underpinning the currently high satisfaction with Red Snapper populations and regulations. In the broader perspective and longer term, angler satisfaction is likely to continually evolve with perceptions of management and access to the fishery.

ACKNOWLEDGMENTS

This study was funded by Mississippi–Alabama Fisheries Sea Grant as a postaward supplement to the Great Red Snapper Count. We thank D. Kulaw, S. Sagarese, and M. Jepson for reviewing our survey, as well as N. Yoon for assistance. There is no conflict of interest declared in this article.

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SUPPORTING INFORMATION

Additional supplemental material may be found online in the Supporting Information section at the end of the article

E. Phase I Workshop Report, Phase I & II Request for Proposals

Phase I Red Snapper Experimental Design Workshop Summary Report

January 10-12, 2017 New Orleans, Louisiana

Workshop Scope

A 2.5-day workshop (Appendix 1) was held in New Orleans, Louisiana, on January 10-12, 2017, to discuss six experimental designs developed through a competitive request for proposals (RFP) issued on May 16, 2016 (Appendix 2). During the workshop design recommendations were developed and will be used as a basis for a \$12-million funding request to conduct a one-time estimate of age 2+ red snapper by habitat type in U.S. Gulf of Mexico waters (Appendix 1).



Figure 1. Workshop participants on January 10, 2017. The workshop was sponsored by the Mississippi-Alabama Sea Grant Consortium on behalf of the National Oceanic and Atmospheric Administration's (NOAA) National Sea Grant College Program and NOAA National Marine Fisheries Service.

The six experimental design reports were submitted to the Mississippi-Alabama Sea Grant Consortium (MASGC) on December 5, 2016. Four fishery stock assessment experts reviewed the experimental design reports using review criteria provided by the project steering committee. Each expert reviewer prepared a written assessment of each report. The steering committee also reviewed the six experimental design reports and the assessments of them prepared by the four expert reviewers. The final experimental design will be guided by the six experimental design reports, expert reviews of design reports, and the project steering committee.

This workshop summary includes recommendations for the final experimental design. This report and the six experimental design reports are available at: <http://masgc.org/red-snapper>.

Workshop Participants

Workshop participants (Appendix 3) included 57 university scientists, state fisheries agency scientists and managers, Sea Grant and National Oceanic and Atmospheric Administration (NOAA) National Marine Fisheries Service (NMFS) employees, and fisheries consultants. Each of the five Gulf of Mexico states was represented.

Background

In FY 2016, Congress directed the National Sea Grant College Program to use \$5 million of its budget to support Gulf of Mexico red snapper fisheries data collections, surveys and assessments, independent of NMFS stock assessment and related efforts. Congress also directed NMFS to use \$5 million of its FY 2016 appropriation for complementary research on red snapper, including applications of advanced sampling technologies potentially useful in improving red snapper stock assessments. Sea Grant and NMFS are working together through a joint steering committee to design an effective research program and to ensure its results can be used to develop an independent estimate of Gulf of Mexico red snapper stock abundance.

In March 2015, more than 60 people from academia, state management agencies, commercial and recreational fishing sectors, NMFS and Sea Grant attended a workshop in New Orleans, Louisiana. Among them, they had more than 1,000 years of red snapper work experience. The purpose of the workshop was to identify and prioritize research and data collection efforts that would improve the accuracy of Gulf of Mexico red snapper stock assessments. Workshop recommendations focused on creating a Gulf of Mexico-wide tagging and advanced technology program capable of accurately sampling red snapper abundance across several different habitat types.

The recommendations from the March 2015 workshop formed the basis of an MASGC competitive RFP (Phase I) to describe alternative experimental designs for use in a large-scale study to determine red snapper abundance (Phase II). Six Phase I experimental design projects received funding totaling \$543,763.

Workshop Content Summary

Day 1

Day 1 consisted of one-hour presentations from each of the six experimental design project teams. Key points from each team presentation are provided below.

Red Snapper Data Collection Spatial Modeling and Population Assessment in Northern Gulf of Mexico. Principal Investigator: Peter Rubec, Florida Fish & Wildlife Conservation Commission.

1. Collect catch, effort and size composition data for red snapper on commercial and recreational vessels from Florida to Texas and associated environmental data (temperature, depth, bottom type).
2. Map bottom circulation, bottom type and bathymetric habitats.
3. Develop Habitat Suitability Models (HSM) that relate catch rates to environmental conditions.

4. Link the HSM to the habitat maps and create maps of abundance (based on Catch Per Unit Effort-CPUE indices) using GIS.
5. Estimate seasonal population numbers for juvenile and adult red snapper from the abundance maps.
6. Apply operations management to plan, organize and coordinate estimation of the red snapper population in the U.S. Gulf of Mexico.

Change-in-Ratio Methods for Estimating Recreational Exploitation Rate and Absolute Abundance of Gulf of Mexico Red Snapper. Principal Investigator: Sean Powers, University of South Alabama.

1. The project team recommended dividing Gulf of Mexico red snapper habitats into three types:
 2. Artificial reef structures, platforms and other known areas of high fish density
 3. Known, natural, low relief reefs
 4. Areas of featureless or unknown bottom type

Based on habitat type, the team proposed using change-in-ratio (CIR), index-removal (IR) and removal estimators to estimate red snapper abundance. The CIR and IR methods involve a survey, a partial depletion of the population or a component of the population (e.g., legal-size animals) and a post-depletion survey. The removal method includes a series of two or more fishing (e.g., longline) sets at each sampling location and noting the progressive decline in catch per set.

Design of a Multidisciplinary Study to Estimate Red Snapper Population Size, Population Connectivity, and Mortality Rates in the US Gulf of Mexico. Principal Investigator: James Cowan, Louisiana State University.

The project team undertook a simulation modelling exercise as the basis for its recommended sampling design. The team focused its effort on obtaining a Gulf-wide estimate of abundance while addressing the need to consider habitat stratification in its design.

The team identified the sample universe using generalized additive model (GAM) to calculate the sampling framework and set of sampling methodologies; and provided a discussion of the interaction between sample costs and levels of uncertainty. Team members proposed to estimate red snapper populations using tag-recapture and video/acoustic methods. They identified assumptions that could affect the uncertainty of the results and identified how their results could be transformed into an estimate of absolute abundance of red snapper.

A summary of key points from the presentation include:

1. Estimate the population size of age 2+ red snapper in the U.S. waters of the Gulf of Mexico using tagging and video-based counts.
2. Divide the northern Gulf into 3 arc-second squared sampling units (~35 million) between 10 and 160 m depths. These sampling units would then be partitioned into 15 strata representing broad boundaries from west to east and 3 depth zones (10-40 m, 40-100 m, 100-160 m). Plotted shipwrecks, obstructions, oil rigs.
3. Create density estimates of red snapper from ROV, acoustic, and catch surveys.
4. Use a delta log-normal generalized additive model (GAM) to estimate expected red snapper density based on physical characteristics. Used conventional and genetic mark recapture methods.
5. Discussed sampling design and evaluation.

An Stratified Random Survey, Tagging Study (Conventional and Telemetry), Fish Health Evaluation, and Genomics Study of Red Snapper, *Lutjanus campechanus*, in the Northern Gulf of Mexico. Principal Investigator: Stephen Szedlmayer, Auburn University.

The project team would carry out a Gulf-wide fishery independent survey of red snapper using a stratified random sampling of three depth strata. Two sampling approaches were recommended. The first would use hydroacoustics and remotely-operated vehicle camera surveys and side-scan sonar. Age and growth analyses would be included in this approach. The second approach would consist of a tagging study using telemetry, conventional tagging and environmental DNA (eDNA).

An Experimental Design to Estimate Absolute Abundance of Red Snapper in the U.S. Gulf of Mexico. Principal Investigator: Greg Stunz, Texas A&M University.

The project team included the following design recommendations:

1. A stratified random sampling framework using four ecological regions and two sub regions with three depth strata.
2. Abundance estimates – Advanced technologies:
 - a. ROV paired with bioacoustics
 - b. Camera-Based Assessment Survey System (C-BASS)
3. Directed Studies:
 - a. High-reward tag-recapture
 - b. Change-in-ratio
 - c. Fixed cameras
 - d. Vertical and bottom longline
 - e. Catch-survey-catch methodology
4. Biological sample collection
5. Design optimization tool
 - a. Coefficient of variation (CV) and cost estimates
 - b. Scalable without sacrificing geographic coverage

Methods for the Determination of High Precision Estimates of Red Snapper Abundance in the Gulf of Mexico. Principal Investigator: Robert Leaf, the University of Southern Mississippi.

The Leaf team described the expected precision of a regional abundance estimate of age-2+ red snapper using a two-year conventional tagging and recapture study. The team constructed an individual-based simulation model parameterized using values derived from expert opinion and the literature. The team analyzed the binary-recapture probabilities in different experimental design scenarios and characterized the associated outputs.

Morning of Day 2

During the morning of Day 2, project teams, steering committee members and external report reviewers discussed the main points of each report. Several challenges were identified during the discussion in designing a protocol(s) to comprehensively and accurately sample red snapper in the Gulf of Mexico (Phase II). Five grand challenges were identified:

1. Funding: Cost estimates from the reports ranged from \$6-30M.
2. Time frame: Completing the Phase II project within one year is extremely unlikely. At least two years would be needed for a comprehensive study, including adequate time for data analysis.
3. Habitat mapping: Identifying all red snapper habitat in the Gulf of Mexico will be costly. The use of existing red snapper habitat mapping data could reduce the cost.
4. Overall complexity of program: Managing a regional, multi-institutional consortium will be an important component of Phase II.
5. Stakeholder engagement: At least one team identified the need for better stakeholder engagement on red snapper stock assessments. One reviewer pointed out the need for adequate stakeholder engagement across all program aspects (e.g., design, implementation and analysis). Without adequate stakeholder engagement, the validity of the final survey results will be challenged. Over the long-term, increased stakeholder engagement, science synthesis and communication is needed.

Afternoon of Day 2 and Morning of Day 3

After lunch on Day 2, all project team participants departed. The steering committee and external report reviewers began the process of synthesizing the results from the six experimental design reports and recommending final design components for the Phase II study. The focus during the afternoon of Day 2 and morning of Day 3 was to critique design criteria identified during the workshop. By the end of the workshop, the steering committee and external report reviewers drafted recommendations based on the six experimental design reports, discussions among workshop participants, expert written reviews of each design and discussion between expert reviewers and the steering committee.

The recommendations are organized into five sections: general, geographic scale and sampling depths, habitat types, working with fishing industries and sampling methods. Key points under each section are provided below. NOTE: The final recommendations will be included in the Phase II RFP.

General

1. A single RFP for \$12 million (including \$2.5 million in required non-federal match) is recommended. The RFP should require a project design that includes mark-recapture tagging and advanced sampling technology.
2. Lead investigators must be from a university within the Gulf of Mexico region.
3. A Letter of Intent (LOI) will be required to submit a proposal. The LOI will allow MASGC the needed time to identify reviewers while full proposals are being developed.
4. A full proposal narrative of no more than 25 pages should be adequate to allow investigators to fully describe their approach.
5. In addition to the 25-page narrative, include:
 - a. A two-page description of how the project consortium will be managed
 - b. A two-page description of how an additional \$10 million in funding would be used to increase the precision of the stock assessment. There is a possibility of an additional \$10 million being made available to this effort under the FY 2017 appropriations to NOAA's NMFS and the National Sea Grant College Program. As of the writing of this summary report, these appropriations are not yet made.
6. Investigators will have three months to put together the Phase II proposal.
7. Project duration can be up to 2 years: 6 months to prepare and 18 months to implement. Additional time may be needed for complete data analysis.
8. A coefficient of variation (CV) of 0.3 for the abundance estimate is the planning target and the proposals should describe the expected precision of the proposed approach.
9. Any sampling method providing a relative abundance must be converted to absolute abundance of 2+ red snapper as both total numbers of fish at length and total biomass.
10. Proposals must include methods and approaches to account for fish growth, recruitment, movement and mortality over the survey period.
11. Proposals must include approaches for collecting biological samples during surveys (e.g., otoliths, tissues for genetics, reproductive tissues)
12. Proposals must include a data management plan to store, access and protect raw and processed data.
13. Fish health studies are a low priority because project funding is limited and disease work may not contribute directly to a stock assessment.
14. Future developments in genetic sequencing may mean genetic tagging will become a viable and cost effective individual marking option for red snapper.

Geographic Areas and Sampling Depths

15. Utilize 2-4 geographic areas (Appendix 4 provides an example from Stunz, et al.). At a minimum, the study should be divided into an Eastern and Western Gulf sub-regions with the division being made near the Mississippi River. Two additional strata per sub-region should be considered for the purposes of looking at spatial difference in age structure, growth and mortality. Rationale should be provided for proposed boundaries to be able to detect differences between strata.
16. Eastern boundary of the study areas should be the Dry Tortugas and the western boundary is Texas border with Mexico.
17. Sampling should be within the depth range of 10-150 meters.

Habitat Types

18. Habitat suitability maps (HSMs) are not sufficiently comprehensive to provide complete mapping of red snapper habitat. However, HSMs may be appropriate to inform targeted sampling.
19. At a minimum, there should be three habitat classifications:
 - a. Known artificial reefs
 - b. Known natural reefs
 - c. Unknown bottom: unconsolidated uniform bottom, unknown natural reefs and unknown artificial structure.
20. Include sources of locations of known natural and artificial reefs and include a description of the process for identifying habitat types to be sampled.
21. Proposals must include a sampling plan to support the spatial allocation of sampling and to estimate the expected precision of the results.
22. Proposals are not to include further habitat mapping. However, the successful project team will seek out high-resolution habitat maps to leverage the funds available for this program. A component of the proposal could include the synthesis of habitat maps from various sources.
23. Reserve around \$200K to develop a model-based approach at the end of the project to identify future stock assessment strategies (multi-method/multi-area).

Working with the Fishing Industries

24. Strongest proposals will work directly with the commercial and recreational fishing industries. Fishermen should be included from the start of program. It is possible to hire commercial fishermen and allow them to catch fish and then sell them under the red snapper Individual Fishing Quota (IDQ) program. This approach could lower vessel use costs. It also is possible for charter boat captains to lease quota from commercial fishermen. This would require an exempted fishing permit from the NMFS Southeast Regional Office.
25. Strongest proposals will include a communication strategy to ensure the fishing community, resource managers and other stakeholders are regularly updated on the status of the project.

Sampling Methods

26. Sampling methods must involve the use of advanced technologies (acoustics and video), depletion ratio surveys, and mark-recapture.
27. Regardless of the technology used, validation and bias correction of the principal survey method will be essential using a combination of fish capture (hook-and-line) and video methods when acoustics is the primary method, and hook-and-line when video is the primary survey method.
28. For all methods, investigators will need to provide detailed steps for calibration and how to avoid biases and address uncertainties.
 - a. A sample size to cost determination should be included using an approach like Cowan, et al. (Appendix 5).
 - b. A simulation analysis must be conducted and results included in the proposal to understand the sensitivity of the estimates to some of the more obvious sources of bias associated with a mixed survey spatial allocation design.
29. The use of mark-recapture will be essential to:
 - a. Determine local scale abundance estimate validation, accounting for selectivity bias
 - b. Growth, movement and mortality estimation.
30. The steering committee does not recommend mark-recapture methods as the primary Gulf-wide method for two reasons:
 - a. It is unlikely to be feasible to tag and release snapper over depth ranges greater than 75 meters with conventional tagging technologies due to high barotrauma mortality.
 - b. It is doubtful a Gulf-wide mark-recapture program could achieve a sufficiently random distribution of tags across the whole region.
 - i. Random distribution would be necessary because red snapper do not move rapidly enough to mix throughout the Gulf,
 - ii. Red snapper fishing is concentrated in localized areas of the Gulf.
31. Recommended sampling methodologies by habitat type. Regardless of survey method, all surveys should be completed within a few months to minimize possible bias from movement, mortality, recruitment and growth.
 - a. Known natural and artificial reef habitats: Depletion and mark-recapture methods emerged as the most appropriate methods to implement on known artificial and known natural habitats. The depletion method is based on short-term depletion experiments on the selected habitats. The mark-recapture method would require the use of acoustics as the primary source of quantitative abundance data. The depletion approach could be complemented by simultaneous mark-recapture methods. The acoustic method would need to be paired with another method like optical methods fish collection by hook-and-line.
 - i. As a minimum mark-recapture designs will need to account for known sources of bias (e.g., tag-loss, release mortality, trap-shyness).

ii. Where possible the fishing industry should be involved in tag recovery, including so investigators should consider high-dollar tag rewards (\$25-50K) in their proposal budget.

ii. Different sizes and types of natural and artificial reefs have different average numbers of red snapper. The proposal must address these differences in selecting habitats to sample.

b. Unknown habitat: This strata is the large majority of the bottom area in the Gulf of Mexico and fish numbers per unit area are expected to be much lower than on the reefs. However, the large areas mean that total red snapper abundance will be significant. Survey methods used must be able to cover large areas, and must be able to accurately measure the number of fish per unit area. Towed video or towed acoustic technologies are recommended for unknown habitats

i. Fish, including red snapper, react to observing platforms. Proposals must address the calibration of towed technology to calculate the number of red snapper per unit area sampled.

ii. The unknown habitat areas will likely have large numbers of unknown high density reefs

iii. Known natural and known artificial reefs and structures falling within the unknown habitat type will need to be excluded from the unknown habitat estimate. An alternate random survey position should be used when a random survey site falls on a known reef complex. Likewise, the physical area (and or number) of known reefs must be removed from the total unknown habitat area estimate prior to scaling up the survey estimates.

Phase II Project Timeline

RFP released in late February or early March 2017

Proposals due June 2017

Proposal reviews July 2017

Technical review panel meeting in mid-August 2017

Project start October 2017

Project end date September 2019

Next Steps

A RFP will be released to implement the stock assessment (Phase II). It is expected that this RFP will include both tagging and advanced technology components. Total funding for the stock assessment will be up to \$9.5 million plus \$2.5 million in non-federal match for the Sea Grant share. Selection of the successful Phase II proposal is expected by September 2017, and work on this project will begin in October 2017. This one-time estimate will be considered an independent Gulf-wide red snapper stock abundance estimate.

There were three short-term action items:

1. Deciding if the Sea Grant and NMFS funding can be combined into a single RFP.
2. Send the four written reviews conducted by the external review team to the principal investigators of each of the six experimental design reports.
3. Produce a workshop summary (this report). The report will be sent to workshop participants and published on MASGC's web and social media sites.
4. Draft Phase II RFP by end of January.

Conclusions

The workshop confirmed the complexity of a project of this scale. It is an ambitious undertaking. Although unprecedented and challenging, a one-time estimate of absolute abundance by habitat type can produce reliable and valid results. To succeed, a well-thought-out research program using appropriate tagging and advanced technology methods will require excellent science, multi-institutional collaboration and strong project management abilities.

Appendix 1: Workshop Agenda

Phase I Red Snapper Workshop Agenda

January 10-12, 2017

Hilton New Orleans Riverside Two Poydras Street

New Orleans, LA 70130

Tuesday, January 10		
9:00 a.m.	Registration	Kay Bruening
9:30	Introductions, Workshop Goals, Agenda Overview	LaDon Swann
	Presentations by Project Teams	Jim Berkson
10:00	Red Snapper Data Collection Spatial Modeling and Population Assessment in Northern Gulf of Mexico	Peter Rubec
11:00	Change-in-Ratio Methods for Estimating Recreational Exploitation Rate and Absolute Abundance of Gulf of Mexico Red Snapper	Sean Powers
Noon	Lunch	
1:00 p.m.	Design of a Multidisciplinary Study to Estimate Red Snapper Population Size, Population Connectivity, and Mortality Rates in the US Gulf of Mexico	Rob Ahrens
2:00	A Stratified Random Survey, Tagging Study (Conventional and Telemetry), Fish Health Evaluation, and Genomics Study of Red Snapper, <i>Lutjanus campechanus</i> , in the Northern Gulf of Mexico.	Steve Szedlmayer
3:00	Break	
3:30	An Experimental Design to Estimate Absolute Abundance of Red Snapper in the U.S. Gulf of Mexico	Greg Stunz
4:30	Methods for the Determination of High Precision Estimates of Red Snapper Abundance in the Gulf of Mexico	Robert Leaf
5:30	Closing comments	Jim Berkson and others

Wednesday, January 11		
8:00 a.m.	Welcome and recap	LaDon Swann
8:15	Group Discussion Common ground among designs Identify essential design elements Discuss implementation challenges	
10:15	Break	
10:30	Group Discussion (continued)	
Noon	Lunch with everyone	
1:30 p.m.	Reviewers and project steering committee reconvenes	
	Discussion with report reviewers and steering committee	LaDon Swann
3:00	Break	
3:30	Develop initial draft of Phase II design	
5:00	Discuss framework for Thursday morning	LaDon Swann
5:15	Adjourn for the day	
Thursday, January 12, 2017		
8:30 a.m.	Report reviewers and steering committee reconvenes to discuss Phase II design	LaDon Swann
10:00	Break	
10:15	Finalize Phase II design	
Noon	Lunch and Adjourn	

Experimental Design Request for Proposals

Request for Proposals: Red Snapper (*Lutjanus campechanus*)
Experimental Design for Population
Estimates in the Gulf of Mexico Region



Funding Opportunity Title: Fiscal Year (FY) 2016 Red Snapper (*Lutjanus campechanus*)
Experimental Design for Population Estimates in the Gulf of Mexico Region

Funding Source: The National Oceanic and Atmospheric Administration's (NOAA) National Sea Grant College Program and NOAA Fisheries. The research competition will be managed by the Sea Grant Programs in the Gulf of Mexico region.

Announcement Type: Notice of request for proposals (RFP)

Release Date: May 16, 2016

Funding Opportunity Summary: This notice advises the public that the Mississippi-Alabama Sea Grant Consortium (MASGC), on behalf of the four Sea Grant Programs in the Gulf of Mexico region and NOAA Fisheries, is accepting proposals to develop an experimental design(s) that will be incorporated into larger advanced technology and mark-recapture requests for proposals planned for Fiscal Year 2017 (Federal). The design will be used to assess the population of red snapper on artificial reefs and other structures and as the basis for a Gulf-wide estimate (with estimates also produced for natural habitats) of absolute abundance. The design may include traditional tagging methods and/or advanced technology for large-scale field projects to be used in red snapper stock assessments. Project initiation is scheduled for September 2, 2016. Award period is September 2, 2016, through December 5, 2016, no extensions.

Eligibility: MASGC welcomes proposals from individuals, institutions of higher education, nonprofit organizations, businesses, and tribal, state and local governments. The proposal principal investigator (PI) must be located within a U.S. Gulf of Mexico state. Co-investigators

may be located in other U.S. regions. Federal partners may participate as uncompensated collaborators. No person shall be excluded on grounds of race, color, age, sex, national origin or disability from participation in, denied benefits of, or be subjected to discrimination under any program or activity receiving financial assistance from MASGC.

Funding Levels: MASGC anticipates funding approximately five proposals from the \$500,000 available to support the design phase.

Deadlines: A Letter of Intent (LOI) is required in order to submit a full proposal and is due by 5 p.m. CST on Friday, June 3, 2016. Full proposals are due by 5 p.m. CST on Friday, July 15, 2016. Submissions after the deadline will not be reviewed or considered for funding.

Funding Priorities

MASGC invites proposal submissions that will recommend an experimental design for estimating the abundance of red snapper (*Lutjanus campechanus*) in the U.S. portion of the Gulf of Mexico. The red snapper is a popular target of sportfishers and the commercial fishing industry throughout the Gulf of Mexico. Historical overharvesting resulted in a depleted population, but under current management measures the population is recovering, with full recovery expected by 2032. Some controversy surrounds the current stock assessment for red snapper, particularly with regard to accuracy of population estimates on artificial reefs and other structures considered to be difficult to sample using trawl surveys. Given this, interest exists in the development of an independent estimate of red snapper abundance in the U.S. portion of the Gulf of Mexico.

Input leading to this funding request was obtained at a workshop held March 2-3, 2016, in New Orleans, Louisiana. The guidance received at the workshop was useful in understanding how a U.S. Gulf-wide mark-recapture tagging program or synoptic survey might take advantage of large-scale traditional tagging and advanced technology by habitat type (including artificial reefs and other structures) to provide an independent estimate of the red snapper population. The independent estimate from the tagging/survey program will be compared to the estimate obtained from NOAA's current stock assessment approach to evaluate its accuracy.

Only the primary and secondary objectives will be considered for this competition.

1. **The primary objective** is to assess the population of red snapper on artificial reefs and other structures, and to provide a Gulf-wide estimate (with

estimates also produced for natural habitats) of absolute abundance of fish Age-2 and older in the U.S. Gulf of Mexico (by age or age-groups).

2. **The secondary objective** is to estimate biological parameters, such as growth and natural mortality rates (by age or age-groups).

To accomplish this, a multi-step process will be followed, beginning with experimental design planning (Phase I supported by this request for proposals) and followed by implementation (Phase II and the subject of request(s) for proposals in FY 2017). A valid and reliable experimental design will be critical due to the unprecedented scale and complexity of any tagging/survey study designed to estimate fish abundance Gulf-wide. The Phase I experimental design will engage the Gulf scientific community and other Gulf stakeholders before proceeding with Phase II. The Phase I experimental design will also ensure results obtained from Phase II can be used for comparison and possible integration with NOAA's stock assessment.

In Phase I, funding will be provided for designs of studies that will provide preliminary estimates of absolute abundance Gulf-wide and by habitat type (artificial/natural) within two years of the commencement of the Phase II study. Precise estimates of abundance with a coefficient of variation of approximately 30% are desired. The design should consider that significant funding will be available, but the design should be scalable in terms of sampling intensity (not geographic scale) if the original design exceeds the available funds.

Study designs developed through this competition will be reviewed at a workshop to be held in December 2016, and a final study design will be selected. Members of funded project teams will also participate in the workshop. The final experimental design used for the Phase II RFP may result from a combination of the designs. The final design will then provide the basis for Phase II (design implementation) that will be funded through request for proposals in FY 2017. Success in receiving Phase I funding does not guarantee or obligate funds in Phase II to the PI(s), even if a portion of or the entire experimental design that a PI(s) develops in Phase I is identified for implementation in Phase II.

Considerations in the Ultimate Design

It is recognized that obtaining a U.S. Gulf-wide or habitat-specific estimate of absolute abundance for red snapper will prove challenging. All of the approaches proposed to date, whether based on tag recaptures, cameras or acoustics, have technical challenges to overcome. Optical platforms (fixed cameras, ROVs, towed arrays) are routinely used to produce indices of local density, but estimation of local absolute abundance requires knowing the fraction of the population that is detected by the camera and how it changes with fish behavior (avoidance/attraction), habitat type and water clarity. Acoustic methods can provide biomass

estimates if red snapper can be distinguished from other species, but they are complicated by features that block backscatter (e.g., the dead zone near the seabed and obstructions around platforms) or side lobes that can “create fish” on steep slopes. A significant amount of ground-truth measurements on known fish may be needed, and research is required to develop classification algorithms. Tagging approaches are challenged by the high rate of release mortality associated with fish caught at depth or hooking-related injuries, tag shedding and

non-reporting of recaptured tags. Genetic methods (individual genetic tag-recapture or parental recaptures) may also prove to be an approach for monitoring red snapper and samples should be taken during the study, but further research may be required to identify genetic markers before the samples can be analyzed. In order to meet these challenges, a successful study design will likely need to employ a combination of approaches and take advantage of existing resources in the Gulf of Mexico.

Proposals selected for development should, therefore, address the following:

If tagging is included then provide:

1. Recommended types of tags and corresponding field tagging protocols
2. Sampling plan that ensures the tagged population will be representative of the untagged population at large, specifically accounting for
 - a. the Gulf-wide geographic range of red snapper
 - b. stratification by different habitat types (recognizing that different age classes occupy natural reefs, artificial structures and low-relief bottom)
 - c. high site fidelity of red snapper
 - d. mechanisms to mitigate or account for post-release mortality (e.g., depth-related barotrauma)
3. Systematically designed scientific release and scientific recovery effort structured in a way to maximize potential for obtaining robust estimates of abundance
4. Recreational and/or commercial fishery-based recoveries to augment information from the scientific release/recovery program
5. Estimation of biological parameters, such as growth, natural mortality rates and release mortality rates of red snapper (by age or age-groups)
6. Identify challenges with using the approach(es) to estimate red snapper populations and present alternative approaches to overcome the challenges

If advanced technology surveys are included then provide:

1. Recommended technology (e.g., cameras, acoustic profilers) and field implementation protocols
2. Sampling plan that ensures the surveyed population will be representative of the unsurveyed population at large, specifically accounting for
 - a. the Gulf-wide geographic range of red snapper
 - b. artificial and natural habitat types (recognizing that different age classes occupy natural reefs, artificial structures and low-relief bottom)
 - c. high site fidelity of red snapper
3. Approaches to dealing with fish attraction/avoidance
4. Identify challenges with using the approach(es) to estimate red snapper populations and present approaches to overcome the challenges

In all cases provide:

1. Description of how areas of different habitat types (e.g., natural reefs, artificial structures and low-relief bottom) will be estimated
2. Description of how the data in the study will be transformed into an estimate of absolute abundance of red snapper throughout the U.S. Gulf of Mexico region, and on areas of natural reefs, artificial structures and low-relief bottom, and what the expected precision would be for these estimates
3. Description of the logistical challenges to implementing large-scale surveys and tagging studies in the Gulf of Mexico, including the varying environmental conditions in different regions of the Gulf and how those challenges can be met, including possible contingency plans
4. Approaches for collecting biological samples during surveys (where appropriate), scientific tagging studies and from the commercial/recreational fisheries (e.g., otoliths, tissues for genetics, reproductive tissues)
5. Possible ways to allow citizens or regional consortia to provide regional support without compromising the ability to obtain Gulf-wide red snapper population estimates
6. Schedule of work from initial fieldwork through analysis and report preparation
7. Cost estimate for a full study and how this cost could be scaled to lower levels (and the impacts on precision)
8. How the design could be adapted to become part of a regular process for estimation of red snapper abundance in the U.S. Gulf of Mexico by resource managers
9. Description of a multidisciplinary, integrated approach for conducting the research across a broad geographic scale. Multi-state, multi-institutional/agency and interdisciplinary projects are strongly encouraged, but not required

2016 Timeline

- May 16 (Monday) – Request for proposals released
- June 3 (Friday) – Letters of intent due
- July 15 (Friday) – Full proposals due
- August 12 (Friday) – Notification of funding
- September 2 (Friday) – Project initiation
- December 5 (Monday) – Project ends and final report due

Contacts for Additional Information

For additional information, contact LaDon Swann (swannld@auburn.edu or 251-648-5877). Contact Loretta Leist (loretta.leist@usm.edu) for proposal guidance or Devaney Cheramie (devaney.cheramie@usm.edu) on fiscal matters.

Proposal Development Instructions

Letter of Intent

A Letter of Intent (LOI) is required to be eligible to submit a full proposal to MASGC. The LOI should be submitted to MASGC as a PDF file attached to an email message to Research Coordinator, at rc@masgc.org. The LOI should include a project title, names and work affiliation of investigators and a short description of the proposed approach. The LOI must be no more than 2 pages. There will be no formal review of LOIs. The LOI will help expedite the review process and is due on Friday, June 3, 2016, by 5 p.m. CST.

Full Proposal

The full proposal must be submitted to MASGC as a PDF attached to an email message to Research Coordinator, at rc@masgc.org. The proposal submission deadline is 5 p.m. CST on Friday, July 15, 2016. Proposal guidelines, required forms and other information can be found at the following website: <http://masgc.org/funding/red-snapper>.

Required Proposal Elements

Each of the following sections and sub-sections are required proposal elements. Omission of any element from I-V will result in the proposal being disqualified.

Proposals must include:

- I. 2016 Red Snapper Phase 1-Experimental Design Project Summary Form 90-2 Completed and unsigned copy of the cumulative 2016 Red Snapper Phase 1-Experimental Design Title/Cover Form (MS Word)
- II. In a single file
 - A. Signed 2016 Red Snapper Phase 1-Experimental Design Title/Cover form (signed by institutional authority)
 - B. Project Narrative (maximum of 10 pages)
 1. Rationale
 2. Scientific and Professional Merit
 - a. Hypotheses
 - b. Objectives
 - c. Approach
 - i. A sampling plan
 - ii. A plan for data analysis
 - iii. A detailed funding estimate to implement experimental design
 - d. Links to Other Projects
 3. Expected Benefits
 4. End-users, Partners and Co-Sponsors
 - C. Literature Cited (no page limit)
 - D. Curriculum Vitae (2 pages per investigator)

- E. Current and Pending Support for Each Investigator (NSF, NIH or USDA formats are acceptable)
- F. Project Schedule
- III. MASGC Budget Form 90-4 (MS Excel)
- IV. MASGC Budget Justification (MS Excel)
- V. (Optional) List of people who should not review the proposal (MS Word)

Description of Each Proposal Element

I. 2016 Red Snapper Phase 1-Experimental Design Project Summary Form 90-2

MASGC suggests completing this form as the final step in writing the proposal to concisely summarize what is stated in the project narrative.

II. 2016 Red Snapper Phase 1-Experimental Design Title/Cover Form

Submit signed 2016 Red Snapper Phase 1-Experimental Design Title/Cover Form

III. Project Narrative (Maximum length, 10 pages)

Maximum length is 10 pages and single-spaced on 8.5" x 11" paper with one-inch margins. Times New Roman or an equivalent serif typeface with a 12-point or larger font should be used. Tables and figures are included in the page limit. Paginate the narrative with page numbers right-justified in the footer.

Literature citations and CVs are not included in the 12-page limit.

No appendices are permitted. Citations in the narrative should follow your disciplinary literature format.

1. Rationale

Use the research literature and/or preliminary research to describe the problem or opportunity at hand. Document the magnitude of the situation and the relevance of the issue or problem in the Gulf of Mexico region. Describe how this work would add to the body of knowledge in the research area.

The rationale section needs to address both the scientific rationale for the project and quantify from a practical standpoint why the issue is a high priority. Describe what makes this project innovative and why this topic is important. The goal of the proposal should flow logically from this discussion. The overarching approach (e.g., tagging, advanced technologies, combination) should be included under the rationale.

2. Scientific and Professional Merit

Describe in detail the overall project design and include enough detail to demonstrate the technical qualities of the proposed approach so that the salient features can be quantitatively assessed by those who review the proposal. This section must include sub-sections for hypotheses; objectives; approach; and links to other projects. In the proposal provide a subheading for each of the following:

a. Hypotheses: Include all hypotheses related to the proposed work. These must be presented in bulleted format.

b. Objectives: The objectives should be a numbered list and each objective should begin with the word "To" followed by a verb. Be specific and brief. Proposals that state objectives in a way that is specific, measurable, attainable, realistic and time-bound will fare best during the review process. Be realistic and do not list more objectives than can be accomplished.

c. Approach: Provide specific details on how the proposal will develop a sampling plan and a plan for data analysis. Include proposed methods, approaches and techniques that will be used to meet the stated objectives. Proposals should describe major aspects of the project, such as controls, replication, sampling, surveys, etc. Include information about facilities, equipment, personnel, management and interactions with other institutions or other resources that are directly applicable to the proposed project. A budget estimate to implement the proposed experimental design is also requested.

d. Links to Other Projects: Describe how this project could interface with other related research or similar projects that you or others are leading. The links to other projects may be local, statewide, regional or national in scope. Please be specific in identifying and explaining these links. Clearly distinguish how the proposed work relates to or is associated with any current or pending funding.

3. Expected Benefits

Describe the overall impacts of the completed project and how results can be immediately applied to inform Phase II (implementation) of the regional red snapper estimate of absolute abundance. Describe how the results of the project can be applied to improve governmental and other management decisions, improve technological or economic efficiency and/or benefit community members, industry or others. Be as specific as possible.

4. End-users, Partners and Co-Sponsors

Successful application of the research results will depend on the inclusion of end-users, partners and, in many cases, co-sponsors. This section should identify approaches to involve the recreational and commercial fishing industries. Also, describe their role and how they will be part of the planning and implementation of Phase II (design implementation) of the project, how they will be brought into the execution of the project, and/or how they will use the results.

III.D. Literature Cited (no page limit)

Provide complete reference information, per your disciplinary literature format. Citations should include author, date, title, source and page number. Up-to-date citations are expected.

III.E. Curriculum Vitae

Up to a two-page CV that includes evidence of each investigator's position, education, qualifications and experience in the field.

III.F. Current and Pending Support for Each Investigator

For all investigators on the project, include current and pending extramural sponsored research projects using NSF, NIH or USDA formats that include the title, sponsor, total budget, FTE devoted to the project and duration for each entry.

III.G. Project Schedule Form

Milestones are specific actions that will be undertaken to accomplish the objectives whereby progress toward the goals and/or outcomes is realized. Examples of milestones are data collection, analyzing samples, engagement with end-users and presentation/publication of results. Mark with an "X" the appropriate year(s) and month(s) expected for individual milestones identified for the proposed work.

IV. MASGC Budget Form 90-4

Complete one budget form for the project. Sub-award recipients must complete a budget form for their portion of the project. Label each budget form where indicated to appropriately describe the budget year and sub-award recipient.

V. MASGC Budget Justification Form

Investigators must use the MASGC Budget Justification Form. Complete one overall MASGC Budget Justification form. Sub-award recipients will be requesting funds must complete a budget justification form. Label each budget justification form with the budget year and sub- award recipient.

VI. (Optional) List of people who should not review the proposal

Although not required, investigators are welcome to submit a list of people who should not review their proposal for any reason. This list will be kept confidential. Also consider including scientists and other people with whom you would have a conflict of interest in reviewing the proposal.

Proposal Submission Information

Electronic mail submissions of the proposal in a PDF format are preferred and should be addressed to "Research Coordinator" (rc@masgc.org). If an electronic mail submission is not possible, please contact Loretta Leist at loretta.leist@usm.edu for instructions for submitting a hard-copy.

Evaluation of Proposals

Proposals are expected to be highly integrated, multidisciplinary projects that address the research need identified in this request. Multi-state, multi-institutional/agency and interdisciplinary projects are strongly encouraged, but not required.

Proposals will be evaluated using merit reviews from national experts, followed by a review by a Technical Review Panel (TRP). The TRP includes scientists from universities around the U.S. and federal employees who have the necessary technical expertise. The TRP will recommend placement of each proposal into one of three categories (“fundable,” “maybe fundable” and “not fundable”) based on their reviews and the merit reviews. The funding request will be closed in the event no proposals are identified as “fundable” by the TRP.

The top ranked “fundable” proposal(s) will be recommended for funding and will be funded as resources permit. The final funding decision will be made in consultation with the four Gulf of Mexico Sea Grant Programs and with concurrence from the NOAA National Sea Grant Office and NOAA Fisheries.

Evaluation Criteria

All proposals will be evaluated by external reviewers and the TRP based on the following criteria:

1. **Rationale (10%)** – Evaluates how well the proposed project addresses this RFP.
2. **Scientific and Professional Merit (30%)** – Assesses whether there is a clearly stated testable hypothesis, if the approach is technically sound and/or innovative, whether there are clear objectives, if methods are appropriate, and whether the research will advance the state of the science or discipline. Determines the degree to which approaches are used to solve problems or focus on new resources, timely issues or opportunities. Proposed budgets will also be evaluated under this criterion.
3. **Expected Benefits (30%)** – Evaluates the overall impacts of the completed project and whether results can be immediately applied to inform Phase II (implementation) of the regional red snapper estimate of absolute abundance.
4. **End-users, Participants and Co-Sponsors (10%)** – Assesses the degree to which users or potential users of the results of the proposed project can be brought into the planning and implementation of Phase II.

5. **Investigator Qualifications (20%)** – The degree to which the applicant and identified collaborators possess the necessary education, training and/or experience to execute the proposed activity. This assessment will be primarily based on the investigator(s) CV(s). This criterion will also assess the stage of career development and record of productivity with previous funding.

Post-Project Selection Requirements

PIs of selected project(s) will be required to submit additional materials prior to project initiation. These include:

1. Applicant response to any significant review comments.
2. Letter of commitment from the institutions involved in the project. Letters of commitment will also be required for each sub-award recipient, co-sponsor and unfunded collaborator identified within the proposal. Letters of commitment from sub- award recipients must be signed by the appropriate institutional authority.
3. Consent Form – Intellectual Property.
4. Form CD-512 or CD-511 (Certification Regarding Lobbying).
5. Standard Form 424B (Assurances – Non-Construction Programs).
6. Participate in a workshop with investigators from other funded projects. Please include travel costs estimates for a two-day meeting in a city with a major airport in the Gulf of Mexico region (i.e. Tampa, New Orleans or Galveston)
7. Additional materials may be requested as needed.

NOAA Data Sharing Plan

Environmental data and information collected and/or created under NOAA grants/cooperative agreements must be made visible, accessible and independently understandable to general users, free of charge or at minimal cost, in a timely manner except where limited by law, regulation, policy or security requirements. PIs of selected project(s) will be required to submit an acceptable Data Sharing Plan prior to funding.

Participant List

Name	Affiliation	Email address
Investigators (26)		
Greg Stunz	Harte Research Institute, Texas A&M University – Corpus Christi	greg.stunz@tamucc.edu
Robert Leaf	University of Southern Mississippi	robert.leaf@usm.edu
Steve Szedlmayer	Auburn University	szedlst@auburn.edu
Sean Powers	Dauphin Island Sea Lab	spowers@disl.org
Peter Rubec	FWC/Florida Fish & Wildlife Research Institute	Peter.Rubec@myfwc.com
Rob Ahrens	University of Florida	rahrens@ufl.edu
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John Hoenig	Virginia Institute of Marine Science	hoenig@vims.edu
Liese Carleton	Virginia Institute of Marine Science	lcarleton@vims.edu
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Judd Curtis	Harte Research Institute, Texas A&M University – Corpus Christi	judd.curtis@tamucc.edu
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Kevin Boswell	Florida International University	kevin.boswell@fiu.edu
Reviewers (4)		
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Richard Starr	Cal State	starr@mlml.calstate.edu
Patrick Sullivan	Cornell	pjs31@cornell.edu
Steering Committee (10)		
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Name	Affiliation	Email address
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William Wise	New York Sea Grant	william.wise@stonybrook.edu
LaDon Swann	Mississippi-Alabama Sea Grant Consortium	swanndl@auburn.edu
State Agencies (11)		
Chris Blankenship	Alabama Marine Resources Division	chris.blankenship@dnr.alabama.gov
Kevin Anson	Alabama Marine Resources Division	Kevin.Anson@dnr.alabama.gov
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Robert Shipp	University of South Alabama	rshipp@southalabama.edu
Robert Twilley	Louisiana Sea Grant	rtwilley@lsu.edu
Marc Santora	NOAA/NMFS	marc.santora@noaa.gov
Loretta Leist	Mississippi-Alabama Sea Grant Consortium	loretta.leist@usm.edu
Kay Bruening	Mississippi-Alabama Sea Grant Consortium	kay.bruening@usm.edu



**Request for Proposals: Red Snapper
(*Lutjanus campechanus*) Abundance
Estimate in the U.S. Gulf of Mexico Region**



Funding Opportunity Title: Red Snapper (*Lutjanus campechanus*) Absolute Abundance Estimate in the U.S. Gulf of Mexico Region

Announcement Type: Notice of request for proposals (RFP)

Release Date: March 14, 2017

Funding Source: The National Oceanic and Atmospheric Administration's (NOAA) National Sea Grant College Program and NOAA National Marine Fisheries Service (NMFS)

Funding Type: Funding will be provided to successful applicant(s) through a contract with the Mississippi-Alabama Sea Grant Consortium's (MASGC) fiscal host at the University of Southern Mississippi's Office of Sponsored Programs Administration.

Funding Opportunity Summary: This notice advises the public of a funding opportunity to develop an independent abundance estimate of Age-2 and older red snapper in the U.S. waters in the Gulf of Mexico. The successful applicant will determine the absolute abundance of the red snapper population by habitat type including artificial reefs, natural reefs and unclassified habitats. The design must include mark-recapture tagging and advanced technology methods. The award period will be from October 1, 2017, through September 30, 2019. The grant program is managed MASGC for the National Sea Grant College Program and the NMFS.

Eligibility: MASGC welcomes proposals from institutions of higher education. The proposal principal investigator (PI) must be located within a U.S. Gulf of Mexico state. Co-investigators, including state agencies, non-governmental organizations and the fishing industry, may be in any U.S. region. Federal partners may also participate as uncompensated collaborators. No person shall be excluded on grounds of race, color, age, sex, national origin or disability from participation in, denied benefits of, or be subjected to discrimination under any program or activity receiving financial assistance from MASGC.

Funding Levels: MASGC anticipates funding one consortium proposal at level of \$9.5 million plus a non-federal match requirement of \$2.5 million.

Reporting: Semi-annual progress reports will be required.

Deadlines: A Letter of Intent (LOI) is required to submit a full proposal and is due by 5 p.m. Central Time on Friday, April 7, 2017. Full proposals are due by 5 p.m. Central Time on Friday, June 9, 2017. Submissions after either deadline will not be reviewed or considered for funding.

Funding Priority

Program Objective: Provide an independent absolute abundance estimate of Age-2 and older red snapper in the U.S. waters in the Gulf of Mexico by habitat type including artificial reefs, natural reefs and unclassified habitats.

MASGC invites proposal submissions to estimate the abundance of red snapper in the U.S. waters in the Gulf of Mexico using the design criteria described in this funding request. The red snapper is economically important to sportfishers and the commercial fishing industry throughout the Gulf of Mexico. Historical overharvesting resulted in a depleted population, but under current management measures the population is recovering, with full recovery expected by 2032. The current stock assessment for red snapper may undersample fish in certain habitat types, particularly on artificial reefs and other structures where sampling is difficult. Given this, there is a need to obtain an independent estimate of red snapper abundance in the U.S. waters in the Gulf of Mexico.

Design Guidelines

Guidance for this funding request was obtained through a previous research competition in which experimental designs were developed. The results of six proposed designs and input from stock assessment experts were critical in identifying appropriate methods for conducting a Gulf-wide absolute abundance estimate using mark-recapture tagging and advanced technology methods by habitat type (including artificial reefs and other structures) to provide an estimate of the red snapper population.

General guidance includes:

1. Projects can be up to 2 years: no more than 6 months to prepare and the remaining time (no less than 18 months) to implement and complete data analysis.
2. Investigators should include a power analysis in their proposal showing the expected coefficient of variation (CV) of the abundance estimates from their sampling plan (a CV < 0.3 is desired, but may be difficult to achieve).
3. Relative abundance estimates must be converted to an estimate of absolute abundance.

Other guidelines were developed for Geographic Areas and Sampling Depths, Habitat Types, Working with the Fishing Industries and Sampling Methods. Applicants must follow these guidelines:

Geographic Areas and Sampling Depths

1. Proposals should utilize at least two geographic areas. The study area must be divided into Eastern and Western Gulf sub-regions with the division near the Mississippi River to align with the current NOAA stock assessment. At least two additional strata per sub-region should be considered for the purposes of looking at spatial differences in age structure, movement and mortality. A rationale should be provided for the proposed boundaries including consideration of the ability to detect differences between strata.
2. The eastern boundary of the study is the Dry Tortugas and the western boundary is the Texas-Mexico border.
3. Sampling should be distributed sufficiently across a depth range of 10-150 meters to provide age-structured abundance estimates for Age-2 and older red snapper in that depth range.

Habitat Types

4. Habitat suitability maps (HSMs) are not sufficiently comprehensive to represent all red snapper habitat in the Gulf of Mexico. However, HSMs may be appropriate to inform targeted sampling.
5. At a minimum, there should be known artificial reefs, known natural reefs and unknown/uncharacterized bottom habitat classifications. Depth or other stratifications within each of these may improve statistical performance of the chosen sampling methods.
 - a. Known artificial reefs. There are thousands of known and mapped artificial reefs where red snapper are found.
 - b. Known natural reefs. Natural hard bottom features are widely distributed throughout the Gulf of Mexico.
 - c. Unknown/uncharacterized bottom. This stratum should include all habitats that fall outside the domains of known artificial and natural reefs. It is recognized that the bottom in many of these areas is made up of unconsolidated sediments of various types and hold low densities of red snapper. However, these areas are vast in extent and may include a significant number of red snapper. Uncharacterized bottom will also contain uncharted artificial reefs and natural reefs.
6. Include a description of the process for identifying habitat types to be randomly sampled.
7. Seek out high-resolution habitat maps to leverage the funds available for this program. A component of the proposal can include the synthesis of habitat maps from various sources. Include the sources of the locations of known natural and artificial reefs.
8. Proposals must use power analysis/simulations to determine the percent of each habitat category necessary to sample and the expected precision (CV) of the overall estimate for the eastern and western Gulf, separately.

Working with the Fishing Industries

9. Investigators should work directly with the commercial and recreational fishing industries. Engagement with fishermen should be included from the start of program and be a key component of your proposal. It is possible to hire commercial fishermen to assist with catching and tagging fish, as well as keep and sell fish using Individual Fishing Quotas (IFQ) that would otherwise die from discard mortality or that are kept for biological sampling purposes. This could offset some boat charter costs.
10. Proposals will include an outreach strategy to ensure the fishing community, resource managers and other stakeholders are regularly updated on the status of the project.

Sampling Methods

11. It is not expected that a single sampling method is capable of providing one absolute abundance measurement in each habitat type. The sampling methods considered most likely to succeed are:
 - a. Depletion method coupled with mark-recapture for artificial and small natural reefs that have high densities of red snapper. A diverse and broadly distributed

set of reefs of various types and sizes would need to be sampled to extrapolate to all known reefs.

- b. A combination of acoustics and visual advanced technology surveys could be used on larger reefs. If all known large reefs cannot be sampled, the sampled reefs need to be representative and well-distributed. Acoustics could provide total fish counts while visual surveys could provide species composition for larger natural reefs.
 - c. Because of the geographic size of the unknown bottom category, this habitat type will need a sampling strategy different than the methods used for known artificial and natural reefs. Sampling tools such as acoustics and towed cameras appear most promising to sample this stratum across the entire Gulf. Known reefs in this category should not be sampled, but randomly sampled unknown reefs should be sampled.
12. For all methods, investigators will need to provide detailed steps for calibration and how to avoid sampling biases.
 13. A simulation analysis or power analysis must be conducted and results included in the proposal to understand the sensitivity of the estimates to some of the more obvious sources of bias associated with a mixed survey spatial allocation design. Investigators must clearly lay out all of the assumptions of their methods.
 14. Tagging and depletion methods
 - a. For known artificial reefs, an effective strategy for obtaining a total abundance estimate for a single reef or close cluster of reefs is a mark-recapture tagging method such as the Petersen mark-recapture coupled to a depletion method. Sampling assumptions for the selected mark-recapture method and depletion method must be addressed. Tag survey analysis will need to account for known sources of bias (e.g., tag-loss, release mortality, reporting rates) and this accounting should be based on measured rates for these factors. Where possible the fishing industry should be involved in tag recovery. A sample size to cost determination should be included.
 - b. For tagging and depletion methods, additional consideration should be given to:
 - i. Validation of acoustics (mortality and movement), visual, double tagging and catchability
 - ii. Archival tags and high-dollar tags need to be included.
 - iii. When sampling, collect tissue samples and archive for genetic work using future or existing funding sources outside this funding request.
 - iv. Collect otoliths to determine age structure. The added expense of collecting otoliths may require the use of a more imprecise estimate using length frequency data.
 - v. Maintain spatial and temporal consistency.
 15. Advanced technology methods
 - a. Cameras on remotely operated vehicles (ROV) is an option on natural reefs larger than 90 meters.

- b. Dual use of sonar and towed cameras is an option for sampling larger natural reefs.
- c. ROVs is an option for sampling small artificial reefs.
- d. Towed cameras is an option for unknown bottom. A rapidly towed video technology like the Camera-Based Assessment Survey System (C-BASS) should be considered for this habitat type, but other acoustic and optical platforms may be feasible. Data processing and analysis time would be substantial for all technologies relying on camera and video imagery and this needs to be accounted for in the budgets. Information on known automated image analysis software can be provided on request.
- e. Camera deployment vehicles are known to repel or attract some species of fish, and to have a range of detection that is difficult to quantify depending on lighting and water clarity. To address these challenges a specific calibration experiment is necessary to demonstrate calibration of camera observations into measurements of red snapper per unit bottom area.

Phase II Timeline

- RFP released on March 14, 2017
- Letter of Intent due April 7, 2017
- Proposals due June 9, 2017
- Notification of funding decisions on September 1, 2017
- Project initiation on October 1, 2017
- Project end date on September 30, 2019

Contacts for Additional Information

For additional information, contact LaDon Swann (swannld@auburn.edu or 251-648-5877). Contact Loretta Leist (loretta.leist@usm.edu) for submission guidance or Amanda Seymour (amanda.k.seymour@usm.edu) for budget questions.

Letter of Intent Instructions

A Letter of Intent (LOI) is required to be eligible to submit a full proposal to MASGC. The LOI should be submitted to MASGC to Loretta Leist, MASGC Research Coordinator at: Loretta.leist@usm.edu. The LOI should include the project title, names and work affiliation of investigators and a short description of the proposed approach. The LOI must be no more than 2 pages. There will be no formal review of LOIs. The LOI will help expedite the process for identifying full proposal reviewers and is due on Friday, April 7, 2017, by 5 p.m. Central Time.

Full Proposal Development Instructions

The full proposal must be submitted to MASGC through eSeaGrant: <http://eseagrant.masgc.org>. User instructions for eSeaGrant, proposal development instructions, required forms and other information can be obtained at: <http://masgc.org/red-snapper/RFP>. The proposal submission deadline is 5 p.m. Central Time on Friday, June 9, 2017. Applicants will receive a confirmation email after submitting a proposal. If you do not receive a confirmation email, please contact Loretta Leist (loretta.leist@usm.edu or 228-238-8835). Changes can be made to proposal until the closing date and time.

Required Proposal Elements

Each of the following sections and sub-sections are required proposal elements. **Omission of any element from I-XIII will result in the proposal being disqualified.** Instructions for each section and sub-section are available through eSeaGrant.

Proposals must include:

- I. 2017 Red Snapper Phase II- Project Summary Form 90-2
- II. Completed 2017 Red Snapper Phase II Cover Form
- III. Project Narrative: a full proposal narrative of **no more than 25 pages** (A-D) to fully describe the approach.
 - A. Rationale: Use the research literature and/or preliminary research to describe the problem or opportunity at hand. Document the magnitude of the situation and the relevance of the issue or problem in the Gulf of Mexico region. Describe how this work would add to the body of knowledge in the research area. The rationale section needs to address both the scientific rationale for the project and quantify from a practical standpoint why the issue is a high priority. Describe what makes this project innovative and why this topic is important. The goal of the proposal should flow logically from this discussion. The overarching approach including the use of tagging and advanced technologies should be included under the rationale.
 - B. Scientific and Professional Merit: Describe in detail the overall project design and include enough detail to demonstrate the technical qualities of the proposed approach so that the salient features can be quantitatively assessed by those who review the proposal. This section must include sub-sections for hypotheses; objectives; approach; and links to other projects.
 1. Hypotheses: Include all hypotheses related to the proposed work. These must be presented in bulleted format.
 2. Objectives: The objectives should be a numbered list and each objective should begin with the word "To" followed by a verb. Be specific and brief. Proposals that state objectives in a way that is specific, measurable, attainable, realistic and time-bound will fare best during the review process.
 3. Approach: Provide specific details for developing and implementing the sampling plan and a plan for data analysis. Include proposed methods, approaches and techniques to meet the stated objectives. Proposals must

describe major aspects of the project, such as controls, replication, sampling surveys, validation, assumptions and other information needed to adequately understand the proposed approach. The approach must describe the reliability and validity of the sampling method(s) for estimating absolute abundance. Include information about facilities, equipment, personnel, management and interactions with other institutions or other resources that are directly applicable to the proposed project.

- C. Expected Benefits: Describe the overall impacts of the completed project and how results can be applied to improve governmental and other management decisions, improve technological or economic efficiency and/or benefits to community members, industry or others.
 - D. End-users, partners and co-sponsors: Successful application of the research results will depend on the inclusion of end-users, partners and, in some cases, co-sponsors. This section should identify approaches to involve the recreational and commercial fishing industries.
- IV. A 2-page description of how the overall project will be managed and coordinated.
 - V. A 2-page description of how an additional \$10 million in funding, pending FY17 appropriations, would be used to improve the abundance estimate.
 - VI. Curriculum Vitae: Two pages per investigator using National Science Foundation (NSF), National Institutes of Health (NIH), United States Department of Agriculture (USDA) or similar formats.
 - VII. Project Schedule: A detailed timeline of major milestones of the proposed project.
 - VIII. Data Management Plan: Proposals must include a data management plan to store, access and archive raw and processed data.
 - IX. Literature Cited (no page limit): Use any standard format for peer reviewed publications
 - X. Current and Pending Support for each investigator using NSF, NIH, USDA or similar formats.
 - XI. MASGC Budget Form 90-4: A budget estimate to implement the proposed experimental design.
 - XII. MASGC Budget Justification: A description of each item listed in the budget.
 - XIII. Letters of support from end-users, participants and co-sponsors.
 - XIV. (Optional) List of people who should not review the proposal.

Question-and-Answer Webinar

One webinar will be held to discuss this funding opportunity on March 31, 2017, from 1-2:30 p.m. Central Time. Please visit the MASGC red snapper funding webpage (<http://masgc.org/red-snapper/RFP>) for instructions on how to participate in the webinar. The webinar will be recorded and posted on the MASGC funding webpage after the webinar.

Evaluation of Proposals

Proposals are expected to be highly integrated and multidisciplinary projects that address the program objective identified in this request. Multi-state and multi-institutional projects involving the fishing industries are strongly encouraged.

Proposals will be evaluated using merit reviews from national fisheries experts, followed by a review by a Technical Review Panel (TRP). The TRP includes scientists from universities and fisheries agencies around the U.S. and federal employees who have the necessary technical expertise. The TRP will recommend placement of each proposal into one of three categories (“fundable,” “maybe fundable” and “not fundable”) based on their reviews and the merit reviews. The funding request will be closed in the event no proposals are identified as “fundable” by the TRP.

The top ranked “fundable” proposal(s) will be recommended for funding and will be funded as resources permit. The final funding decision will be made in consultation with the four Gulf of Mexico Sea Grant Programs and with concurrence from the NOAA National Sea Grant Office and NOAA NMFS.

Evaluation Criteria

All proposals will be evaluated by external reviewers and the TRP based on the following criteria:

1. **Rationale (10%)** – Evaluates how well the proposed project addresses this RFP.
2. **Scientific and Professional Merit (50%)** – This section will be evaluated to determine the degree to which approaches will meet the program objective of the funding request. This section will also assess whether there is a clearly stated testable hypothesis, whether there are clear objectives, if the approach is technically sound, if methods are appropriate and whether the research will advance the science of stock assessments. Proposed budgets will also be evaluated under this criterion.
3. **Expected Benefits (15%)** – Evaluates the overall impacts of the completed project and whether results can be applied to inform red snapper resource managers, the fishing industry and other stakeholders.
4. **End-users, Participants and Co-Sponsors (10%)** – Assesses the degree of engagement with the fishing industry or other stakeholders in the implementation of the proposed project.
5. **Investigator Qualifications (15%)** – The degree to which the applicant and identified collaborators possess the necessary education, training and/or experience to execute the

proposed project. This assessment will be primarily based on the investigator(s) CV(s). This criterion will also assess the stage of career development and record of productivity with previous funding.

Post-Project Selection Requirements

Applicants selected for funding will be required to submit additional materials prior to project initiation. These include:

1. Applicant response to any significant review comments.
2. Consent Form – Intellectual Property.
3. Form CD-512 or CD-511 (Certification Regarding Lobbying).
4. Standard Form 424B (Assurances – Non-Construction Programs).
5. NOAA Data Sharing Plan.
6. Participate in one or more conference calls with program managers.
7. Additional materials may be requested as needed.

NOAA Data Sharing Plan

Environmental data and information collected and/or created under NOAA grants/cooperative agreements must be made visible, accessible and independently understandable to general users, free of charge or at minimal cost, in a timely manner except where limited by law, regulation, policy or security requirements. Applicants of selected project(s) will be required to submit an acceptable Data Sharing Plan before project initiation.

1. About the Sea Grant Programs in the Gulf of Mexico Region

The Sea Grant programs in the Gulf of Mexico region represent four of the 33 Sea Grant Programs around the United States. Sea Grant is a National Oceanic and Atmospheric Administration (NOAA) sponsored partnership with institutions of higher learning engaged in research, communications, education, extension service and legal advisory activities to enhance the value and sustainability of the nation's ocean and coastal resources for the benefit of the public.

Title: An Experimental Design for Estimating Absolute Abundance of Red Snapper in the
U.S. Gulf of Mexico

FINAL REPORT

Submitted To:

Mississippi-Alabama Sea Grant Consortium 2016
Red Snapper Phase 1 – Experimental Design

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Introduction to the Design

General Overview

This design addresses one of the most pressing issues currently facing Gulf of Mexico (GOM) fisheries management – estimating absolute abundance of Red Snapper. The *overarching goal* is to provide an experimental design for carrying out an intensive research initiative that estimates Red Snapper absolute abundance. The *rationale* for this work is that having an estimate of Red Snapper abundance across the U.S. GOM will allay much of the controversy surrounding the contentious management and build confidence in our understanding of the population dynamics for this species across their range and distribution among habitats. The detailed design and implementation information here will allow managers to make the most informed decisions related to this controversially managed species.

To accomplish this ambitious task, we assembled a multidisciplinary team that included experts from across the entire U.S. Gulf region. These individuals have extensive experience with Red Snapper along with some of the most robust data sets, ongoing research programs, sampling techniques, and specific analytical skills available in the GOM. Through a series of two-in-person workshops, several subtask workgroup meetings, and many conference calls, we were leveraged these capabilities along with our own and other ongoing research to develop this design. Thus, the primary deliverables for this design project are fourfold:

- (1) A framework for a Gulf-wide, stratified random sampling design;
- (2) A detailed design description for both standard methods and advanced technologies across all major habitat types for Red Snapper;
- (3) Key directed tagging and survey studies to determine specific data needs; and most importantly,
- (4) A Design Optimization Tool that generates Coefficients of Variation (CVs) for scalable sampling effort and cost estimation without sacrificing regional coverage.

A key feature of this document is that it is a “living” design, in that it can be adapted to account for the habitat nuances at each GOM region or sub-region identified here as well as the marginal costs and sampling effort required for these regions. This adaptability and scalable approach is primarily accomplished through the Design Optimization Tool.

Goals and Objectives

One major obstacle to gaining a comprehensive and accurate estimate of the Red Snapper abundance in the GOM has been the absence of directed sampling effort in structured habitats, including active oil/gas platforms and their associated infrastructure (e.g., pipelines), man-

made artificial reefs, and other structures where this species occurs. Lack of abundance data from these areas has been a key factor leading to controversy in this fishery. Thus, developing a sampling design that targets oil/gas structures, artificial reefs, natural hard bottom structured habitats, and unconsolidated bottom to complement existing random stratified sampling programs and assessments in a scalable and cost-effective framework are needed to better understand the population dynamics of Red Snapper.

Goal: Our overarching goal is to provide a design that will estimate the absolute abundance of Red Snapper in the U.S. GOM. This goal can be met by addressing the following objectives.

- Objective 1:** Provide a scalable design in terms of cost and effort to estimate the absolute abundance of Red Snapper on artificial reefs, natural hard bottom, and other habitats to provide a Gulf-wide estimate of absolute abundance of fish Age-2 and older in the U.S. GOM.
- Objective 2:** Provide a sampling framework from which habitat-specific biological parameters such as growth and natural mortality rates by specific age groups can be derived.
- Objective 3:** Engage the GOM scientific community and other Gulf stakeholders.
- Objective 4:** Ensure the design will result in estimates that will be used for comparison and integration into NOAA's Red Snapper stock assessment.

Experimental Design Overview and Structural Framework Summary

Red Snapper distribution and abundance across the U.S. GOM landscape is very heterogeneous. Thus, estimating Red Snapper absolute abundance is complex, and to do so requires expertise in multiple areas of fisheries and statistical sciences, as well as a sampling regime using both traditional and “advanced technology” methodologies across all habitat types in the U.S. GOM. Thus, we strongly recommend a design that uses a combination of sampling approaches at the core, but also takes advantage of and explores the wealth of existing data resources throughout the GOM when developing a comprehensive sampling design. At the core of our approach is a stratified random sampling design that can be used across all U.S. GOM regions (see Fig 1). We then recommend some key directed studies to determine specific abundance questions or estimate variability among habitat types that allow refining of the absolute abundance estimate. To accomplish this task, we used a detailed habitat analysis to generate four U.S. GOM regions based on their detailed geography and ecological zonation patterns. Details are thoroughly described below, but briefly, the structural framework of the design for each regional zone includes shallow, mid, and deep-depth strata. For each depth strata the following corresponding habitats will be sampled: artificial reefs (both large and small), natural banks, and unconsolidated bottom. The gear used for this sampling is also described in detail below but includes video surveys by either a Remotely Operated Vehicle (ROV) coupled with bioacoustics techniques over artificial reefs and natural habitat, or a Camera-Based Assessment Survey System (C-BASS) over both natural banks and low-relief unconsolidated bottom that includes oil and gas infrastructure (e.g., pipelines). We cannot overemphasize the importance of sampling unconsolidated bottom to this design. For example, we hypothesize Red Snapper occur in relatively low density over this habitat type as a whole, but it likely contains the vast majority of Red Snapper biomass (see below for details) due to the vast coverage of this

low-relief habitat. The conventional naming of these areas as low-relief “unconsolidated bottom” is somewhat misleading, because these vast areas certainly contain enough structured habitat (e.g., exposed oil/gas pipelines, ephemeral mud features) and uncharted natural or man-made features to maintain Red Snapper. Thus, it is imperative that any design fully capture this unconsolidated bottom, where both sampling and mapping is most needed. The current state of mapping resolution and variance estimates for known Red Snapper habitat is insufficient (or does not exist) in many cases, and this represents a great challenge. As a result, we recommend preliminary studies to refine variance estimates for these habitat types. Moreover, this design precludes detailed habitat mapping in the GOM, which is much needed. For the implementation phases, we also recommend an intensive data mining exercise to include regional expertise that can elucidate more habitat-specific information for each region, and update the Design Optimization Tool before much of the sampling commences, or at least concurrently for a more efficient design.

There is likely Red Snapper biomass in the U.S. GOM that has not been accounted for nor quantified. For example, we have recently become aware of a large abundance of Red Snapper in “non-traditional” areas occurring at deep depths along the continental shelf slope on bathymetric features such as salt domes, seamounts, and other natural features. Fish abundance on these areas may be of high value, but the habitat features do not make it conducive to efficiently sample, particularly in a stratified random construct. We have provided a section entitled “ancillary index-based methods” that provide designs and sampling protocols for these areas to account for these fish. Thus, given the geographic diversity and wide-range of the Red Snapper population, we feel strongly that there is not one single approach that can estimate their absolute abundance. There is also a clear need for more directed studies to allow for validation of visual survey data or answer specific questions about the variability in abundance among similar structures. These techniques include high-reward tag and recapture, change-in-ratio (CIR), bottom longline (BLL), vertical longline (VLL), and catch-survey-catch (CSC) methods. While these methods are not conducive for full incorporation into the core stratified design, they are nonetheless of high-value for calibrating the absolute abundance estimation.

More detailed information and cost analyses for each design has also been provided. Additionally, there is likely a wealth of information regarding habitat features and Red Snapper abundance from previous and ongoing research. Many research groups are currently carrying out similar regional studies (including many of our own) that would be valuable to this estimation. Moreover, the final implementation should include funds to carry out a rigorous mapping exercise that includes detailed assembly of all known habitats and their areal coverages and any associated Red Snapper abundance estimates from both published and unpublished sources. By combining information from these new and ongoing studies, assembling historical data sources, and overlaying with the most detailed habitat mapping as possible, we are confident these studies can provide an accurate estimate of Red Snapper absolute abundance in the U.S. GOM. Finally, and most importantly, working with Dr. Lynne Stokes, an expert statistician in the field of Sampling Design/Optimization, we have developed a Red Snapper Design Optimization Tool. For any design scenarios, this decision support tool can be easily manipulated to generate CVs for scalable sampling effort and their associated costs without sacrificing regional coverage. Because estimates of variance are uncertain in many cases, we have provided a range of design scenarios. Consideration of the scenario options provided will be a key component in finalizing any design of this magnitude to ensure adequate sampling effort with desired variance estimates are obtained, while also retaining a feasible cost framework for the project.

The key features of this design include:

1. A stratified random sampling structural framework.
2. Abundance estimation using advanced technologies:
 - a. ROV paired with bioacoustic technologies
 - b. C-BASS
3. Directed studies:
 - a. High-reward tag-and-recapture
 - b. Change-in-ratio
 - c. Fixed cameras
 - d. Vertical longline
 - e. Catch-survey-catch method
 - f. Bottom longline
4. Opportunistic biological sample collection.
5. A design optimization decision support tool.
6. Final cost estimates that are scalable without sacrificing geographic coverage.

Establishing a Stratified Random Sampling Design

The GOM contains extensive geographic variability in physicochemical and geological (substrate) conditions, which creates substantial differences in habitat types and associated Red Snapper density across the basin. The western relative to the eastern region contains relatively little natural hard bottom structure, and consists predominately of silt and mud. As Red Snapper abundance is thought to be disproportionately related to structured habitat, it is essential that the geographic extent and composition of these areas across the GOM are accurately quantified. Thus, the first step in constructing a robust stratified design should begin with an exercise in data mining to gather the latest and most comprehensive information on GOM bathymetry, as well as compile an inventory of all standing and reefed oil/natural gas platforms, and other artificial structures that currently exist in the GOM. Assessing these comprehensive data resources will enable researchers to calculate the areal extent of natural hard bottom substrates versus unconsolidated bottom habitats (i.e., mud/sand), and quantify the number of existing artificial reef structures. Combining this information with available physicochemical, geological, and bathymetric information will allow meaningful ecological boundaries to be drawn across the GOM, and allow regions to be divided into appropriate geographic and bathymetric strata serving as the framework for the stratified random design in which the various sampling methods will occur. We conducted an initial analysis based on the above framework using currently available datasets (described in section b below), but we highly recommend further data compilation to fully assess appropriate strata during project implementation.

Habitat Mapping and Data Mining

While a coordinated synoptic study that occurs simultaneously across the U.S. GOM is certainly needed, it would be unwise not to utilize the similar but smaller-scale work that many research groups

have performed or are ongoing. While these disparate studies are smaller- scale, regional, and typically habitat-specific, they are nevertheless very valuable to this study. For example, these data may be very important even if only for validation purposes or to provide much needed variance estimates. This group strongly recommends the final implementation phase for this absolute abundance estimation should include funds to carry out a rigorous, detailed mapping inventory and meta-analysis that includes detailed assembly of all known habitats (natural and artificial), their areal coverages, and any associated Red Snapper abundance estimates (and the variance) from both published and unpublished sources. A detailed habitat inventory is essential for a comprehensive study such as this, but these data are not readily available and additional mapping is not within the scope of this study. However, much of these data are located with Federal agencies and private industry (e.g., BOEM, U.S. Navy, oil and gas industry, and other similar sources). For example, BOEM maintains the mapping of deep substructures on the ocean floor; however, the surface features may be available because they are not typically of interest to industry but are certainly important for this project. For example, there are approximately 44,000 km of pipelines in the GOM (Appendix A), and many of these exposed areas at the seabed surface and are known to harbor Red Snapper. There is also valuable information contained in vessel monitoring system (VMS) tracks (Appendix A) from the commercial Red Snapper fishery. Additionally, research groups have high resolution multi-beam and side-scan sonar imagery that could be accessed. These examples demonstrate the available data that might prove to be very valuable in characterizing the GOM habitats. Thus, this group strongly recommends an approach that uses a combination of new sampling strategies at the core, but also takes advantage of the wealth of existing data resources throughout the GOM in developing a comprehensive design. By combining information from ongoing studies, assembling historical data sources, and overlaying with the most detailed habitat mapping as possible, we are confident these studies would improve the estimate of Red Snapper absolute abundance in the U.S. GOM.

Habitat Mapping and Defining Ecological Boundaries

Multivariate models based on physicochemical (sea surface temperature, salinity, dissolved oxygen) and geological conditions (substrate type, % gravel, sand, silt, clay, mud) as well as the presence of artificial reefs (density) within the northern GOM were used to identify major geographic boundaries and regions for the purpose of developing our experimental design. Explanatory variables derived from several sources (usSEABED 2006, GCOOS 2016) were first assigned to cells at a resolution equal to 0.05° , and then natural geospatial associations were determined using grouping analysis within the Mapping Clusters toolset of ArcGIS. Benthic substrate was based on the usSEABED (2006) dataset obtained from the USGS and bathymetry data was obtained from GCOOS (2016). Continuous raster layers of bottom dissolved oxygen, turbidity, and salinity were kriged from data points collected on SEAMAP trawl surveys throughout the Gulf from 2008-2014 to create a continuous raster surface. Grouping analysis is a classification-based procedure that maximizes similarities among geographic cells, leading to natural groupings of cells that share common environmental attributes. Four major groupings or regions were identified in the northern GOM (Fig 1), with the specific location of geographic boundaries influenced by explanatory variables included in the models. Within each of the four major regions, sub-regions were identified using both grouping analysis and bathymetry (breakdown of sub-regions presented in Region 1 shown in Fig 2). Other plausible grouping scenarios for determining strata are possible and are presented in Appendix F. As an example, one option included 3 explanatory variables (salinity, substrate type, and artificial reef density), while a second option included 4 explanatory variables (substrate type, bottom salinity, bottom turbidity, artificial

reef density). Next, the areal coverage of all habitat types within each strata in Region 1 was estimated (Table 1), and this information was then used to assign sampling effort among the major habitat types sampled as part of the stratified random design (Fig 3).

The example presented here only represents Region 1 for the GOM. For the purposes of this design, we assumed that the other Gulf regions were similar, and that costs would not be drastically different. Nevertheless, we do know these regions vary, but a rigorous habitat analysis of the entire GOM was beyond the scope of this design; however, a similar exercise would need to be undertaken by experts for the other three regions that are acutely aware of the habitat nuances associated with those regions for calculating the number of structures and habitat area.

Table 1. Areal extent (km²) of natural hard bottom and unconsolidated bottom, and number of artificial reef structures divided between upper and lower Region 1 split by bathymetric zones.

	Region 1					
	Upper			Lower		
	< 20m	20-50m	>50m	< 20m	20-50m	>50m
Natural hard bottom (km ²)	200	362	369	5	5	294
Unconsolidated bottom (km ²)	15074	14987	10925	5441	14875	13537
Artificial reef small (# structures)	1250	1250	5	1250	1250	5
Artificial reef large (# structures)	6	231	231	5	48	30

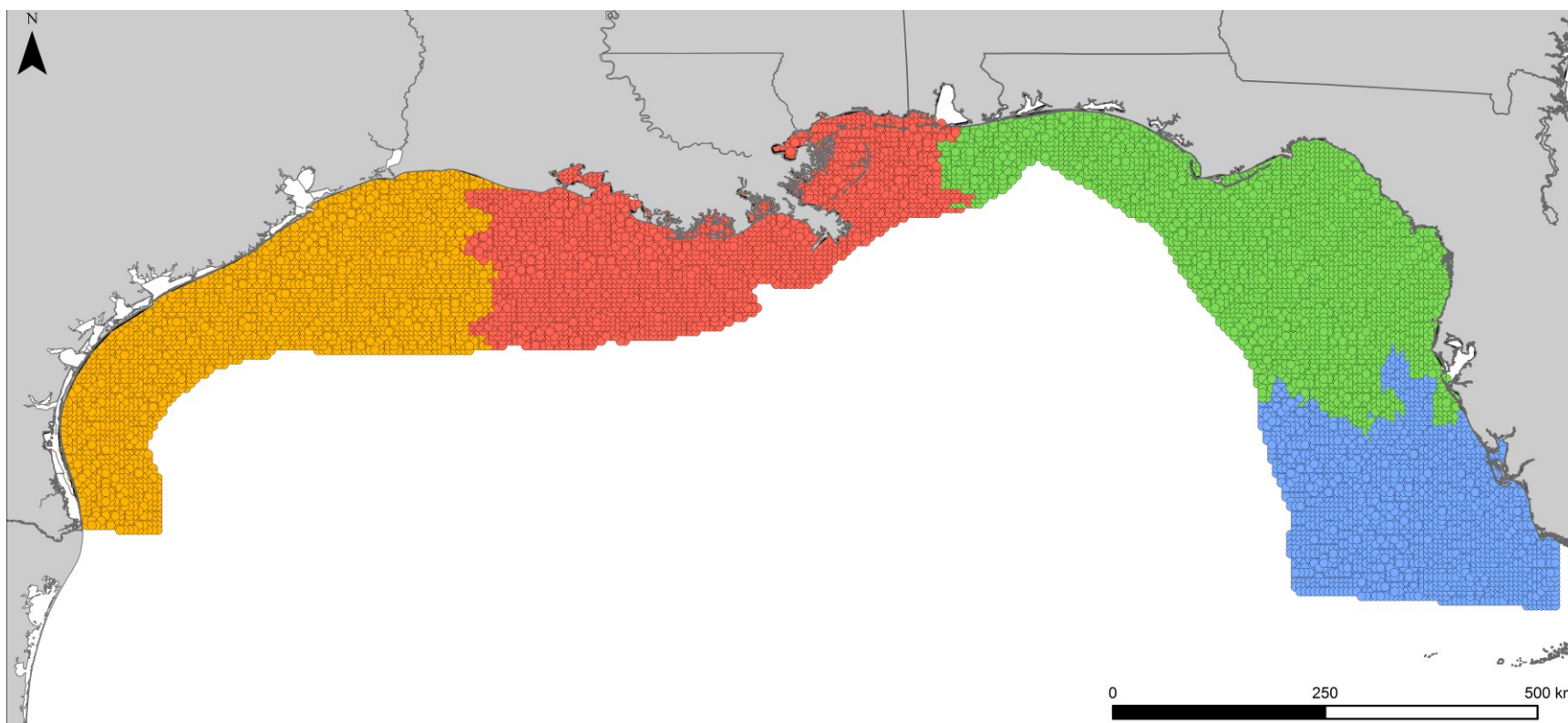


Fig 1. The four major groupings or regions identified in the northern Gulf of Mexico, with the specific location of geographic boundaries influenced by explanatory variables included in the grouping models.

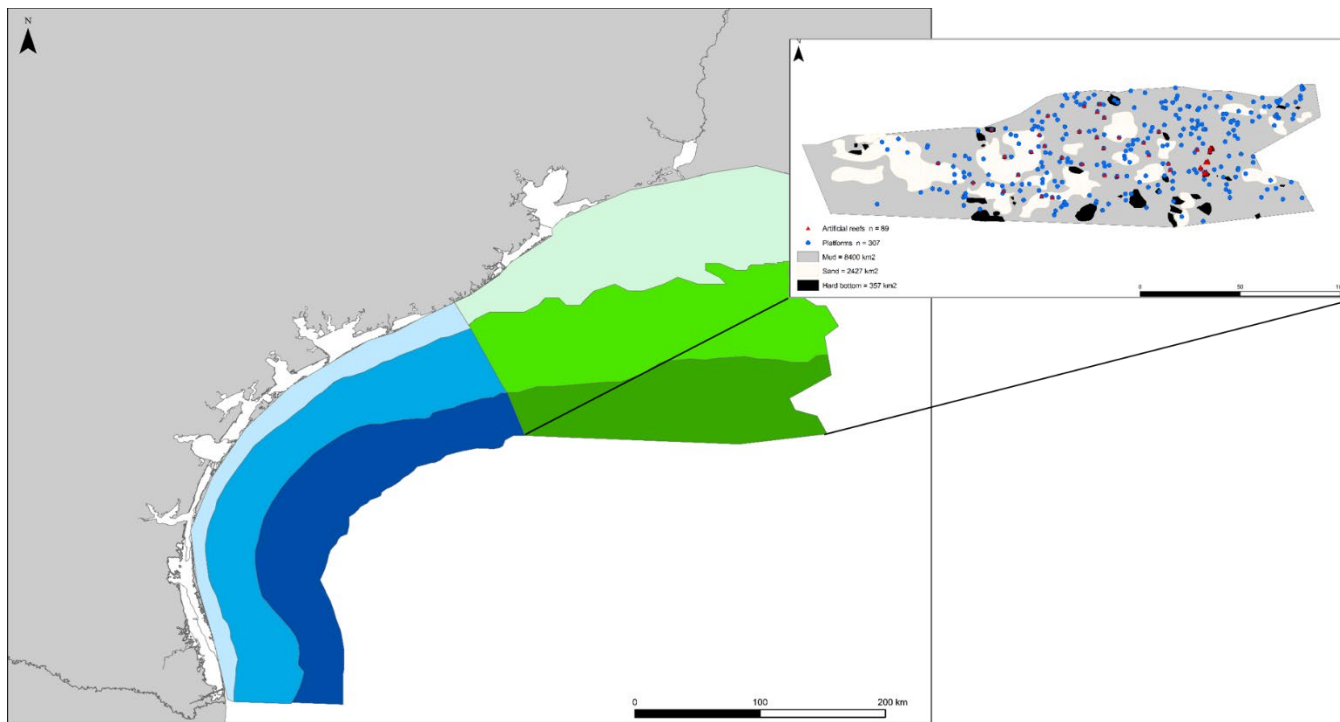


Fig 2. Six strata of Region 1 as determined by ecological mapping and grouping analysis. Inset: geospatial distribution and coverage of habitat types in one selected strata that includes areal coverage of unconsolidated bottom (sand and mud) and natural banks (hard bottom), and the count of artificial reef structures (large and small).

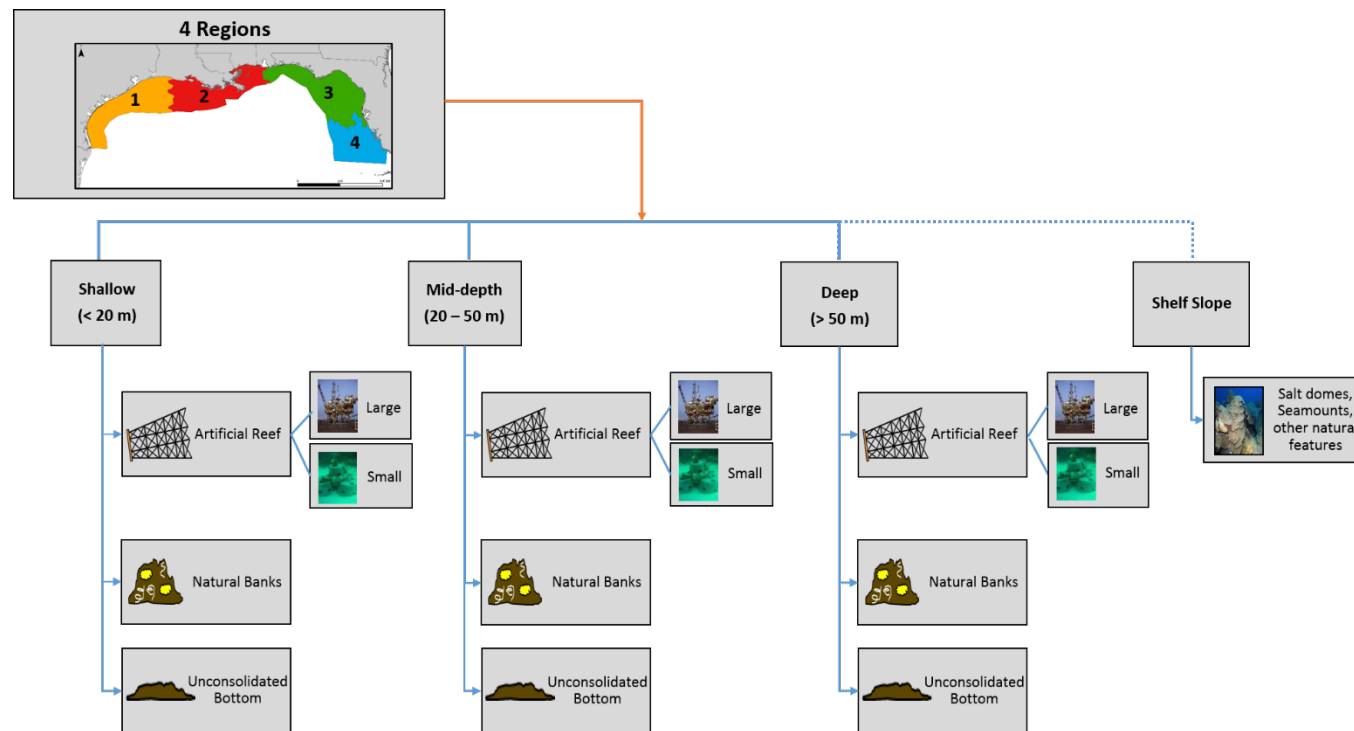


Fig 3. Schematic flow chart representation of the stratified random design of a sub-region from region 1. Each of the 4 regions across the GOM is broken down into 2 sub-regions, and each sub-region contains 3 depth strata (shallow, mid-depth, and deep) to comprise 6 strata for each region. Habitat types are broken down into artificial reef (large and small), natural banks, and unconsolidated bottom for each depth strata. Associated with each region, though not built into the design, are other natural features in deeper waters on the shelf slope. These may include salt domes and seamounts that may hold substantial biomass of Red Snapper but have challenging sampling logistics, and will be opportunistically sampled.

Red Snapper Abundance Estimation

With the robust stratified random design described, the second stage sampling describes specific details on the sampling methodologies being used for Red Snapper abundance estimation. These methods are broken into three categories: (a) exploitation/abundance approaches, (b) density estimates using advanced technologies, and (c) ancillary index-based methods. Methods (b) and (c) strictly adhere to the stratified random design described above for determining necessary sample sizes for efficiently partitioning the variance and sampling effort among the various geographic region and habitat types. The exploitation methods rely on fishing “hotspots” with certain levels of effort provided by the recreational sector that is constrained both spatially and temporally. Thus, this method requires some flexibility in design and may operate independently from the stratified random design used for density estimates, but these studies are still very powerful and will supplement the design by providing additional, independent estimates that will increase our confidence and precision in providing overall GOM-wide estimates of absolute abundance.

Exploitation/Abundance Approaches

Two approaches to determine abundance based on exploitation are presented below. The first method involves an extensive tag-and-recapture program revolving around the federal recreational open season. The initial tagging approach will be scientific-based to ensure appropriate sample design, controls for tagging effects, and addresses assumptions inherent in tagging studies. Recapture of marked individuals will incorporate recreational and commercial fishery stakeholders along with the scientific community to maximize the potential in recovery efforts and provide a mechanism for stakeholder involvement into the abundance estimation process. This involvement allows citizens and regional consortia to provide regional support without compromising the ability to obtain accurate and precise GOM-wide abundance estimates.

The change-in-ratio method requires areas where both recreational fishing and removals are high, making the Red Snapper recreational fishery an ideal sector for data collection using this method. Change-in-ratio was developed and submitted in a separate proposal by PIs Powers, Drymon, Hoenig, and Carleton specifically for the Alabama Artificial Reef Zone, but this method can be easily applied to other recreational fishing “hotspots” across the GOM where effort and removals are high. We are partnering with this group to execute their design in other regions of the GOM. Change-in-ratio methods are briefly described in the section below. For comprehensive details and methodology, see proposal submission by PIs Powers, Hoenig, Drymon, and Carleton.

High Reward Tag-and-Recapture

Sampling Environment - Gulf-wide, artificial reefs and natural hard bottom areas where both recreational fishing effort and removals are high. Here the northern GOM is represented as an example, but this method can easily be scaled to “hotspots” across the GOM.

Sampling Method - High dollar reward tagging studies should be used to improve precision of Red Snapper abundance estimates on artificial and natural structured bottom habitat in areas of high Red Snapper density. Improving the precision of abundance estimates for these areas should increase precision of the total abundance estimates for the northern GOM if abundance is high in these areas. The use of the directed recreational fishing season is an opportunity to use the high-dollar reward estimates of exploitation to obtain abundance estimates by dividing an estimate of Red Snapper harvest for these areas by the estimate of the exploitation rate from high reward tagging.

Protocol - The tagging approach should carry out one-year studies at each area by releasing tagged fish into the population immediately prior to the opening of the recreational fishing season. Reward tagging studies rely on anglers to report the capture of tagged fish, for which they receive a relatively large monetary reward. Because of the short duration of the recreational fishing season (<50 days, 2010 – 2014), commercial harvest and natural mortality can be assumed negligible. The exploitation rate (i.e., proportion of the stock harvested; fishing mortality) could, therefore, be estimated simply as the proportion of the tagged Red Snapper harvested and reported by recreational anglers during the fishing season, after accounting for tag-loss, tagging mortality, and angler reporting rate.

We recommend employing a high-dollar reward approach, so that the reporting rate recaptured fish by anglers can be assumed to be 100% or very nearly so. Anglers will receive a reward of \$300 for reporting the capture of a tagged fish. We anticipate that this reward amount will elicit a 100% reporting rate based on inflation-adjusted estimates from previous studies (Nichols et al. 1991; Denson et al. 2002; Taylor et al. 2006). Tag loss will be estimated by double tagging 30% of tagged Red Snapper. Tag loss rates can then be inferred from the proportion of double-tagged fish that are reported as harvested with only one tag remaining.

Tagging mortality estimates should be taken from previous and ongoing studies (for example, Curtis et al. 2015 and others) and from published meta-analyses of tagging mortality of Red Snapper (Campbell et al. 2014) and will depend on the water depth at the point of release for each fish. High dollar tagging studies should be employed only in areas with substantial inventory of existing natural and artificial structures. Structures on which to tag fish should be randomly selected from inventory lists of structures from side-scan sonar images or published lists of waypoints. This approach will ensure that sufficient numbers of Red Snapper can be captured, tagged, and released efficiently, and that tagged fish can be distributed throughout these high-density study areas. Fish should be tagged only in depth zones that do not exceed 30 m to reduce barotrauma and subsequent tagging mortality.

A robust study should tag 500 fish per year at each study area. Fish should be tagged on artificial and natural structures only, and thus the exploitation and abundance estimates will pertain only to fish located on those structures. No more than five fish should be tagged at each structure to guard against non-independence of the fates of individual tagged Red Snapper. Thus 100 structures should be selected in each study area. Fish should be tagged with individually numbered 150-mm long yellow dart tags. The reward amount along with instructions for removing the tag and phone and email contact information should be printed on each tag. Tagged fish should be released with a descender device to reduce barotrauma and each release video should be recorded at depth to assess the condition of released fish.

Tagging should take place during a one month period just prior to the opening of the Federal directed Red Snapper recreational fishing season.

Exploitation rates should be estimated using a closed population probability model that uses a Bayesian estimation approach via MCMC sampling. The Bayesian approach will facilitate the propagation of uncertainty in literature-based tagging mortality estimates by formulating informative prior distributions for tagging mortality rates. The model will assume a multinomial sampling distribution for the observed fates (reported as captured with one tag, reported with two tags, not reported) of tagged fish. The model will estimate the exploitation rate, tag loss rate, and tagging mortality rate.

Abundance from the tagging studies should be estimated by dividing harvest estimates by exploitation rate estimates. Harvest estimates for each study area that receives a high-dollar tagging study should be obtained by adding language to dockside interviews and telephone effort surveys to determine the amount of time anglers spent fishing in these well-defined areas. During angler interviews, communication of latitude/longitude coordinates should occur. For example, use maps shown to anglers and/or describe the boundaries of each study area to elicit accurate fishing location responses from anglers. Harvest (and its variance) can then be estimated separately for each study area for ultimate use in the abundance estimation. Abundance estimates can be obtained by dividing harvest estimates by the exploitation rate estimates. Uncertainty in abundance can be obtained by dividing 1,000 samples of harvest drawn from its estimated probability distribution by 1,000 samples of the exploitation rate taken from its posterior distribution from the Bayesian model. The mean and variance of these 1,000 quotients should then be computed and interpreted as the abundance estimate for Red Snapper residing on structured habitats in each area.

We conducted statistical power analyses to estimate the expected coefficient of variation of these abundance estimates. Tagging of 500 Red Snapper results in a CV of the estimated exploitation rate of approximately 10% under realistic exploitation rates of around 15%, tagging mortality of 15%, and tag loss rates of 15% (Sackett and Catalano *accepted*). If we assume that the CVs of the harvest estimates would be approximately 30%, then our simulations suggest that CVs of the abundance estimates would typically be around 15%. Costs for a single tagging study in one area would include: \$30,000 for tag rewards and \$170,000 for capturing, tagging, and releasing fish for a total project cost of \$200,000 per study area.

Change-In-Ratio Method

Sampling Environment - Gulf-wide, artificial reefs and natural hard bottom areas where recreational fishing effort is high and removals are high. Here the northern GOM is represented as an example, but this method can easily be scaled to “hotspots” across the GOM.

Protocol - The change-in-ratio (CIR) method can be used to estimate exploitation and abundance of legal- and sublegal-sized fish in areas where removals are high. Change-in-ratio is based on the idea that selective harvest of legal-size individuals shifts the population ratio of legal to sub-legal fish. This method requires fishery-independent surveys before and after the fishing season (see “Vertical Longline” section below for details). Estimates of exploitation rate can be calculated without knowing removals. An estimate of absolute abundance requires knowing the absolute removals in an area; however, an estimate of relative abundance simply requires knowing the fraction of removals (taken in the entire U.S. GOM) that occurs in an area. The CIR method has been used in preliminary work for Red Snapper on artificial reefs within the Alabama Artificial Reef Zone. Using this method, an unbiased estimate of exploitation can be

obtained via $u = \frac{p_1 - p_2}{p_1(1-p_2)}$ where p_1 and p_2 represent the proportion of legal-size fish in the pre-

and post-season survey, respectively (Seber 1982, Pollock and Hoenig 1998). If the survey gear is size-selective, then p_1 and p_2 should be corrected to reflect this.

Advantages – The change-in-ratio method can provide both exploitation and absolute abundance with relatively few assumptions necessary. Computationally, the method is straightforward. Conceptually, the method is also straightforward, making it easy to convey to stakeholders. It has successfully been conducted (proof of concept), and generated consistent and comparable results with other approaches (e.g., catch-survey-catch). The change-in-ratio method can be combined within a likelihood framework to obtain more precise estimates of abundance (Chen et al. 1998).

Limitations – These methods only work in areas where removal are high and occur over a brief period, so that emigration and natural mortality is negligible. You may need to account for gear selectivity, which affects your choice of sampling gear options. Growth is not accounted for, although it is presumably negligible if conducted in a short timeframe.

Density Estimates using Advanced Technologies

One of the most difficult aspects in estimating an absolute abundance is determining which method(s) are most capable of providing an unbiased and accurate count of individuals considering the myriad of challenges associated with each sampling gear. This is further complicated by the large variation of habitat types, bathymetry, and physical conditions (i.e., depth, visibility, etc.) across the GOM. Certain gear types may excel at sampling artificial reef habitats but fail when more expansive areal coverage is required for estimating density over unconsolidated bottom habitat. We recommend a combination of advanced ROV surveys with built-in bioacoustic methods coupled with video-surveys from a towed camera system known as C-BASS (Camera-Based Assessment Survey System). The ROV/bioacoustic surveys will focus on artificial reef habitats (large and small) and natural hard bottom habitats, where the areal footprint is relatively small. The towable C-BASS excels at efficiently sampling large areal expanses of relatively low relief structure and will be used to sample low relief natural hard bottom areas and the extensive area of unconsolidated bottom habitat. Details for these recommended methods are described in the following sections.

ROV/USBL/Bioacoustic Surveys

Sampling Environment - Gulf-wide: artificial reefs and natural hard bottom. This combination of methods allows for density estimation in areas with a variety of visibility conditions.

Sampling Method - In-situ abundance, density, and biomass information of Red Snapper on large artificial reefs (standing platforms, rigs-to-reefs structures, ships), small artificial reefs (concrete pyramids and culverts, tanks, chicken coops) and natural banks (or other large-scale natural habitats) within the study area should be estimated using a complimentary micro-remotely operated vehicle (micro-ROV) and bioacoustic survey approach. Current work on large artificial reefs in the NW GOM is being successfully conducted with a VideoRay Pro 4 micro-ROV equipped with a compass, depth sensor, temperature sensor, auto-depth holding capabilities, forward facing color camera (520 line, 0.1 lux), LED array for illumination, and red laser scaler to estimate fish size (8 cm between lasers). Additionally a Tritech MicronNav Ultra Short Baseline (USBL) Positioning System is used to track the vehicle in real-time and

record survey tracks.

Because this VideoRay Pro 4 system does not record high-definition footage, a forward facing GoPro® camera is also mounted on the ROV float block. Footage from these devices is used to solely supplement identification, with all counts conducted within the VideoRay field of view.

In the Northern GOM, surveys on small structures have been conducted with a similarly outfitted VideoRay ROV as well as a Seabotix five-thruster LBV300-5 ROV equipped with parallel red lasers spaced 3 cm apart. For ROV-based density estimates conducted across the GOM, we suggest the use of identical or similar micro-ROVs. The use of identical/similar equipment will minimize variability between surveys as these small ROVs will generate similar luminescence, noise, and physical disturbance (see Limitations section). For bioacoustics surveys, dual frequency identification sonar (ARIS explorer 1200) acoustic techniques should be used to derive these metrics. The application of the ARIS explorer systems provides enhanced real-time acoustic images of fishes and their associated habitat using advanced technology that has improved upon DIDSON sonar technology. The dual frequency operation of 1.8 MHz and 1.1 MHz will allow for video-quality resolution over a wide-range of depths with a maximum rating of 300 m. The benefit of the ARIS sonar over a traditional video technique is the ability to capture acoustic images of fish in highly turbid and light limited environments, allowing for detailed abundance and biomass estimates in environmental conditions typical of coastal waters in Louisiana and north Texas.

Protocol – Large Artificial Reef Survey - Upon arrival at a sampling site, a bioacoustic survey should be performed. These surveys would consist of three replicate sets at each reef with a deployment time of 10 min for each set. The sonar should be attached to a fixed frame and deployed approximately 2-3 m deep into the water directly above the habitat. After these initial bioacoustic surveys, an ROV survey should be conducted using methods adapted from Streich (2016; described below) which used video-based surveys to estimate Red Snapper density at large artificial reefs and natural banks using standardized transects. Abundance estimates from ROV should be standardized by estimating the area surveyed (e.g., area surveyed = mean visual field width x transect length). Visual field width should be estimated using the laser scale to measure the field of view at approximately fixed intervals along transects. Measurements should then be averaged to provide a mean visual field width for each transect. Distance surveyed should be confirmed using the USBL position data.

At large artificial reefs, 40-m transects (the approximate length of a toppled rigs-to-reefs structure) should be surveyed. During these transects, the ROV would travel forward at a constant speed along an approximately straight path and maintain a consistent distance from the artificial structure (e.g., 2-3 m). These criteria will minimize double counting fish and allow better estimates of surface area surveyed – thus, providing more accurate density estimates. Generally, transects at large artificial reefs should be located along piles or crossbeams (standing platforms or rigs-to-reefs structures) or along the length of the structure (ships) to provide a natural navigation aid for the ROV pilot. Because Red Snapper are a demersal species (Gallaway et al. 2009), and with previous micro-ROV work on large artificial reefs in the NW GOM indicating detection depths of approximately 40-60 m (Stunz, unpublished data), transects should also be located as close to the seafloor as possible (as deep as visibility allows) to maximize effort within Red Snapper preferred habitat. To control field of view, only Red Snapper within 1 m of the exterior of the artificial reef should be counted (i.e., those inside the crossbeams and piles should not be counted). Furthermore, camera angles will be kept consistent throughout the survey and lights should remain off. In some circumstances, lights may be necessary and if so, a documented log of variables should be maintained. Three independent transects should be performed at each structure to account for potential sampling variability. A second set of bioacoustics surveys should be completed after the micro-

ROV survey at selected sites during the study. This additional data will help to clarify fish attraction and avoidance issues with the ROV technology.

Protocol - Small Artificial Reef Survey - On a single artificial reef, an ROV is deployed and positioned on the bottom within 5 meters of the target feature. Target features sampled with this protocol include small artificial pyramids (less than 3 m vertical relief) tanks, chicken coops, cement drums, etc. The ROV heading, depth, range to target, GPS position (for the boat) and start time of the video are recorded for the feature. Video should be recorded for two minutes at the designated heading (in degrees); then the ROV should be flown to the opposite side of the feature for two additional minutes for sampling as described above (i.e. on the bottom, within 5 meters of the feature). The second heading and range to feature is recorded (~180 degrees from first heading). Finally, the ROV should be positioned ~1 meter above the feature for a slow 360-degree spin and a vertical view of the structure. After recording is complete, the video stop time is recorded. Total time for video recording should be between 7-10 minutes. A minimum count (a.k.a. MaxN) of Red Snapper is then generated for each ROV deployment. The minimum count method is a standard way of generating an index from video data because it represents an absolute minimum number of fish at that station while avoiding the issue of double counting (Bacheler and Shertzer, 2015). Red Snapper lengths can be obtained in instances where the fish are illuminated by the ROV mounted lasers, spaced 3 cm apart.

Because the entire reef can potentially be seen in the video, there is potential to generate an absolute abundance with this method. An estimate of the reef area included in the video analysis will need to be determined to calculate a mean density for these small-scale structures. A second set of bioacoustics surveys should be completed after the micro-ROV survey at selected sites during the study. This additional data will help to clarify fish attraction and avoidance issues with the ROV technology.

Protocol - Natural Bank - As described above for the other habitat types, a bioacoustic survey should be performed upon arrival at a sampling site. Transect placement on natural banks should be guided by georeferenced multibeam maps of bank bathymetry as available. Because natural banks are considerably larger than artificial structures, a grid should be overlain onto multibeam or other existing imagery of the selected natural bank or reef in ArcGIS (ESRI 2015). Grid cells should be sequentially numbered, and three cells should be randomly selected for sampling before each sampling trip using a random number generator. Within a selected cell, a weight attached to the ROV tether will be lowered to the seafloor, and the ROV should be deployed with a predetermined length of leash. Using the weight as a starting point, three orthogonal 40-m transects with specific compass headings will be traversed with at least one of these spanning the maximum available vertical relief of the bank (i.e., directly up the bank slope). After the three transects are completed, the boat would move to the next selected grid cell and repeat the procedure. During each transect, the micro-ROV should maintain a consistent speed, heading, and distance from benthos, lights will remain off and camera angle will be maintained. A second set of bioacoustics surveys should be completed after the micro-ROV survey at selected sites during the study. This additional data will help to clarify fish attraction and avoidance issues with the ROV technology.

Protocol - Video Review - Recorded ROV video should be examined in the lab by two independent viewers. Viewing should begin as soon as the ROV enters the water and end when the ROV surfaces. Fish should be identified to the lowest possible taxon (typically species), enumerated, and recorded each time they enter the field of view. If fish counts differ, the two viewers should jointly examine the video to reach a consensus. For density estimation at large artificial reefs and natural banks, the final counts from each 40-m ROV transect should be summed and divided by the area surveyed to generate a Red Snapper density estimate for each transect (no. of individuals m⁻²). At small structures, an estimate of the reef area

included in the video analysis will need to be determined to calculate a mean density. Using the proportional abundance of Red Snapper observed during an ROV survey, Red Snapper abundance can also be estimated from the total fish abundance estimated during the bioacoustic surveys.

Advantages - There are multiple advantages to using a combination of visual and bioacoustics technologies. Density estimates can be obtained using these survey methods with simple updates in technology (USBL) and methodology. These non-invasive methods can also be applied to a variety of habitat types including large/small artificial reefs, natural banks, and low-relief natural hard bottom. The small size of the micro-ROV mentioned above allows for ease of deployment (no winch, 12V battery for power source) off a small vessel. Additionally, issues such as fish attraction and avoidance should be less than with a larger, working class ROV (less noise, lower light intensity, etc.). For Gulf-wide surveys, the costs associated will be much less than with a working class ROV that requires a large research vessel for deployment and operation. The addition of bioacoustics technology also gives researchers the ability to capture acoustic images of fish in highly turbid and light limited environments, allowing for detailed abundance and biomass estimates in environmental conditions typical of certain regions of the NW GOM.

Limitations - Perhaps the greatest limitation to using ROV technology Gulf-wide is the visibility constraint in many nearshore and near-bottom environments. For the NW GOM, this does appear to have a seasonal timeframe with late summer/early fall allowing access to clearest water. Sampling with visual methods such as ROV should be limited to this time of year for those environments. Additional methods (bioacoustics, vertical line, tagging studies, etc.) should be implemented to allow for alternate/complimentary estimation techniques that additionally provide validation.

Fish avoidance and attraction can also be problematic if those effects are unknown for the species of interest. Although this has been noted for many species (see Stoner et al 2008), no published data exist for Red Snapper. Previous ROV work in the NW GOM (Stunz, unpublished) has commonly sighted Red Snapper on various habitat types, however the effects of the associated noise, lighting, etc. have not been specifically evaluated. Qualitative observations suggest that Red Snapper show limited avoidance or attraction to the micro-ROV. In the proposed work, the maintenance of detailed descriptions of equipment and operating modes will allow for comparative analysis across the GOM and teams will need to use similar gear. The use of bioacoustic surveys both pre- and post-survey will help evaluate behaviors of fish in both the near and far field area.

Contingency Plans - In areas where poor visibility interferes with ROV visual data collection, baited vertical longline fishing surveys may be used to validate species identifications from the acoustic data. At each reef location, two vertical longline reels should be used for a combined total of 5-10 longline sets per reef with a soak time of 5 min for each set. Explicit vertical longline sampling methods are described below in section c.

C-BASS

Sampling Environment - Gulf-wide; the towed camera method using C-BASS will allow for density estimates across a variety of habitats including expansive “unconsolidated” bottoms and natural reef habitats.

Sampling Method - Stratified density and total abundance for Red Snapper on large expanses of “unconsolidated” bottom (open mud, sand, pipelines, ephemeral mud features and lumps, etc.) and natural reef habitats within the study area will be estimated using video-surveys from a towed camera system known as C-BASS (Camera-based Assessment Survey System). The C-BASS is a towbody equipped

with cameras and other instrumentation which has been designed to be towed off the stern of research vessels at a constant speed and consistent altitude above the seafloor for several hours at a time. Survey operations with C-BASS can be done using small (> 100 feet) to mid-size (150-200 feet) research vessels that are equipped with a winch- controlled four conductor cable capable of loads >2,000 kg. The system is intended to be towed at 1.0 - 2.0 meters per second under both daytime and nighttime conditions at altitudes of less than 5 meters when the bottom rugosity permits. The C-BASS has a maximum operating depth of 200 m. Power to the C-BASS comes through two of the conductors in the cable which are connected to the ship's generators. The other two conductors are used for communications which affords the pilots of the system real-time control of the cameras and other sensors via a custom web-based user interface. The C-BASS is currently configured with ports for up to six video cameras, a laser scale, four LED lights, a CTD, fluorometer, altimeter, compass, internal monitoring sensors, and a DIDSON sonar, all of which can be monitored real time. It has also been equipped with an onboard computer and 1TB of storage. The C-BASS video system is capable of efficiently sampling large areas, a modest cost to build and operate, and relatively easy to use.

Protocol - Transect placement on unconsolidated bottoms and natural reefs will be guided by geo-referenced multibeam maps of habitat bathymetry when available but will generally employ a stratified random design. Upon arrival at a sampling station, C-BASS will be lowered from the ship's winch to a depth of 10-15 m. Once proper functioning of cameras, other sensors, and communication with the vessel is established, the C-BASS is lowered to the desired towing altitude above the seafloor (optimally 2-3.5 m) and towed 1.0 - 2.0 meters per second for the duration of the deployment. The C-BASS is typically deployed for 3-6 hr durations which results in 18-36 km transects. All footage from the video systems is analyzed by first counting and identifying all observed fishes in the imagery. Red Snapper counts will be binned into one minute intervals from the continuous video recorded for each transect.

To estimate fish density from the towed camera data, the total area viewed during each transect is calculated which requires knowing the average width and length of the transect for each minute of the survey. Transect width is determined by measuring how many "laser widths" crossed the width of the field-of-view at various altitudes (Grasty 2014). The total distance covered for each transect is then calculated by averaging the speed over ground (in knots) of the ship for each minute, converting this value to meters/second, and using this result to estimate the distance covered. The product of transect width and length covered per minute provides the approximate area in which counts are made for each transect and can be used to estimate Red Snapper density.

Using the raw counts of Red Snapper for each minute in each transect, abundance estimates can be extrapolated using stratified random sampling statistics. The raw counts are divided by the area covered during the respective one minute interval in which they are made and this density estimate is converted to number of individuals per square kilometer. These values are then sorted into groups based on which stratum they are associated with (e.g., unconsolidated bottom or natural reef) and the densities within each stratum were averaged (the number of samples taken for each stratum is the number of minutes spent in that habitat type). These average densities are then used to estimate absolute abundance of Red Snapper within the different strata following methods outlined in Cochren (1977).

Advantages - There are multiple advantages to using the C-BASS towed camera system. One of the greatest advantages of the C-BASS over other video-based methods such as ROVs, AUVs, or fixed cameras is that it can cover a much larger habitat area at a moderate cost. Density estimates can be readily obtained using this method, and the arrangement and presence of numerous other sensors and equipment allows pairing other habitat characteristics with density estimates. In addition to estimating reef fish densities, imagery can be used to assign habitat types in the area(s) being surveyed for stratifying population estimates by habitat type. Imagery can also aid in ground-truth of acoustic

backscatter and grab sampling, two common assessment techniques for creating benthic habitat maps. This method can also be applied to a variety of habitat types including oil or gas pipelines, open mud/sand, natural banks or other hard bottom. The C-BASS has been tested aboard multiple vessels and its performance was stable regardless of vessel thereby demonstrating the versatility of the C-BASS.

Limitations - Perhaps the greatest limitation to using C-BASS technology Gulf-wide is the visibility constraint in many nearshore and near-bottom environments. For the NW GOM, this does appear to have a seasonal timeframe with late summer/early fall allowing access to clearest water. Sampling with visual methods such as C-BASS should be limited to this time of year for those environments. Additional methods (e.g., bottom longline area fished) should be implemented to allow for alternate estimation techniques. Fish avoidance and attraction can also be problematic if those effects are unknown for the species of interest. Although this has been noted for many species (see Stoner et al 2008), no published data exist for Red Snapper. An analysis by Grasty (2014) showed that most fish showed a neutral (fish not attracted or repelled from C-BASS) or weak negative (fish moves away from camera system but remains within the camera field-of-view).

Contingency Plans - In areas where poor visibility interferes with C-BASS visual data collection, bottom longline area fished methods can be used to estimate Red Snapper abundance. At each planned transect location, two bottom longline sets should be performed.

Ancillary Index-Based Methods

While we highly recommend the above described methods that use advanced technologies to estimate absolute abundance for Red Snapper, there are several ancillary sampling methodologies that can be used to supplement the density estimations described above. These methods may be especially useful in areas where visibility may impede visual-based surveys, and can serve as contingency plans when optimal conditions or requirements for sampling are not possible. Although these ancillary methods generate indices of relative abundance of Red Snapper, the resulting data will allow us to calibrate and reduce the variability (CV) of the density calculations across the Gulf of Mexico by addressing critical data gaps. Additionally, the GOM shelf slope should be considered in the overall design as these deep habitats potentially harbor Red Snapper that are typically not sampled with previously described sampling techniques. While estimating the abundance of Red Snapper in these deep habitats is beyond the scope of this project, we recommend that the methods described below (particularly Fixed Camera arrays and BLL sampling) are used as time and resources allow. Studies on these deep habitats could be as simple as assessing species presence/absence, as very little information about Red Snapper use of these deep habitats exists. Such data could be analyzed in conjunction with the density of snapper on shallower, natural habitats where better population estimates are possible, and this certainly would require assuming similarities in abundance.

However, these studies would allow us to begin to make inferences about densities of snapper on these deeper habitats on the shelf slope in the GOM, where they are now known to occur.

Fixed Cameras Visual Surveys

Sampling Environment - This supplemental technique can be used Gulf-wide, but only in areas with acceptable visibility (75% transmissivity).

Sampling Method - Red Snapper abundance and size can be assessed using a fixed 4-camera underwater video array on various habitats in the GOM (unconsolidated bottom, natural banks, and artificial reefs) at a broad range of depths from shallow waters (20 m) to deep waters (60 m) of the shelf. Previous studies

in the GOM (e.g., NOAA, Wells and Cowan 2007) have used arrays consisting of four Sony DCR-VX1000 digital video camcorders housed in aluminum underwater housings. Cameras are positioned orthogonally to one another at a height of 25 cm above the bottom to provide a nearly 360° view. Each camera has a 72.5° viewing angle with a viewing distance of 5 m, resulting in an estimated viewing area of 70.4 m³ (Rademacher and Render 2003). For this method to be effective, it is critical that transmissivity is at least 75% which will confirm the viewing distance of 5 m (Gledhill and Lyczkowski 1994). Finally, two parallel beam lasers placed 10 cm apart are attached below each camera to aid in estimating lengths of observed fish to the nearest cm. Although traditionally fixed camera arrays used for reef fish assessments have consisted of equipment described as in the above text, other less costly alternatives can be considered which will reduce both equipment and deployment costs as these less bulky arrays can be deployed off smaller vessels. Updates in camera technology (High Definition cameras such as Go Pros) can allow researchers to devise a similar system with less expensive, commercially available equipment.

Protocol - The fixed-camera array is deployed for 30-min and is baited with a single Atlantic menhaden (*Brevoortia tyrannus*), which is replaced after each deployment. All video samples should be taken during daylight hours (30 minutes after sunrise to 30 minutes before sunset). In order to estimate the visibility, optical backscatter measurement will need to be taken at each site. This can be done with a Sea-Bird SBE-25 CTD during the camera array soak period.

Continuous 20 min segments of one video are examined for fish abundances at each deployment. Videos are chosen based upon the optimal view of the habitat of interest combined with the best visibility (i.e., in focus, good orientation relative to the current). Gledhill (2001) determined this continuous 20 min method to be optimal for reducing error in abundance estimates for sampling the taxa present, and for minimizing logistical constraints such as available time at sea. Start time begins once the camera array is on the bottom and after sufficient time elapsed for the water column to clear. All Red Snapper are identified and counted, and the minimum count (MIN), the maximum number of a species observed at any one time on the video, is recorded to gain a relative abundance for each habitat/depth. Estimates of total length (TL) are made only at MIN counts to eliminate repeated measurements of the same fish. Maximum counts (MAXIM) are also made to obtain total counts of each fish species seen over the 20 min segment of the video analyzed.

Advantages - Fixed camera methodology is a practical method to characterize relative abundance of Red Snapper over a variety of substrate types, particularly because structurally complex habitat types with high relief (natural and artificial reefs) require non-invasive sampling techniques. In addition, the logistical simplicity of dropping the camera array for a 30min period makes this an appropriate method if multiple deployments over distant sites are needed.

Limitations - A limitation of this methodology is that it may be size-selective for larger Red Snapper, which may underestimate the total number of individuals at each habitat. Thus, recent studies (Wells and Cowan 2007) recommend pairing fixed camera systems with another gear type (trawls) which are more effective at sampling smaller individuals. Similar to the other visual techniques proposed (ROV and C-BASS), visibility is a constraint in many nearshore and near-bottom environments. Additionally, the expense of using this method may be cost-prohibitive, as a large research vessel is required to deploy the large camera system described in detail above. However, if less costly modifications were implemented to make it deployable off of small vessels it would be much easier to implement regionally and gulf-wide. Finally, this approach can only be used to generate an index of relative abundance, because it is a baited sampling technique that only generates MIN counts.

Vertical Longline

Sampling Environment – Gulf-wide; this method is best suited for discrete areas with high concentrations of fish, such as large and small artificial reefs or small natural hard bottom habitats, but is otherwise widely scalable across the GOM.

Protocol - Vertical Longline is a sampling technique that can be used to capture fish to aid alternate methods of abundance estimation (e.g., catch-survey-catch, change-in-ratio), and serve as a method for biological sample collection. Additionally, vertical longline represents the most common harvest gear in the commercial fishery for Red Snapper. Three manual, hand cranked reels are fished simultaneously; each monofilament backbone is assigned a different circle hook size (8/0, 11/0, and 15/0) with 10 gangions spaced equally along the backbone. All hooks are baited with Atlantic mackerel (*Scomber scombrus*). After a five-minute soak time, gear is brought to the surface and hook size, species identification, condition, size (standard, fork and stretched total lengths), and weights as well as station information (location, bottom type, depth, time of day) are recorded. The gear configuration and sampling procedure described above have been adopted by NOAA SEAMAP as a standardized method for vertical longline sampling throughout the GOM.

Advantages – Vertical longline sampling allows for direct quantification of fish (via catch) and is a method already extensively employed by many GOM SEAMAP partners to develop long standing time series of catch data. This technique can be used in conjunction with multiple other techniques described (catch-survey-catch, change-in-ratio methods) to estimate absolute abundance. Catch obtained from this method provides specimen collection for biological samples (otoliths, gonads, fin clips, tissue, etc.).

Limitations - In and of itself, this approach can only be used to generate an index of relative abundance, and not absolute abundance. However, this does allow for calibration of other methodologies, and can be used in combination with other methods to provide absolute abundance, and should be used where appropriate.

Catch-Survey-Catch Method

Sampling Environment - This method is best suited for shallow areas with small artificial reefs; as such, it could be scaled to several areas within the GOM. This is particularly true for Alabama and Florida, yet particularly problematic for areas like Texas and Louisiana.

Protocol - Catch-survey-Catch (CSC) is a proportional or ratio-based method similar to the Change-in-Ratio method that uses vertical longline sampling in combination with ROV surveys. On a single artificial reef, an ROV is deployed immediately before and after fishing with vertical longline gear. A minimum count (a.k.a. MaxN) of Red Snapper is generated for each ROV deployment both before and after fishing with vertical longline gear. The minimum count method is a standard way of generating an index from video data because it represents an absolute minimum number of fish at that station while avoiding the issue of double counting (Bacheler and Shertzer, 2015). The number of fish removed by the VL gear is known, and any reduction in the index can be attributed to this removal. The abundance of fish at a single reef can be quantified using the minimum count as an index via the index-removal method. Dividing the total Red Snapper removed by the vertical longline by the proportion removed in the before/after video index gives an estimate of Red Snapper on each structure. Mean abundance or biomass at each site can be multiplied by the total number of reefs in the area of interest to estimate a

total abundance in the area.

Advantages – Vertical longline sampling in and of itself is solely capable of generating an index of relative abundance; however, when used in combination with ROV surveys using this method can provide an estimate of absolute abundance. Further advantages include the need for relatively few assumptions to be made, the method is computationally straight forward, and conceptually straight forward, which makes it easy to convey to stakeholders. This technique has been successfully conducted previously (illustrating proof of concept) and resulted in comparable and consistent estimates with other approaches (e.g., Index removal, Change-in-ratio methods). Catch obtained from the vertical longline component of this method provides specimen collection for biological samples (otoliths, gonads, fin clips, tissue, etc.).

Limitations – There are several limitations with this method. It will only work in areas where the sampling units are discrete and relatively small (i.e., single reef pyramids or small natural reefs). Larger habitat types such as oil/gas platforms or large natural banks encompass too great a scope for this method to be feasible. Similar to ROV and fixed camera methods, it will only work in areas where camera gears are feasible – relatively shallow depths, and necessitating a certain level of visibility for success. Also, this method assumes that the index generated from the camera is directly related to CPUE of the vertical longline.

Bottom Longline

Sampling Environment - This method is best suited for large areas with relatively low concentrations of fish, but is otherwise widely applicable across the GOM. Applicable habitat types include natural hard bottom and to an extent nearby unconsolidated bottom, but would not be feasible on artificial reef habitats.

Protocol - Bottom Longline is a sampling technique that can be used to capture fish to aid alternate methods of abundance estimation (e.g., catch-survey-catch, change-in-ratio, and index-removal methods). A monofilament mainline (426 kg or 1000 lb test, 4 mm diameter, 1 nautical mile length) is deployed off the stern through a block. High flier buoys and bullets are used at the start and end of each set. Five kg (11 lb) weights (start, mid-set, end set), and 3.66 m (12 ft) gangions (332 kg or 700 lb test, 3 mm diameter) with 15/O circle hooks are clipped to the mainline as it is deployed. Vessel speed ranges from 4 – 5.5 knots during deployment. One hundred hooks are fished for one hour (soak time). Soak time is determined from the time the last high flier buoy and bullet were deployed during the set until the first high flier buoy and bullet are retrieved to begin the haulback. Haulback speed is approximately 3.5 - 4 knots.

Gangions are baited with Atlantic mackerel (*Scomber scombrus*, 12 kg or 27 lb per set) cut to fit the circle hooks. All fishes that can be safely handled are boated, measured to the nearest mm (standard, fork and stretched total lengths) and weighed to the nearest 0.1 kg. For complete details see Drymon et al. 2013.

Advantages – Bottom longline sampling allows for direct quantification of fish (via catch) and is a method already extensively employed by many GOM SEAMAP partners to develop long standing time series of catch data. This technique can be used in conjunction with multiple other techniques described (Catch-Survey-Catch, Change-In-Ratio, Index-Removal methods) to estimate absolute abundance. Spatially, this method offers the opportunity to sample much greater area than other capture gears (e.g., vertical longline). Lastly, catch obtained from this method provides specimen collection for biological samples (otoliths, gonads, fin clips, tissue, etc.).

Limitations - In and of itself, this approach can only be used to generate an index of relative abundance,

and not absolute abundance. However, this does allow for calibration of other methodologies, and should be used where appropriate.

Final Design and the Design Optimization Tool

We have developed a Design Optimization Tool in Microsoft Excel that allows the user to explore alternative sampling designs along with the costs and associated Coefficient of Variations (CVs). The tool requires the user to supply information about strata sizes, estimated means, and standard deviations of Red Snapper for each stratum. With this information, the tool allows the user to experiment by specifying different scenarios such as varying sample sizes that will achieve the desired CV. If the user would like to find the design that minimizes cost, they can also supply the cost per sampled unit parameter for each stratum, and the tool will solve for costs. With this information, the Excel tool can produce the optimal design; i.e., the minimum cost design that achieves the target CV. The problem defined in equations (1) – (4) below is an optimization problem that can be solved with a nonlinear program (NLP). The Design Optimization Tool we have produced makes use of a built-in NLP function (called Solver) in Excel. We have prepared a brief tutorial illustrating how to use the decision support tool, along with a description of the data sources we used to construct our preliminary models in Appendix B, along with screenshot examples in Appendices C-F. All examples below were produced specifically for Region 1.

Stratified Random Design

Two sampling frames were developed to implement the sample design. The first is a frame of artificial reef structures, including standing oil/gas platforms, reefed platforms, and pyramids in the GOM in Region 1. The second is an areal frame, consisting of all natural hard bottom habitat including low relief unconsolidated bottom habitat for Red Snapper in Region 1. The sampling units in the first frame are the structures themselves. The sampling units in the second frame are transects, defined by selecting a random starting point and direction. The total abundance of Red Snapper is assumed to be the sum of those in the structures and those in the area outside the structures. Thus, our estimator of total Red Snapper will be derived by summing estimates of total Red Snapper from samples selected from each frame.

An efficient sample design will require dividing both sampling frames into strata consisting of sampling units that are similar. A key component of this will require local knowledge of habitat and structure distribution, and why we are also specifying a series of workshops to refine these data. In our illustrative example of sample design, the area covered is the GOM Region 1 as defined in Section II. b., and this obviously would need to be performed for other regions. For this population, we selected several stratifying variables. First was geography, which applied to both frames. The geographical strata were defined by two factors: upper and lower coast of Region 1 and distance from shore/depth (see Figure 2). Besides geography, other stratifying variables that differ between frames may also be chosen. Whatever stratifying variables are chosen, the total number of units in each and in the entire frame must be known. In our example, the structures in the frame were stratified by size (large and small) and the areal frame was stratified by type of habitat, described as unconsolidated bottom and natural hard bottom.

The final estimator of total Red Snapper will be the sum of the two total estimates made from the samples from the two frames. These two estimators have different forms. For a stratum in the structure frame, the total Red Snapper is estimated using an expansion estimator, defined as the number of

structures in the stratum times the average number per structure in the sample. For a stratum in the areal frame, the total Red Snapper is estimated using a ratio estimator, defined as the area of habitat in the stratum times the density of Red Snapper in the transects of the sample. The density is calculated as the total Red Snapper in all sample transects divided by their total area. If all transects are identical in size, this will be a simple mean rather than a ratio, but the estimation process is general enough to accommodate transects of varying sizes, by design or by circumstance.

Statistical Modeling

To examine how to select the sample sizes to achieve adequate precision, notation for these estimators and their variances are needed. This notation will be illustrated with notation specific to the proposed sample design for the western Region 1. The goal is to produce a sample design that will produce an estimate of total Red Snapper in the region that has a coefficient of variation (CV) that does not exceed 0.3. To determine the sample size needed for such an estimate, we express the CV of \hat{t} as a function of the number of sampled units in each stratum. Then we must determine stratum sample sizes that allow \hat{t} to achieve the target CV. Many designs will achieve this goal; however, we would like to find the most efficient qualifying design. That is, we would like to find the sample design that achieves the target CV with minimum cost.

Let N_{gl} and N_{gs} denote the number of large and small structures, respectively, in geographic area $g = 1, \dots, 6$. Likewise, let A_{gn} and A_{gu} denote the area of natural hard bottom and unconsolidated bottom in geographic area g . Then the estimator of total Red Snapper is the sum of the estimators for the large and small structures and natural hard bottom and unconsolidated bottom areas:

$$\begin{aligned}\hat{t} &= \hat{t}_{Sl} + \hat{t}_{Ss} + \hat{t}_{An} + \hat{t}_{Au} \\ &= \sum_{g=1}^6 N_{gl} \bar{y}_{gl} + \sum_{g=1}^6 N_{gs} \bar{y}_{gs} + \sum_{g=1}^6 A_{gn} \hat{d}_{gn} + \sum_{g=1}^6 A_{gu} \hat{d}_{gu}\end{aligned}\quad (1)$$

where \bar{y}_{gl} and \bar{y}_{gs} are the sample means for Red Snapper per large and small structure and

\hat{d}_{gn} , \hat{d}_{gu} are density estimates (using the same units as A_{gn} and A_{gu}) made from sampled transects in the natural hard bottom and unconsolidated bottom habitats. The density estimate for each stratum is

$$\hat{d} = \frac{\text{sum of Red Snapper in all transects sampled in stratum}}{\text{sum of area of all transects sampled in stratum}}. \quad (2)$$

We denote the costs of sampling a single unit in the g^{th} geographic stratum of the structure frame as c_{gl} and c_{gs} for the large and small structures, and as $c_{gn}(a)$ and $c_{gu}(a)$ for transects of area a in the natural bank and unconsolidated bottom habitats, respectively. The means and standard deviations of Red Snapper per structure in the structure strata are denoted by

μ_{gl} , μ_{gs} and σ_{gl} , σ_{gs} . The densities and standard deviations in the areal strata are denoted by d_{gn} , d_{gu} and $\sigma_{t,gn}$, $\sigma_{t,gu}$. These standard deviations are defined so that they are scaled to a single unit of area (density-based). That is, if the raw transect to transect SD for a transect of size a is denoted by $\sigma_{t,gu}(a)$, then our standardized SD is $\sigma_{t,gu} = \sigma_{t,gu}(a)/a$. For the purposes of these computations, we are

assuming that all transects have the same area; however, variation in transect size could be accommodated if needed.

The CV of \hat{t} is the ratio of the estimator's standard error to t itself, which we assume to be the total of Red Snapper in the two habitats together with those associated with the structures; i.e.,

$$t = \sum_{g=1}^6 N_{gl}\mu_{gl} + \sum_{g=1}^6 N_{gs}\mu_{gs} + \sum_{g=1}^6 A_{gn}d_{gn} + \sum_{g=1}^6 A_{gu}d_{gu}.$$

Since \hat{t} is the estimator of total from a stratified random design, its variance is (Lohr 2010, p.79),

$$V(\hat{t}) = \sum_{g=1}^6 \left[\frac{N_{gl}^2 \sigma_{gl}^2}{n_{gl}} \left(1 - \frac{n_{gl}}{N_{gl}} \right) + \frac{N_{gs}^2 \sigma_{gs}^2}{n_{gs}} \left(1 - \frac{n_{gs}}{N_{gs}} \right) + \frac{A_{gn}^2 \sigma_{t,gn}^2}{m_{gn}(a)} \left(1 - \frac{m_{gn}(a)}{M_{gn}(a)} \right) + \frac{A_{gu}^2 \sigma_{t,gu}^2}{m_{gu}(a)} \left(1 - \frac{m_{gu}(a)}{M_{gu}(a)} \right) \right], \quad (2)$$

where n_{gl} and n_{gs} are the number of structures sampled from the frame of large and small structures and $m_{gn}(a)$ and $m_{gu}(a)$ are the number of transects (of size a) that are sampled from the natural and unconsolidated areal frames. The notation $M_{gn}(a)$ and $M_{gu}(a)$ are used to denote the number of transects in the entire stratum population; i.e., $M_{gn}(a) = \frac{A_{gn}}{a}$.

a

With this notation, we can describe the sample design problem as follows. Find samplesizes n_{gl} , n_{gs} , $m_{gn}(a)$, $m_{gu}(a)$ for $g = 1, \dots, 6$ such that

$$CV(\hat{t}) = \sqrt{V(\hat{t})/t} \leq 0.3. \quad (3)$$

There are many designs that will satisfy this standard. The best design is defined as the onesatisfying (3) that also minimizes the cost:

$$C = \sum_{g=1}^6 (c_{gl}n_{gl} + c_{gs}n_{gs} + c_{gn}m_{gn}(a) + c_{gu}m_{gu}(a)). \quad (4)$$

Nonsampling errors

The calculations of sample size using the solver tool are based on the assumption that number of Red Snapper observed in the sampled units (either transect or structure) are accurate. In reality, this is probably over-optimistic, and there are measurement errors in the reported values. These errors can cause either additional variance or bias, or both, in the measurements. Bias occurs if the count of Red Snapper consistently over- or underestimates the true number present in the sampling unit. This can occur due to low visibility, to edge effects in the transects (consistently including or excluding fish on the edge of the field of view), and other limitations discussed in the individual sampling methods above. Added variance can occur because fish are not stationary, and their numbers present in a transect do vary as they move, or due to random miscounts of coders of video.

Some of these sources of error can be mitigated by careful data collection. For example, having more than one coder review the video, and taking their average as the measurement will reduce the added variance due to random miscounts. But other sources of variance may not be eliminated so easily. It would be advantageous to embed some experiments in the data collection process to assess the magnitude of the measurement errors that can be anticipated. If they are substantial, then the experimental data may be used to adjust the estimates from the sample design. One example is to include a capture-recapture experiment at one or more of the sampled structures. Then the usual method of obtaining an estimate of the number of Red Snapper in the structure could be compared with the capture-recapture estimate to assess whether or not there is a substantial bias in the former. A second example is a special study designed to assess the impact of low visibility on counts of Red Snapper. This approach is common for researchers measuring abundance of birds, and these methods, which attempt to build detection models by using data on distance from the observer to the detected birds, are known as distance sampling methods (Norvell et al. 2003). These methods allow a principled way for estimates to be adjusted upward to account for the underestimation due to difficulty of detection.

Finally, it might be prudent to reduce the target CV for the sample size calculation problem to provide a protection against any unforeseen increase in variance due to unanticipated measurement error. For example, a sample size determined to achieve a CV of 0.25 (or less) rather than 0.3 could account for increased variation that was not included in the estimates from past studies.

Scalable Sampling Effort and Cost Estimates

The design optimization tool we have developed provides a flexible and adaptable framework that allows for numerous scenarios that optimize sample size and where sampling effort should be targeted based on specific input parameters. Here, we have described four design scenarios in which we make certain assumptions and modified the parameters accordingly. Spreadsheet screenshots of the tool and calculated cost estimates are enclosed as Appendices. The various scenarios described above have resulted in sampling effort estimates that can be used to calculate associated costs. For each scenario, a cost estimate table has been generated for Region 1 that is subdivided by sampling method. Each table also includes costs for expanding the methods Gulf-wide (all 4 regions) assuming similar level of effort and habitat types, and a grand total which includes all sampling methods across all regions (Appendix C-F). The cost per unit for each method is the full cost to process a sample and includes personnel (field, post-processing, etc.), vessel time, fuel cost, and other disposable incidentals (i.e., bait, tags) for the various methods. Fixed costs are estimated for all necessary sampling equipment, and represent a one-

time purchase cost (Appendix H). Once on site, effort includes replicate surveys where necessary, and this expense is accounted for under the overall cost estimate for each site. Tagging studies have all associated costs included related to fishing “hot spots” that will be identified regionally. Although 2 and 3 “hot spot” sites (High Reward Tag and Recapture and Change-In-Ratio method, respectively) are mentioned in the text and the Cost Estimate Tables, the number of sites may be scaled up or down depending on regional analyses.

Scenario 1 (Appendix C) represents the cost-optimal model, whereby sample sizes are minimized to reduce costs but still achieve an overall regional CV of 0.3. Sampling effort is directed towards natural hard bottom strata where the greatest proportion of density exists based on preliminary estimates generated from project PIs and other documented data sources. The Cost Estimate Table for Scenario 1 was generated through the Data Optimization Tool using existing data sets as described above. The grand total for implementing this scenario across the GOM is approximately \$ 8 million. This includes sampling using all methods Gulf-wide (advanced technology – \$ 1.7 million, ancillary methods – \$ 1.2 million, and tagging approaches – \$ 5.2 million).

Scenario 2 (Appendix D) is a hypothetical scenario in which we assumed that 30% of the Red Snapper were in the unconsolidated habitat, but their distribution is patchy. Specifically, we assumed that they occur in only 10% of the area of the habitat, causing considerable clustering and an increase in variance between sample units. To model the standard deviation resulting from this distribution, we assumed that the density of red snapper per transect is defined as

$$Y = \begin{cases} 0 & \text{with probability } \pi \\ X & \text{with probability } 1 - \pi \end{cases}$$

where X is the density of Red Snapper in non-empty transects, which is assumed to have mean m and standard deviation s . Thus the mean of Y can be shown to be $\mu_Y = (1 - \pi)\mu$ and the standard deviation of Y can be shown to be

$$\sigma_Y = \sqrt{\mu^2\pi(1 - \pi) + \sigma^2(1 - \pi)}. \quad (5)$$

For modeling purposes, we set m so that the total Red Snapper in the unconsolidated habitat (Area of habitat * μ_Y) composes 30% of total RS when $\pi = 0.9$. Then we set s so that the ratio $s/m = 1.38$, which is the same as that in the non-zero transects of preliminary C-BASS sampling data. Then using equation (5), this provided a modeled value for the standard deviation for the hypothesized Red Snapper distribution and sampling plan. The Cost Estimate Table for Scenario 2 resulted in a grand total for implementation Gulf-wide of \$12.6 million. This includes sampling using all methods Gulf-wide (advanced technology – \$ 4.1 million, ancillary methods – \$ 3.3 million, and tagging approaches – \$ 5.2 million).

Scenario 3 (Appendix E) is another hypothetical distribution of Red Snapper, where one assumes equal proportion of Red Snapper across all habitat types. Large and small artificial reef strata are determined to each contain half of the overall proportion of total artificial reefs.

Since the number of such reefs are assumed known, we can establish the mean per reef (large and small). We then assumed that the ratio between standard deviation and mean per structure was the same as in previous samples ($\sigma/\mu = 1.625$ for small and $= 1.5$ for large structures). The cost estimate table for Scenario 3 with a completely equal sampling design across all strata, totaled over \$ 21 million. This includes sampling using all methods Gulf-wide (advanced technology – \$ 9 million, ancillary methods – \$ 7.6 million, and tagging approaches – \$ 5.2 million).

Scenario 4 (Appendix F) represents a balanced design where all habitats across all strata are sampled equally, regardless of the proportion of abundance, until each individual strata meets a CV of 0.3. This results in a region-wide CV much lower (~0.18), but also dramatically increases cost estimates.

The Cost Estimate Table for Scenario 4 totaled over \$ 31 million. This includes sampling using all methods Gulf-wide (advanced technology – \$ 14.3 million, ancillary methods – \$ 12 million, and tagging approaches – \$ 5.2 million).

Sampling Timeline

We have provided an example project timeline for the implementation Phase II to determine the absolute abundance of Red Snapper in the U.S. GOM (Appendix G). We based our timeline on a 2-year project to complete all field sampling, analyses, and final determination of Red Snapper abundance. For simplicity, we chose an arbitrary start date of January 2018 and input our proposed sampling design and how it would be accomplished in two years. This constrained timeline would only allow for one year of field collections as the second year would be needed for the large-scale video and data analyses, as well as for data integration, end-user workshop, and final report preparation. We caution against such a short timeline allowing for only one year of data collection. Thus, we would highly recommend modifying this timeline into a 3-year project to allow for 2 years of field collections, which would provide a much more robust dataset by incorporating temporal changes in the final analyses. However, we understand extending the sampling into two years will increase the cost of the overall project. The Design Optimization Tool could be used to ensure sufficient sampling in each year to keep the overall project costs to a minimum.

Arriving at Absolute Abundance Estimate for the GOM

To arrive at a GOM-wide estimate of absolute abundance will require a regional cooperative approach that draws upon the knowledge and sampling expertise of multiple scientists in these different geographic regions. The distinct regional differences in habitat types, geography, and bathymetry necessitates a regional approach. We prescribed four ecologically-distinct regions, and we have delineated the key habitat feature using Region 1 as an example. This includes density estimates using advanced technologies, exploitation/abundance approaches, and ancillary index-based methods (as well as other contributed methods not outlined in this proposal), and this sampling methodology should occur in each of these four regions using the stratified random sampling framework to obtain the best possible regional estimates of abundance. Once we have obtained precise and confident estimates of regional abundance, the overall GOM estimates of absolute abundance may then be calculated through the summation of all regions.

Crucial to these ‘best estimates’ will be the contribution of regional expertise from scientists, managers, and other stakeholders. The design optimization tool we have presented that determines sample sizes per strata, cost estimates, and focuses our targeted effort is conditional upon the best possible data entering the model. We suggest organizing a coupled before-and-after workshop to solicit and refine this information, as well as obtain and feedback from participants on the variety of components specified in this design. The beginning workshop should focus around collecting the best possible regional mapping data available from a comprehensive list of sources along with associated Red Snapper abundance and variability for model parameterization and data input. Outcomes of this workshop will be a final robust stratified random sampling design for each region that generates a directed and targeted sampling approach, inclusive of sample sizes per strata to minimize CV, and an appropriate cost estimate for carrying out this sampling across the GOM. The follow-up workshop should invite other stakeholders as participants to provide objective feedback on the abundance estimates that have been generated through

this process. Galvanizing support and confidence in these estimates from fishery stakeholders will ensure regulations are well- understood and maintain the level of transparency necessary for effective management of the stock.

Biological Sampling

There is potential to opportunistically collect valuable biological samples as a result of some of the abundance studies. Although directed biological tissue sampling is not prescribed due to the financial and broad geographic scale of the study, several of the sampling methodologies proposed will result in specimen collection in many areas that are much needed by the Southeast Data, Assessment and Review. We recommend researchers collect as many fish tissue samples as feasible. These could include tissues such as sagittal otoliths (age structure), muscle tissue (stable isotopes), fin clips (genetic signatures), stomach contents (diet composition), and gonads (fecundity and sex ratios). Additionally, samples could include tissues specifically for an on-going GOM-wide analysis of polycyclic aromatic hydrocarbon (PAH) exposure. These include liver, bile, gonad, and muscle tissue samples. Although funding for sample analysis is not included in the cost estimates in this project outline, samples can be housed and analyzed as additional funding and data needs are identified in various regional areas. During the implementation phases, investigators should work closely with the NOAA Southeast Fisheries Science Center as well as state agencies to ensure any potential for any fish sample collection is accomplished.

Stakeholder Input and End-User Sponsors and Co-Sponsors

Other key objectives of this design are to engage the Gulf scientific community and other Gulf stakeholders and to ensure results in Red Snapper abundance estimates will be used for comparison and integration into NOAA's Red Snapper Stock Assessment. A project of this magnitude will require important partnerships with state, Federal, and academic scientists, other federal and state partners, and end users of these data. In fact, these groups are already involved in this design process by providing advice and consultation during its development.

The primary end users will be stock assessment scientists at the Southeast Fisheries Science Center (SEFSC), the Southeast Regional Office (SERO), the GOM Fishery Management Council (GMFMC), and Science and Statistical Committee (SSC). However, partnerships with both the recreational and commercial fishing industry should be developed and will be essential to create constituent buy-in. There is a wealth of opportunity and potential for these relationships to develop during the implementation phase. We offer a few recommendations on how to facilitate this process.

For example, the final implementation design should fully involve representatives from the SEFSC assessment team and the SERO office. This will open a line of communication and ensure the outcome of the study will generate parameters suitable for integration into current stock assessment in a meaningful way. Our research team is uniquely suited to facilitate this type of interaction during the development of this design given our active involvement with the fishery management process. Our design was developed with these outcomes in mind – to produce a useable product for stock assessment. For example, three of the PIs participate with the Gulf Council's management committees. PIs Drymon and Powers are members of the SSC (PI Powers former Chair) and PI Stunz serves on the GMFMC as well as former SSC member. Jim Tolan with Texas Parks and Wildlife was a collaborator on this design

and is a member of the SSC. Thus, our team is acutely aware of the management process and can facilitate the transfer of information to be incorporated into the Red Snapper stock assessment.

While not directly involved in the technical aspects of scientific data collection, constituents' participation represents a strategic engagement opportunity between the scientific/management community and Gulf stakeholders. In our opinion, developing these relationships during the implementation phase is critical. Our team of investigators routinely partners with willing and enthusiastic individuals (citizen scientists) to help collect meaningful data that would otherwise be too expensive or time-intensive to obtain. These partnerships are important in not only informing the general public about ongoing research in their community but, in many cases, creating a vested interest by the public in understanding and conserving our natural resources. We have several design components that easily facilitate participation for recreational and commercial anglers. The primary component of this design that includes stakeholder participation is the high-reward tagging study that will be performed regionally throughout the GOM. While scientific tagging during the initial fishing effort is imperative, recapture of the fish is not. Thus, we will rely on commercial and recreational anglers to catch and report tagged fish. To ensure high reporting, we will heavily incentivize reporting of these captured fish with high monetary rewards, which has been very successful in other studies. Certainly, a major benefit from this involvement is that anglers become engaged in the study and thus the fishery. Awareness campaigns regarding the high-reward tagging study also offer the opportunity to engage the general and angling public about this study.

Conclusion and Summary

This design addresses one of the most pressing issues currently facing Gulf of Mexico fisheries management – estimating absolute abundance of Red Snapper, but it also represents a very challenging undertaking, especially given the complexities of the question and dynamics of the population. While these challenges are described in detail in each section, we certainly feel they can be overcome to provide a robust estimate of Red Snapper absolute abundance.

However, this design is the initial step and will require a very large effort from key experts in the field across the GOM. For example, to accomplish this ambitious task, we assembled a multidisciplinary work group that included leading Red Snapper experts from across the entire US GOM region. These individuals have extensive experience with Red Snapper along with some of the most robust data sets, ongoing research programs, sampling techniques, and specific analytical skillsets available in the GOM. However, there exists a large amount of data and expertise that would need to be drawn upon to fully develop and implement this design during the data collection phase. Thus, in developing a comprehensive design this group strongly recommends an approach that uses a combination of directed sampling at the core, but also takes advantage of the wealth of existing data resources and other complementary studies throughout the GOM. By combining information from these new studies, assembling historical data sources, and overlaying with the most detailed habitat mapping available, we are confident these data will improve the estimate of Red Snapper absolute abundance.

A project of this magnitude will also require key partnerships with academic scientists, federal and state partners, and end-users of these data. Once a final design is selected, we recommend a series of pre-workshops with these user groups be conducted to gather all known data sources (both published and unpublished). This will accomplish two goals. First, it can serve to refine GOM habitat mapping that is essential and a fundamental basis for the estimate. Second, assembling a team of experts that provide

informative data will further refine our regional variability estimates – a critical but often unknown parameter in many cases that currently constrains the design. We also recommend, where necessary, to carry out directed pilot studies to generate estimates of variability as needed. These refined values fine-tune the Design Optimization Tool to generate the best estimates of abundance for each ecological zone. These groups should work closely and stay informed throughout the 2-yr study. Upon completion of the sampling phase, we recommend this group meet again to evaluate the data and reach consensus on a final absolute abundance estimation. Together, having these partnerships and buy-in from all partners will help to form the most robust estimate of Red Snapper abundance across the U.S. GOM.

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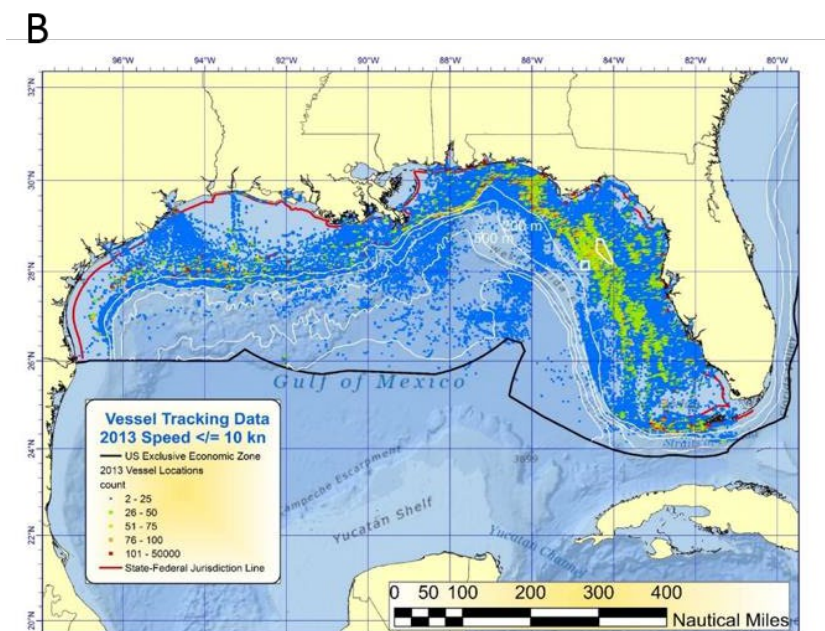
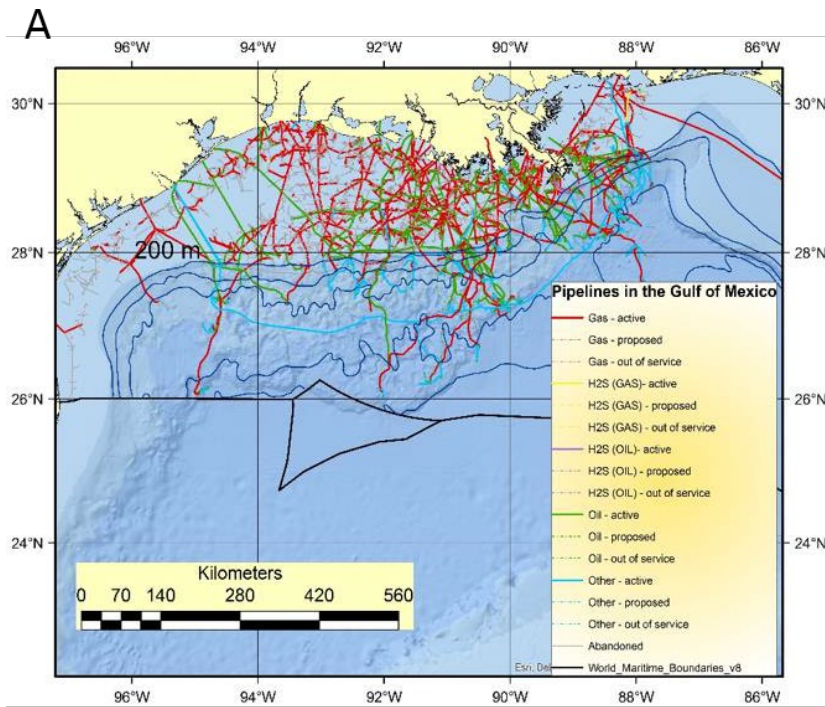
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Appendices

Known structures

Examples of data resources that can be integrated into ecological mapping exercise to determine extent and areal coverage of habitat types in the GOM. (A) Oil/gas pipelines off the TX/LA GOM. (B) Vessel tracking data for the GOM region (2013).



How to use the Decision Optimization Tool

A screenshot of the decision optimization tool and cost estimate worksheet are included Appendices. To use the tool, the user inputs population size, mean, standard deviation, and cost of sampling a unit for each stratum. These inputs are provided in Tables A through D of the Excel worksheet, respectively. The user must also specify the target CV (in cell E1), and the size of the transect to be used (in cell E2). We illustrate use of the tool with the Texas example, providing the source of data used to parameterize the model for this region.

Table A. Population Size. Within this table, estimates of the amount of each habitat type are shown for each sub-stratum of the western Gulf/Texas region. Estimates were primarily derived using the USGS usSEABED (2006) and GCOOS bathymetry datasets and other existing habitat maps. Natural hard bottom (reefs, banks, outcroppings) and unconsolidated bottom (open mud/sand, pipelines, unknown natural features) are entered as the estimated areal extent (i.e., footprint) in km². Artificial reef small includes the estimated number of small structures (reef pyramids, culverts, etc.) in each sub-stratum, while artificial reef large includes the number of standing oil and gas platforms and the number of sites containing large artificial reefs (ships, decommissioned oil and gas platforms, etc.) in each sub-stratum.

Table B. Mean. In this table, estimates of mean Red Snapper density (e.g., natural hard bottom) or total abundance (e.g., artificial reef small) are entered for each habitat type within each sub-stratum of the western Gulf/Texas region. Therefore, a density estimate derived from C-BASS transects over natural habitats in the eastern Gulf was used (Grasty 2014; 1568/km²). For the lower Texas coast deep stratum, an estimate was available from ROV transects on natural banks in the region (Streich 2016; 21,844/km²). This estimate was also used for the upper coast deep stratum because no estimate was available. No estimates were available for unconsolidated bottom off Texas, so an estimate from C-BASS transects in the eastern Gulf was used (Grasty 2014; 7/km²) for all sub-strata. The number of Red Snapper on small artificial reefs was estimated from ROV surveys of artificial structures off the coast of Alabama (Powers and Drymon, unpublished data; 8/small structure). This estimate was used for all sub-strata because no estimates were available for small structures within the Texas region. The number of Red Snapper on large artificial reef sites was estimated from ROV surveys from the lower Texas coast (Streich 2016; 2,242/reef site). This estimate was used for all sub-strata.

Table C. Standard deviation. In this table, standard deviation for the density or abundance estimates in Table B are entered. For natural hard bottom in the two shallower sub-strata (i.e., ≤ 50 m) of the upper or lower coast no estimates of Red Snapper density (or standard deviation) were available. Thus, the standard deviation for the C-BASS estimate in Table B was used (Grasty 2014; 2017/km²). Standard deviation for the two deep sub-strata was derived from ROV transects conducted on natural banks on the lower coast (Streich 2016; 27,004/km²). For unconsolidated bottom, standard deviation was estimated using from the C-BASS data from

Table B (12/km²), and this estimate was used for each sub-stratum. Given the uncertainty surrounding artificial reefs, we used an empirically derived relationship of mean abundance and standard deviation to estimate standard deviation for small artificial reefs and large artificial reefs in each sub-stratum (standard deviation was typically 1.5 times the mean). Using this relationship, the small artificial reef standard deviation was estimated to be 13/structure while the large artificial reef standard deviation was estimated to be 3,363/reef site.

Table E. Sample Sizes. For any sample sizes proposed, CV and associated cost are computed, and appear as separate tabs in the workbook. Estimated costs are given per sampling site. Sampling at each site may include up to 3 replicates (transects, surveys, sets, etc.) depending on technology/method. Estimated costs include personnel cost, vessel time, fuel cost, and post-processing costs for the various methods. Fixed Camera estimates are for modified Go Pro version. If larger-scale version used, this cost will increase substantially due to vessel requirements. Sampling at various habitat types is determined by the method and its applicability to those specific habitats. The user can fill in these cells to examine the performance of any proposed sample design. Alternatively, the user can ask Solver to determine the most efficient design; i.e., to find the sample sizes that satisfy the NLP in (1) – (4). To do that, the user must first fill in some candidate values for sample sizes; say for example, fill in all 2's in the cells of Table E. Then the user will invoke Excel's Solver to solve the NLP that has been embedded in the tool. To do that, on the Excel menu click **Data** → **Solver**. This reveals the specifications for the NLP, including the quantity to be minimized (cost), variables (stratum sample sizes), and the constraints. The target CV is one of the constraints (CV ≤ 0.3). The others specify that stratum sample sizes must be smaller than their population sizes, that all sample sizes must be at least 1, and are integers. Then the user clicks the **Solve** button. The program will search for the optimum solution. When an adequate solution is found, the interface reports that, and the user clicks **OK** to retain it. Sometimes a solution cannot be found, which is also reported. There are a variety of causes for this outcome, many of which can be remedied by adjusting settings of Solver. For more detail on how this can be done, see Stokes and Plummer (2004).

Design Optimization Tool – Scenario 1: Optimal Cost

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Table A. Pop size

	Region 1						Region 1 = total transects					
	Upper			Lower			Upper			Lower		
	<20m	20-50m	>50m	<20m	20-50m	>50m	<20m	20-50m	>50m	<20m	20-50m	>50m
Natural hard bottom (km^2)	200	362	369	5	5	294	1428571	2585714	2635714	35714	35714	2100000
Unconsolidated bottom (km^2)	15074	14987	10925	5441	14875	13537	107671429	107050000	78035714	38864286	1.06E+08	96692857
Artificial reef small (# structures)	1250	1250	5	1250	1250	5						
Artificial reef large (# structures)	6	231	231	5	48	30						

Table B. Mean

	Region 1						#RS	prop RS
	Upper			Lower				
	<20m	20-50m	>50m	<20m	20-50m	>50m		
Natural hard bottom (km^2)	1568	1568	21844	1568	1568	21844	15,379,468	90%
Unconsolidated bottom (km^2)	7	7	7	7	7	7	523,873	3%
Artificial reef small (# structures)	8	8	8	8	8	8	40,080	0%
Artificial reef large (# structures)	2242	2242	2242	2242	2242	2242	1,235,342	7%
Prop RS	3%	7%	54%	0%	1%	41%	17,178,763.00	100%

Table C. Standard deviation

	Region 1					
	Upper			Lower		
	<20m	20-50m	>50m	<20m	20-50m	>50m
Natural hard bottom (km^2)	2017	2017	27004	2017	2017	27004
Unconsolidated bottom (km^2)	12	12	12	12	12	12
Artificial reef small (# structures)	13	13	13	13	13	13
Artificial reef large (# structures)	3363	3363	3363	3363	3363	3363

Table E. Sample size

	Region 1						total sample
	Upper			Lower			
	<20m	20-50m	>50m	<20m	20-50m	>50m	
Natural hard bottom transects	1	1	7	1	1	7	18
Unconsolidated bottom transects	1	1	1	1	1	1	6
Artificial reef small (# structures)	1	1	1	1	1	1	6
Artificial reef large (# structures)	1	1	1	1	1	1	6
	total=						36
	Var that=						5026754.897

Var that= 1.9606E+11 1.1666E+12 1.48E+13 4854796161 57742644191 9.04E+12

E(that)= 442570 1200427 8654853 67137 229581 6584195 17178763
 CV= 1.00047687 0.89976486 0.444536 1.03782366 1.0466763 0.456662 0.292614486

SAMPLING METHOD - Advanced Technology	REGION	HABITAT TYPE	NUMBER OF SITES	COST PER UNIT	TOTAL COST
ROV/Bioacoustics	1	Natural Hardbottom	18	\$ 6,200.00	\$ 111,600.00
		Artificial Reef (Small)	6	\$ 5,000.00	\$ 30,000.00
		Artificial Reef (Large)	6	\$ 6,200.00	\$ 37,200.00
ROV/Bioacoustics Cost - Region 1					\$ 178,800.00
<hr/>					
C-BASS*	1	Natural Hardbottom	18	\$ 10,000.00	\$ 180,000.00
		Unconsolidated Bottom	6	\$ 10,000.00	\$ 60,000.00
C-BASS Cost - Region 1					\$ 240,000.00
Total - Advanced Technology - Region 1					\$
					Total Cost - Adv. Tech - 4 regions
					\$
SAMPLING METHOD - Supplemental Methods	REGION	HABITAT TYPE	NUMBER OF SITES	COST PER UNIT	TOTAL COST
Catch-Survey-Catch	1	Artificial Reef (Small)	6	\$	34,500.00
				5,750.00	\$
VL and ROV Depletion Cost - Region 1					\$ 34,500.00
<hr/>					
Bottom Longline	1	Natural Hardbottom	18	\$ 5,750.00	\$ 103,500.00
		Unconsolidated Bottom	6	\$ 5,750.00	\$ 34,500.00
BLL Area Fished Cost - Region 1					\$ 138,000.00
<hr/>					
Fixed Camera (*modified Go Pro version)	1	Natural Hardbottom	18	\$ 3,500.00	\$ 63,000.00
		Unconsolidated Bottom	6	\$ 3,500.00	\$ 21,000.00
		Artificial Reef (Small)	6	\$ 3,500.00	\$ 21,000.00
		Artificial Reef (Large)	6	\$ 3,500.00	\$ 21,000.00
Fixed Camera Cost - Region 1					\$ 126,000.00
Total - Supplemental Methods - Region 1					\$
					Total Cost - Suppl. Methods - 4 regions
					\$
SAMPLING METHOD - Tagging	REGION	HABITAT TYPE	NUMBER OF SITES	COST PER UNIT	TOTAL COST
5.2	1	Fishing Hot Spots (Fed. Season)	3	\$ 200,000.00	\$ 600,000.00
Change-in-Ratio Method	1	Sites w/in Hot Spot zones (Fed. Season)	2	\$ 350,000.00	\$ 700,000.00
Total - Tagging - Region 1					\$ 1,300,000.00
					Total Cost - Tagging - 4 regions
					\$
Grand Total - All methods - Region 1					\$
					Grand Total - All methods - 4 regions
					\$

Design Optimization Tool – Scenario 2: 30/10 Constraint

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	Region 1						Region 1 = total transects					
	Upper			Lower			Upper			Lower		
	<20m	20-50m	>50m	<20m	20-50m	>50m	<20m	20-50m	>50m	<20m	20-50m	>50m
Natural hard bottom (km^2)	200	362	369	5	5	294	1428571	2585714	2635714	35714	35714	2100000
Unconsolidated bottom (km^2)	15074	14987	10925	5441	14875	13537	107671429	107050000	7.8E+07	3.9E+07	1.1E+08	9.7E+07
Artificial reef small (# structures)	1250	1250	5	1250	1250	5						
Artificial reef large (# structures)	6	231	231	5	48	30						

	Region 1						#RS	prop RS
	Upper			Lower				
	<20m	20-50m	>50m	<20r	20-50m	>50m		
Natural hard bottom (km^2)	1568	1568	21844	1568	1568	21844	15,379,468	65%
Unconsolidated bottom (km^2)	95.4	95.4	95.4	95.4	95.4	95.4	7,139,641	30%
Artificial reef small (# structures)	8	8	8	8	8	8	40,080	0%
Artificial reef large (# structures)	2242	2242	2242	2242	2242	2242	1,235,342	5%
Prop RS	8%	11%	43%	2%	7%	34%	23,794,530.60	100%

	Region 1					
	Upper			Lower		
	<20m	20-50m	>50m	<20m	20-50m	>50m
Natural hard bottom (km^2)	2017	2017	27004	2017	2017	27004
Unconsolidated bottom (km^2)	578	578	578	578	578	578
Artificial reef small (# structures)	13	13	13	13	13	13
Artificial reef large (# structures)	3363	3363	3363	3363	3363	3363

	Region 1						total sample
	Upper			Lower			
	<20m	20-50m	>50m	<20m	20-50m	>50m	
Natural hard bottom transects	1	1	12	1	1	10	26
Unconsolidated bottom transects	11	11	8	4	11	10	55
Artificial reef small (# structures)	1	1	1	1	1	1	6
Artificial reef large (# structures)	1	1	1	1	1	1	6

total= **93**

Var that=	7.0645E+12	7.956E+12	1.4E+13	2.4732E+12	6.746E+12	1.2E+13	7108727.967
E(that)=	1775111.6	2525277.8	9620623	548121.4	1544531	7780866	23794530.6
CV=	1.49731732	1.11695963	0.38696	2.86914007	1.681614663	0.4532	0.298754705

SAMPLING METHOD - Advanced Technology	REGION	HABITAT TYPE	NUMBER OF SITES	COST PER UNIT	TOTAL COST
ROV/Bioacoustics	1	Natural Hardbottom	26	\$ 6,200.00	\$ 161,200.00
		Artificial Reef (Small)	6	\$ 5,000.00	\$ 30,000.00
		Artificial Reef (Large)	6	\$ 6,200.00	\$ 37,200.00
ROV/Bioacoustics Cost - Region 1					\$ 228,400.00
C-BASS*	1	Natural Hardbottom	26	\$ 10,000.00	\$ 260,000.00
		Unconsolidated Bottom	55	\$ 10,000.00	\$ 550,000.00
C-BASS Cost - Region 1					\$ 810,000.00
Total - Advanced Technology - Region 1					\$
Total Cost - Adv. Tech - 4 regions					\$

SAMPLING METHOD - Supplemental Methods	REGION	HABITAT TYPE	NUMBER OF SITES	COST PER UNIT	TOTAL COST
Catch-Survey-Catch	1	Artificial Reef (Small)	6	\$	34,500.00
				5,750.00	\$
VL and ROV Depletion Cost - Region 1					\$ 34,500.00
Bottom Longline	1	Natural Hardbottom	26	\$ 5,750.00	\$ 149,500.00
		Unconsolidated Bottom	55	\$ 5,750.00	\$ 316,250.00
BLL Area Fished Cost - Region 1					\$ 465,750.00
Fixed Camera (*modified Go Pro version)	1	Natural Hardbottom	26	\$ 3,500.00	\$ 91,000.00
		Unconsolidated Bottom	55	\$ 3,500.00	\$ 192,500.00
		Artificial Reef (Small)	6	\$ 3,500.00	\$ 21,000.00
		Artificial Reef (Large)	6	\$ 3,500.00	\$ 21,000.00
Fixed Camera Cost - Region 1					\$ 325,500.00
Total - Supplemental Methods - Region 1					\$
Total Cost - Suppl. Methods - 4 regions					\$

SAMPLING METHOD - Tagging	REGION	HABITAT TYPE	NUMBER OF SITES	COST PER UNIT	TOTAL COST
High Reward Tag and Recapture	1	Fishing Hot Spots (Fed. Season)	3	\$ 200,000.00	\$ 600,000.00
Change-in-Ratio Method	1	Sites w/in Hot Spot zones (Fed. Season)	2	\$ 350,000.00	\$ 700,000.00
Total - Tagging - Region 1					\$ 1,300,000.00
Total Cost - Tagging - 4 regions					\$ 5,200,000.00
Grand Total - All methods - Region 1					\$
Grand Total - All methods - 4 regions					\$

Design Optimization Tool – Scenario 3: Equal Proportions

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	Region 1						Region 1 = total transects					
	Upper			Lower			Upper			Lower		
	<20m	20-50m	>50m	<20m	20-50m	>50m	<20m	20-50m	>50m	<20m	20-50m	>50m
Natural hard bottom (km^2)	200	362	369	5	5	294	1428571	2585714	2635714	35714	35714	2100000
Unconsolidated bottom (km^2)	15074	14987	10925	5441	14875	13537	107671429	107050000	7.8E+07	3.9E+07	106250000	96692857
Artificial reef small (# structures)	1250	1250	5	1250	1250	5						
Artificial reef large (# structures)	6	231	231	5	48	30						

	Region 1						#RS	prop RS
	Upper			Lower				
	<20m	20-50m	>50m	<20m	20-50m	>50m		
Natural hard bottom (km^2)	784	784	10922	784	784	10922	7,689,734	33%
Unconsolidated bottom (km^2)	103	103	103	103	103	103	7,708,417	33%
Artificial reef small (# structures)	767.5	767.5	767.5	767.5	767.5	767.5	3,845,175	17%
Artificial reef large (# structures)	7106.5	7106.5	7106.5	7106.5	7106.5	7106.5	3,915,682	17%
Prop RS	9%	18%	35%	3%	10%	25%	23,159,007.50	100%

	Region 1					
	Upper			Lower		
	<20m	20-50m	>50m	<20m	20-50m	>50m
Natural hard bottom (km^2)	2017	2017	27004	2017	2017	27004
Unconsolidated bottom (km^2)	790	790	790	790	790	790
Artificial reef small (# structures)	2533	2533	2533	2533	2533	2533
Artificial reef large (# structures)	21320	21320	21320	21320	21320	21320

	Region 1						total sample
	Upper			Lower			
	<20m	20-50m	>50m	<20m	20-50m	>50m	
Natural hard bottom transects	1	2	21	1	1	17	43
Unconsolidated bottom transects	25	25	18	9	25	22	124
Artificial reef small (# structures)	7	7	1	7	7	1	30
Artificial reef large (# structures)	1	10	10	1	2	2	26

total= **223**

Var that= 7.273E+12 9.6184E+12 1.1E+13 3.4862E+12 7.44973E+12 9.1E+12 6936244.61

E(that)= 2711436 4428445.5 6800932 1559250.5 2836532 4822412 23159007.5

CV= 0.99461823 0.70032418 0.4918 1.19746495 0.962238354 0.62544 **0.299505262**

SAMPLING METHOD - Advanced Technology	REGION	HABITAT TYPE	NUMBER OF SITES	COST PER UNIT	TOTAL COST	
ROV/Bioacoustics	1	Natural Hardbottom	43	\$ 6,200.00	\$ 266,600.00	
		Artificial Reef (Small)	30	\$ 5,000.00	\$ 150,000.00	
		Artificial Reef (Large)	26	\$ 6,200.00	\$ 161,200.00	
ROV/Bioacoustics Cost - Region 1					\$	
					577,800.00	
<hr/>						
C-BASS*	1	Natural Hardbottom	43	\$ 10,000.00	\$ 430,000.00	
		Unconsolidated Bottom	124	\$ 10,000.00	\$ 1,240,000.00	
C-BASS Cost - Region 1					\$	1,670,000.00
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Total - Advanced Technology - Region 1					\$	2,247,800.00
						Total Cost - Adv. Tech - 4 regions \$ 8,991,200.00
<hr/>						
SAMPLING METHOD - Supplemental Methods	REGION	HABITAT TYPE	NUMBER OF SITES	COST PER UNIT	TOTAL COST	
Catch-Survey-Catch	1	Artificial Reef (Small)	30	\$ 5,750.00	\$ 172,500.00	
		VL and ROV Depletion Cost - Region 1				
<hr/>						
Bottom Longline	1	Natural Hardbottom	43	\$ 5,750.00	\$ 247,250.00	
		Unconsolidated Bottom	124	\$ 5,750.00	\$ 713,000.00	
BLL Area Fished Cost - Region 1					\$	960,250.00
<hr/>						
Fixed Camera (*modified Go Pro version)	1	Natural Hardbottom	43	\$ 3,500.00	\$ 150,500.00	
		Unconsolidated Bottom	124	\$ 3,500.00	\$ 434,000.00	
		Artificial Reef (Small)	30	\$ 3,500.00	\$ 105,000.00	
		Artificial Reef (Large)	26	\$ 3,500.00	\$ 91,000.00	
Fixed Camera Cost - Region 1					\$	780,500.00
<hr/>						
Total - Supplemental Methods - Region 1					\$	
						Total Cost - Suppl. Methods - 4 regions \$
<hr/>						
SAMPLING METHOD - Tagging	REGION	HABITAT TYPE	NUMBER OF SITES	COST PER UNIT	TOTAL COST	
High Reward Tag and Recapture	1	Fishing Hot Spots (Fed. Season)	3	\$ 200,000.00	\$ 600,000.00	
Change-in-Ratio Method	1	Sites w/in Hot Spot zones (Fed. Season)	2	\$ 350,000.00	\$ 700,000.00	
Total - Tagging - Region 1					\$	1,300,000.00
						Total Cost - Tagging - 4 regions \$ 5,200,000.00
<hr/>						
Grand Total - All methods - Region 1					\$	
						Grand Total - All methods - 4 regions \$

Design Optimization Tool – Scenario 4: Fully Balanced Design

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	Region 1						Region 1 = total transects					
	Upper			Lower			Upper			Lower		
	<20m	20-50m	>50m	<20m	20-50m	>50m	<20m	20-50m	>50m	<20m	20-50m	>50m
Natural hard bottom (km^2)	200	362	369	5	5	294	1428571	2585714	2635714	35714	35714	2100000
Unconsolidated bottom (km^2)	15074	14987	10925	5441	14875	13537	107671429	107050000	78035714	38864286	1.06E+08	96692857
Artificial reef small (# structures)	1250	1250	5	1250	1250	5						
Artificial reef large (# structures)	6	231	231	5	48	30						

	Region 1						#RS	prop RS
	Upper			Lower				
	<20m	20-50m	>50m	<20m	20-50m	>50m		
Natural hard bottom (km^2)	1568	1568	21844	1568	1568	21844	15,379,468	90%
Unconsolidated bottom (km^2)	7	7	7	7	7	7	523,873	3%
Artificial reef small (# structures)	8	8	8	8	8	8	40,080	0%
Artificial reef large (# structures)	2242	2242	2242	2242	2242	2242	1,235,342	7%
Prop RS	3%	7%	54%	0%	1%	41%	17,178,763.00	100%

	Region 1					
	Upper			Lower		
	<20m	20-50m	>50m	<20m	20-50m	>50m
Natural hard bottom (km^2)	2017	2017	27004	2017	2017	27004
Unconsolidated bottom (km^2)	12	12	12	12	12	12
Artificial reef small (# structures)	13	13	13	13	13	13
Artificial reef large (# structures)	3363	3363	3363	3363	3363	3363

	Region 1						total sample
	Upper			Lower			
	<20m	20-50m	>50m	<20m	20-50m	>50m	
Natural hard bottom transects	16	16	16	16	16	16	96
Unconsolidated bottom transects	16	16	16	16	16	16	96
Artificial reef small (# structures)	16	16	16	16	16	16	96
Artificial reef large (# structures)	16	16	16	16	16	16	96
	total=						384
	otal=						3
Var that=	1.219E+10	7.0464E+10	6.24E+12	250209398	3099774659	3.94E+12	204550.45
E(that)=	442570	1200427	8654853	67137	229581	6584195	7178763
CV=	0.24946597	0.22113029	0.288666	0.23560792	0.242509702	0.301522	0.186541397

SAMPLING METHOD - Advanced Technology	REGION	HABITAT TYPE	NUMBER OF SITES	COST PER UNIT	TOTAL COST	
ROV/Bioacoustics	1	Natural Hardbottom	96	\$ 6,200.00	\$ 595,200.00	
		Artificial Reef (Small)	96	\$ 5,000.00	\$ 480,000.00	
		Artificial Reef (Large)	96	\$ 6,200.00	\$ 595,200.00	
ROV/Bioacoustics Cost - Region 1					\$	
1,670,400.00						
<hr/>						
C-BASS*	1	Natural Hardbottom	96	\$ 10,000.00	\$ 960,000.00	
		Unconsolidated Bottom	96	\$ 10,000.00	\$ 960,000.00	
C-BASS Cost - Region 1					\$	1,920,000.00
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Total - Advanced Technology - Region 1					\$	3,590,400.00
					Total Cost - Adv. Tech - 4 regions	\$ 14,361,600.00
<hr/>						
SAMPLING METHOD - Supplemental Methods	REGION	HABITAT TYPE	NUMBER OF SITES	COST PER UNIT	TOTAL COST	
Catch-Survey-Catch	1	Artificial Reef (Small)	96	\$ 5,750.00	\$ 552,000.00	
		VL and ROV Depletion Cost - Region 1				
<hr/>						
Bottom Longline Area Fished	1	Natural Hardbottom	96	\$ 5,750.00	\$ 552,000.00	
		Unconsolidated Bottom	96	\$ 5,750.00	\$ 552,000.00	
BLL Area Fished Cost - Region 1					\$	1,104,000.00
<hr/>						
Fixed Camera (*modified Go Pro version)	1	Natural Hardbottom	96	\$ 3,500.00	\$ 336,000.00	
		Unconsolidated Bottom	96	\$ 3,500.00	\$ 336,000.00	
		Artificial Reef (Small)	96	\$ 3,500.00	\$ 336,000.00	
		Artificial Reef (Large)	96	\$ 3,500.00	\$ 336,000.00	
Fixed Camera Cost - Region 1					\$	1,344,000.00
<hr/>						
Total - Supplemental Methods - Region 1					\$	3,000,000.00
					Total Cost - Suppl. Methods - 4 regions	\$ 12,000,000.00
<hr/>						
SAMPLING METHOD - Tagging	REGION	HABITAT TYPE	NUMBER OF SITES	COST PER UNIT	TOTAL COST	
High Reward Tag and Recapture	1	Fishing Hot Spots (Fed. Season)	3	\$ 200,000.00	\$ 600,000.00	
Change-in-Ratio Method	1	Sites w/in Hot Spot zones (Fed. Season)	2	\$ 350,000.00	\$ 700,000.00	
Total - Tagging - Region 1					\$	1,300,000.00
					Total Cost - Tagging - 4 regions	\$ 5,200,000.00
<hr/>						
Grand Total - All methods - Region 1					\$	7,890,400.00
					Grand Total - All methods - 4 regions	\$ 31,561,600.00

Timeline

Example of an estimated 2-yr project timeline for implementation. We based the below timeline around a 2-yr project to complete all field sampling, analyses, and final determination of Red Snapper abundance. For simplicity, we chose an arbitrary start date of January 2018 and input our proposed sampling design and how it would be accomplished in two years.

		JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
2018	Project Start	Funds Disbursed											
		Planning Workshop											
	Design: Stratified Random Sampling		Habitat Mapping and Ecological Boundaries										
			Parameterization of Design Optimization Tool										
	Field Collections: Exploitation/Abundance					High Reward Tags Fish tagging		High Reward Tags - Recapture data collection					
						Change-in-Ratio VLL		Change-in-Ratio VLL					
	Field Collections: Density Estimates							ROV/Bioacoustics					
							C-BASS						
Field Collections: Ancillary Index-Based Methods							BLL (Biological Sample Collection)						
							Fixed Cameras						
							VLL/ROV Depletion (Biological Sample Collection)						
Analyses										ROV/C-BASS/Fixed Camera Video Analysis			
										High Reward Tagging Analysis			
										Change-in-Ratio Analysis			
2019	Analyses	ROV/C-BASS/Fixed Camera Video Analysis											
		High Reward Tagging Analysis											
		Change-in-Ratio Analysis											
					Data Integration and Final Analyses								
Project End										End-User Workshop			
										Report Writing			
													Final Report

Equipment costs

Fixed costs for equipment necessary for sampling methods.

EQUIPMENT - Advanced Technologies	COST
VideoRay Pro 4 with laser scaler	\$ 57,170.00
Tritech Micron Nav USBL with integration kit	\$ 23,080.00
Aris explorer 1800 with rotator	\$ 100,000.00
C-BASS	\$ 250,000.00
Total - Adv. Tech Equipment - Region 1	\$ 430,250.00

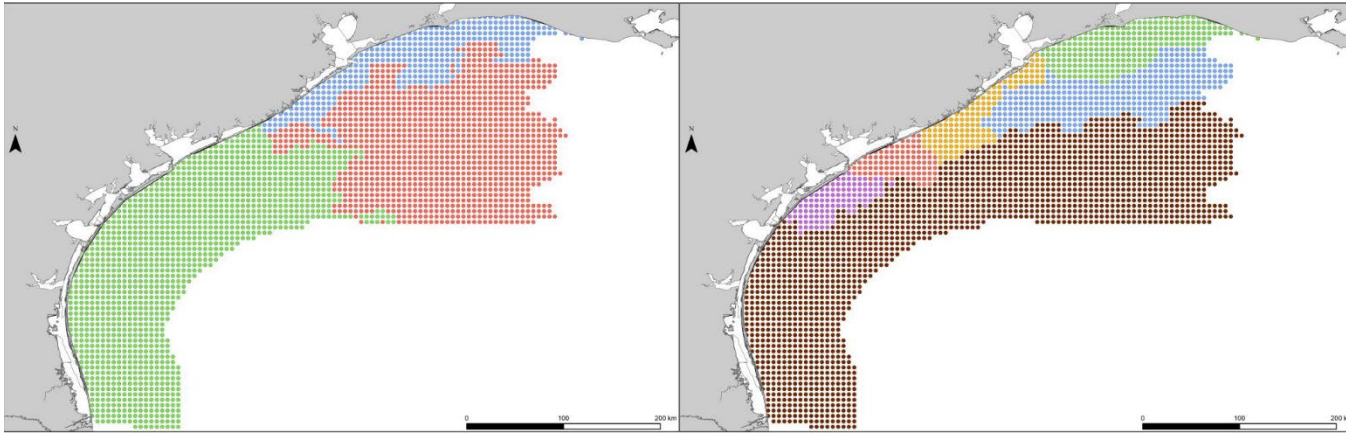
Total - Adv. Tech Equip. - 4 regions \$ 1,721,000.00

EQUIPMENT - Supplemental Methods	COST
Vertical Line Gear (plus ROV - see cost above)	\$ 15,000.00
Bottom Longline Gear	\$ 5,000.00
Fixed Camera (Go Pro modified design)	\$ 10,000.00
I - Supplemental Method equipment - Region 1	\$ 30,000.00

Total - Supp. Methods Equip. - 4 regions \$ 120,000.00

Fixed Camera (NOAA design) \$ 100,000.00 * if preferred method

Alternate Ecological Mapping Option



F. Tag Return Questionnaire

Recorder Name: _____

Date Received: _____

1. What is the tag number? _____ Double tag? _____
2. What is the tag color? _____
3. What date was the fish caught (YYYY/MM/DD)? _____ / _____ / _____
4. Did you keep the tag(s): Y or N (circle one)
5. Did you KEEP or RELEASE the fish? (circle one)
6. Which of the following best describes the type of fishing that you were doing (circle one)
 - a. commercial
 - b. recreational hook and line from a private boat
 - c. recreational hook and line from a charter or 'for hire' boat
 - d. recreational spear fishing from a private boat
 - e. recreational spear fishing from a charter or 'for hire' boat
- 6a. If you answered "c" or "e" for question 6, what was the name of the charter boat?

7. If you had a fishing license for the trip, which state was it from? _____
8. Which state were you off when you captured the fish? _____
9. Which state did your trip originate from? _____
10. Which port/dock did your trip originate from? _____
11. What was the approximate depth at which the fish was caught? _____
12. Was the fish caught on a published or unpublished reef? _____
13. Was the fish caught on natural habitat, an artificial reef, or an unknown bottom type?
14. If you answered "artificial reef" to question 13, enter the type of artificial structure that best describes the artificial reef? (circle one)
 - a. wreck
 - b. oil/gas platform (active or decommissioned)
 - c. pipeline
 - d. other (cube, chicken coup, rubble, sunken debris, etc): _____
15. What was the name of the artificial reef site, if applicable?

16a. What was the latitude and longitude of the capture location (all of the information entered into this survey will be kept strictly confidential). Please include at least degrees and minutes or decimal degrees to the second decimal place:

Lat: _____ **Long:** _____ (ask 16b if unknown)

16b. Describe the location where the fish was caught by filling in the following sentence:

“The fish was caught _____ miles to the N NNE NE ENE E ESE SE SSE S SSW SW WSW W

WNW NW NNW (circle one or enter degrees heading here _____) of _____ (enter port name or landmark here)”

18. Approximate length of the fish? _____

19. Approximate weight of the fish? _____

20. Were you interviewed by a law enforcement officer during or after your trip? Yes / No

21. How did you find out about the high reward red snapper tagging program?

a. agency or university website

b. social media

c. signage posted a boat ramps

d. word of mouth

e. called the number on the tag

d. other (describe here: _____)

22. Did you become aware of the high reward snapper tagging program BEFORE or AFTER catching a tagged fish?

23. What is the name, address, phone, and email of the person who caught the tagged fish?

NAME: PLEASE MAIL TAG TO:

PHONE: Attn: Dannielle Kulaw

Texas A&M University-Corpus

Christi

EMAIL: 6300 Ocean Dr. Unit 5869

Corpus Christi, TX 78412

ADDRESS: