**A multidisciplinary approach to estimating red snapper,** *Lutjanus campechanus***, behavioral** 

## **response to mobile camera and sonar sampling gears**

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We examined the potential for Gulf of Mexico red snapper (RS) behavior to bias count estimates 25 collected with a remotely operated vehicle (ROV), towed camera sled (TCS), subsurface towed 26 acoustic sled (TAS), or SCUBA diver at artificial reef sites. Near-  $(\leq 5 \text{ m})$ , mid-  $(\leq 15 \text{ m})$ , and far-field (≤100 m) responses were examined using stationary stereo cameras, a horizontal acoustic profiler, and three-dimensional acoustic telemetry, respectively. Survey gears were deployed sequentially for 15 minutes with each gear immediately preceded by a 15-minute control period. 30 Near-field data (mean RS minute<sup>-1</sup>) indicated counts were 7.3 times higher with the diver present and 1.9 times higher with the ROV. The TCS had a significant interaction effect with time on mean RS count in the near- and mid-field, as well as depth and acceleration. The TAS had no effect on RS behavior at any scale. Far-field data showed no significant effect of any gear on mean RS distance to reef. Overall, results indicate RS respond neutrally to survey gears at 35 medium ( $\leq 15$  m) to large ( $\leq 100$  m) spatial scales, but small-scale ( $\leq 5$  m) spatial attraction may bias RS counts with benthic survey gears, primarily by individuals near the periphery of the surveyed area approaching the gear.

#### **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). Many types of stock assessment models rely on time series of catch and effort data to estimate total population size, with indices of abundance from both fishery-dependent and -independent data included when possible to track population trends (Chen et al., 2003; Haddon, 2010; Hutchings et al., 2010; Maunder and Punt, 2013). Indices of abundance, while useful, can only track relative interannual changes but are not typically scaled to total abundance. In contrast, absolute abundance estimates provide 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). Rather than working backwards to estimate stock abundance using landings, demographic, and relative abundance data via an assessment model, absolute abundances are derived through direct estimation *in situ*. Expanded direct counts also provide a method to scale time series of relative indices to an absolute abundance estimate, which then can be used to understand historic stock size, as well as current stock productivity. Habitat-specific density estimates are 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). Deploying mobile survey gears allows estimation of the area swept, with common mobile survey gears including subsurface acoustic profilers (Kotwicki et al., 2013; Davison et al., 2015), stationary or mobile digital video camera systems (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). Subsurface acoustic profilers are best suited for estimating fish abundance over large areas in simple habitats with low species-diversity (Lawson and Rose, 1999; Kotwicki et al., 2013; Davison et al., 2015). Stationary camera systems are often effective in sampling relatively small areas of complex habitat (Somerton and Gledhill, 2005; Watson et al., 2005; Schramm et al., 2020), while towed cameras or remotely operated vehicles (ROVs) are effective for sampling either large or small areas of simple or complex habitat depending on the sled or ROV design (Somerton and Gledhill, 2005; Schramm et al., 2020). Sub-surface 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. Estimating the area viewed with stationary cameras is often possible, but estimating fish density (i.e., number per area) can be problematic because it is difficult to determine the spatial origin of observed fish on video, especially for baited camera rigs, and extended deployments increase the likelihood of double counting individuals (Harvey et al., 2007; Langlois et al., 2010; Schramm et al., 2020).

Reef fish densities are especially difficult to estimate due to myriad factors influencing the ability to detect and accurately count 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 identification. With optical methods in clear water, one can 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 avoidance or attraction behaviors that alter fish spatial distribution at scales larger than the sampled area which are not detectable without secondary sampling gear or an established calibration (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 rig but may expand their distribution to avoid a rapidly approaching mobile sampling gear.

Here, our objective was to assess behavioral responses to mobile video and acoustic sampling gears commonly used to survey reef fishes in the northern Gulf of Mexico (nGOM). 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 age-2+ abundance in US waters of the GOM. We estimated the behavioral response of red snapper to a mini ROV, a towed camera sled (TCS), a towed acoustic sled (TAS), and a diver (hereafter included when referring to mobile gears) at multiple scales. In the far field (up to 100 m from a reef), acoustic telemetry provided information on red snapper distance from reef, height off bottom, and acceleration to evaluate if fish were entering or exiting the surveyed area. Mid-field (up to 15 m from a reef) responses were examined with count data collected with a stationary, epibenthic horizontal-beam acoustic



Proposed study reefs were first surveyed with the 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), an array of 70 Vemco (Bedford, Nova Scotia, Canada) VR2Tx acoustic receivers was deployed on September 25-26, 2019. Receivers were deployed 470 m apart in a 11.9 km<sup>2</sup> Vemco Positioning System (VPS) array such that all sampling reefs were located within the array and >500 m from the rectangular array perimeter (Fig. 1B). Receiver spacing and overall VPS array design was intended to provide high-resolution red snapper geoposition 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 and range tests within this region (Dahl et al., 2020; Bohaboy et al., 2020). All acoustic receivers had internal synchronization transmitters set to 160 dB and were attached to the top of 2-m PVC support pipes with heavy duty UV stabilized nylon cable ties (250-lb tensile strength). An additional paracord safety line (550-lb tensile strength) was attached between each receiver and the 40-kg cement base (~0.5 m diameter) that anchored the PVC support pipe to the seafloor. 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 echo sounder (AIRMAR series) and chart plotter (Garmin GPSMAP series) for receiver recovery at the end of the experiment.

148 Red snapper  $(n = 50)$  were captured with hook-and-line at 5 study reefs  $(n = 10$  fish per reef) within the acoustic array on October 28-29, 2019 and Vemco V9AP acoustic tags were externally attached 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 153 codes, acoustic tags also transmitted acceleration  $(m·s<sup>-2</sup>)$  and depth converted pressure data  $(m)$ , with the latter utilized to estimate depth occupied by tagged fish. Tags were attached externally using the method of Bohaboy et al. (2020) to minimize handling time, avoid surgery required for internal tagging, and facilitate quicker post-tagging acclimation. Following tagging, fish were attached to a descender device clamped onto their lower jaw, returned to depth, and released. The descender device was deployed with a small-diameter (~2.54 cm) handline rope and set to release fish at 4 atm (33 m). Two GoPro (Hero5) digital cameras in underwater housings were attached in line with 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). The first camera was mounted 1 m above (oriented downward toward the seabed) and the second camera 1 m below (oriented upward toward the sea surface) the descender device.

# *2.2. 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 and released at the end of October 2019. This provided at minimum a two-week acclimation period following tagging. This was determined to be adequate as 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 was deployed to the seabed. The camera stand was equipped with four GoPro (Hero5) digital cameras housed in rigid waterproof cases. Stereo cameras were arranged in two pairs each with a baseline distance of 75 cm; the upper camera pair was mounted 15 cm above the bottom pair. The upper pair of stereo cameras was positioned 10° upward (oriented towards the surface) from horizontal while the lower pair were positioned parallel with the seafloor. All four cameras were positioned with a 10° toe-in angle and were set to 2.7k resolution at a frame rate of 60 fps. 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 and single stereo camera system calibration. 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 and accurate stereo measurements during video analyses in the laboratory (Garner et al. 2021). A benthic, stationary multibeam imaging sonar (500 kHz Mesotech M3) secured atop a 1-m tall tripod was deployed 15 m from each study reef 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 193 configuration, the sampled beam volume was approximately  $9,300 \text{ m}^3$ . A 1-hour acclimation period followed deployment of the stereo-camera stand and M3, after which divers positioned the stereo-camera stand 5 m from the reef and the M3 15 m from the

90° headings relative to each other. GoPro Hero5 cameras have a vertical and horizontal field of

reef in their terminal position using a transect tape. The camera stand and M3 were positioned at

198 view of 49.1° and 64.6°, respectively, which results in a 29.0 m<sup>2</sup> (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 200 viewing window of 19.3 m<sup>2</sup> (4.6 m x 4.2 m) to track red snapper movements and collect length 201 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°) following the methods of Delacy et al. (2017) and Garner et al. (2021). The diver began the calibration by positioning the checkerboard at 5 m distance (i.e., 205 adjacent to the reef) oriented towards the centerline of the camera stand and then swam slowly 206 forward while tilting the checkerboard forwards, backwards, to the right, and to the left  $(20^{\circ})$ range from perpendicular in each direction) in decreasingly large circular motions until the diver was 1 m from the camera stand (Delacey et al. 2017; Garner et al. 2021). The diver then repeated the same motions while swimming backwards and away from the camera stand towards the reef. The entire calibration procedure at each site required <5 mins to complete. The circular checkerboard movements allowed the checkerboard to be viewed simultaneously by each camera pair during each transect while tilting the checkerboard increased contrast between paired images extracted in the laboratory during camera calibration.

Red snapper behavioral experiments commenced once the diver completed positioning the M3. Behavioral experiments at each reef site consisted of four 15-minute gear deployment periods and three 15-minute control periods (range: 14-21 min depending on haulback times) without mobile gears occurring in an alternating fashion (i.e., control, gear, control, gear etc). The diver treatment, which could not be randomized because it always preceded the other three survey gears, consisted of the 15-minute period immediately following positioning the stationary gears and the 15-minute control period immediately following the diver exiting the water. Each

of the three mobile survey gears (i.e., ROV, TCS, TAS) were then deployed in a randomized 222 order with each preceded by a control period. Thus, the diver and the first mobile gear deployed at each site had a shared control period. The 15 min prior to diver deployment could not be used as the control period for the diver because the stereo cameras and M3 had not yet been positioned.

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 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 6.4 m thick PVC board "fins" attached for stability that carried a downward facing echosounder (70 kHz).

After positioning the stationary equipment, the diver proceeded to follow a mock point count method for a 15-min period (Bohnsack and Bannerot 1986; Patterson et al. 2009). During ROV deployments, it was flown 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 following the same mock survey protocol as the diver. 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 of 1-2 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 246 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.

### *2.3. Data processing*

Data stored on acoustic receivers were downloaded onto a laptop in the field and the digital files were transmitted to Innovasea, Inc. in Dalhousie, Nova Scotia, Canada for post-processing with proprietary software (Espinoza et al. 2011; Smedbol et al. 2014). Geoposition (latitude and 256 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). Tags that were persistently stationary on the seafloor were assumed to have been shed. Tags that recorded acceleration values well above the mean for red snapper and with dramatic changes in geoposition were deemed predated if not viewed directly on video during the fish's return to depth with the descender device.

Video data were post-processed in the laboratory to estimate fish abundance and fork length (FL) to the nearest mm and track fish movements in response to mobile gear deployment. While 265 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 control period, red snapper were counted from one camera of the top pair and one from the bottom pair. Camera-specific counts were estimated/annotated for each minute of each gear deployment and control period with counts defined as the maximum number of red snapper viewed during each minute of each 15-min period. Video or sonar data were not collected from any of the mobile gears or the diver; count data were taken only from the stationary camera stand and M3.

Red snapper tracking analysis performed on stereo camera video data utilized 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 280 the checkerboard (n = 50) were extracted for calibration. Calibration files had  $\langle 1\%$  error for all 281 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 origin point (0,0) corresponding to the center point 292 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, acoustic 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 x 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 thereby produces a time series of fish abundance (non-specific to species) associated with each site and used to compare with coincident estimates of counts from stereo-camera videos. Targets that met the minimum length criteria were enumerated in each ping and summed across each 1-minute interval so that abundance estimates could be compared with those derived from the cameras. To derive red snapper-specific abundance, the minute-specific count was then multiplied by the corresponding minute-specific proportion of red snapper observed on digital video. Video data indicated artificial reef study sites had low diversity (~5 species per site), and red snapper were the numerically dominant species at >30 cm TL, and other species were viewed infrequently. Thus, we were confident that partitioning echosounder fish abundance data using this method was robust.

*2.4. Statistical analyses*

A generalized linear model (GLM) was fit 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 geoposition estimates and the center point of each reef site. We excluded geoposition 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 (Bohaboy et al., 2020). Geoposition estimates of red snapper >100 m from the study reef being examined were also excluded from statistical analysis of red snapper geoposition for the series of gear deployments at that reef. Depth data recorded on acoustic tags were converted to height off bottom (HOB; bottom depth – tag depth, m). 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) with the general equation:  $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + v + \omega + e$ ,  $Y \sim \text{Gamma}(u, v)$  where *Y* is the response variable specified with a gamma distribution and link function specified 330 as the log of the response value. Data values were  $\geq 0$  with a right skewed distribution and non-331 constant variance. The explanatory variables were gear presence  $(X_1;$  deployment versus pre-332 deployment), time elapsed  $(X_2; 1$  to 15 min), and the interaction term. Fish ID and site were

specified as random effects (intercepts) and are indicated by υ and ω, respectively. Model

coefficients were exponentiated to allow interpretation on the original response scale. Separate

models were computed for each survey gear type (i.e., diver, ROV, TCS, or TAS). Residual diagnostic plots were utilized to examine model fit.

Red snapper count data derived from 1-min segments of M3 sonar and stereo-camera video were also analyzed with GLMMs but with the response variable for video samples being the mean number of red snapper observed per min, which was assumed to be Poisson-distributed with mean λ. For cameras, count data were computed as the average of the per-min counts between one top and bottom camera from each site. Separate GLMMs were estimated for each gear treatment where 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, except that statistical models could not be estimated for the diver deployments due to interference from bubbles in the water column.



353 Mean FL  $(\pm 95\% \text{CI})$  of tagged red snapper was 448.1 mm  $(\pm 27.4 \text{ mm})$  with individuals ranging from 325 to 620 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 (floated) 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 likely depredated, 2 (3.3%) shed their tags, and 6 (12%) tags were never 360 detected within the array. Results from GLM analysis indicated neither fish FL ( $p = 0.335$ ) nor 361 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.

There was a minimum of 4 and maximum of 7 acoustically tagged red snapper with unique ID tags present at each of the 5 survey reefs during gear deployments. In total, 1,004 geoposition estimates were logged during gear deployment periods among all red snapper behavioral experiments, which was 60.8% of acoustic pings emitted by tags during deployment periods. However, only 184 geoposition estimates 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 estimated to be 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 min 1-15) (Stands treatment), or well after (post-deployment min 16-60) deployment of the stereo camera and M3 sonar stands during the acclimation period (Acclimation) (Fig. 2; S Table 1). There was 377 a significant effect of minute on red snapper acceleration ( $p = 0.007$ ) but the magnitude of the coefficient (1.02) was minimal. Analysis of red snapper counts per min 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 equilibrated to background 381 levels after  $\approx$  20-30 min during the 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 (S 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 the 388 diver ( $p = 0.372$ ), ROV ( $p = 0.299$ ), or TAS ( $p = 0.458$ ) was present as compared to control 389 periods, but the interaction between the TCS and minute was significant ( $p = 0.002$ ; S Table 3). Fish acceleration decreased during ROV and TAS gear deployments and increased during the diver deployments compared to control periods (Fig. 4C) but was not significantly different (S Table 4). Fish acceleration showed an overall decrease during TCS deployments (coefficient = 393 0.26) but increased during the deployment period (TCS\*Minute term coefficient = 1.19; p <0.001).

Analysis of stereo-camera video data indicated red snapper counts per minute were significantly greater during some gear deployments relative to their respective control periods (S 397 Table 5; Fig. 5). The presence of the diver (p  $\leq 0.001$ ), ROV (p = 0.001) or the TCS (p = 0.002) significantly affected mean red snapper counts. Mean per minute counts were 7.29 times higher during diver deployments, 1.89 times higher during ROV deployments, and 0.53 times lower during TCS deployments (S Table 5). The interaction term for TCS (TCS\*minute) was significant (p <0.001) indicating a positive increase in RS per minute during deployments. The TAS did not have a significant effect on RS mean counts per minute.

Red snapper counts estimated with the M3 sonar (Fig. 6) were similar to count estimates derived from video samples (Fig. 5) but results of statistical analyses differed. In contrast to models for near-field counts, statistical models for sonar-derived red snapper count estimates 406 indicated no significant difference in counts per min for the ROV ( $p = 0.517$ ). Furthermore, the TCS had a significant positive overall effect on mean fish during deployment (coefficient = 3.34; 408 p  $\leq 0.001$ ) and a negative effect on mean fish per minute (coefficient of TCS\*Minute interaction 409 = 0.88;  $p \le 0.001$ ; S Table 6). The TAS had no significant effect on RS or counts per min and no significant interaction terms (p > 0.05; S Tables 5 and 6). Visual inspection of mean red snapper 411 counts per min derived from M3 sonar data show relatively stable mean  $(\pm SE)$  counts per min across all three gears except during mins 3 and 4 for the TCS where mean red snapper counts 413 were 6.3 ( $\pm$ 4.2) and 9.1 ( $\pm$ 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) fish per min for the TCS gear treatment (Fig. 6, column B). Tracking data estimated for red snapper from stereo-camera video suggest behavioral responses in the near field in response to some survey gears. Overall, observed fish tended to be between 0.5 and 2 m above the seabed and within 3-4 m of reef modules. During TAS deployments, most red snapper were loosely aggregated above the reef with a few individuals in very close proximity (Fig 7D). During diver and ROV deployments, nearly all fish were aggregated above reefs, while fish were less tightly aggregated around the reef but nearer the seabed during TCS deployments (Fig. 7C). 

**4. Discussion** 

Study results indicate that red snapper behavioral responses to the mobile survey gears examined in this study were observed in the near field at the smallest scale but not in the far field where fish would be considered entering or leaving the survey area. Therefore, we infer that none of the mobile survey gears examined would be likely to introduce substantial bias into estimates of red snapper because the number of red snapper associated with a reef site during a survey is constant. However, individuals just beyond the periphery of the area viewed during the survey could become identifiable and positively bias count data by approaching mobile gear. Video data reveal that red snapper can be inquisitive towards and approach foreign objects, like the stereo camera and M3 sonar stands, which might be interpreted as attraction when viewed 435 only with gears that have small sampling volumes (10s of  $m<sup>3</sup>$ ) that are much 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). Generally, it is challenging to infer much about red snapper movement behavior from near-field video data alone because water clarity in the nGOM can be limited to <5 m at habitats closer to river outflows or after strong rain events, which makes it difficult to continuously track individuals seen on video (Stoner et al., 2008). High-resolution 3D acoustic telemetry provides critical movement information at spatial scales (100s of m) beyond the visual field of optical equipment and provides a robust evaluation on the potential for fish behavior to bias count data. 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 near-field (10-20 m) 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 red snapper to fishery-independent mobile survey gears. We saw no evidence of avoidance behavior by red snapper in response to the presence of any of the survey gears used in this study. Startle responses were not observed on digital video and mean acceleration data were similar among nearly all gear treatments, as well as between paired gear deployment and control periods. Telemetry-derived geoposition data did not indicate an increase in mean distance from survey reefs, which would have been indicative of large-scale avoidance unobservable on 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 detection entirely. 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 cryptic, do not exhibit schooling behavior, are active with relatively low (0.5

 $m\text{-}sec^{-1}$  swimming speeds, and are readily distinguishable from other taxa and most congeners, thus allowing them to be confidently identified and enumerated.

Based on our video observations, gear attraction (positive bias) would be a more important potential issue than gear avoidance when conducting red snapper surveys, especially surveys 476 designed to estimate absolute abundance. 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 m 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. Such a contraction of their distribution could arise through inquisitive actions towards the gear or through a flight response that concentrates them nearer to reef structure. Despite this potential for positive bias, neither telemetry data nor benthic sonar indicated any large- or medium-scale attraction of red snapper to the initial stereo camera or M3 stand deployments or during deployment of the survey 487 gears. Fish did display positive bias at the smallest-scale  $(<5 m)$  when the diver or ROV were deployed, presumably due to LEDs associated with the ROV's electronics, general curiosity, or disturbance of potential benthic food items by the diver's fins. However, when the different data sources are examined together, the potential for bias is likely to be relatively small because it was only observed at the smallest scale and only affected by individuals near the periphery of the viewed area.

# Red snapper behavioral responses were more complex for the TCS given 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 they 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. A towed camera sled deployed in a single unidirectional transect over relatively great distances with forward-facing cameras would not record following behavior and thus avoid that source of numerical bias when estimating animal density for the area surveyed. However, individuals near the periphery approaching the gear would have a similar effect on counts as during diver or ROV deployments. Geoposition estimates of acoustically tagged red snapper indicated following behavior was over very short distances as we did not observe an increase in distance from the reef during TCS surveys nor an increase in the variance associated with the distance of tagged red snapper from the reef compared to other treatments.

Red snapper swimming behavior was not affected by the TAS at any scale. 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; Kotwicki et al., 2013; Kotwicki 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; Kotwicki 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 reactions to 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 effect of the TAS on red snapper position or movement metrics.

Red snapper counts were higher when the stereo camera and M3 sonar stands were first deployed, but that effect dissipated over a relatively short time period (10s of min). The stands were the first gear introduced at each of the survey sites and they disturbed the sediment when they landed on the 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 sentiment and thus possibly exposed benthic prey fauna. This could explain the persistent rather than fleeting attraction of fish to the divers as well as the greater magnitude of the effect compared to the ROV.

Overall, study results indicate that none of the survey gears used in this study were likely to elicit a strong behavioral response that would substantially bias count estimates at relevant spatio-temporal scales. However, there are two caveats to this interpretation. First, stereo camera and M3 sonar stands always were deployed first at each reef site in our multidisciplinary attempt to estimate the effect of mobile survey gears on red snapper behavior. It is unknowable from our design whether red snapper would have displayed different behavior in response to any one of

the mobile survey gears if it had been the first or only gear deployed at a reef. Field surveys typically consist of only one survey gear type per sample site. Although future studies could test potential attraction issues with only a single response measurement method per site (i.e., telemetry, acoustic sonar, or stereo cameras), which in hindsight perhaps should have been done at additional study reefs, it was important to measure the behavioral response at multiple scales. 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 magnitude 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 move >50 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 directed at mostly non-reef prey is consistent between the habitat types.

In conclusion, results from this study indicate that the mobile survey gears typically used to collect density estimates at scales necessary for population assessment (i.e., ROV, TCS, or TAS) had minimal effects on mid or far field red snapper behavior. Therefore, we found minimal evidence for the major potential source of error: large-scale movements away from or towards survey reefs that would significantly bias red snapper abundance or density estimates. Small-scale movements within the area surveyed could positively bias count estimates made with mobile gears operating near reef structure or the seafloor, but this would likely involve few fish relative to the viewed area (i.e., only fish near the periphery of view). 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 the issue of detectability is important depending on whether optical 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.

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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 km<sup>2</sup>) 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, red snapper 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<sup>2</sup>) 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  $\pm 95\%$  CIs of 5-min time bins. The vertical gray line 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<sup>2</sup>) of acoustically tagged red snapper that were near the survey site  $(\leq 100 \text{ m})$  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 control 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 predeployment period for each gear treatment. Error bars indicate ±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 ±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.















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Minute