Temporal Persistence of Red Grouper (*Epinephelus morio*) Holes in the Steamboat Lumps MPA and the Analysis of Associated Fish Assemblages from Towed Camera Data

By

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This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the <u>Version of Record</u>. Please cite this article as <u>doi:</u> 10.1002/TAFS.10154

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4 Article type : Article
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7 <A> Introduction

8 The discourse around fisheries management in the Gulf of Mexico often centers on the large 9 and complex Red Snapper Lutjanus campechanus stocks found within its waters (Morrison 2016). 10 However, historic grouper landings in the Gulf of Mexico, composed mainly of Red Grouper *Epinephelus morio*, have far surpassed those of Red Snapper (Simmons et al. 2015). This trend holds 11 12 when considering just the eastern Gulf of Mexico where landings revenue has and continues to be 13 dominated by Red Grouper over the other reef fish fisheries in the region (NOAA Fisheries 2016). 14 In 2004, Red Grouper were classified as a 'near-threatened' species by the International Union for 15 the Conservation of Nature (IUCN). This designation was updated in late-2018 to 'vulnerable' 16 meaning that this species' global mature population is continuing to decline (Brule et al. 2018). The 17 outlook is better for the Red Grouper population in the Gulf of Mexico as the most recent stock 18 assessment has found the population is rebuilding (SEDAR 2015). Knowing more about this 19 species' population dynamics and habitat use is therefore imperative to evaluate their population 20 status and develop effective management strategies for one of the most important fisheries in the Gulf of Mexico. 21

22 Red Grouper are a long-lived species (Beaumariage and Bullock 1976; Burgos et al. 2007) that 23 are also known ecological engineers (Coleman and Williams 2002; Coleman et al. 2010). In certain 24 areas, juvenile to adult life stage individuals have been found to actively clear and maintain large 25 holes on the seafloor for several years (Scanlon et al. 2005; Coleman et al. 2010). This behavior 26 creates habitat for themselves as well as small reef fishes in areas that are otherwise very low in 27 species diversity and structural complexity (Ellis 2015). In the Steamboat Lumps Marine Protected 28 Area (SL-MPA; Fig. 1), the creation and continued excavation of these holes by Red Grouper is a 29 notable example of this behavior. Aside from these holes, the surrounding seafloor within the SL-

MPA is largely comprised of flat, sandy expanses with some low-relief features (Gardner et al. 2005). The grouper holes in this area were extensively analyzed by Wall et al. (2011) and a large portion of this study was devoted to determining if the holes in the SL-MPA were being maintained, abandoned, or newly excavated between 2006 and 2009. The purpose of the present study is to continue analyzing these trends with an additional multibeam bathymetry dataset collected in 2017. This purpose of this new analysis was to further elucidate the long-term persistence of these bioengineered features.

37 [Figure 1]

Past assessments of Steamboat Lumps' grouper hole species assemblages have been carried out using stationary cameras, submersibles, and ROV's (Gledhill and David 2004; Coleman et al. 2010). In this study, the second objective was to collect video data using a towed camera system in an attempt to observe Red Grouper presence and species diversity of larger reef fishes within the holes.

43 Methods

44 The new bathymetric dataset used in this study was collected in April 2017 by the Continental 45 Shelf Characterization, Assessment, and Mapping Project (C-SCAMP) using a Reson Seabat 7125. This system is a high-resolution, dual-frequency multibeam swath sonar with 512 overlapping 46 beams. It was operated at 400 kHz with a 140° swath which provides an across-track receive beam 47 48 width of 0.5° and an along-track transmit beam width of 1°. Navigation and motion compensation 49 data were collected with an Applanix POS MV OceanMaster system with Fugro Marinestar (Fugro 50 2016). The POS MV OceanMaster with Marinestar enabled has a horizontal accuracy of 10 cm with 51 95% certainty and a vertical accuracy of 15 cm with 95% certainty (Applanix 2017). The roll and 52 pitch have a 0.01° accuracy. An AML Oceanographic Minos•X with an SV•X change sound velocity sensor was used for sound velocity profile correction. 53

The previously collected data as well as the full data analysis from the Wall et al. (2011) publication were obtained directly from Dr. Carrie Wall. This included the bathymetry surfaces used for analysis as well as all of the georeferenced, individual grouper hole data (i.e. latitude/longitude, depth, etc.). In order to do a comparison analysis, the bathymetric data from April 2017 were processed in Caris HIPS & SIPS 10 to exceed International Hydrographic Organization (IHO) standards 1B (IHO 2008). The resulting bathymetry surface using the 2017 data could be processed

to a 0.5 m x 0.5 m grid size. However, for direct comparison, the 2017 multibeam data were
processed to 3 m x 3 m grid size to match the 2006 and 2009 surfaces.

62 The 3 m x 3 m grid multibeam bathymetry surface for 2017 was then imported into ArcMap 10.4.1 (ESRI 2016) along with the Wall et al. (2011) bathymetric surfaces from 2006 and 2009. The 63 64 same 1.64 km² comparison extent from Wall et al. (2011) was then identified for the 2017 65 bathymetric surface in order to begin analysis (white bounding box; Fig. 1). Grouper holes present in 66 2017 within this boundary were identified and counted using the slope surface derived from the 67 2017 bathymetric surface, as slope more clearly delineates the hole depressions within the area (Fig. 68 1). The density of grouper holes for all three years within the overlapping area was then calculated 69 for comparison. Holes that were present in one dataset but not the other within the comparison 70 extent were noted separately as 'Newly Formed' or 'Filled In'. The amount of multibeam data 71 collected in 2017 (8.5 km²) surpassed what was collected in 2006 and 2009 enabling a better 72 understanding on the spatial extent of grouper holes in the SL-MPA. Grouper holes throughout the 73 entirety of this newly mapped area, beyond those already analyzed within the comparison extent, 74 were identified and enumerated as well.

75 Average hole depths, widths, and heights throughout the comparison region were measured and 76 calculated (Fig. 2). These attributes were compared to the hole depths and widths measured by Wall 77 et al. (2011) with the purpose of determining if the holes were being actively maintained (same depth 78 or deeper) or abandoned (shallower). Only holes from the 2017 dataset that could be directly linked 79 to ones present in 2009 within the comparison area were measured. To identify these holes, the 80 'Near' function in the ArcGIS analysis toolbox was employed which identified holes from the 2009 81 and 2017 datasets which were within 5 m of one another (ESRI 2016). The holes identified from the 82 2009 dataset were then used select the matching holes from 2006 which were initially identified by 83 Wall et al. (2011) to include in the hole structure trend analysis.

84 *[Figure 2]*

For each dataset, hole depths were determined using the profile tool in CARIS BASE Editor 5.1 (CARIS 2018) and each profile was taken such that it intersected the deepest portion of the hole. The hole height was calculated by subtracting the depth of the sides of the hole from its maximum depth; if a hole had sides that were at different depths, hole height was based on the deeper side of the hole so as to not positively skew hole heights potentially caused by any piling of sediment during

90 excavation. Hole widths were measured from where the slope went from essentially flat (0° to 1.5°) 91 to inclined (>1.5°) on either side of the hole. This differed from the method used by Wall et al. 92 (2011) so 2006 and 2009 hole heights and widths were recalculated from their respective bathymetry 93 surfaces using the described approach. The slopes were calculated by dividing half the width by the 94 height of the hole. The new measurements of the height, width, and slope of each hole were then 95 averaged. Two t-tests were used to determine if the average structural components changed 96 significantly between 2006–2009 and 2009–2017.

97 An aspect of the present study that was not part of Wall et al.'s (2011) work was the visual 98 surveys completed in April 2017 in concert with the collection of the multibeam bathymetry dataset. 99 These surveys were completed using a towed platform known as the Camera-Based Assessment 100Survey System (C-BASS; Lembke et al. 2017) which is towed at speeds of 3 to 4 knots as it collects continuous video and environmental sensor data near the seafloor (3 to 4 m high). Approximately 101 four hours of seafloor imagery were collected along 27.1 km of transect throughout the grouper hole 102 103 area in the SL-MPA (Fig. 3). The video was then analyzed for fish presence within and near each observed hole. All fishes observed in the imagery were enumerated and identified to the lowest 104 105 taxonomic level possible. Any individual fish that was smaller than approximately the size of a 106 bigeye (Priacanthidae spp.) or squirrelfish (Holocentridae spp.) was binned into a "Small NoID" (no identification) category. Example imagery from this survey is available as a video (S.1) which can be 107 108accessed via the online version of this article in the Supplemental Information section.

- 109 [Figure 3]
- 110 <C> Results

In Wall et al. (2011), 181 holes in 2006 and 231 holes in 2009 were detected from the multibeam bathymetry data within the area of overlap (Table 1). Using the 2017 multibeam bathymetry data, 3 m x 3 m grid, a total of 317 grouper holes were detected within the comparison area. This number increased to 340 when the 0.5 m x 0.5 m grid was analyzed. Within the 1.64 km² comparison area, this results in densities of 193 holes per km² and 207 holes per km² for the 3.0meter and 0.5 m grids, respectively. These results indicate a trend of increasing hole density from 2006 to 2017.

118 *[Table 1]*

When the entire 2017, 0.5 m x 0.5 m grid bathymetry surface was considered, several areas containing grouper holes outside of the comparison boundary became apparent (Fig. 4) and a total of 456 holes were identified within this 8.5 km² area. The locations outside of the comparison area were concentrated to the north and west of its bounds with some just adjacent to its northwest and southeast borders. The broader 2017 dataset illustrates that the originally mapped region in 2006 and 2009 aptly covered the greatest concentration of grouper holes.

125 [Figure 4]

126 A total of 188 holes within the comparison area could be linked to holes measured in 2006 and 2009. Based on this subset, the average hole height in 2017 was similar to what was 127 observed in 2006, but less than that estimated from the 2009 data (Table 2). The average widths of 128 129 the holes decreased in 2009 from 2006 but then increased in size by 2017. The slopes of the holes increased from 2006 to 2009 but then decreased in 2017 and were more comparable to the 2006 130 131 average value. A t-test was used to evaluate statistically significant differences in the average height, width, and slopes between 2006–2009 and 2009–2017. All six pairs of average values were found to 132 be significantly different within each category (p < 0.05). 133

134 [Table 2]

When examining the individual changes in widths for each of the 188 holes from 2009 to 2017, a majority experienced a growth in size of approximately 2% to 39% of their width in 2009 (Table 3). For those that decreased in size, most shrank by 70% to 90%. The number of holes that were present in 2009 but not 2017, and vice versa, were also counted ("Filled In" and "Newly Formed"; Table 3). Overall, there was a greater number of newly formed holes (61) than filled in holes (31) by 2017.

141 [Table 3]

In the four hours of C-BASS imagery collected, a total of 95 holes were captured on film. Of these, 63 holes (~66%) had fish present and no fish were detected in the remaining 32 (Table 4; note: there were likely smaller-bodied fishes, such as Gobidaee spp. or Opistognathidae spp., present but which could not be observed from the towed platform). Of the 63 holes where the towed system imagery detected fish, 19 holes were observed to have Red Grouper present and 7 had an unidentified Grouper (Epinephelinae spp.) species individual (Table 4). This means that just over

40% of the holes observed had an Epinephelinae spp. individual present. Lastly, of the holes in
which fish were detected, 84% (53 of the 63 holes) had at least one Lionfish (Pterois) spp. individual
in or near the hole and approximately 35% (22 of the 63 holes) had more than two Lionfish spp.
individuals present (Table 4). The maximum number of Lionfish spp. individuals observed within
and near a single Red Grouper hole was 24 individuals.

153 *[Table 4]*

A more detailed look at each hole where fish were observed is presented in Table 5. There were frequently "Small NoID" individuals observed within these macrohabitats. Excluding Grouper spp. and Lionfish spp., bigeyes (Priacanthidae spp.) were the next most frequently encountered species with approximately 11% of the holes having at least one individual present. The other species of larger reef fishes (i.e. angelfishes (Pomacanthidae spp.), butterflyfishes (Chaetodontidae spp.), Red Snapper, and triggerfishes (Balistidae spp.) tended to be rarely observed on the C-BASS imagery with only 1% - 4% of the 95 holes seen having any detectable individuals.

- 161 *[Table 5]*
- 162 <D> Discussion

163 The work presented here was not an exhaustive study into the efficacy of Steamboat Lumps 164 as a marine protected area. However, the results of this research in tandem with the work done by 165 Wall et al. (2011) show that over the last 11 years, the density of Red Grouper holes has continued 166 to increase within the boundaries of the study area. Though the SL-MPA was intended to protect 167 spawning aggregations of Gag Mycteroperca microlepis (Coleman et al. 2004), we have documented that 168 there are likely positive side-effects for the local Red Grouper population. This result is even more 169 impactful when you consider that these holes are used by Red Grouper for spawning as well as 170 habitation (Wall et al. 2011). As the Red Grouper population in the Gulf of Mexico continues to 171 recover from past exploitation (SEDAR 2015), this can serve as an example of the importance of 172 properly implemented marine protected areas. To truly comment on the efficacy of the SL-MPA to 173 support a growing local population, data on Red Grouper populations and hole habitat outside of 174 the MPA are necessary. A subsequent analysis to assess the level of illegal fishing that occurs within 175 the SL-MPA (Gledhill and David 2004) could also help to determine if the increase in holes is in fact 176 due to the effective protection of this habitat.

177 Although the density of holes within the SL-MPA appears to be increasing, analysis of the 178 hole characteristics did not result in such a clear trend. The statistically significant changes in hole 179 width, height, and slope between years demonstrates that these habitats are dynamic and experience 180 significant growth and contraction on fairly short time scales (3 to 11 years). A longer time series is 181 necessary to determine whether average hole widths, heights, and slopes increasing, decreasing, or if 182 they go through continuous cycles of growth and reduction.

Despite an unquantifiable level of fish detection, the towed camera video survey showed that 183 184 Red Grouper continue to persist in the SL-MPA and that several other species of reef fishes are 185 commonly found among the Red Grouper holes. Work is ongoing to determine the species and 186 habitat-specific "catchabilities" for the C-BASS. As such, these data cannot yet estimate the true 187 densities of the observed reef fishes because we do not know how efficiently the C-BASS captured 188 their presence within and near the holes in the SL-MPA. This limitation could be addressed in future 189 endeavors by combining the towed camera work with a remotely operated vehicle (ROV). This type 190 of visual survey equipment has significantly better maneuverability to allow for more extensive observations of the holes. 191

192 Though C-BASS's detection ability for most reef fishes is likely affected by reactive behavior, 193 as is the case for visual surveys in general (Stoner et al. 2008), we believe that Lionfish spp. are an 194 exception to this condition. Previous studies on Lionfish have reported how minimally reactive this 195 species is towards divers, and that at times they can actually be fairly aggressive (Whitfield et al. 196 2007). Based on this information, and the hundreds of hours of C-BASS imagery that have been collected over the past five years where Lionfish are frequently observed, we believe that Lionfish 197 198 are minimally reactive towards the system. It is important to note that Lionfish commonly reside in 199 crevices and under overhanging rocks where towed video imagery cannot sample effectively. 200 Therefore, the observations of Lionfish on C-BASS imagery are likely underestimated, but 201 determining to what degree requires further habitat-specific surveys. Nonetheless, the Lionfish 202 observations were a notable part of the towed camera work as nearly 85% of the 94 holes observed 203 with the C-BASS had at least one Lionfish spp. individual present in or near the hole (Table 4).

The spread of Lionfish spp. began along the East Coast of Florida in the early 1980's (Morris, Jr. and Whitfield 2009) but this species wasn't detected in the Pulley Ridge MPA – an area ~400 km south of the SL-MPA – until 2010 (Harter et al. 2017). The last published study using a visual-based survey to evaluate the reef fishes found in the SL-MPA was completed in 2002, fifteen

years prior to this 2017 survey (Gledhill and David 2004). The area was scheduled to be resurveyed
in 2010 but due to funding issues, SL-MPA was removed from the cruise plan (David and Gledhill
2012). However, Lionfish were observed in 2013 from C-BASS video data collected in the SL-MPA
grouper holes area (Grasty 2014). It is therefore likely that Lionfish spp. did not start colonizing the
SL-MPA until after 2010, but took less than four years to populate the grouper holes.

213 Understanding the effects of invasive Lionfish spp. on the native reef fishes in the Gulf of Mexico and how to effectively address their impacts on the Gulf's ecosystems are ongoing 214 215 endeavors for scientists and fisheries managers (Johnston et al. 2017; Marshak et al. 2018; Harris et 216 al. 2019). A recent study on the grouper holes in the Pulley Ridge MPA by Harter et al. (2017) did 217 not find conclusive evidence of a negative effect of the Lionfish spp. on the other reef fishes that 218 were part of the assemblage. As they state in their work, this was an uncommon finding as other 219 studies examining the impact of Lionfish spp. on the Atlantic, Caribbean, and Gulf of Mexico 220 regions have found evidence to the contrary (Whitfield et al. 2007; Ballew et al. 2016; Hixon et al. 221 2016). The authors proposed that this apparent neutral existence of Lionfish with the native fishes 222 may be due in part to high colonization of the holes by reef fishes resulting from potential high prey 223 availability which could allow it to coexist with native species. Additional visual survey work in 224 tandem with diet studies would be needed to assess if the same holds true for the grouper hole habitat in the SL-MPA. 225

226 Since towed systems can cover more area in a shorter amount of time than some tethered 227 and autonomous platforms (Lembke et al. 2017), the C-BASS facilitated a quick means of collecting 228 data on what species were observed among the holes (i.e. presence confirmation along the transect). 229 Small modifications to towing operations of the C-BASS such as decreased towing speed and 230 upgraded side cameras are expected to result in increased species resolution and potentially increased 231 detection abilities of the larger reef fishes. However, C-BASS would still not be ideal for 232 characterizing small reef fish populations and experiences some level of species-specific bias due to 233 reactive behavior from the larger reef fishes. Additional video data from an ROV or stationary 234 camera system survey are necessary to determine which holes are truly occupied vs. unoccupied, and 235 to estimate the abundance of the other reef fishes. This additional methodology would complement 236 C-BASS' ability to quickly assess the presence of larger reef fish, namely groupers, and the non-237 reactive, invasive Lionfish spp., and therefore provide a full analysis of the species composition in 238 and near grouper holes. As we have demonstrated here, repetitive acoustic and visual surveys can be

highly valuable for documenting and better understanding population changes over time, particularlyin this critical dynamic habitat.

241 <E> Supplemental Information

242 Supplement: Video from the C-BASS (towed camera) survey in the SL-MPA in April 2017

243 Supplemental Video S.1. This video is a compilation of several clips of imagery that were collected

244 with the C-BASS in the Steamboat Lumps MPA during the April 2017 survey. The clips presented

in this video are examples of what the holes look like on towed camera footage and also depict

246 typical Red Grouper Epinephelus morio encounters with the C-BASS. Each Red Grouper in the video

has been indicated by a red circle and the playback has been slowed to 0.5x for easier viewing.

248 Although they are present, no other species are indicated by the circles.

249

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