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## Shelf-scale Mapping of Fish Distribution Using Active and Passive Acoustics

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Shelf-Scale Mapping of Fish Distribution Using Active and Passive Acoustics

by

Carrie C. Wall

A dissertation submitted in partial fulfillment  
of the requirements for the degree of  
Doctor of Philosophy  
College of Marine Science  
University of South Florida

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Keywords: Red grouper, *Epinephelus*, West Florida Shelf, toadfish, spawning

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Dedication

To Ryan.

## Acknowledgements

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## ABSTRACT

Fish sound production has been associated with courtship and spawning behavior. Acoustic recordings of fish sounds can be used to identify distribution and behavior. Passive acoustic monitoring (PAM) can record large amounts of acoustic data in a specific area for days to years. These data can be collected in remote locations under potentially unsafe seas throughout a 24-hour period providing datasets unattainable using observer-based methods. However, the instruments must withstand the caustic ocean environment and be retrieved to obtain the recorded data. This can prove difficult due to the risk of PAMs being lost, stolen or damaged, especially in highly active areas. In addition, point-source sound recordings are only one aspect of fish biogeography. Passive acoustic platforms that produce low self-generated noise, have high retrieval rates, and are equipped with a suite of environmental sensors are needed to relate patterns in fish sound production to concurrently collected oceanographic conditions on large, synoptic scales. The association of sound with reproduction further invokes the need for such non-invasive, near-real time datasets that can be used to enhance current management methods limited by survey bias, inaccurate fisher reports, and extensive delays between fisheries data collection and population assessment.

Red grouper (*Epinephelus morio*) exhibit the distinctive behavior of digging holes and producing a unique sound during courtship. These behaviors can be used to identify red grouper distribution and potential spawning habitat over large spatial scales. The goal of this research was to provide a greater understanding of the temporal and spatial

distribution of red grouper sound production and holes on the central West Florida Shelf (WFS) using active sonar and passive acoustic recorders. The technology demonstrated here establishes the necessary methods to map shelf-scale fish sound production. The results of this work could aid resource managers in determining critical spawning times and areas.

Over 403,000 acoustic recordings were made across an approximately 39,000 km<sup>2</sup> area on the WFS during periods throughout 2008 to 2011 using stationary passive acoustic recorders and hydrophone-integrated gliders. A custom MySQL database with a portal to MATLAB was developed to catalogue and process the large acoustic dataset stored on a server. Analyses of these data determined the daily, seasonal and spatial patterns of red grouper as well as toadfish and several unconfirmed fish species termed: 100 Hz Pulsing, 6 kHz Sound, 300 Hz FM Harmonic, and 365 Hz Harmonic.

Red grouper sound production was correlated to sunrise and sunset, and was primarily recorded in water 15 to 93 m deep, with increased calling within known hard bottom areas and in Steamboat Lumps Marine Reserve. Analyses of high-resolution multibeam bathymetry collected in a portion of the reserve in 2006 and 2009 allowed detailed documentation and characterization of holes excavated by red grouper. Comparisons of the spatially overlapping datasets suggested holes are constructed and maintained over time, and provided evidence towards an increase in spawning habitat usage. High rates of sound production recorded from stationary recorders and a glider deployment were correlated to high hole density in Steamboat Lumps. This research demonstrates the utility of coupling passive acoustic data with high-resolution bathymetric data to verify the occupation of suspected male territory (holes) and to

provide a more complete understanding of effective spawning habitat. Annual peaks in calling (July and August, and November and December) did not correspond to spawning peaks (March – May); however, passive acoustic monitoring was established as an effective tool to identify areas of potential spawning activity by recording the presence of red grouper.

Sounds produced by other species of fish were recorded in the passive acoustic dataset. The distribution of toadfish calls suggests two species (*Opsanus beta* and *O. pardus*) were recorded; the latter had not been previously described. The call characteristics and spatial distribution of the four unknown fish-related sounds can be used to help confirm the sources. Long-term PAM studies that provide systematic monitoring can be a valuable assessment tool for all soniferous species. Glider technology, due to a high rate of successful retrieval and low self-generated noise, was proven to be a reliable and relatively inexpensive method to collect fisheries acoustic data in the field. The implementation of regular deployments of hydrophone-integrated gliders and fixed location passive acoustic monitoring stations is suggested to enhance fisheries management.

## Chapter 1: Introduction

Sound has long been recognized as an important means of animal communication (Darwin 1896). Many species of fish produce sound as a normal component of their behavioral repertoire, often in association with courtship, spawning, parental, aggressive and territorial activities (e.g., Breder 1968, Fish & Mowbray 1970, Lobel et al. 2010). One mechanism some species of fish use to produce sound involves the innervation of muscles on or around the swimbladder (sonic muscles) (Fish & Mowbray 1970, Tavolga 1971). Serranids (e.g., grouper), Batrachoidids (toadfish, midshipman), Triglids (searobins), and Sciaenids (e.g., drum) all produce sound this way. Most fish calls are species-specific and repetitive, which enable sound production to be used for identifying species distribution and behavior. In addition, sound can be used to determine when and where reproductive activities occur, which provides valuable information on spawning habitat and timing that can aid fisheries managers in maintaining population stability.

Recent research has shown male red grouper (*Epinephelus morio*) produce sound during courtship and territorial behavior (Nelson et al. 2011). Red grouper comprise a large commercial and recreational fishery with approximately 150,000 pounds of commercial landings and over 130,000 recreationally-caught red grouper reported in 2008 (SEDAR 2009). In addition, red grouper act as ecosystem engineers by excavating depressions (or holes) in areas of flat sandy bottom. The approximately 5 m diameter holes are used by males for courtship of females thus defining the holes as critical spawning sites. Further, the holes provide suitable habitat for themselves and structure

for other commensal, typically reef-associated species (Scanlon et al. 2005, Coleman et al. 2010).

Spawning habitat is likely to experience increased human disturbances as intense fishing in shallow areas drives fish population sizes down and fisheries move offshore (Koslow et al. 2000, Coleman & Koenig 2010). Thus, locating source populations and spawning habitat essential to sustain fishery production and conservation is a critical consideration for fisheries management (Coleman et al. 1996, Crowder et al. 2000). Further, when fishermen target spawning aggregations, a large fraction of reproductively active fish can be removed, which in turn can disturb the sex transition mechanisms and skew the sex ratios by removing a higher percentage of males from the population due to their larger size and more aggressive behavior towards fishing lures (Gilmore & Jones 1992, Coleman et al. 1996, Koenig et al. 1999). As the impact of fishing increases on this commercially important species, effective management tools become essential for conservation.

High-resolution active sonar (i.e., side-scan sonar and multibeam sonar) can be used to detect excavated holes (Scanlon et al. 2005, Allee et al. 2011). Passive acoustic devices can be used to record red grouper sound production. The research presented in the succeeding chapters, which formulates this doctoral dissertation, employed both acoustic methods to identify the range and calling patterns of red grouper in addition to other soniferous fish in the eastern Gulf of Mexico. The goals of this work were to provide large-scale, long-term knowledge of the spatial and temporal distribution of red grouper sound production and potential spawning habitat to resource managers to aid in determining critical spawning times and areas.

Chapter 2 discusses the distribution and dynamics of red grouper holes identified using two multibeam sonar surveys conducted in the Steamboat Lumps Marine Reserve three years apart (2006 and 2009). In addition, acoustic communication ranges of red grouper were estimated as a means to understand the proximity of neighboring holes and thus the groupers' social system. Chapter 3 develops the application of hydrophone-integrated glider technology as a platform to detect and map fish sounds in the ocean over a large spatial scale. Oceanographic parameters, such as temperature and fluorescence, measured by the glider are compared to the concurrently recorded acoustic data, which includes calls from red grouper, toadfish, and three unknown fish-related sounds. Chapter 4 incorporates passive acoustic data collected on multiple glider missions and at numerous fixed location recorders deployed during periods between 2008 and 2011. These data were analyzed to determine the daily and seasonal patterns of red grouper sound production, and to define more precisely the range of potential spawning habitat for this species. Chapter 5 outlines the habitat ranges, and daily and seasonal patterns in calling for the remaining sounds identified in Chapter 3 using the same acoustic dataset analyzed in Chapter 4. Sound occurrence was compared to environmental data to understand the variability in seasonal calling and help determine the sources of the unknown fish sounds.

## Chapter 2: Spatial and Temporal Variability of Red Grouper Within Steamboat Lumps Marine Reserve, Gulf of Mexico

### Note to Reader

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### Abstract

Red grouper, *Epinephelus morio*, act as ecosystem engineers by excavating depressions (or holes) in areas of flat sandy bottom, which provide suitable habitat for themselves and for numerous other species. To understand the spatial extent of the holes, which serve as spawning habitat, and determine how that habitat changes, high-resolution multibeam sonar data were collected in overlapping areas in 2006 and 2009 within the Steamboat Lumps Marine Reserve. This marine reserve was established in 2000 and is located in the eastern Gulf of Mexico. Vertical profiles of the holes visually identified from the multibeam datasets were extracted to characterize hole shape and determine how the height, width, and slope of each hole changed over time and space. Results from this analysis indicate an increase in hole density from 110 to 141 holes per km<sup>2</sup> from 2006 to 2009, respectively, with 181 holes detected in 2006 and 231 holes detected in 2009.

Height and slope also increased between 2006 and 2009. The changes in these parameters and the 151 holes identified in the same location between the years suggest hole shape is varied due to red grouper maintenance and that holes are constructed and maintained over time. The increase in number and density of holes from 2006 to 2009 demonstrates multiyear habitat mapping using active acoustic sonar is an effective method to monitor the presence and extent of red grouper spawning populations.

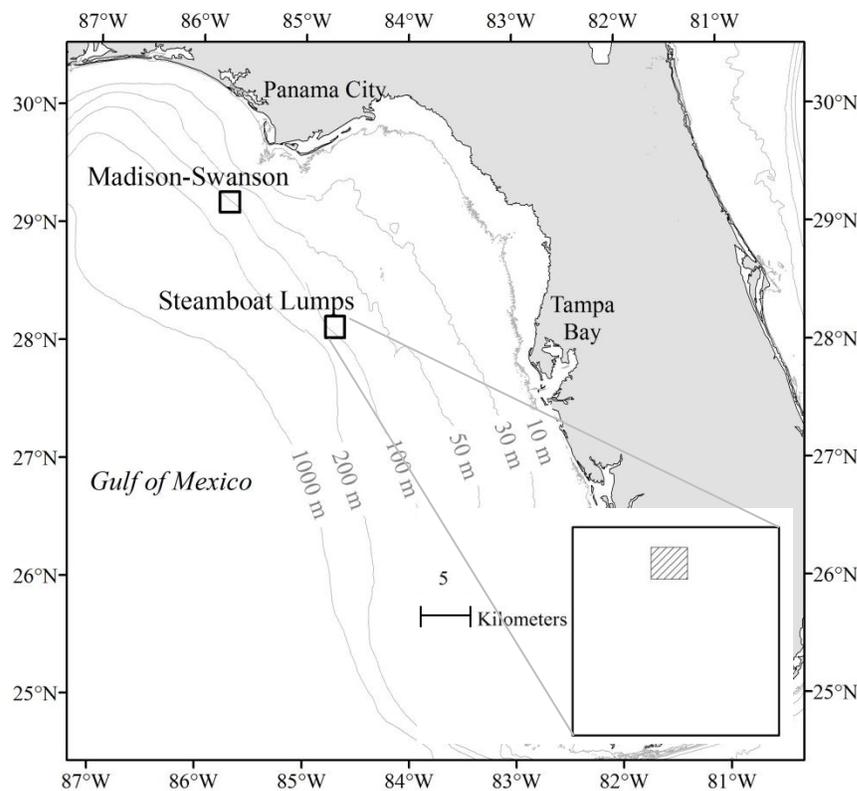
## Introduction

Like all grouper species, red grouper, *Epinephelus morio*, are slow-growing, late-maturing, relatively stationary, and long-lived. Red grouper are protogynous hermaphrodites that change sex from female to male between five to ten years of age (Moe 1969, Jory & Iversen 1989, Heemstra & Randall 1993, Musick 1999, Coleman et al. 2000, Sadovy 2001). These are characteristics that should make them vulnerable to overexploitation, especially in the Gulf of Mexico where there is a strong fishery. However, red grouper may be relatively resilient to fishing pressure because this species forms small polygamous spawning groups dispersed over large areas instead of large spawning aggregations common to other grouper species (Coleman et al. 1996). Still, red grouper have experienced a truncated age structure and are currently considered near threatened by the International Union for Conservation of Nature (IUCN) (SEDAR 2009, Coleman & Koenig 2010, IUCN 2010).

Red grouper spawn offshore (~70 m depth) during the late winter to early spring for approximately four months, with spawning peaks in April and May (Jory & Iversen 1989, Koenig et al. 2000). During this time, a female approaches a male, who exhibits high site fidelity, in his “home territory” (Coleman et al. 2010). If the male successfully courts the female, they ascend the water column to spawn.

Such offshore spawning habitat is likely to experience increased human disturbances as intense fishing in shallow areas drives fish population sizes down and fisheries move offshore (Koslow et al. 2000, Coleman & Koenig 2010). Thus, locating

mature fish populations and spawning habitat essential to population stability is a critical consideration for fisheries management (Coleman et al. 1996, Crowder et al. 2000). To mitigate fishing pressure on grouper aggregations during spawning, in June 2000 two marine reserves covering 200 square nautical miles were established on the shelf break (50 – 120 m deep) of the northeastern Gulf of Mexico (Figure 2.1) – Madison Swanson (N29° 06' – N29 ° 17'; W085° 38' – W085° 50') and Steamboat Lumps (N28° 03' – N28° 14'; W084° 37' – W084 ° 48') Marine Reserves (Coleman et al. 2004a).



**Figure 2.1.** Madison-Swanson and Steamboat Lumps Marine Reserves. Inset: 2006 and 2009 multibeam data were collected in the thatched square within the Steamboat Lumps Marine Reserve (black box).

Two important red grouper behaviors have been documented recently in these marine reserves i) sediment excavation (Scanlon et al. 2005, Coleman et al. 2010) and ii) sound production (Nelson et al. 2011). In this paper, we focus on excavation in the

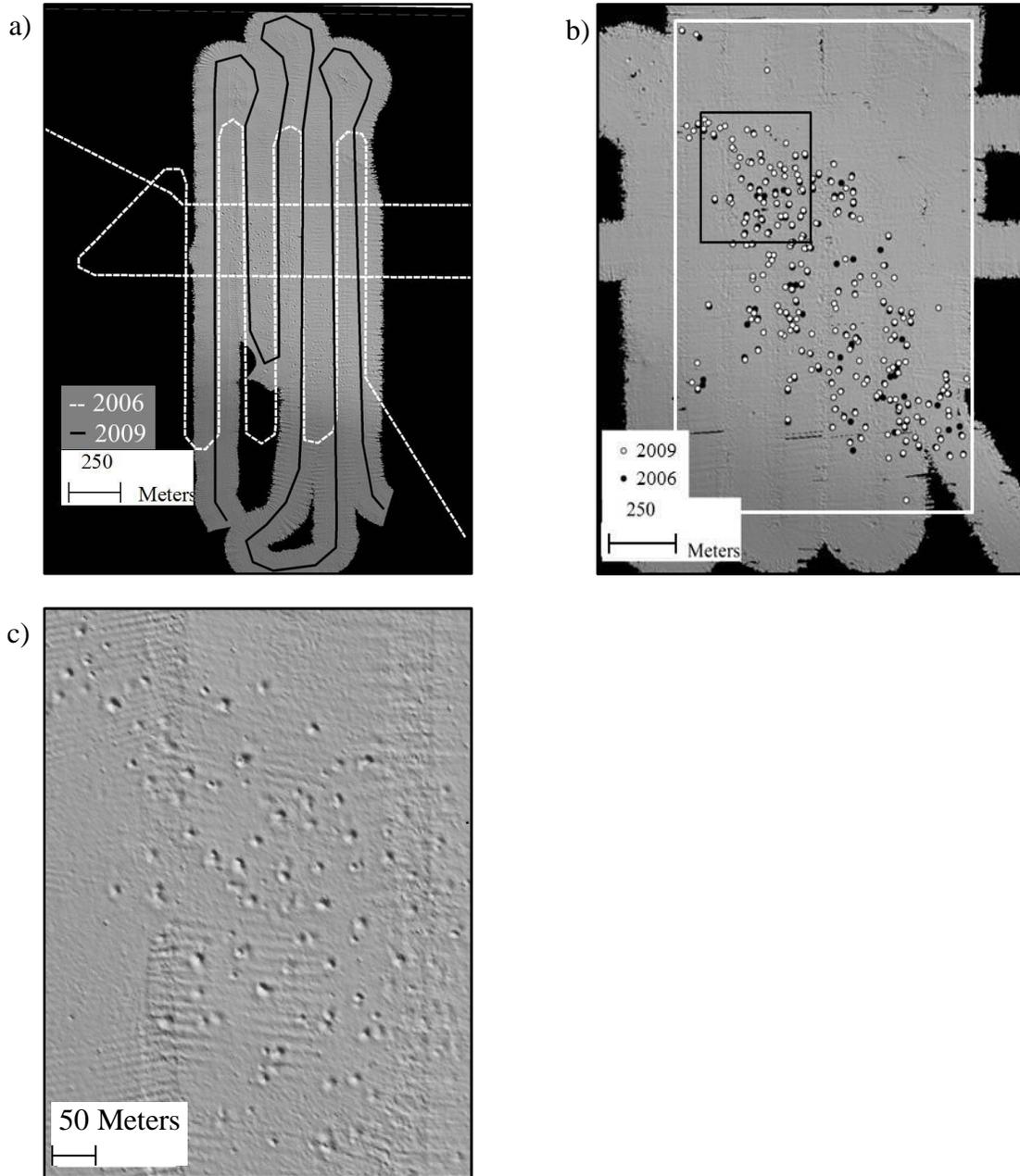
Steamboat Lumps Marine Reserve and potential red grouper communication ranges in relation to the two-dimensional spacing of the holes.

In continental shelf areas with a sedimentary bottom, red grouper excavate large (5 – 25 m diameter) depressions (or holes) that they use as home territories (Scanlon et al. 2005). Red grouper excavate by carrying mouthfuls of sediment from within a depression to a short distance away and then deposit the sediment by flushing it through their opercles (Scanlon et al. 2005, Coleman et al. 2010). In the Steamboat Lumps Marine Reserve, holes are mainly observed to be dug and maintained by males who use this habitat as their “home territory” and for spawning. Further inshore, juvenile (female) red grouper also exhibit this behavior (Coleman et al. 2010). Hole excavation is mainly found in areas where relief such as rock outcroppings is not present (Coleman et al. 2010). Excavation uncovers loose rocks such as cemented carbonate nodules, which provide an important source of substrate and refuge for organisms in areas where it was not previously available (Scanlon et al. 2005). Habitat preferences based on substrate composition influence the distribution of many marine organisms, especially benthic species (Day et al. 1989, Coleman & Koenig 2010). Additionally, the probability of observing other species is higher at holes where red grouper are present (“active sites”) compared to holes where red grouper are not present (“inactive sites”) (Coleman et al. 2010).

Holes can be observed using high-resolution acoustic sonar (e.g., side-scan sonar or multibeam sonar) (Scanlon et al. 2005, Allee et al. 2011; Figure 2). In addition, the swim bladder in fish, including red grouper, can be detected using sonar due to acoustic reflections resulting from the density differences between gases in the swim bladder and

the surrounding seawater (Misund 1997). Therefore, the application of active acoustic technology can provide high-resolution information on the changes in bathymetry (including holes), as well as the presence of fish.

The goals of this project were to study the distribution and dynamics of red grouper holes using two multibeam sonar surveys conducted three years apart. Additionally, we aimed to quantify the percentage of holes potentially occupied by red grouper and estimate grouper acoustic communication ranges as a means to indicate marine reserve success and understand the groupers' social system.



**Figure 2.2.** Multibeam bathymetry data collected in the Steamboat Lumps Marine Reserve. a) 2009 multibeam data overlaid by the vessel tracklines of 2006 (dotted white line) and 2009 (black line). b) 2006 multibeam data overlaid with red grouper holes detected in 2006 (●, N=181) and 2009 (white circles, N=231). White box indicates where hole profiles were extracted. Black box indicates area of inset in (c). c) Close up image of holes. Latitudinal bands are artifacts of the sonar swath overlap. Latitude and longitude are not shown to protect the location of the holes.

## Methods

*Study Area.* The West Florida Shelf (WFS) extends over 200 miles from the Florida coast between the Florida Keys and the Mississippi River delta creating a wide, gently sloping shelf. The inner WFS consists of a nearly flat, drowned and partially dissolved lithified carbonate (karst) platform covered by a thin layer of carbonate-siliciclastic sediment (Hine 1997, Brooks et al. 2003b). Five Holocene facies, or sediment veneers, have been identified overlying the bedrock of the central WFS: organic-rich mud, muddy sand, shelly sand, mixed siliciclastic/carbonate, and fine quartz sand (Edwards et al. 2003, Robbins et al. 2008). The distribution of each sediment type is highly varied along the inner central WFS and reflects both low accumulation rates and the lack of a single dominating source, all of which come from within or along the perimeter of the catchment (Brooks et al. 2003a). Scarped hard bottom systems are the only natural relief (< 4 m) (Obrochta et al. 2003). The lack of active coral reefs in this region is attributed to the effects of the high-nutrient, low-salinity Mississippi River discharge entrained in the Loop Current (Hallock 1988, Gilbert et al. 1996). Detailed descriptions of the WFS geology are provided in Randozzo and Jones (1997) and Jarrett (2003).

*Bathymetry Mapping.* Red grouper spawning habitat was mapped using a Kongsberg (Kongsberg, Norway) EM3000 multibeam swath sonar. The EM3000 operates at 300 kHz with 127 overlapping beams. Beam width is 1.5 x 1.5 degrees with beam spacing of 0.9 degrees producing a 130 meter swath transverse to ship heading.

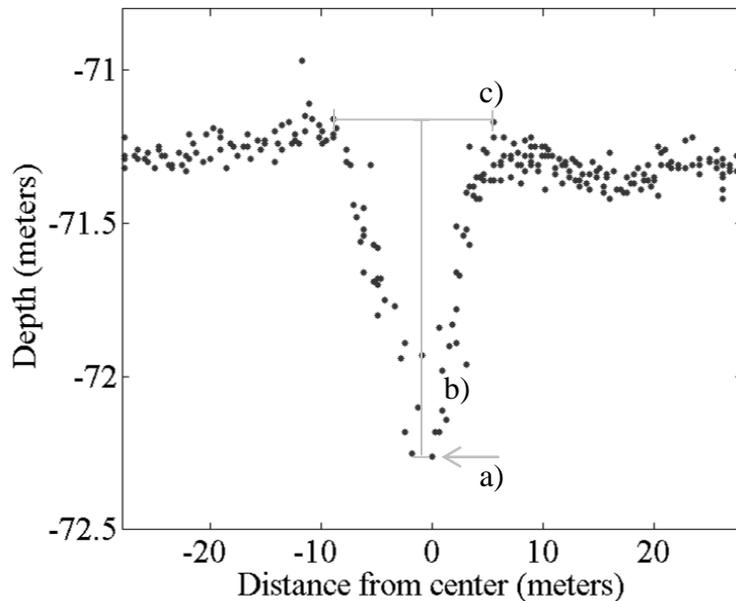
The vertical uncertainty of the EM3000 in a water depth of 100 m is 10 cm RMS with a 20 cm accuracy and 1 m positioning accuracy using an Applanix POSMV 320 system upgraded to a L1/L2 band that provides 0.02 degrees RMS roll, pitch, and heading accuracy. Heave accuracy is 5 cm or 5% of the heave amplitude. Tide data were used to normalize sea level to a mean low low water (MLLW) chart datum.

Multibeam data were collected in overlapping portions of the Steamboat Lumps Marine Reserve on 27 July 2006 and 23 April 2009 (see Figure 2.1). The survey tracks were retraced to replicate the data collection process (see Figure 2.2). The specific site chosen here corresponded to the study site of another project that focused on passive acoustic monitoring of red grouper sound production. Therefore, this site was a location of opportunity but, from that previous work, red grouper were known to be present. Due to the high cost of ship time and the lack of funding, only one small portion of the reserve was monitored as a pilot study. While analysis of multiple areas in Steamboat Lumps over time would have allowed for the detection of red grouper habitat usage throughout the reserve, we were not capable of such a study chosen for this pilot “proof-of-concept” program.

The multibeam data were displayed and calibrated using CARIS HIPS and SIPS 7.0 software. Corrections for roll, pitch, heave, and tide were applied. Since tide data were not available for 2009, a static offset of 0.53 m, the mean difference between the two datasets at 100 randomly selected locations, was applied to allow direct comparison to the 2006 data. The short survey period, 103 minutes, allowed the offset to be static and did not need to account for any significant tidal ebb or flow. Further details on the method used to determine this offset are provided in the next paragraph. Depth

thresholds were applied to remove data out of the range of depths encountered during the survey. Data were further filtered using a threshold of 3 standard deviations away from the moving mean depth. A vertical exaggeration of 5 and sun angle of 45° were applied to visualize the features (see Figure 2.2).

*Hole Profiles.* Two-dimensional vertical profiles of data points that crossed each hole visually identified from the multibeam data were extracted for both years. The profiles best represent the characteristics of the hole including the deepest point (Figure 2.3). These data were used to determine the location (latitude and longitude), depth (the distance from the tide-corrected surface to the bottom of the hole, height (vertical distance from the depth of the hole edge to the depth of the hole center), width (distance across the hole), and slope (height divided by  $\frac{1}{2}$  the width) of each hole. The above-hole depth, hole height plus hole bottom depth, was calculated to determine an offset in the depth calibration between the two data sets. Although the static offset applied to the 2009 data greatly improved its alignment to the 2006 dataset, any error in the actual absolute depth measurement will not affect the hole characteristics that are measured (height, slope and width) as they are determined by the difference of very precise (not necessarily accurate) depth measurements. For further discussion and analysis of this type of approach, see Wolfson et al. (2007).



**Figure 2.3.** Example of a hole profile. The data points or measurements for a) depth, the distance from tide-corrected surface to hole bottom; b) height, the distance from the top of the hole to the bottom of the hole, and c) width are indicated. Slope is calculated by dividing height (b) by  $\frac{1}{2}$  the width (c). Above-hole depth is calculated by subtracting height (b) from depth (a). The data point (or ping) above the left rim of the hole is suspected to be a result of fish presence. Note the exaggeration in the vertical scale.

Due to differences in survey extent between the two years (e.g., data were collected further south in 2009 than in 2006), only profiles within overlapping datasets were used. Areas in the data where sonar swaths overlap interfere with an accurate representation of the bathymetry and inhibit proper detection and hole characterization. Therefore, only areas with adequate bathymetry data coverage were used to detect holes.

To identify corresponding holes in 2006 and 2009, holes detected in 2006 that were within 10 meters of those detected in 2009 were assumed to be potentially the same hole and were inspected visually. Ten meters was chosen as a conservative estimate and encompassed the vast majority of holes coinciding between the years. This analysis was completed using ESRI (Environmental Systems Research Institute) ArcGIS 10 software and was used to account for any georeferencing inconsistencies between the two datasets.

The height, width, and slope of these holes were compared to determine changes from 2006 and 2009. Significant changes in these parameters over the three year period were tested using a paired t-test.

Profiles were also analyzed to determine if a hole had been abandoned or was less defined (inactive) from 2006 to 2009, or if a hole had been created or was better defined (active) in 2009 compared to 2006.

As the multibeam data consist of discrete data points (or pings), a ten-term polynomial was fitted to each profile to create a continuous cross-section. This analysis was done to mathematically characterize the general shape of the holes. Unfiltered (raw data) pings floating 10 cm above the seafloor along the hole profile were assumed to result from the presence of fish because they are distinct from the underlying seafloor (see Figure 2.3). To determine if a hole was active or inactive and to characterize the shape of active and inactive holes, I quantified the number of non-seafloor associated pings per profile and compared this count to the hole's polynomial-derived shape and slope. The slope that characterized the steepness of the hole was calculated from each polynomial by subtracting the hole depth at five meters to the left of the hole center (a placement always located within the hole) from the hole depth at the center, and then dividing by five meters (the horizontal distance from the hole depth to the hole center). I then determined if the hole slopes differed significantly as a result of height or number of non-seafloor associated pings.

*Hole Distance and Red Grouper Source Level.* The distance from the deepest point of each profile to the deepest point of the nearest profile was calculated in ArcGIS for 2006 and 2009. Histograms of between-hole distances for both years were created in

MATLAB (Mathworks). Male red grouper produce sound during courtship and territorial behavior (Nelson et al. 2011). To determine the potential communication network within the study area, the relationship between estimated grouper communication range and distances between the holes was analyzed. The intensity of sound produced by red grouper from one meter away, also known as source level (SL), was assumed to be equal to the most intense received level recorded of a sound produced by a red grouper over many hours of recordings (Nelson et al. 2011). Although red grouper are a benthic species and the substrate will interact with the propagation of sound, a cylindrical model (Urick 1983) is not practical due to the depth of the water column (~100 meters) where red grouper produce sound without constraint from an air-water interface. Therefore, I applied a spherical spreading model as a conservative estimate of transmission loss ( $TL_{\text{spherical}}$ ),

$$TL_{\text{spherical}} = - 20 \log(R), \quad \text{Eq. 1}$$

where R is range in meters. With this model, I calculated the maximum acoustic communication range given SL and noise floor level (NL),

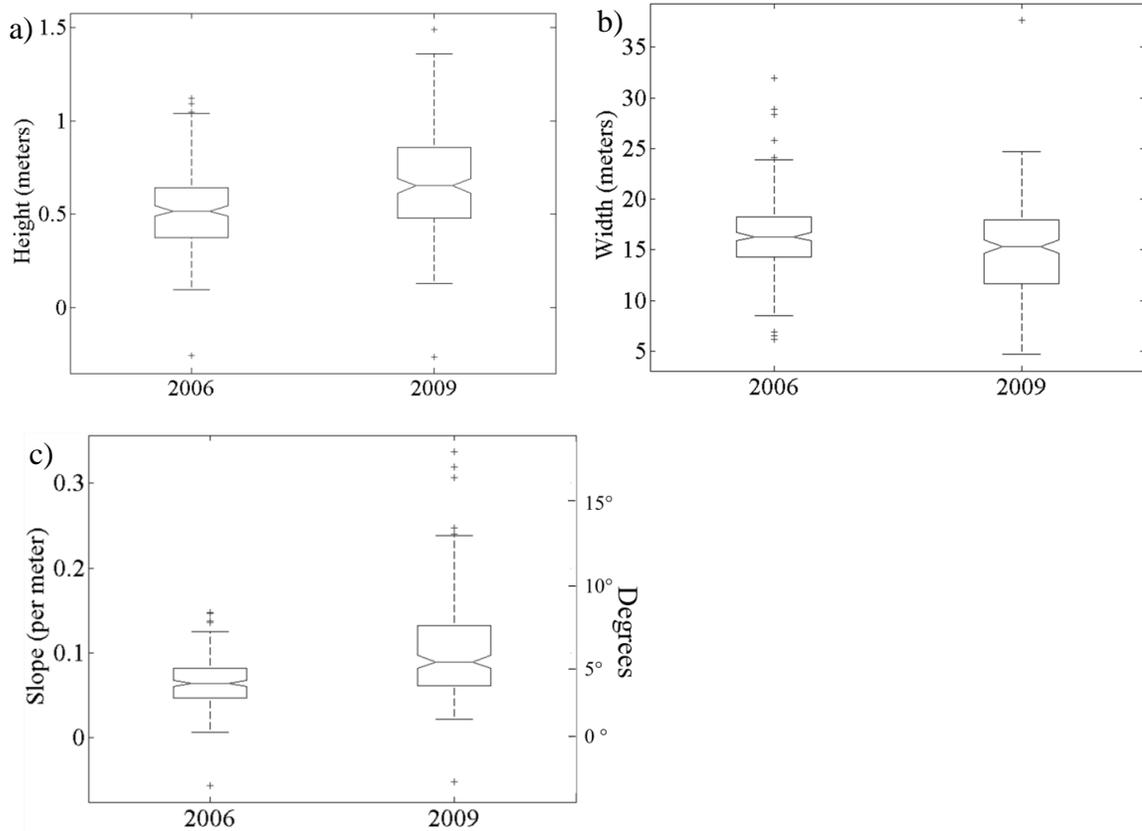
$$R = 10^{(SL-NL)/20}, \quad \text{Eq. 2}$$

where distance is in meters and SL-NL represents the signal-to-noise ratio (SNR).

## Results

*Hole Profiles.* There were 219 profiles extracted from the 2006 data (1.88 km<sup>2</sup> surveyed) and 278 profiles from the 2009 data (2.81 km<sup>2</sup> surveyed). Thus, the grouper hole density over the areas surveyed was 116 holes per km<sup>2</sup> in 2006, and 98 grouper holes per km<sup>2</sup> in 2009. After restricting the study area to where the two datasets overlapped and removing profiles due to their inability to be potentially detected in the other dataset, there were 181 holes in 2006 and 231 holes in 2009 covering approximately 1.64 km<sup>2</sup>. These constraints resulted in a density of 110 and 141 grouper holes per km<sup>2</sup>, respectively. Height and slope of the holes increased significantly from 2006 to 2009 (Figure 2.4).

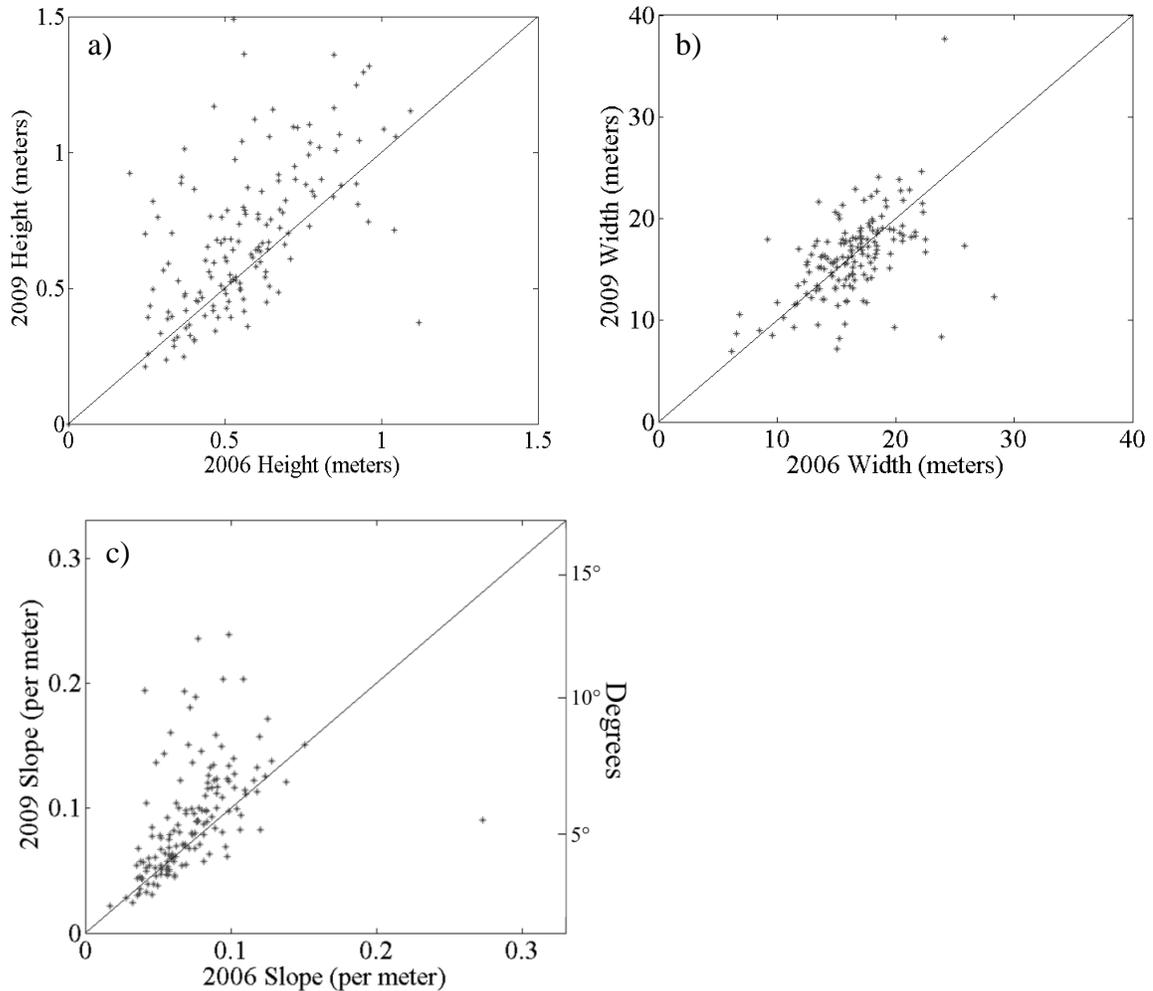
The 10 meter buffer analysis found 151 profiles to be directly comparable between the two datasets. In comparisons of height, width and slope for corresponding holes, only height and slope were significantly different over the three years (Table 2.1, Figure 2.5). Regression of the above-hole depth of the directly comparable 2006 and 2009 holes identified a close fit between the datasets, with an R<sup>2</sup> of 0.9. Additionally, the mean of the absolute value of the residuals was 0.1 m.



**Figure 2.4.** Boxplot of a) height, b) width and c) slope hole profiles. The central mark is the median, the box boundaries are the 25<sup>th</sup> and 75<sup>th</sup> percentiles, the whiskers extend to the 95<sup>th</sup> percentile and the outliers are plotted individually. With 95% confidence, medians are significantly different if the notch intervals do not overlap. N=181 in 2006; N=231 in 2009.

**Table 2.1.** Paired t-test analysis for directly comparable holes. N=151.

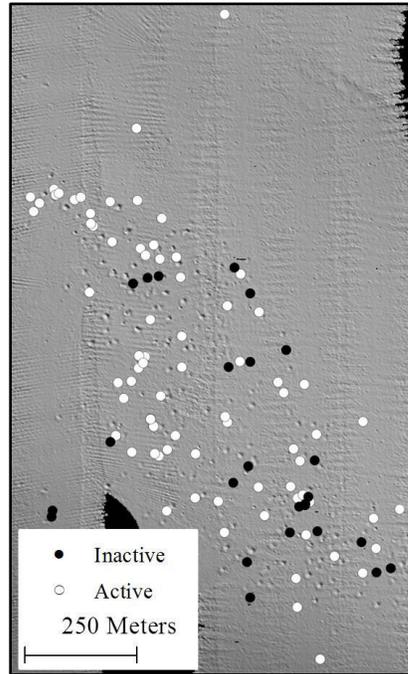
	Height	Width	Slope
2006 Mean	0.6	16.4	0.07
2009 Mean	0.7	16.3	0.09
Standard Deviation	0.2	3.7	0.04
P-Value	< 0.001	0.8	< 0.001



**Figure 2.5.** Corresponding hole a) height, b) width and c) slope. The black line shows a 1:1 ratio. Data points above this line indicate an increase in that parameter from 2006 to 2009. N=151.

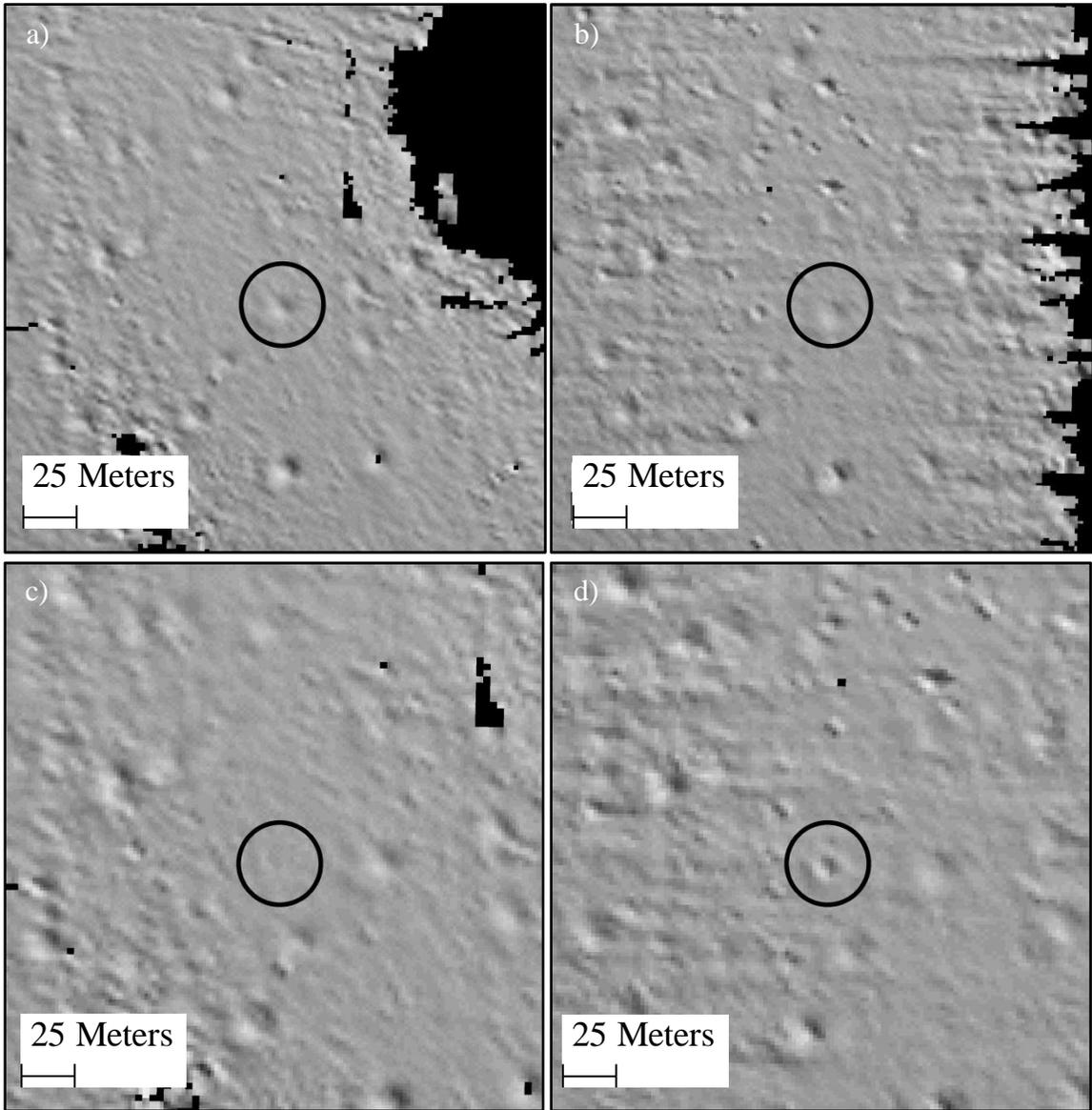
The number of new holes detected in 2009 was greater than the number abandoned after 2006 (Figure 2.6). Twenty-three holes (out of 181, 13%) were identified in 2006 and not in 2009 (Figure 2.7a, 2.7b). Conversely, 77 holes (out of 231, 33%) were identified in 2009 and not in 2006 (Figure 2.7c, 2.7d). There were 158 total non-seafloor associated pings found among the 23 inactive holes, with a median of five non-seafloor associated pings per hole (SD=6). This value is in comparison to the 473 total non-

seafloor associated pings found among the 77 active holes, with a median of six non-seafloor associated pings per hole (SD=4).

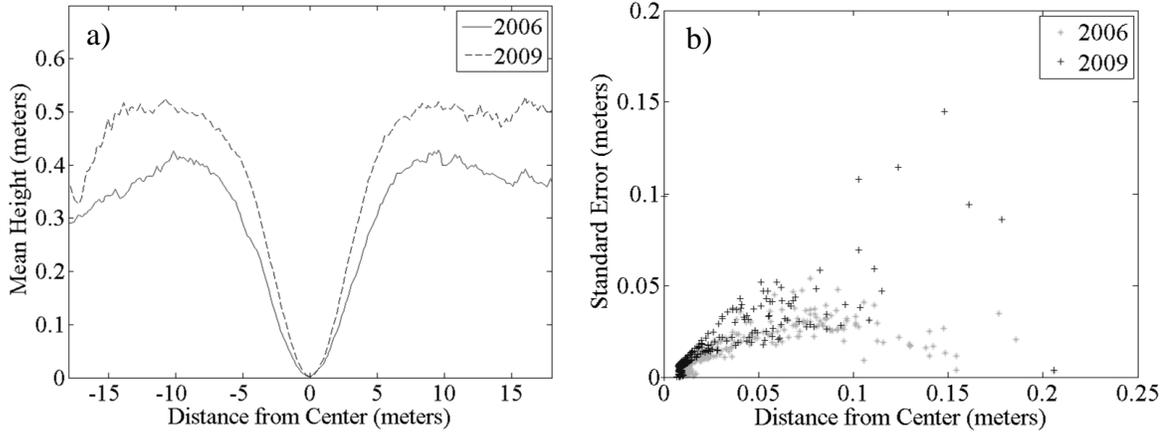


**Figure 2.6.** 2009 multibeam data with holes that filled in (inactive) or were new or deeper (active) between 2006 and 2009. N=23 for inactive holes (●) and 77 for active holes (white circles).

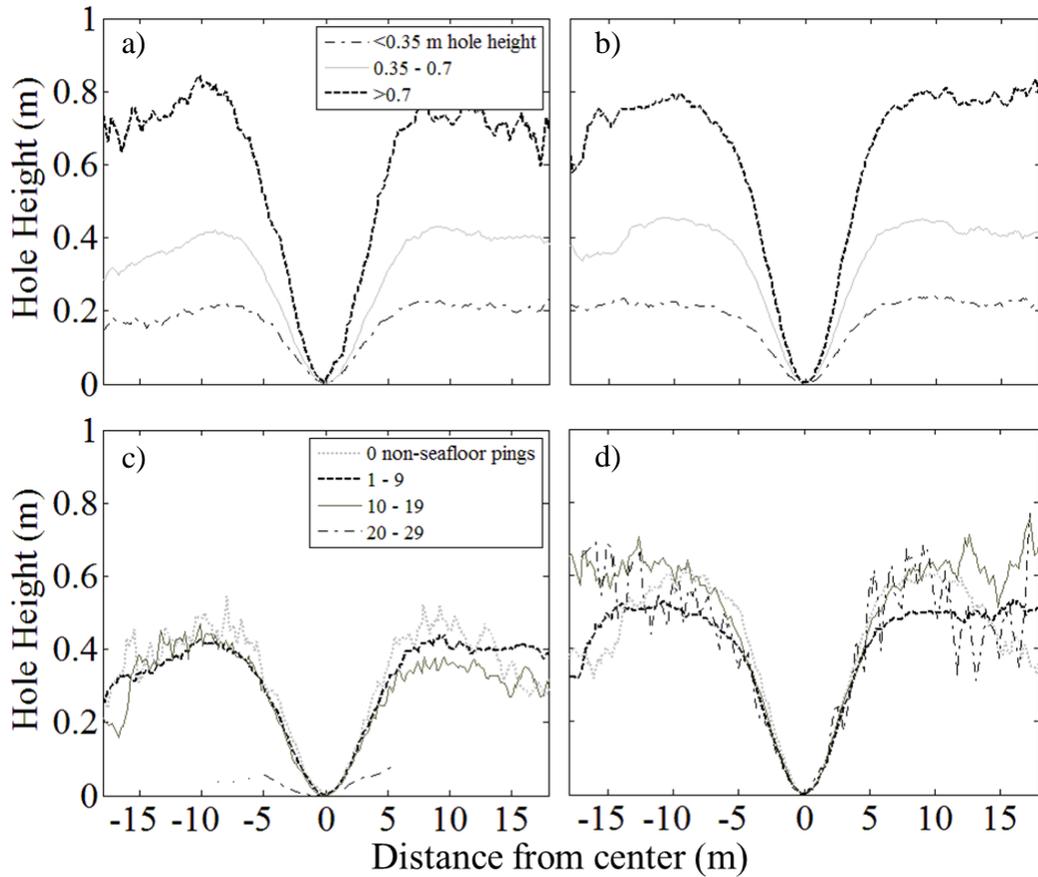
The mean of the hole polynomials calculated for each year showed an overall increase in the height and slope of the hole from 2006 to 2009 (Figure 2.8a). However, the shape of the polynomials was variable (Figure 2.8b). To reduce this variability, the polynomials were separated into three categories of height: < 0.35 m, 0.35 – 0.70 m and > 0.70 m (Figure 2.9a, 2.9b), and into four categories of non-seafloor associated pings: 0, 1 – 9, 10 – 19, 20 - 29 (Figure 2.9c, 2.9d), and the mean polynomial by group was calculated. Holes with greater height had a steeper slope. However, the hole shape did not appear to be correlated to the number of non-seafloor associated pings, and therefore potential fish presence.



**Figure 2.7.** Examples of changes in holes (black circle) between the three years. A hole that is detected in a) 2006 is less detectable in b) 2009 and a hole less detectable in c) 2006 is detected in d) 2009.

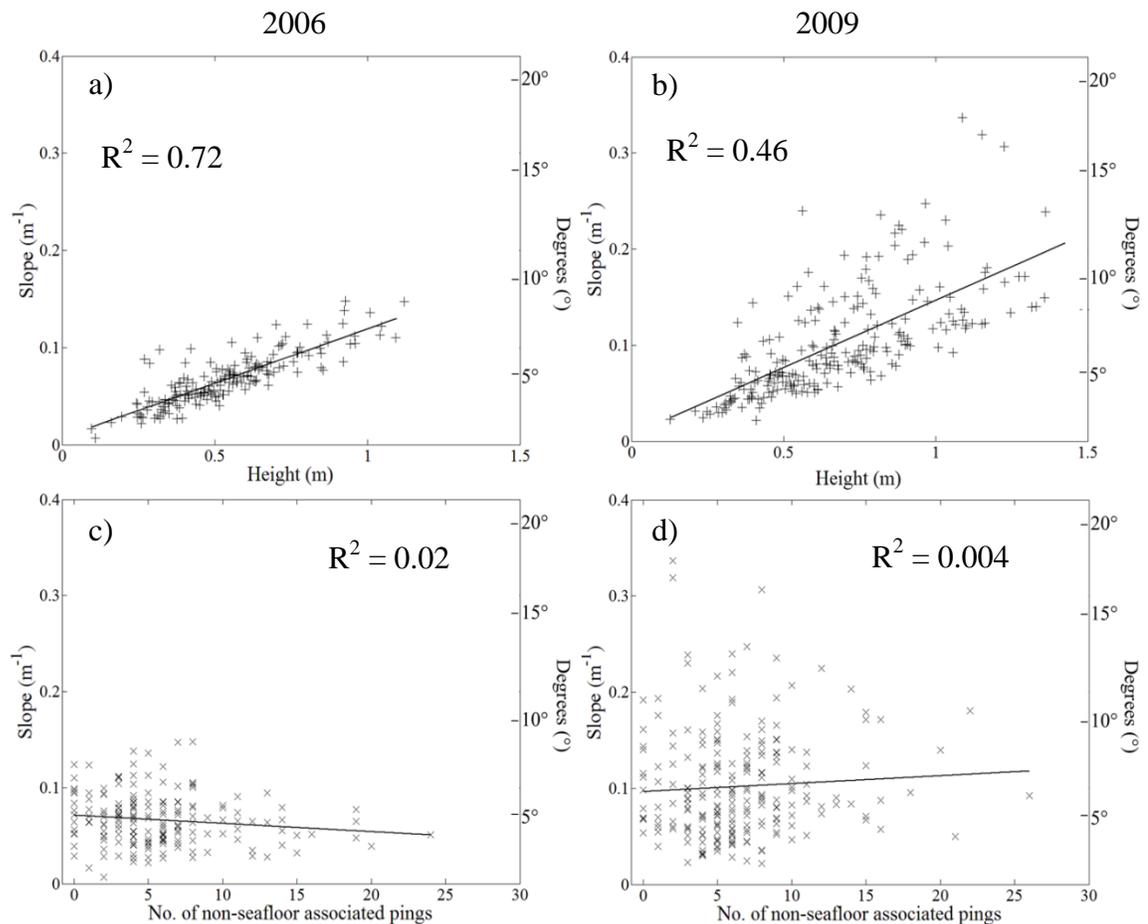


**Figure 2.8.** Results from the ten-term polynomial applied to each hole profile. a) Mean of the polynomials by year. Note the exaggeration in the vertical scale. b) Standard error of the mean of the polynomials by year.



**Figure 2.9.** Mean polynomial calculated from a subset of polynomials sorted by hole height for a) 2006 and b) 2009 and sorted by the number of non-seafloor associated pings for c) 2006 and d) 2009. Note the exaggeration in the vertical scale.

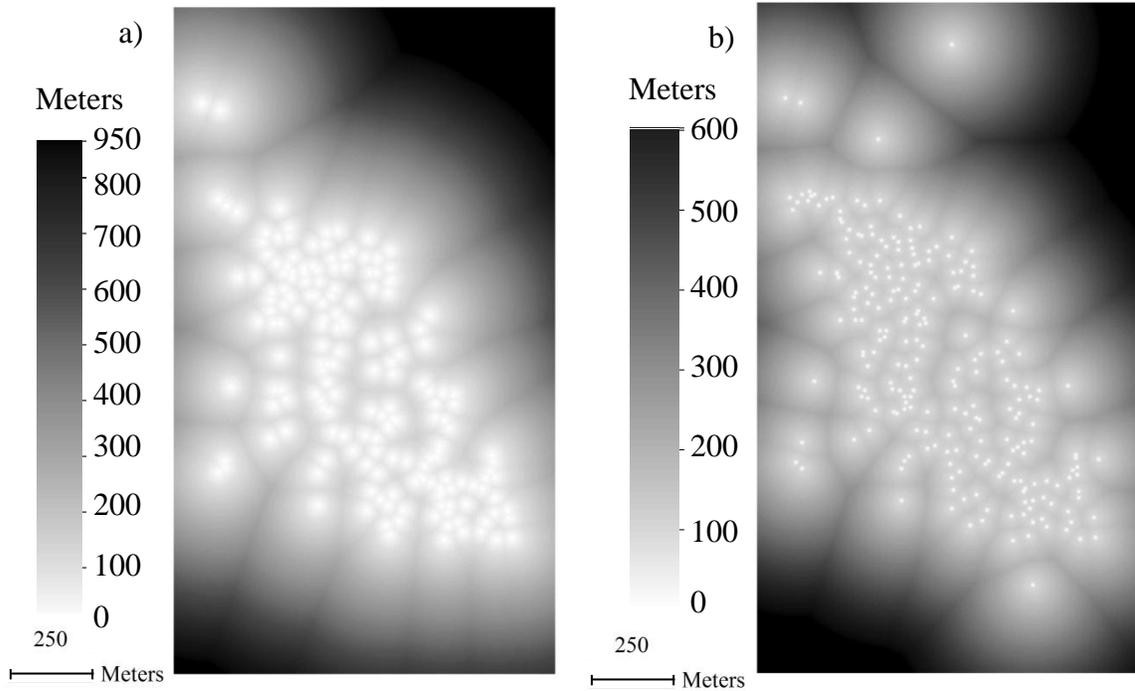
Linear regression of the slope and height for 2006 and 2009 show a positive correlation and relatively good fit (Figure 10a, 10b). The regression of slope and number of non-seafloor associated pings show a poor fit and low correlation (Figure 10c, 10d). Over 90% of the profiles for 2006 (164 out of 181) and 2009 (214 out of 231) contained at least one non-seafloor associated ping. Slopes corresponding to holes with 0 non-seafloor associated pings (potentially inactive holes) did not differ from slopes corresponding to holes with at least one non-seafloor associated ping (potentially active holes).



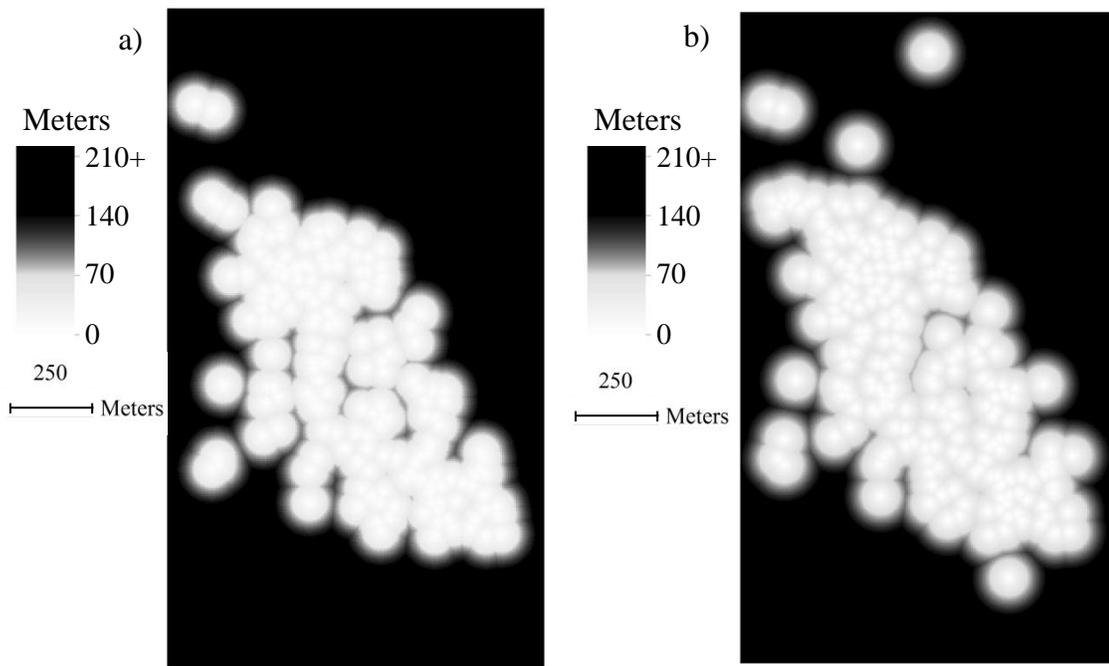
**Figure 2.10.** Hole slope and height in a) 2006 and b) 2009 and number of non-seafloor associated pings for c) 2006 and d) 2009. Linear regression (black line) and R<sup>2</sup> of the fit are also shown. Non-seafloor associated pings indicate relative fish abundance.

*Hole Distance and Red Grouper Source Level.* The sound pressure level (SPL) thresholds of red grouper hearing within the frequency range of red grouper sound production (100 – 300 Hz) are estimated to be 100 dB re 1  $\mu$ Pa based on hearing thresholds of gag grouper, *Myceteroperca microlepis* (S. Larsen and D. A. Mann unpubl data). Therefore, with a median NL of 105 dB re 1  $\mu$ Pa, the noise floor will limit communication distance, rather than hearing thresholds. With an estimated SL of 142 dB re 1  $\mu$ Pa (Nelson et al. 2011), sound produced by one red grouper is calculated to be detected by another red grouper up to 70 meters away. Due to the short transmission distance and low acoustic frequency, acoustic attenuation due to absorption is negligible.

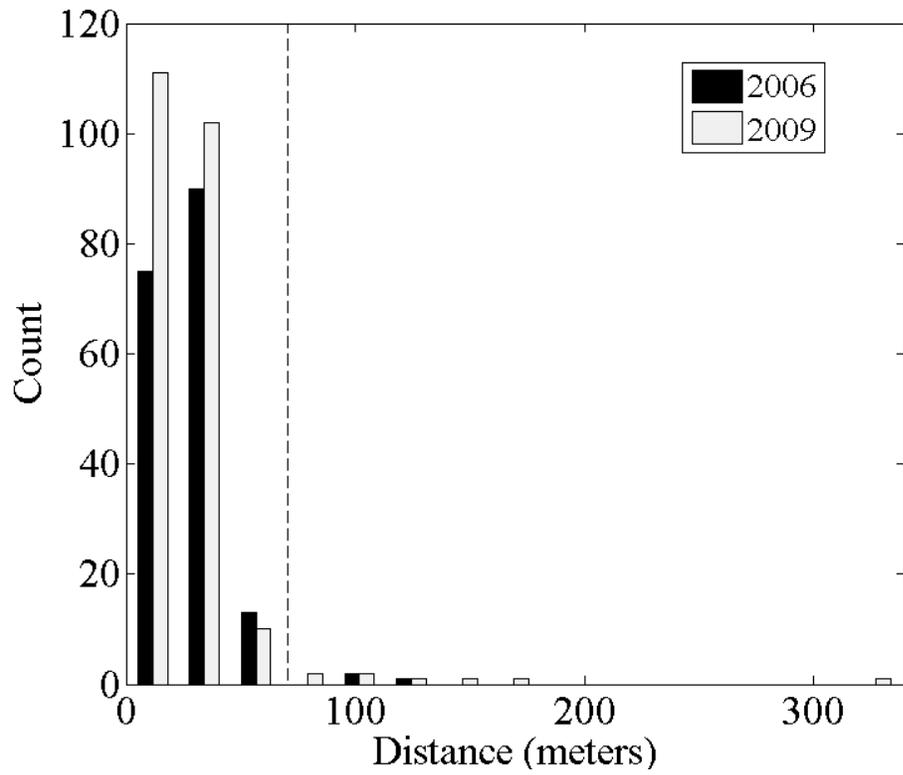
Maps of the distance estimated between the deepest point of the hole profiles in 2006 and 2009 show the holes cluster towards the center of the study area (Figure 2.11). The effective acoustic communication network between holes, based on the maximum range estimate of 70 m, is illustrated in Figure 2.12. Histograms of the between-hole distances show over 95% of holes are located within 70 m of their nearest neighbor (Figure 2.13). The median distance between nearest holes is 26 m (SD=15) and 24 m (SD=28) for 2006 and 2009, respectively.



**Figure 2.11.** Nearest neighbor distance in meters between holes detected in a) 2006 and b) 2009.  $N=181$  and  $N=231$  for 2006 and 2009, respectively. Note the difference in scale.



**Figure 2.12.** Red grouper communication network based on a range estimate of 70 m for a) 2006 and b) 2009. Distances greater than 70 m are suspected to be outside of the effective communication range among the red grouper holes and are shown in black.



**Figure 2.13.** Histogram of distances between nearest holes identified in 2006 (black) and 2009 (gray) binned into 20 m intervals. The dashed line indicates 70 m, the extent of the red grouper communication range. Over 95% of the holes in both years are within 70 m of their nearest neighbor.

## Discussion

High-resolution multibeam bathymetry data collected in 2006 and 2009 in a portion of the Steamboat Lumps Marine Reserve allowed detailed documentation and characterization of holes excavated by red grouper. Analysis of these data showed a significant increase in number, height and slope of holes over this three year period. Direct comparisons of holes detected in both 2006 and 2009 indicated significant changes in height and slope. The changes in these parameters suggest hole shape could be varied due to maintenance by red grouper and that holes are constructed and maintained over time (Coleman et al. 2010). Further, low sediment accumulation rates in the Gulf of Mexico would prevent quick infill and shape modification of holes in the absence of red grouper (Brooks et al. 2003a).

Active vents are generally steeper and deeper than inactive vents indicating that increased height in conjunction with slopes greater than the angle of sediment repose might signify active hole occupation (Saleem 2007). Although overall hole slope increased significantly from 2006 to 2009, the lack of correlation between hole slope and number of non-seafloor associated pings suggests the shape does not change significantly if unoccupied unless bad pings are poor indicators of fish. Ground-truth data are needed in concert with simultaneous multibeam data collection to determine if hole occupation can be established based solely on the presence of non-seafloor associated pings.

Despite having collected bathymetry data in depths ranging from 69 m to 81 m, the median depth for all holes was 71.2 m with a standard deviation of 0.6 m. The reason

for the clustering of holes within this depth range is unknown. Initially, we suspected the clustering to be related to constraints of bottom composition preventing hole excavation since sediment type distribution is highly varied throughout the WFS (Brooks et al. 2003a). Yet, backscatter data, which is useful for identifying bottom type (Dartnell & Gardner 2004), collected concurrently with the bathymetry data indicated uniform sediment distribution in our study area. If more than just social behavior is at hand, additional factors such as water temperature, bottom currents and Loop Current intrusions may be influencing the location of red grouper holes in this area.

Scanlon et al. (2005) calculated hole density in Steamboat Lumps to be 250 holes per km<sup>2</sup> from side-scan sonar data collected in 2000. This value is roughly double the hole density measured with multibeam sonar in this study (110 and 141 per km<sup>2</sup> in 2006 and 2009). The specific 0.4 km<sup>2</sup> area they surveyed did not directly overlap with the area surveyed in this paper. In addition, the hole density calculated by Scanlon et al. (2005) focused on a subset of data that was heavily populated with holes and then extrapolated the density estimate throughout the study area. I calculated hole density over the entire survey area, which consisted of dense and sparse areas of holes. Scanlon et al. (2005) classified a grouper hole visually from the interpolated raster created from side-scan sonar data. By examining the multibeam data on the data point level, I was able to exclude artifacts and errant pings that appeared to be a hole when solely examining the backscatter raster data. Although it aides visually, applying a vertical exaggeration to interpolated bathymetry maps can trick the eye into believing a hole exists when it is actually an artifact of the data. This density discrepancy could be compounded further by the differences in sonar technologies used. Interpreting the backscatter shadows in side-

scan sonar data can be difficult due to the angular uncertainty, their dependence on the direction of the boat, and the varying grazing angles, which can change throughout a survey and across-track. The backscatter shadows can be misleading because they can result from changes in seafloor geology or biology in addition to relative depth. With multibeam data, shadows can be created in software during post processing and will provide a consistent “grazing” angle across the regardless of depth. Side-scan sonar offer more refined backscatter data to determine bottom composition, and higher resolution when towed close to the bottom compared to hull-mounted multibeam sonar.

The increase in number of holes detected from 2006 to 2009 is consistent with increases in hole density and habitat usage, potentially the result of an increased grouper population. I attempted to identify if red grouper or other species were present within or near a hole using non-seafloor associated pings. The percentage of potentially inactive holes (0 non-seafloor associated pings) decreased from 9% in 2006 to 5% in 2009, which also supports an increase in active holes. As fish very close to (< 10 cm) or on the bottom become indistinguishable from the bottom structure by the multibeam sonar, the values determined from this method are likely conservative and more holes may be occupied than can be identified using non-seafloor associated pings. It is also possible that inactive holes still have other fish using the exposed habitat. Ground-truth data are necessary before concrete conclusions regarding increases in the number of active holes can be made.

The communication network maps created from assuming a 70 m limit to red grouper acoustic communication shows an increase in communication overlap within the cluster of holes found in the center of the study area from 2006 to 2009. The numbers of

holes in communication solitude also increased. Sound production may just be used for short-range communication suggesting that fish have to move to be heard over large areas. Females likely need to travel during mate choice, which is consistent with observations of females swimming towards holes occupied by males.

Red grouper, a large commercial fishery in the Gulf of Mexico, are established “ecological engineers” whose behavior provides structure and protection for other reef fish and invertebrates (Jones et al. 1994, Coleman & Williams 2002). Sustaining red grouper populations is therefore important at both the species and ecosystem levels (Jones et al. 1994, Wright & Jones 2006). I believe I have identified an increase in spawning habitat usage within a portion of the marine reserve over a three year period. It is anticipated that populations in the reserve would increase naturally in the absence of fishing (Claudet et al. 2010). Poaching is known to occur within the reserve (C. C. Koenig pers. comm.) and the rate of increase in red grouper population may not be as high as it could be (Russ & Alcala 2004). Regardless, the results of this research provide evidence towards the potential benefit of such reserves (Pauly et al. 2002, Jennings 2009, Lester et al. 2009, Babcock et al. 2010).

Conducting a similar analysis outside of the marine reserve is a necessary next step to understand changes in habitat usage by fished red grouper populations. Initial analysis of multibeam data collected across the WFS indicates numerous areas containing putative grouper holes (Coleman et al. 2010, Allee et al. 2011, D. F. Naar unpubl data). Reserve- and shelf-wide mapping of red grouper habitat would be a time and cost intensive endeavor due to the relatively narrow swath width that can be collected with

multibeam sonar in shallow water. However, small subsets of data over time would provide highly informative glimpses into large-scale changes in habitat use.

## Chapter 3: Shelf-Scale Mapping of Sound Production by Fishes in the Eastern Gulf of Mexico Using Autonomous Glider Technology

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### Abstract

Autonomous gliders are a relatively new technology for studying oceanography over large time and space scales. A hydrophone was integrated into the aft cowling of a glider and used in a one week, shelf-scale deployment on the West Florida Shelf to detect and map fish sounds in the ocean over a large spatial scale. In addition to red grouper and toadfish sounds, at least 3 unknown biological sounds suspected to be produced by fish were identified through manual analysis of the acoustic files. The biogeography of these fishes was identified by mapping the occurrence of sounds along the glider track. Sounds produced by red grouper and toadfish were detected throughout the day predominately in bottom depths greater than 40 m. Conversely, the three unknown biological sounds were detected exclusively at night over varying bottom depths. Glider technology provides a reliable and relatively inexpensive method to collect acoustic and

environmental data over large spatial scales while maintaining a high rate of successful retrieval.

## Introduction

Sound has long been recognized as an important means of animal communication (Aristotle 1910). In the ocean, the range of acoustic communication often exceeds the range of visibility (Urlick 1983, Hawkins 1993, Bass & Clark 2003). Sound production in fishes is used for communication and has been shown to be associated with courtship, spawning, parental, aggressive and territorial behavior (Lobel et al. 2010). Most fish sounds are species-specific and repetitive, which enables passive acoustic recordings of sound production to be used to identify their distribution and behavior.

Passive acoustic monitoring (PAM) systems can record large amounts of acoustic data. These systems are often moored in a specific area for days to years recording sounds from biological, physical (e.g., noise generated from wave action) and anthropogenic (e.g., vessel traffic) sources (Urlick 1983, Mellinger et al. 2007, Locascio & Mann 2008, Luczkovich et al. 2008, Dudzinski et al. 2009). PAM systems collect data in remote locations under potentially unsafe seas throughout a 24-hour period providing large datasets that are unattainable using observer-based methods (Rountree et al. 2006). Since sound is associated with reproduction in many species, an important application of PAM is to determine when and where reproductive activities occur for fish and marine mammals (Mann & Lobel 1995, Lobel 2002, Gannon 2008, Van Parijs et al. 2009, Lobel et al. 2010).

Sound alone cannot paint the full picture of biogeography. Knowledge of the ocean environment is necessary to fully understand animal behavior and distribution.

Thus, platforms containing environmental sensors can be used to record data to relate patterns in sound production to oceanographic conditions (Baumgartner & Fratantoni 2008, Mann & Grothues 2008). The suite of environmental and optical sensors in autonomous ocean gliders provides a three-dimensional view of physical and biological processes over time and space as the glider moves through the water column (Webb et al. 2001, Schofield et al. 2007, Castelao et al. 2008). Simultaneous collection of sound and environmental data can fill the gap left by PAM systems between acoustic signal and the environment in which that sound was produced. To this end, several groups have recently demonstrated passive acoustic glider data collected from integrated hydrophones (Moore et al. 2007, Baumgartner & Fratantoni 2008, Haun et al. 2008, Ferguson et al. 2010, Matsumoto et al. 2011). Their research has focused largely on marine mammal acoustics or ambient noise.

The purpose of this study was to detect and map fish sounds in the ocean over a large spatial scale using glider technology. For this study a hydrophone was integrated into the aft cowling of a Slocum electric underwater glider (Teledyne Webb Research), and used to record sound and other oceanographic parameters, such as temperature, salinity, and chlorophyll concentration, during a deployment off west-central Florida.

## Methods

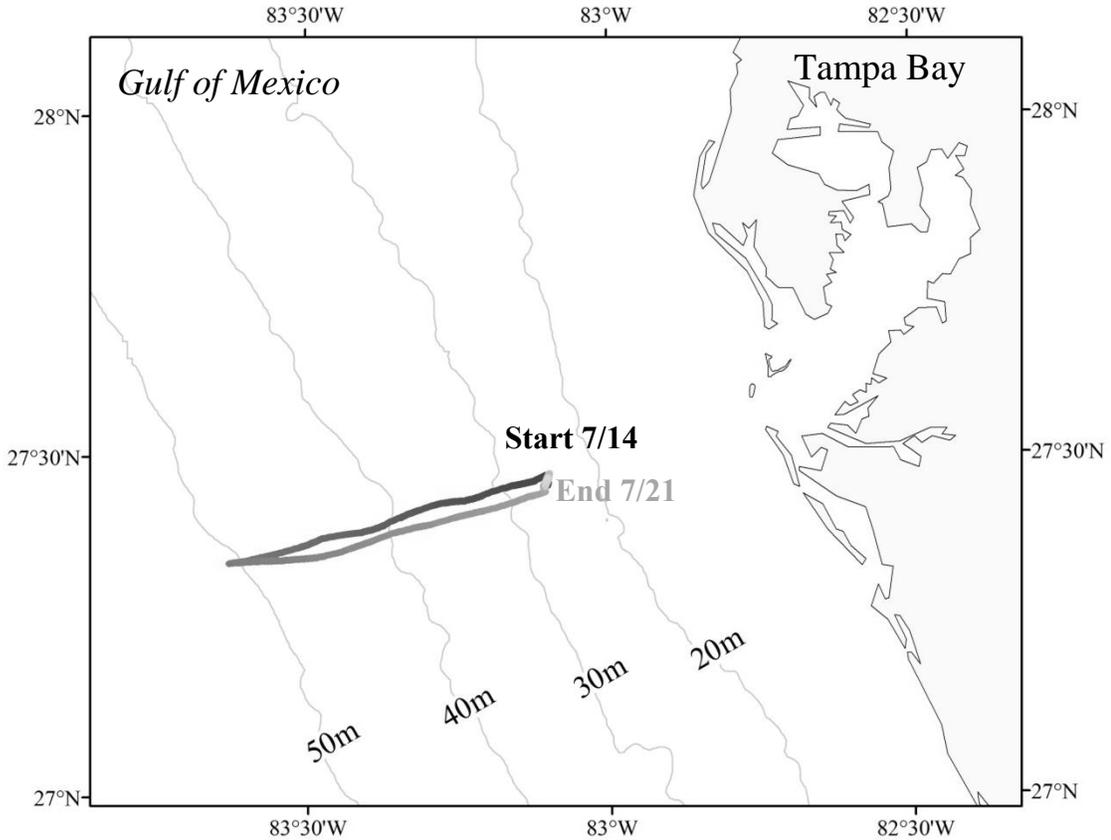
Slocum gliders are buoyancy driven electric autonomous underwater vehicles 1.8 m in length and shaped like a winged torpedo (Webb et al. 2001, Schofield et al. 2007). They can traverse over 600 km using a single set of alkaline batteries and contain sensors that measure temperature, salinity, depth averaged currents (i.e., currents averaged vertically from near the surface to near the bottom), surface currents, fluorescence, and apparent and inherent optical properties (Schofield et al. 2007). In this study, these measurements were taken approximately 15 times a minute as the glider ascended and descended through the water column roughly 330 times a day, depending on water depth. In addition to this suite of sensors, we integrated a hydrophone that extended 10 cm from the aft endcap within the water-flooded aft cowling of the glider. No part of the hydrophone extended outside of the main glider body.

The glider's digital acoustic recording system, Digital SpectroGram recorder (DSG; Loggerhead Instruments), recorded sound for 25 seconds every 5 minutes at a sample rate of 70,000 Hz. This duty cycle optimized the collection of acoustic data for the allotted 16 GB SD card storage space. The DSG is a low-power acoustic recorder controlled by script files on the SD card in concert with the on-board real-time clock. The clock, which is synced to the clock on-board the glider's computer, is highly accurate with temperature compensated drift. The DSG file system is an advanced data file structure that stores embedded time stamps with the raw data allowing each file to remain time-aligned with UTC and with the glider data.

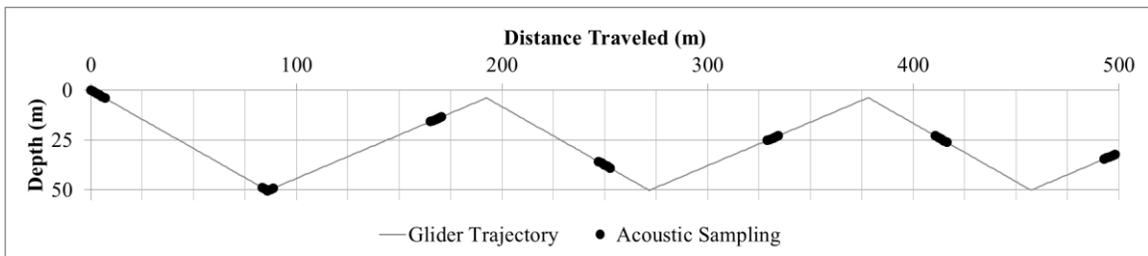
Forward propulsion in the glider is created by varying the vehicle buoyancy. The wings enable forward movement as buoyancy changes causing the glider to move

downward when it is denser than the water, and upward when less dense, creating sawtooth-like profiles through the water column. The absence of a drive motor and propellers minimizes mechanical noise produced by the glider. Efforts were made prior to deployment to reduce electronic noise by including dual capacitive filtering (1500  $\mu$ F and 1  $\mu$ F) on the power supply coming from the glider's battery.

The glider was deployed on the West Florida Shelf on July 14, 2009. The mission started west of the mouth of Tampa Bay in 25 m water depth, continued westward to approximately 50 m water depth, and then returned to the start location where it was retrieved on July 21, 2009 (Figure 3.1). During this mission 1,989 acoustic files were recorded over the 135 km track. Each acoustic recording is represented as a file encompassing a 25 second period of time. Sounds identified will be reported as a percentage of occurrences in all of the files since the recordings are discontinuous, and the location of the source and detection range of the glider is unknown. On average, the glider moved approximately 75 to 85 m between acoustic recordings (Figure 3.2). The figure presented demonstrates the approximate spatial distribution of the acoustic samples during the deepest portion of the deployment. This plot of the glider's horizontal and vertical progression was calculated using the horizontal ( $0.273 \pm 0.026 \text{ m s}^{-1}$  [mean  $\pm$  SD]), vertical ascent ( $0.112 \pm 0.030$ ), and descent ( $0.160 \pm 0.035$ ) speeds averaged over the entire deployment. Exact profiles and sample spacing would be similar, though the spacing tends to be larger in deeper water where the glider generally moves faster and slower in shallower water.



**Figure 3.1.** Position data from a glider mission in the eastern Gulf of Mexico from July 14 – 21, 2011, during which time acoustic data were collected. Grey contours are bathymetric depth.



**Figure 3.2.** Typical glider trajectory during the deployment (see Figure 3.1), calculated from average horizontal, ascent, and descent speeds, with positions of acoustic sampling over a 30 minute period. The acoustic data were recorded for 25 seconds every five minutes.

Metadata collected throughout the deployment included glider depth in the water column, bottom depth, UTC time stamp, roll, pitch, and heading. Latitude and longitude

were collected when the glider was at the surface. The position of the glider when not at the surface was estimated from the surface latitude and longitude coordinates using linear interpolation and a 10-point moving average.

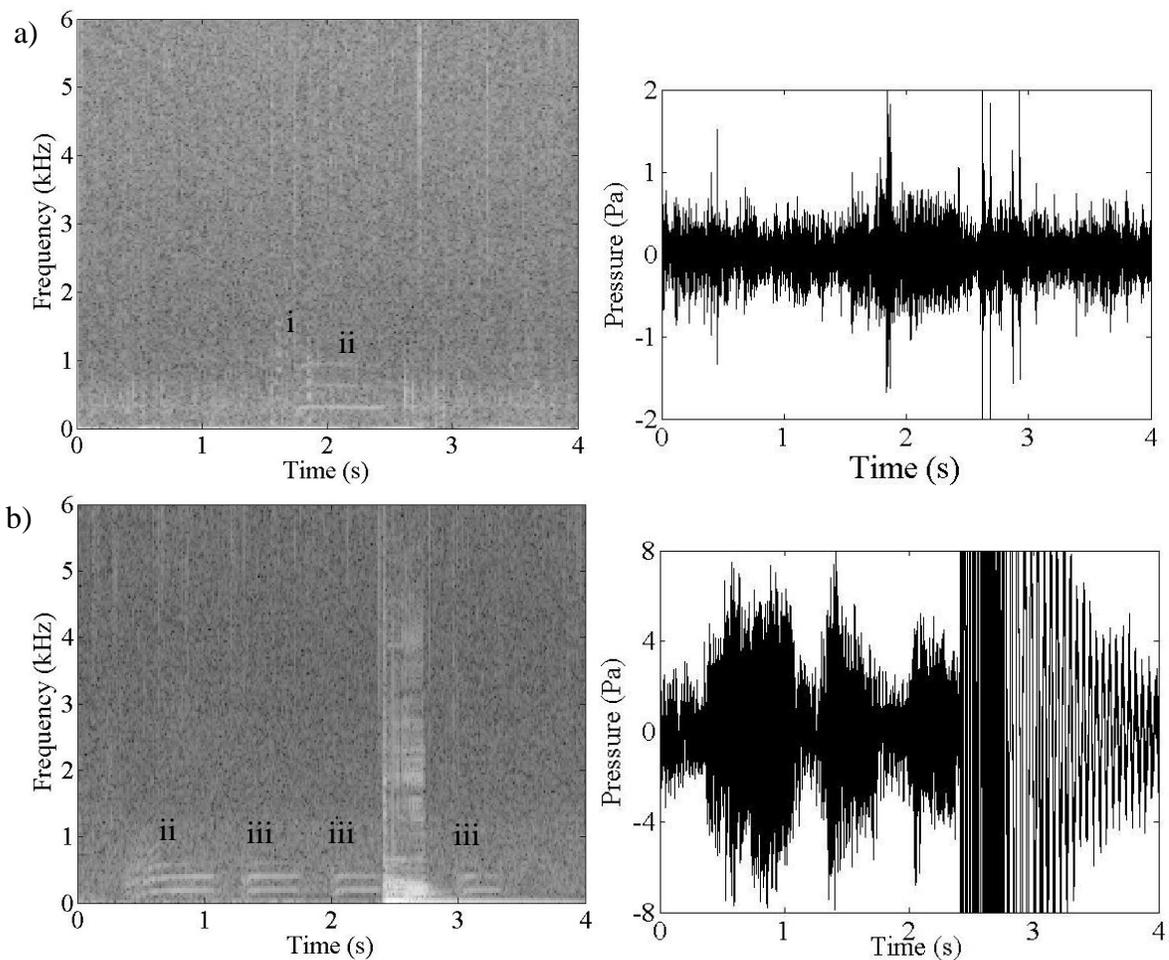
Fish sounds were identified manually because automated detection methods were hampered by the presence of noise from the gliders' altimeter, pump, rudder, and at-surface iridium satellite link. Sounds from known and unknown sources were mapped using the interpolated positions and acoustic file time stamp to determine spatial and temporal ranges. Temperature, salinity and chlorophyll concentration data were compared to the sounds identified in the acoustic recordings, the time of the recording, and the bottom depth when the sound was recorded. Spectrograms were generated from 2,048 point Hann-windowed fast Fourier transforms (FFT) with 50% overlap. Analyses were completed using MATLAB (Mathworks) and ESRI (Environmental Systems Research Institute) ArcGIS 10 software.

## Results

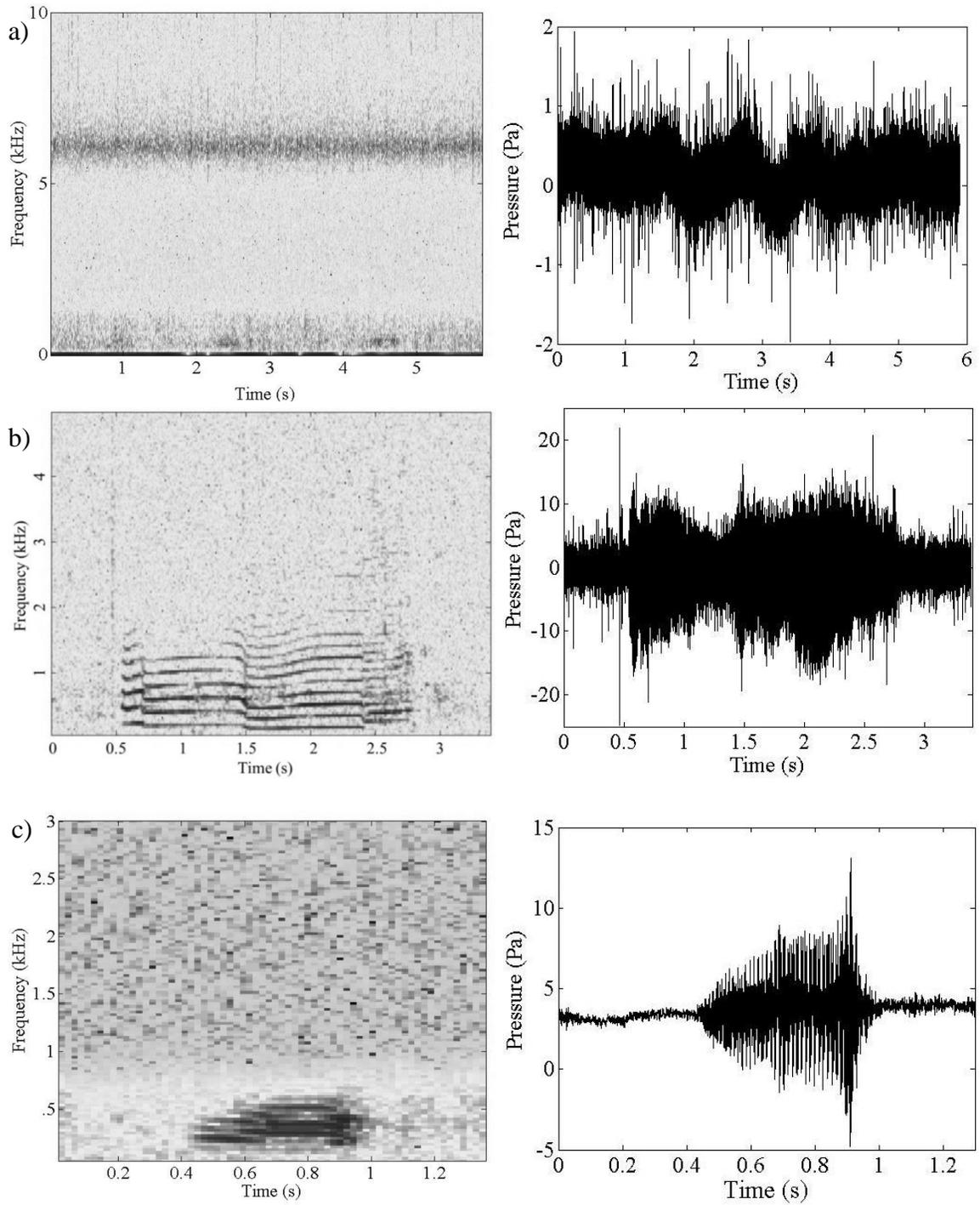
Snapping shrimp from the family Alpheidae were frequently recorded on the glider, and may act as an indicator of the presence of hard bottom. Red grouper (*Epinephelus morio*) and toadfishes *Opsanus* spp. were among the most frequent fish sounds identified in the files. Based on location and slight differences between the calls, it is suspected the toadfish species was leopard toadfish, *O. pardus*, as opposed to the inshore gulf toadfish, *O. beta* (Figure 3.3). The fundamental frequency and call duration for *Opsanus* recordings in this study were (mean  $\pm$  SD)  $190 \pm 0$  Hz and  $2.34 \pm 0.36$  s, respectively (N=10). This is in comparison to a fundamental frequency of 350 Hz and call duration of  $1.23 \pm 0.22$  s for *O. beta* (Tavolga 1958, Thorson & Fine 2002). Furthermore, initial grunts were not present in these toadfish recordings, unlike *O. beta*.

Three additional suspected fish sounds were also common. The first unknown sound included a 200-500 Hz wide band around 6 kHz (this will be termed the ‘6 kHz Sound’) (Figure 3.4a). This sound appeared continuously between sunset and sunrise (“night”) and ranged from 5.9 to 6.4 kHz, with the dominant frequency at 6 kHz. The second unknown sound was a frequency modulating harmonic with an average peak frequency of 300 Hz (‘300 Hz FM Harmonic’) (Figure 3.4b). The duration of this sound was approximately 2.25 s with an average fundamental frequency of 150 Hz. These calls typically contained four abrupt changes in frequency. Peak frequencies for frequency modulated sections were 690 Hz, 612 Hz, 531 Hz, and 399 Hz, with a fundamental frequency of 230 Hz, 204 Hz, 177 Hz and 133 Hz, respectively. Harmonics reached up to

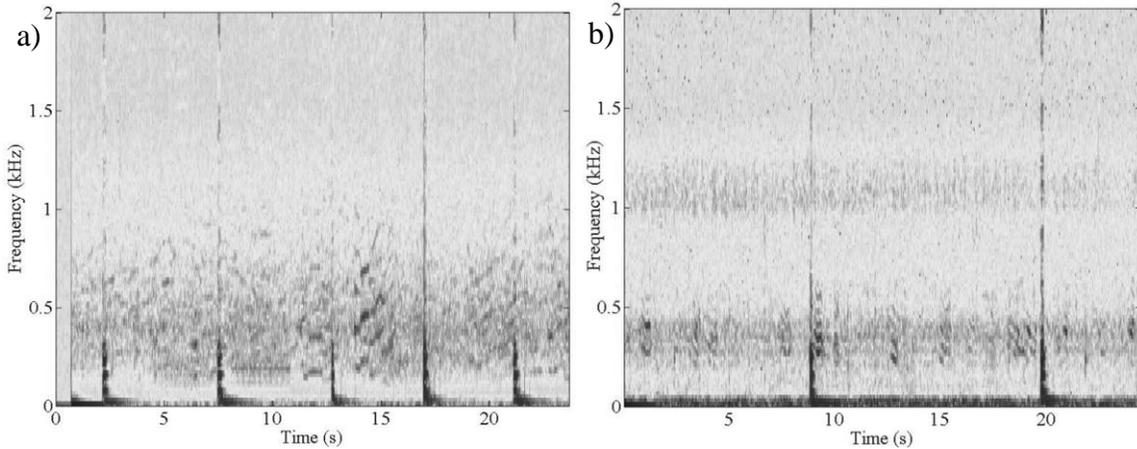
2,478 Hz. The third unknown sound was a tonal harmonic with a peak frequency of 365 Hz ('365 Hz Harmonic') (Figure 3.4c). This sound was 0.51 s long (S.D=0.1, N=100) with a 73 Hz fundamental frequency and the highest harmonic detected at 732 Hz. Extensive overlap of calling was common in both 300 Hz FM Harmonic and 365 Hz Harmonic sounds and often made identification of individual calls difficult (Figure 3.5). Whistles and echolocation produced by dolphin species, most commonly bottlenose dolphins (*Tursiops truncatus*), were also identified in the files.



**Figure 3.3.** Spectrogram (left) and waveform (right) of sounds from a) *Opsanus beta* recorded off Tampa Bay (D. Mann, unpub data) and what is suspected to be b) *O. pardus* recorded by the glider. Grunt (i), initial tone (ii), and succeeding tones (iii) are identified. In b), at 2.5 s, noise from a rudder adjustment masks frequencies below 1 kHz.



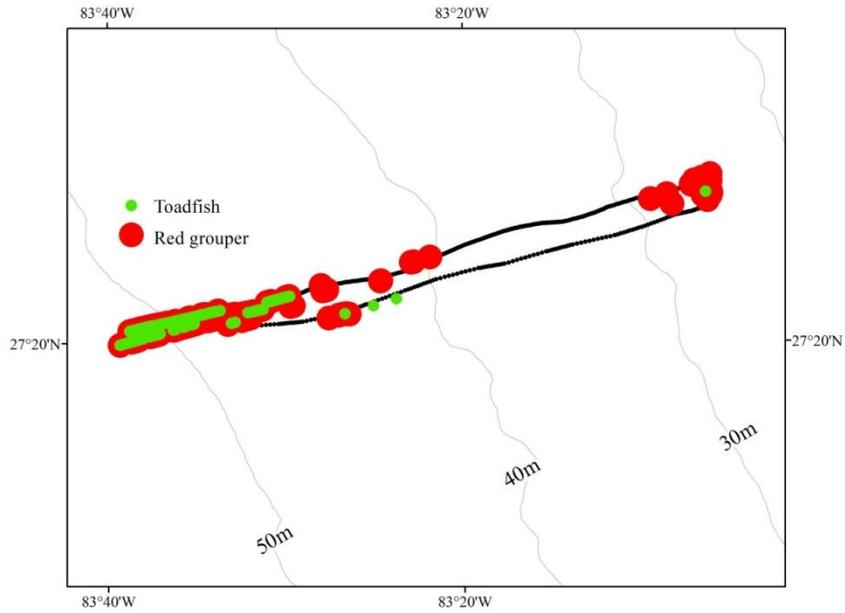
**Figure 3.4.** Spectrogram (left) and waveform (right) of a) 6 kHz Sound, b) 300 Hz FM Harmonic and c) 365 Hz Harmonic. Note the differences in frequency and time scale.



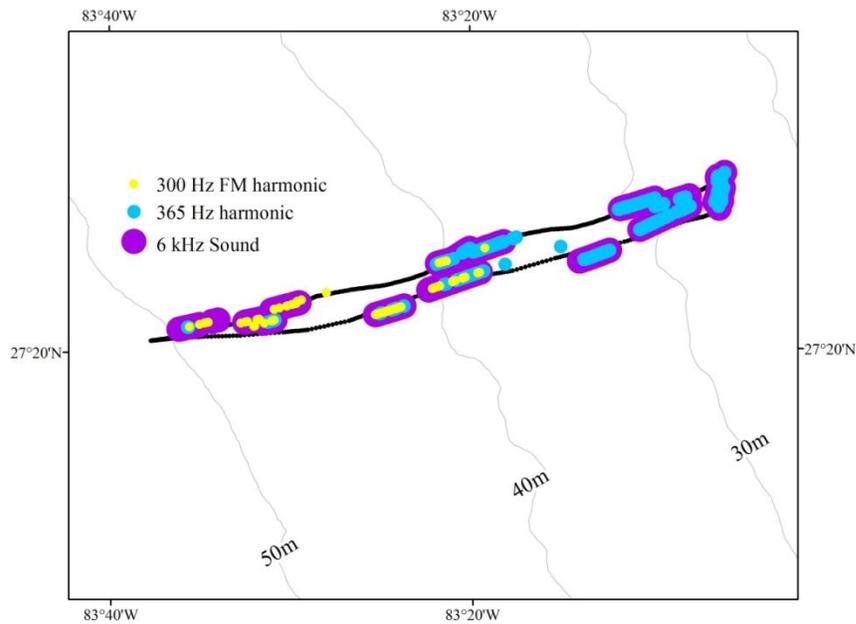
**Figure 3.5.** Spectrographic example of overlapping calls from the a) 300 Hz FM Harmonic between 100 and 1,000 Hz and b) 365 Hz Harmonic between 150 and 500 Hz. Broadband noise from the glider's rudder is present every 5 to 10 seconds in the files.

Overall, the 6 kHz Sound was detected in 32% (628/1989) of the total files, the 365 Hz Harmonic was detected in 18% (349/1989) of the files, red grouper were detected in 9% (181/1989) of the files, toadfish were detected in 8% (158/1989) of the files, and the 300 Hz FM Harmonic was detected in 4% (87/1989) of the files. Spatial patterns in sound production for known sources (Figure 3.6) and unknown sources (Figure 3.7) were determined from the interpolated glider position. Red grouper (136/181; 75%), toadfish (158/158; 100%), and the 300 Hz FM Harmonic (82/87; 94%) were predominantly found where bottom depths were greater than 40 m. Although they were detected throughout the glider track, the 6 kHz Sound (330/628; 53%) and the 365 Hz Harmonic (234/349; 66%) were more common where the bottom depth was shallower than 35 m. The diurnal pattern of sound production was discerned by mapping the occurrence of sounds by time of day (Figure 3.8). The 6 kHz Sound (628/628), 300 Hz FM Harmonic (87/87) and the 365 Hz Harmonic (347/349) sounds were detected over 99% of the time at night.

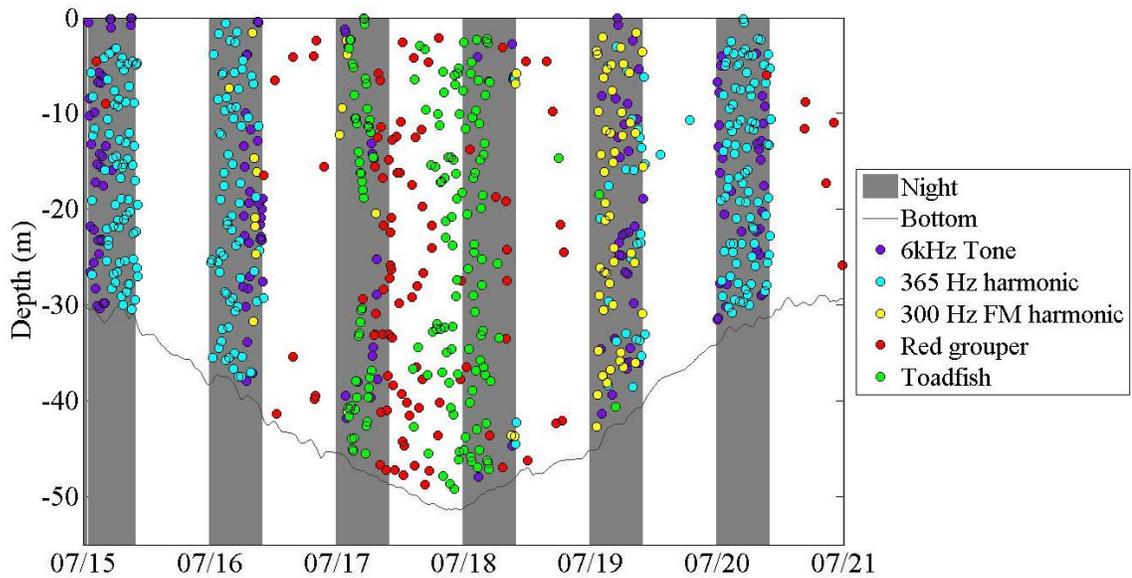
Conversely, only 35% (65/181) of the files containing red grouper sounds and 67% (106/158) of the files containing toadfish sounds were detected at night.



**Figure 3.6.** Position along the interpolated glider track (•) of two known sounds, toadfish (●) and red grouper (●). N=158 and 181, respectively.



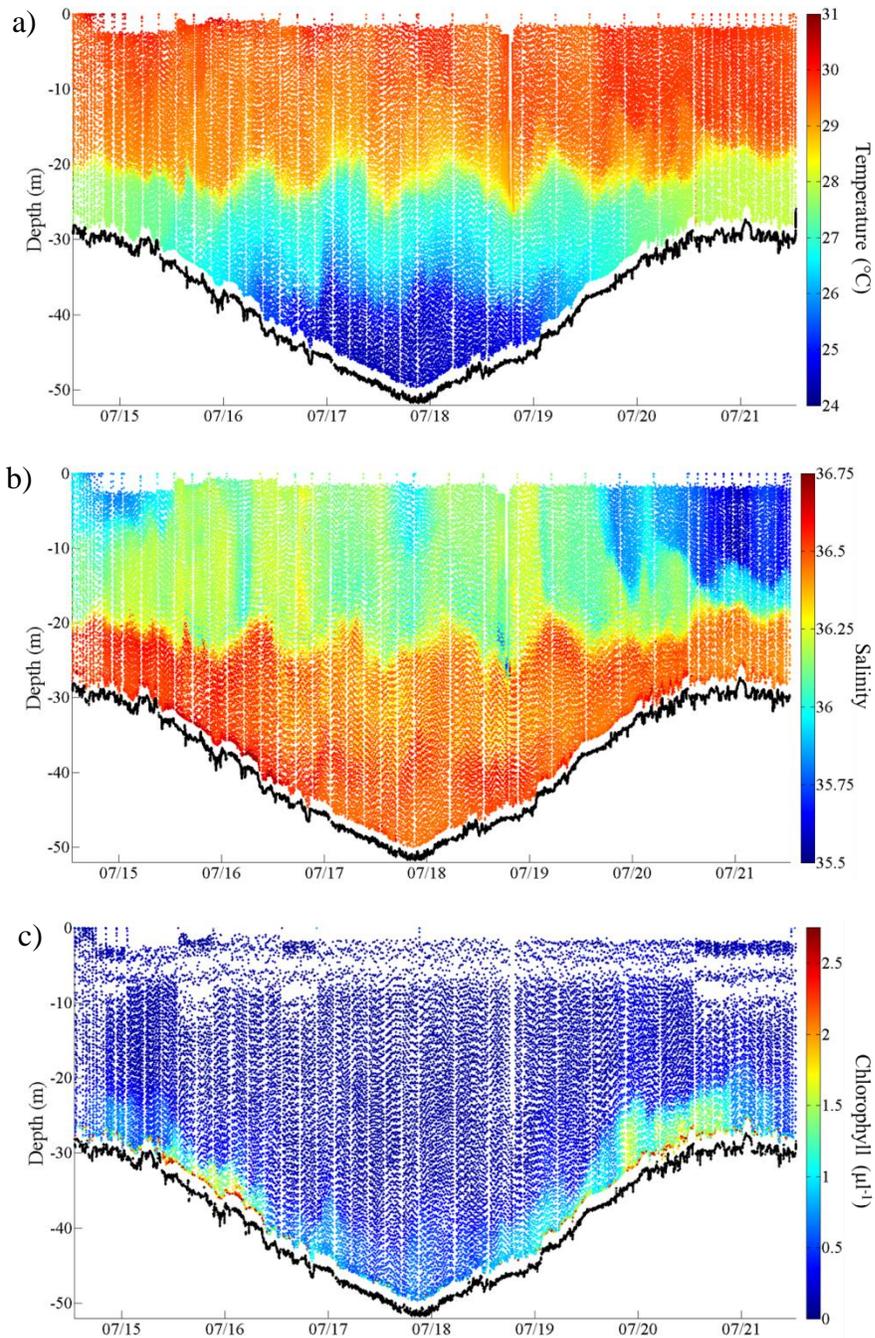
**Figure 3.7.** Position along the interpolated glider track (•) of three unknown sounds, 300 Hz FM harmonic (●), 365 Hz harmonic (●) and 6 kHz Sound (●). N=87, 349, and 628, respectively.



**Figure 3.8.** Occurrence of fish sounds identified in the glider acoustic files by time and depth of the glider in the water column at the time of the recording. The black line indicates the bottom depth measured by the glider’s altimeter and illustrates the glider’s offshore and then onshore track during the mission. Grey bars indicate night (sunset through sunrise).

A strong thermocline and pycnocline were present near 20 m depth (Figure 9a; Figure 9b). Pockets of increased chlorophyll concentration were detected near the seafloor between 30 and 35 m depth (Figure 9c). Temperature, salinity and chlorophyll concentration measurements that correspond to known and unknown sounds were compared to the associated bottom depth and hour of sound production (Table 1). Only environmental data measured when the glider was within 5 m of the bottom depth were incorporated into the median and standard deviation calculations for the demersal red grouper and toadfish. The deeper bottom depth, decreased temperature, and increased salinity and chlorophyll concentrations reflect the conditions surrounding the expected habitat for red grouper and toadfish recorded along the glider’s track. The location of the sources for the 6 kHz Sound, 300 Hz FM Harmonic and 365 Hz Harmonic are unknown. Therefore, the temperature, salinity and chlorophyll concentration measurements

associated with these sounds were incorporated without concern for the glider's position in the water column and typically resulted in higher standard deviations.



**Figure 3.9.** Environmental data measured along the spatial track by sensors on the glider included a) temperature ( $^{\circ}\text{C}$ ), b) salinity, and c) chlorophyll ( $\mu\text{g l}^{-1}$ ). The black line indicates the bottom depth measured by the glider's altimeter.

**Table 3.1.** Median and standard deviation of bottom depth (Depth), local hour of sound production (Hour), temperature (Temp), salinity (Salinity) and chlorophyll concentration (Chl) associated with known and unknown fish sounds. Only temperature, salinity and chlorophyll concentration measurements recorded when the glider was within 5 m of the bottom depth were calculated for red grouper (N=30) and toadfish (N=20).

	Depth (m)	Hour	Temp (°C)	Salinity	Chl ( $\mu\text{g l}^{-1}$ )	N
Red Grouper	48.6 $\pm$ 8.5	10 $\pm$ 6	24.6 $\pm$ 1.7	36.5 $\pm$ 0.1	0.6 $\pm$ 0.2	181
Toadfish	49.6 $\pm$ 2.4	17 $\pm$ 9	24.5 $\pm$ 0.3	36.5 $\pm$ 0.0	0.6 $\pm$ 0.1	158
6 kHz Sound	32.9 $\pm$ 6.6	4 $\pm$ 9	28.9 $\pm$ 1.5	36.2 $\pm$ 0.3	0.2 $\pm$ 1.4	628
300 Hz FM Harm	42.8 $\pm$ 3.0	5 $\pm$ 10	27.7 $\pm$ 1.7	36.3 $\pm$ 0.2	0.2 $\pm$ 0.3	87
365 Hz Harmonic	32.1 $\pm$ 4.8	5 $\pm$ 9	29.1 $\pm$ 1.2	36.2 $\pm$ 0.3	0.3 $\pm$ 1.8	349

## Discussion

A hydrophone-integrated glider was deployed successfully for one week in the eastern Gulf of Mexico in 2009 covering 135 km. During this time, nearly 2,000 acoustic files were recorded. Manual analysis of these files identified the frequent occurrence of sounds produced by red grouper and toadfish along with three unknown sources suspected to be fish (6 kHz Sound, 300 Hz FM Harmonic, 365 Hz Harmonic).

Red grouper and toadfish produce sound throughout a 24 hour period mainly in depths greater than 40 m. These characteristics support the finding that red grouper, who prefer deeper water, call throughout the day and night (Nelson et al. 2011). These findings also illustrate the ability of the glider's hydrophone to record demersal species (red grouper and toadfish) regardless of the glider's location in the water column within this 50 m depth range. The 6 kHz Sound, the 300 Hz FM Harmonic and the 365 Hz Harmonic were only detected at night, and the 6 kHz Sound and the 300 Hz FM Harmonic were predominately detected in depths shallower than 40 m. Though unknown, the impact of masking between 100 to 2,400 Hz from call overlap by the 300 Hz FM Harmonic and the 365 Hz Harmonic is potentially significant.

The *Opsanus* species recorded during the glider mission produced a characteristic toadfish 'boatwhistle'. But the depth range (25-50 m) of the glider track and the low fundamental frequency (190 Hz) of the recorded sound distinguishes it from the nearshore (< 5 m) *O. beta* and *O. tau*, which have been described extensively (Tavolga 1958, Gray & Winn 1961, Winn 1964, Thorson & Fine 2002, Locascio & Mann 2008). I

suspect that *O. pardus*, the offshore species of toadfish present in the Gulf of Mexico, is the source of the stereotypic toadfish calls present in the glider's acoustic recordings, although its acoustic signal has not been described. Locascio & Mann (2008) reported nocturnal calling of *O. beta* with peaks at sunrise and sunset. Though not described in their paper, *O. beta* sound production was also observed during the day (J. Locascio pers. comm), which is consistent with the lack of a strong diel periodicity observed in the suspected *O. pardus* calling.

The results from this initial work can be used to help determine the source of the three unknown suspected fish sounds. A preliminary analysis of families of sound-producing fishes in the Gulf of Mexico using published literature (Fish & Mowbray 1970, Hoese & Moore 1998) and unpublished sound recordings identified nearly 90 genera likely to make sound (C. Wall unpubl data). Discussions with colleagues and *a priori* knowledge of behavior and habitat make Atlantic midshipman *Porichthys plectrodon* a likely candidate for the 300 Hz FM Harmonic, which is similar to the 'growl' call of the plainfin midshipman, *Porichthys notatus* (Brantley & Bass 1994, A. Bass pers. comm). The documented sound production of *Prionotus carolinus* establishes searobin species (e.g., Blackwing searobin *Prionotus rubio*) as likely candidates for the 365 Hz Harmonic (Connaughton 2004). I suspect the 6 kHz Sound is related to gas release in clupeids (e.g., Gulf menhaden *Brevoortia patronus*) (Nøttestad 1998, Wahlberg & Westerberg 2003, Wilson et al. 2004, Doksæter et al. 2009, Knudsen et al. 2009). Based on the nocturnal characteristics and depth preferences identified in the study, and the reduced list of candidates, efforts can be honed with the aid of fixed acoustic arrays and a video

observation system (Rountree 2008) to increase the chances of successful identification in future work.

The three environmental parameters shown, temperature, salinity and chlorophyll concentration, provide an initial glimpse into the environment in which the recorded sounds were produced. Stratification of the water column is attributed to the high heat flux and a lack of strong storms (>15 knot winds) in the area, which effectively mix density layers (He & Weisberg 2002, Virmani & Weisberg 2003). The fundamental frequency of some soniferous fish (including *Opsanus* sp.) changes with temperature making concurrent environmental data essential in understanding sound production (Fine 1978, Brantley & Bass 1994, Connaughton et al. 2000). Further, the link between sound production and spawning necessitates mapping temporal and spatial ranges of sound as a non-invasive proxy for identifying potential spawning habitat (Fine 1978, Brantley & Bass 1994, Mann & Lobel 1995, Lobel 2002, Locascio & Mann 2008, Van Parijs et al. 2009, Rountree & Juanes 2010, Nelson et al. 2011).

Glider technology provides a reliable and relatively inexpensive method to collect acoustic data in the field while maintaining a high rate of successful retrieval (Schofield et al. 2007). Moored PAM systems can be effective but have greater risk of being lost, stolen or damaged, especially in highly active areas (Luczkovich et al. 2008, Dudzinski et al. 2009). In addition, the spatial coverage and suite of environmental and optical conditions measured concurrently by the glider provide detail of the ocean environment and acoustic scene that cannot be discerned from stationary PAM methods that record only sound (Rudnick et al. 2004).

Remotely operated vehicles (ROV) have also been used as a platform for passive acoustic studies (Rountree & Juanes 2010). This application, while taking advantage of video observation, was greatly limited by the self-generated noise of the ROV, which frequently masked suspected sound production (Rountree & Juanes 2010). Although the glider does not have the ROV's capability to maintain a fixed position, the absence of a mechanical propulsion system allows the glider to produce significantly lower noise. To date, I do not know the extent, if any, in changes in fish behavior as a result of the glider presence. Based on the low level of self-generated noise, I suspect the impact is likely less than an ROV but more than a stationary acoustic array (Stoner et al. 2008).

Red grouper are a large commercial and recreational fishery in the Gulf of Mexico. In 2008, approximately 150,000 pounds of commercial landings and over 130,000 recreationally-caught red grouper were reported (SEDAR 2009). In addition, red grouper are established as "ecological engineers" whose behavior provides structure and protection for other reef fish and invertebrates (Jones et al. 1994, Coleman & Williams 2002). Sustaining red grouper populations is therefore important at both the species and ecosystem levels (Jones et al. 1994, Wright & Jones 2006). However, the current methods used to assess and manage fisheries are limited by survey bias, inaccurate fisher reports, and extensive delays between fisheries data collection and population assessment (NRC 2006). These limitations preclude real-time stock assessment and create a potentially harmful lag in accounting for the impact of overfishing and episodic environmental events, such as red tides (SEDAR 2009). Additional methods that collect near real-time stock assessment data and use a no-take approach are needed to effectively manage species with greater immediacy, which will aid in maintaining long-term

population stability and fishing activities. I suggest the implementation of regular deployments of hydrophone-integrated gliders as a possible method for enhancing fisheries management.

## Chapter 4: Temporal and Spatial Mapping of Red Grouper (*Epinephelus morio*) Sound Production on the West Florida Shelf

### Abstract

The goals of this project were to determine daily, seasonal and spatial patterns of red grouper (*Epinephelus morio*) sound production on the West Florida Shelf (WFS). Passive acoustic recordings were made across the WFS during periods from 2008 to 2011 using passive acoustic recorders in fixed locations and incorporated into autonomous underwater vehicles (AUVs). The longest time series of sound production (~1 year) was recorded in the Steamboat Lumps Marine Reserve. Red grouper sound production was observed 24 hours-a-day and throughout all months in which data were recorded. Increased calling was correlated to sunrise and sunset, and peaked in late summer (July and August) and early winter (November and December). Sounds were primarily recorded in waters approximately 15 to 93 m deep, with increased calling within known hard bottom and a marine reserve area offshore. Satellite-derived sea surface temperature (SST) and SST anomaly values were positively correlated to red grouper sound production, while chlorophyll concentration was negatively correlated. The results of this study do not indicate a strong correlation between red grouper sound production and known peaks in spawning (March – May). Although passive acoustic monitoring of this species may not provide insight into the timing of spawning throughout the year, it is an

effective tool to identify areas of potential spawning habitat. Passive acoustic mapping is an ideal tool for a long-term, large-scale study of fish abundance and behavior.

## Introduction

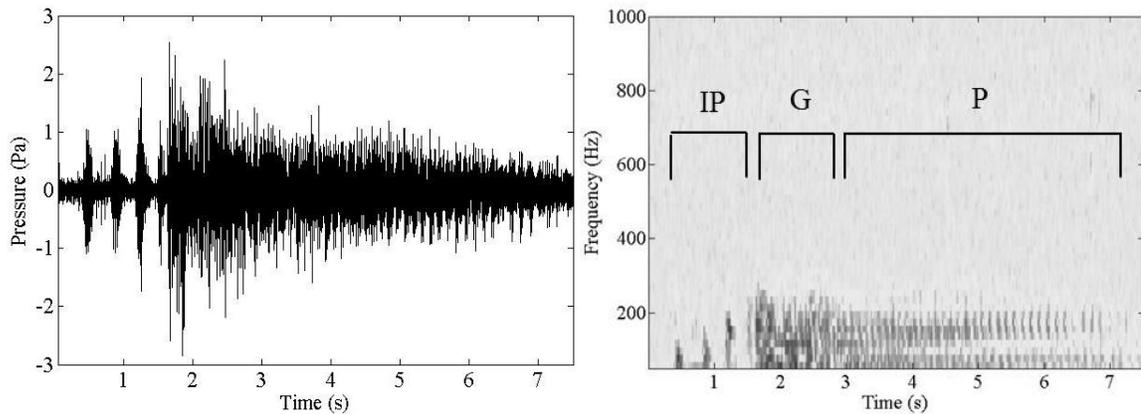
Red grouper (*Epinephelus morio*) comprise a large commercial and recreational fishery in the Gulf of Mexico. In 2008, approximately 150,000 pounds of commercial landings and over 130,000 recreationally-caught red grouper were reported (SEDAR 2009). Red grouper are “ecological engineers” who excavate pits and expose structure that serves as habitat for themselves, and other reef fish and invertebrates (Jones et al. 1994, Coleman & Williams 2002, Coleman et al. 2010). Sustaining red grouper populations is clearly an important management goal (Jones et al. 1994, Wright & Jones 2006). However, effective management first requires improving our understanding of the life history characteristics and reproductive behavior of these fish.

Like all grouper species, red grouper are slow-growing, late-maturing, relatively stationary, and long-lived. Red grouper are protogynous hermaphrodites that change sex from female to male (Moe 1969). Collins et al (2002) estimated that 50% sex-transition for red grouper populations in the eastern Gulf of Mexico occurred at an age of 13 years; however, this varies considerably (Moe 1969, Jory & Iversen 1989, Heemstra & Randall 1993, Musick 1999, Coleman et al. 2000, Sadovy 2001). Immature females are found year-round inshore (7 – 27 m depth), while immature females, mature females and males, and transitional fish are found offshore (30 – 90 m depth) (Brulé et al. 1999). This is consistent with increased size of red grouper with distance from the shore (Burns 2009), adult site fidelity (Coleman et al. 2010), and offshore (~70 m depth) spawning (Coleman et al. 1996).

Red grouper form small polygamous spawning groups dispersed widely throughout the West Florida Shelf (WFS) from late winter to early spring, peaking from March to May (Jory & Iversen 1989, Coleman et al. 1996, Brulé et al. 1999, Collins et al. 2002, Koenig et al. 2000). These small groups, compared to large aggregations common to many grouper species, and high fecundity (or ability to reproduce) (Sadovy 2001, Collins et al. 2002) enable red grouper to be relatively resilient to fishing pressure (Coleman et al. 1996). Yet, populations have experienced a truncated age structure and are currently considered near threatened by the International Union for Conservation of Nature (IUCN) (SEDAR 2009, Coleman & Koenig 2010, IUCN 2010). To date, most analyses of spawning populations derive from invasive, point-source, gonadosomatic index (GSI) examinations (Moe 1969, Jory & Iversen 1989, Coleman et al. 1996, Johnson et al. 1998, Brulé et al. 1999, Collins et al. 2002), leaving our knowledge of the spatial range of spawning habitat largely undefined.

Relatively recent developments in the field of marine bioacoustics allow passive acoustic technology to effectively monitor soniferous fish over a wide range of habitat, depths, and time periods (Mann & Lobel 1995, Lobel 2002, Luczkovich et al. 2008, Van Parijs et al. 2009, Lobel et al. 2010, Locascio & Mann 2011). Passive acoustic monitoring (PAM) systems can record large amounts of acoustic data and their application to soniferous fish (100~2,000 Hz) studies have been successfully demonstrated using moored devices (e.g., Locascio & Mann 2008, Nelson et al. 2011) and autonomous vehicles (Wall et al. 2012a). Furthermore, acoustic recordings allow marine mammal sounds (15~150,000 Hz) and anthropogenic noise (50~100,000 Hz) to be collected as well.

Recent PAM research has shown male red grouper produce sound during courtship and territorial behavior (Nelson et al. 2011). The mechanism for sound production is extrapolated from laboratory observations of Nassau grouper (*E. striatus*) in which bilateral muscles located behind the opercles and in contact with the surface of the swimbladder contract rapidly causing the swimbladder to vibrate, which results in a loud “grunt” (Hazlett & Winn 1962, Fish & Mowbray 1970). Nelson et al. (2011) catalogued calls from *in situ* recordings as introductory pulses, a grunt, and, at times, a pulse train. Figure 4.1 shows an example of a red grouper call recorded in this study. Nelson et al (2011) measured peak frequencies at 180 Hz with call duration positively correlated to the number of pulses present, and the highest sound pressure level (SPL) was 142 dB re 1  $\mu$ Pa RMS, which provides a rough estimate of source level. Red grouper were observed to call throughout the day and night with peaks near sunrise and sunset (Nelson et al. 2011).



**Figure 4.1.** Waveform (left) and spectrogram (right) of red grouper sound. This example shows a call with four introductory pulses (IP), a grunt (G) and a pulse train (P). The spectrogram was created using a 2,048 point Hann-windowed fast Fourier transform with 50% overlap.

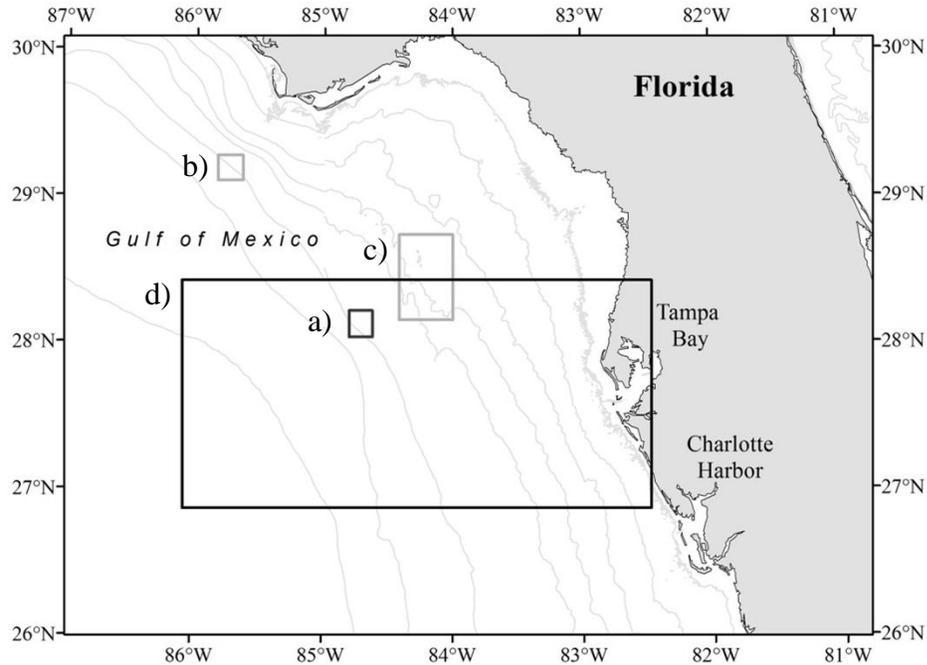
Although Nelson et al. (2011) did not observe spawning coincident with sound production, potentially due to the video's limited field-of-view, the frequent occurrence of calls during a peak-spawning month, the associated courtship behavior, and the crepuscular calling suggest that sonic activity may be related to reproductive behavior (Mann & Lobel 1995, Lobel 2002, Mann & Locascio 2008, Sadovy De Mitcheson et al. 2008, Locascio & Mann 2011).

The goals of this project were to determine daily and seasonal patterns of red grouper sound production, and map sound production on the WFS to define more precisely the range of potential spawning habitat for this species. Approximately one year of sound production was recorded in the Steamboat Lumps Marine Reserve. This is presented in concert with recordings from across the WFS collected using fixed recorders and AUVs, specifically Slocum gliders, outfitted with hydrophones. To determine potential environmental influences on calling patterns, spatial and temporal variability in sound production was compared to variation in environmental parameters (e.g., water temperature and bottom type).

## Methods

*Study Area.* The WFS extends over 200 miles off the west Florida coast and features a wide, gently sloping shelf (Figure 4.2). The inner WFS consists of a nearly flat, drowned and partially dissolved lithified carbonate (karst) platform covered by a thin layer of sediment (Hine 1997, Brooks et al. 2003b). Sediment types overlying the bedrock are highly varied and range from organic-rich mud, muddy sand, shelly sand, mixed siliciclastic/carbonate, and fine quartz sand (Edwards et al. 2003, Robbins et al. 2008). To mitigate fishing pressure on grouper aggregations during spawning, in June 2000 two marine reserves covering 200 square nautical miles were established on the shelf break (50 – 120 m deep) of the northeastern Gulf of Mexico – Madison Swanson (N29° 06' – N29 ° 17'; W085° 38' – W085° 50') and Steamboat Lumps (N28° 03' – N28° 14'; W084° 37' – W084 ° 48') Marine Reserves (Coleman et al. 2004a). Like much of the WFS, the Steamboat Lumps Marine Reserve (Steamboat Lumps) lacks geologic relief. However, “holes” excavated by male red grouper create structure and uncover carbonate nodules in the otherwise flat, sandy bottom of Steamboat Lumps (Scanlon et al. 2005, Coleman et al. 2010, Wall et al. 2011). The Florida Middle Grounds is a 1,193 km<sup>2</sup> area east of Steamboat Lumps, approximately 200 km northwest of Tampa Bay. This area consists of two north-northwesterly parallel ridges separated by a valley. It is home to stony corals that provide extensive hard bottom and structure for numerous species of reef fish, algae, sponges, mollusks, crustaceans and echinoderms (NOAA

2002, Coleman et al. 2004b). Artificial reefs and wrecks are common between the Florida Middle Ground and the coast, on the mostly flat sediment bottom.



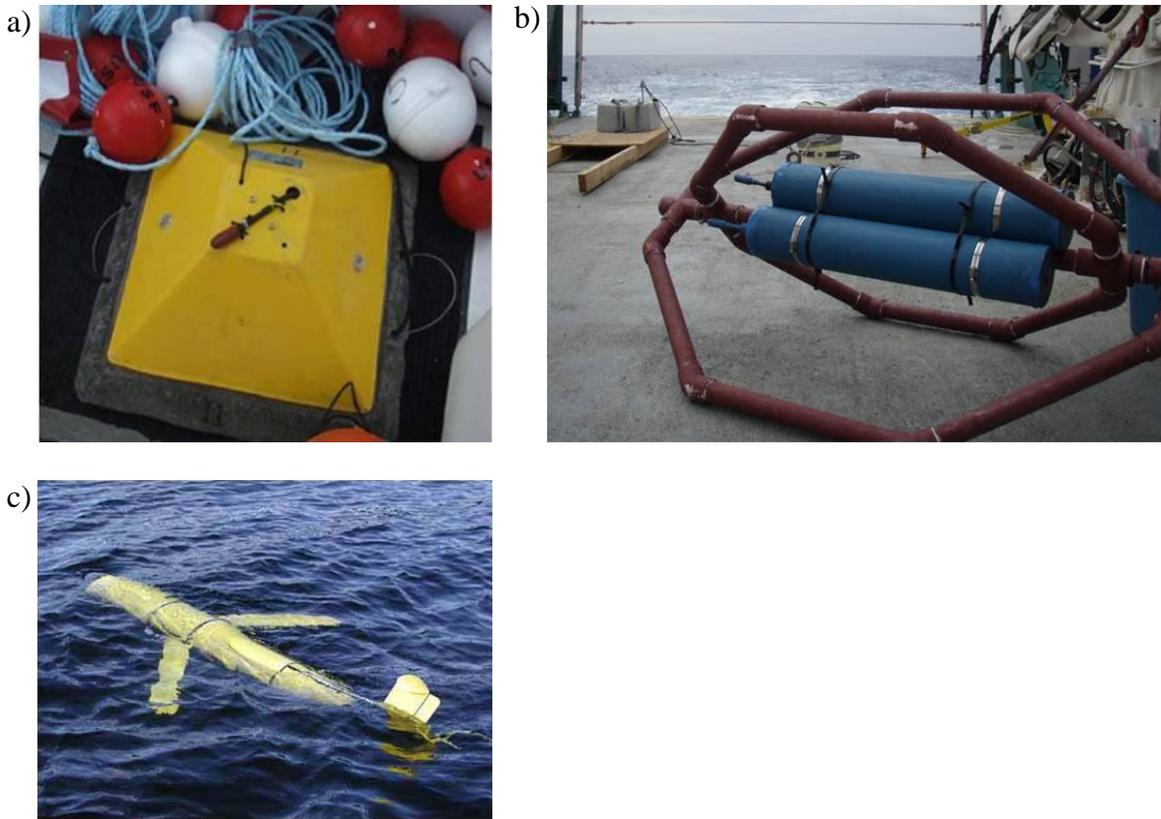
**Figure 4.2.** Study area within the eastern Gulf of Mexico. Boxes indicate boundaries of the a) Steamboat Lumps and b) Madison Swanson Marine Reserves, c) the Florida Middle Grounds, and d) the study area where acoustic data were collected. Grey lines indicate the 10, 20, 30, 40, 50, 100, 200 and 1,000 m isobaths.

*Data Collection.* All acoustic data were recorded using Digital Spectrogram Recorders (DSG; Loggerhead Instruments). The DSG is a low-power acoustic recorder controlled by script files stored on a secure digital (SD) memory card (16 GB or 32 GB) and an on-board real-time clock. The DSG clock is highly accurate with temperature compensated drift. The DSG file system is an advanced data file structure that stores embedded time stamps with the raw data, allowing each file to remain in synchrony with other glider or mooring data. Hydrophone (HTI-96-MIN, sensitivity -186 dBV (June and July 2008) or -170 dBV (June 2009 and Glider),  $\pm 3$  dB from 2 Hz-37 kHz, High Tech, Inc) signals were digitized with 16-bit resolution by the DSG recorders.

Stationary Recorders. Moored acoustic recorders were deployed on the WFS for one month to one year between 2008 and 2010. Several designs were implemented as this project progressed from short-term, nearshore pilot studies to a year-long, shelf-wide deployment (Dudzinski et al. 2009). Initial deployments conducted for one month in June 2008 and two to five months in July 2009 (N=5 and 18, respectively) employed bottom-mounted, trawl-resistant casings in which the hydrophone was exposed through a fiberglass flat-top pyramid-shaped cover; the cover was connected to a 1 m<sup>2</sup> cement base with stainless steel cables (Figure 4.3a). A PVC tube located inside the casing provided a water-tight housing for the DSG and battery packs; the DSG was connected to the hydrophone through a bulkhead connector. During these deployments, the DSG recorded sound for 10 seconds every hour at a sample rate of 50 kHz.

A larger deployment aimed at recording sound for one year at 63 sites on the WFS occurred between June 2009 and May 2010. The recorders were deployed in a grid 20 km apart from the coast (10 m depth) to approximately 150 km offshore (100 m depth). The greater depth precluded the continued use of bottom-mounted recorders. Therefore, a mid-water column design was implemented in which the PVC tubes, which housed the hydrophone, DSG and battery packs, were hose-clamped to polypropylene line 10 m (or shallower) below the water surface. The polypropylene line extended from the water surface where it was connected to surface and subsurface buoys down to the seafloor where it was connected to the bottom mooring constructed from two cement-filled cinderblocks joined with chain. To protect against impact from boats and shrimp trawls, PVC cages consisting of four semicircular, buoyant arms surrounded one to two PVC tubes (Figure 4.3b). Additional recorders were attached directly to the

polypropylene line near the mooring blocks in deep water (> 30 m) to ensure the recording of demersal red grouper. DSGs recorded sound for 6 to 8 seconds every hour at a rate of 36.4 kHz or 50 kHz. Sample rate and frequency varied slightly among sites in an attempt to optimize recording longevity and storage capacity of the SD card. All PVC material was covered with anti-fouling paint.



**Figure 4.3.** Recorder designs deployed for the study. a) Trawl-resistant housing containing the DSG (yellow) with exposed hydrophone and cement base (grey) deployed in June and July 2008. b) Mid-water column housing with protective PVC arms (maroon) and PVC tubes encasing the DSG (blue) with exposed hydrophone employed for the June 2009 deployment. c) Hydrophone-integrated Slocum glider.

Several additional recorders were deployed in Steamboat Lumps (N=7, 71 – 73 m depth) and at nearshore sites to specifically target red grouper (“RG”) (N=6, 15 – 40 m depth) between April 2009 and May 2010. PVC tubes housing the hydrophone, DSG and

battery packs were hose-clamped to polypropylene line within 1 meter from the mooring blocks. Surface and subsurface floats attached to the polypropylene kept the line and recorder upright in the water column. All Steamboat Lumps recorders were deployed within 0.2 km of each other and, due to the close proximity, are considered one site in the analyses. These recorders sampled 10 seconds every 6 minutes at 20 kHz.

Hydrophone-integrated Gliders. A hydrophone was integrated into the aft cowling of four Slocum electric underwater gliders (Teledyne Webb Research) for passive recording of sound with an on-board DSG board while concurrently collecting a suite of environmental and optical parameters (Wall et al. 2012a) (Figure 4.3c).

Slocum gliders are buoyancy driven autonomous underwater vehicles 1.8 m in length and shaped like a winged torpedo (Webb et al. 2001, Schofield et al. 2007). They can traverse over 600 km using a single set of alkaline batteries and contain sensors tailored toward scientific applications (Schofield et al. 2007). The University of South Florida (USF) gliders used in this study measure temperature, salinity, depth-averaged currents (i.e., currents averaged vertically from near the surface to near the bottom), surface currents, fluorescence, dissolved oxygen, and apparent and inherent optical properties. These measurements were taken as the glider ascended and descended through the water column. The glider's DSG recorded sound for 25 seconds every 5 minutes at a sample rate of 70 kHz. The DSG clock is synchronized to the clock on-board the glider's computer and thus the environmental sensors.

Glider deployments of one to four weeks in length began in April 2009 and continued through April 2011 covering a range of depths (up to 984 m) depending on the

deployment path. Gliders were run and maintained at the USF, Center for Ocean Technology.

Environmental Data. At some of the stationary sites, *in situ* temperature was also recorded using HOBO® data loggers. The temperature loggers were attached to the polypropylene line near the PVC cages and, when present, to bottom recorders for the mid-water deployment. They were also affixed to all recorders deployed in Steamboat Lumps. Temperature was recorded every 12 minutes and analyzed using HOBOWare Pro software and MATLAB (Mathworks).

Satellite-derived sea surface temperature (SST), SST anomaly, and chlorophyll *a* concentration (Chl) data were obtained for periods and areas in which acoustic data were recorded. SST data were derived from infrared data collected by NASA's Moderate Resolution Imaging Spectroradiometers (MODIS) onboard satellites Aqua and Terra. Chl were calculated from visible data collected by ORBIMAGE's Sea-viewing Wide Field-of-View Sensor (SeaWiFS) using standard SeaDAS processing. SST anomaly data were calculated as the difference between weekly mean SST and corresponding weekly mean climatology based on SST data from 2001 to 2010. Time series data for each of these parameters were calculated for each stationary site. All satellite data processing was performed using IDL (Research Systems, Inc).

High-resolution bathymetry data were collected in Steamboat Lumps in 2009 using a Kongsberg EM3000 (300 kHz) multibeam sonar (Wall et al. 2011). These data show holes excavated by male red grouper and indicate areas of potential spawning habitat. The locations of red grouper sound production in Steamboat Lumps identified

within the acoustic data were compared to the locations of holes identified in the 2009 multibeam data.

Sunrise and sunset, and lunar cycle data were obtained from June 2009 to May 2010 (USNO 2012a, b). Daily and seasonal patterns in red grouper sound production were compared to sunrise and sunset, and lunar phase. Locations of red grouper sound production were compared to bottom sediment data (Jenkins 2011).

### *Data Catalogue*

Stationary Recorders. All acoustic data and associated metadata were catalogued in a custom MySQL database and the files stored on a 192 TB Sun Fire X4400 server called Ocean Observing Metadata Archive (OOMA) using a MATLAB interface. OOMA is stored at the USF, College of Marine Science. Metadata included hydrophone sensitivity, latitude and longitude coordinates of the recorder site, water depth, sample rate, file size, UTC timestamp of the recording, file path and file name on the server, and UTC timestamp of the recorder deployment and recovery times.

Hydrophone-integrated Gliders. Acoustic data collected by the glider were also catalogued in the database and stored on the server. Metadata collected throughout the deployment included glider depth in the water column, bottom depth, UTC time stamp, roll, pitch, and heading. Latitude and longitude position were collected using GPS satellites when the glider was at the surface. The position of the glider when not at the surface was estimated from the surface latitude and longitude coordinates using linear interpolation and a 10-point moving average.

*Data Analysis.*

Stationary Recorders – Automatic Detection. An automatic detection algorithm was developed to identify red grouper calls within the time domain of sound files. The number of introductory pulses and presence of a pulse train vary among calls, so the algorithm targeted the statistical parameters of one introductory pulse and the grunt.

Each acoustic file was first resampled at a lower rate (1/10 the original sample rate with lowpass filtering) to enable faster processing. The RMS of three bands, each 130 Hz wide, were used to measure proxies of red grouper sound level (Band 1; 50-180 Hz), sound level of other fish calls (Band 2; 270–400 Hz), and the level of ambient and anthropogenic noise (Band 3; 869–999 Hz). For automated detection within Band 1, data were normalized to the maximum signal level, rectified and enveloped with a 75 ms window. High-amplitude, narrow-band pulses that result from hydrophone interference were removed to reduce noise and increase the ability of the algorithm to accurately detect the more subtle introductory pulse and grunt. Once noise-related pulses were removed, data were normalized again and signal values above a threshold, representing potential grunts, were identified. For each “grunt”, the duration, time in the file, peak frequency, and 3 dB bandwidth were calculated. The threshold value was determined from previous analyses of files containing red grouper to optimize the detection of calls with high signal to noise and ignore low-amplitude noise. The duration of any prior “grunts”, representative of introductory pulses (“introductory pulse intervals”), were also identified. The peak frequency and 3 dB bandwidth were calculated from an FFT (fast Fourier transform) of the signal with a 5 Hz resolution.

A MySQL table was created to catalogue each file that contained “grunts”. Catalogued information included file attributes (file timestamp, location), “grunt” attributes (duration, time in file, peak frequency, 3 dB bandwidth and introductory pulse intervals), and RMS values (Band 1, Band 2 and Band 3). These data were then subjected to a suite of parameter restrictions to select for red grouper and minimize false detections due to noise. Parameter restriction values were determined from red grouper call statistics calculated in Nelson et al. (2011) and from a training library recorded during this study (1,306 files containing red grouper calls; 1,476 files containing “noise”). Grunt duration was set between 0.35 and 0.71 s, peak frequency was set between 78 and 194 Hz, 3 dB bandwidth was set to 140 Hz or less, introductory pulse intervals were set to 0.49 and 0.79 s, and RMS band values were set to 0.34 or less, 5.8 to 9.6, and 5.8 to 6.1 for Band 1, Band 2, and Band 3, respectively. Files with detections that met these restriction criteria were then manually verified.

When run on the training library, the detection algorithm accurately detected red grouper 44% of the time (true positives) and falsely detected red grouper 4% of the time (false positives). Although the level of true positives is low, a conservative detector was deliberately sought in order to significantly reduce the number of false positives. The algorithm showed positive results when applied to the Steamboat Lumps dataset. Manual verification of files with detections that met the restriction criteria showed 92.5% contained red grouper sounds (7.5% false detection rate). Similar results were observed for one RG site (RG3) (10% false detection rate).

The detection algorithm was applied to the acoustic files stored on OOMA using MATLAB scripts run on a computer cluster. Analysis of the restriction criteria were run

using MATLAB programs directly on OOMA. Visual verification of files that met the restriction criteria were analyzed using MATLAB on a local machine.

Stationary Recorders – Manual Analysis. Although the detection algorithm showed promise for sites offshore and/or highly populated with red grouper, inshore sites that were more susceptible to boat traffic, equipment noise (e.g., movement of the PVC tube), and less likely to have red grouper proved to be very difficult for the detection algorithm to detect red grouper with high accuracy and avoid false positives. Therefore, files collected at the remaining stationary sites were analyzed manually by visually inspecting spectrograms to identify the acoustic presence of red grouper. Spectrograms were created using 2,048 point Hann-windowed FFTs with 50% overlap. Due to the large number of acoustic files recorded throughout the deployments, only files recorded between 1600-2200 hours (local time), which represented 57,414 files, were reviewed. Red grouper call 24 hours-a-day ensuring this timeframe will reveal their sound production if present (Nelson et al. 2011). The RG recorders recorded more frequently (10 seconds every 6 minutes) than the other stationary recorders (6 to 10 seconds every hour). To reduce sampling bias, one file per hour of the RG recorders was analyzed.

Hydrophone-integrated Gliders. Acoustic files recorded during the glider missions contained extensive electrical and mechanical noise, which similarly prevented the successful application of the detection algorithm. Therefore, all files were analyzed manually. Detections were binned into one hour intervals over the glider track. Temporal binning also resulted in spatial binning as the glider moved continuously. Therefore, the interpolated coordinates of the file closest to the 30 minute mark were

used to display the spatial position for that one hour bin. Hourly bins and data display were completed using MATLAB and ArcGIS (ESRI).

Water temperature and fluorescence were compared to the results of the acoustic analysis. Although environmental data were collected more frequently than acoustic data, gaps within the environmental measurements persisted. To reduce these gaps, the in-water temperature and fluorescence data were smoothed using a ten-point window and then linearly interpolated.

All red grouper sounds in the acoustic files, whether identified by the automated detection algorithm or visually from spectrograms, were binned by hour and month and normalized by the total number of files analyzed per hour and per month (“call per unit effort”), to show daily and seasonal patterns without a sampling bias. To better understand how the daily calling patterns changed throughout the year, a matrix of the number of calls detected per hour for each month was created. Histograms of the percentage of files containing red grouper recorded between 1600-2200 hours per month were calculated for each recorder recovered in June 2009 to illustrate the duration and areas in which acoustic files were recorded and how red grouper calling changed throughout the year.

Environmental Data. Daily counts of files containing red grouper sounds were extracted from the Steamboat Lumps analysis. An FFT was applied to the time series of daily counts to determine whether there were cyclical peaks in calling. These peaks were then compared to the cyclical pattern of lunar phases to determine if it influenced red grouper calling. Acoustic detections of red grouper from stationary recorders were binned by week and correlated to SST anomaly values as well as weekly median SST and

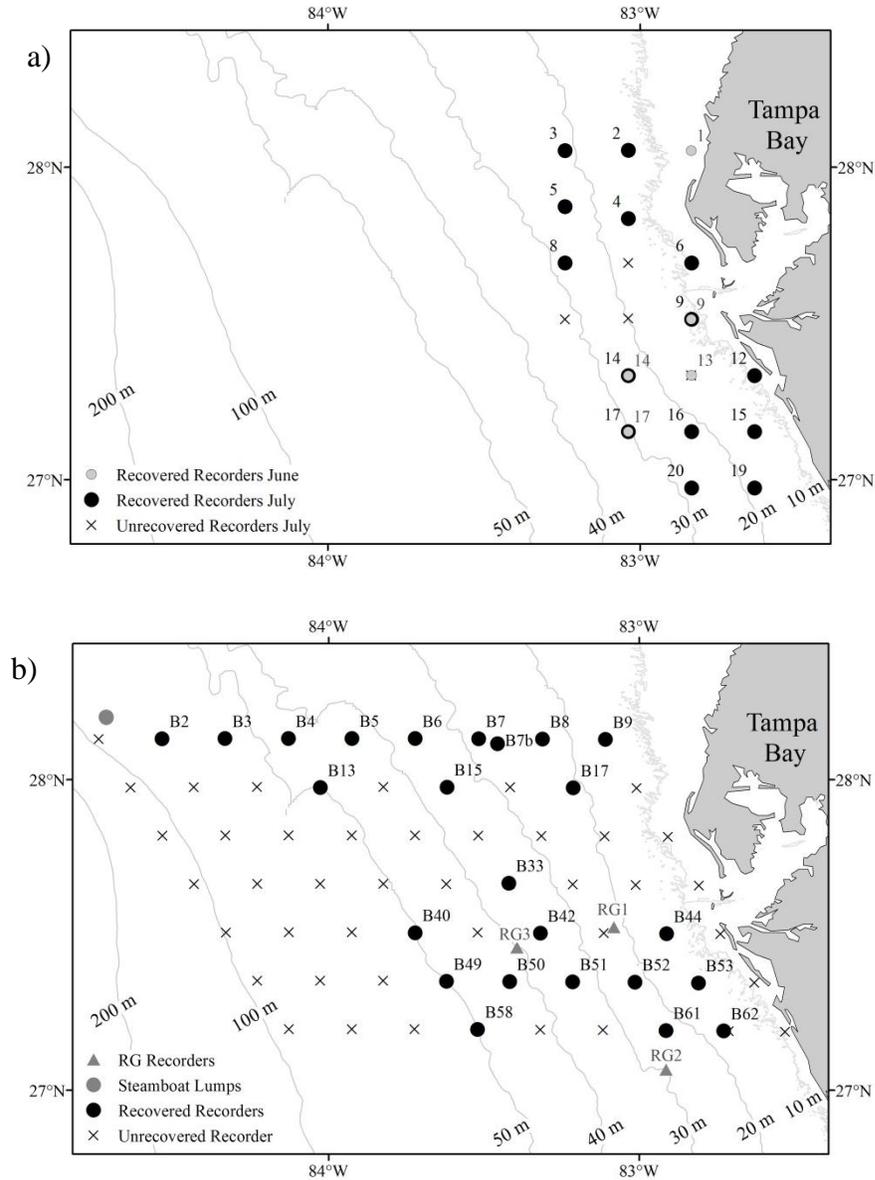
Chl. Separate calculations were made for detections from Steamboat and RG3, and the manually analyzed stationary recorders. Red grouper observations in the glider acoustic files were examined for correlations to on-board water temperature and fluorescence measurements. Glider data were also binned weekly. Statistical analyses were completed using MATLAB.

## Results

### *Data Collection.*

Stationary Recorders. Nineteen out of 23 recorders deployed in 2008 were recovered (5 from June; 14 from July; 83% recovery rate). A total of 16,482 files were recorded from these stationary sites. Of the 63 recorders deployed in 2009, 29 were recovered (46% recovery rate) resulting in an additional 121,524 acoustic files. Four recorders deployed in Steamboat Lumps (162,877 files) and four recorders deployed at the RG sites (101,862 files) were successfully recovered (57% and 67% recovery rate, respectively). After removing files recorded before and after the deployment period, the total acoustic library collected during this study consisted of 377,728 files. Figure 4.4 illustrates the location of recovered and unrecovered stationary recorders. The recording duration of each recorder is outlined in Table 4.1. Some recorders stopped recording before deployment or ended prematurely. Upon retrieval, some of these recorders showed battery corrosion and failure due to a leak in the PVC tube. When battery leaks were not evident, software errors are suspected to be the cause.

Hydrophone-integrated Gliders. Fifteen glider missions were conducted between April 2009 and April 2011 (Figure 4.5). Throughout these missions, 25,760 files were recorded over the various glider tracks. All gliders deployed were successfully retrieved, however, acoustic recordings stopped before recovery due to filled storage space on the SD card for some of the longer missions (Table 4.2).



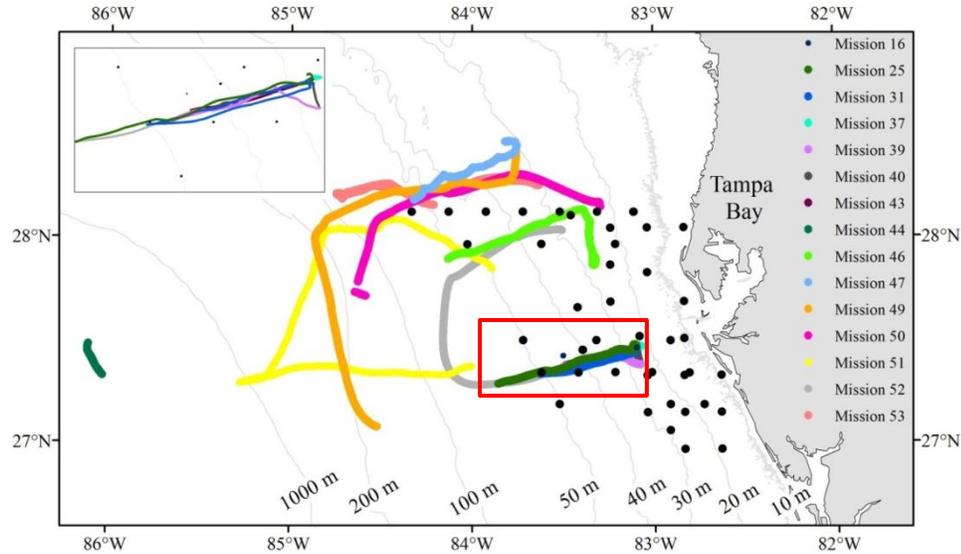
**Figure 4.4.** Map of the stationary acoustic recorders. a) Positions of recovered recorders deployed in June (●) and July 2008 (●), and unrecovered recorders deployed in July 2008 (X). Station labels for the June 2008 deployment are in grey. b) Positions of recovered (●) and unrecovered recorders (X) deployed in June 2009, and recorders deployed to target red grouper inshore (RG) (▲) and in Steamboat Lumps (●). B5 and B5b, B6 and B6b, B8 and B8b, B9 and B9b, and B33 and B33b are located at the same site and only one station label is shown.

**Table 4.1.** Recovered stationary recorder deployment information. Noted are the recorder station numbers (Station), recorder deployment (Deployed) and recovery (Recovered) times, number of days recording (Days Recorded), and water depth at the site (Depth). DSGs recorded for the duration of the deployment (--) unless otherwise reported in “End Recording”. No Data indicates the recorder stopped working before deployment or only collected “stuttered files”, a recording format not incorporated in this study. Inshore recorders deployed to specifically target red grouper are noted as “RG”.

Deployment	Station	Deployed	Recovered	End Recording	Days Recorded	Depth (m)
June 2008	1	6/11/2008	9/16/2008	--	98	4
	9	6/10/2008	6/26/2008	--	17	11
	13	6/10/2008	6/26/2008	--	17	13
	14	6/10/2008	6/26/2008	--	17	24
	17	6/10/2008	6/26/2008	--	17	31
July 2008	2	7/23/2008	11/13/2008	9/26/2008	66	4
	3	7/23/2008	11/13/2008	8/15/2008	24	21
	4	7/23/2008	12/5/2008	9/27/2008	67	12
	5	7/28/2008	12/5/2008	9/4/2008	39	22
	6	7/23/2008	12/5/2008	9/3/2008	43	9
	8	7/28/2008	12/31/2008	--	157	27
	9	7/21/2008	12/31/2008	No Data	0	9
	12	7/21/2008	12/31/2008	No Data	0	10
	14	7/21/2008	12/31/2008	No Data	0	26
	15	7/29/2008	12/31/2008	10/28/2008	92	14
	16	7/21/2008	12/31/2008	No Data	0	24
	17	7/21/2008	12/31/2008	No Data	0	31
	19	7/29/2008	12/31/2008	No Data	0	18
20	7/29/2008	9/15/2008	--	49	29	
June 2009	B2b	10/13/2009	6/10/2010	--	241	72
	B3	6/1/2009	10/13/2009	7/15/2009	45	59
	B4	6/1/2009	10/13/2009	8/23/2009	84	46
	B5	6/1/2009	9/3/2009	8/13/2009	74	42
	B5b	9/3/2009	5/20/2010	--	260	42
	B6	6/1/2009	9/3/2009	No Data	0	35
	B6b	9/3/2009	5/20/2010	5/1/2010	235	35
	B7	6/1/2009	9/3/2009	No Data	0	28
	B7b	8/27/2009	5/18/2010	3/28/2010	214	28
	B8	6/1/2009	8/3/2009	--	64	24
	B8b	8/27/2009	5/18/2010	--	265	24
	B9	6/1/2009	9/3/2009	--	95	15
B9b	8/27/2009	5/18/2010	5/8/2010	255	15	

**Table 4.1 Cont.**

	B13	6/3/2009	5/25/2010	2/14/2010	257	49
	B15	6/4/2009	8/1/2009	--	59	35
	B17	6/5/2009	5/18/2010	3/3/2010	244	24
	B33	6/4/2009	4/21/2010	2/21/2010	263	36
	B33b	6/4/2009	4/21/2010	No Data	0	36
	B40	6/3/2009	6/2/2010	--	365	40
	B42	6/4/2009	6/2/2010	3/3/2010	273	35
	B44	6/5/2009	11/24/2009	6/30/2009	26	13
	B49	6/3/2009	4/22/2010	--	324	49
	B50	6/4/2009	4/22/2010	--	325	44
	B51	6/4/2009	9/23/2009	--	112	33
	B52	10/13/2009	5/20/2010	--	220	24
	B53	6/5/2009	11/24/2009	10/3/2009	121	13
	B58	6/3/2009	4/22/2010	9/22/2009	112	49
	B61	6/4/2009	5/6/2010	7/9/2009	36	23
	B62	6/4/2009	11/24/2009	--	174	15
RG	RG1	4/23/2009	8/18/2009	--	118	16
	RG2	4/11/2009	8/18/2009	--	130	30
	RG3	4/23/2009	8/18/2009	--	118	39
	RG4	4/23/2009	8/25/2009	No Data	0	39
Steamboat Lumps	RG 7	4/23/2009	10/12/2010	9/20/2009	163	72
	RG 7b	11/17/2009	10/12/2010	5/20/2010	185	72
	RG 8	4/23/2009	10/12/2010	9/20/2009	163	73
	RG 8b	11/17/2009	10/12/2010	5/16/2010	181	72



**Figure 4.5.** Map of interpolated tracks for hydrophone-integrated glider missions between 2008 and 2011. Red box indicates the area highlighted in the inset (grey box). Recovered stationary recorders (●) are show for reference.

**Table 4.2.** Hydrophone-integrated glider deployment information. Noted are the mission number (Mission), glider deployment (Deployed) and recovery (Recovered) times, number of days acoustic data were recorded (Days Recorded), distance the glider traveled (Distance) and maximum water depth reached during the deployment (Max Depth). All DSGs recorded for the duration of the mission (--) unless otherwise noted in “End Recording”.

Mission	Deployed	Recovered	End Recording	Days Recorded	Distance (km)	Max Depth (m)
16	4/9/2009	4/12/2009	--	4	51	45.1
25	6/2/2009	6/15/2009	6/7/2009	6	238	78.3
31	7/14/2009	7/21/2009	--	8	136	50.2
37	9/22/2009	9/24/2009	--	2	11	28.2
39	10/8/2009	10/14/2009	--	7	106	45.4
40	10/8/2009	10/21/2009	10/12/2009	5	209	95.4
43	4/20/2010	5/4/2010	4/23/2010	4	230	76.7
44	5/23/2010	5/25/2010	--	3	138	182.5
46	5/27/2010	6/8/2010	--	13	237	183.6
47	6/8/2010	6/14/2010	6/11/2010	4	98	57.5
49	7/13/2010	8/10/2010	7/29/2010	17	467	181.1
50	9/27/2010	10/9/2010	--	12	205	162.6
51	10/12/2010	10/30/2010	10/21/2010	10	384	984.1
52	1/31/2001	2/12/2011	--	13	225	92.4
53	3/29/2011	4/15/2011	4/14/2011	17	228	86.1

Environmental Data. Thirteen *in situ* water temperature data loggers were retrieved from the June 2009 deployment and two were retrieved from the Steamboat Lumps deployment. The difference between *in situ* temperature and satellite-derived SST for corresponding timestamps was calculated for each data logger (Table 4.3). Data collected at the cage depth (approximately 10 m) were within 1 °C of the SST, whereas data collected at greater depths showed greater disparity from the SST. The greatest variation between *in situ* and SST (~5 °C) was observed at the deepest recorder depth (72.5 m), which was located in Steamboat Lumps. However, SST were positively correlated to *in situ* temperature from Steamboat Lumps (RG8 and RG7b) throughout the period acoustic data were recorded (April 23, 2009 to May 17, 2010;  $r=0.58$ ,  $p=0$ ).

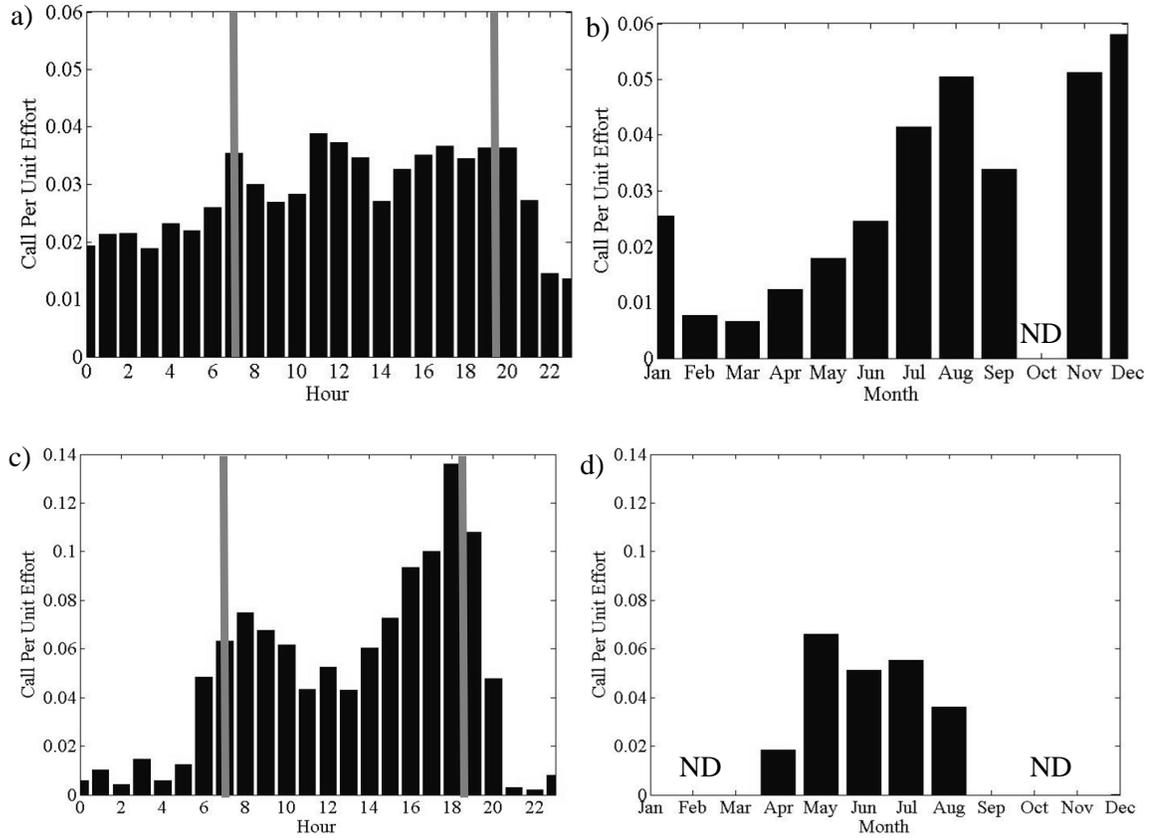
**Table 4.3.** *In situ* temperature data compared to SST. Shown are the station names (Station), the depth of the temperature logger (Logger Depth), deployment (Deployed) and recovery (Recovered) period for each site, and median (Median) and standard deviation (Std) of the absolute difference between SST and *in situ* temperature for corresponding times ( $\Delta^{\circ}\text{C}=\text{SST} - \text{logger}$ ).

Station	Logger Depth (m)	Deployed	Recovered	Median ( $\Delta^{\circ}\text{C}$ )	Std
B3 - Cage	10	6/1/2009	10/13/2009	0.5	0.6
B3 - Bottom	58.6	6/1/2009	10/13/2009	5.7	3.5
B4 - Cage	10	6/1/2009	10/13/2009	0.5	0.6
B4 - Bottom	45.7	6/1/2009	10/13/2009	4.3	3.0
B5 - Cage	10	6/1/2009	9/3/2009	0.6	0.7
B5 - Bottom	42.1	6/1/2009	9/3/2009	2.4	1.8
B6 - Cage	10	6/1/2009	9/3/2009	0.7	0.7
B7 - Cage	10	6/1/2009	9/3/2009	0.7	0.7
B8 - Cage	10	6/1/2009	8/3/2009	0.9	0.8
B9 - Cage	10	6/1/2009	9/3/2009	0.5	0.6
B15 - Cage	10	6/4/2009	8/1/2009	0.8	1.0
B15 - Bottom	34.7	6/4/2009	8/1/2009	1.7	1.2
B51 - Cage	10	6/4/2009	9/23/2009	0.7	0.9
RG8 - Bottom	72.5	4/23/2009	10/12/2009	5.7	4.1
RG7b - Bottom	72.5	11/17/2009	10/12/2010	4.0	4.6

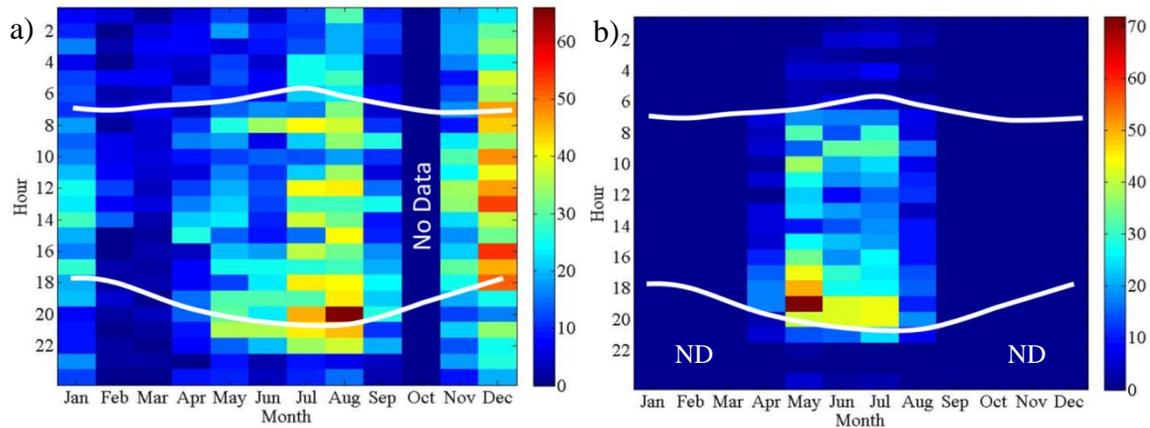
### *Data Analysis*

Stationary Recorders – Automated Detection. In Steamboat Lumps, red grouper produced sound throughout the day and night, and during all months sound was recorded (Figure 4.6a,b). Daily peaks in calling were observed just after the local yearly mean sunrise ( $7:05 \pm 0:20$  [mean  $\pm$  SD]) and just before mean sunset ( $19:40 \pm 1:03$ ), as well as mid-day (1100-1300 hours). Monthly calling increased progressively from spring to summer (March to August), decreased in the fall, and peaked again in early winter (November to December). Diurnal peaks in calling appear to coincide largely with the seasonal shift in sunrise and sunset times (Figure 4.7a). The most files containing red grouper detected per hour per month were observed at 19:00 in August (66/69; 96%).

Hourly and monthly sound production patterns at RG3 were compared to Steamboat Lumps (Figure 4.6c,d and 4.7b). Crepuscular peaks were more pronounced for this inshore station, with only 20% of calling occurring at night compared to 38% for Steamboat Lumps. The limited deployment period of RG3 (April to August) does not allow for a comparison of seasonal variation. Yet, RG3 did not show the same trend of increasing call rate from May to July that was observed at Steamboat Lumps (see Figure 4.6).

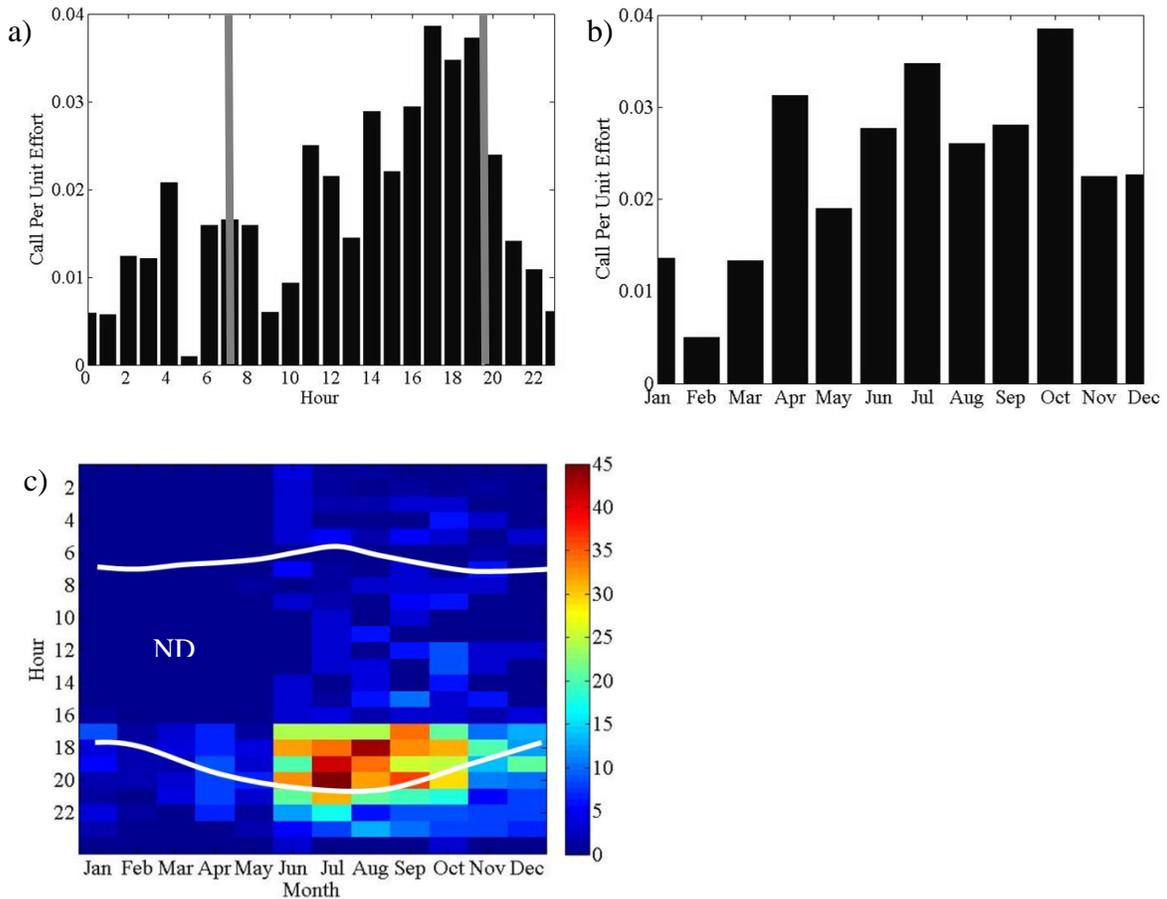


**Figure 4.6.** Histograms of red grouper calls identified by the detection algorithm. Calls recorded in Steamboat Lumps were binned a) hourly and b) monthly. Calls recorded at the inshore red grouper-targeted site (RG3) were binned c) hourly and d) monthly. All times are local (EST). Grey lines indicate mean sunrise (7:05) and sunset (19:40) throughout the year. No acoustic data (ND) were recorded in October in Steamboat Lumps or January to March and September to December inshore.



**Figure 4.7.** Matrix of red grouper calls per hour for each month from a) Steamboat Lumps and b) RG3. The color bar indicates total number of files containing red grouper per hour for each month. White lines indicate sunrise (top) and sunset (bottom) times. No acoustic data (ND) were recorded in October in Steamboat Lumps or January to March and September to December inshore. Note the slight difference in scale of the color map.

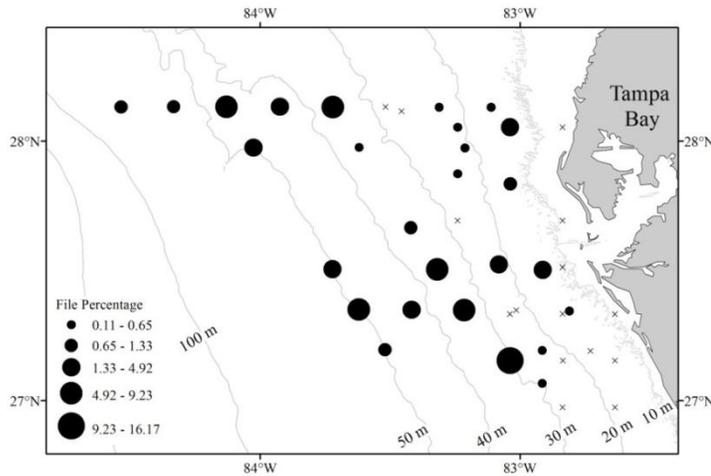
Stationary Recorders – Manual Analysis. Red grouper calls identified through manual analysis of acoustic data recorded between 1600 and 2200 hours were supplemented with files analyzed during the preliminary analysis of the detection algorithm on the entire dataset. Although the manual analysis effort focused mainly on files recorded between those evening hours, it is evident that red grouper calls were present throughout the entire 24-hour day (Figure 4.8a). Calling peaked in the late afternoon to early evening (1600–1900 hours). A seasonal pattern in sound production is more difficult to discern with calling largely steady from April to December, with small peaks in April, July and October (Figure 4.8b). In February, red grouper sound was observed very infrequently. For the seven hours manually analyzed, the majority of calling by month was observed between June and October, with peaks in July (45/752 files; 6% during 2000 hours) and August (43/835 files; 5% during 1800 hours) (Figure 4.8c).



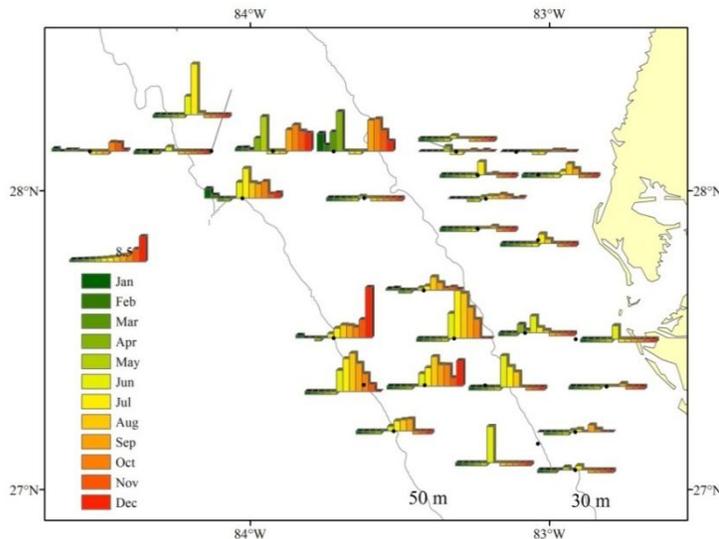
**Figure 4.8.** Red grouper sound production for manually analyzed stationary recorders. a) Hourly and b) monthly bins of the number of files that contained red grouper, divided by the total number of files analyzed. c) Matrix of the number of files per hour per month. Grey lines indicate mean sunrise (7:05) and sunset (19:40) throughout the year. White lines indicate sunrise (top) and sunset (bottom) times. The color bar indicates total number of files containing red grouper per hour for each month. ND indicates no acoustic data were analyzed.

The spatial area over which red grouper sounds were identified is illustrated in Figure 4.9. The symbol size is proportional to the percentage of files red grouper were present in compared to the number of files analyzed. The highest percentages of files containing red grouper are found between the 30 and 50 m isobaths. This is further illustrated by the map of monthly percentage of red grouper calling determined for each recorder recovered from the June 2009 deployment (Figure 4.10). Bars below the x-axis

indicate no data were recorded. Red grouper sound production in the winter months was only observed at recorders deployed between 30 and 50 m depths. However, as no data were recorded by the inshore (< 30 m) recorders during this period the correlation between depth and winter sound production calling remains unknown.

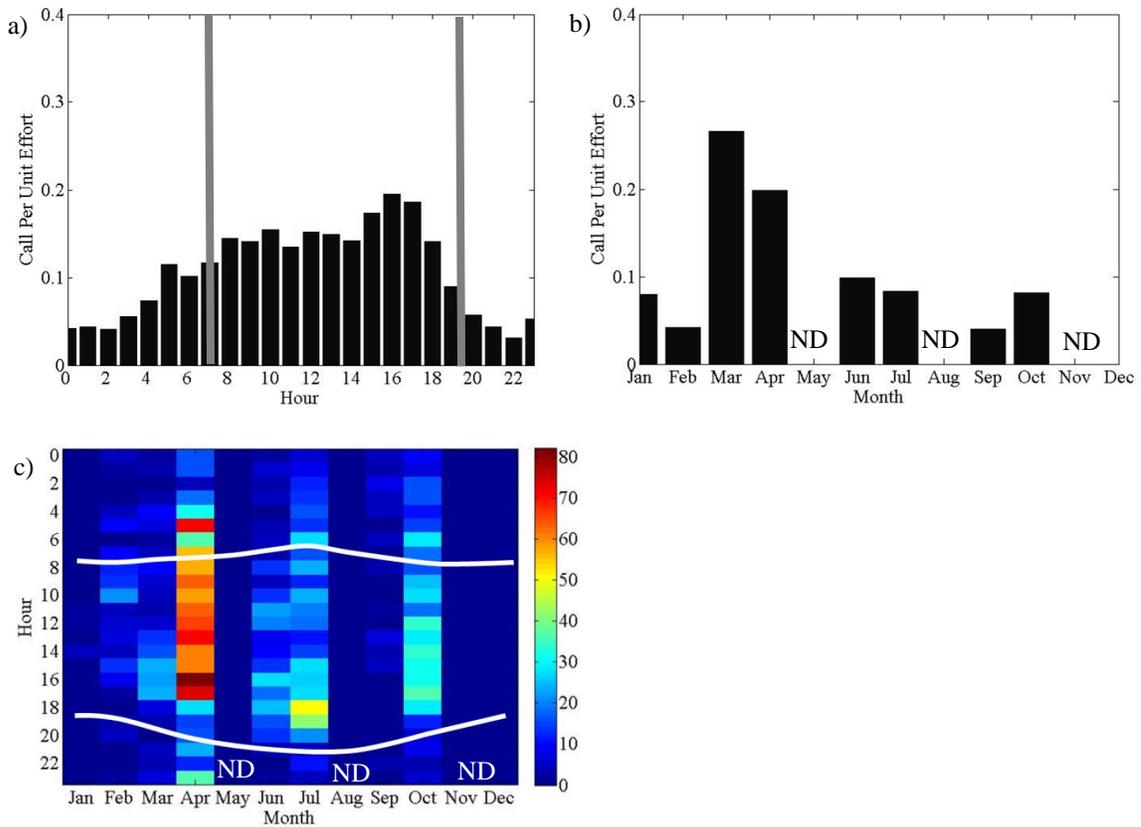


**Figure 4.9.** Red grouper calls identified in manually analyzed stationary recorders. Symbol size is proportional to the percent of files red grouper were detected, out of the total number of files analyzed per site. Recorder locations in which no red grouper calls were identified are also shown (x).



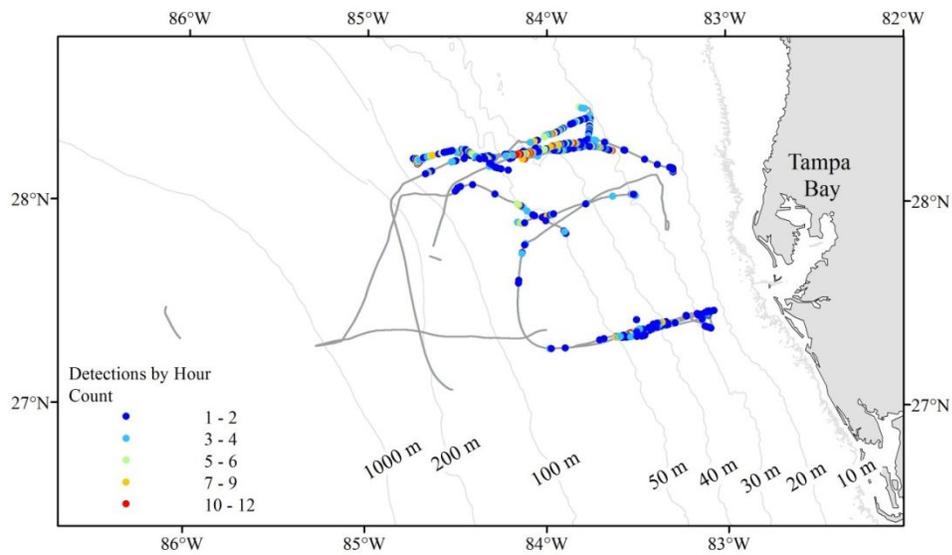
**Figure 4.10.** Histograms of monthly red grouper sound production for recorders recovered in June 2009. Bar height indicates the percent of files containing red grouper recorded between 1600-2200 hours per month. The bar height is negative where no data were recorded.

Hydrophone-integrated Gliders. Glider missions were run during all months except May, August, November and December, and data were collected during all hours of the day (Figure 4.11). Daily peaks in calling are apparent at dusk; however, the relative increase is lower compared to Figure 4.6b. Most calling occurs between sunrise and sunset with only 23% observed at night. Throughout the months in which gliders were deployed, March and April show the highest number of red grouper calls (182/684 and 1,036/5,227 or 0.27 and 0.20 calls per unit effort, respectively).

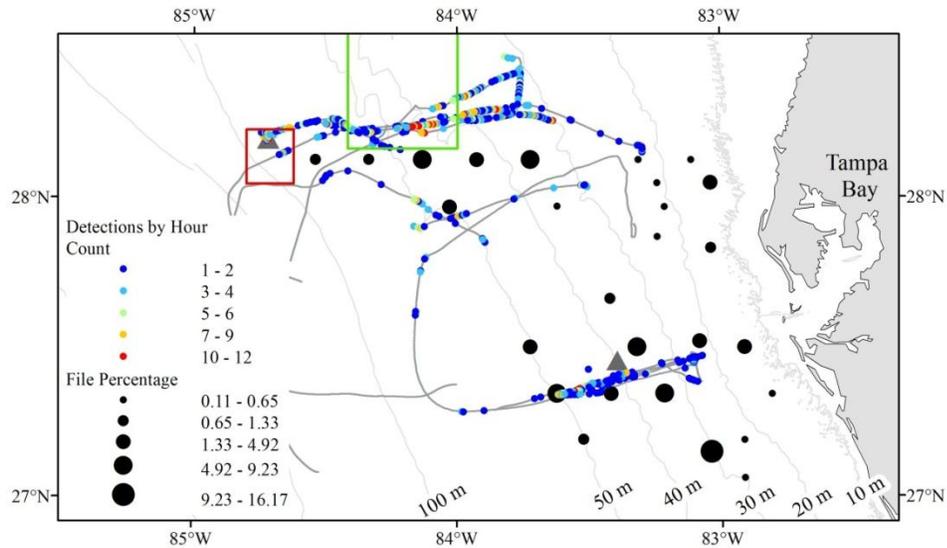


**Figure 4.11.** Red grouper sound production identified in the glider acoustic data. a) Hourly and b) monthly bins of the number of files that contained red grouper are compared to the total number of files analyzed. c) Matrix of the number of files per hour per month that contained red grouper sounds. Grey lines indicate mean sunrise (7:05) and sunset (19:40) throughout the year. No acoustic data (ND) were collected in May, August, November or December.

Hourly bins of red grouper sounds recorded along the interpolated track for each glider mission are shown in Figure 4.12. The bin values represent the number of files that contained red grouper sound within that hour. Since files are recorded every 5 minutes, the maximum number possible is 12. The results of the manual analysis of the stationary recorders were overlaid on the hourly bins of red grouper detections (Figure 4.13). Directly west of Tampa Bay, all recordings identify that most red grouper calls are detected between the 30 to 50 m isobaths. In the northern portion of our study area, red grouper are consistently observed from 30 to 93 m water depths. No red grouper were detected in depths greater than 93 m.

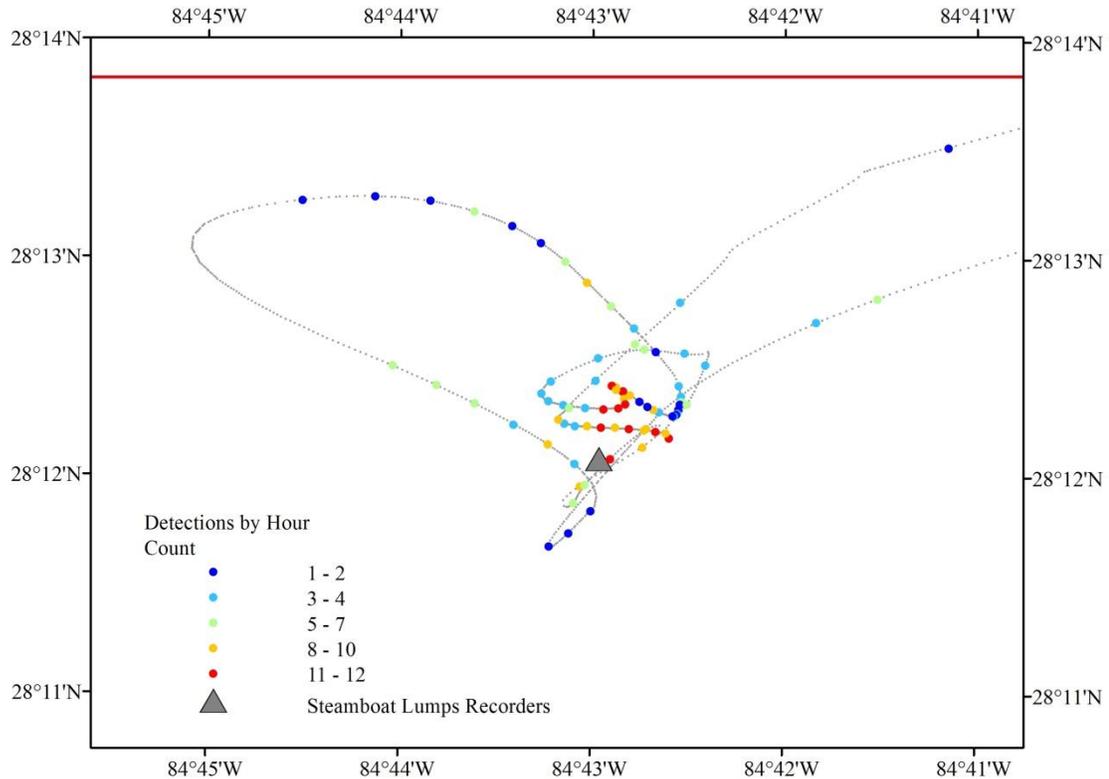


**Figure 4.12.** Interpolated glider tracks (●) with hourly bins of red grouper call detections (colored dots). The values represent the number of files within that hour that contained red grouper.



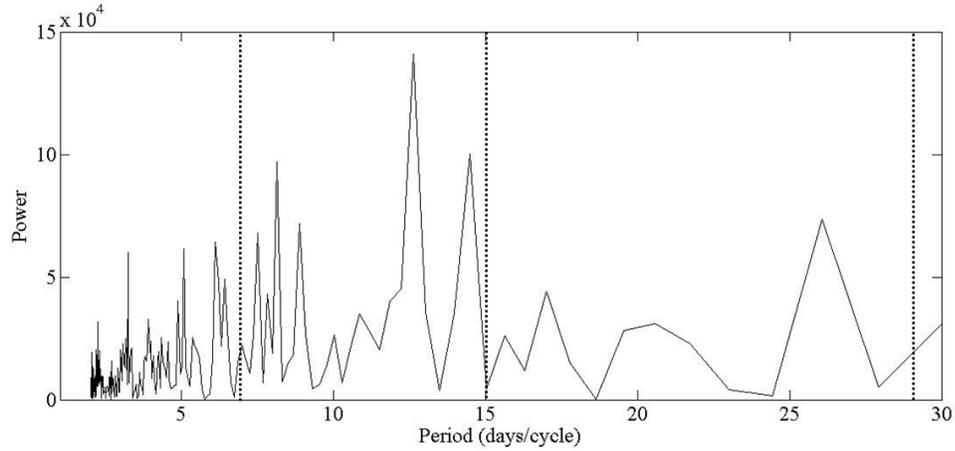
**Figure 4.13.** Red grouper call detection rates from glider data (Detections by Hour) and manually analyzed stationary data (File Percentage). Locations of the Steamboat Lumps and RG3 recorders (▲), the boundaries of Steamboat Lumps (red box), and the lower boundary of the Florida Middle Grounds (green box) are also shown.

The track for Mission 53 deployed March 29 – April 15, 2011 was specifically selected to overlap the area within Steamboat Lumps where stationary recorders were deployed. A portion of this glider track within Steamboat Lumps and associated red grouper detections binned by hour is illustrated in Figure 4.14. Hourly bins with the highest number of files that contained red grouper sounds were detected closest to the stationary recorder site. Areas further away showed less calling per hour.

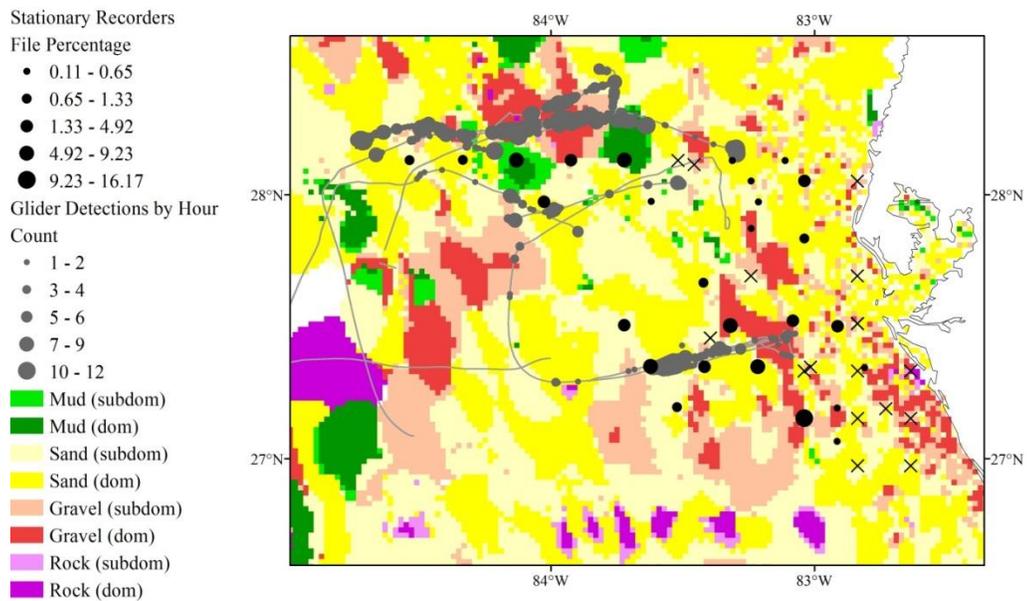


**Figure 4.14.** Red grouper detection counts binned by hour for Mission 53, deployed March 29 -April 14, 2011, within Steamboat Lumps. Location of the interpolated glider track (●) and stationary recorders deployed in Steamboat Lumps (▲) are also shown. The red line denotes the northern boundary of Steamboat Lumps.

Environmental Data. Peaks in red grouper sound production did not correspond to the approximately 7 day cycle of the four moon phases (first quarter, new moon, last quarter or full moon), the approximately 15 cycle between any two moon phases (e.g., new or full moon) or the approximately 29 cycle of a single moon phase (Figure 4.15). The location of red grouper sound production varies over gravel, mud and sand bottom types (Figure 4.16). Rock bottom is outside of the study area boundaries or beyond the depths in which red grouper sound were observed.



**Figure 4.15.** FFT of the daily counts of files containing red grouper sound collected in Steamboat Lumps. Vertical dashed lines indicate sub-lunar and lunar cycles of moon phase (7, 15 and 29 days).

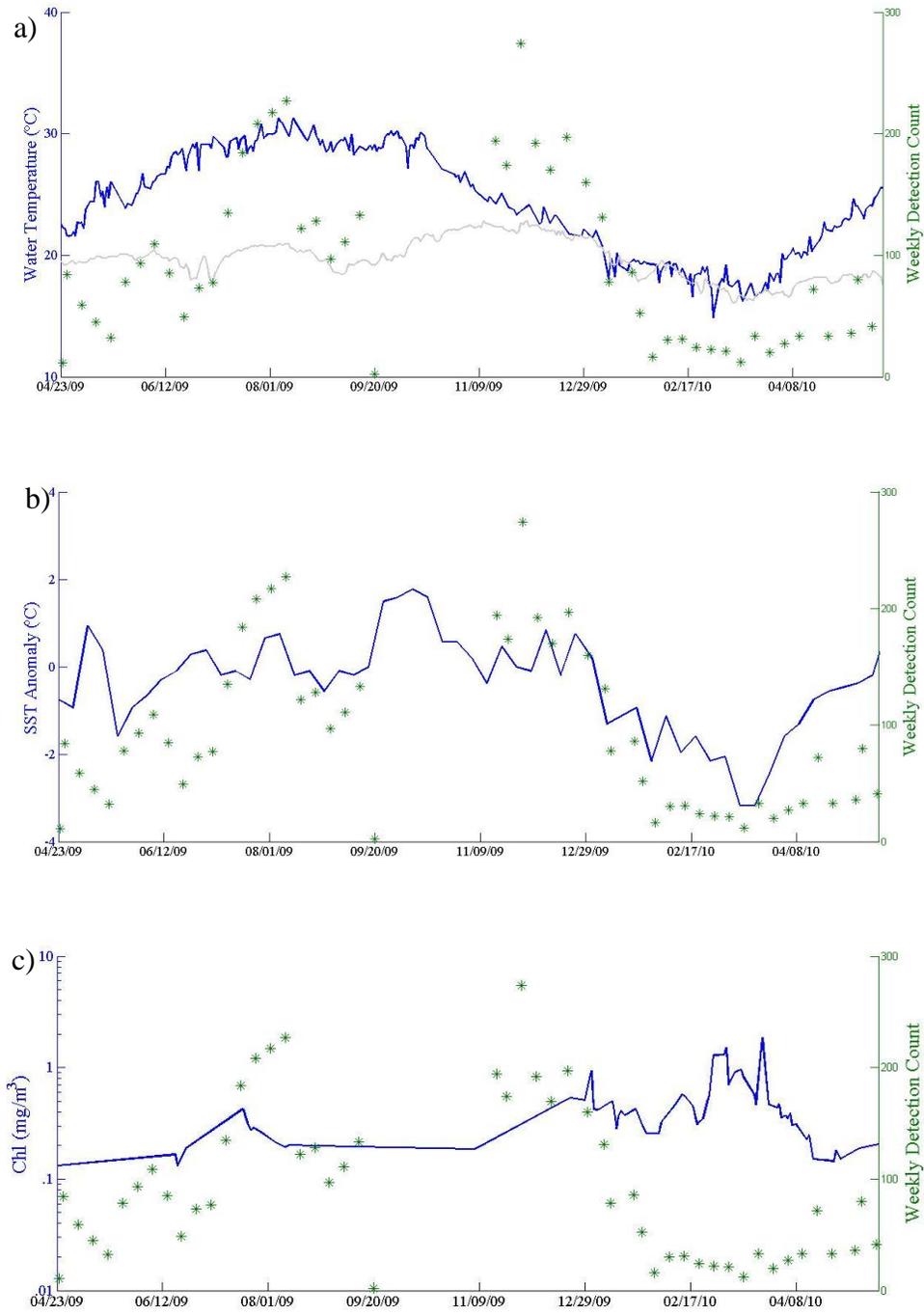


**Figure 4.16.** Red grouper sound production with onto bottom type from Jenkins (2011). Red grouper call observations from manually analyzed stationary data (File Percentage) and glider data (Detections by Hour) are presented. Interpolated glider tracks (•) and stationary recorders where no red grouper were detected are also shown (x). Dominant bottom types (dom) indicate at least 66% of the sediment is composed of that bottom type. Subdominant (subdom) indicates that area has 33% or more of that bottom type.

Weekly-binned red grouper detection counts from the stationary sites were significantly correlated to SST, SST anomaly, and Chl (Table 4.4). Figure 4.17 illustrates the positive correlation of weekly detection counts to SST and SST anomaly, and the negative correlation to Chl. Bottom temperature collected by *in situ* temperature loggers in Steamboat Lumps shows a similar but more muted pattern of seasonal temperature changes compared to SST (Figure 17a). Water temperature and fluorescence collected by the glider were not significantly correlated to red grouper calls observed in the glider acoustic files.

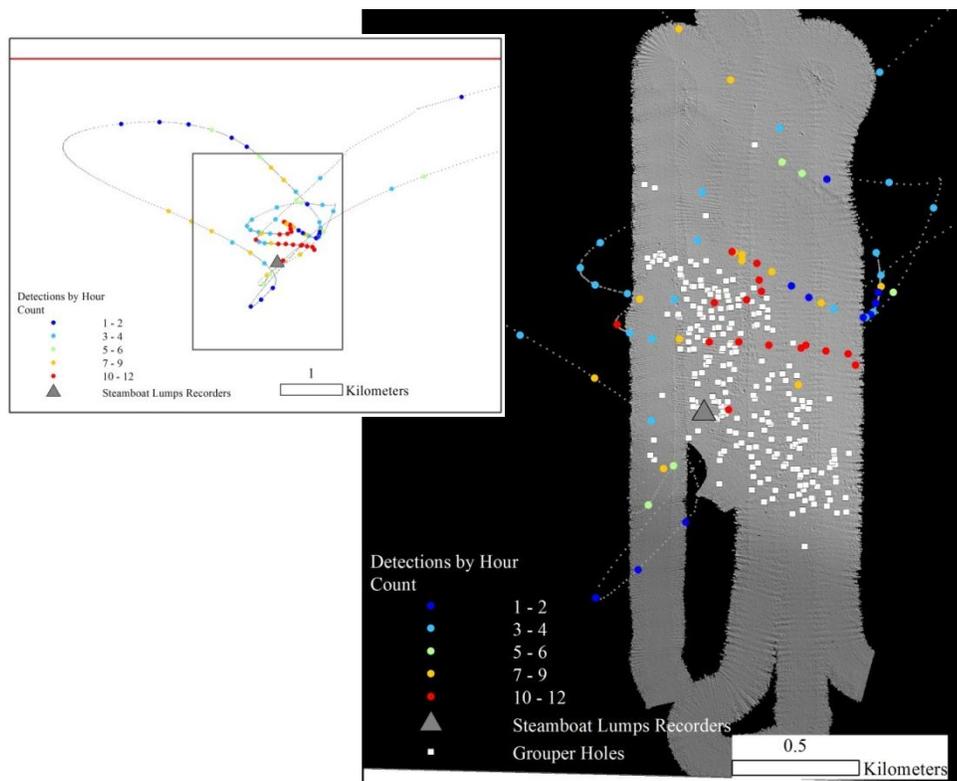
**Table 4.4.** Correlation of weekly median SST (°C), Chl (mg m<sup>-3</sup>) and SST anomaly (°C) values, and weekly red grouper detection counts. Red grouper calls are separated by stationary recorder data that were automatically detected (Automated Detection) and analyzed manually (Manual Analysis), and glider data (Glider). Water temperature (Water temp; °C) and fluorescence (Fluor; µg L<sup>-1</sup>) collected from on-board sensors are compared with the glider acoustic data. The number of files containing red grouper sounds (N) and the number of weeks within a year that red grouper sounds were present (N (weeks)), and pairwise linear correlation coefficients (r) are noted. P-value indicates if the red grouper count and environmental parameter are significantly correlated.

Site	N	N (weeks)	Parameter	r	p-value
Automated Detection	6,148	50	SST	0.65	<<0.05
			Chl	-0.46	<0.05
			SST anomaly	0.67	<<0.05
Manual Analysis	1,333	66	SST	0.74	<<0.05
			Chl	-0.45	<0.05
			SST anomaly	0.54	<<0.05
Glider	2,581	21	Water temp	-0.37	0.19
			Fluor	0.15	0.61



**Figure 4.17.** Time series from Steamboat Lumps. Weekly red grouper detection counts (\*) are shown with associated a) SST (blue line) and *in situ* bottom temperature (gray line), b) SST anomaly and c) Chl.

Holes excavated by male red grouper in Steamboat Lumps have been identified from multibeam data collected in 2009 (Wall et al. 2011). The location of these holes, the position of stationary Steamboat Lumps recorders deployed in 2009, and the track and red grouper detection counts by hour identified from Mission 53 glider data are illustrated in Figure 4.18. Red grouper hourly counts coincide largely with areas of higher hole density. The number of red grouper files per hour decreased in areas away from the densely located holes. The location of the stationary recorders deployed in Steamboat Lumps appears to be within the densely populated area of holes, and thus potentially sound-producing male red grouper.



**Figure 4.18.** Red grouper detection counts identified in Mission 53, binned by hour, overlaid on multibeam data collected in Steamboat Lumps in 2009. Location of the Mission 53 interpolated track (●), stationary recorders deployed in Steamboat Lumps (▲) and the grouper holes identified from multibeam data (□) that were presented in Wall et al. (2011) are also shown. The grey box within the inset shows where multibeam data were collected.

## Discussion

Red grouper sound production was observed throughout the day and night, and during all months in which data were recorded. Peaks in calling were correlated to sunrise and sunset. The varying gradients in crepuscular peaks, namely between Steamboat Lumps and RG3, are attributed to the bottom depth in which sound was produced. Red grouper are a demersal species and cues from changes in sunlight will likely be muted with increased depth. Sound production peaked in late summer (July and August) and early winter (November and December).

GSI data collected from red grouper in the eastern Gulf of Mexico indicate that spawning occurs in the late winter to late spring (March to May) with a peak in April (Moe 1969, Coleman et al. 1996, Collins et al. 2002). This is corollary to the peaks in yearly sound production identified in this study. A ten year dataset (1991-2001) of female GSI show variation within spawning peaks (36 – 82% of active females observed between March and May), and active females were observed, though infrequently, throughout the year (Collins et al. 2002). Collins et al. (2002) also observed an overall lack of reproductive synchrony among female and male red groupers, which is attributed to pair-spawning within small groups thus limiting the numbers of actively spawning fish. This finding is supported by a shift in spawning period for male red grouper (December to April) and female red grouper (January to March) in the southern Gulf of Mexico (Brulé et al. 1999). Compared to the eastern Gulf of Mexico, these earlier spawning peaks are attributed to the variation in water temperature from northern to

southern Gulf of Mexico, suggesting that to some degree spawning is related to water temperature (Coleman et al. 1996, Brulé et al. 1999, Collins et al. 2002). The lack of synchrony between yearly sound production peaks observed in this study and spawning peaks supports the lack of reproductive synchrony but also suggests that red grouper do not produce sound solely during courtship. Territorial behavior is another potential cause (Mann & Lobel 1995, Nelson et al. 2011). However, further research that incorporates long-term video monitoring in addition to acoustic recordings is needed to understand better seasonal peaks in red grouper sound production.

The lack of any strong seasonal peak in calling for the stationary recorders that were manually analyzed between 1600-2200 hours is attributed to the varying levels of hourly call production throughout the year. The nearly year-long, fine-resolution analysis conducted in Steamboat Lumps demonstrates that most sound production occurs between sunrise and sunset, and the hourly range in which calling occurs changes throughout the year as the times of sunrise and sunset change. For example, from November to February, it appears the majority of calling occurs before 1600 and would have been missed by the manual analysis. By analyzing a static range of hours throughout the year in this study, it is likely that the seasonal variation in calling will not be effectively observed.

The spatial range over which red grouper sounds were detected is quite extensive, from approximately 15 to 93 m bottom depth. For most of the study area, the majority of red grouper sound production occurred in waters between 30 and 50 m deep. Along the northern boundary of the study area, red grouper sounds were detected predominately in water depths between 40 and 93 m. Preferential habitat in the Florida Middle Grounds

(hard bottom) and Steamboat Lumps (excavated sediment) are suspected to be the cause for the slight shift in depth range in this region. The lack of sound production in depths beyond 100 m suggests that red grouper are not likely present in these areas, although red grouper are expected to inhabit depths up to 400 m (Moe 1969). It should be noted that areas where no red grouper sound was recorded does not implicitly mean that no red grouper are there. It is possible, especially nearshore, where immature females are common (Brulé et al. 1999), that non-soniferous, female red grouper may be present.

Nelson et al. (2011) recorded RMS received sound pressure levels (SPL) of red grouper calls from 110 to 142 dB re 1  $\mu$ Pa. Since acoustic recorders were placed very near (< 2 m) occupied red grouper holes, 142 dB re 1  $\mu$ Pa will serve here as a source level estimate. If a conservative spherical spreading loss model is assumed (Urick 1983), this same call would be reduced to 102 dB re 1  $\mu$ Pa 100 meters away from the source. We suspect that the stationary recorders, especially the bottom-mounted designs, would have comparable received SPL of red grouper calls due to the similarity in instrumentation and environment. Gliders, however, traverse the water column and produce self-generated noise. The maximum RMS received SPL of red grouper calls (N=167) recorded from Mission 31 (July 14 – 21, 2009) was 106 dB re 1  $\mu$ Pa. The average RMS received SPL for background noise within the same bandwidth was 80 dB re 1  $\mu$ Pa (SD=4 dB re 1  $\mu$ Pa). The background noise in this area would ensure that the Nelson et al. (2011) source level estimate could be detected by the recorder 100 m away. Applying the same spreading loss model to the glider acoustic data, the 106 dB re 1  $\mu$ Pa red grouper call would be reduced to 66 dB re 1  $\mu$ Pa at a distance of 100 m – well below the average background noise. Therefore, red grouper would have to be within

40 m of the glider to be detected. Of course, these estimates are rudimentary and exact source level calculations are needed in order to determine, accurately, each design's detection range.

File attributes (hour and month of recording) and associated environmental data (depth, SST, SST anomaly and Chl) all influence red grouper sound production observed in this study. Glider water temperature and fluorescence sample means, however, were not significantly correlated to red grouper calls. These measurements are made as the glider ascends and descends the water column and therefore most of the variation recorded is a result of vertical stratification. Conversely, SST and Chl provide a consistent view of the ocean surface. Although red grouper live on the seafloor and can be up to 100 m away from the ocean surface, it is possible to characterize the seasonal cycles of the areas where fish live using the surface satellite-derived information. The positive correlation of weekly red grouper counts to SST may be a reflection of season as red grouper peaked in the spring and summer (when SSTs are high). However, SST anomaly data indicate red grouper detection counts were positively correlated to changes in surface temperature without the bias of seasonal fluctuations.

SST data indicate that the summer of 2008 and particularly the winters of 2009 and 2010 were anomalously cold. This is supported by the median SST anomaly values associated with stationary recorder files that contained red grouper sounds for each year (June to September 2008: -0.28; April to December 2009: -0.09, and January to May 2010: -0.94) and is illustrated in Figure 18b. The positive correlation between red grouper detection counts and SST anomaly data suggest that anomalously cold temperatures do negatively influence sonic activity. However, as the time series of the

three years' datasets do not overlap nor are there glider missions that cover the same area in the same month among the years, it is difficult to determine the degree to which anomalous temperatures may have shifted daily and/or monthly distributions of red grouper sound production. Additional multi-year data are needed.

Steamboat Lumps is established red grouper spawning habitat. Previous research in this area has employed point-source acoustic monitoring techniques (Nelson et al. 2011). The use of glider technology allows the ability to monitor large areas over a relatively short period of time, providing a near synoptic view of sound production within this invaluable habitat. High-resolution multibeam bathymetry data collected in 2009 show the locations of grouper holes (Wall et al. 2011). Holes are excavated by male red groupers and used as their home territory from which they court females for reproduction. The stationary recorders were purposely deployed in Steamboat Lumps where red grouper were previously observed (C. Koenig pers. comm). Numerous nearby holes identified from the multibeam data and the extensive amount of sound production recorded confirms this. Although glider Mission 53 (March 29 to April 15, 2011) occurred about two years after stationary data and multibeam data were collected, areas of high hourly call counts were typically observed near hole locations. This correspondence supports that red grouper maintain these holes over time (Coleman et al. 2010, Wall et al. 2011).

Passive acoustic monitoring allows for vast amounts of acoustic data to be recorded over large spatial and temporal scales. Several PAM designs were employed during this study, most proving to be successful. Variation in recovery rates among the stationary deployments are a result of design and the environment in which the recorder

was deployed (Dudzinski et al. 2009). The use of a surface expression, such as a buoy, can facilitate retrieval but also leaves the equipment prone to removal by contact with boat propellers or territorial fishermen. These are the most likely reasons for unrecovered inshore recorders. Retrieval of offshore recorders was difficult due to the increased transit distance and necessary field conditions for recovery (calm wind and seas). In addition, a large amount of biofouling accumulated on the polypropylene lines causing the surface, subsurface and cage containing the DSG to sink to depths below normal scuba diving limits (> 40 m). Despite these setbacks, nearly 1 TB of acoustic data was retrieved comprising extensive information on the sound production of red grouper, many other species of fish (Wall et al. 2012a), marine mammals, and anthropogenic noise (Wall et al. 2012b). To ensure future success in PAM recovery, we suggest additional deployment designs, such as the use of acoustic releases, be explored to reduce the issues encountered in this study.

Recreational and commercial ship traffic are common throughout the study area, with increased numbers nearshore (Wall et al. 2012b). Broadband, high energy noise associated with boats can impede the detection of red grouper sounds. However, the low percentage of files that contain boat noise recorded during the stationary (2.6%; 1,647/63,993 files) and glider deployments (1.6%; 403/25,129 files) suggests that the impact is not significant. Wall et al. (2012a) identified extensive nocturnal chorusing of other fish species within the study area. Masking from these species is more likely to limit detection of red grouper calling at night, however, the extent of masking is not known.

The results of this study indicate a lack of strong correlation between red grouper sound production and known peaks in spawning. Although acoustic monitoring of this species may not provide insight into the timing of spawning throughout the year, it is an effective tool to identify areas of potential spawning activity by recording the presence of potentially reproductively mature male red grouper. By mapping sound production and thus male red grouper, areas of potential spawning habitat become immediately apparent. As demonstrated in this study, acoustic data can be coupled with high-resolution bathymetric data to verify the occupation of suspected male territory (holes) and provide a complete understanding of effective spawning habitat. In addition, long-term passive acoustic studies that provide systematic monitoring can be a valuable assessment tool for multiple species on the WFS.

## Chapter 5: Large-Scale Passive Acoustic Monitoring of Fish Sound Production on the West Florida Shelf

### Abstract

Toadfish and four unknown fish-related sounds were identified in a visual analysis of passive acoustic recordings that were made in fixed locations and using autonomous underwater vehicles (AUVs) in the eastern Gulf of Mexico during periods between 2008 and 2011. The goals of this research were to map the spatial and temporal occurrence of these five sounds over the west Florida shelf. Variation in sound production was correlated to environmental parameters (bottom depth, bottom type, temperature, and chlorophyll concentration) to understand the variability in seasonal calling and to help discern the sources of the unknown fish sounds. Toadfish boatwhistles were recorded throughout the entire day, with the majority of calling observed between 1500-0400 hours. Annual peaks coincided with the suspected spawning period in the late spring to early summer. The four unknown sounds were termed: '100 Hz Pulsing', '6 kHz Sound', '300 Hz FM Harmonic', and '365 Hz Harmonic'. The 100 Hz Pulsing has the characteristics of a cusk-eel call but it occurs in a much lower frequency range (100-500 Hz). The majority of sound production was observed at night (sunset to sunrise) with annual peaks in the spring and fall. The 6 kHz Sound was observed exclusively at night between 15 to 50 m depths, and occurrence reduced significantly in the winter (December to February). The peak frequency of the 6

kHz Sound was positively correlated to satellite-derived sea surface temperature (SST) and negatively correlated to chlorophyll concentration. The 300 Hz FM Harmonic was observed largely (89%) at night and appeared offshore (40-200 m). The 365 Hz Harmonic was observed 98% of the time at night and in waters less than 40 m deep. Calling was largely consistent throughout the year with a small peak in the summer (June – September). Fundamental frequency of the 365 Hz Harmonic increased significantly with increasing SST, while call duration decreased significantly. Potential sources of the unknown sounds are presented.

## Introduction

Soniferous fish use sound for communication associated with parental, courtship, spawning, aggressive and territorial behavior (Lobel et al. 2010). Most fish calls are species-specific and repetitive; this enables sound production to be used for identifying species distribution and behavior. Recent developments in passive acoustic technologies have facilitated marine bioacoustics studies to effectively monitor soniferous fish over a wide range of habitat, depths and time periods (Mann & Lobel 1995, Lobel 2002, Luczkovich et al. 2008, Van Parijs et al. 2009, Lobel et al. 2010, Locascio & Mann 2011). Passive acoustic monitoring (PAM) of fish has been successfully demonstrated using moored devices (e.g., Locascio & Mann 2008, Nelson et al. 2011) and integrated into autonomous vehicles (Wall et al. 2012a). Further, the acoustic recordings allow not only sound-producing fish (100~2,000 Hz) to be recorded, but also marine mammals (3,000~200,000 Hz) and anthropogenic noise (50 Hz ~100,000 Hz).

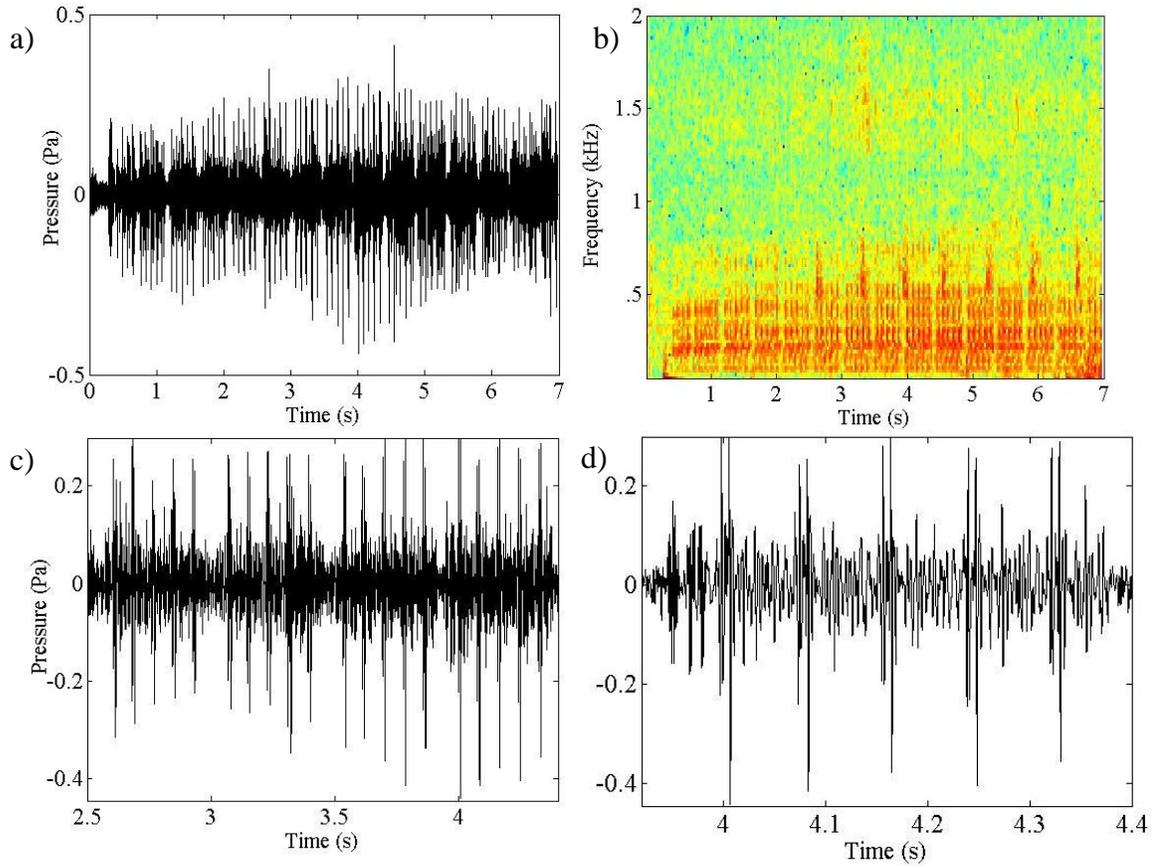
The research presented here expands on a pilot study of acoustic data collected by a hydrophone-integrated Slocum glider, which demonstrated the utility of this technology as a platform for passive acoustic monitoring (Wall et al. 2012a). The glider was deployed off Tampa Bay for one week during which time sounds from several identifiable fish (including, red grouper, *Epinephelus morio*, and toadfish, *Opsanus* sp.) were recorded. In addition, at least three unknown biological sounds suspected to be produced by fish were also recorded. Since the initial study, multiple glider deployments and several deployments of stationary acoustic recorders have been conducted for the

purpose of mapping the spatial and temporal patterns of red grouper, and marine mammals. This paper discusses large-scale, long-term sound production of one known (toadfish) and four unknown fish-related sounds that were commonly recorded in the acoustic data.

As described in Wall et al. (2012a), toadfish sounds recorded off Florida are suspected to be produced by two species: *Opsanus beta* in nearshore habitats and *O. pardus* in offshore habitats. The sounds produced by these two species are similar in that they are typical to toadfish ‘boatwhistles’, but they do have distinct features (Wall et al. 2012a). Three of the unknown sounds presented here have been previously described. They consist of: 1) a 200-500 Hz wide band around 6 kHz (‘6 kHz Sound’) that appears continuously between sunset and sunrise; 2) a frequency modulated harmonic with an average peak frequency of 300 Hz (‘300 Hz FM Harmonic’) approximately 2.25 s in length and typically containing four abrupt changes in frequency, and 3) a tonal harmonic with a peak frequency of 365 Hz (‘365 Hz Harmonic’) and 0.51 s in length (Wall et al. 2012a). All unknown sounds were observed only at night. The fourth unknown sound, which was not described in Wall et al. (2012a), consists of a series of pulses (pulse train) with a fundamental frequency of approximately 100 Hz (‘100 Hz Pulsing’) (Figure 5.1). Average call duration is  $4.5 \pm 1.5$  s (N=27) and harmonics are present up to approximately 650 Hz. Pulse trains typically consist of five pulses, however, four pulses were observed in some recordings.

The goals of this research were to identify habitat ranges for the five sounds by mapping sound production, and determine the daily and seasonal patterns in calling.

Sound occurrence was compared to environmental data to understand the variability in seasonal calling and to help discern the sources of the unknown fish sounds.



**Figure 5.1.** Example of the ‘100 Hz Pulsing’ sound. a) Waveform and b) spectrogram of the full signal. Close up of the waveform shows c) repeated 5 pulse trains and d) the detail of a single 5 pulse train. The spectrogram was created using a 2,048 point Hann-windowed fast Fourier transform with 50% overlap.

## Methods

### *Data Collection.*

Acoustic Data. Acoustic data were collected across the West Florida Shelf (WFS) off west-central Florida in the eastern Gulf of Mexico (see Figure 4.1). All acoustic data were recorded using the digital acoustic recording system, Digital SpectroGram recorder (DSG; Loggerhead Instruments). Detailed descriptions of the stationary recorder and hydrophone-integrated glider designs are provided in Wall et al. (2012a). Stationary acoustic recorders were deployed in June 2008 for one month, in July 2008 for two to five months, and in June 2009 for approximately one year (N=5, 18, and 63 recorders, respectively). In addition, a hydrophone was integrated into the aft cowling of several Slocum electric underwater gliders (Teledyne Webb Research) to record sound while concurrently collecting a suite of environmental and optical parameters. Fifteen glider missions one to four weeks in duration were conducted on the WFS between April 2009 and April 2011.

Environmental Data. Satellite-derived sea surface temperature (SST) and chlorophyll a concentration (Chl) data were collected for periods and areas in which acoustic data were recorded. SST was derived from infrared data collected by NASA's Moderate Resolution Imaging Spectroradiometer (MODIS) onboard satellites Aqua and Terra. Chl was calculated from visible data collected by ORBIMAGE's Sea-viewing Wide Field-of-View Sensor (SeaWiFS) using standard SeaDas processing. Time series data were calculated for each stationary site. All satellite data processing was performed

using IDL (Research Systems, Inc). Sunrise and sunset, and lunar cycle data were obtained between June 2008 and April 2011 (USNO 2012a, b).

### *Data Analysis*

Acoustic Data. Acoustic files collected at the stationary sites were analyzed manually as spectrograms to visually identify fish sounds. Spectrograms were created using 2,048 point Hann-windowed fast Fourier transforms (FFTs) with 50% overlap. Due to the large number of acoustic files recorded throughout the deployments, only files recorded between 1600-2200 hours (local time) were reviewed for determining the spatial and seasonal distribution of sound occurrence. Initial research identified that time frame encompasses sound production for all sounds of interest (Wall et al. 2012a). For analysis of daily variability, this dataset was supplemented by files recorded during all hours that were manually analyzed during a preliminary analysis of an automated red grouper detection algorithm on the entire dataset.

All acoustic files recorded during the glider missions were analyzed manually. Files in which sounds of interest were visually identified were binned into one hour intervals over the glider track.

All sounds identified in the acoustic files were binned by hour and month and normalized by the total number of files analyzed per hour and per month (“call per unit effort”) to show daily and seasonal patterns without a sampling bias. For the fixed recorders, only sites where at least one sound was detected were included in the normalization. To better understand how hourly calling patterns changed throughout the year, a matrix of the number of calls detected per hour for each month was created for the stationary data and the glider data. Separating these two datasets for this analysis shows

where gaps in data collection exist and helps to better understand relative increases and decreases in calling. These analyses were completed using MATLAB (Mathworks). Spatial maps of the data were created using ArcGIS (ESRI).

Visual analysis of the stationary recorder dataset indicated that characteristics of the 6 kHz Sound and 365 Hz Harmonic changed over time. To quantify these changes, the frequency of the 6 kHz Sound was estimated from FFTs with 100 Hz resolution applied to files in which the sound was present without interfering noise (e.g., boat or mechanical noise). The frequency between 3-7 kHz with the highest amplitude was extracted for each file, along with the associated amplitude. To reduce error from noise in the frequency and amplitude data, outliers (three standard deviations away from the mean) were removed, a non-linear interpolation was applied and the data were then smoothed using a 20-point moving average. Only stationary sites that recorded sound for over six months were included to ensure seasonal variation was incorporated. Long-term sound production is displayed as a composite spectrogram in which 100 Hz resolution FFTs are applied to each file and then placed together chronologically to create an image. The duration and fundamental frequency of 365 Hz Harmonic calls, with signal to noise ratios of at least 6 dB, were measured in the frequency domain. These analyses were completed using MATLAB.

Environmental Data. Trends in SST and Chl, and in-water temperature and fluorescence were compared to sound production patterns identified from the stationary recorders and glider missions, respectively. The in-water temperature and fluorescence data were linearly interpolated and then smoothed using a ten-point window for comparison to acoustic data. Frequency and amplitude of the 6 kHz Sound were

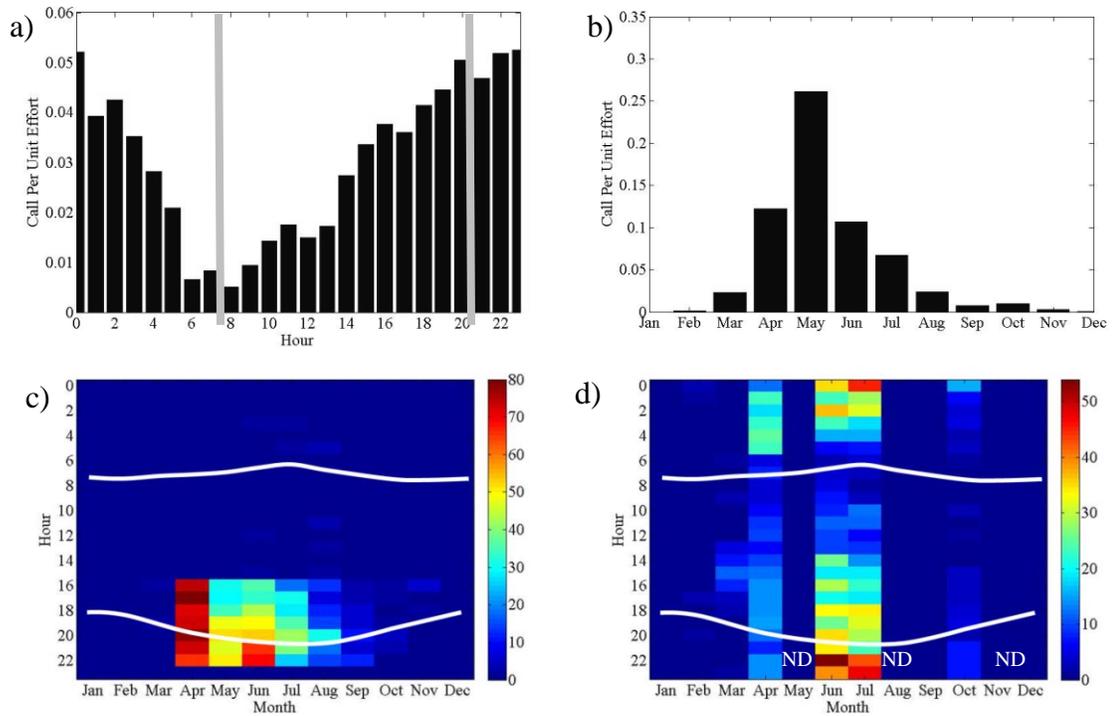
correlated to SST and Chl. Call duration and fundamental frequency of the 365 Hz Harmonic were correlated to SST and Chl. Weekly counts of files containing each sound were extracted from a stationary recorder selected for each sound that had a high occurrence of calls and a long time series (toadfish: recorder RG3; 100 Hz Pulsing: recorder B5b; 6 kHz Sound: recorder B52; 300 Hz FM Harmonic: recorder B2b; 365 Hz Harmonic: recorder B42). An FFT was applied to the time series of weekly counts to identify the presence, if any, of cyclical peaks in calling. These peaks were then compared to the cyclical pattern of the moon phases to determine if variation in sound production was correlated to lunar phase. Daily and seasonal patterns in sound production were compared to sunrise and sunset.

## Results

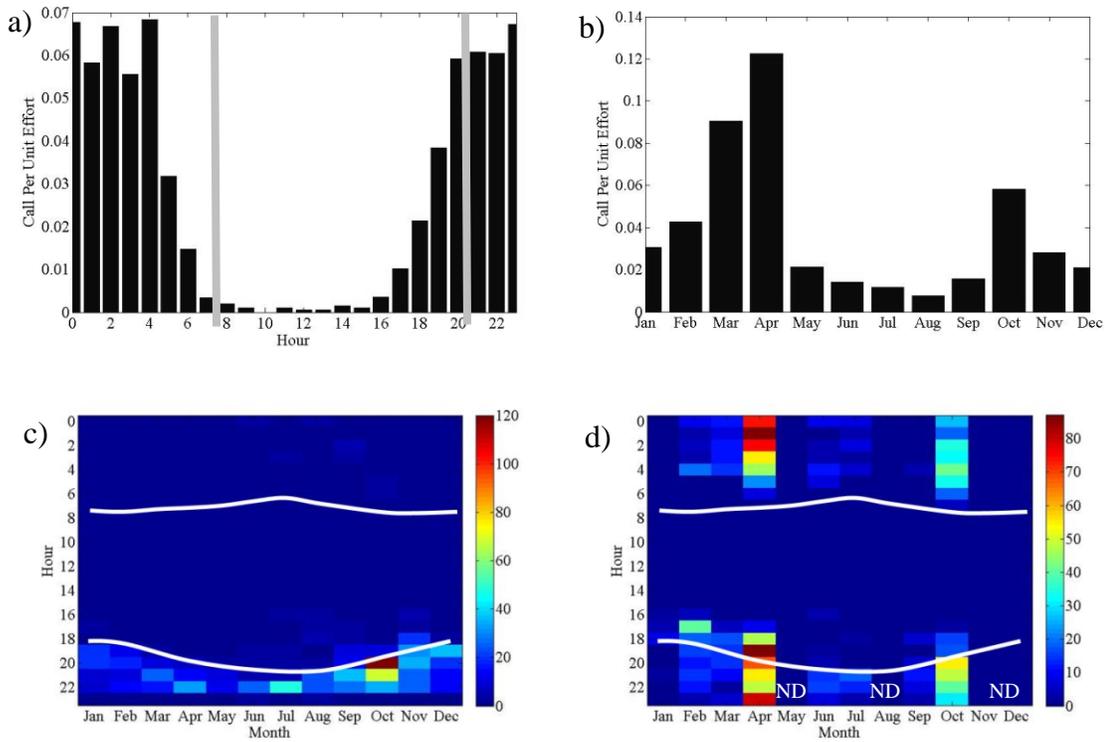
*Data Collection.* The acoustic library collected from stationary recorders deployed at various periods between 2008 and 2010 consisted of 377,728 files. 25,760 files were recorded throughout the fifteen glider missions conducted between April 2009 and April 2011. Acoustic data were collected by the stationary recorders during all months and all hours. Gliders recorded acoustic files during all hours but not in the months of August, November and December. Detailed descriptions of the location and duration of recordings are provided in Chapter 4.

### *Data Analysis*

Acoustic Data. The hourly and monthly distribution of toadfish calling is illustrated in Figure 5.2. Calling occurred throughout the day, with a drop in sound production in the early morning (6am – 9am). Annual peaks in calling occurred between April and June, and became rare from September to February. The 100 Hz Pulsing was observed largely at night (94% occurrence between 1800-500 hours) and in early spring (March – April), with a secondary peak in October (Figure 5.3).



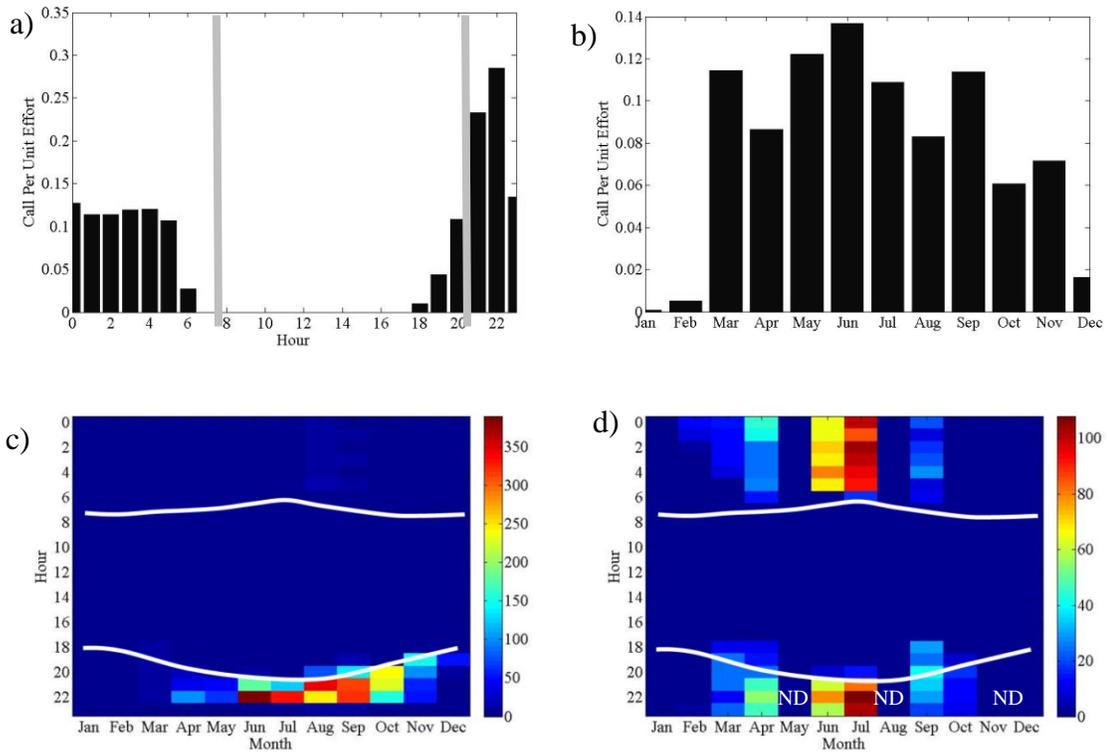
**Figure 5.2.** Toadfish sound production. a) Hourly and b) monthly bins of the number of files that contained toadfish compared to the total number of files analyzed. Matrix of the number of files per hour per month that contained toadfish sounds identified from c) stationary recorders (from 1600-2200 hours) and d) glider missions. Grey lines indicate mean sunrise (7:05) and sunset (19:40) throughout the year. White lines indicate sunrise (top) and sunset (bottom) times. No acoustic data (ND) were collected by the glider in May, August, November or December. Note the differences in scale.



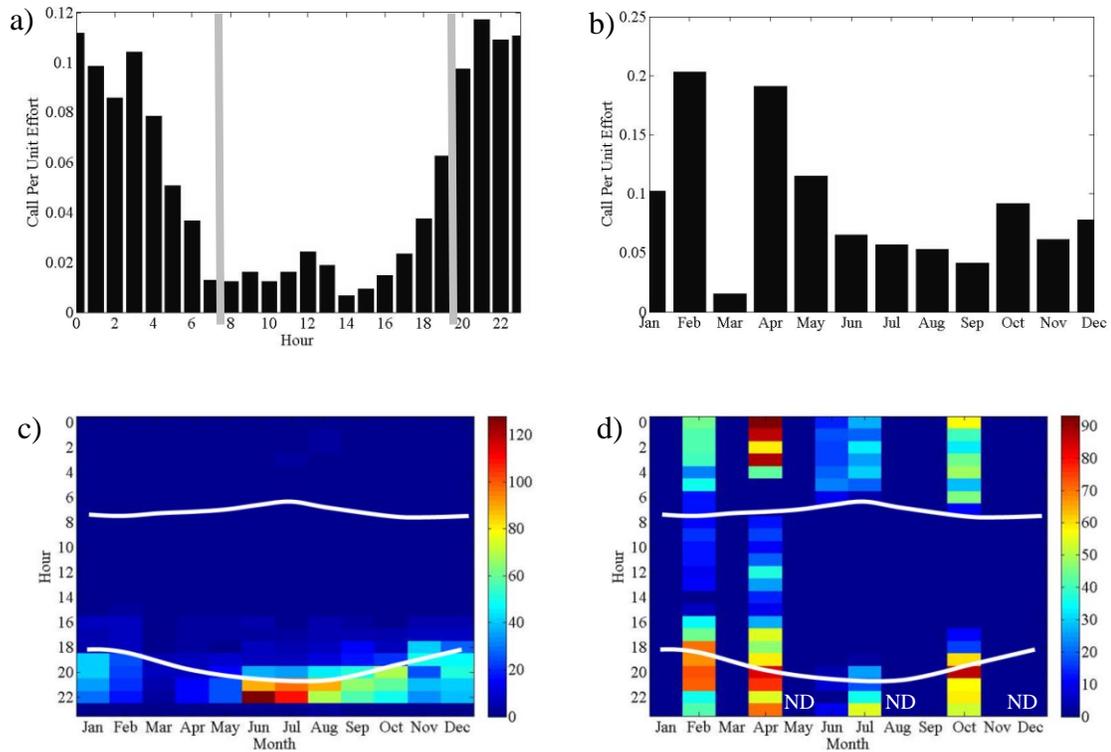
**Figure 5.3.** 100 Hz Pulsing sound production. a) Hourly and b) monthly bins of the number of files that contained 100 Hz Pulsing divided by the total number of files analyzed. Matrix of the number of files per hour per month that contained 100 Hz Pulsing sounds identified from c) stationary recorders (from 1600-2200 hours) and d) glider missions. Grey lines indicate mean sunrise (7:05) and sunset (19:40) throughout the year. White lines indicate sunrise (top) and sunset (bottom) times. No acoustic data (ND) were collected by the glider in May, August, November or December. Note the differences in scale.

The 6 kHz Sound was observed exclusively at night (100% occurrence between 1800 – 600 Hours) with less than 2% of the observations occurring in the winter (December to February; Figure 5.4). The 300 Hz FM Harmonic appeared largely at night (89% occurrence between 1800-600 hours) with a secondary peak mid-day (1200-1300 hours) (Figure 5.5a). Annual peaks were observed in February, April and October with an abrupt decrease in March (Figure 5.5b). The stationary data identified peaks in calling in June and July; however, peaks in February, April and October are identified the glider data (Figure 5.5c,d). This discrepancy is attributed to the 24-hour range in which glider

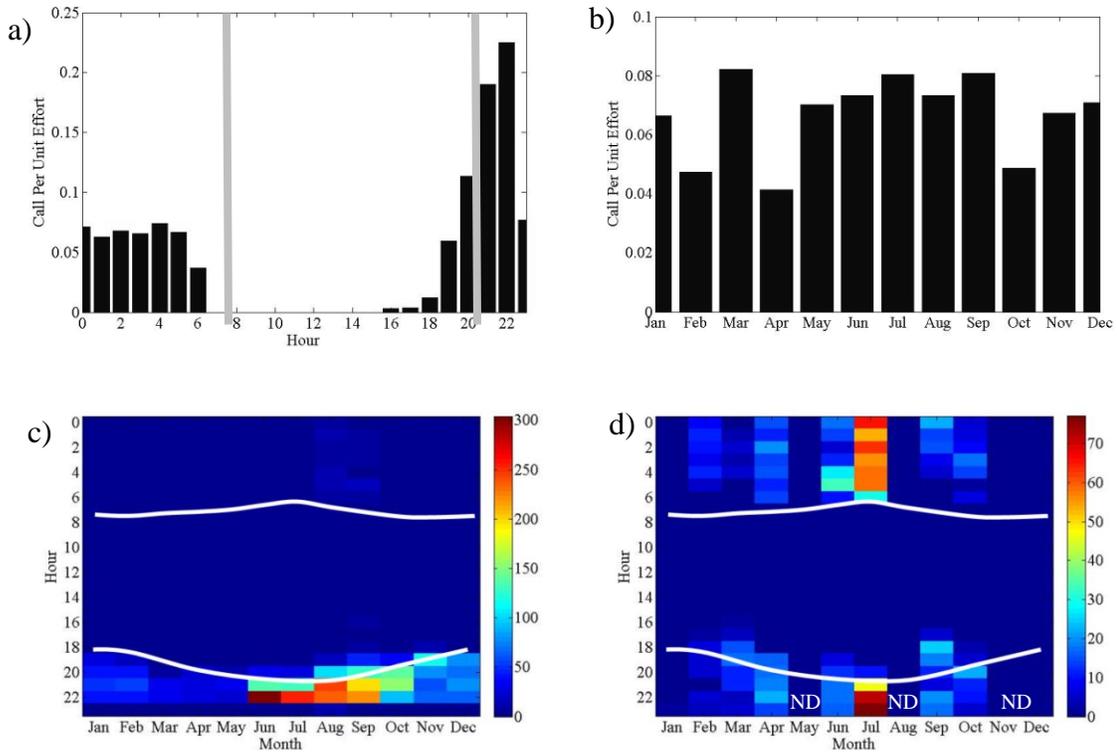
data were analyzed compared to the narrow evening hours in which the stationary recorder data were analyzed, and is supported by the daytime calling observed only in February and April. The 365 Hz Harmonic was observed almost exclusively at night (98% occurrence between 1900-600 hours) and calling was largely consistent throughout the year with a small peak in the summer (June – September; Figure 5.6).



**Figure 5.4.** 6 kHz Sound sound production. a) Hourly and b) monthly bins of the number of files that contained the 6 kHz Sound divided by the total number of files analyzed. Matrix of the number of files per hour per month that contained the 6 kHz Sound identified from c) stationary recorders (from 1600-2200 hours) and d) glider missions. Grey lines indicate mean sunrise (7:05) and sunset (19:40) throughout the year. White lines indicate sunrise (top) and sunset (bottom) times. No acoustic data (ND) were collected by the glider in May, August, November or December. Note the differences in scale.



**Figure 5.5.** 300 Hz FM Harmonic sound production. a) Hourly and b) monthly bins of the number of files that contained the 300 Hz FM Harmonic divided by the total number of files analyzed. Matrix of the number of files per hour per month that contained the 300 Hz FM Harmonic identified from c) stationary recorders (from 1600-2200 hours) and d) glider missions. Grey lines indicate mean sunrise (7:05) and sunset (19:40) throughout the year. White lines indicate sunrise (top) and sunset (bottom) times. No acoustic data (ND) were collected by the glider in May, August, November or December. Note the differences in scale.



**Figure 5.6.** 365 Hz Harmonic sound production. a) Hourly and b) monthly bins of the number of files that contained the 365 Hz Harmonic divided by the total number of files analyzed. Matrix of the number of files per hour per month that contained the 365 Hz Harmonic identified from c) stationary recorders (from 1600-2200 hours) and d) glider missions. Grey lines indicate mean sunrise (7:05) and sunset (19:40) throughout the year. White lines indicate sunrise (top) and sunset (bottom) times. No acoustic data (ND) were collected by the glider in May, August, November or December. Note the differences in scale.

Spatial distributions of the five sounds of interest are illustrated in Figure 5.7. Toadfish sound production is slightly variable on the WFS, with higher densities in the northern and central part of the study area between 30 to 50 m depths (56% of observations; Figure 5.7a). Toadfish were not detected in acoustic files recorded in depths greater than 83 m. 100 Hz Pulsing sound production was much more widespread with calling observed to some degree at almost all stationary recorders (Figure 5.7b).

Glider data indicated higher numbers of files containing this sound in the northern part of our study area. Sounds were present in waters up to 170 m deep.

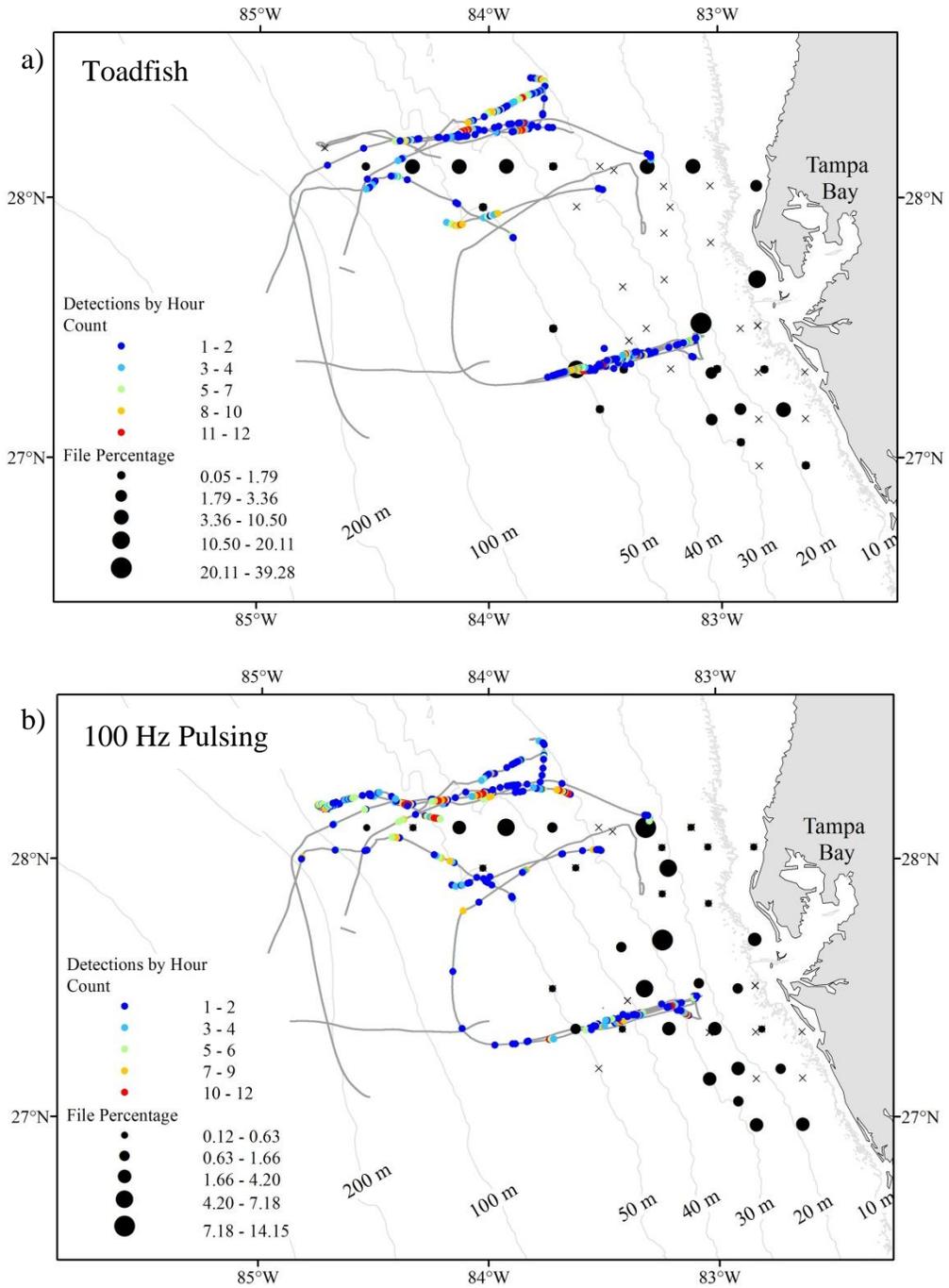
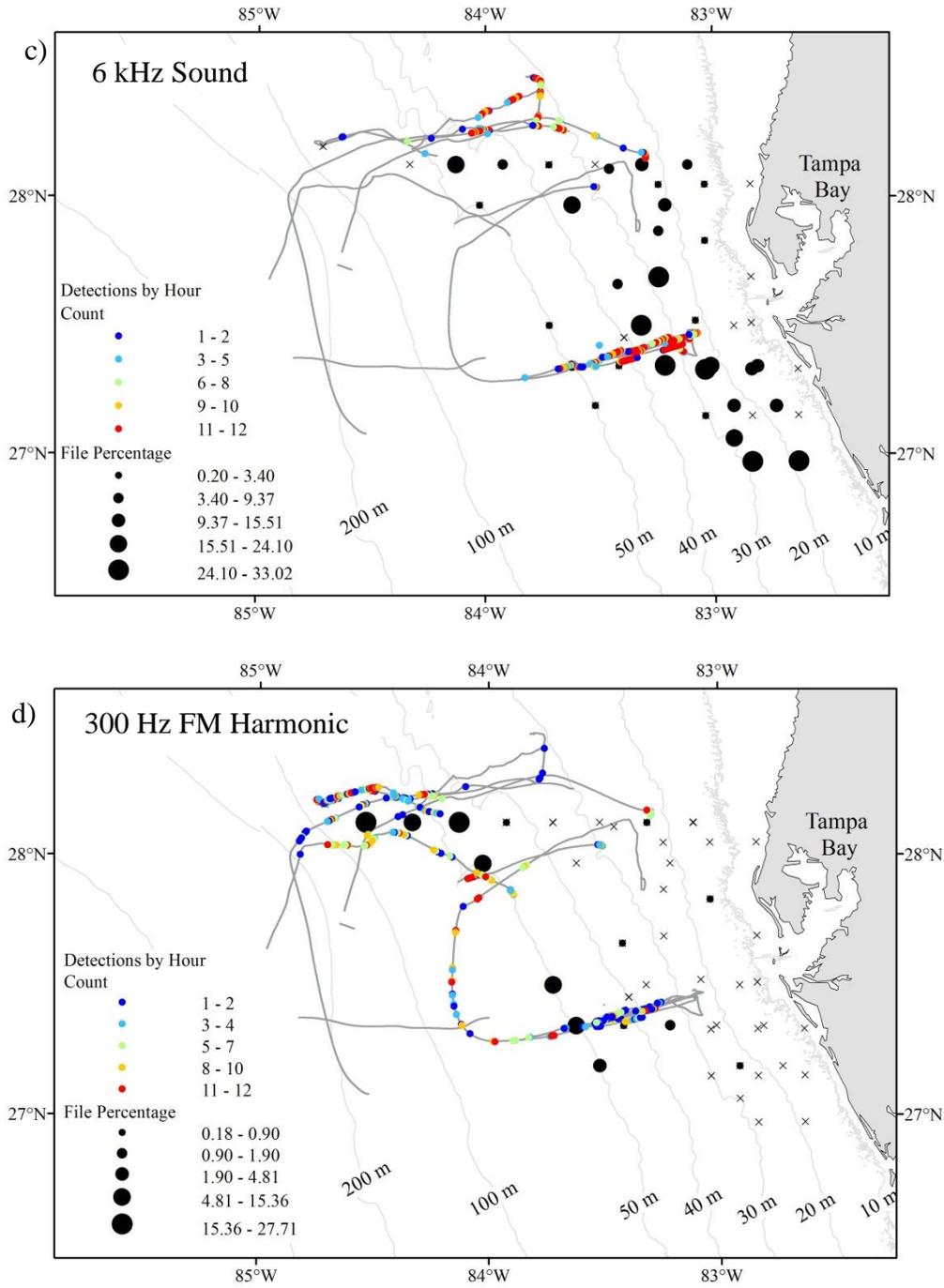
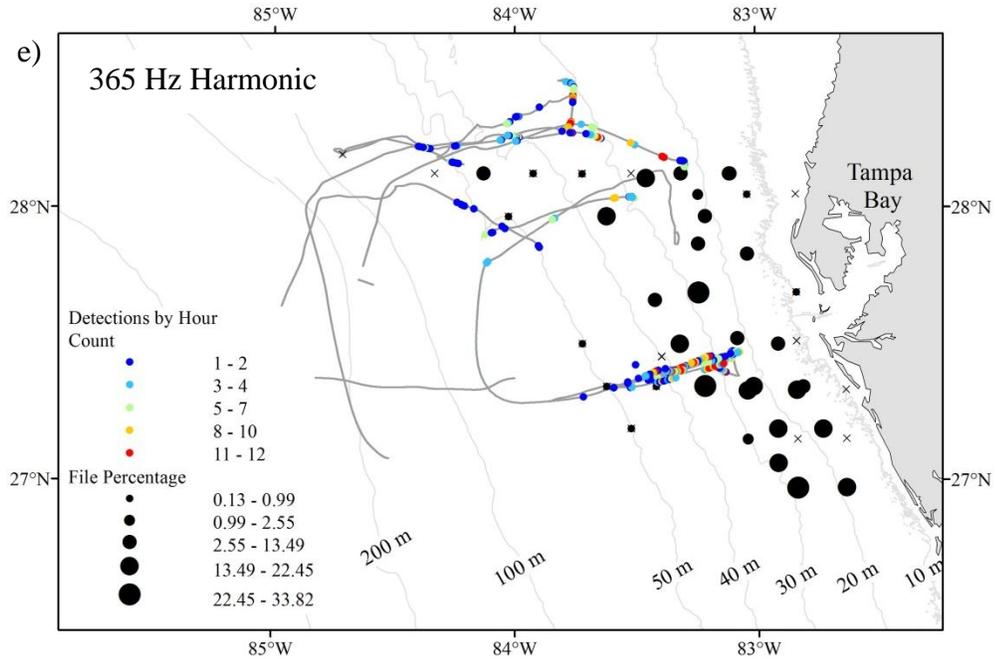


Figure 5.7 Cont.



**Figure 5.7 Cont.**



**Figure 5.7.** Sound distribution for a) toadfish, b) 100 Hz Pulsing, c) 6 kHz Sound, d) 300 Hz FM Harmonic and e) 365 Hz Harmonic. Black symbol size is proportional to the percent of files that contained this sound, compared to the number of stationary recorder files analyzed. Stationary recorders where this sound was not observed are also shown (x). Colored dots indicate the number of files per hour collected by the hydrophone-integrated glider that contained this sound overlaid on the interpolated glider tracks (•). Note differences in scale.

The majority of 6 kHz sound was observed between 15 to 50 m water depths (94% of observations; Figure 5.7c). The glider track within this range in which no 6 kHz Sound was detected was deployed in the winter (Mission 52: 1/31 – 2/12, 2011). The 300 Hz FM Harmonic appears almost exclusively offshore, 40 to 200 m depth (91% of observations; Figure 5.7d). Conversely, the majority of the 365 Hz Harmonic sound was detected inshore (92% of observations occurred in waters less than 40 m deep; Figure 5.7e).

**Table 5.1.** Temperature and chlorophyll concentration for the time and location of toadfish, 100 Hz Pulsing, 6 kHz Sound, 300 Hz FM Harmonic, and 365 Hz Harmonic sounds. Shown are the median and standard deviation (std) values, the pairwise linear correlation coefficients (r) of weekly binned detection counts to associated environmental parameters, and the number of files (N) for each sound. <sup>1</sup>Files derived from stationary acoustic recorders and environmental data are remotely sensed. <sup>2</sup>Files derived from gliders and environmental measurements were taken with on-board sensors.

		SST <sup>1</sup> (°C)	Water Temp <sup>2</sup> (°C)	Chl <sup>1</sup> (mg m <sup>-3</sup> )	Fluor <sup>2</sup> (µg L <sup>-1</sup> )
Toadfish	Median	26.7	21.5	0.3	0.3
	std	3.3	4.2	1.0	0.6
	r	-0.1	0.6	-0.2	-0.4
	p-value	0.6	0.02	0.2	0.2
	N	1,849	566	1,849	566
100 Hz Pulsing	Median	26.3	20.9	0.5	0.5
	std	5.0	4.2	0.6	0.5
	r	0.5	-0.5	-0.1	0.4
	p-value	<< 0.05	0.1	0.7	0.1
	N	1,135	966	1,135	966
6 kHz Sound	Median	29.0	21.4	0.5	0.4
	std	2.9	4.7	0.3	0.4
	r	0.8	0.2	-0.3	-0.2
	p-value	<< 0.05	0.6	0.1	0.5
	N	4,022	853	4,022	853
365 Hz FM Harmonic	Median	27.4	20.3	0.2	0.4
	std	4.0	3.7	0.3	0.6
	r	0.7	-0.8	-0.5	0.7
	p-value	<< 0.05	< 0.05	<< 0.05	0.03
	N	2,153	890	2,153	890
300 Hz Harmonic	Median	28.7	24.8	0.5	0.4
	std	4.8	4.6	0.5	0.4
	r	0.8	0.2	-0.4	-0.2
	p-value	<< 0.05	0.5	< 0.05	0.56
	N	3,858	525	3,858	525

Environmental Data. Observations of each fish-related sound were binned weekly and correlated to the median value of associated environmental parameters (Table 5.1). SST was positively correlated to 100 Hz Pulsing, 6 kHz Sound, 365 Hz FM

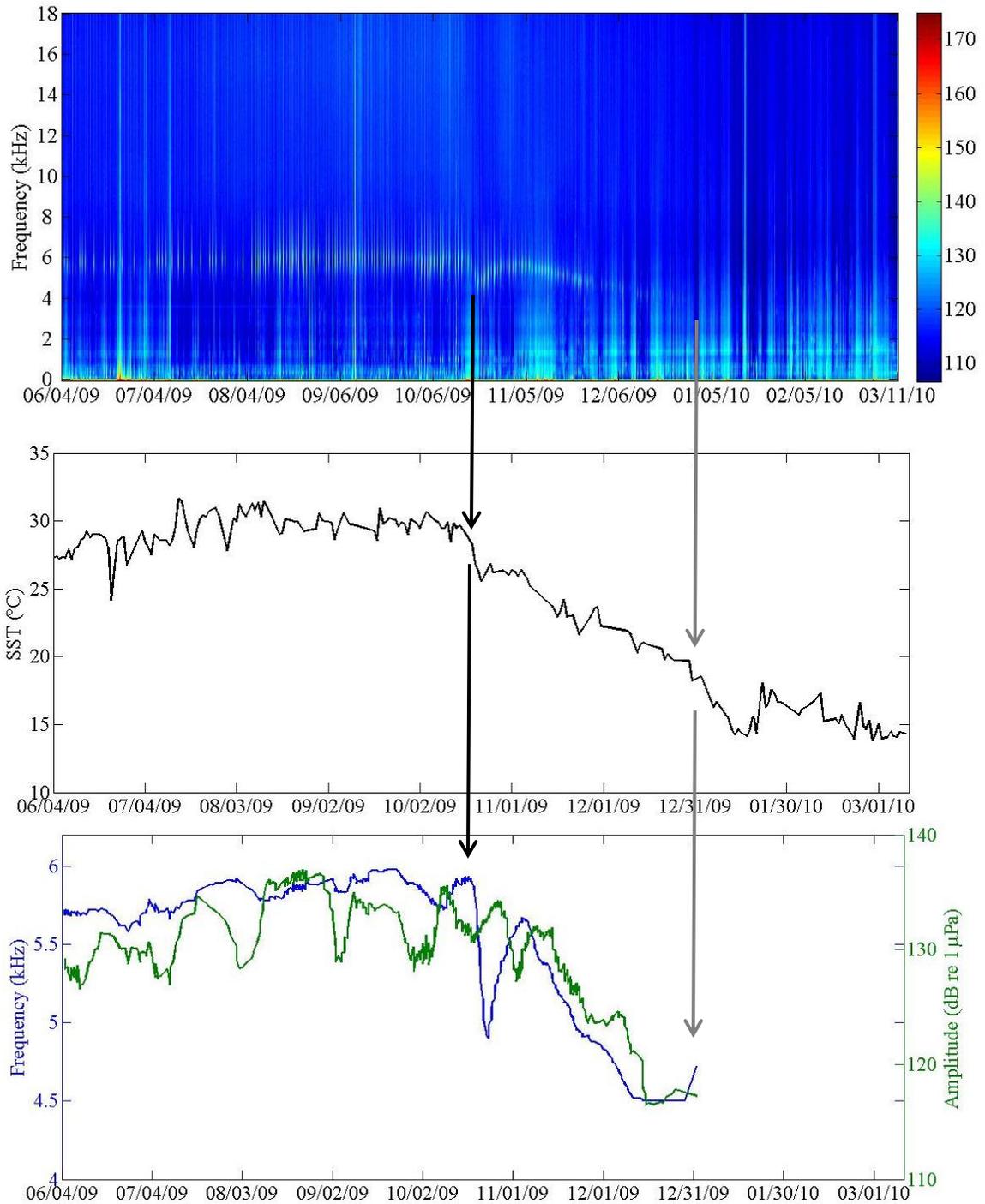
Harmonic and 300 Hz Harmonic sounds. Glider-measured water temperature was positively correlated to toadfish calls and negatively correlated to 365 Hz FM Harmonic sounds. Chl was negatively correlated to 365 Hz FM Harmonic and 300 Hz Harmonic sounds. Glider-measured fluorescence measurements were not significantly correlated to any of the sounds. SST and water temperature appear to vary by about 5 °C. This is likely due to the glider measuring temperature within the water column where measurements are subject to vertical stratification compared to the SST measurements where only the surface of the water is analyzed. Among the five sounds, 6 kHz Sound had the highest median SST value (29.0 °C), which may correspond to the lack of observations in the winter that would otherwise drive the overall associated temperatures down. Variability in chlorophyll measurements likely results from the spatial (stationary and glider data) and vertical (glider data) range in which data are collected.

The frequency and amplitude associated with the 6 kHz Sound were significantly correlated to SST and Chl values (Table 5.2). Only ten stationary sites recorded sound for over six months (B5, B6, B7, B9, B17, B33, B42, B52, B61, and B62). SST is positively correlated to frequency and amplitude while Chl is negatively correlated. Figure 5.8 illustrates the seasonal variation in the 6 kHz Sound, namely as SST decreases the frequency of the 6 kHz Sound also decreases. The small increase in amplitude with decreasing temperature in January through March is likely associated with increased background noise and not a direct result of changes in 6 kHz Sound amplitude.

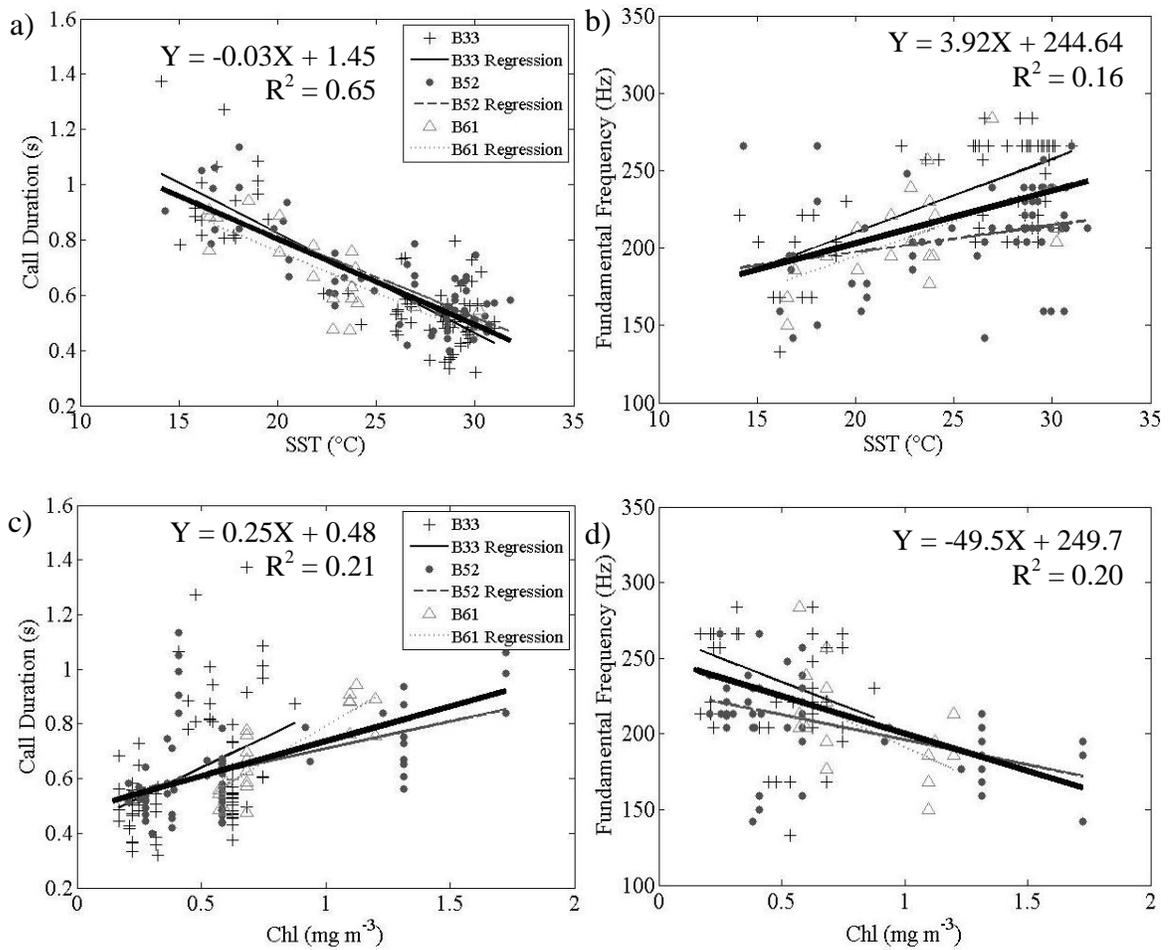
**Table 5.2.** 6 kHz Sound frequency and amplitude correlation to environmental data. Station sites (Station), number of files examined (N), and mean (Mean) and standard deviation (std) of each site's frequency and amplitude are shown. Pairwise linear correlation coefficients of frequency and amplitude values to associated SST and Chl were calculated for each site. Coefficients that were significantly correlated are in bold ( $p < 0.05$ ). Only stationary recorders that collected data for over six months are shown.

Station	N	Frequency (Hz)				Amplitude (dB)			
		Mean	std	SST	Chl	Mean	std	SST	Chl
B5	126	4268	571	<b>0.30</b>	0.11	137	571	<b>0.27</b>	-0.02
B6	87	4642	1047	<b>0.93</b>	<b>0.21</b>	125	105	<b>-0.59</b>	-0.10
B7	135	5516	341	<b>0.90</b>	<b>-0.48</b>	144	341	<b>0.83</b>	<b>-0.45</b>
B9	123	5392	462	<b>0.95</b>	<b>-0.49</b>	149	462	<b>0.61</b>	<b>-0.34</b>
B17	244	5628	278	<b>0.85</b>	<b>-0.56</b>	128	278	<b>0.33</b>	<b>-0.46</b>
B33	266	5357	392	<b>0.76</b>	<b>-0.57</b>	128	392	<b>0.58</b>	<b>-0.37</b>
B42	340	5483	335	<b>0.65</b>	<b>-0.54</b>	129	335	<b>0.62</b>	<b>-0.49</b>
B52	402	5527	430	<b>0.92</b>	<b>-0.75</b>	131	430	<b>0.68</b>	<b>-0.56</b>
B61	237	5665	312	<b>0.83</b>	<b>-0.60</b>	131	312	<b>0.42</b>	0.03
B62	174	5721	291	<b>0.80</b>	<b>-0.44</b>	135	291	<b>0.68</b>	0.00

SST and Chl data were compared to the duration and fundamental frequency of 156 365 Hz Harmonic calls selected for high signal to noise ratios ( $6^+$  dB) from three stationary recorders (Figure 5.10). Call duration decreased and fundamental frequency increased with increasing SST while call duration increased and fundamental frequency decreased with increasing Chl. The regression slopes are shown for each recorder, and from all recorders combined (thick black line). For simplicity, the fit of the regression ( $R^2$ ) and the slope were calculated using data from all three recorders. The  $R^2$  values for call duration and fundamental frequency in relation to SST is 0.65 and 0.15, respectively, and to Chl is 0.21 and 0.20, respectively.



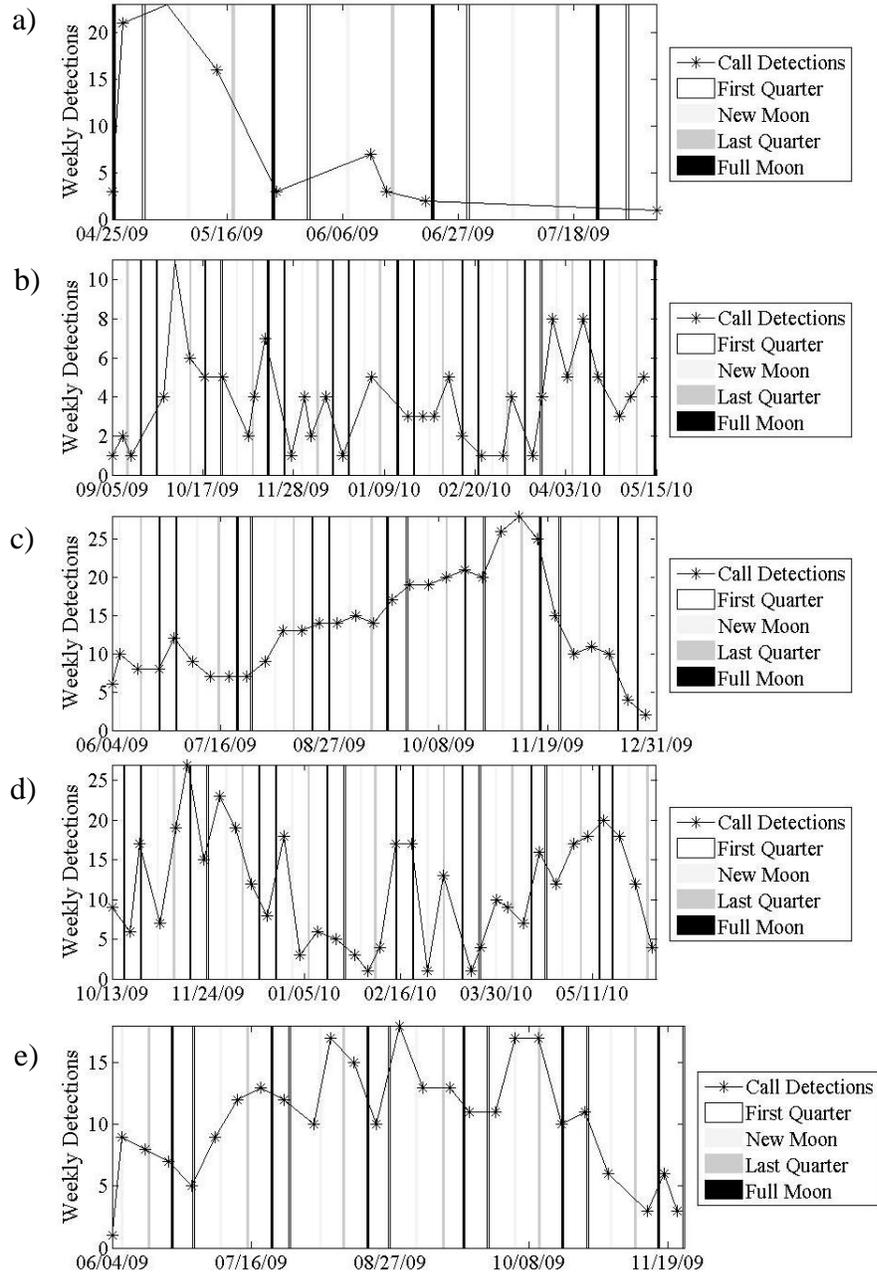
**Figure 5.8.** Time series of the 6 kHz Sound and SST for one stationary recorder. (top) Composite spectrogram, (middle) associated SST and (bottom) frequency and amplitude of the 6 kHz Sound derived from the composite spectrogram (top). Increases in amplitude between 5-6 kHz represent the 6 kHz Sound. Black arrow indicates a decrease in frequency and increase in amplitude of the 6 kHz Sound and concurrent drop in SST. Grey arrow indicates the last day the 6 kHz Sound was detected (January 1, 2010).



**Figure 5.9.** SST and Chl associated with 365 Hz Harmonic call parameters. SST and a) call duration and b) fundamental frequency, and Chl and c) call duration and d) fundamental frequency were calculated from three stationary recorders, B33, B52 and B61 (N=71, 65, and 20, respectively). Regression coefficients and  $R^2$  values are shown for the slope calculated from all data points (thick black line; N =156).

Weekly detection counts calculated for toadfish, 100 Hz Pulsing, 6 kHz Sound, 300 Hz FM Harmonic and 365 Hz Harmonic were not correlated to moon phase (Figure 5.10). Cyclical calling peaks calculated from FFTs appear at 4, 17, 23, 20, and 19 days/cycle for toadfish, 100 Hz Pulsing, 6 kHz Sound, 300 Hz FM Harmonic and 365 Hz Harmonic, respectively. This further supports the lack of correspondence to moon phase cycles, which occur at approximately 7 days for all four moon phases (first quarter, new

moon, last quarter or full moon), approximately 15 days for any two moon phases (e.g., new or full moon), and approximately 29 days for a single moon phase.



**Figure 5.10.** Time series of weekly detection counts and moon phase at a fixed location site. The total number of files, per week, containing a) toadfish, b) 100 Hz Pulsing, c) 6 kHz Sound, d) 300 Hz FM Harmonic and e) 365 Hz Harmonic sounds (\*) are shown with the occurrence of each moon phase (vertical lines). Note the differences in scale.

## Discussion

Here we determined the diurnal and seasonal calling patterns of five fish-related sounds in addition to outlining the spatial distribution of each acoustic signal using passive acoustic technology. The sounds included toadfish and four unknown (100 Hz Pulsing, 6 kHz Sound, 300 Hz FM Harmonic, and 365 Hz Harmonic) fish-related sources. The results of this research provide insight into the habitat ranges and potential spawning patterns of several fish species in addition to determining the influence of environmental data on sound production.

The boatwhistle of the male toadfish is established as a courtship call to attract mates (Gray & Winn 1961, Breder 1968, Fine 1978, Hoffman & Robertson 1983, Barimo et al. 2007). Toadfish boatwhistles were recorded throughout all hours of the day with the majority of calls observed between 1500-0400 hours. This is consistent with Gulf toadfish, *Opsanus beta*, calling patterns (Breder 1968). Sound production was predominately observed from late spring to early summer (April – July), which coincides with the spawning season of oyster toadfish, *O. tau*, which is found off the east coast of Florida (May to July in 17.5 °C – 27 °C, with maximum reproductive activity throughout June and early July) (Gray & Winn 1961, Fine 1978). In Biscayne Bay, FL, gonadosomatic index (GSI) data indicate that *O. beta* spawning peaks from February to April (Malca et al. 2009). Increased water temperatures associated with the more southern latitude of Biscayne Bay is likely responsible for the earlier spawning season of *O. beta* (Gray & Winn 1961). The single peak in toadfish sound production presented

here further supports that *Opsanus* sp. spawn once per year instead of twice as reported in Breder (1941).

Boatwhistle sounds detected inshore (< 10 m) may be produced by the inshore gulf toadfish, *O. beta*, found in shallow waters on the east coast of southern Florida and the Gulf of Mexico (Thorson & Fine 2002). Conversely, those detected offshore (> 10 m) are likely from the offshore leopard toadfish, *O. pardus*, whose call characteristics were first described in Wall et al. (2012a). Further analysis is needed to discern the exact transition of Gulf toadfish to leopard toadfish with increasing depth.

The characteristics of the 100 Hz Pulsing are strikingly similar to cusk-eel sound production (Mann et al. 1997), however, it occurs at a much lower frequency range (100-600 Hz). Cusk-eels use extrinsic sonic muscles to produce rapid pulse trains with a peak frequency of 1,200 Hz (Mann et al. 1997). However, this mechanism is complicated by the use of modified vertebra and a highly modified swimbladder, which contains a rocker bone and vibrating membranes (Parmentier et al. 2010). To date, the exact sound-producing method is not entirely understood. *Ophidion rochei* calls were characterized to contain most of their energy below 500 Hz (Parmentier et al. 2010). This is consistent with the 100 Hz Pulsing frequency range and, based on the similarity in waveforms, suggests that the source of the 100 Hz Pulsing is likely cusk-eel. Cusk-eel sound production is associated with courtship and spawning, and may be important for communication since spawning occurs at night (Mann et al. 1997, Sprague et al. 2001, Mann & Grothues 2008). The largely nocturnal calling of the 100 Hz Pulsing is commensurate with cusk-eel sound production patterns. Fish assemblage data collected in the summer and fall of 2008 to 2010 by Southeast Area Monitoring Assessment

Program (SEAMAP) identified *O. holbrooki*, *O. beani*, and *Lepophidium jeannae* to be common off west-central Florida (SEAMAP 2012). If the 100 Hz Pulsing is made by cusk-eel species using a mechanism that produces sound at this lower frequency, the wide depth distribution and largely nocturnal calling suggest *L. jeannae* is a likely source. The infrequent daytime calling observed suggests *Ophidion* species may also contribute to the overall sound production.

Annual peaks in 100 Hz Pulsing sound production indicate that, if sound is associated with courtship and spawning as it is in cusk-eels, reproductive activity is potentially highest in the spring and fall. This is consistent with overall spawning periods (March to July or August and October to late November) identified in four cusk-eel species found off Texas (Retzer 1991). Retzer (1991) noted wider depth ranges and longer spawning periods for the strictly nocturnal *Lepophidium* species compared to the nocturnal and diurnal *Ophidion* species. The timing of the spring and fall spawning is suspected to take advantage of currents that move eggs and larvae inshore, as opposed to the summer currents that move offshore (Robins & Lea 1978, Standard & Chittenden 1985, He & Weisberg 2002, 2003).

Wall et al. (2012a) identified potential candidates for three of the unknown sounds. The 6 kHz Sound source was suspected to be related to gas release from Clupeid schools (Nøttestad 1998, Wahlberg & Westerberg 2003, Wilson et al. 2004, Doksæter et al. 2009, Knudsen et al. 2009), the 300 Hz FM Harmonic is potentially produced by Atlantic midshipman, *Porichthys plectrodon* (which is similar to *P. notatus* recorded by Brantely & Bass (1994)), and the 365 Hz Harmonic is possibly from a searobin species

(e.g., blackwing searobin, *Prionotus rubio*) (which is similar to *P. carolinus* recorded by Connaughton (2004)).

If the 6 kHz Sound does result from Clupeid gas release, round sardine, *Sardinella aurita*, scaled sardine, *Harengula jaguana*, and Atlantic thread herring, *Opisthonema oglinum* are common species present on the inner WFS (Pierce & Mahmoudi 2001, SEAMAP 2012). The purely nocturnal occurrence of this sound may relate to the diurnal vertical migration of these fishes as schools disperse at night and reassemble during the day (Knudsen et al. 2009, Hawkins et al. 2012). Buoyancy in physostome fishes, such as Clupeids, is controlled by adjusting swim bladder pressure through the exchange of gas in the blood, and the capture and release of gas through the pneumatic duct (known as the ‘gasspuckerreflex’) (Fange 1976). Buoyancy-regulation may be controlled by numerous autonomic neurons that innervate the swimbladder muscles (Finney et al. 2006). In addition, the sphincter surrounding the opening of the pneumatic duct is a primary control point for the gasspuckereflex in many cyprinids (Nilsson 1971, Finney et al. 2006).

As fish are exothermic organisms, ambient temperature alters the rate swimbladder-associated muscles are innervated in some species, with decreasing temperature decreasing the rate of neuron synapsis, and thus frequency of muscle contractions (Fine 1978, Connaughton et al. 2000, Connaughton et al. 2002). This supports the positive correlation between the presence, peak frequency and amplitude of the 6 kHz Sound to SST. The approximately 2 kHz range over which the peak frequency of this sound was observed suggests that a change in nomenclature is imperative and that temperature directly affects the mechanism of sound production. Therefore, changes in

the contraction rates of buoyancy-regulating muscles and size of the sphincter may play a role in altering the frequency and/or amplitude of the 6 kHz Sound. It should be noted that Clupeid gas release is just one plausible hypothesis and further research is needed to determine the source of this sound with any certainty.

The acoustic distribution of the 300 Hz FM Harmonic was noted mainly offshore (> 40 m depth) with the greatest abundance in the northwest corner of the study area. This spatial range is consistent with Atlantic midshipman collected off west-central Florida from 2008 to 2010 (SEAMAP 2012). Plainfin midshipman (*Porichthys notatus*) produce several sounds directly and indirectly associated with courtship and spawning: long-duration ‘hums’, short-duration ‘grunts’, and ‘growls’ (Brantley & Bass 1994, Sisneros 2009). The growl is a multiharmonic, long duration (>1 s) sound with gradual changes in fundamental frequency (Sisneros 2009). This call most closely describes the 300 Hz FM Harmonic. The growl is produced at the beginning of the breeding season when Type I reproductive males are establishing nesting sites and are highly territorial and aggressive (Sisneros 2009). The plainfin midshipman breeding season occurs from late spring to summer (April to August). This supports the seasonal peaks in 300 Hz FM Harmonic sound production in February, April, June and July. While the midshipman growl is a very likely candidate for the 300 Hz FM Harmonic, several discrepancies are noted. Type I plainfin midshipman males build and defend nests positioned under rocky shelters in the intertidal zone (Brantley & Bass 1994, Sisneros 2009). However, the 300 Hz FM Harmonic was mainly observed offshore (40 – 200 m). In addition, to my knowledge, the hum and grunt calls, which are also produced by Type I males, were not observed. Differences in sound production and habitat preferences between plainfin and

Atlantic midshipman may be the cause for this disagreement; however, further research is needed.

The 365 Hz Harmonic sound was present mainly inshore (< 40 m depth) and in the northern portion of the study area. This spatial range is consistent with blackwing searobin, barred searobin (*Prionotus martis*) and bighead searobin (*P. tribulus*) collected off west-central Florida from 2008 to 2010 (SEAMAP 2012). Connaughton (2004) described the sound production mechanism of Northern searobin, *P. carolinus*, as alternating contractions of paired sonic muscles. The fundamental frequency of northern searobin (200-280 Hz) is comparable to that of the 365 Hz Harmonic (mean fundamental frequency: 223 Hz, SD: 36 Hz) and both species show an increase in fundamental frequency with increasing temperature (Connaughton 2004). Variability between SST and call duration, especially among the different sites, is likely due to the temperature measurement reflecting only the ocean surface and not the bottom (ambient) temperature. The effect of temperature on call characteristics has also been observed in weakfish (*Cynoscion regalis*) and oyster toadfish (Fine 1978, Connaughton et al. 2000, Connaughton et al. 2002). In both species, fundamental frequency increases with increasing temperature. Similar to the 365 Hz Harmonic, weakfish pulse duration is inversely proportional to temperature.

Peak spawning for northern searobin and striped searobin (*P. evolans*), in the mid-Atlantic Ocean extends from May to July (Richards et al. 1979) or May to September in offshore waters (McBride et al. 2002, McBride 2002). Leopard searobin (*P. scitulus*), bluespotted searobin (*P. roseus*), and barred searobin spawn on the WFS during spring and late summer (Ross 1980, 1983). These spawning periods are consistent with the 365

Hz Harmonic summer peak in sound production (June – September). In addition, bighead searobin spawn on the inner (< 42 m) WFS from fall to early spring (Ross 1983), which could account for the secondary peaks in 365 Hz Harmonic sound production in March and early winter (November and December). Ross (1980) demonstrated that leopard searobin swimbladder size decreases with breeding behavior and maximal gonadal development, especially for female fish. Although the intrinsic sonic muscles of searobin cause the fundamental frequency to be potentially independent of body size (Connaughton et al. 2000, Fine et al. 2001, Connaughton et al. 2002), the effect of the seasonal changes in swimbladder size on overall sound production (Fine et al. 1977, Connaughton et al. 1997) is not known.

Atlantic midshipman and blackwing searobin are just a few sound-producing species present on the WFS. A preliminary analysis of families of soniferous fishes in the Gulf of Mexico using published literature (Fish & Mowbray 1970, Hoese & Moore 1998) and unpublished sound recordings identified nearly 90 genera are likely to make sound based on anatomy (C. Wall unpubl data). This leaves the list of potential sources of sound described in this study rather vast. SEAMAP (2012) data show Jackknife fish (*Equetus lanceolatus*), cubbyu (*Equetus umbrosus*), and bluespotted searobin are all common on the WFS, with bluespotted searobin extending furthest offshore (~100 m depth). It was determined that these species are possibly soniferous via a dissection that showed both *Equetus* species have extrinsic sonic muscles and bluespotted searobin have intrinsic sonic muscles.

Passive acoustic monitoring systems record acoustic data over large spatial and temporal scales. Since sound is associated with reproduction in many species, an

important application of PAM is to determine when and where reproductive activities occur for fish (Mann & Lobel 1995, Lobel 2002, Gannon 2008, Van Parijs et al. 2009, Lobel et al. 2010). The employment of stationary and autonomous PAM resulted in acoustic data for not only the original target species (red grouper) but incidental low-frequency sounds as well, which provided valuable information into the broader acoustic scene. From these data, a greater understanding of the spatial and temporal patterns of sound associated with five fish-related sources (toadfish, 100 Hz Pulsing, 6 kHz Sound, 300 Hz FM Harmonic, and 365 Hz Harmonic) developed. These data on the spatial and temporal occurrence of these sounds will be useful for more directed studies to verify the sound producers. Five additional unknown, suspected fish sounds (e.g., “grunts” and “pulses”) were observed in the acoustic files but were not presented here. Further research in confirming the source of all unknown sounds is essential to advancing the field of fish bioacoustics and communication.

## Chapter 6: Conclusion

The research presented here employed active and passive acoustics to determine the shelf-scale distribution and long-term calling patterns of fish on the West Florida Shelf (WFS). Acoustic recordings were made during periods from 2008 to 2011 using fixed location passive acoustic recorders and multiple hydrophone-integrated gliders. Analyses of these data helped determine the daily, seasonal and spatial patterns of red grouper, toadfish, and four unknown fish-related sounds (100 Hz Pulsing, 6 kHz Sound, 300 Hz FM Harmonic, and 365 Hz Harmonic).

The spatial range over which red grouper sounds were detected was quite extensive (15-93 m bottom depth) with most calling recorded in waters between 30 and 50 m deep. Along the northern boundary of the study area, red grouper sounds were detected mainly in water depths between 40 and 93 m. Preferential habitat in the Florida Middle Grounds (hard bottom) and Steamboat Lumps Marine Reserve (excavated sediment) is suspected to be the cause for the shift in depth range in this region.

Steamboat Lumps is established as red grouper spawning habitat. High-resolution multibeam bathymetry data collected in a portion of the reserve in 2006 and 2009 allowed detailed documentation and characterization of holes excavated by red grouper that are used by males as spawning sites. Analysis of these data suggested that holes are constructed and maintained over time, and provided evidence towards an increase in spawning habitat usage, which would confirm the potential benefit of such reserves.

Red grouper sound production observed from a glider mission (Mission 53 March 29 to April 15, 2011) that specifically targeted Steamboat Lumps was compared to the multibeam bathymetry data collected in the reserve in 2009. The results indicated areas along the glider track with high hourly call counts were typically observed near holes identified in the multibeam data. This suggests that the holes are occupied by red grouper and, due to the two year lag between the datasets, further supports that the holes are maintained over time. This research demonstrates the utility of coupling passive acoustic data with high-resolution bathymetric data to verify the occupation of suspected male territory (holes) and to provide a more complete understanding of effective spawning habitat. Although annual sound production peaks (July to August and November to December) were not correlated to spawning peaks (March to May), passive acoustic monitoring (PAM) is an effective tool to identify areas of potential spawning activity by recording the presence of potentially reproductively mature male red grouper.

Understanding the timing and location of spawning adults is essential to fisheries management. Environmental factors, such as tides, currents, lunar phase, primary productivity, and temperature, can play a role in initiating spawning activity (e.g., Peebles 2002). Even after spawning, these environmental factors continue to influence the survival of pelagic eggs and larvae (e.g., Houde 1989). To link spawning events to large-scale and potentially transient oceanographic conditions, monitoring methods must be of a commensurate scale (i.e., cover large spatial areas and temporal periods). Passive and active acoustics are one tool that can be used to achieve these requirements. As these technologies continue to progress, additional developments will further their efficacy for fish ecology studies.

The distribution of toadfish calls suggests two species (*Opsanus beta* and *O. pardus*) were recorded; the latter being first described here. The spatial range and call characteristics identified from the large-scale acoustic dataset can be used to help determine the sources of the four unknown sounds. Despite occurring in a much lower frequency range (100-500 Hz), the 100 Pulsing has the characteristics of a cusk-eel call and is suspected to be produced by cusk-eel species present in the eastern Gulf of Mexico. The 6 kHz Sound is hypothesized to be the result of gas release from Clupeids (Wahlberg & Westerberg 2003, Wilson et al. 2004) due to its nocturnal occurrence and high frequency range (4-6 kHz), which suggests a method of sound production alternative to sonic muscle contraction or stridulation. The 300 Hz FM Harmonic is similar to the multi-harmonic growl of the Plainfin midshipman (*Porichthys notatus*) recorded by Brantely & Bass (1994) and may result from Atlantic midshipman (*P. plectrodon*), which are present in the eastern Gulf of Mexico. Lastly, the 365 Hz Harmonic is possibly from a searobin species (e.g., blackwing searobin, *Prionotus rubio*) due to its similarity to the Northern searobin (*P. carolinus*) call recorded by Connaughton (2004). Further research in confirming the source of all unknown sounds is essential to advancing the field of fish bioacoustics and communication.

Fisheries management methods that collect near real-time stock assessment data and use a no-take approach are needed to effectively manage species with greater immediacy and will aid in maintaining long-term population stability and fishing activities. Long-term passive acoustic studies that provide systematic monitoring can be a valuable assessment tool for soniferous species. Here, glider technology was proven to be a reliable and relatively inexpensive method to collect fish acoustic data while

maintaining a high rate of successful retrieval. In addition, the multibeam sonar mapping of habitat combined with a large-scale passive acoustic survey is a transformational approach to fisheries-independent sampling of adult reproductive populations. Therefore, the implementation of regular deployments of hydrophone-integrated gliders and fixed location PAM stations, and shelf-scale habitat mapping is suggested as a possible method for enhancing fisheries management, protecting known adult reproductive populations and contributing to an ecosystem-based management regime.

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## About the Author

Carrie Wall received a Bachelor's of Science in Biology at Salisbury University and a Bachelor's of Science in Environmental Marine Science at the University of Maryland Eastern Shore in 2002. While studying abroad at James Cook University in Australia in 2000 Carrie developed a long-lasting fascination with marine science.

In 2004, Carrie began her Master's in Marine Science at the University of South Florida under the tutelage of Dr. Frank Muller-Karger. Her work in Dr. Muller-Karger's Institute for Marine Remote Sensing focused on understanding recreational catch of king mackerel in relation to satellite-derived oceanographic parameters. Shortly after completing her Master's, Carrie began her doctoral work in Dr. David Mann's Marine Sensory Biology Lab studying large-scale sound production by fish, namely red grouper. Working in these labs has afforded Carrie many positive opportunities such as cruises, conferences, publications, and workshops. Carrie currently lives on Vancouver Island in British Columbia with her husband, dog and two cats.