Sound production and behavior of red grouper (Epinephelus morio) on the West Florida Shelf

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Sound Production and Behavior of Red Grouper (*Epinephelus morio*)
on the West Florida Shelf

by

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A thesis submitted in partial fulfillment
of the requirements for the degree of
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DEDICATION

I would like to dedicate this thesis to my mom, Roberta Cauthron, for her unwavering support and encouragement, and for taking the time to look up all the words she didn’t know. To Gay Allison, for her absolute faith in me, and for always being a mother, even though she didn’t have to. To my husband and best friend, Eric Montie, whose never-ending enthusiasm and scientific curiosity made it impossible to be anything but excited about my research. And finally, to my dad, Eric Nelson, for sharing his love of science and fascination with nature, and for being the greatest father a girl could have.
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Sound Production and Behavior of Red Grouper (*Epinephelus morio*) on the West Florida Shelf

Misty D. Montie

**ABSTRACT**

Red grouper (*Epinephelus morio*) are long-lived, commercially important, soniferous fish belonging to the family Epinephelidae. Found throughout the western North Atlantic and Gulf of Mexico, they are protogynous hermaphrodites, and peak spawning occurs from March through May. Unlike many grouper species, red grouper do not form large spawning aggregations; rather, they form small polygynous groups, and remain in relatively close proximity to rocky depressions excavated in the sandy bottom by males. This excavation activity creates structure and habitat for a wide variety of species, and as a result, red grouper are a keystone species on the West Florida Shelf. While extensive life-history information exists, largely from fishery catches, little is known about sound production or behavior of red grouper in their natural environment. Passive acoustic recordings combined with simultaneous digital video recordings were used to investigate sonic activity and behavior of red grouper on the Steamboat Lumps and Madison-Swanson marine reserves on the West Florida Shelf. Red grouper were found to produce a unique series of low-frequency (180 Hz peak) pulses, consisting of 1-4 brief (0.15 s) broadband pulses and a 0.5-2 s down-swept “buzz” (i.e., short call); occasionally these were followed by a rapid series of 10-50 broadband pulses (i.e., pulse
train). Sound production was observed throughout the day and night, but most sounds occurred between sunrise and sunset, with a noticeable increase during late afternoon. Behaviors associated with sound production included territorial displays and courtship interactions, indicating that sound production is likely related to spawning activity. Thus, monitoring red grouper using passive acoustics could be an effective tool in fisheries management and conservation efforts.
INTRODUCTION

Red grouper (*Epinephelus morio*) are long-lived, commercially important members of the family Epinephelidae, and are found throughout the western North Atlantic and Gulf of Mexico. Recent evidence indicates that red grouper modify their local environment by excavating sediments to expose rocky depressions on the seafloor (Coleman *et al.*, 2010); this “ecosystem engineering” creates habitat for a wide variety of species, and red grouper may serve as keystone species in the Gulf of Mexico (Coleman and Williams, 2002; Coleman *et al.*, 2010). Despite their economic and ecological significance, little is known about the *in situ* behavior of red grouper. The bulk of information available is based largely on samples collected from or dependent on fishery catches, captive individuals, or assumptions extrapolated from closely related species.

Red grouper in the United States are managed as part of the shallow-water grouper (SWG) complex, which also includes gag (*Mycteroperca microlepis*), black (*M. bonaci*), yellowmouth (*M. interstitialis*) and yellowfin grouper (*M. venenosa*), red hind (*E. guttatus*), rock hind (*E. adscensionis*), and scamp (*M. phenax*) (SEDAR, 2006). South Atlantic and Gulf of Mexico populations are geographically isolated, and are managed as separate stocks (Burgos *et al.*, 2007). In 2008, the National Marine Fisheries Service reported commercial red grouper landings of 5,578,037 pounds (2,530,200 kg) for the west coast of Florida alone, which were worth $13,459,803. This catch composed
80% of the United States Gulf of Mexico grouper fishery (NMFS, 2010). Starting in 2008, several regulatory changes were implemented to reduce overfishing of gag grouper. These changes were offset by regulations aimed at increasing harvest of red grouper, which included: i) increasing the annual red grouper total allowable catch by 15%, from 6.56 million pounds (mp; 2,975,600 kg) to 7.57 mp (3,433,700 kg) gutted weight, ii) establishing a seasonal SWG fishery closure from February through March, aimed at reducing gag harvest by 26%, while increasing red grouper harvest by 17%, and iii) lowering the minimum size limit for red grouper from 20 inches (50.8 cm) to 18 inches (45.7 cm) total length (GMFMC, 2008). While current assessments indicate that the Gulf of Mexico red grouper stock is neither overfished nor undergoing overfishing, overfishing has occurred as recently as 2004 (SEDAR, 2006), and a red tide mortality event in 2005 likely resulted in a subsequent population decrease (SEDAR, 2009a). The South Atlantic stock was overfished and overfishing was occurring in 2009 (SEDAR, 2009b). Responsible harvesting and effective management are essential to the long-term sustainability of any fishery. Specifically, reducing fishing pressure on reproductively active individuals will support successful spawning and facilitate strong recruitment to the population. Exploring new methods of monitoring habitat use and reproductive activity are important factors in developing these regulations.

Like many epinephelids, red grouper are protogynous hermaphrodites; female to male transition occurs between 5-10 years of age, at an annual transition rate of approximately 15% (Jory and Iversen, 1989). The red grouper reproductive season extends from January through July, with a peak in spawning activity occurring between
March and May (Moe, 1969; Johnson et al., 1998; Collins et al., 2002; Burgos et al., 2007). Limited findings suggest that, unlike many grouper species, red grouper do not form large spawning aggregations (Brule et al., 1999); rather, they form small polygynous groups (Coleman et al., 1996), and remain in relatively close proximity to limestone outcroppings or rocky depressions excavated in the sandy bottom by males (Moe, 1969; Bullock and Smith, 1991; Coleman and Williams, 2002; Scanlon et al., 2005; Coleman et al., 2010). Red grouper spawning behavior is believed to be similar to that of coney (Cephalopholis fulva) and graysby (C. cruentatus), which has been described as nonmigratory, polygynous pair-spawning (Sadovy et al., 1994; Coleman et al., 1996). Male coney (Family: Epinephelidae) are territorial, and spawning with multiple females occurs daily, just prior to sunset (Heemstra and Randall, 1993). Details of red grouper spawning behavior, however, remain largely unknown.

Red grouper larvae are pelagic, and juveniles can be found on near-shore reefs, grass beds and estuaries (Moe, 1969; Burgos et al., 2007). With the onset of sexual maturity, typically around 5 years of age, individuals migrate into deeper waters of the continental shelf and shelf edge (Moe, 1966; 1969). Adults are not thought to undertake long-distance seasonal migrations. Tagging studies have shown that 87% of individuals remained within 10 miles of the tagging location, and 61% remained within one mile (SEDAR, 2006). Given that red grouper distribution appears to be relatively stable, large-scale mapping throughout the Gulf of Mexico would provide valuable information to fishery managers. Red grouper are soniferous, and passive acoustic recordings would be a cost-effective way to perform this mapping.
The mechanism of red grouper sound production is believed to be similar to the closely-related Nassau grouper, *E. striatus*, and results from rapid contraction of bilateral muscles behind the opercles, which causes the swim bladder to vibrate (Hazlett and Winn, 1962). Fish and Mowbray (1970) describe a simple “boom” sound generated during competitive feeding among several captive adult red grouper. They also describe sounds of numerous other epinephelid fishes, including Nassau grouper, rock hind, red hind, speckled hind (*E. drummondhayi*), Warsaw grouper (*E. nigritus*), and goliath grouper (*E. itijara*). Low frequency booms, thumps, knocks, rasps and grunts were observed. However, most observations were made by mechanical or electrical stimulation of captive individuals, which provides little insight into the full repertoire of possible sounds or the associated behaviors. Fishes produce sounds in a variety of contexts, including territorial defense (Ladich, 1997), courtship (Myrberg *et al.*, 1986; McKibben and Bass, 1998) and spawning (Lobel, 1992; Mann *et al.*, 1997; Amorim *et al.*, 2003), but very few published reports identify the specific *in situ* behavior associated with sound production.

Several studies in the 1960s utilized underwater acoustic-video recorders to observe sound production in fishes, primarily to identify species-specific sounds (Steinberg *et al.*, 1965; Myrberg, 1973). Laboratory studies have used video to observe sound production and behavior in captive fish (McKibben and Bass, 1998; Malavasi *et al.*, 2009; Maruska and Mensinger, 2009). Commercially available digital recorders, microcomputers and high-capacity data storage devices have greatly expanded the potential applications of this technology. This study used passive acoustic recordings
combined with simultaneous digital video recordings to explore the behavior of red grouper in their natural environment, focusing on sound production and its potential relationship to spawning. Primary objectives were to 1) describe sounds produced by red grouper in situ, 2) relate sound production to specific behaviors, and 3) describe the daily periodicity of red grouper sound production.
MATERIALS AND METHODS

Study Sites

Recorders were deployed within the Madison-Swanson (MS) and Steamboat Lumps (SL) marine reserves on the West Florida Shelf (Figure 1). These reserves were established in 2000, and are closed year-round to all commercial and recreational reef fishing (GMFMC, 2003). While the reserves were implemented primarily to reduce fishing pressure on aggregations of gag and scamp (Coleman et al., 2004), red grouper also occur within reserve boundaries (Scanlon et al., 2005). In MS, red grouper are commonly associated with low-relief carbonate rock outcroppings in the sandy bottom; in SL, red grouper are typically found in excavated solution holes, which can be observed in high-resolution sidescan sonar images (Coleman et al., 2010).

Field operations were carried out aboard the M/V Liberty Star from May 5-15, 2008. Sidescan bathymetry maps were used to guide the ship to areas likely to have red grouper. A Deep Ocean Engineering Phantom S2 remotely operated vehicle (ROV) was used to provide real-time video transmission to the control ship, allowing for verification of red grouper presence. This initial assessment enabled placement of recorders close to fish, in water depths of up to 90 meters (Table 1).
Video and Acoustic Recorders

Autonomous recorders captured digital video using low-light, wide-angle black and white cameras. Custom cylindrical PVC housings with a clear acrylic plate covering one end were mounted on aluminum tripods weighted with lead. Four housings contained Chasecam PDR100 Solid State Digital Recorders (Chase Product Development, Inc., La Mesa, CA) with 4 internal Lithium AA batteries, while the fifth contained an Archos 605 WIFI Portable Media Player (Archos Inc., Greenwood Village, CO). Additionally, each unit had an SSC-108WXXB .0003 Lux Low Light B/W 420 Line Board Lens camera (Advance Security Products, Belleville, IL) mounted to the acrylic plate. Two High Tech, Inc. HTI-96-MIN Series hydrophones (Gulfport, MS; sensitivity = -164 ± 1 dB re: 1V/μPa; flat response between 2 Hz-37 kHz) were also connected to each video housing unit, one directly attached to the back plate, and the second attached to a 1.4 m tether. The two channels of audio were recorded continuously at a sample rate of 44.1 kHz, and along with video data, were saved to A-Data Speedy 32-GB compact flash memory cards (A-DATA Technology Co., Ltd., City of Industry, CA), except in the case of the Archos recorder, which had 30-GB internal memory. Each unit was powered by 8 D-cell batteries connected in series. Foam padding was packed into each housing prior to sealing, to prevent equipment movement or damage. Silica packs were used to absorb moisture and prevent condensation from interfering with the video. Video recorders were placed manually by deep-sea scuba divers around active red grouper holes (i.e., holes observed by the ROV to have resident red grouper), with the cameras directed at the hole. Two to five units were simultaneously deployed at different
locations around a given site, and recorded audio and video continuously for up to 24 hours (Table 1).

Additional custom Digital SpectroGram (DSG) audio recorders were deployed to provide a longer time-series for periodicity analyses: four on Steamboat Lumps with a duty-cycle of 2.5 minutes every 10 minutes for five days, and three recorders at Madison-Swanson with a duty cycle of eight minutes every 10 minutes for two days (Table 1). These units consisted of a cylindrical PVC housing, a single HTI-96-MIN hydrophone (sensitivity = -186 ± 1 dB re: 1V/μPa; flat response between 2 Hz-37 kHz), micro-computer, circuit board, and were powered by 6 D-cell alkaline batteries. Single-channel audio was recorded at a sample rate of 50 kHz, and was saved on Patriot 16-GB SanDisk secure digital flash memory cards (SanDisk Corporation, Milipitas, CA). Individual recording units were attached to anchored line using steel gangion snaps and cable ties placed approximately three meters above the anchor, with a single float 30 meters above the unit to prevent sinking, as well as surface buoys for re-location and retrieval (Figure 2). Units were deployed from the deck of the ship in the general vicinity of active grouper holes.

Analysis of Sounds

Audio portions of video recordings were used for descriptive analyses, as these units were placed in closest proximity to fish, and therefore contained the clearest and most consistent sounds. Audio tracks were separated from each MPEG video file using
Ulead VideoStudio 11.0 (Corel Corporation, Ottawa, Canada, www.ulead.com), and saved as 16-bit WAV files. MPEG files recorded during nighttime hours were often highly compressed, and were manually divided into two-hour sections prior to analysis. Files longer than 10 hours were split into two separate files using Servant Salamander 2.51 (ALTAP, Novy Bor, Czech Republic, www.altap.cz) before they could be opened in Ulead.

Each WAV file was initially analyzed using Raven Pro 1.3 (Cornell Laboratory of Ornithology, Ithaca, NY, www.birds.cornell.edu/raven) software. Spectra were generated with a Hann window and discrete Fourier transform (DFT) size of 4096 samples, and frequencies from 0-1200 Hz were viewed in 30-second windows. Individual red grouper sounds were manually selected (selection boxes included approximately two seconds beyond the end of each call, to serve as a measure of background noise) and saved as separate 16-bit WAV files with a sample rate of 44.1 kHz. The Batch Channel Exporter was then used to create separate WAV files for each channel.

MATLAB 7.7 (The Mathworks, Inc., Natick, MA, www.mathworks.com) was used to resample each call to 4410 Hz. All video recorders were calibrated by presenting 0.1 V peak test sine waves at 10, 20, 50, 100, 150, 200, 400, 700, 1,000 and 1,500 Hz, and determining the frequency response. For each recorder, the unique frequency response was used to create a custom finite-duration impulse response (FIR) filter, which was then applied to the resampled WAV files from that recorder. Signal-to-noise ratios
(SNRs) were calculated for each corrected file, and all files with SNR > 2 were included in subsequent analyses. Start-time and root-mean-square (RMS) amplitude were then generated for each file. Files from the same site and time were categorized as replicates (i.e., the same call was recorded on more than one unit), and calls with the highest RMS amplitude for each replicate were included in final analysis.

Additional manual analysis in Raven was performed on each call: spectra (Hann window, DFT size: 1024 samples, 0-2205 Hz) were used to generate peak frequency measurements. Waveform plots were used to measure durations and inter-pulse intervals. Custom MATLAB programs were used to calculate all other measurements, including amplitudes, bandwidths and sound pressure levels.

Behavioral Analysis

Analysis of video footage associated with sounds was performed using Ulead. Video from all recorders was visually inspected for each sound event included in the descriptive analysis. Although only the best replicate was included in the sound analysis, all replicates were included in the behavioral analysis, as fish were not necessarily visible in footage from all units. Observations were made for at least 10 s before and after each sound, and included the number of fish observed and a general description of behavior at the time of sound production. Sex of individuals was noted if possible, based on distinct display coloration patterns: males have darkening along the dorsum, and females exhibit
several broad white vertical bands along the body (C. Koenig, pers. comm.). Behavioral observations were combined and summarized for each replicate.

Periodicity Analysis

Periodicity of sound production was measured by manually browsing spectrograms from DSG recordings using Raven (Hann window, DFT = 4096 samples, 0-1000 Hz, 30-second increments). Each spectrogram was visually analyzed, and all red grouper sounds were logged, generating a total number of calls recorded for each file. Of 3,644 files recorded, 1,002 were excluded from analysis because background noise was present at levels likely to mask detection of red grouper calls. Engine noise from the Liberty Star was the largest contributor to background noise, although abrasion of the PVC housings by the steel gangions also produced intermittent noise sufficient to cause masking. Call rates were calculated by dividing the total number of calls per recording by the duration (e.g. 12 calls/2.5 minutes = 4.8 calls/minute). End time was used to categorize each recording into 30-minute and 1-hour time-of-day “bins” (e.g. 13:00-13:29; 08:00-08:59). Call rates and counts for each bin were then averaged across all days to calculate mean number of calls and call rate for that time of day. When comparing number of calls from both SL and MS, only calls recorded in the first 2.5 minutes of MS files were included.
RESULTS

Description of Sounds

Red grouper were found to produce a unique series of low-frequency pulses, and two distinct variations were observed. Short calls were composed of one to four brief pulses followed by a down-swept “buzz” (Figure 3). Pulse train calls comprised a short call immediately followed by a rapid series of broadband bursts (Figure 4). Applying the SNR > 2 threshold resulted in 167 short calls, 100 of which were randomly selected for analysis, and 16 pulse trains, all of which were included in analysis.

Descriptive statistics were calculated for each call type separately (Table 2). Inferential statistical comparisons between call types were not performed because there was no way to know how many individual fish may have been recorded. Fast Fourier transforms (FFTs) of all calls were averaged to generate a mean FFT (Figure 5), showing a dominant frequency around 180 Hz. This 180 Hz frequency peak is also reflected in the distribution of peak frequency measurements (Figure 6). Energy below 50 Hz was likely due to vessel noise, and inclusion of this energy in peak frequency measurements explains the lower mean peak frequencies (150 Hz for short calls, 131 Hz for pulse trains) reported in Table 2. Six-dB bandwidth measurements (Figure 7) indicate most energy lies between 50-310 Hz. Root-mean-square (rms) received sound pressure levels (SPLs)
ranged from 110-142 dB re: 1μPa (Figure 8). While actual source levels were not obtained, the proximity of recording units to fish suggests that maximum received levels (e.g. 142 dB re: 1μPa, SNR\(_{dB} = 37\)) may serve as a close approximation. For example, fish observed directly in front of a recorder, at an estimated range of <3 m, produced a pulse train sound at 127 dB re: 1μPa\(_{rms}\). Call duration increased with the number of pulses, and varied from 1-3 s for short calls and from 3-22 s for pulse trains (Figure 9).

Behavior

Video analysis was performed for all 116 calls included in the descriptive analysis. Of these calls, 56 sounds occurred at night, while 35 sounds had no red grouper visible in video footage. Red grouper behavior observed during sound production fell into two categories: territorial activity and courtship interactions. Territorial activity included patrolling, where a male swims in a repeated pattern around and above a hole (n = 12), and color changes (e.g. darkening of dorsum; n = 3). Courtship interactions consisted of a male and female observed swimming together (n = 10). Rapid swimming with direct physical contact between a male and female occurred during four of these interactions, although no apparent spawning was observed. A single male was observed for all sounds associated with territorial activity; one male and one female were present during courtship interactions. Short calls were associated with all behaviors, and pulse trains were associated with patrolling, color change and courtship with direct body contact. A summary of each sound-behavior event is given in Table 3.
Diel Periodicity

Red grouper were found to produce sounds at all times of day and night, and showed a strong diel pattern of sound production (Figure 10). Calling increased just before dawn, dropped off briefly after sunrise, then increased throughout the day, peaking in the late afternoon before dropping off again after sunset (Figure 11). While both MS and SL exhibited similar patterns of sonic activity, SL had higher daytime calling rates, and MS had a later afternoon peak. Only short calls appeared to exhibit a daily pattern; calls with pulse trains were found at low levels through both day and night (Figure 12). Without an accurate assessment of fish abundance, statistical comparisons between sites cannot be made. The increase in call rates could be attributed to greater numbers of fish, rather than increased activity of individuals; it is interesting to note, however, that nighttime call rates were similar at both locations.
DISCUSSION

*In situ* sounds produced by red grouper are similar to low-frequency pulsed sounds reported for other free-ranging epinephelids, including Nassau grouper and red hind (Moulton, 1958; Steinberg *et al.*, 1962; Steinberg *et al.*, 1965), as well as goliath grouper (Mann *et al.*, 2009). The frequency range and pulse duration are consistent with agonistic sounds produced by captive red grouper described by Fish and Mowbray (1970), but the overall call structure was found to be more complex.

No direct observations of spawning were made. This could be explained by a) red grouper do not produce sound during the act of spawning; or b) spawning occurred during one of the 91 sounds produced either at night or while red grouper were not visible in video footage. Additional video analysis is necessary to confirm whether sound production is directly related to spawning. Nevertheless, red grouper were found to produce sounds during a known peak-spawning month (May). This finding, combined with observations of calls made during territorial displays and courtship, suggests that sonic activity may be linked to reproductive behavior. This relationship is further supported by evidence that sounds are produced by males during patrolling and interaction with females, which is similar to behavior of the closely-related red hind (Mann and Locascio, 2008).
Crepuscular peaks in calling activity have been observed in several species that also typically spawn at dawn and/or dusk. Mann and Lobel (1995) found that damselfish (Dascyllus albisella) sound production peaked at dawn and corresponded with spawning activity. Winn et al. (1964) found that longspine squirrelfish (Holocentrus rufus) sound production peaked at both dawn and dusk, and was related to territorial displays.

Evening spawning has been observed in tiger grouper (M. tigris) (Sadovy et al., 1994), leopard grouper (M. rosacea) (Erisman et al., 2007), halfmoon grouper (E. rivulatus) (Mackie, 2007) and dusky grouper (E. marginatus) (Hereu et al., 2006), but information on patterns of sound production for these species is not available. However, if red grouper do spawn in the evening, observed increases in late afternoon calling activity would suggest that sound production is linked to spawning. The results of this study indicate that passive acoustics could potentially be used to monitor red grouper reproductive activity.

The significance of this work lies in the potential for using passive acoustics as a method for monitoring fish populations. Locascio and Mann (2008) demonstrated the utility of passive acoustic monitoring for studying reproductive activity in soniferous fishes, and its applicability over a broad range of spatial and temporal scales. Passive acoustics could be used in defining spawning grounds for those species that produce sounds directly associated with spawning behavior (Mok and Gilmore, 1983; Luczkovich et al., 1999). Numerous studies suggest that fisheries closures, particularly of spawning sites, may be an effective management strategy. Red hind in the U.S. Virgin Islands have benefitted from seasonal closures of a spawning aggregation site, with size increases of...
spawning adults and a favorable shift in female-to-male sex ratio (Beets and Friedlander, 1998; Nemeth, 2005). Additionally, Nemeth (2005) reported that establishing a permanent marine protected area surrounding the same aggregation site resulted in a 60% increase in stock density and biomass. Coleman et al. (2000) recommended establishing networks of large Marine Protected Areas (MPAs) for managing species – including red grouper – that may be highly susceptible to overfishing. Similarly, Koenig et al. (2000) emphasized the importance of protecting habitat by establishing reserves, which would then allow researchers to better understand production in un-fished areas.

However, the widespread distribution, protracted spawning season, and lack of seasonal movement of red grouper make designation of critical habitats and effective fishery closures challenging. Small areal closures may not protect sufficient numbers of fish, while large and/or widespread closures are difficult to enforce. Deployment of passive acoustic recorders throughout the known range of red grouper could provide a detailed map of their distribution. If sound production proves to be closely linked to spawning behavior, habitat use could then be more clearly defined. This approach would enable identification of critical habitats and, ultimately, designation of reserves for red grouper.

Red grouper, along with all marine organisms that rely on sound for communication, may be impacted by increasing levels of anthropogenic sound in the ocean. Oil and gas exploration, vessel traffic, scientific research, and military activity all contribute acoustic energy to the marine environment (Hildebrand, 2009). Low-
frequency components attenuate least, and therefore travel farthest, so those species relying on low-frequency sounds to communicate – like red grouper – are most likely to be affected. Shipping noise has risen to such levels that global deep water ambient noise has increased 10- to 100-fold for frequencies below 300 Hz over the last 50 years (Ross, 2005). At a minimum, this noise will likely cause an increase in signal masking below 300 Hz. Masking occurs when noise levels increase (relative to a signal of interest) to the point that a receiver is no longer able to discriminate between the signal and background noise. Masking may interfere with social communication, predator avoidance, prey detection, and other important signals. Vasconcelos et al. (2007) demonstrated that vessel noise negatively affected hearing and conspecific communication in the Lusitanian toadfish (*Halobatrachus didactylus*). Codarin et al. (2008) report that masking occurred in the presence of ship noise for drum (*Sciaena umbra*) and damselfish (*Chromis chromis*).

Recent research suggests that ocean acidification due to increasing atmospheric carbon dioxide is likely to result in decreased sound absorption for frequencies below 10 kHz (Hester *et al*., 2008; Brewer and Hester, 2009). Ocean acidification could exacerbate the already increasing levels of ocean noise, and amplify the potential for masking of acoustic cues. Long-term noise exposure effects have been examined in terrestrial animals, but ocean noise research has focused almost entirely on marine mammals (Wright *et al*., 2007; Clark *et al*., 2009). Very little is currently being invested in studying lower-profile species such as fish. Understanding the acoustic
communication of red grouper will lay the foundation to determine their susceptibility to noise pollution.

The results presented in this study offer new insight into the acoustic behavior of red grouper in their natural environment and provide a framework for future passive acoustic research. Better understanding of spatial and temporal patterns of red grouper reproductive activity will enable implementation of management practices aimed at optimizing spawning and recruitment, while at the same time maintaining a productive fishery. As a keystone species, red grouper play an important role in maintaining biological diversity and abundance. Given the value of red grouper, both in terms of human economics and ecosystem balance, effective conservation and management strategies are crucial.
### Table 1  Location, deployment, and data collection information for all recorders.

<table>
<thead>
<tr>
<th>Unit</th>
<th>Location</th>
<th>Depth (m)</th>
<th>Deployment Date &amp; Time (EST; 2008)</th>
<th>Retrieval Date &amp; Time (EST; 2008)</th>
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<th>Audio</th>
<th>Sample Rate (kHz)</th>
<th>Duty cycle</th>
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<td>70.4</td>
<td>12-May 12:51</td>
<td>13-May 09:05</td>
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<td>70.4</td>
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<td>13-May 09:05</td>
<td>Yes</td>
<td>Stere</td>
<td>44.1</td>
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</table>

1 Steamboat Lumps Marine Reserve  
2 Madison Swanson Marine Reserve
Table 2 Descriptive statistics for red grouper calls.

| Call Type | Measure                      | Mean | SD  | Min. | Max. | CV (%)
|-----------|------------------------------|------|-----|------|------|-------
| Short (n=100) | Peak Frequency (Hz)       | 150  | 51  | 39   | 190  | 34
|          | 6-dB Bandwidth (Hz)        | 148  | 57  | 3    | 260  | 38
|          | Call Duration (s)          | 1.9  | 0.3 | 1.4  | 2.8  | 14.9
|          | # Pulses/Call              | 4    | 1   | 3    | 7    | 19
|          | Mean IP\(^1\) Pulse Duration (ms)   | 161  | 33  | 67   | 244  | 20
|          | Mean IP\(^1\) Inter-pulse Interval\(^4\) (ms) | 355  | 51  | 272  | 440  | 14
|          | CP\(^2\) Pulse Duration (s) | 0.7  | 0.2 | 0.5  | 1.2  | 21.0
|          | CP\(^2\) Peak Frequency (Hz) | 112  | 56  | 35   | 237  | 50
|          | Peak Amplitude (mV)        | 114  | 118 | 13   | 733  | 104
|          | Received SPL\(^5\) (dB re: 1\(\mu\)Pa\(_{rms}\)) | 124  | 6   | 110  | 142  | 5
| Pulsetrain (n=16) | Peak Frequency (Hz)       | 131  | 63  | 13   | 185  | 49
|          | 6-dB Bandwidth (Hz)        | 118  | 62  | 30   | 179  | 53
|          | Call Duration (s)          | 7.7  | 5.4 | 2.5  | 22.4 | 70.9
|          | # Pulses/Call              | 33   | 16  | 11   | 57   | 50
|          | Mean IP\(^1\) Pulse Duration (ms)   | 153  | 43  | 92   | 224  | 28
|          | Mean IP\(^1\) Inter-pulse Interval\(^4\) (ms) | 370  | 43  | 286  | 448  | 12
|          | CP\(^2\) Pulse Duration (s) | 1.8  | 0.2 | 1.5  | 2.2  | 11.8
|          | CP\(^2\) Peak Frequency (Hz) | 89   | 55  | 35   | 202  | 62
|          | Mean PT\(^3\) Pulse Duration (ms)   | 113  | 42  | 50   | 195  | 37
|          | Mean PT\(^3\) Inter-pulse Interval\(^4\) (ms) | 201  | 76  | 105  | 391  | 38
|          | Peak Amplitude (mV)        | 88   | 49  | 24   | 184  | 56
|          | Received SPL\(^5\) (dB re: 1\(\mu\)Pa\(_{rms}\)) | 122  | 4   | 116  | 128  | 3

\(^1\) IP: introductory pulses  
\(^2\) CP: central pulse  
\(^3\) PT: pulse train  
\(^4\) Inter-pulse intervals were measured as the time from the first positive peak of one pulse to the first positive peak of the subsequent pulse.  
\(^5\) SPL: root-mean-square (rms) sound pressure level, dB relative to a reference pressure of 1\(\mu\)Pa  
\(^6\) CV = coefficient of variation, calculated as 100*(SD/Mean)
Table 3  Sound properties and observed behaviors of red grouper.

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<th>Recorder</th>
<th>Time (EST)</th>
<th>Type¹</th>
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<th>Peak Frequency (Hz)</th>
<th># Pulses</th>
<th>Dur (s)</th>
<th>Sex</th>
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<td>courtship w/ dbc</td>
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¹ pt: pulse train
² RL: received sound pressure level, dB relative to a reference pressure of 1 μPa
³ dbc: direct body contact
Figure 1 Locations of marine reserves on West Florida Shelf where acoustic and video recorders were deployed. Black triangles represent deployment sites.
Figure 2  Diagram of DSG audio recorder setup in the water column. Note: distances not to scale.
Figure 3  (A) Waveform, (B) spectrogram (FFT = 1024 samples) and (C) band sound pressure level (BSPL; frequency resolution = 10 Hz) of a typical red grouper short call. Arrows in (B) indicate divisions of call for descriptive measures: IP = introductory pulses; CP = central pulse.
Figure 4  (A) Waveform, (B) spectrogram (FFT = 1024 samples) and (C) band sound pressure level (BSPL; frequency resolution = 10 Hz) of a red grouper call with a pulse train. Arrows in (B) indicate divisions of call for descriptive measures: IP = introductory pulses; CP = central pulse; PT = pulse train.
Figure 5  Average fast Fourier transform (FFT) for red grouper calls (n = 116). Raw files were resampled to 4,410 Hz and filtered using a finite-duration impulse response (FIR) correction filter unique to each recorder. Number of points used in each FFT was 110,211, corresponding to the number of points in the longest WAV file; amplitude values for each frequency bin (0.04 Hz) were scaled to the maximum amplitude in each file above 100 Hz, to ensure scaling to signal peaks rather than noise.
Figure 6  Distribution of peak frequency values for all red grouper calls (n = 116). Counts in each column represent number of files having peak frequency within 10-Hz bins.
Figure 7  Distribution of 6-dB bandwidth for red grouper calls (n = 116). Counts in each column represent the number of calls having 6-dB bandwidth values within 10-Hz bins.
Figure 8  Received levels (root-mean-square; dB relative to reference pressure of 1μPa) of red grouper calls (n = 116). Counts in each column represent number of calls having received sound pressure levels within 2-dB bins.
Figure 9  Number of pulses in red grouper calls versus total call duration (n = 116). Circles represent short calls; triangles represent calls with pulse trains. Duration=0.22*#Pulses+0.85, R²=0.824
Figure 10  Time series of red grouper sound production at Steamboat Lumps and Madison-Swanson Marine Reserves, May 7-14, 2008. Number of calls represents the mean number of calls for each 10-minute time bin for all recorders at each location. For Steamboat Lumps (4 recorders), all calls were included from 2.5 minute files recorded every ten minutes; for 8-minute Madison-Swanson recordings (3 recorders), only calls in the first 2.5 minutes were counted. Gaps in each time series are due to recordings containing high levels of background noise likely to mask detection of red grouper calls, which were excluded from analysis.
Figure 11  Diel periodicity of red grouper sound production at Steamboat Lumps and Madison-Swanson Marine Reserves. Mean +/- SD call rate for all recordings within 30-minute time bins. Steamboat Lumps (red triangles) call rates were calculated from 2.5-minute recordings every ten minutes; Madison-Swanson (blue circles) values were calculated from 8-minute recordings every ten minutes. Shaded areas represent night (sunrise: 0550 EST; sunset: 1920 EST).
Figure 12  Periodicity of red grouper sound production by call type at (A) Steamboat Lumps and (B) Madison-Swanson. Number of calls represents the mean number of calls in all recordings from each 60-minute time bin, with black bars representing calls with pulse trains and grey bars representing short calls. Steamboat Lumps duty cycle was 2.5-minute recordings every ten minutes; Madison-Swanson was 8-minute recordings every ten minutes. Shaded areas represent night (sunrise: 0550 EST; sunset: 1920 EST).
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Misty D. Montie was born in Missoula, MT, and earned a B.A. degree in Environmental Biology from the University of Montana in 2000. She has received two Honorable Mentions from the National Science Foundation Graduate Research Fellowship Program, and is the recipient of a National Defense Science and Engineering Graduate Fellowship.