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A passive acoustic survey of fish sound production at Riley's Hump within Tortugas South Ecological Reserve: implications regarding spawning and habitat use

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Abstract—Passive acoustic recorders were used to monitor sound production indicative of the use of spawning habitat by groupers (Serranidae) at Riley's Hump, which is located in the Tortugas South Ecological Reserve (TSER), part of the Florida Keys National Marine Sanctuary. Sound production by black grouper (*Mycteroperca bonaci*), red grouper (*Epinephelus morio*), and red hind (*E. guttatus*) was recorded year-round and at all times of day but occurred more often in the evening during the winter–spring spawning period than during other times of the day and year. This pattern for these species is consistent with results of previous studies that documented the association of sound production with reproductive behavior at spawning sites. Distinct diel and seasonal patterns of sound production by the longspine squirrelfish (*Holocentrus rufus*) and bicolor damselfish (*Stegastes partitus*) also were recorded. Riley's Hump is a documented spawning site for mutton snapper (*Lutjanus analis*), and recordings of black grouper, red grouper, and red hind indicate that it is used for reproductive purposes by these species as well. These results showed the importance of the TSER and the need for continued research to understand its role in the recovery and sustainability of managed fish populations.

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Because most large groupers support large commercial and recreational fisheries, there is an increasing focus on the conservation and management of their stocks and habitats. Fundamental to management decisions about these species is information on trends in population abundance and distribution, life history, and habitat use. Data that are used to understand these topics typically are generated from long-term time series based on visual surveys by divers, active acoustic surveys, mark and recapture studies, or subsampling of commercial catches. Recognition and protection of critical habitat, both essential for the sustainability of groupers and other reef fishes, have been realized through the establishment of marine protected areas and reserves (Roberts et al., 2005). An important consideration when selecting a location for a marine reserve is whether the area is used as a spawning aggregation site (Koenig et al., 2000). In many cases, fish spawning aggregation sites were first discovered by commercial fisherman and later established as marine reserves when their conservation value was understood. Such sites become prime locations for implementing long-term field studies to evaluate the efficacy of reserves for population recovery and to learn more about the behavior and dynamics of spawning aggregations (Burton et al., 2005).

Groupers, as the name implies, form seasonal spawning aggregations at traditional sites. The structure and size of these aggregations vary by species and may directly influence their vulnerability to overfishing. For example, Nassau grouper (*Epinephelus striatus*) and red hind (*E. guttatus*) form few, large aggregations (Whaylen et al., 2004; Kadison et al., 2009), a characteristic that increases their risk to recruitment overfishing. Because of this vulnerability, it has been important to consider spawning locations of red hind in the establishment of marine protected areas and seasonal closures in the U.S. Caribbean (Nemeth, 2012). Evidence from Belize indicates that black grouper (*Mycteroperca bonaci*) form numerous medium-size aggregations (<200 individuals) at various locations among offshore atolls (Paz and Sedberry, 2008). In contrast, red grouper (*E. morio*) do not form large aggregations but instead appear to use more discretely formed spawning sites, where individual male territories are indicated by shallow pits excavated in the sediment (Coleman and Koenig, 2010; Nelson et al., 2011).

At many traditional aggregation sites, a variety of species may co-occur and form seasonal reproductively active communities; hence these sites are of value for conservation and research purposes (Heyman et al., 2001). The establishment of marine reserves at such locations provides an effective approach for the management of stocks in multispecies fisheries (Huntsman et al., 1999; Ault et al., 2008a).

In addition to the more traditional methods, passive acoustics represents a relatively new and underused approach to survey fish populations at spawning sites. Sound production is common among many fishes and is associated most often with courtship and spawning behaviors (Mok and Gilmore, 1983). Because sounds are species-specific, once the source has become positively identified, the information can be referenced to all future recordings to identify the presence of a given species at a monitoring site. Time series from the acoustic monitoring of fish sound production, therefore, can be used as a proxy to document the timing and location of reproductive behavior (Locascio and Mann, 2008). Recording technologies now allow multiyear deployments during which short periods of data (e.g., tens of seconds) may be recorded every few minutes. The trend in recording technologies becoming more sophisticated and less costly to acquire and deploy will continue and result in the collection of larger, synoptic acoustic data sets at more locations.

Groupers are among the most economically important fishes currently being studied with passive acoustics, and accomplishments from such monitoring are still few but increasing. Thus far, the sounds of Atlantic goliath grouper (*E. itajara*), red hind, red grouper, yellowfin grouper (*M. venenosa*), Nassau grouper, and black grouper have been positively identified and correlated with known spawning seasons (Mann et al., 2009; Mann et al., 2010; Nelson et al., 2011; Schärer et al., 2012, 2013). Other grouper species are also likely to produce sound, and these sounds await discovery. Only one study has attempted to quantify population size of a grouper species (red hind) with the use of passive acoustics in combination with visual surveys made by divers (Rowell et al., 2012).

Riley's Hump was a historically productive commercial fishing ground, particularly for mutton snapper (*Lutjanus analis*) (Burton et al., 2005). Anecdotal input from fishermen and the recommendations of a 25-member working group of commercial and recreational fishermen, divers, conservationists, scientists, concerned citizens, and representatives from government agencies led to the creation of the Tortugas South Ecological Reserve (TSER), a research-only marine reserve, in 2001 to protect the overexploited population of mutton snapper. Mutton snapper use Riley's Hump as a spawning aggregation site in the late spring and early summer months (NOAA[1]; Domeier, 2004; Burton et al., 2005). Since the inception of the TSER and the protection of the aggregation of mutton snapper at Riley's Hump, increased numbers of mutton snapper have been seen in visual surveys at Riley's Hump, at downstream locations along the Florida Keys reef tract (Ault et al., 2013), and in recreational headboat fishery landings (Brennan[2]). Many grouper species also inhabit Riley's Hump, but their use of the site for reproductive purposes has not been documented. The primary purpose of this study was to conduct an acoustic survey of Riley's Hump to document grouper sound production, which is generally used as a proxy for reproductive behavior.

Materials and methods

Riley's Hump, a geologic feature of approximately 10 km^2, marks the western extent of the south Florida reef tract and lies entirely within the TSER (Fig. 1, A and B). The limestone composition at Riley's Hump is typical of the sedimentary geology of the Gulf of Mexico, and its surface ranges from sandy bare areas to rugose hard bottom and low-relief outcroppings. Depths range from approximately 30 m on the hump to approximately 60 m immediately adjacent to it (Mallinson et al., 2003). Relief is highest along the edges, especially from the northeast to southern edges in a clockwise direction. The steepest vertical drop-off is located along the south–southwestern edge, which also has been observed to have the highest fish densities (Burton et al., 2005). The benthic community is composed of hard and soft corals, gorgonians, and a variety of sponges (Weaver et al., 2006).

Acoustic digital spectrum recorders (Loggerhead Instruments[3], Sarasota, FL) were deployed at 7 locations on Riley's Hump during multiple periods from 2010 through 2012 (Table 1; Fig. 1). These locations included 3 previously established study sites (12, 12A, and 15), where visual surveys of fishes were conducted during prior years, along with 4 new sites established for this study, including 3 sites on Riley's Hump (RH1, RH2, and RH3) and a deepwater site off the southwestern edge of Riley's Hump (RHDW) at a depth of approximately 60 m. Digital spectrum recorders were programmed to record 10 s of sound every 10 min at a

[1] NOAA. 2000. Draft supplemental environmental impact statement/draft supplemental management plan for the Tortugas Ecological Reserve, 250 p. Mar. Sanctuaries Div., Off. Ocean Coast. Resour. Manage., Natl. Ocean Serv., NOAA, Silver Spring, MD. [Available at website.]

[2] Brennan, K. J. 2013. Personal commun. Beaufort Laboratory, Southeast Fisheries Science Center, National Marine Fisheries Service, NOAA, Beaufort, NC 28516-9722.

[3] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

15,094 Hz sample rate; they were moored to steel rebar anchored in the limestone substrate. All recorders were deployed and recovered by scuba divers, except for the deployment of the deepwater recorder (RHDW), which was dropped in a weighted housing unit from the swim platform of the MV *Spree* and recovered by scuba divers. Visual surveys were conducted along transects of 50 m by divers to document the presence of grouper species at acoustic monitoring sites (12, 12A, 15, RH1) during April 2010 and January and February 2011. All field operations were conducted from the MV *Spree*.

Each 10-s acoustic file was analyzed in MATLAB, vers. R2009B (The Mathworks Inc., Natick, MA) with a fast Fourier transform to generate a power spectrum from which the band sound pressure level (SPL) in 100-Hz-wide bins was calculated. Patterns in fish sound production on daily and seasonal scales were examined in plots of power spectra and, for grouper species, by direct counts of calls in a subsample of 10,000 files randomly selected from the entire database of acoustic recordings. The number of calls counted for each grouper species in the subsample was normalized as a ratio of the number of calls to number of files reviewed for each month at each site. Spectrograms of acoustic recordings were reviewed with Adobe Audition, vers. 2.0 (Adobe Systems Inc., San Jose, CA) to identify species present in the recordings or previously undescribed calls. Daily sound patterns were estimated by binning the number of calls for each species (sites combined) into three 8-h periods (0000–0800, 0800–1600, and 1600–0000, all local time) and comparing them by means of tests for analysis of variance. Acoustic time-series data were examined for peaks associated with lunar phases.

Custom underwater audio and video (A/V) systems were used to verify sources of fish sound production and to understand the behavioral context associated with that sound production. The recording system included a low-light, 0.001-lux, black-and-white flat lens board camera and 2 HTI 96-MIN hydrophones (sensitivity −164 dB reference pressure [re]: 1V/µPa) (High Tech Inc., Long Beach, MS) that recorded to a ChaseCam deck (Chase Vision, LLC, Cleveland, TN). Each A/V system was deployed in a clear, waterproof housing unit and placed on the seafloor overnight at select sites where long-term acoustic recorders were deployed. The A/V system recorded continuously to compact flash memory cards for approximately 20 h. A Sony HDR-XR100 video camera (Sony Corp., Tokyo) fitted with an HTI 96-MIN hydrophone was used during visual surveys conducted by divers to record fish sound production and associated behavior. Audio and video data were reviewed with Corel Video Studio software, vers. X6 (Corel Corp., Ottawa, Ontario) and with Adobe Audition software to identify species-specific acoustic signals and associated behavioral context.

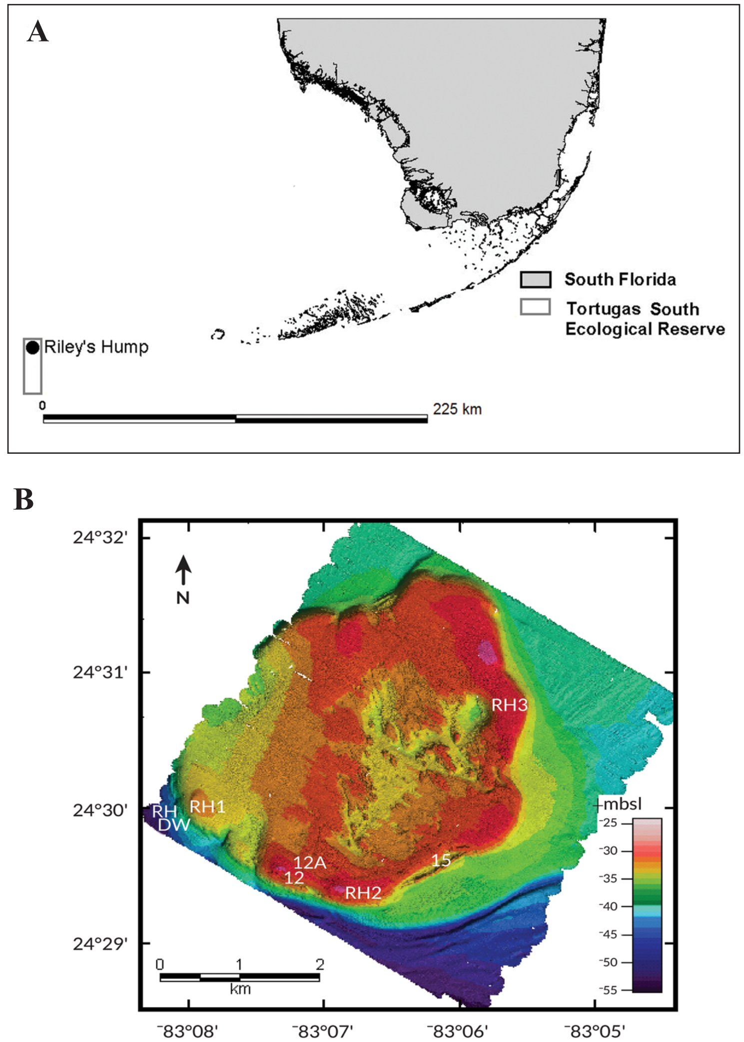

Figure 1

(**A**) Location of Riley's Hump within the Tortugas South Ecological Reserve and in relation to mainland Florida, and (**B**) locations of acoustic monitoring sites on Riley's Hump indicated on the multibeam bathymetric image of this geologic feature. The scale of the image represents meters below sea level (mbsl). The deepwater site (RHDW) that would be on the left side of the image is located a bit farther west than what is indicated in this figure. The depth of this site was approximately 60 m; depths at all other acoustic montioring sites located on Riley's Hump were approximately 30 m. Bathymetric image is taken from figure 7 of Mallinson et al., 2003.

Table 1

Record of deployment locations, periods, and number of records used to monitor sound production by groupers and other fish species at Riley's Hump (RH), Tortugas South Ecological Reserve, Florida Keys National Marine Sanctuary. All recorders were programmed to record 10 s of sound every 10 min at a sample rate of 15,094 Hz. At the deepwater site (RHDW), located off the southwestern edge of Riley's Hump, the recorder was deployed at a depth of 60 m.

Site	Deployment Period	Days	Records
12	29 April–28 June 2010	60	8640
12	18 January–14 July 2011	177	25,488
12A	29 April 2010–16 July 2011	443	63,792
15	29 April 2010–9 December 2011	589	84,816
RH1	18 January 2011–17 June 2012	516	74,304
RH2	18 January 2011–17 June 2012	516	74,304
RH3	18 January 2011–21 June 2012	516	74,304
RHDW	17 July 2011–13 June 2012	332	47,808

Results

Patterns in fish sound production recorded at all study sites were classified into 3 frequency ranges: <200 Hz, 300–400 Hz, and 500–800 Hz. Identifiable sounds produced in the lowest frequency range (<200 Hz) were associated mainly with 3 grouper species: red grouper, red hind, and black grouper. Positive identification of these species in the recordings was based on previous descriptions of their sounds (Mann et al., 2010; Nelson et al., 2011; Schärer et al., 2013) and on additional evidence from this study of sound production by black grouper documented with the A/V systems. Sound production by each of these species was greatest during the evening period (1600–0000) but was not significantly different from any of the 3 time periods (black grouper: F=3.1, P=0.05; red grouper: F=1.4, P=0.24; and red hind: F=4.5, P=0.64) (Fig. 2). Diel variability in SPLs during the winter–spring period ranged from about 5 to 10 dB SPL (re: 1 µPa) above daily background levels in the frequency range used by groupers (<200 Hz) at all sites during 2011 and 2012 except RHDW. This

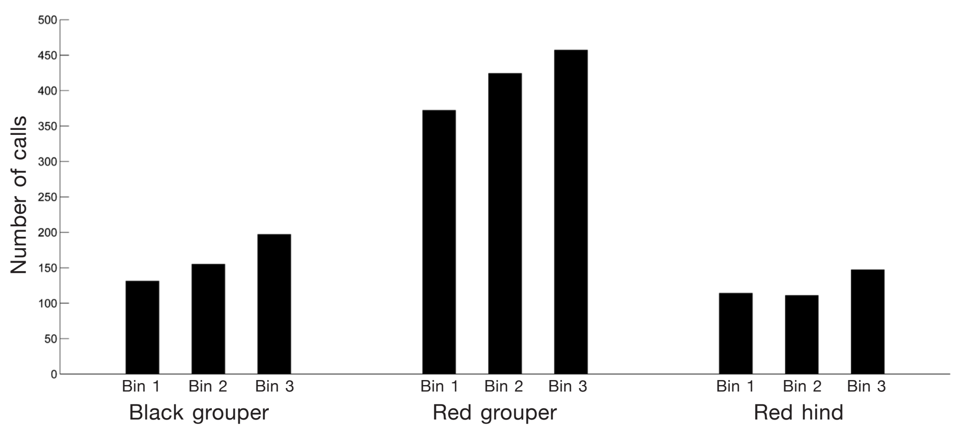

Figure 2

Diel pattern of sound production by black grouper (*Mycteroperca bonaci*), red grouper (*Epinephelus morio*), and red hind (*Epinephelus guttatus*) at Riley's Hump, Tortugas South Ecological Reserve, Florida Keys, in 2012. Data are distributed in 3 bins of 8 h each (bin 1=0000–0800; bin 2=0800–1600; bin 3=1600–0000) and are the sum total of calls identified in analysis of 10,000 files randomly selected from the entire database of acoustic recordings. The number of calls is greatest during the evening period for these species, but significant differences do not exist between time periods for any species based on the methods used in this study.

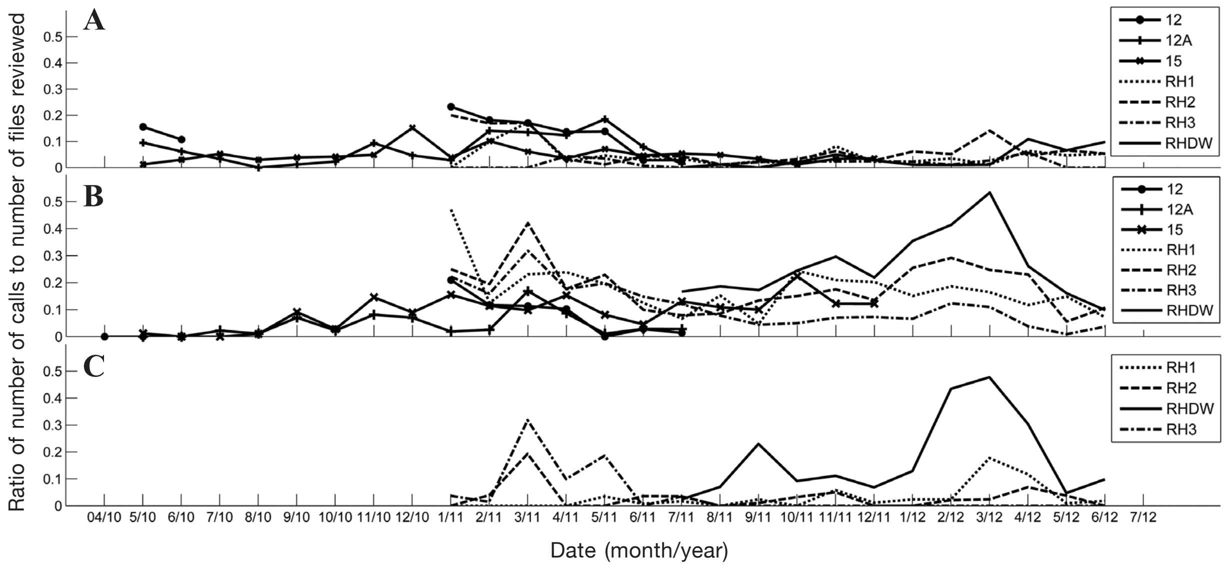

Figure 3

Time series of the ratio of the number of calls of 3 grouper species, (**A**) black grouper (*Mycteroperca bonaci*), (**B**) red grouper (*Epinephelus morio*), and (**C**) red hind (*Epinephelus guttatus*), recorded at Riley's Hump, Tortugas South Ecological Reserve, Florida Keys, 2010–2012 to the number of files reviewed in a subsample of 10,000 randomly selected audio files. For each species, call volume increased during the winter–spring reproductive season. The 2012 season had a greater call volume and appears more protracted than the 2011 season for red grouper and red hind; the higher numbers of calls in 2012 may represent the presence of more fish species at these sites in 2012 than in 2011. Red grouper and red hind both demonstrated a preference for the deepwater site (RHDW) in 2012 and for sites RH2 and RH3 in 2011, respectively. Black grouper were recorded at all sites yet did not produce particularly high numbers of calls at a specific site, although, overall, most sound production by this species was recorded at site RH2 during both years.

resulted in relatively poor signal-to-noise ratios which prevented identification of a clear diel pattern in the power spectra results associated with groupers. Recordings of red hind at the RHDW site were the only exception to this and diel patterns in the power spectra associated with this species were clearly discernible from background levels.

Sound production by each grouper species occurred year-round, but levels were highest from January through May and typically peaked in March for each species during 2011 and 2012 (Fig. 3). Black grouper were recorded at all sites; the highest standardized number of calls was recorded at site 12A in 2011 and at site RH2 in 2012. A modestly higher number of black grouper calls were recorded in 2011. Red grouper were recorded at all sites; the highest number of calls by this species was recorded at site RH2 in 2011 and at site RHDW in 2012. Red hind were recorded only at sites RH1, RH2, and RH3 and at site RHDW; the highest number of calls by this species was recorded at site RH3 in 2011 and at site RHDW in 2012. The seasonal pattern of calls by black grouper indicates a more even distribution over the winter–spring period than that of the seasonal pattern of sound production by red grouper and red hind, which peaked more sharply in March and April of each year. Sound pressure levels recorded at site RHDW reached amplitudes that were 15 dB SPL (re: 1 µPa) greater than daytime background levels and were positively associated with the last quarter moon phase. All 3 grouper species were recorded at site RHDW, but sound production was dominated at this site by the red hind (Figs. 3 and 4).

Red and black groupers and red hind were the dominant grouper species recorded at Riley's Hump, but other low-frequency, pulsed and modulated tonal calls were occasionally discovered during review of audio files. Three instances of calls by yellowfin grouper were positively identified in recordings made at site 12A during November 2010 by comparison with documented characteristic sound production of yellowfin grouper (Schärer et al., 2012). Several instances of a call type that resembled that of the Nassau grouper were also noted in recordings made during winter–early spring at site RHDW. Both the yellowfin grouper and Nassau grouper were observed in visual surveys conducted by divers at Riley's Hump during this study. More detailed studies of sound production of Nassau grouper are in progress, and the results will be useful for quantifying the presence of this species in the recordings made at Riley's Hump.

Additional information on sound production by black grouper was documented in this study. Two

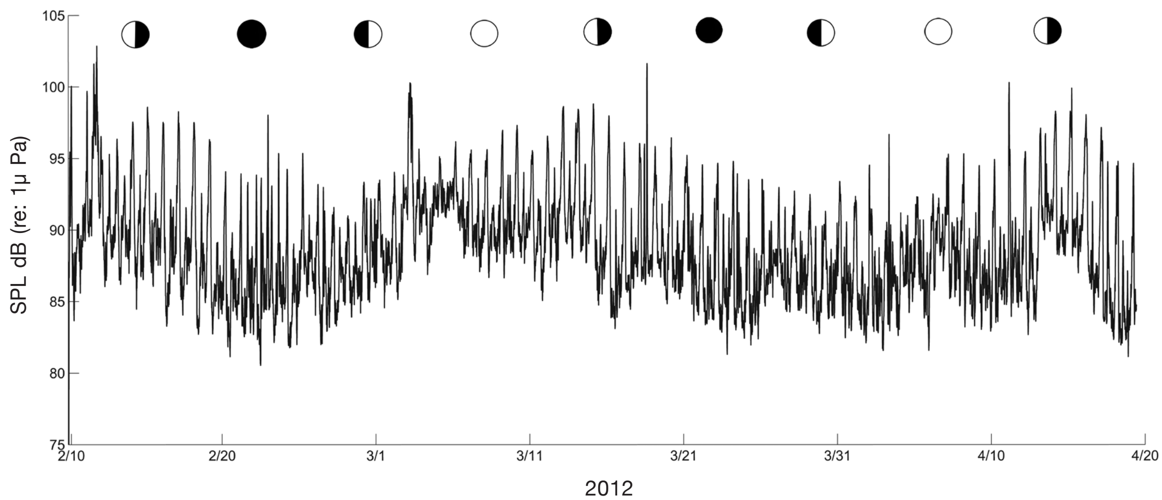

Figure 4

An example of seasonal periodicity of sound pressure levels (SPLs) in the time series of acoustic data for the frequency range <200 Hz recorded at the deepwater site (RHDW) at a depth of 60 m at Riley's Hump, Tortugas South Ecological Reserve, Florida Keys, 2010–2012. Red grouper (Epinephelus morio), black grouper (Mycteroperca bonaci), and red hind (Epinephelus guttatus) all were recorded at this site, but the red hind was the dominant sound producer during the period shown. Sound production occurred during all lunar phases, but peaks were coincident with the last quarter moon phase. Lunar symbols represent moon phases as follows: new moon, ●; first quarter moon, ◐; full moon, ○; and last quarter moon, ◑.

variations of the call of black grouper were identified. One variation, BGV1, was composed of a relatively long, frequency-modulated tonal portion only. The other variation, BGV2, was composed of an initial, shorter-duration frequency-modulated tone followed by several individual pulses and concluded with a longer frequency-modulated tonal portion characteristic of the BGV1 (Fig. 5, A–D). The long, frequency-modulated tonal portion common to both variations (n=20) had a mean duration of 5.2 s (standard deviation [SD] 1.2) and was modulated between 60–120 Hz at a mean rate of 170 ms (SD 0.03. The highest received root-mean-square (RMS) SPL for the long, frequency-modulated portion of the call was estimated at 149.9 dB RMS SPL (re: 1 µPa). The highest received RMS SPL for the introductory portion of the call only and the overall combined portions of the call were 143.3 and 144.3 dB SPL (re: 1 µPa), respectively.

This call type was identified 76 times in the audio track of the recordings made with the remote A/V systems. Black grouper appeared in the video from the A/V systems during 18 of the 76 times either call variation was identified and 10 additional times within 25 s of the call being made. No other grouper species were recorded on video at or near the time during which either variation of this call was produced. Other grouper species were verified at these sites by divers during visual surveys, including the Nassau grouper, yellowmouth grouper (*M. interstitialis*), scamp (*M. phenax*), yellowfin grouper, rock hind (*E. adscensionis*), and coney (*Cephalopholis fulva*).

In most cases, only a single black grouper appeared in the video when a call of either variation (BGV1 or BGV2) was made and only once was interaction between 2 fish recorded (site 12A). On this occasion, one fish with a blotched pattern approached another more monochromic fish from below and behind and briefly made contact as it passed under the rear portion of the other's body. The 2 fish then swam slowly away in opposite directions and out of the video frame (Fig. 6, A–D). The blotch-patterned fish swam toward the camera and out of the frame, and within 10 s a relatively high amplitude (149.9 dB SPL) BGV1 call was recorded. This behavior could indicate possible courtship, but it could also represent a territorial display.

On the morning that the A/V system that made this recording was deployed (27 April 2010) divers reported seeing several black grouper at this site (12A) swimming together in a daisy chain pattern high in the water column. Apparent courtship behavior between 2 black grouper was also observed during a visual survey conducted at site RH1 on 19 January 2011. On this occasion, a large, light-colored black grouper was observed to approach a smaller black grouper from behind and swim alongside it for a few moments and then to rub and shake its body against it for about 2 s. No sound associated with this behavior was heard by divers, but it may have gone unnoticed. Similar courtship behavior of black grouper was described by Paz

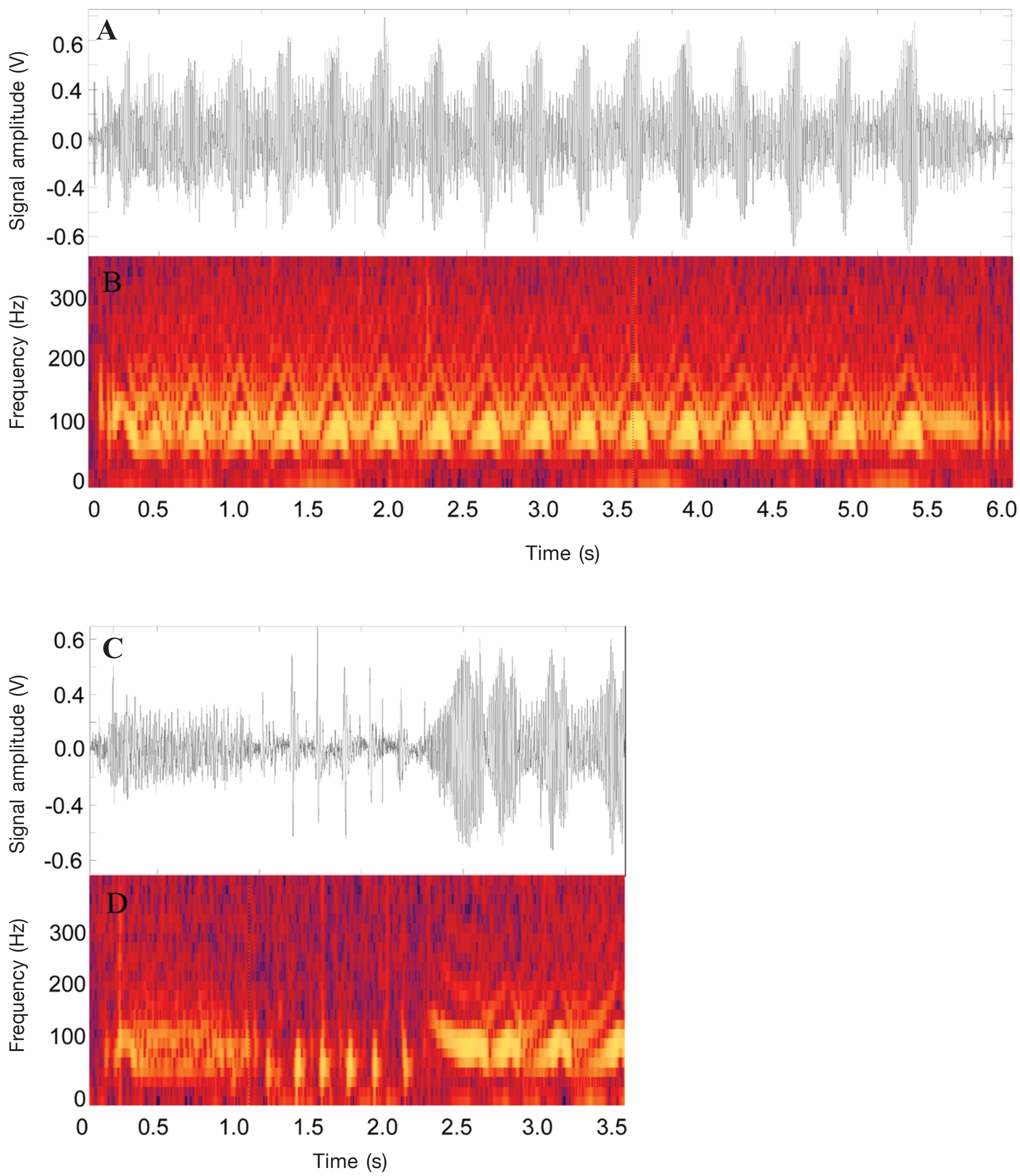

Figure 5

Examples of acoustic recordings of black grouper (*Mycteroperca bonaci*) sounds made at site 12 at Riley's Hump, Tortugas South Ecological Reserve, Florida Keys in April 2012: (**A**) in waveform and (**B**) as a spectrogram of black grouper call variation 1 (BGV1), which is frequency modulated between 60–120 Hz at a rate of about 0.2 s. Average duration of this variation was 5.2 s (SD 1.2, *n*=20), and highest received levels recorded were 149.9 dB SPL (re: 1 µPa). (**C**) Waveform and (**D**) spectrogram of black grouper call variation 2 (BGV2), which contains an introductory portion composed of a short frequency-modulated period followed by individual pulses and then the longer frequency-modulated portion common to BGV1. The BGV2 call was uncommon in field recordings. How the context of the 2 call variants differs is unknown. The longer frequency-modulated end portion of the BGV2 call is truncated because the programmed 10-s recording period elapsed during the call.

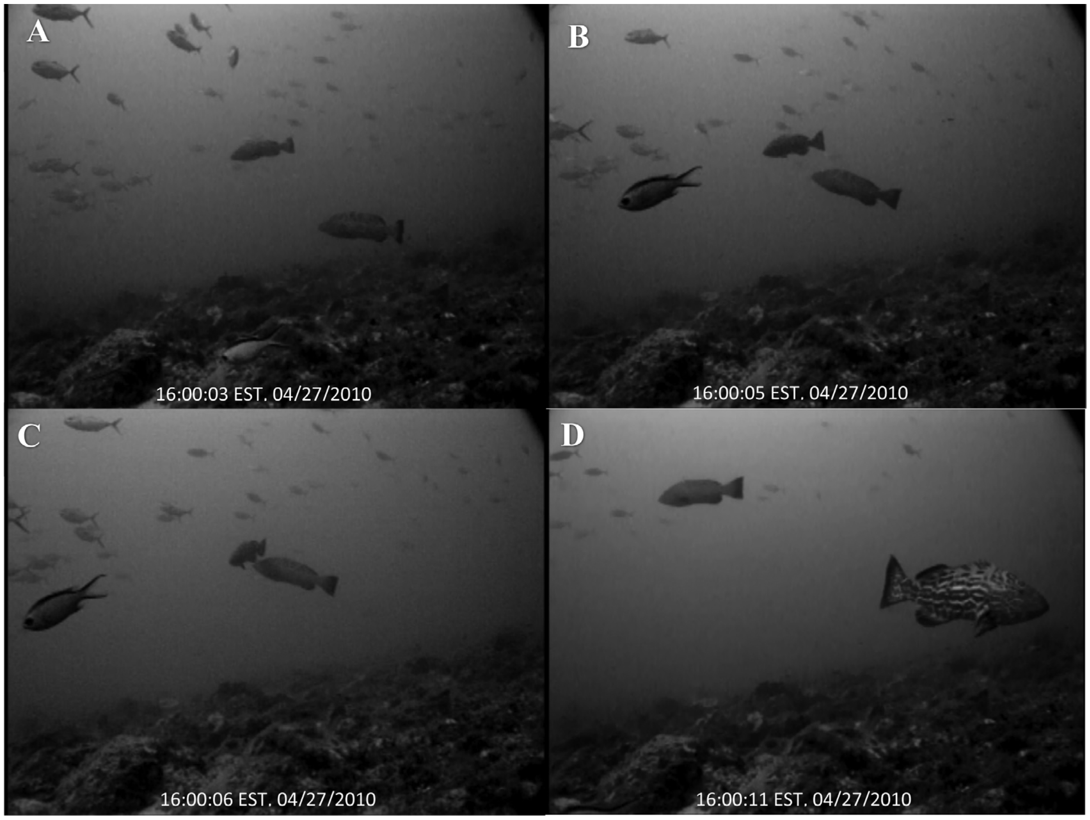

Figure 6

Interaction between black grouper (*Mycteroperca bonaci*) was captured only once in video recordings made at Riley's Hump, Tortugas South Ecological Reserve, Florida Keys, and that interaction is shown in these video stills from videos taken at site 12A on 27 April 2010: (**A**) and (**B**) a blotch-patterned black grouper (right side of figure) approaches a second black grouper from below. (**C**) The approaching fish passes directly beneath the rear portion of the second fish and briefly makes contact, but no additional interaction occurs. (**D**) The 2 fish swim away from each other in opposite directions and a BGV1 call is produced a few seconds later. This interaction may have been courtship or territorial associated behavior. When the audio and video system was deployed at this site, divers reported seeing several black grouper midway in the water column swimming together in a generally circular pattern, similar to behavior observed in a spawning aggregation documented in Belize.

and Sedberry (2008) at spawning aggregation sites of black grouper in Belize, where these authors also noted that the blotched-color phase was seen during the morning of the day that spawning occurred.

Sound production in the frequency range of 300–400 Hz was dominated by the longspine squirrelfish (*Holocentrus rufus*). This finding was validated by an analysis of recordings made with the handheld Sony video camera fitted with a hydrophone and also by comparison with descriptions made by Winn et al. (1964). This species produced a pulsatile call with received SPLs of 6.0–8.0 dB (re: 1 µPa) above daytime background levels. The diel pattern was crepuscular with slightly higher SPLs reached during the evening than during the morning. Patterns in the SPLs and timing of this call type began in early spring and continued through late summer and early fall. These patterns were similar among sites and between years, and they were not associated with a lunar period (Fig. 7).

Sound production in the frequency range of 500–800 Hz was also dominated by a pulsatile call, typical of the family Pomacentridae and attributed to the bicolor damselfish (*Stegastes partitus*). Some energy associated with this call extended above and below the range of 500–800 Hz but was minimal by comparison. Sound production in this range was considered to be from a different source than that of the signal produced in the range of 300–400 Hz by the longspine squirrelfish because plots of each signal indicated they were out of phase with each other (i.e., not temporally synchronized). Sound production and behavior by this species were also recorded by the remotely deployed A/V sys-

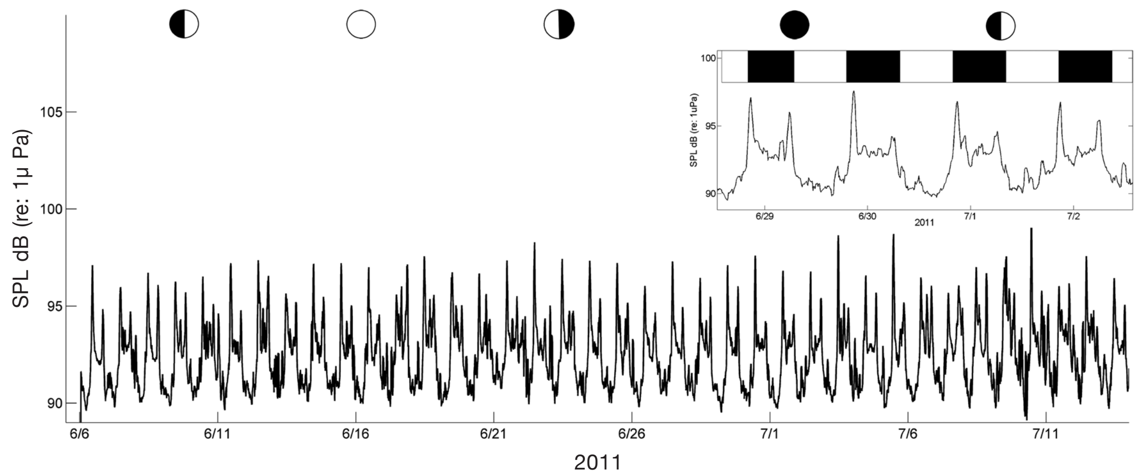

Figure 7

An example of sound production by longspine squirrelfish (*Holocentrus rufus*), measured in sound pressure levels (SPLs), from the time series of acoustic data recorded in the frequency range of 300–400 Hz at site RH1 at Riley's Hump, Tortugas South Ecological Reserve, Florida Keys, in 2011. The seasonal period of sound production in this frequency range occurred from early spring through late summer and early fall, beyond the limits of the x-axis. Illustrated more closely in the inset figure, with bars that indicate periods of day and night, diel periodicity of sound production by this species is defined by a crepuscular pattern. No lunar periodicity is evident. Lunar symbols are defined as follows: new moon, ●; first quarter, ◐; full moon, ○; and last quarter moon, ◑.

tems. This call type occurred at all sites between late March and mid-July and had an associated lunar period that began on or within 2 days of the full moon and continued to about the first quarter moon of the following lunar cycle, a period of approximately 18–20 days. Nightly maximum SPLs associated with this call were about 25 dB above daytime background levels during peak season, and they increased rapidly just after the full moon and decreased rapidly near the new moon (Fig. 8). The relatively high signal-to-noise ratio indicates that the source was close to the hydrophone.

Discussion

In this study, the common occurrence of sound production by red grouper, black grouper, red hind, longspine squirrelfish, and bicolor damselfish was documented at Riley's Hump along, as well as the rare occurrence of sound production by yellowfin grouper and possible sound production by Nassau grouper. Several other relatively uncommon call types were recorded and noted during review of audio files and may be identified to source in future research. Temporal patterns in sound production by red grouper and red hind were similar to the patterns observed in analysis of previous recordings made at sites in the Gulf of Mexico and Puerto Rico, patterns in which sound production was positively correlated with spawning season (Mann et al., 2010; Nelson et al., 2011). The last quarter lunar phase associated with maximum vocalizations of red hind in this study was consistent with the lunar period in sound production of red hind reported from the western coast of Puerto Rico (Rowell, et al,. 2012). The temporal association of a lunar phase with aggregations of red hind has been shown previously to vary between sites. The significance of this finding is not well understood, but it may be associated with patterns in local currents (Nemeth, 2012). Patterns in lunar periodicities associated with sound production by black grouper require more detailed analysis of time-series data and are the subject of a future study.

In these prior studies, observations of grouper behavior associated with sound production did not include actual spawning; rather, they included courtship interactions and territorial behavior during which the presumed male was the sound producer. In our study, apparent courtship or territoriality between 2 black grouper was recorded on video, and one observation of similar behavior was made during a visual survey conducted on 19 January 2011 at site RH1. Because spawning is rarely observed, the exact timing and context of sound production in relation to gamete release is not well understood for grouper species, but sound production does generally correlate well with the reproductive period on a seasonal basis (Locascio and Mann, 2011a).

In this study, more calls of red grouper and red hind were recorded over a longer seasonal period in 2012 than in 2011. The more protracted period of sound pro-

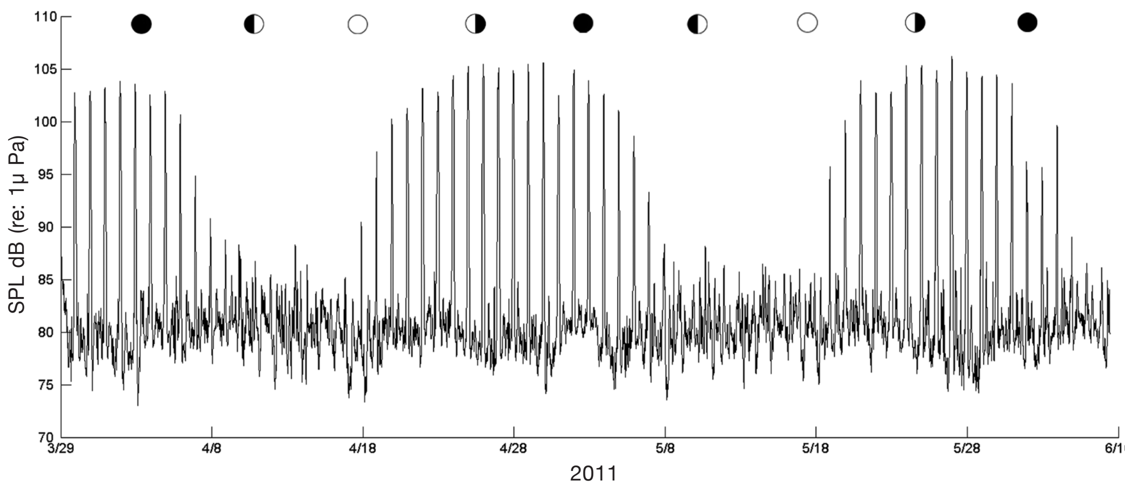

Figure 8

An example of sound production attributed to the bicolor damselfish (*Stegastes partitus*) from the time series of acoustic data recorded in the frequency range of 500–800 Hz at site RH2 at Riley's Hump, Tortugas South Ecological Reserve, Florida Keys, in 2011. The seasonal period of sound production in this frequency range occurred from early spring through mid-summer. Sound pressure levels (SPLs) increased and decreased gradually over a period of about 18 days, beginning near the new moon. Lunar symbols are defined as follows: new moon, ●; first quarter, ◐; full moon, ○; and last quarter moon, ◑.

duction in 2012 by these species could indicate that a longer spawning season occurred during that year and possibly also that a greater number of fishes were present. During 2012, both species demonstrated a preference for site RHDW, which was not monitored in 2011 in our study. This site is located near the base of the steepest vertical relief of Riley's Hump, a habitat feature of aggregation sites associated with relatively high densities of fishes (Kobara et al., 2013).

The spawning season of the red grouper occurs approximately from March through July in the Gulf of Mexico and peaks between March and June, although there is some variability with latitude. This timing is consistent with peak levels of sound production recorded in this study. This species is not considered currently to be overfished or experiencing overfishing (Lowerre-Barbieri et al.[4]).

The spawning season of the black grouper is reported to occur from December through March in the Gulf of Mexico, although Crabtree and Bullock (1998), on the basis of gonad condition, suggested that spawning may occur year-round. The results of our study show that sound production of black grouper occurs year-round, but at levels higher from December through May than during other periods. The black grouper is not considered presently to be overfished or experiencing overfishing (SEDAR[5]).

Red hind in the U.S. Caribbean form spawning aggregations associated with various lunar phases from December through March (Mann et al., 2010; Nemeth, 2012). In our study at Riley's Hump, seasonal and lunar periods in sound production by red hind were similar to the periods observed in studies conducted in the U.S. Virgin Islands, although the timing of peak levels occurred 1–2 months later in the year at Riley's Hump. Results of a stock assessment conducted during 2013–2014 (SEDAR[6]) indicate that the red hind is not overfished or experiencing overfishing in the U.S. Caribbean, but this notion was not strongly conclusive on the basis of available data.

Although most sound production by each grouper species reported here occurred during the winter and spring, calls also were recorded at other times of the year. It is difficult to conclude an alternative meaning for this finding without concurrent observations of behavior, but one possibility may be that limited spawning occurs during other times of the year. The black grouper, for example, has been reported to remain in sexually mature condition year-round (Crabtree and

[4] Lowerre-Barbieri, S., L. Crabtree, T. S.Switzer, and R.H. McMichael Jr. 2014. Maturity, sexual transition, and spawning seasonality in the protogynous red grouper on the West Florida Shelf. Southeast Data Assessment and Review SEDAR42-DW-07, 21 p. [Available at website.]

[5] SEDAR (Southeast Data Assessment and Review). 2010. SEDAR 19 stock assessment report Gulf of Mexico and South Atlantic black grouper, 656 p. SEDAR, North Charleston, SC. [Available at website.]

[6] SEDAR (Southeast Data Assessment and Review). 2014. SEDAR 35 stock assessment report U.S. Caribbean red hind, 350 p. SEDAR, North Charleston, SC. [Available at website.]

Bullock, 1998). Another possible explanation is that sound production is associated with other forms of behavior besides courtship and spawning. For example, red grouper excavate and maintain pits in the sediment that are used by other species, and red grouper are believed to have strong site fidelity to these engineered features (Coleman and Koenig, 2010; Wall et. al, 2011). Given these circumstances, sound production may be used in other social contexts, such as territorial or agonistic interactions.

The calls recorded in our study that were attributed to black grouper are consistent with the stereotypical characteristics of calls by groupers (low frequency, modulated, and long duration) and agree with descriptions of sound production of black grouper from recordings made in Puerto Rico (Schärer, et al., 2013). Although apparent courtship or territorial behavior was followed closely by a BGV2 call in only one video segment, the black grouper was the only grouper species appearing in the video recorded at or near the time that a call of either variation was produced, strongly indicating that this species was the source. Additionally, for black grouper, the relatively high received RMS SPLs of the call variations indicate that the source was close to the hydrophone, and, in these cases, black grouper appeared in the video within a few seconds of these call types. A source level (i.e., decibels of SPL at 1 m from source) can be roughly estimated with a spreading loss model (Urick, 1983) and a received SPL of a call. In our study, a spherical model that estimates a 6-dB loss per distance doubling was used with the highest received SPL of 149.9 dB RMS SPL (re: 1 µPa), which was recorded when black grouper appeared to be only a few meters from the A/V system. Adding 6 dB to the highest received level is equivalent to 1 distance doubling, placing the source 2 m away from the A/V system and resulting in an estimated source level of 155.9 dB RMS SPL (re: 1 µPa). Adding 12 dB to the highest received level would be equivalent to 2 distance doublings and would place the source 4 m away from the A/V system with an estimated source level of 161.9 dB RMS SPL (re: 1 µPa), and so on. For reference, estimates of source levels produced by black drum (*Pogonias cromis*) are 165 dB RMS SPL (re: 1 µPa) (Locascio and Mann, 2011b). Extrapolated estimates of source levels for black grouper that were calculated with the spherical spreading loss model seem reasonable in the context of source levels reported for black drum.

Calls of black grouper contain a frequency-modulated feature, a mechanism associated with sound production that speeds up and slows down over the duration of a call, but the structure of calls of black grouper are unique from the calls of red grouper, yellowfin grouper, and red hind in that they do not feature a long frequency down-sweep. The BGV2 call was differentiated from the BGV1 call by a series of initial pulses. This difference also exists between call types of the red grouper (Nelson et al., 2011), but the significance of those differences is not understood for either species. Sounds of most grouper species documented thus far, with the exception of the call of the Atlantic goliath grouper, give evidence of a complex structure relative to the more common pulsatile structure of many fish calls. The mechanisms associated with the sound production of groupers have been reported only in general terms as bilateral muscles that work in conjunction with the swim bladder (Hazlett and Winn, 1962). A more detailed analysis of the mechanisms and processes responsible for sound production of groupers and the associated behavioral context is warranted given the unusual modulated tones.

Estimates of acoustic communication ranges for fish require data on source levels and hearing thresholds, along with site-specific information on loss of signal transmission and on background levels. Locascio and Mann (2011b) estimated that the acoustic communication range of black drum was 33–108 m on the basis of direct measurements of each of these parameters and found that the range for this species was limited by background levels rather than by hearing thresholds. Data for the complete suite of these parameters do not exist for any grouper species; however, based on the highest received levels of 142.0 dB SPL (re: 1µPa) reported by Nelson et al. (2011) for red grouper and of 149.9 dB SPL (re: 1µPa) recorded in our study for black grouper, a reasonable estimate of communication ranges of groupers in a noisy reef habitat would be on the order of tens of meters.

Spawning sites of black grouper are not well known in the United States. Only 2 probable spawning sites have been documented in the literature, one in the Florida Keys Marine Sanctuary (Eklund et al., 2000) and the other at Mona Island, Puerto Rico (Schärer et al., 2013). In addition to demonstrating that Riley's Hump is also a likely spawning site for black grouper, the information generated in this study can be used to help document other spawning aggregation sites and provide opportunities to learn more about the role of sound production by this species.

The source of sounds produced in the range of 300–400 Hz was verified as longspine squirrelfish by comparing descriptions of the call of this species made by Winn et al. (1964) and that made in our study with a handheld A/V system. Recordings were made as longspine squirrelfish emitted sounds just before retreating into their den after they were approached by a diver. This agonistic behavior is associated with sound production by this species. This behavioral context, together with this species' strong site fidelity to dens (Ménard, et. al, 2008) and its nocturnal behavior, may explain the crepuscular pattern observed in sound production by this species in our study. Other less commonly observed holocentrid species that occupy Riley's Hump s include the longjaw squirrelfish (*Neoniphon marianus*) and blackbar soldierfish (*Myripristis jacobus*). Sound production by these species has not yet been documented in the field, but on the basis of their taxonomy it is likely that these species do produce sounds.

Acoustic signals generated in the range of 500–800

Hz were attributed to the bicolor damselfish on the basis of analysis of A/V recordings and comparison with descriptions of the acoustic signature and associated behavior of this species by Myrberg (1972). Sound production, termed *chirping*, is used in conjunction with short vertical ascents and dives, termed *dipping*, by males to attract females to nest sites. Lunar patterns in sound production were very similar to those patterns described for spawning by this species. Schmale (1981) reported that most spawning by bicolor damselfish occurred between the full moon and just after the first quarter moon—timing that corresponds to the onset and sustained period of sound production by bicolor damselfish that was recorded in our study. Sound production was sustained at high levels beyond the first quarter moon to about the time of the new moon and then decreased sharply. Hatching occurs for this species near the time of the new moon (Schmale, 1981); therefore, the prolonged period of sound production that extended past the time of spawning is likely associated with nest guarding. The bicolor damselfish is among the most abundant pomacentrids reported in visual surveys made by divers at Riley's Hump as part of our study. Other common pomacentrids at the study sites included the blue chromis (*Chromis cyanea*) and purple reeffish (*C. scotti*), neither of which have been documented as sound producers, but it is a possibility that they produce sounds given the common use of sound by this family.

The seasonal and lunar timing of the sounds produced at 500–800 Hz do overlap somewhat with the period of reproductive aggregation of mutton snapper at Riley's Hump in May–July. However, on many occasions when videos of large schools of mutton snapper were recorded by remote A/V systems, as well as video of courtship and spawning behavior (senior author, unpubl. data), the call type of this species was not recorded coincidentally, and there is yet no published evidence of sound production by snappers.

The potential of Riley's Hump as a source of mutton snapper larvae for the Florida Keys and southeastern Florida was demonstrated by Domeier (2004). Its upstream location in the Florida Reef Tract positions Riley's Hump as the starting point of a larval pathway that could populate downstream juvenile habitats throughout the Florida Keys and southeastern Florida. In addition to being a documented spawning aggregation site for mutton snapper, Riley's Hump is a location where divers have observed courtship behavior of permit (*Trachinatus falcatus*), and it has been recorded with the use of A/V systems. The general geomorphology of Riley's Hump is consistent with features of multispecies spawning aggregation sites described by Heyman and Kobara (2010). The results of this study provide additional documentation of the importance of Riley's Hump as a multispecies spawning site and a possible source of larval recruits for populations of red hind, red grouper, and black grouper.

Ault et al. (2008b) reported spawning potential ratios of 0.8% and 17.7% for black grouper and red grouper in the Florida Keys, respectively. These values are far below the federally defined benchmark of 30% for sustainability of these species, especially for the black grouper. Although a managed species, the red hind is not currently targeted commercially in the southeastern United States but is targeted in the U.S. Caribbean; this species was the focus of a stock assessment recently held by Southeast Data Assessment and Review (SEDAR[6]). Estimates of spawning potential ratios for this species are unavailable.

Results from this study indicate that at least 3 economically important grouper species use Riley's Hump as a reproductive habitat, further indicating its importance as part of a marine reserve and the need for continued research to understand its significance on the recovery of fish populations in the southeastern United States. Sonic tagging of groupers at Riley's Hump during the spawning season could provide useful information on whether they are resident or transient and on their geographic range, especially in regard to the reserve boundaries and level of connectivity that may exist among regional populations. For example, estimated mean home ranges of black grouper and red grouper in the Dry Tortugas were 1.44–7.72 km^2; a range of areas slightly smaller than that of Riley's Hump and considerably smaller than the total area (206 km^2) of the TSER (Farmer and Ault, 2011).

Remote monitoring of fish behavior with passive acoustics was especially effective for assessment of the use of spawning habitat in the deep water adjacent to the highest vertical relief associated with Riley's Hump. Little is known about fish use of this deepwater habitat because most research has been conducted on the hump at depths <37 m. Black grouper, red grouper, and red hind all used site RHDW, and it was the preferred site of red grouper and red hind during the 2012 spawning season. Divers also reported that they saw a school of 50–100 cubera snapper (*Lutjanus cyanopterus*) during the dive to recover the acoustic recorder at site RHDW in July 2012.

In addition to providing the first evidence of the use of Riley's Hump by groupers for reproductive purposes, we provide evidence of the value of the use of passive acoustics for exploring long-term monitoring of habitat use by important sound-producing fish. Such efforts will continue to be useful for fishery biologists and ecologists but will require the skilled management of large data sets and additional work for the documentation of sound production by other species.

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