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DESCRIPTION OF A NEW SOUND PRODUCED BY NASSAU GROUPER AT SPAWNING AGGREGATION SITES^S

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INTRODUCTION

The continued discovery of sounds produced by fishes has increased our understanding of acoustic communication in a number of behavioral contexts, including predator defense, distress, agonism, and reproduction (Ladich and Myrberg 2006, Lobel et al. 2010). During reproductive periods, numerous species produce particular sounds when defending territories, protecting access to mates, and exhibiting courtship displays (Lobel et al. 2010, Schärer et al. 2014, Tricas and Boyle 2014), which may convey information about body size and fitness (Colley et al. 2009, Vasconcelos et al. 2012) and influence competitive outcomes and reproductive success (Ladich et al. 1992, Vasconcelos et al. 2012). With an understanding of the acoustic repertoire of species, the monitoring of fish sounds can provide insight into species diversity in different habitats (Parsons et al. 2016, McWilliam et al. 2018), abundances of regional populations (Rowell et al. 2012, Rowell et al. 2017), and spatio-temporal patterns of different behaviors such as spawning (Erisman and Rowell 2017, Rice et al. 2017). Thus, the identification of new sounds and their behavioral contexts is a priority for fish bioacousticians (Rountree et al. 2006).

Endangered Nassau Grouper (*Epinephelus striatus*; Epinephelidae) produce two sounds in association with courtship and distress which have been used to document patterns of reproduction (Figure 1; Supplemental Sound files S1, S2; Fish and Mowbray 1970, Schärer et al. 2012, Rowell et al. 2015). The ability of Nassau Grouper to produce multiple sounds indicates that the species may produce additional sounds associated with different behaviors that have yet to be fully documented. In this study, we sought to identify additional sounds produced by Nassau Grouper and describe the associated behaviors using autonomous video and acoustic recorders. Here we describe a new sound that was recorded at 2 Nassau Grouper spawning sites.

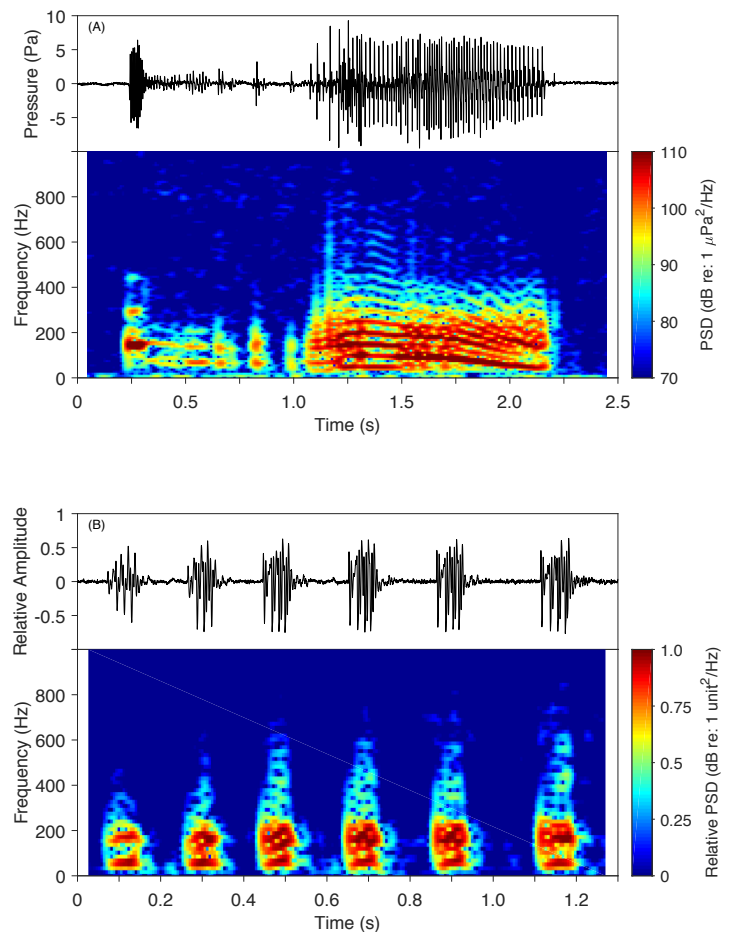


FIGURE 1. Ociolograms (black and white) and spectrograms (color) of sounds produced by Nassau Grouper (*Epinephelus striatus*) in association with (A) courtship and (B) distress behaviors. Relative amplitude is provided in (B). PSD = pressure spectral density level. The figure is adapted from Schärer et al. (2012) by permission from Inter-Research [Endangered Species Research, copyright 2012]. The sound files used to generate (A) and (B) are provided as Supplemental Sound files S1 and S2, respectively, and are best heard with external speakers or headphones.

^SThis article is based on a presentation given in November 2017 at the 71st annual Gulf and Caribbean Fisheries Institute conference in San Andrés, Columbia

MATERIALS AND METHODS

Study sites

Audio–video recordings were made at Bajo de Sico, Puerto Rico, to identify new sounds produced by Nassau Grouper. Bajo de Sico, located 27 km off the west coast of Puerto Rico, is a submerged seamount where <100 Nassau Grouper aggregate between January and April to spawn after the full moon (Schärer et al. 2012). Examples of new sounds for acoustic characterization were initially identified from long–term, audio–only recordings collected at the Grammanik Bank, located 12 km south of St. Thomas, United States Virgin Islands. Over 200 Nassau Grouper aggregate

to spawn at the Grammanik Bank after the full moon in the months of January to May (Nemeth et al. 2006, Rowell et al. 2015).

Identification of new sound

Between 25–30 March 2014, a synchronous audio–video recorder (Open Cam; Loggerhead Instruments, USA) was deployed at Bajo de Sico at a depth of 50 m, where courtship had been observed by divers since 2012 (Schärer et al. 2012). The recorder consisted of a HackHD video camera and single low–frequency hydrophone (HTI–96–min; High–Tech; sensitivity = -164 dBV/ μ Pa, 2 Hz to 30 kHz). Audio and video were recorded for 4 to 20 min durations at

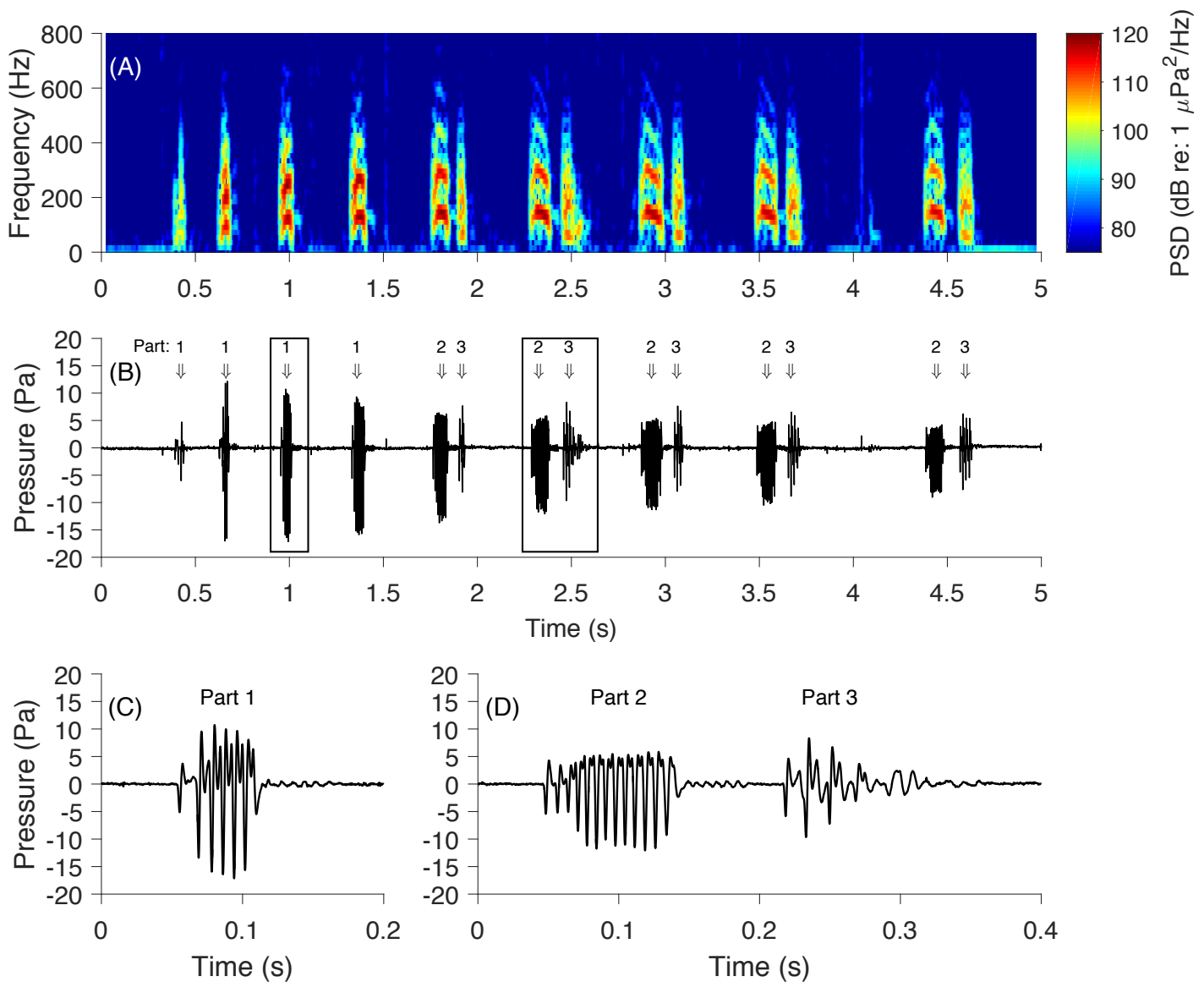


FIGURE 2. Visual representations of the new sound identified during an agonistic behavior between 2 Nassau Grouper during the spawning season. (A) A spectrogram of the acoustic signal which depicts the frequency composition of different parts of the sound as a function of time. (B) An oscillogram which shows the amplitude of the signal as a function of time. Individual parts (1, 2, 3) are indicated with arrows. (C) Close-up of Part 1 from boxed area in panel B. (D) Close up of Parts 2 and 3 from boxed area in panel B. PSD = pressure spectral density level. The sound file used in the generation of the figure is provided as Supplemental Sound file [S3](#) and is best heard with external speakers or headphones.

9 programmed intervals (07:30, 08:00, 15:00, 16:00, 16:30, 17:00, 17:30, 18:00 and 18:25 Atlantic Standard Time (AST); Coordinated Universal Time (UTC) – 4) to target known periods of courtship and sound production (Schärer et al. 2012). Video was recorded at a rate of 30 frames/sec with a resolution of 1920 x 1090, and stored onto a 64 GB micro SD card as MPEG-4 video with an audio sampling rate and resolution of 48 kHz and 32-bit, respectively. Resulting audio–video recordings were viewed with headphones to identify new sounds produced by Nassau Grouper. For a new sound to be attributed to Nassau Grouper, we required that it co-occurred with a visible behavior among conspecifics in close proximity to the audio–video recorder. Upon the identification of a new sound produced by Nassau Grouper, the associated behaviors were described, and the audio was extracted from the recording to permit the identification of the new sound within the long-term acoustic dataset for subsequent characterization.

Characterization of new sound

From 20 January to 31 May 2011, ambient sound was recorded at the spawning aggregation site of Nassau Grouper within the Grammanik Bank, using a long-term acoustic recorder, to monitor known courtship-associated sounds (DSG–Ocean; Loggerhead Instruments, USA). The self-contained acoustic recorder housed a single hydrophone (HTI-96–min; High-Tech; sensitivity = -186 dBV/ μ Pa, 2 Hz to 30 kHz) and recorded 20 sec of audio every 5 min at a sampling rate of 9.523 kHz. As part of previous efforts to describe patterns of courtship-associated sounds (e.g., Schärer et al. 2012, Rowell et al. 2015), examples of unknown sounds had been noted but not fully documented. After the discovery of a new sound produced by Nassau Grouper at Bajo de Sico, previous notes were consulted and used to select examples for characterization. As the new sound consisted of 3 parts (i.e., Parts 1, 2, and 3), oscillograms and spectrograms (Kaiser window, Fast Fourier Transform (FFT) length = 2048) were used to measure count statistics for each part of the sound. The duration (msec) of each part and intervals (msec) between successive parts were measured. Pressure spectral density levels (dB re: $1 \mu\text{Pa}^2/\text{Hz}$; PSD; 1 Hz resolution) were used to measure the dominant frequencies and the 3 dB and 6 dB bandwidths (Hz) of parts. All analyses were conducted in Matlab (The Mathworks, USA).

RESULTS AND DISCUSSION

Identification of new sound

On 29 March 2014 at 18:15 AST, the audio–video recorder at Bajo de Sico captured an agonistic interaction accompanied by a new sound (Supplemental Video file S1; Figure 2) that differed from other sounds produced by Nassau Grouper (Figure 1; Schärer et al. 2012). In the video clip, a single bicolored fish was filmed following an apparently gravid, dark phased fish. The pair was approached by

a second bicolored fish, and an agonistic exchange ensued between the two bicolored fish concurrent with the new sound. During the interaction, the original bicolored fish stopped following the dark phased fish and appeared to attack the intruder to defend its position near the dark phased fish. After the acoustic–physical interaction, the original bicolored fish returned to following the apparently gravid fish while the unsuccessful competitor swam away from the pair. The aggressive behaviors observed had been previously documented in competitive interactions between bicolored adult fish (Colin 1992), but sound production accompanying the agonistic display was previously unknown.

Based on video documentation of the sound and corresponding behaviors, we speculate that the sound was produced by a male defending access to a spawning capable female, but we acknowledge that the sound may also be produced within other behavioral contexts. The agonistic behavior observed in concert with the sound occurred in the presence of an apparently gravid, dark phased fish, a color phase thought to be exclusive to females (Colin 1992). Thus, it is probable that the dark phased fish was a hydrated female, considering that our observations were made in the evening hours during the spawning period. The agonistic exchange occurred between two bicolored fish, a color phase that can be found in males and females ready to spawn (Colin 1992, Archer et al. 2012). When assuming the presence of an apparently gravid female coupled with the reformation of a pair after the competitive exchange, the two bicolored fish were likely males competing for access to a mate, and the sound likely originated from the defender, as documented in other species, to express competitive fitness (Colley et al. 2009, Tricas and Boyle 2014). However, future work is needed to confirm sex-specific coloration patterns and the sound producing capabilities of males and females (Archer et al. 2012, Ladich 2015).

Characterization of new sound

The new sound was comprised of 3 parts and commenced with a variable number of grunts (Part 1; mean = 2.8/sound; range = 0–9), separated by a mean interval of 332 msec (range = 167–579 msec). Part 1 was followed after a mean interval of 413 msec (range = 281–546 msec) by the combined repetition of Parts 2 and 3 (mean = 3.6/sound; range = 1–9), which audibly resembled the sound of a human heartbeat (Figure 2; Supplemental Sound files S3, S4). The mean interval between Part 2 and Part 3 was 63 msec (range = 23–141 msec), while the mean interval between combined Parts 2 and 3 (e.g., end of Part 3 to the start of next Part 2) was 483 msec (range = 261–913 msec). Part 1 was shorter in duration than Parts 2 and 3 on average (Table 1; not statistically tested). However, over the progression of the sound, Part 1 increased in duration and dominant frequency, evolving harmonically into what we have defined as Part 2. Dominant frequencies within parts were variable,

TABLE 1. Characterization statistics of the 3 part sound produced by Nassau Grouper (*Epinephelus striatus*) during an agonistic behavior observed in this study. Statistics of sounds associated with courtship and distress, as estimated by Schärer et al. (2012), are provided for comparison. Dominant frequencies and bandwidths represent the frequencies and distributions of the largest amplitudes of acoustic pressure for each part of the sound. CI_{95} = 95% confidence interval.

Behavior	Mean \pm CI_{95}	n	Min	Max	Reference
Agonism					This study
Part 1					
Duration (msec)	82.8 \pm 5.8	70	22.6	159.7	
Dominant frequency (Hz)	119 \pm 8	70	66	215	
3 dB bandwidth (Hz)	22 \pm 3	70	9	71	
6 dB bandwidth (Hz)	35 \pm 6	70	12	176	
Part 2					
Duration (msec)	131.3 \pm 5.4	81	84.6	214.3	
Dominant frequency (Hz)	128 \pm 4	81	67	155	
3 dB bandwidth (Hz)	16 \pm 2	81	8	42	
6 dB bandwidth (Hz)	24 \pm 3	81	12	60	
Part 3					
Duration (msec)	160.1 \pm 17.1	81	31.4	426.6	
Dominant frequency (Hz)	106 \pm 12	81	39	257	
3 dB bandwidth (Hz)	13 \pm 2	81	2	47	
6 dB bandwidth (Hz)	22 \pm 4	81	3	100	
Courtship					Schärer et al. 2012
Duration (msec)	1600 \pm 60.3	95	900	2300	
Dominant frequency (Hz)	99 \pm 7	95	51	206	
3 dB bandwidth (Hz)	22 \pm 2	95	4	54	
Distress					Schärer et al. 2012
Duration (msec)	90 \pm 4.1	91	60	110	
Dominant frequency (Hz)	77 \pm 6	91	36	169	
3 dB bandwidth (Hz)	56 \pm 6	91	10	166	

but Part 2 had the highest mean dominant frequency (not statistically tested). The new sound conformed to the frequency ranges of previously documented sounds of Nassau Grouper but differed in terms of spectral structure and temporal characteristics (Table 1; Figures 1, 2). Individual parts of the new sound were repeated in sequence and were shorter than the duration of courtship sounds. While repetitive, distress sounds lack distinct parts and have shorter intervals between repetitions compared to the new sound.

SUMMARY AND CONCLUSIONS

This study identified a new sound produced by Nassau Grouper in association with, although potentially not exclusive to, an agonistic interaction at a spawning aggregation. We have also provided a behavioral and acoustic de-

scription for identification of this sound in future studies. The discovery of a third type of sound produced by Nassau Grouper further highlights the importance of acoustic communication coupled with visual displays in fishes, and enhances our ability to decipher patterns of different behaviors (Ibrahim et al. 2018). Furthermore, identification of a new sound increases the ability to document the presence of this endangered species at spawning sites. Future efforts may reveal that the sound is produced within additional behavioral contexts during and outside of spawning seasons, such as the defense of territories or food resources (Ladich and Myberg 2006). Continued efforts to catalogue the sounds and behaviors of species like Nassau Grouper will increase our ability to monitor and understand fish behaviors.

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