

**Red hind *Epinephelus guttatus* vocal repertoire characterization, temporal patterns and call detection with micro accelerometers**

By:

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## **Abstract**

Passive acoustic monitoring has been used to study groupers that produce courtship associated sounds (CAS) when they aggregate to spawn. This technique has revealed patterns of sound production during red hind (*Epinephelus guttatus*) spawning aggregations with extremely high temporal resolution. In particular, it has been shown that groupers can have a varied vocal repertoire, however, detailed studies of the number, types and periodicity of CAS are lacking. The purpose of this study was to characterize and understand in detail the CAS and other vocalizations in *E. guttatus* and their respective behavioral context, using a combination of field and laboratory studies. During the 2017 spawning season (Dec – Feb), red hind were held in a 57,000-liter tank equipped with a low frequency acoustic recorder and video cameras to record behavior. Additionally, the experimental set up included an open accelerometer to detect sound in smaller devices, which can enable scientist to track individual fish vocalizations in the field. Field recordings from a simultaneous spawning aggregation were used to quantitatively characterize and compare the sound types recorded by audio and video during captivity. Five sound types were characterized: four from captivity and an additional one from the field recordings - ‘chorus’. These sounds consisted of variations and combinations of low (50-450hz) pulses, Grunt/Grunt Trains and tones. Some vocalizations exhibited diel and lunar oscillations, and for these both field and captivity recordings peaked daily at 1800 AST and at 8 days after the full moon. The open accelerometer was successful in recording sound but only at minimal range. Standardizing characterization of call types, coupled with this improved technology will facilitate automating call detections. Such improved data will further help relate call types to behavior,

test hypotheses relating calling behavior to fitness and mate choice, determine the existence of regional dialects (indicating connectivity within the Caribbean) and assist management in monitoring and assessing grouper aggregations. This research was accepted for review by the Journal of Fish Biology.

## **Resumen**

Las acústicas pasivas han sido esencial para el estudio de los meros durante sus agregaciones de desove. Estos meros producen sonidos asociados a la reproducción lo cuales han sido utilizados para demostrar los patrones reproductivos de mero cabrilla (*Epinephelus guttatus*) con gran resolución temporal. Varios estudios han demostrado que los meros poseen una gran variedad de vocalizaciones. Actualmente hay una carencia de estudios sobre la cantidad, tipos y periodicidad de estas vocalizaciones asociadas a la reproducción. Por lo tanto, el propósito de este estudio es caracterizar y comprender en detalle las vocalizaciones asociadas a la reproducción y otras llamadas de *E.guttatus* y su comportamiento correspondiente utilizando una combinación de métodos en cautiverio y en el campo. Durante la temporada de reproducción del 2017 (Dic-Feb), se recolectaron meros cabrilla de una agregación de desove y colocados en un tanque de 5700-litros. Este tanque fue equipado con grabadoras de baja frecuencia y video para grabar los sonidos y sus comportamientos correspondiente. Además, el tanque contaba con un micro acelerómetro para detectar sonidos en equipos más pequeños que puedan ser introducidos dentro de la cavidad del pez y utilizados para rastrear las vocalizaciones de un individuo en el campo. Para comparar, caracterizar y cuantificar las vocalizaciones grabados en cautiverio, se utilizaron grabaciones obtenidas de la agregación de desove de la misma

temporada, donde provinieron los individuos estudiados en cautiverio. Se reconocieron y caracterizaron cinco vocalizaciones en total; cuatro en cautiverio y una en el campo, el 'coro'. Estas vocalizaciones consisten en variaciones y combinaciones de sonidos de baja frecuencia (50-450hz) en forma de pulso/tren de pulsos, gruñidos/tren de gruñidos y tonos. Algunas vocalizaciones exhibieron las mismas oscilaciones crepusculares y asociadas a la luna en cautiverio tal cual en el campo con máximos de vocalizaciones a las 1800 AST y ocho días después de la luna llena. El micro acelerómetro fue exitoso en grabar vocalizaciones, pero a una distancia mínima. Al estandarizar y caracterizar estas vocalizaciones con su comportamiento respectivo nos ayudara a; establecer relaciones en cuanto el sonido y la aptitud de un individuo, como el sonido afecta a la elección de parejas, determinar la existencia de dialectos (indicando conectividad dentro del Mar Caribe) y asistir en el monitoreo. manejo y evaluación de agregaciones de meros. Este trabajo fue aceptado para revisión por la revista Journal of Fish Biology.

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## **List of symbols and abbreviations**

CAS = Courtship associated sounds

DAFM = Days after the full moon

UVC = Underwater visual census

DSG = Digital spectrogram (recording unit)

FSA = Fish spawning aggregation

PAM = Passive acoustic monitoring

SPCC= Spectrographic Cross Correlation

PCO= Principal Coordinate Ordinance

PERMANOVA= Permutational Analysis of Variance

ANOSIM= Analysis of Similarity

SPL= Sound Pressure Level

N/A= Not applicable

F-RMS= Filtered Root Mean Square

AST= Atlantic Standard Time

## **Appendix List**

Available upon request:

Electronic Supplementary Materials, ESM\_EGU\_CAS\_A.

Electronic Supplementary Materials, ESM EGU\_B

Electronic Supplementary Materials, ESM EGU\_C

Electronic Supplementary Materials, ESM EGU\_MVM

Electronic Supplementary Materials, ESM EGU\_PulseTrain



## Introduction

Red hind *Epinephelus guttatus* are commercially important, soniferous grouper that are seasonally protected in USVI and PR. Passive acoustic monitoring has been used to study groupers that produce courtship associated sounds (CAS) when they aggregate to spawn. This technique has revealed patterns of sound production during red hind (*Epinephelus guttatus*) spawning aggregations with extremely high temporal resolution. In particular, it has been shown that groupers can have a varied vocal repertoire, however, detailed studies of the number, types and periodicity of CAS are lacking. This is due to the difficulty of obtaining simultaneous audio video recordings of this behaviors since spawning aggregations occur in discrete periods of time, often far from shore where wind/sea conditions limit the accessibility and divers are logistically restrained to make observations in a limited time period. Therefore, the objectives of this study are to use the knowledge of the red hind spawning behavior to set up a laboratory facility designed to stimulate production of CAS and use this facility to (i) identify CAS call types and temporal patterns of sounds produced during reproductive activity use bioacoustics to quantitatively characterize and compare sounds produced by red hind during reproductive activity, (ii) use video recordings to associate sounds with specific behavior, and (iii) detect fish vocalizations with open accelerometer tags. Field acoustic recordings from an aggregation site were then used to (iv) quantitatively characterize and compare sounds, and (v) determine their temporal patterns. This research demonstrated, characterized and identified courtship associated sounds and behavior on a commercially important and overfished grouper along with demonstrating the use of micro accelerometers for fish sound detection. Standardized

classification coupled with the ability to distinguish among the vocalizations and the ability to use accelerometers, allows for the search for distinct dialects occur across the Caribbean, sets a precedent for call rate studies in grouper and the factors that affect it, enables the use of gliders and or drifters to assess habitat distributions and increases the acoustic library for correction in soundscapes studies.

### **Literature Review**

Groupers (Teleostei: Epinephelidae: Epinephelini) include many species that are considered important top-level predators of coral reefs worldwide. Groupers are generally long lived, slow-growing, and a few species form large aggregations (Erisman et al. 2009), as part of their reproductive strategy. Transient spawning aggregations known in the Caribbean region last for a few days during two or three lunar cycles, often during the winter when water temperatures reach minima between 26-27.5 C. For Epinephelids, their longevity, slow growth rates, late sexual maturation, and spawning strategy, especially the predictability of the spawning aggregations in space and time, makes them particularly vulnerable to exploitation. One particularly common species in the Caribbean, the red hind (*Epinephelus guttatus*), is a mid-sized protogynous grouper, which has been subject to overfishing specifically during spawning aggregations after significant declines in Nassau grouper (*Epinephelus striatus*) populations, resulting in high exploitation rates of red hind in Puerto Rico (Matos-Caraballo & Sadovy 1990, Sadovy 1993, Matos-Caraballo 1997), United States Virgin Islands (USVI) (Beets & Friedlander 1992) and Bermuda (Luckhurst & Ward 1996).

During reproductive events some species of groupers, including *E. guttatus* (red hind), *Epinephelus striatus* (Nassau grouper), *Epinephelus morio* (red grouper), *Mycteroperca bonaci* (black grouper), *Mycteroperca venenosa* (yellowfin grouper), and *Epinephelus itajara* (goliath grouper), produce courtship associated sounds (CAS) (Mann et al. 2009; Schärer et al. 2012a; Schärer et al. 2012b; Mann et al. 2010, Nelson et al. 2011). Passive acoustics have been used to study grouper spawning site usage and temporal patterns of fish reproduction in Puerto Rico, the USVI, Cayman Islands and Florida (Sanchez et al. 2017; Mann et al. 2009, 2010; Schärer et al. 2012a,b; Schärer et al. 2014; Locasio et al. 2016; Rowell et al. 2012, 2015). It has been proposed that the sounds are generated by the rapid contraction of muscles that reverberate the swim bladder, as was documented for Nassau grouper (Hazlet & Winn 1960). The CAS produced by red hind are of low frequency (0-500 Hz) and are produced during the spawning season associated with territorial or agonistic confrontations and towards gravid females during presumed courtship behaviors (Mann et al. 2010). Visual observations coupled with passive acoustics has been used to categorize sounds with associated behavior in epinephelids like the antagonistic sounds of *E.striatus*, *M. jordani*, *E.morio* the latter use lek- like systems of reproduction.

In red hind reproduction, females are determinate spawners, and larger females may spawn more than once a year (Sadovy et al. 1994b). Males arrive at the spawning aggregation site before the females and establish territories that are defended during agonistic and territorial confrontations followed by courtship towards females (Colin et al. 1987; Shapiro et al. 1993). Changes in coloration are also part of their behavioral displays when males exhibit a light anterior ventral coloration and dark posterior ventral coloration with a barred pattern and barred lips (Shapiro et al. 1993;Ojeda-Serrano 2002) (Figure 1, picture taken during female-male

interactions). After males establish territories, small groups of three to five females become established at each male's territory (Colin et al. 1987). Within these small groups spawning occurs, a few meters off the bottom, in short spawning rushes at night (Colin et al. 1987).

Red hind remain at the aggregation site during the spawning season and high abundances of females occur in pulses lasting one to three days (Nemeth et al. 2007). These gender specific movements alter quickly the female to male ratio, which can range from 20:1 to 1:1 (Whiteman et al. 2005). These changes in sex ratio during the aggregation period could be the only chance for the female population to assess sex ratio and stimulate sex change (Shapiro et al. 1993). Migrations toward the spawning site can be from 5-33 km since red hind exhibit site fidelity and females inhabit shallow reef areas with overlapping habitat ranges from 112-5636 m<sup>2</sup> (Nemeth et al. 2005). The larger red hind remain in deeper habitat similar to or near their aggregation sites (Nemeth 2006). Density and number of individuals in spawning aggregations vary from a few 100 adults to 80 000 adults, which can occupy an area of 0.015 km<sup>2</sup> to 0.35 km<sup>2</sup>. (Shapiro 1987; Shapiro et al. 1993b; Nemeth 2005). Extensive fishing on red hind spawning aggregations in Puerto Rico and the USVI led to direct management strategies. Seasonal and permanent closure in spawning aggregation sites in St Thomas have proven to improve fish size, abundance and density (Nemeth 2005; Beets & Friedlander 1998). However similar strategies in St Croix and Puerto Rico did not have the same effect (Nemeth et al. 2006). Matos-Caraballo et al. (2005) reported a slight increase in red hind mean fork length from commercial landings data after implementation of seasonal closure in Puerto Rico. But lack of enforcement/compliance and increased effort may have overridden these protective measures (Marshak 2007).

Understanding the social and behavioral context of the aggregations is essential to monitor changes over time and the direct monitoring of red hind spawning aggregations has provided useful information to assess the effectiveness of management measures in the USVI (Beets & Friedlander 1998; Nemeth 2005; Nemeth et al. 2006). However, in Puerto Rico this has proven difficult as multiple known aggregation sites are located offshore, making it challenging to survey consistently and assess changes in spawning stock over time. Additionally, visual observations to associate behavior with sound production are limited by diver time and the ability to discriminate a single source of sound production.

Passive acoustic monitoring of CAS was proposed as an alternative to overcome these limitations (Mann et al. 2010; Rowell et al. 2012). However, passive acoustic studies have been limited by a lack of characterization and standardization of each species' type of CAS, which increases variability and complicates hypothesis testing. Some factors known to affect CAS production and calling patterns, such as the time of day (light-levels), lunar period or day of the aggregation can be controlled by standardizing the period of analysis. Other suspected factors, such as current flow, water temperature, swells or number of individuals need to be directly accounted for. Comparison of sound pressure levels and abundance estimates from red hind (Rowell et al. 2012) and croaker (Rowell et al. 2017; Sprague & Luczkovich 2012) spawning aggregations showed a significant positive relationship. However, for red hind the specifics of this relationship were not constant over years or across spawning sites (Appeldoorn et al. 2013), thereby limiting the potential use of PAM as a relative index of abundance. The limiting factor in converting CAS counts to number of individuals is an understanding of the calling behavior of a single fish during the reproductive period, specifically the call rate of an individual male, which may be subject to

several factors in addition to the number of males and females . Additionally, a methodology to track an individual's sonic activity is needed to estimate site-specific spawning stock.

The objectives of this study are to use the knowledge of the red hind spawning behavior to set up a controlled environment to study their CAS and (ia) identify the acoustic repertoire, (ib) characterize each type of CAS and (ic) determine the temporal patterns of each types of CAS, (ii) describe the behavioral context of CAS, and (iii) determine the feasibility of open accelerometer tags to detect CAS. Simultaneous acoustic recordings from an aggregation site offshore were used to (iv) characterize and compare CAS in the field with captive fish, and (v) determine the correlation between field and captive temporal patterns in CAS. These results lay the groundwork for studying red hind sound production and further analyses considering variability in CAS types and call rate measurements. In an evolutionary context the results of this research allow for comparisons with other groupers, as well as other sound producing fishes. Furthermore, this represents one of the first attempts at using an accelerometer tag to record fish sound production, thus enabling the application of this tool towards the study of fish sonic activity.



Figure 1. Male (larger) with white coloration pattern in dorsal side following a female (smaller). Picture taken during captivity; as a male chased a distended female around, CAS-B was recorded during this interaction.

## **Materials and Methods:**

### *Aquarium facility set up and design*

The acoustic and reproductive behaviors of red hind were studied in a 5700-l (1,500-gallon) tank (Figure 2). The tank was connected to the seawater system of the Magueyes Island Marine Laboratory of the University of Puerto Rico – Mayaguez’s Department of Marine Sciences. The open circulatory system pumped fresh seawater into the tank at a flow rate of 475 l/hr, and the tank was covered at the top with a 40% light-attenuating shade fabric. Inside, the tank was partitioned with 1.5-cm plastic coated wire mesh into a 2-m diameter central staging area surrounded by a 1.5-m wide, 43.98 m<sup>2</sup> external area (Figure 2). The central staging area was designed to act as the home territory of a resident male, so four structures made of coral rubble were added to simulate the caves and flat areas characteristic of the benthic habitat where the fish were captured. Measures that were taken to promote courtship behavior included: (i) a biased sex ratio 1:3 M:F, (ii) timing of capture to coincide with an aggregation in the wild, and (iii) presence of another male near its territory. This facility was stocked with three red hind, one male and two distended females that were captured by scuba divers at the Abrir La Sierra (Figure 3) aggregation site during the 2016-2017 spawning season (DRNA permit 2016-IC-176). Subsequently, an additional male and female were similarly collected and added. Fish were fed California market squid (*Doryteuthis opalescens*) at 0900 AST every three days.

To monitor acoustic activity the tank was equipped with a recording hydrophone (DSG-Ocean Loggerhead sensitivity of 180 dBV $\mu$ Pa<sup>-1</sup>, frequency range 2 to 37 kHz). The hydrophone recorded 59-s files of every minute at a sample rate of 10,000 Hz and sample size of 16-bits. To record

behavior, a Go Pro Hero 3 Video Camera in a waterproof housing was mounted underwater, high on the side to maximize the camera view. During observation periods, the camera recorded six 10-min videos from 0900 to 1000 AST (video was recorded at 59 FPS and audio at 16- bits 480000 Hz) due to battery and equipment limitations. This period of recording was chosen for several reasons; given the known daily periodicity we expected greater calling earlier in the day and toward sunset, in the morning levels were increasing, thus facilitating video recording. Additionally, we hoped this would elicit different call types and since routine maintenance had to be given to maintain proper water quality conditions and maintain recording periods (battery changes), it was thought best to make all the necessary changes during the same time period to minimize changes in red hind behavior due to human presence. Due to limitations caused by the acoustic properties the tank, such as size, location, pump and other external sources of noise, the tank acoustic recordings were used only to identify different vocalizations, validate the CAS time series, and study sound and behavior correlations. Simultaneous recording of video and audio files with identical time stamps allowed the videos to be analyzed for the behavioral context of the sounds produced.

Two open micro accelerometers (Open Accelerometer Tag Loggerhead/Analog Devices accelerometer model adx1345 max output  $\pm 16g$ ) were placed inside the mesh cage. The accelerometers had a detection range of 0.5-m (Loggerhead Instruments, pers. comm.). Therefore, these were placed in the water column by anchoring them to the bottom with a float, placing them 0.5 m from the center of the circle cage to maximize the possibility of sound detection in every dimension inside the mesh without restricting the fish movements. During

observation periods, the accelerometers recorded continuously on the Z-axis every five minutes at 1400 Hz (16-bit).

In the tank, acoustic and video recordings were made under three scenarios. The first consisted of placing the initial male and two females in the staging area to simulate a resident male with two females within his territory. Recordings started on January 12, the day of full moon, two days after acclimation in the tank. On the night of the ninth day of recording (Jan 20), in an effort to further induce calling behavior, the additional distended female was added within the staging area to increase the sex ratio, but the additional male was separated from the rest of the fish by placing it in the external area. This was to present the first male with a potential rival outside his territory. Structure was added to the external area to provide this male with shelter. Lastly, on the morning of the tenth day, the new male was placed inside the circular cage with the other fish to simulate an invading male. As the invading male was quickly chased out by the resident male (forcing him to jump out of the water and over the separating fence), this test was performed three times on three consecutive days (Jan 21-23) to get a sufficient number of recordings. Subsequently, the second male was left in the external area while daily recordings continued. Overall, the fish were held in captivity during the first lunar cycle of 2017, with acoustic and behavioral recordings made from January 12, 2017 to January 25, 2017.

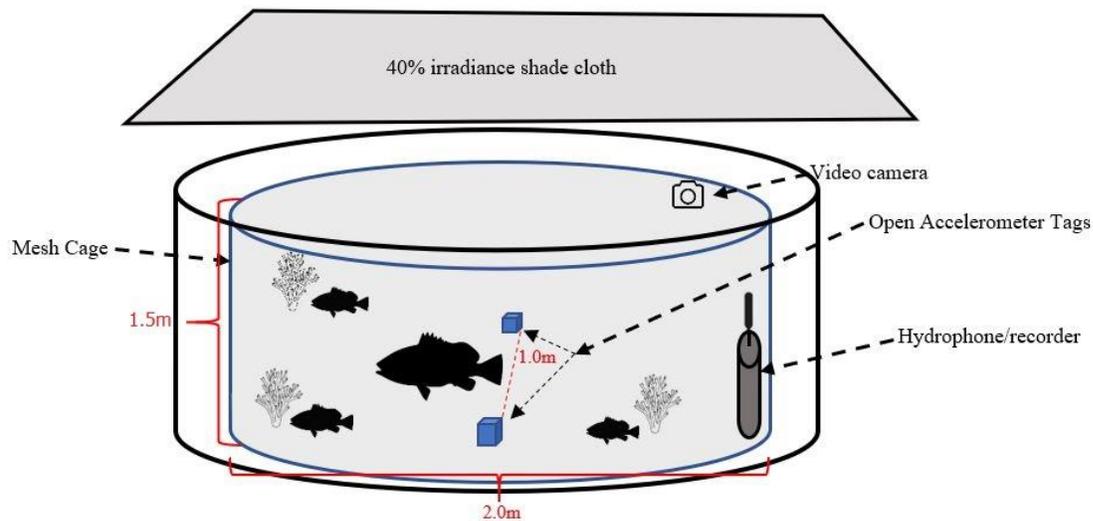


Figure 2. Schematic diagram of tank instrument set up. The 57,00-liter tank was set up with a 2-m diameter and 1.5-m height mesh cage separating an internal staging area and an outer external area. Accelerometers were anchored to the bottom and floated 0.5m from the bottom and 0.5m from the center of the tank. The positioning of the accelerometers was to maximize the range of detection. A DSG ocean loggerhead was used to record sound and a Go Pro video camera to record experiments. The video camera was placed at the top to maximize view. Small fish figures represent distended females larger represents male. Caves, crevices and flat areas are represented by coral figures. All recording of data was during the January 2017 reproductive moon cycle period.

### *Field acoustics records*

Simultaneously, field recordings were taken from the red hind spawning aggregation site where the fish were collected, at Abrir La Sierra, (Figure 3) 14 miles from the Coast of Cabo Rojo (Rowell et al. 2013). Recordings were made using a Loggerhead Instruments DSG-Ocean, with a sensitivity of  $-180 \text{ dBV}\mu\text{Pa}$ -1frequency range 2 to 37 kHz. The hydrophone recorded 20-s files every 5 min at a sample rate of 10,000 Hz and sample size of 16-bits. All the acoustic data were recorded in UTC and converted to AST  $(-4/24)$ . With limited acoustic interference, these data

were used for the quantitative comparison of different vocalizations and to compare CAS time series.

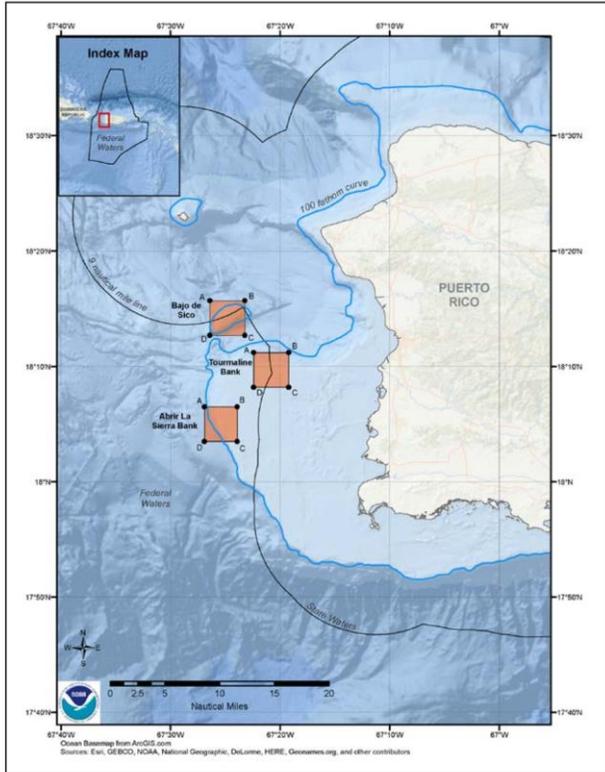


Figure 3. Spawning aggregation sites Abrir la Sierra, Bajo De Sico and Tourmaline Bank. Red boxes indicate perimeters of marine protected areas (MPAs) with seasonal closures in Puerto Rico. Within the Puerto Rico US EEZ. Fish from captivity studies originated from Abrir la Sierra (DRNA permit 2016-IC-176).

### *Quantitative comparison of sounds and parameters*

CAS as reported by Mann et al. (2010) and other sounds were audibly and visually recognized using Raven Pro 1.5 spectrogram parameters with a 1609-point Hann window 3 dB bandwidth = 8.9 Hz with 50% overlap and a 2048-point Discrete Fourier Transform (DFT) window size. Sounds were initially classified into five call types, CAS-A, CAS-B Grunt/grunt train, Pulse/pulse train and chorus based on their relative duration, amplitude, and frequency modulation. Two methods

were used to characterize, describe and compare the calls observed in captivity, but the characterization and analyses were made from the field recordings. Since no measurements were taken from the captivity recordings, no calibration was needed for the tank acoustic properties. Furthermore, only field recorded sounds that had clear structure and minimal background noise were used. Thirty representative samples from each artificially assigned sound type were used for both the characterization and comparison of sounds, except for the Pulse/Pulse Train which only had twenty representative samples. The two methods consisted in using a Principal Coordinate Ordination (PCO) to test for similarity. The first method (Parameters-based PCO) compared sound based on a trained observer's knowledge, and each parameter was tested for similarity. In contrast, the second method (Spectrogram Cross Correlation-PCO (Cortopassi & Bradbury 2000; Rice & Bass 2009)) is an algorithm-generated "blind test" of similarity, where a similarity matrix is produced by an algorithm in the Raven Pro 1.5 software that creates a contingency table comparing pixel by pixel the spectrogram of each call type.

#### *Parameters-PCO-PERMANOVA*

To characterize and describe the calls, seven parameters were measured using Raven Pro 1.5: (1) Peak frequency (Hz) (frequency that has the maximum sound pressure level (SPL) value of the selection), (2) Max Sound pressure level SPL (dB), (3) Total sound duration (s), (4) 90% Bandwidth (Hz) (the frequency that divides the selection into two frequency intervals containing the difference between the 5% and 95% of the frequencies), (5) Total number of pulses (Sum of all pulses that make up a vocalization), (6) Inter-call sound interval (time between successive sounds

that make up a vocalization), and (7) Relative amplitude (Filtered Root Mean Square (F-RMS) amplitude value of selected time and frequency (50Hz-450Hz)). Frequency and power parameters were measured on spectrograms with a 1609-point Hann window, 3 dB bandwidth = 8.9 Hz, with 50% overlap and a 2048-point DFT window size. Time and amplitude parameters were taken from oscillograms. These measurements were then compared in a Principal Coordinate Analysis PCO in Primer 7. They were normalized and analyzed using a resemblance matrix of Euclidian distance. This graphical ordination was accompanied by a one-way ANOSIM with a significance level of (0.1%) and 999 permutations was performed to test for significant differences among call types. Additionally, a PERMANOVA was performed with the same measurements, also normalized and analyzed using resemblance matrix of Euclidian distance with an unrestricted permutation of raw data, a significance level of (0.1%) and 999 permutations.

#### *SPCC-PCO*

Sounds were cross correlated using Raven Pro 1.5 batch correlator function. Sounds were normalized, and a bandpass filter was applied between 50Hz and 450Hz to minimize the effects of ambient noise. Ten representative samples of each call type were used for a total of 25,000 sound comparisons. The resulting similarity matrix was analyzed with a Principal Coordinate Analysis in Primer 7 (PCO) (Cortopassi & Bradbury 2000). Posteriori a one-way ANOSIM with a significance level of (0.1%) and 999 permutations was performed to test for significant differences among call types.

### *Time series analysis*

Only vocalization types that could be identified by audio and visual inspection were counted during the period of study and summed per day or per time-block respectively. Daily counts in captivity represent the sum during a 24-hr period, and time blocks represent four-hours. Recordings from the field represent a sub-sample of all sounds, hence vocalizations type were summed for 96-min of a 24-hour period and 24 min of each four-hour block. To test for differences in the diel pattern a  $X^2$  chi square test was performed to test for independence of call type per time block. Six-time blocks of four hours were used to test for independence.

### *Accelerometer proof of concept*

An additional attempt to record a red hind call using the Open Accelerometer Tag was done on October 25, 2017. A 383mm female red hind was captured off La Parguera shelf edge and placed in a 757-Liter cement tank with the micro accelerometer and the recording hydrophone synchronized by being tapped underwater at the same time. To induce alarm calls the fish was hand held next to both instruments. Holding the fish was enough to produce an alarm call. To describe the alarm call (N=12) in both instruments Raven Pro 1.5 was used to measure four parameters: call duration (s) and amplitude (root mean square (RMS)) taken from oscillogram and peak frequency (Hz) and peak power (dB) taken from spectrogram. Hydrophone spectrograms were made with a 512-point Hann window, 3 dB bandwidth = 28.1 Hz, with 50% overlap and a 512-point DFT window size. Accelerometer spectrograms were made with a 163-point Hann window, 3 dB bandwidth = 12.4 Hz with 49% overlap and a 256-point DFT window size.

## Results

Four different call types were identified in the tank recordings: *Cas-A* or 'woot-woo', *CAS-B* or *Tonal*, *Grunt/Grunt Train* and *Pulse/Pulse Train*. These were subsequently identified, isolated and characterized from recordings made at the spawning aggregation site (Table 1). Video recordings captured behavior associated with three of these calls, *CAS-B* or *Tonal*, *Grunt/Grunt Train* and *Pulse/Pulse Train*. Also recorded in the field was a fifth call type: "*chorus*".

### *Chorus*

This is the first description of natural occurring grouper chorus. It is a continuous sound between 50 Hz-300Hz, consisting of multiple red hind CAS and its combinations. This sound is the result of the vocal interactions among hundreds or thousands of individuals during the spawning aggregation event (Figure 4); thus, it was not recorded in the tank experiments. Additionally, CAS-A can be distinguished and far away calls can be observed as a dot around 225 HZ.

Table 1. Mean value  $\pm$  standard deviation of each parameter for each call type. Sounds were taken from field recordings during a red hind spawning aggregation off the west coast off Puerto Rico.

	Chorus	CAS-A	CAS-B	Grunt/Grunt Train	Pulse/Pulse Train
90% Bandwidth (Hz)	237.15 $\pm$ 49.68	192.37 $\pm$ 11.61	200.51 $\pm$ 20.02	218.10 $\pm$ 29.45	230.72 $\pm$ 35.00
Max Power (dB)	65.00 $\pm$ 4.95	73.21 $\pm$ 3.72	73.71 $\pm$ 5.56	62.73 $\pm$ 6.82	59.44 $\pm$ 5.91
Peak Freq (Hz)	174.80 $\pm$ 48.83	201.17 $\pm$ 18.71	172.69 $\pm$ 32.39	147.47 $\pm$ 43.88	151.37 $\pm$ 42.71
Filtered-RMS-Amplitude	42.67 $\pm$ 17.26	220.29 $\pm$ 133.24	156.41 $\pm$ 135.62	59.12 $\pm$ 54.33	44.48 $\pm$ 28.85
Duration(s)	15.16 $\pm$ 3.35	1.04 $\pm$ 0.27	2.39 $\pm$ 0.46	1.24 $\pm$ 0.25	4.24 $\pm$ 1.59
Intercall-interval (s)	0.00 $\pm$ 0.00	0.17 $\pm$ 0.02	0.18 $\pm$ 0.03	0.54 $\pm$ 0.18	1.00 $\pm$ 0.00
Number of pulses	1.00 $\pm$ 0.00	47.60 $\pm$ 7.21	162.00 $\pm$ 34.52	46.73 $\pm$ 16.64	7.35 $\pm$ 8.77

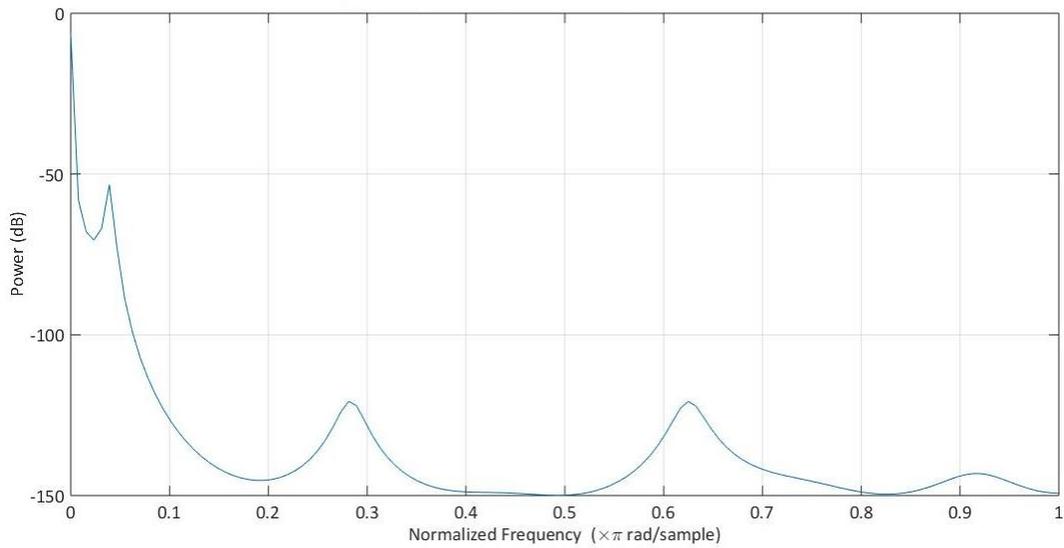
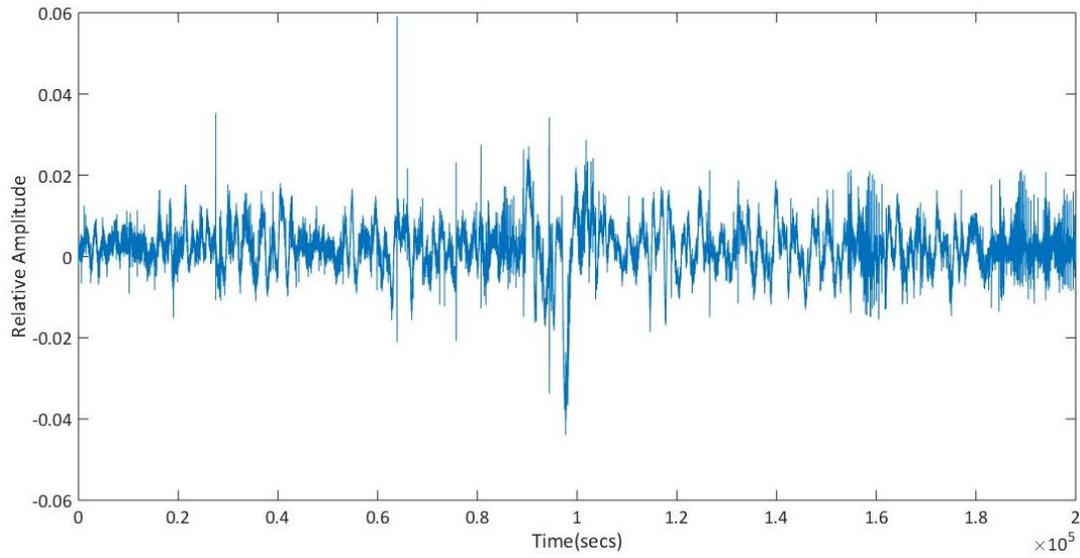
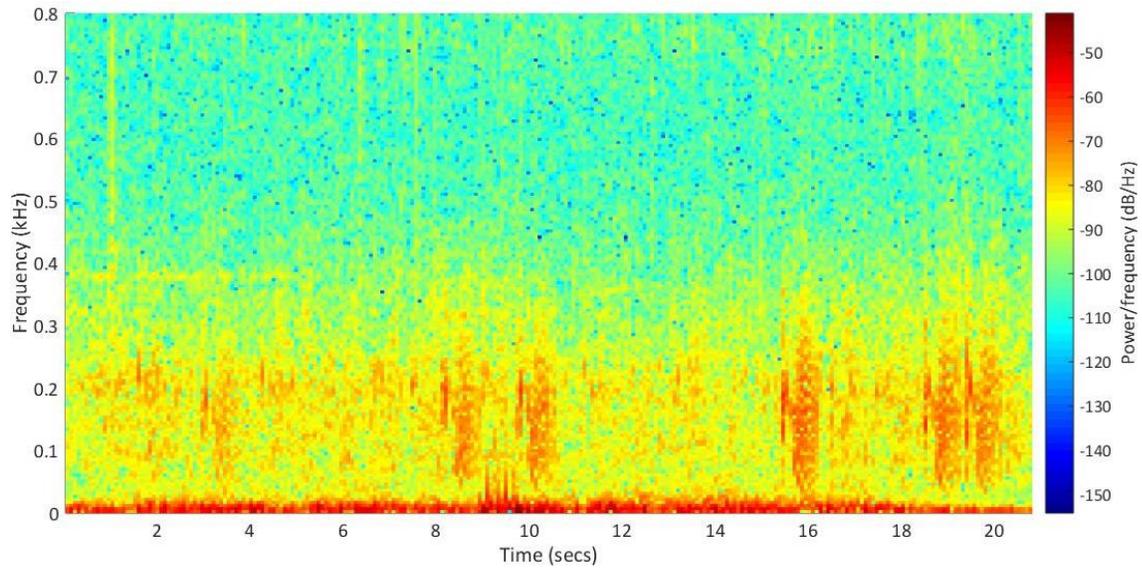


Figure 4. Chorus; From top to bottom, Spectrogram (Kaiser window, fast Fourier transform (FFT) length = 1609 points, overlap = 50%), Oscillogram and Power Spectra. The call was recorded in the field during a red hind spawning aggregation off the west coast off Puerto Rico.

*'woot-woo' or CAS-A*

The CAS-A, or classic “woot-woo” (Mann et al. 2010) is a combination of a short pulse followed by a short tone (Figure 4). This call can be combined with a Pulse/Pulse Train before the call or by producing various continuous short tones after the call modifying the duration of the tone. This call type peaked at 1800 AST and 8 DAFM for both captivity and field data. Associated behavior was captured on video only once. As the female was swimming high in the water column over a cave, she was slowly approached by the male. The female reacted to this motion by swimming towards the bottom of the tank, away from the male. At this point the sound was produced by the male, and the female briefly swam faster and stayed on the bottom [see Electronic Supplementary Information (SuppInfo\_EGU\_CAS-A)].

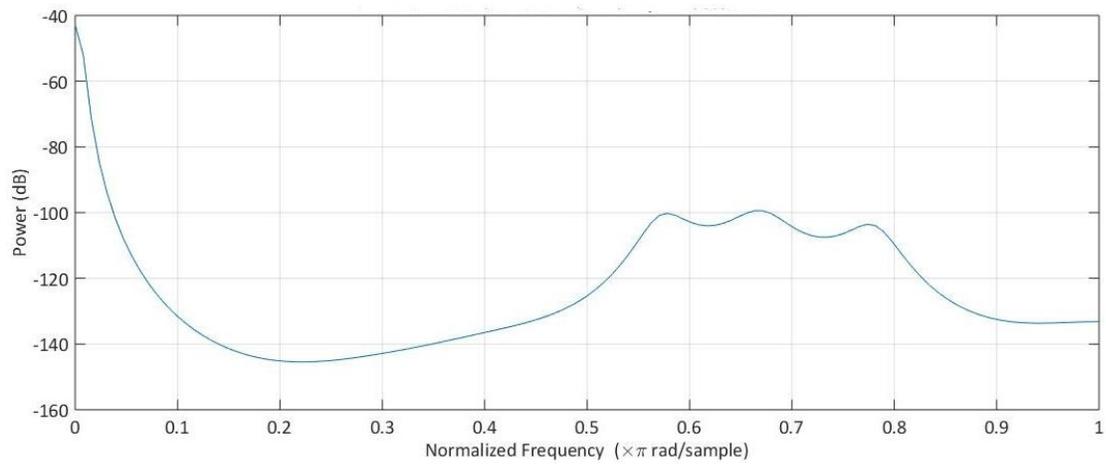
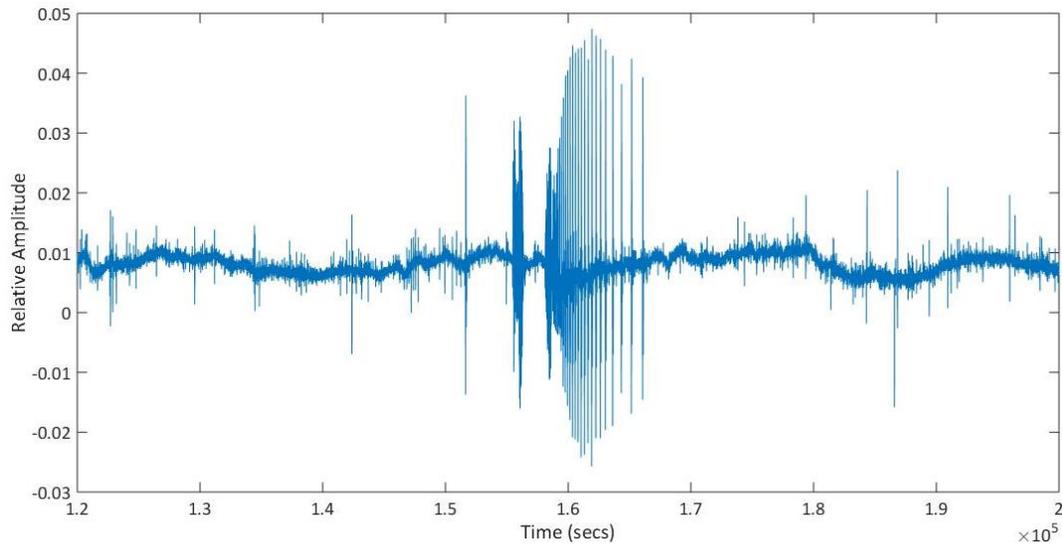
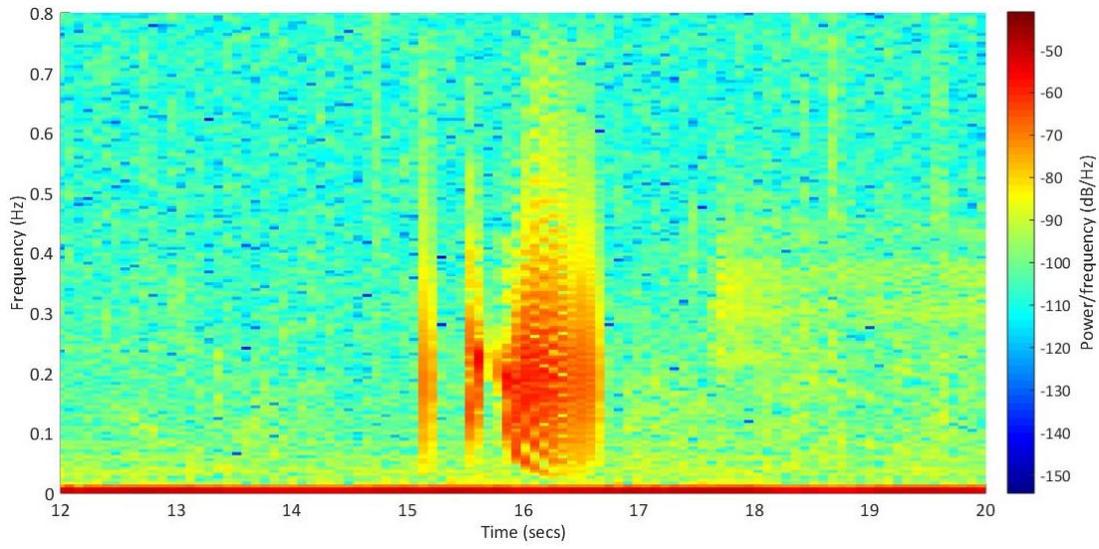


Figure 5. CAS-A or 'woot-woo'; From top to bottom, Spectrogram, (Kaiser window, fast Fourier transform (FFT) length = 1609 points, overlap = 50%) Oscillogram and Power Spectra. The call was recorded in the field during a red hind spawning aggregation off the west coast off Puerto Rico.

#### *CAS-B or Tonal*

This call is composed of a long pulse followed by a long tone (Figure 6). It can be combined with a pulse train before or after the call or with CAS-A in the same manner. The tonal part can also be extended or shortened. The call was recorded during fish interactions [see Electronic Supplementary Material (ESM EGU\_B)] as the resident male, with barred lips and a white forehead coloration (associated to reproduction) (Shapiro et al. 1993), was seen slowly approaching two distended females, then showing its side to an individual female before changing speed and rushing towards the female in a circular pattern until both fish swim away from each other. This event was video recorded before the addition of the second male into the tank.

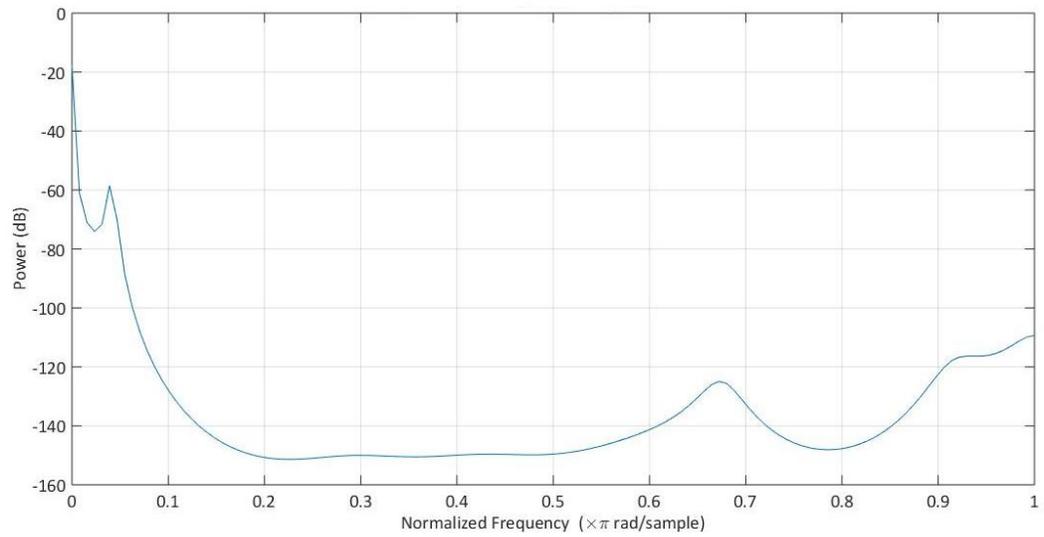
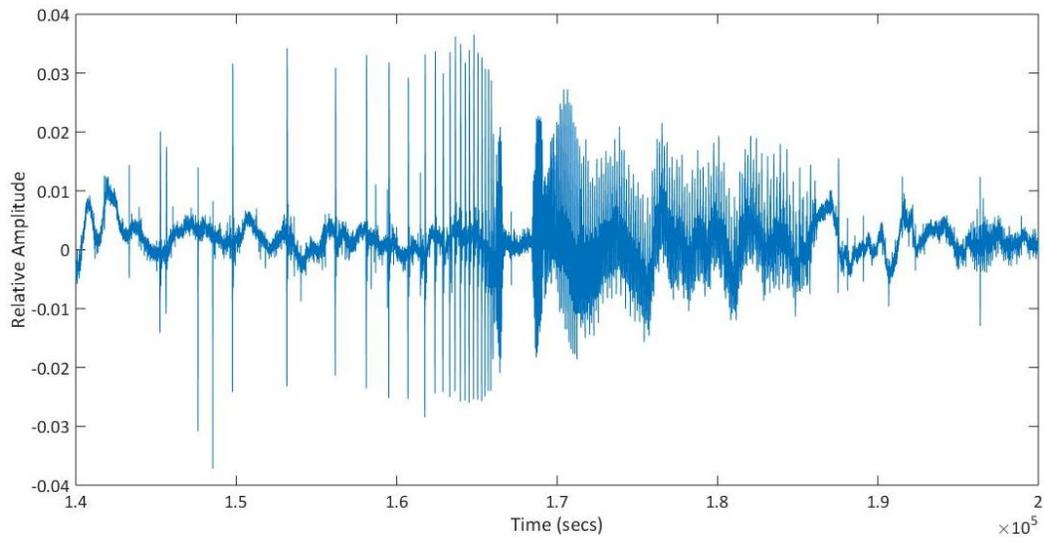
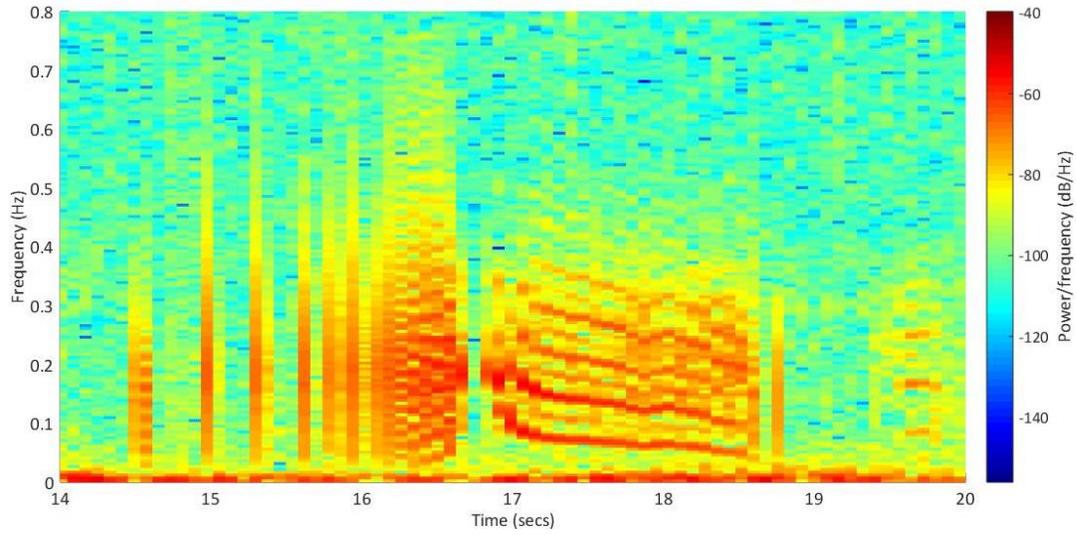


Figure 6. CAS-B or Tonal; From top to bottom, Spectrogram (Kaiser window, fast Fourier transform (FFT) length = 1609 points, overlap = 50%), Oscillogram and Power Spectra. The call was recorded during fish interactions in captivity and in the field during a red hind spawning aggregation off the west coast off Puerto Rico.

### *Grunt/Grunt train*

This call can be produced as a single Grunt/Grunt Train mostly consisting of two or three successive grunts as shown in Figure 7. Video evidence also suggest that a female was recorded producing the single grunt [see Electronic Supplementary Material (ESM EGU\_C)]. She is not seen on camera because she goes behind it and then produces the sound. But two fish, a female and the resident male are observed reacting to the sound, by moving towards the sound source after it was produced. It can be loudly heard in the video but very low in the hydrophone, and the male is seen further away from the camera closer to another hydrophone. When synchronized in time the hydrophone recording shows the same sound as the audio from the video but lower in intensity. Therefore, the sound source was close to the camera and far from the hydrophone. The male was near the hydrophone and far from the camera--opposite in position from the sound source, i.e., the female. It is similar in structure to other grouper sounds like the alarm call as described by Fish & Mowbrey (1970).

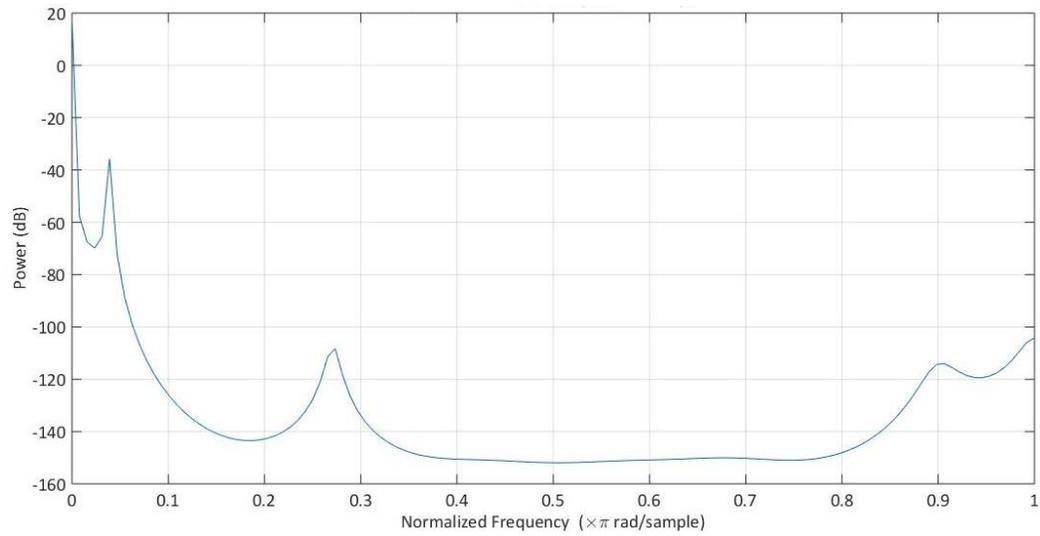
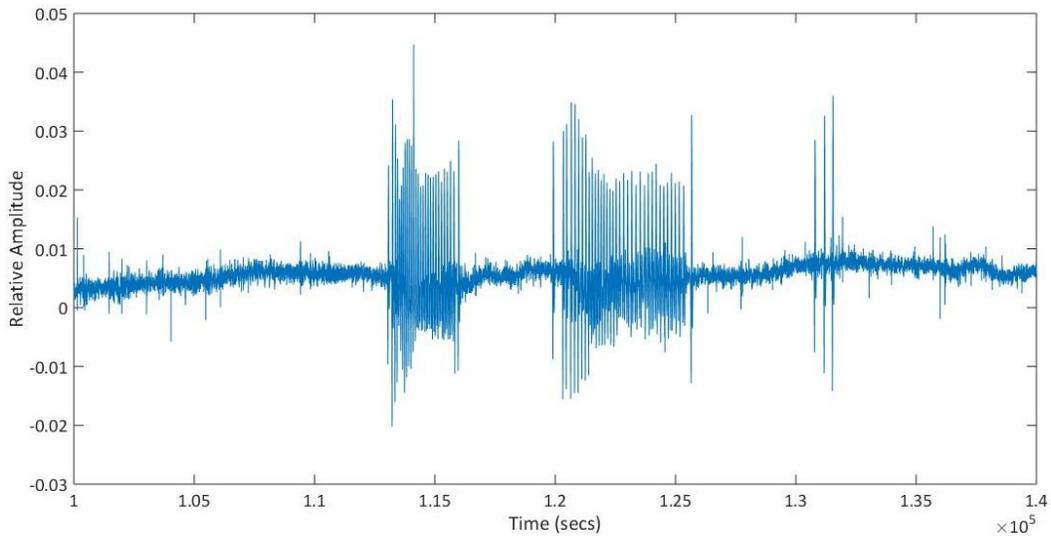
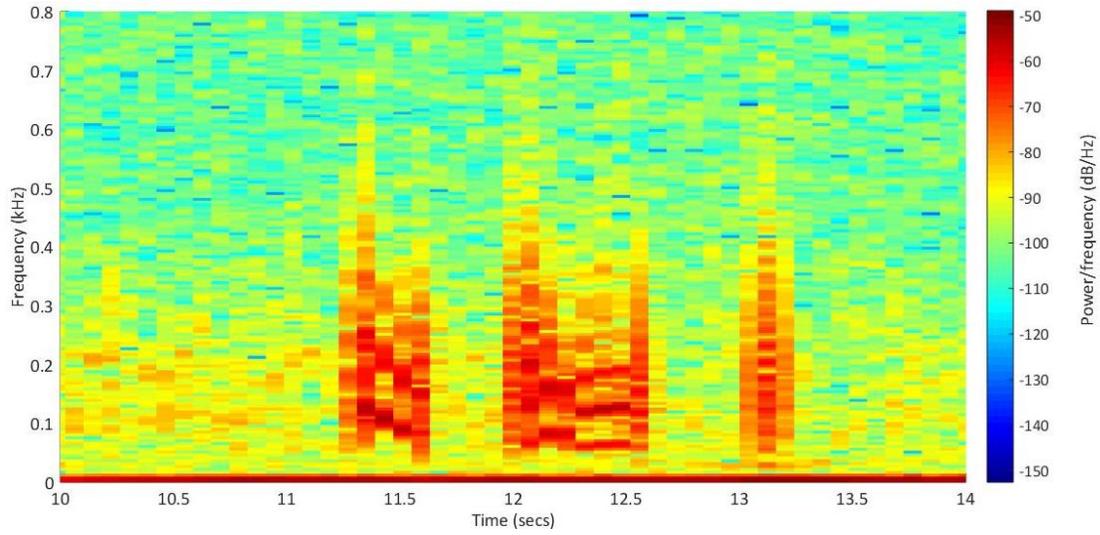


Figure 7. Grut/Grunt train; From top to bottom, Spectrogram (Kaiser window, fast Fourier transform (FFT) length = 1609 points, overlap = 50%), Oscillogram and Power Spectra. The call was recorded during fish interactions in captivity and in the field during a red hind spawning aggregation off the west coast off Puerto Rico.

### *Pulse/Pulse Train*

This call can be produced as a single Pulse/Pulse Train, mostly consisting of multiple consecutive pulses that can be found alone or as part of other calls, usually before or after another call such as CAS-A and Cas-B. Figure 8 shows consecutive short pulses resulting in a pulse train without any other call. Video recordings showed that this call was made during antagonistic behavior by the resident male towards the added male in combination with CAS-B [see Electronic Supplementary Material (ESM EGU\_MVM)]. The resident male is seen with a white forehead and barred lips then showing its side to the non-resident male before changing speed and rushing towards the male as it tried to bite. It was also produced by the resident male in courtship coloration towards a distended female. However, when produced towards the female it was not accompanied by other call types [see Electronic Supplementary Material (ESM EGU\_Pulse Train)].

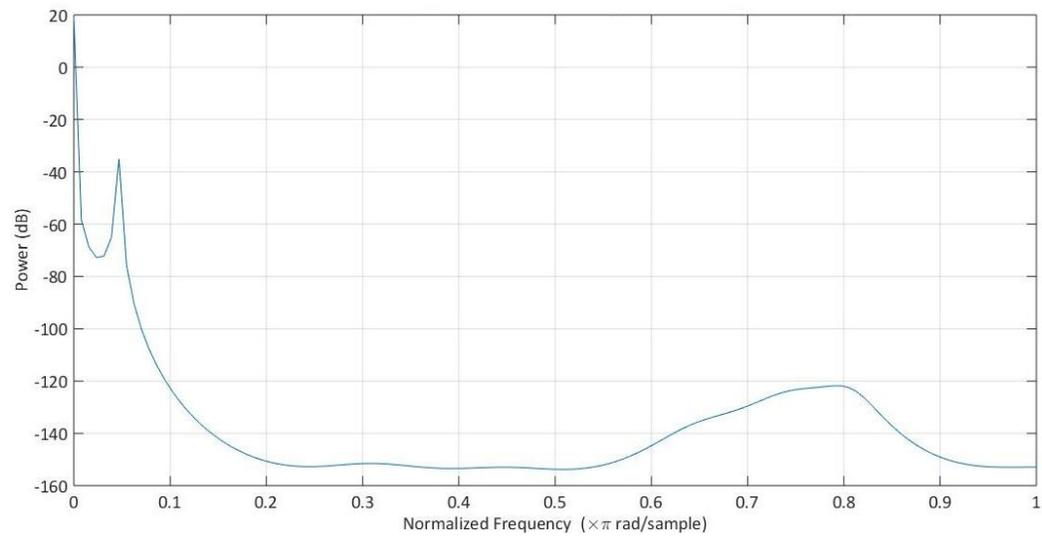
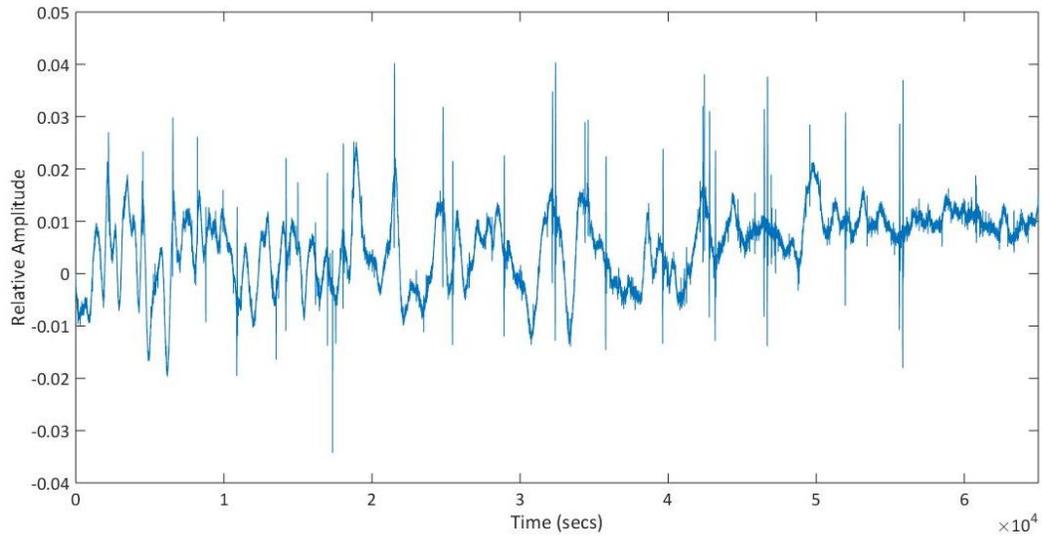
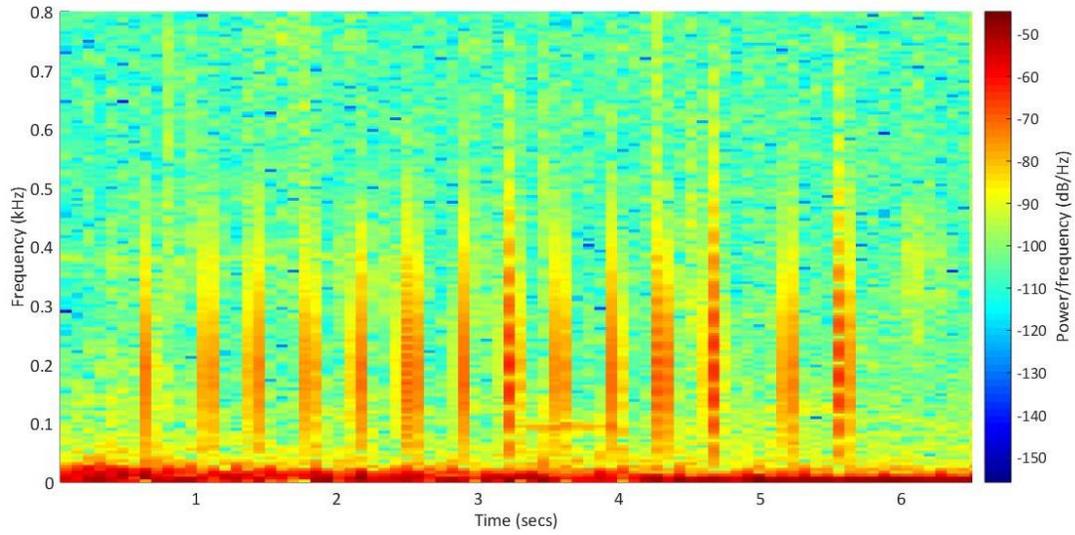


Figure 8. Pulse/Pulse Train; From top to bottom, Spectrogram (Kaiser window, fast Fourier transform (FFT) length = 1609 points, overlap = 50%), Oscillogram and Power Spectra. The call was recorded during fish interactions in captivity and in the field during a red hind spawning aggregation off the west coast off Puerto Rico.

### *Lunar and diel Patterns*

Frequency distributions for daily call counts from Jan 12, 2017 (0 DAFM) to Jan 25, 2017 (13 DAFM) showed that chorus vocalizations in the field peaked on Jan 23, 2017 (11 DAFM) (Figure 9). CAS-A peaked on Jan 20, 2017 (8 DAFM) for both field (Figure 9A) and captivity (Figure 9C) data. There is an increase in CAS-A until peaking on Jan 20, 2017 to a total silence on Jan 21 2017. Additionally, there is a shift in call dominance from CAS-B to CAS-A as the peak in sound production approached. CAS-B showed a peak on Jan 18, 2017 (6 DAFM) in captivity data and followed a bimodal pattern on field data with peaks on Jan 14, 2017(2 DAFM) and Jan 23, 2017 (11 DAFM). The red hind grunt (or grunt train) peaked on Jan 22, 2017 (10 DAFM) in captivity data and on Jan 14, 2017 (2 DAFM) in field data, where it showed a bimodal pattern with another peak on Jan 21, 2017 (9 DAFM). In captivity there was an overall dominance of Pulse/Pulse Train over the Grunt/Grunt Train. Conversely, there was a dominance of the Grunt/Grunt Train over the Pulse/Pulse Train. Finally, the Pulse/Pulse Train peaked on Jan 23, 2017 (11 DAFM) captivity data and on field data it was mostly undetected with a peak on Jan 13, 2017 and Jan 23, 2017 (1 DAFM, 11 DAFM) with only two calls detected.

The diel frequency distribution showed that in the field data the chorus vocalization (field  $X^2= 545.76$ ,  $p < 0.05$ ), CAS-A (field  $X^2= 37.06$ ,  $p < 0.050$ ) and the red hind Grunt/Grunt Train (field  $X^2= 64.79$ ,  $p < 0.05$ ) were not randomly distributed in time (Figure 10). The chorus and CAS -A peaked

at the 1600-1900 AST time block. Additionally, the sum of vocalizations concentrated around the 1600-1900 AST time block. The red hind grunt showed two peaks at night at the 0000-0300 AST and 2000- 2300 AST time blocks. CAS-B (field  $X^2= 17.58$ ,  $p < 0.05$ ) and Pulse/Pulse Train (field  $X^2= 3.63$ ,  $p < 0.05$ ) showed a random but consistent distribution patterns. However, CAS-B was most frequent at night at the 0000-0300 AST and 2000-2300 AST time blocks.

In captivity only CAS-A (Captivity  $X^2= 48.45$ ,  $p < 0.05$ ) and Pulse/Pulse Train (captivity  $X^2= 87.13$   $p < 0.05$ ) were not randomly distributed in time peaking at 1600-1900 AST and 0800- 1100 AST time blocks, respectively. Additionally, in captivity Cas-A and Cas-B both peaked at the same time block. CAS- B (Captivity  $X^2= 23.39$ ,  $p < 0.05$ ) and the red hind grunt (Captivity  $X^2= 20.07$ ,  $p < 0.05$ ) both peaked at 1600-1900 AST time block but did not show a significant diel pattern.

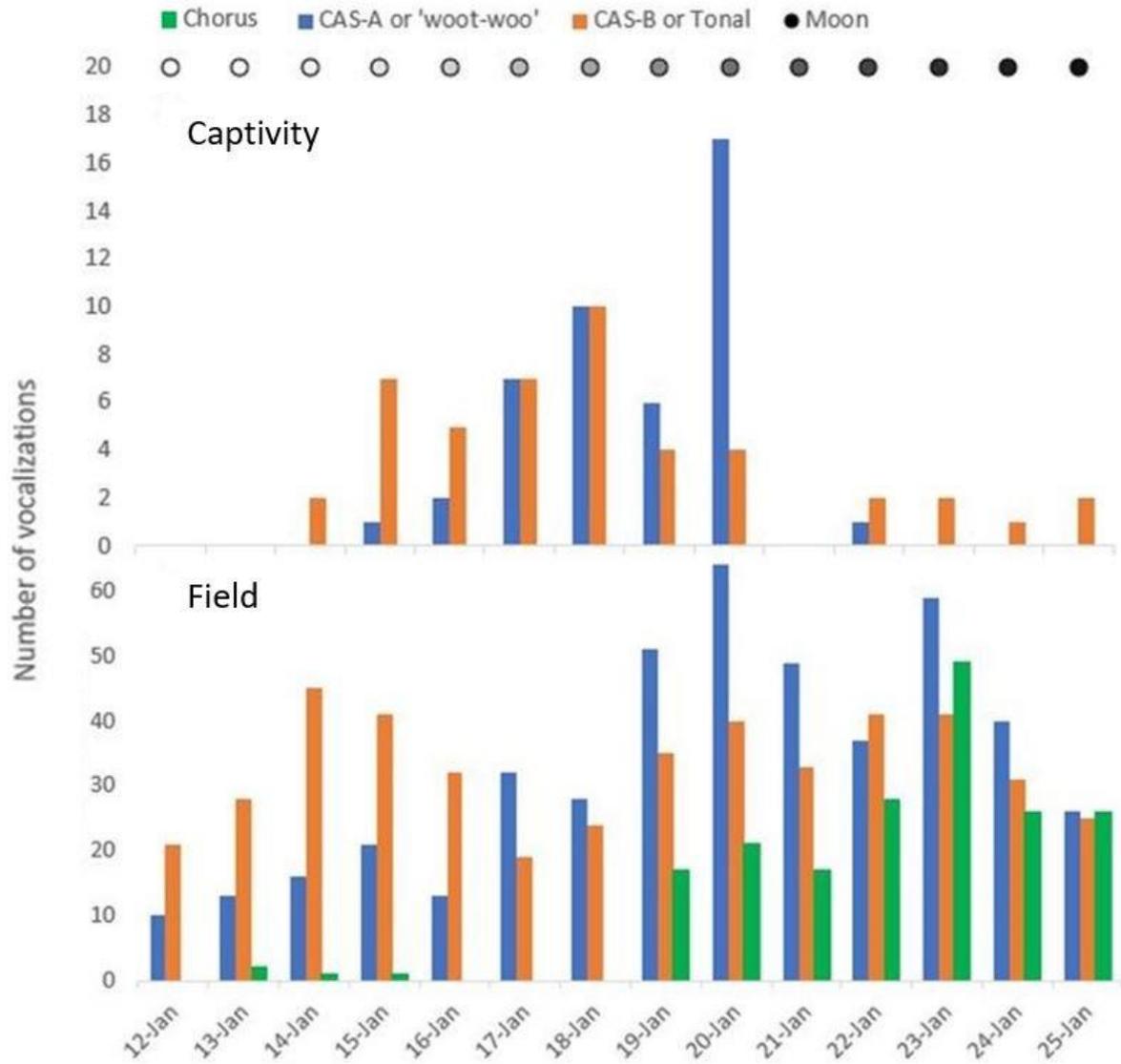


Figure 9. Lunar patterns for different red hind call types; Captivity recordings of CAS-A and CAS-B, top. Field recordings during a red hind spawning aggregation off the west coast off Puerto Rico CAS- A, CAS-B and Chorus bottom. The moon is represented as the circle on top white=full moon, black= 0.7% illumination.

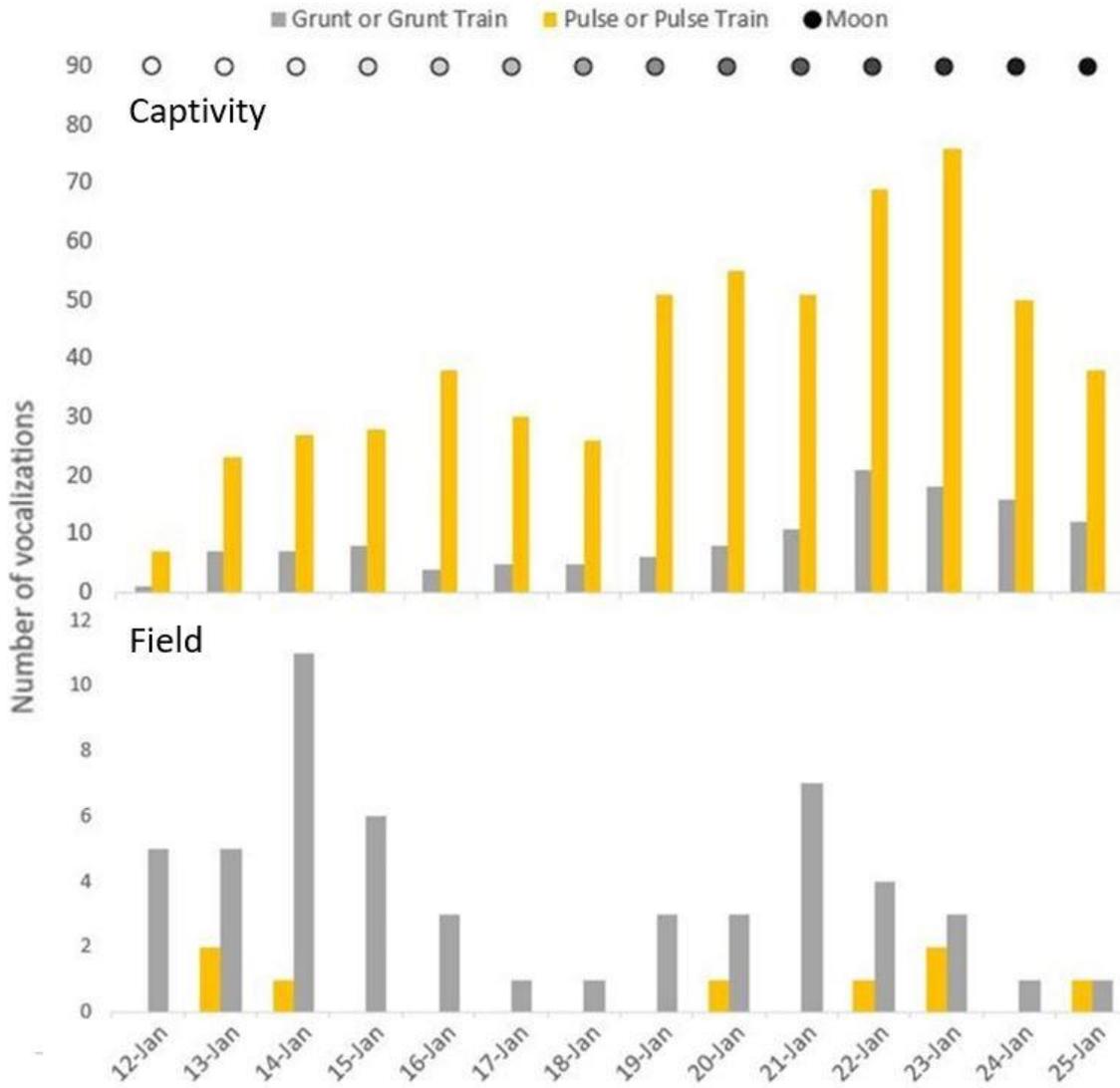


Figure 10. Lunar patterns for different red hind call types; Captivity recordings Grunt/Grunt Train and Pulse/Pulse Train, top. Field recordings of Grunt/Grunt Train and Pulse/Pulse Train during a red hind spawning aggregation off the west coast off Puerto Rico, bottom. The moon is represented as the circle on top white=full moon, black= 0.7% illumination.

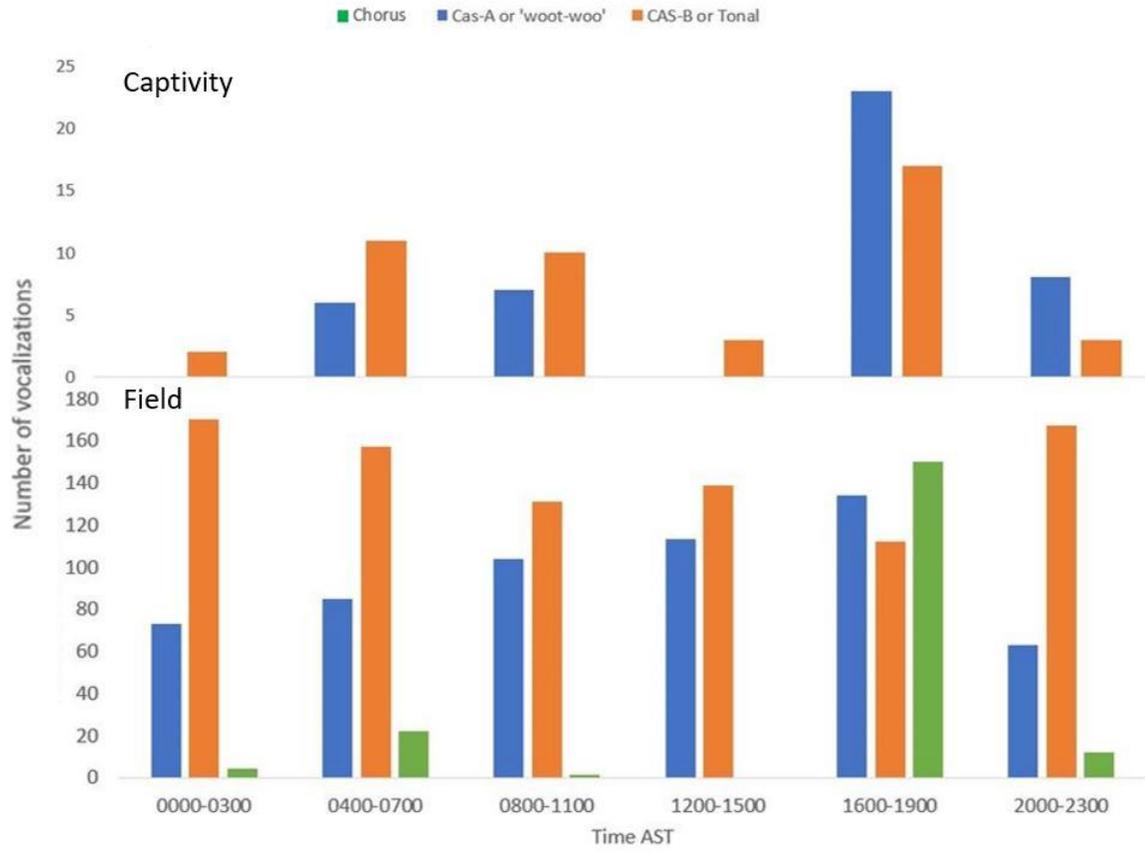


Figure 11. Diel patterns captivity recordings of CAS-A and CAS-B. Field recordings of CAS- A, CAS- B and Chorus during a red hind spawning aggregation off the west coast off Puerto Rico.

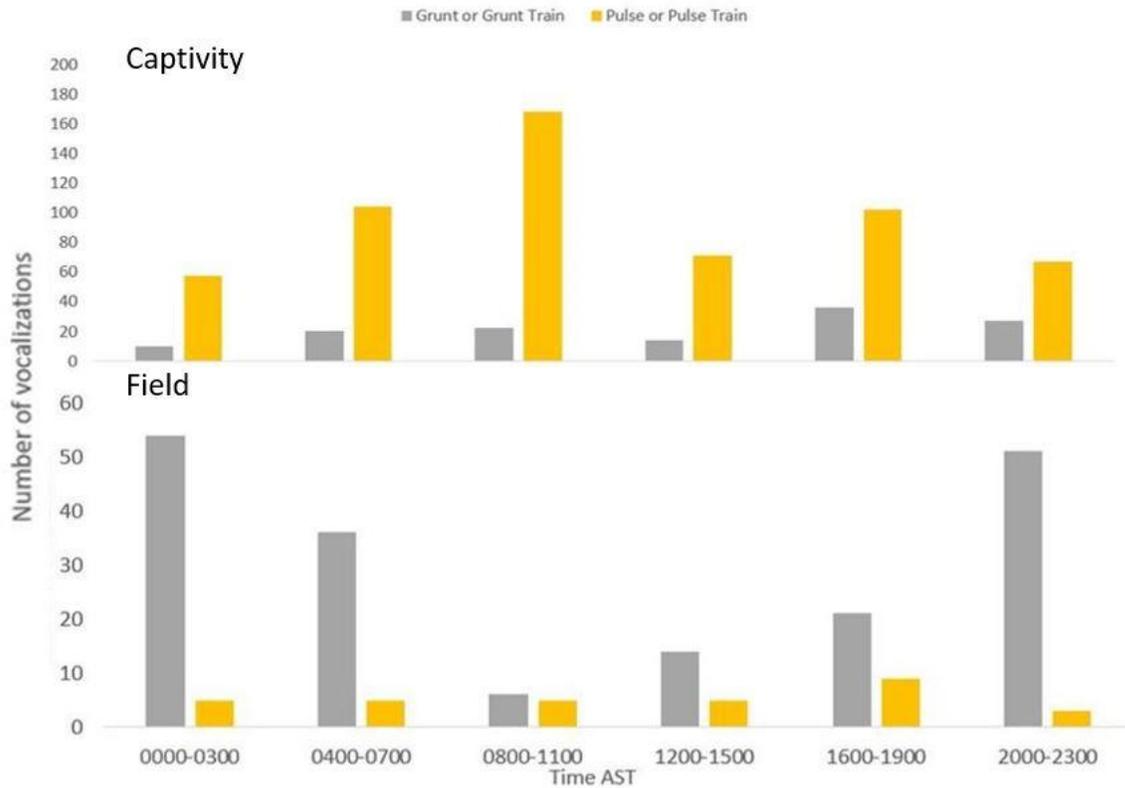


Figure 12. Diel patterns of captivity recordings Grunt/Grunt Train and Pulse/Pulse Train. Field recordings of Grunt/Grunt Train and Pulse/Pulse Train during a red hind spawning aggregation off the west coast off Puerto Rico.

### *Open Accelerometer Tag detection*

No sound was recorded in the open accelerometer tag during the 20-day captivity period. However, in the second experiment both the micro accelerometer and hydrophone recorded the vocalization of the female when being handled (Figure 11). Mean values (N=12) of red hind grunt call parameters; Duration, Peak Frequency, F-RMS Amplitude and Max Power; recorded in captivity by Open Accelerometer Tag and DSG Hydrophone recorder. This grunt was produced by holding the female next to the instruments. Summarized in Table 2.

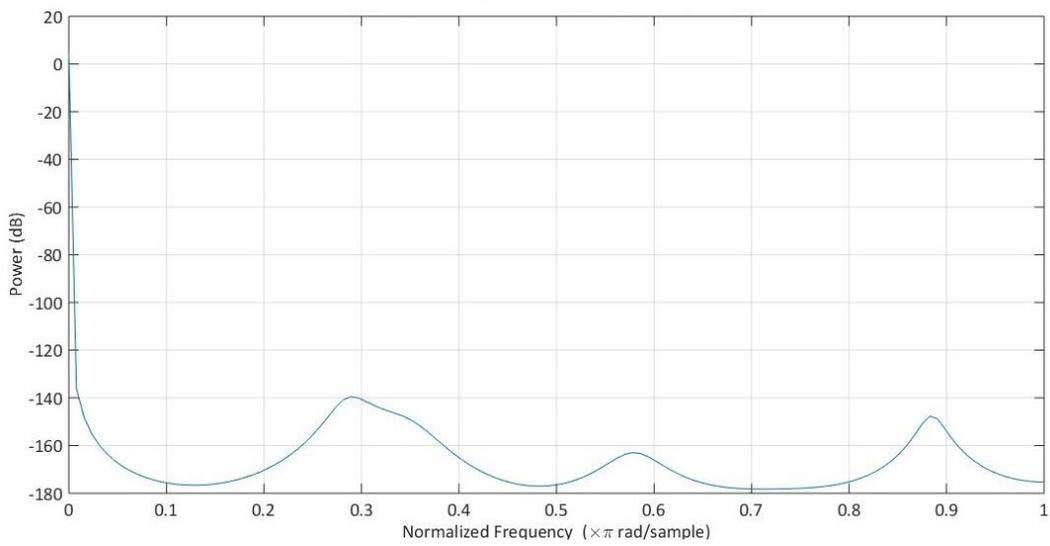
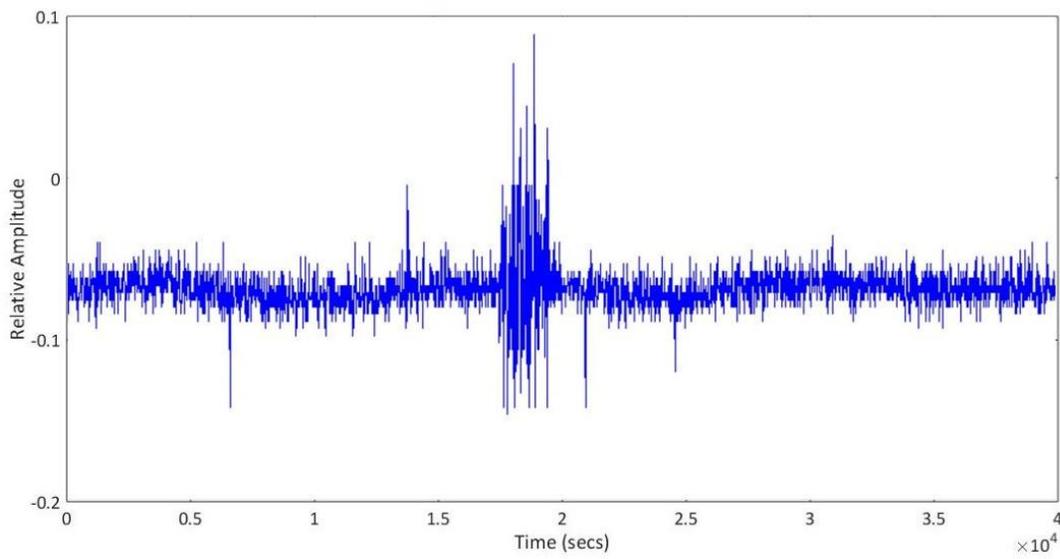
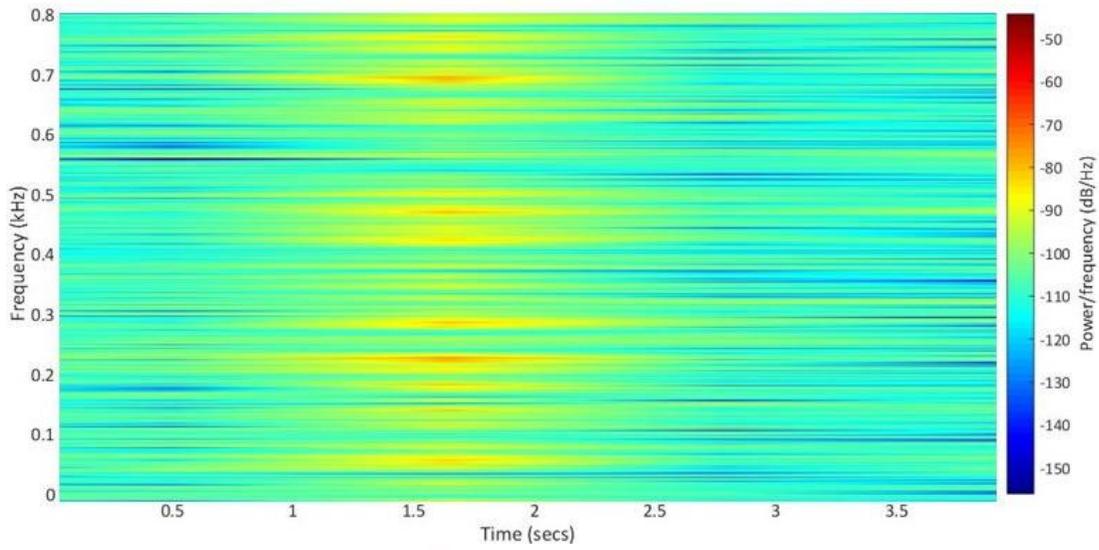
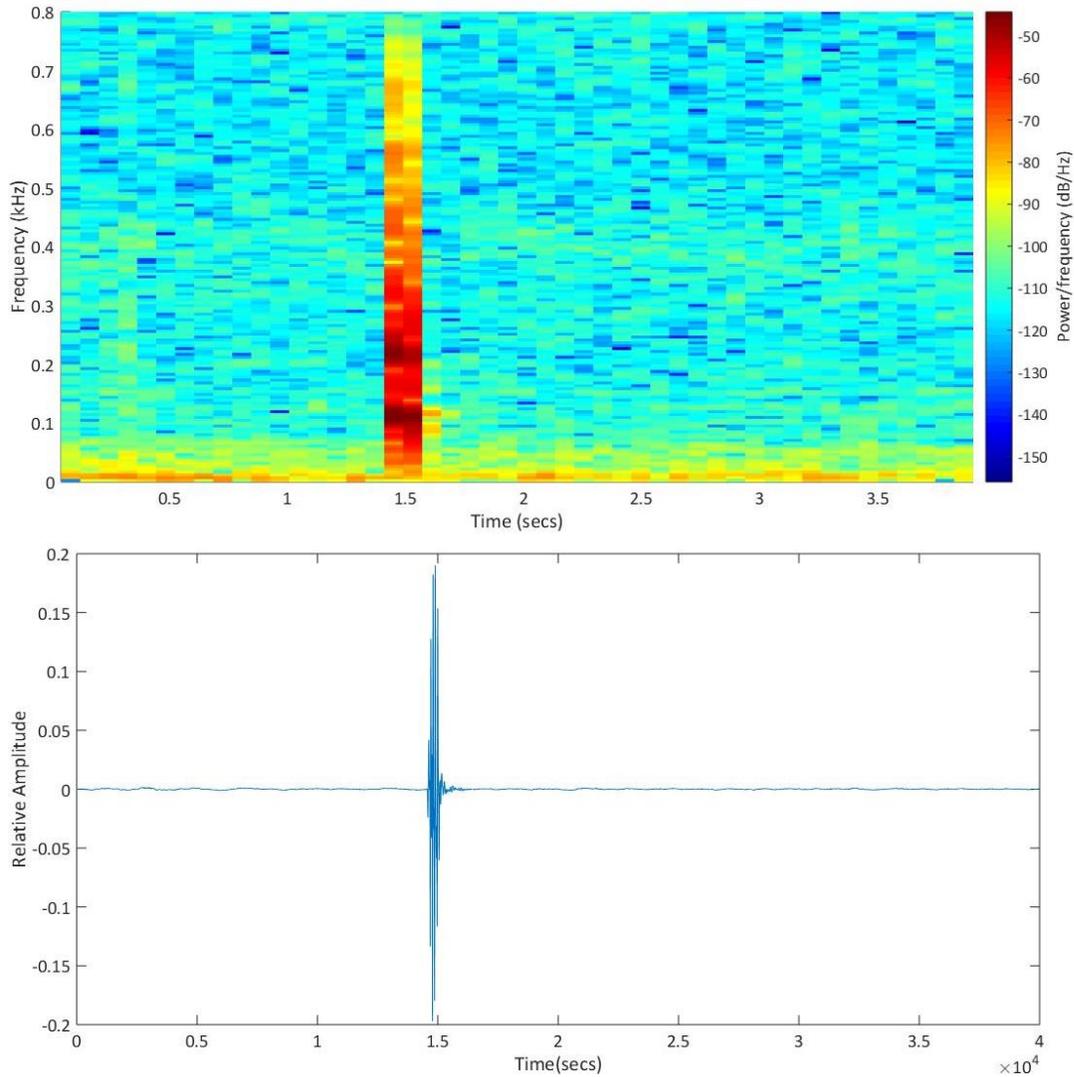


Figure 13. Open Accelerometer recorded red hind grunt call, from top to bottom; Spectrogram (Kaiser window, fast Fourier transform (FFT) length = 1609 points, overlap = 50%), Oscillogram and Power Spectra. Vocalization was produced in captivity and simultaneously recorded on the open accelerometer tag and hydrophone by holding a female red hind was next to the instruments.



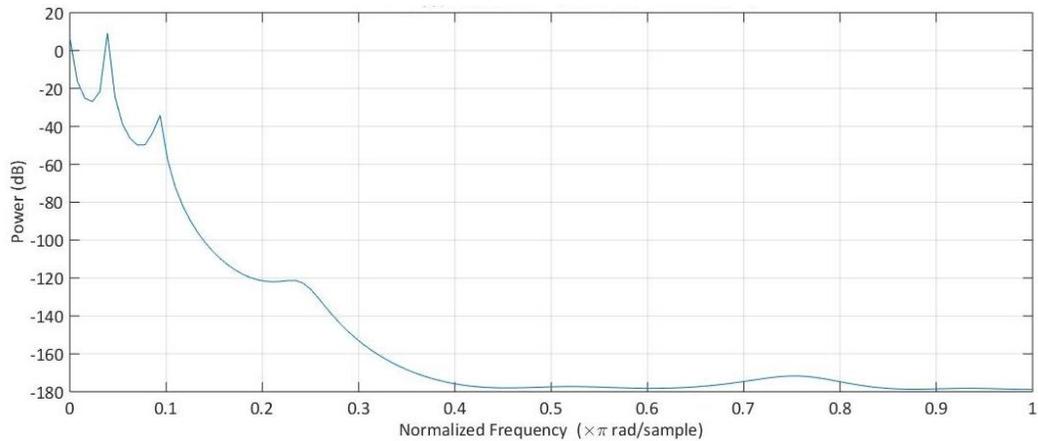


Figure 14. Hydrophone recorded red hind grunt call, from top to bottom; Spectrogram (Kaiser window, fast Fourier transform (FFT) length = 1609 points, overlap = 50%), Oscillogram and Power Spectra. Vocalization was produced in captivity and simultaneously recorded on the open accelerometer tag and hydrophone by holding a female red hind was next to the instruments.

Table 2. Mean values of red hind grunt call recorded in captivity by Open Accelerometer Tag (N=12) and DSG Hydrophone recorder. This grunt was produced by holding the female next to the instruments.

Grunt Vocalization				
	Duration (s) ± SD	Max Power (dB) ± SD	F-RMS-Amplitude ± SD	Peak Frequency (Hz) ± SD
Open Accelerometer Tag	0.11 ± 0.03	30.56 ± 1.56	23.53 ± 2.28	130.32 ± 3.7
Hydrophone Recorder	0.11 ± 0.01	92.2 ± 1.51	1919.35 ± 195.72	126.95 ± 14.89

### *Comparison of red hind vocalizations*

#### *SPCC-PCO*

Spectrographic cross correlation and PCO analysis of the different vocalizations showed three distinct groups (Figure 12). Posteriori One-Way ANOSIM showed significant differences among all call types (Global test  $R=0.542$ ,  $p=0.001$ , Table 3). CAS-A formed a distinct cluster with positive dispersion along PCO1 from 0 to 5, and a small dispersion along PCO2 from -1 to 1. Unlike the

SPCC-PCO, CAS-A did not overlap with the Chorus cluster but overlapped with CAS-B and grunt vocalizations. Cas-B formed a cluster near CAS-A and followed a similar dispersion on PCO1 from 0 to 5 and on PCO2 from -1 to 1.5. The red hind Grunt/Grunt Train formed a cluster on PCO2 from -1 to 2 and on PCO1 from 1 to -2, but many grunts overlapped with all the call types, except the chorus. The red hind Pulse/Pulse Train formed a tight cluster between -3 and 2 on PCO2 and another tight cluster from -2 to 2 on PCO1 overlapping only with the grunt vocalization which was observed as a single sound or as a grunt train of at most three consecutive grunts. The chorus cluster dispersed negatively on PCO2 from -3 to -1 and on PCO1 from -3 to 1, without overlapping with any other sound.

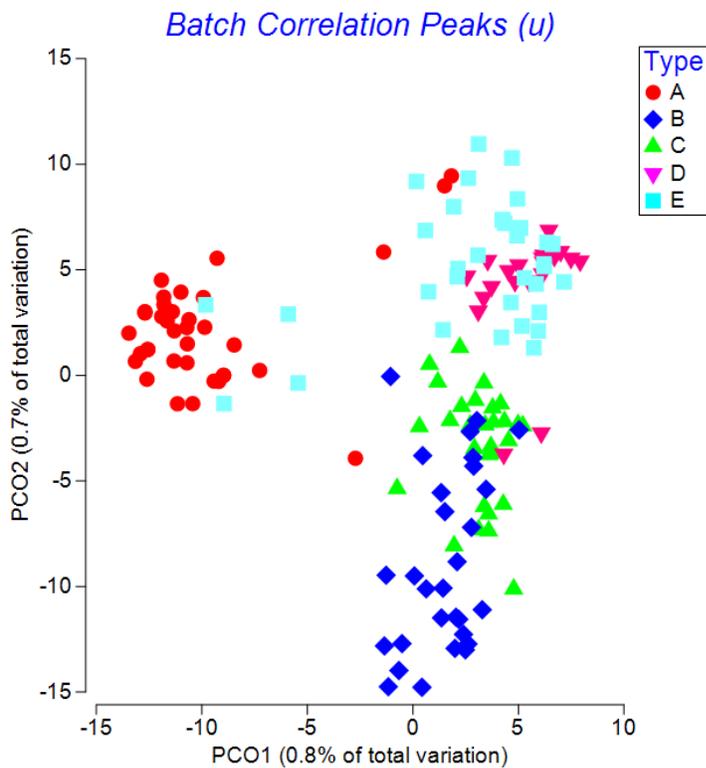


Figure 15. SPCC-PCO for red hind call type classification. A= CAS-A or ‘woot-woo’, B=CAS-B or Tonal, C= Grunt/Grunt Train, D= Pulse/Pulse Train, E= Chorus. All calls were recorded during a red hind spawning aggregation off the west coast off Puerto Rico.

Table 3. R values of SPCC-PCO (N=30) showing significant difference between red hind call types recorded in the field recorded during a spawning aggregation off the west coast of Puerto Rico. A= CAS-A or 'woot-woot', B=CAS-B or Tonal, C= Grunt/Grunt Train, D= Pulse/Pulse Train, E= Chorus.

Groups	R	Significance
D,A	0.912	0.001
D,B	0.805	0.001
C,E	0.72	0.001
E,B	0.713	0.001
B,A	0.652	0.001
D,E	0.537	0.001
C,A	0.524	0.001
E,A	0.514	0.001
C,D	0.444	0.001
C,B	0.371	0.001

#### *Parameters-PCO*

One-way analyses of similarities (ANOSIM), followed by a posteriori-corrected showed that all call types were statistically different from the rest (Global R = 0.704,  $p < 0,001$  and all *a posteriori* comparison at  $p < 0.05$ ; Figure 13). PCO ordinations showed a clear separation among all calling types, especially the *chorus* type, which showed no overlap with any other call type. CAS-A formed a distinct cluster with positive dispersion along PCO1 from 0 to 5, and a small dispersion along PCO2 from -1 to 1. Unlike the SPCC-PCO, CAS-A did not overlap with the Chorus cluster but overlapped with CAS-B and grunt vocalizations. Cas-B formed a cluster near CAS-A and followed a similar dispersion on PCO1 from 0 to 5 and on PCO2 from -1 to 1.5. The red hind grunt formed a cluster on PCO2 from -1 to 2 and on PCO1 from 1 to -2, but many grunts overlapped with all the call types, except the chorus. The red hind Pulse/Pulse Train formed a tight cluster between -3 and 2 on PCO2 and another tight cluster from -2 to 2 on PCO1 overlapping only with the grunt vocalization which was observed as a single sound or as a grunt train of at most three consecutive

grunts. The chorus cluster dispersed negatively on PCO2 from -3 to -1 and on PCO1 from -3 to 1. R values of Parameters-PCO (N=30) showing significant difference between red hind call types recorded in the field during a spawning aggregation off the west coast off Puerto Rico. A= CAS-A or 'woot-woo', B=CAS-B or Tonal, C= Grunt/Grunt Train, D= Pulse/Pulse Train, E= Chorus. Summarized in Table 4.

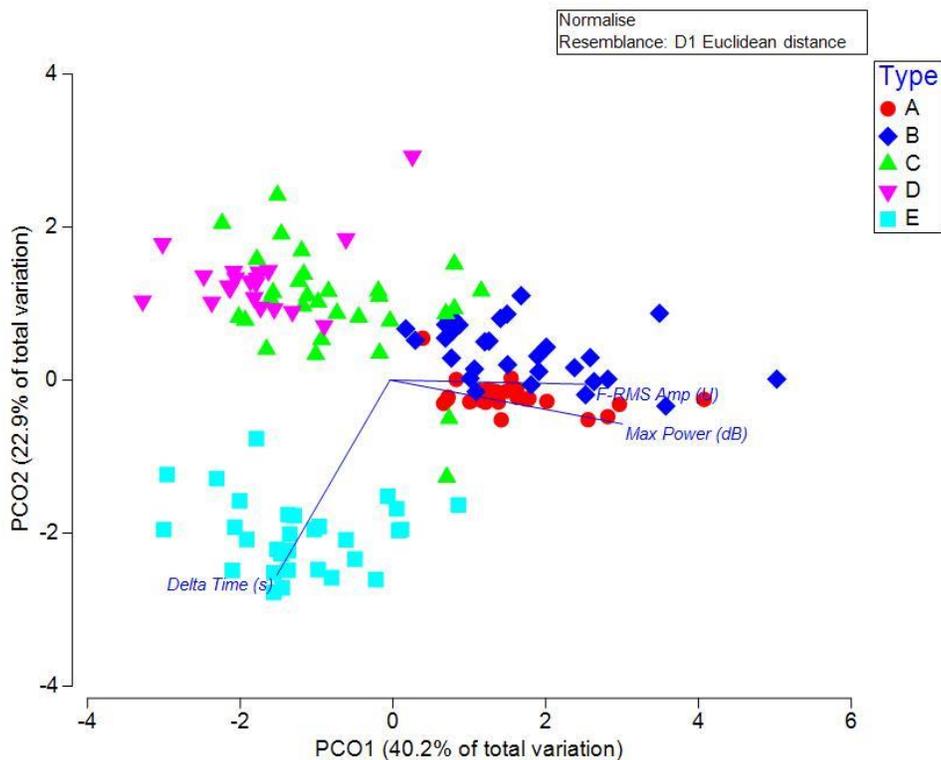


Figure 16. Parameters-PCO for red hind call type classification. A= CAS-A or 'woot-woo', B=CAS-B or Tonal, C= Grunt/Grunt Train, D= Pulse/Pulse Train, E= Chorus. All calls were recorded during in the field during a red hind spawning aggregation off the west coast off Puerto Rico.

Table 4. R values of Parameters-PCO (N=30) showing significant difference between red hind call types recorded in the field during a spawning aggregation off the west coast off Puerto Rico. A= CAS-A or ‘woot-woo’, B=CAS-B or Tonal, C= Grunt/Grunt Train, D= Pulse/Pulse Train, E= Chorus.

Groups	R	Significance
A,D	0.868	0.001
B,D	0.849	0.001
B,E	0.82	0.001
D,E	0.792	0.001
A,E	0.779	0.001
C,E	0.761	0.001
B,C	0.644	0.001
A,C	0.607	0.001
A,B	0.564	0.001
C,D	0.315	0.001

PERMANOVA showed which of the parameters were significantly different between each vocalization type (Table 5). Duration was statistically significant for all call types. F-RMS amplitude was statistically significant for all call types except for the following combinations; grunt/pulse train, grunt/chorus, pulse train/chorus and CAS-A/CAS-B. Max power was statistically significant for all call types except for CAS-A/CAS-B, grunt/pulse train and grunt/chorus. Peak frequency was statistically significant for all call types except for CAS-B/Pulse train CAS-B/Chorus Grunt/Pulse train and Pulse train/Chorus. Intercall/interval was statistically significant for CAS-A/Grunt and CAS-B/Grunt but not statistically significant between CAS-A/CAS-B. The number of pulses was statistically significant for CAS-A/CAS-B and CAS-B/Grunt but not statistically significant between CAS-A/Grunt. 90% Bandwidth was statistically significant for all call types except for grunt/pulse train, grunt/chorus, pulse train/chorus and CAS-A/CAS-B. Intercall/interval and number of pulses was not considered for the PERMANOVA analysis due to the incredible vocal plasticity in red hind, which limits the use of parameters that can quantitatively describe all the vocalization types.

Table 5. PERMANOVA values and significance for each red hind call type comparison from field recorded data during a spawning aggregation A= CAS-A or 'woot-woo', B=CAS-B or Tonal, C= Grunt/Grunt Train, D= Pulse/Pulse Train, E= Chorus and . N/A = Not applicable. Pair wise tests showed which parameters were statistically significant for each pair of vocalization type summarized as \*=Statistically significant difference.

Groups	A,B	A,C	A,D	A,E	B,C	B,D	B,E	C,D	C,E	D,E
Duration (s)	13.565*	2.8526*	10.565*	22.632*	11.844*	5.8722*	20.337*	9.9582*	22.333*	13.317*
p value	0.001	0.005	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Permutations	925	791	982	995	909	967	991	980	995	994
Filtered-RMS-Amplitude	1.8095	6.0319*	5.6936*	7.1193*	3.586*	3.563*	4.48*	1.0833	1.5536	0.27109
p value	0.077	0.001	0.001	0.001	0.001	0.001	0.001	0.265	0.137	0.788
Permutations	954	953	974	964	943	962	932	910	851	853
Max power (Dcb)	0.40251	7.2639*	9.8971*	7.1403*	6.7207*	8.4939*	6.3034*	1.7284	1.4492	3.5251*
p value	0.706	0.001	0.001	0.001	0.001	0.001	0.001	0.067	0.141	0.001
Permutations	362	464	647	395	486	670	446	595	398	570
Peak frequency (Hz)	4.1008*	6.0637	5.5148*	2.7164*	2.4907*	1.9629	0.19361	0.30518	2.242*	1.7108
p value	0.001	0.001	0.001	0.006	0.023	0.052	0.855	0.764	0.035	0.095
Permutations	259	353	401	325	324	371	316	429	386	467
Intercall-Interval (s)	1.8903	11.134*	N/A	N/A	10.715*	N/A	N/A	N/A	N/A	N/A
p value	0.057	0.001	N/A	N/A	0.001	N/A	N/A	N/A	N/A	N/A
Permutations	226	777	N/A	N/A	749	N/A	N/A	N/A	N/A	N/A
Number of pulses	17.469*	0.257	N/A	N/A	16.198*	N/A	N/A	N/A	N/A	N/A
p value	0.001	0.813	N/A	N/A	0.001	N/A	N/A	N/A	N/A	N/A
Permutations	426	122	N/A	N/A	447	N/A	N/A	N/A	N/A	N/A
90% Bandwidth (Hz)	1.8951	4.3778*	5.448*	4.7267*	2.6599*	3.7934*	3.6835*	1.3475	1.7764	0.49173
p value	0.077	0.001	0.001	0.001	0.012	0.001	0.001	0.189	0.075	0.654
Permutations	149	219	319	318	240	333	331	369	326	423

## Discussion

The results and significance of this study are based foremost on the demonstration that red hind can be motivated to produce CAS in captivity, in this case in the presence of distended females. This is what allowed for the identification and classification of different call types and, in some cases, their associated behaviors. These call types could then be identified from field recordings, allowing for quantitative descriptions and comparisons. Comparisons of the temporal call patterns from the tank and field studies also gave insight into potential drivers.

The results demonstrated the ability of red hind to combine and modify known vocalizations to produce quantitatively distinct sounds. Vocalizations were classified into five classes: *Cas-A* or 'woot-woo', *CAS-B* or *Tonal*, *Grunt/Grunt Train* and *Pulse/Pulse Train* and the newly described 'chorus'. All sounds recorded, except for the grunt train later produced by the female, are presumed to have been made by the territorial male. All call types were recorded prior to the introduction of the non-resident male. After its introduction this second male stayed tranquil in its cave where it displayed an overall darker coloration with darker bars associated with submissive and camouflaging behaviors observed in the field.

Unfortunately, fish were not observed during actual spawning events. Indeed, the captured distended females remained distended throughout the recording period. This may be due to the factors not possible to manipulate in this study (e.g., changes in current speed/direction and/or upwelling induced temperature changes; (Appeldoorn et al. 2016, Schärer, unpublished data). Consequently, we still do not have confirmation of sound production associated with the act of spawning, for which unique sounds have been demonstrated to occur in other species of fish. In

*Atractoscion nobilis* (white seabass) there is an association between sound production (drumrolls and thuds) and spawning (Aalbers and Drawbridge 2008), in *Hypoplectrus unicolor* (Butterhamlet) sounds are produced just prior to or simultaneously with gamete release, which may facilitate synchronous gamete release (Lobel 1992), while in *Padogobius martensi* (common goby) and *Knipowitschia punctatissima* (panzarolo goby) spawning vocalizations have been shown to be modified before and during ovideposition (Lugli et al. 1995).

The CAS-A is the same as the previously described “woot-woo” (Mann et al. 2010), while the grunt/grunt train is similar to the ‘grunts’ that recorded Fish and Mowbrey (1970) during feeding times in captivity, but this is the first report of it being recorded from a female. The various sounds mostly differ in duration, power and frequency. CAS-A or ‘woot-woo’, and ‘Chorus’ had the most statistical differences. Standardized classification coupled with the ability to distinguish among the vocalizations allows for the search for distinct dialects occur across the Caribbean. As has been shown in the anemone clownfish *Amphiprion akallopisos* which showed differentiation of antagonistic calls between two populations 6500km apart (Parmentier et al. 2005). The population structure of red hind has not been assessed, but genetic studies on the related Nassau grouper showed the Caribbean to be divided into at least four populations (Jackson et al. 2014). Detection of dialect might suggest barriers to connectivity.

Similarly, multiple calls have been described for other groupers such as *Epinephelus striatus* (Nassau grouper) and *Mycteroperca venenosa* (yellowfin grouper) where three distinct calls have been described for each species (Rowell et al. 2018; Schärer et al. 2012). These studies suggest groupers in general have a more extensive repertoire. Furthermore, the red hind Grunt vocalization looks similar to the antagonistic sound produced by Nassau grouper reported by

Rowell et al. (2018), and due to its simplicity, it could be the basis for comparison to similar vocalizations of other soniferous groupers (Fish and Mowbrey 1970; Rowell et al. 2018). However, to do these comparisons and study the use of sound in groupers, its evolution, and its relationship with local populations the parameters used to describe grouper sounds must be standardized. This will facilitate the quantitative comparisons of sounds, improve passive acoustic monitoring as a tool, and enhance understanding the role of sound communication at spawning sites, including its comparative use across species at multispecies spawning sites (Rowell et al. 2015), it will even facilitate the discovery of new grouper spawning aggregations as more sounds are added to the acoustic library and increases the chances of detection and species recognition (Chérubin et al. 2018).

Studies of anurans (Ryan 1985; Gerhardt and Huber 2002), mammals (Charlton et al. 2007), birds (Catchpole and Slater 2008; Nemeth et al. 2012), and insects (Gray 1997; Gerhardt and Huber 2002; Tregenza et al. 2006) have shown that courtship associated sounds are usually combined with visual displays and play an important role in species recognition and sexual stimulation driving mate attraction and reproductive success. Our understanding of the behavioral context of these sounds in red hind is still at an early stage, but with more complete knowledge it may be possible to extract more information from acoustic datasets. The quantification and association with behavior of these vocalization types will help clarify some of the variability of red hind bioacoustic recordings and what can be inferred from those data, thus allowing behavior during red hind spawning to be studied in greater detail. For example, Connaughton and Taylor (1995) found that patterns of sound production in weakfish, *Cynoscion regalis*, coincide with reproductive condition, while in the Lusitanian toadfish (*Halobatrachus didactylus*), call rate and

pulse period show a strong relationship to male body condition (Amorim et al. 2010), while call rate and call duration are strongly related to number of eggs within nesting sites (Vasoncelos et al. 2012).

Vocalizations involving individual interactions could be important for establishing dominance structures, with obvious implications in mate choice (Donaldson 2019). For example, *Pollymirus isidori* (African electric fish) vocalizations show distinct functions: grunts, moans, and growls are associated with in courtship, but hoots and pops are associated with territory defense, with all varying in intensity depending on the male-male or male-female interaction (Crawford et al. 1986). Vocalizations may serve similar functions within red hind and additionally act as a cue to stimulate sex change. Sounds like CAS-B were recorded on interactions between male-male and male-female, suggesting that these sounds are associated with dominance behavior. In both types of observations, the male changed coloration and showed its side with an erected dorsal fin to then rush the other individual. However, when the interaction was male-male the sounds included pulse trains and the fish was noticeably more aggressive. If this call is related to dominance and not to spawning per se, then the number of such interactions during the reproductive season could also reflect changes in the population sex ratio. Likewise, the Grunt/Grunt train or alarm call, which can be produced by both males and females, is clearly related to individual interactions and could further serve to determine dominance and sex ratio. In this study it was more abundant in the field data when compared to captivity data possibly due to a higher rate of interaction. In the field both the CAS-B and Grunt/Grunt train

vocalizations were randomly distributed across the diel cycle. Despite a general increase in density and increase in number of females during aggregation formation prior to spawning, this

random pattern could result if production of these sounds varies with individual interactions and if dominance hierarchies are established quickly, so that only low rates of vocalizations are needed to maintain these while still reacting to new individuals entering the aggregation.

Overall the acoustic behavior of the resident red hind in captivity followed diel and lunar patterns as described by Mann et al. (2010) in the field with peaks in sound production around 1800 AST 7-10 DAFM. These patterns were driven by two call types, 'woot-woo' and chorus. This suggests that CAS-A, and its accumulative Chorusing, could serve possibly as a mechanism to simulate and synchronize gametogenic activity (Crews et al. 1985, 1986) and subsequent spawning.

The rate of CAS-A call production peaked at the same time during the month (8 DAFM) and day (1800 AST) for both the field and tank data sets. However, there were two big differences between these treatments. One was that the number of individuals in the tank experiment was constant, whereas at the aggregation site the density of red hind increases as the time of spawning approaches, particularly due to the arrival of females to the aggregation site. The second is that there are no currents within the tank, whereas in the field strong tidal currents occur and are thought to be important cues determining the time of the lunar cycle in which spawning occurs (Appeldoorn et al. 2016). Other factors such as light, temperature and hormone levels were left to oscillate naturally. The fact that the natural CAS-A call pattern is reproduced in the tank is significant because the prevailing thought (Mann et al. 2010, Rowell et al. 2012) is that red hind males increase their call rate in response to increasing numbers of arriving females, either by increasing their activity to attract females or increasing their territorial defense against other males, or both, and as such call rate could be used as an indicator of relative abundance. However, Appeldoorn et al. (2013) found that the relationship between call rate and abundance,

while still significant, was not consistent across sites or years, with peak call rates being similar despite large differences in density. The tank recordings further indicate the proposed relationship between call rate and density is too simplistic and offer a potential explanation. CAS- A call rates may be driven by some intrinsic mechanism, a separate external signal, such as lunar light (timing, magnitude), or a combination where the intrinsic mechanisms is entrained by the external signal.

Improving our understanding of the call rate and behavior in red hind and other groupers will require more detailed studies combined with technological innovations. Other studies of fish call rate, such as Lusitanian Toadfish and Pomacentrids, took advantage of easy access and the small territories of these species during reproduction, i.e., they remain on a coral head or in a cave, which makes following sound production of an individual simpler. However, grouper spawning aggregations usually occur at shelf edges and accessibility can be further limited by winter wind/sea conditions. Additionally, at the aggregation groupers move around the spawning aggregation making it impossible to focus on an individual. Accelerometer tags, as an emerging technology, offers the capability to have individualized recorders of sound, with recordings being done independent of sea conditions. Thus, the recognition of call types and the ability to use accelerometers, sets a precedent for call rate studies in red hind and the factors that affect it. For example, the limiting factor in converting CAS counts to number of fish is an understanding of the calling behavior of the fish during the reproductive period, specifically the call rate of an individual male, which may be subject to several factors in addition to the number of other males and females in the aggregation. With the negative effects of overfishing groupers well documented, the ability to remotely monitor individual locations will allow a greater number of

aggregation sites to be concurrently monitored and/or aggregations to be studied in much greater detail. Such data will enhance assessments of site-specific spawning stocks, assess the effectiveness of (population response to) MPAs and other management strategies, and enhance our understanding of fish spawning aggregation sonic behavior. Similarly, with this approach we can test for hypotheses that include mate choice and relationship of sound to fitness at an individual level and test it against the entire aggregation. Finally, by quantitatively characterizing grouper CAS we can analyze and study evolution and use of vocalization in groupers as well as connectivity patterns as has been done in birds and whales.

### **Recommendations**

The results and significance of this study are based foremost on the demonstration that red hind can be motivated to produce CAS in captivity, in this case in the presence of distended females. This is what allowed for the identification and classification of different call types and in some cases their associated behaviors. These call types could then be identified from field recordings, allowing for quantitative descriptions and comparisons. The results demonstrated the ability of red hind to combine and modify known vocalizations to produce quantitatively distinct sounds. Similar research must be done with other groupers (Teleostei: Epinephelidae: Epinephelini) to characterize their respective repertoire. This will enhance on going passive acoustic monitoring in the Caribbean and the information extracted from these datasets. Additionally, these types of experimental set up could be replicated in a larger scale to include more replicates of each interaction and sound associated. Furthermore, facilities were temperature and light levels can be manipulated maybe able to answer which abiotic factors have an effect on grouper spawning. By proving the use of accelerometers in captivity, future studies should include this technology

to follow an individual fish sonic activity and overcome the issues associated with call rate studies. Ultimately, to improve passive acoustic monitoring of groupers an acoustic library has to be created with consistent parameters for sound descriptions across species. By addressing these issues acoustic datasets can be used to remotely determine fish habitat ranges and spawning stock abundances, independent from fisheries data.

## Conclusions

- Red hind (*Epinephelus guttatus*) can be motivated to produce vocalizations in captivity and behaves similarly in the field.
- Red hind (*Epinephelus guttatus*) has a vocal repertoire of five call types; *Cas-A* or 'woot-woo', *CAS-B* or *Tonal*, *Grunt/Grunt Train*, *Pulse/Pulse Train* and *Chorus*.
- Fish vocalizations can be detected with accelerometer tags.
- *Cas-A* and *Chorus* are discreet in time and do not occur randomly during the spawning period both peaking at 1800 AST.
- Female red hind (*Epinephelus guttatus*) can produce sounds; it was believed only males produced sound.
- Red hind (*Epinephelus guttatus*) sound production can increase without an increase in number of individuals.
- Most sonic activity concentrates at 1800 AST.
- Call duration and amplitude were the most useful parameters too distinguish between sound types.

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## **Appendix List**

Available upon request:

Electronic Supplementary Materials, ESM\_EGU\_CAS\_A.

Electronic Supplementary Materials, ESM EGU\_B

Electronic Supplementary Materials, ESM EGU\_C

Electronic Supplementary Materials, ESM EGU\_MVM

Electronic Supplementary Materials, ESM EGU\_PulseTrain