

# Red Hind *Epinephelus guttatus* Vocal Repertoire Characterization, Behavior and Temporal Patterns

## Comportamiento, Patrones Temporales y Caracterización del Repertorio Vocal del Mero Cabrilla, *Epinephelus guttatus*

## Comportement, Modèles Temporels et Caractérisation du Répertoire Vocal du Mérrou Cabrilla, *Epinephelus guttatus*

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### ABSTRACT

Passive acoustic monitoring has been key 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 *E. guttatus* were held in a 5700-liter tank equipped with a low frequency acoustic recorder and video cameras. Acoustic recordings from a simultaneous spawning aggregation at a known *E. guttatus* spawning site were used to quantitatively characterize and compare the sound types recorded in captivity. Five sound types were characterized: four recorded in captivity and an additional one only recorded in the wild labeled 'chorus'. These sounds consisted of variations and combinations of low frequency (50 - 450Hz) pulses, grunts and tones. Some types exhibited diel and lunar oscillations during the spawning season, which both field and captivity recordings peaked daily at 1800 AST and at 8 days after the full moon.

KEYWORDS: Bioacoustics, courtship associated sounds, *Epinephelus guttatus*, fish communication, spawning aggregations

### INTRODUCTION

Red hind (*Epinephelus guttatus*), is a mid-sized, long lived, slow growing, protogynous grouper, which has been subject to overfishing specifically during their transient 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 and Friedlander 1992) and Bermuda (Luckhurst & Ward 1996). During reproductive events red hind 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, Bertucci et al. 2015). Passive acoustics have been used to study red hind spawning site usage and temporal patterns of fish reproduction in Puerto Rico and the USVI, (Mann et al. 2009, 2010, Schärer et al. 2012a,b, Locasio et al. 2016, Rowell et al. 2012, 2015). The courtship associated sounds (CAS) produced by red hind are of low frequency (100 - 500 Hz) and are produced during the spawning aggregation, likely associated with territorial or agonistic confrontations and towards gravid females during presumed courtship behaviors (Mann et al. 2010). Visual observations coupled with passive acoustic recordings has been used to categorize sounds with associated behavior in epinephelids like the antagonistic sounds of *E. striatus*, *M. jordani*, and *E. morio* the latter use lek-like systems of reproduction (Schärer et al. 2012b, Rowell et al. 2019, Nelson et al. 2011). In red hind reproduction, females are determinate spawners, and larger females may spawn more than once a year (Sadovy et al. 1994b, Whiteman et al. 2005). Males arrive at the spawning aggregation site before the females and establish territories that are defended during agonistic and territorial confrontations followed by male courtship displays towards females (Colin et al. 1987, Shapiro et al. 1993). Changes in coloration are also observed during behavioral displays when males exhibit a light anterior ventral coloration and dark posterior ventral coloration with a barred pattern and barred maxilla (Shapiro et al. 1993; Ojeda-Serrano 2002). After males establish territories, small groups of three to five females associate with 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, Shapiro et al. 1993). 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 sex specific movements alter the female to male ratio during brief periods, which can range from 20:1 to 1:1 (Whiteman et al. 2005). These variations 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 range between 5 - 33 km since red hind exhibit site fidelity and females inhabit shallow reef areas with overlapping habitat ranges from 112 - 5,636 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). The density and number of individuals in spawning aggregations varies from 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). 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 such as abundance estimates, density estimates, length frequency distribution and population sex structure to assess the effectiveness of management measures in the USVI (Beets and 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. 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 the gulf corvina (*Cynoscion othonopterus*) (Rowell et al. 2017, Sprague and 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, which may be subject to several factors in addition to the density and sex ratio. The objectives of this study are to use the knowledge of the red hind spawning behavior to study their CAS and:

- i) Identify the acoustic repertoire, characterize each type of CAS, and determine the temporal patterns of each types of CAS,
- ii) Describe the behavioral context of CAS. In addition, the simultaneous acoustic recordings from an aggregation site in the wild were used to
- iii) Characterize and compare CAS in the field with captive fishes, and
- iv) Determine the correlation between field and captive temporal patterns in CAS.

## MATERIALS AND METHODS

### Aquarium Facility Set Up and Design

The acoustic and reproductive behaviors of red hind were studied in a 5700 L tank during the spawning season. The tank was connected to the seawater system at the Maguëyes Island Marine Laboratory of the Department of

Marine Sciences, University of Puerto Rico, Mayaguez. An 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, 44 m<sup>2</sup> external area. The central staging area was designed to act as the home territory isolating one male and the female harem with four artificial habitat structures of coral rubble added to simulate their benthic habitat. Females were added to promote courtship behaviors nearing a 1:3 M:F sex ratio. Fish were captured at the same time as the spawning aggregation formed in local waters (*Personal observation*). This facility was originally stocked with three red hind, one male and two females with distended abdomen captured by scuba divers with nets at a known spawning aggregation site, offshore the west coast of Puerto Rico on January 10, 2017. Subsequently, an additional male and distended female were added on January 20, 2017. The female was added to the staging area while the male was placed outside the staging area with structure to simulate their benthic habitat. Fish were fed one California market squid each (*Doryteuthis opalescens*) at 0900 AST every three days. The tank was equipped with a recording hydrophone (DSG-Ocean Loggerhead sensitivity of -180 dBV $\mu$ Pa-1 frequency range 2 to 37 kHz) to monitor acoustic activity. It was programmed to record 59-s every minute at a sample rate of 10,000 Hz and sample size of 16-bits. This recording schedule was chosen due to battery and memory limitations as well as to avoid interference with red hind behaviors due to human presence. To record behaviors, a Go Pro Hero 3 video camera in a waterproof housing was mounted on the side of the tank to maximize the field of view. During observation periods, from 0900 to 1000 AST, the camera recorded six 10-min videos (59 FPS and audio at 16- bits 480000 Hz) within the limits of battery and memory space. This period of recording was chosen for several reasons; previous research documented higher CAS production near sunrise and sunset, and the morning light levels were best for video recording with ambient light. Additionally, routine maintenance was required to maintain water quality and standardize recording periods (i.e. battery/memory changes). It was determined to make all these changes during the same time period to avoid interference with red hind behaviors due to human presence. Only one day fish were recorded during sunset due to the video limitations aforementioned. Due to acoustic interference caused by the properties the tank, such as size, location, pump and other external sources of noise, these recordings were used to identify different CAS types, validate the CAS time series, and document behaviors associated with sounds, but not for the characterization of vocalizations. 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. In the tank, acoustic and video recordings were made under three different scenarios. The first consisted of placing the initial male and two females in the staging area together simulating a harem. This scenario lasted from January 12, 2019 0 days after the full moon (DAFM), two days after fish were left in the tank

to acclimate and ended on January 19, 2019 (7 DAFM). On the night of the eighth day of recording, 8(DAFM), in an effort to further induce calling behavior, one additional female with distended abdomen was introduced inside the staging area, and an additional male was introduced outside of the staging area separated by the mesh the night of Jan 20, 2019 which also contained artificial structure simulating habitat. This second scenario presented the original male with a second male, that could be sensed through the mesh, but did not have access to the females that were inside the staging area with the male, this scenario lasted from January 20, 2019 (8 DAFM) to January 25, 2019 (13 DAFM). A third scenario was set up on the tenth day of observation (10 DAFM), when the second male was placed inside the staging area simulating an invasion of the first male's territory. The construction of the mesh cage allowed for the invading male to jump out of the water and over the mesh boundary, if chased by the resident male. The observations during the third scenario were performed three times on three consecutive days (Jan 21 - 23, 9 - 11 DAFM) to increase the potential of successful recordings. The second scenario was observed on 5 occasions and the first scenario was observed on 13 occasions. Red hind were held in captivity during the first lunar cycle of 2017, beginning two days before the full moon, which was on Jan 10th, corresponding to 20 days after the winter solstice, and behavioral observations were made from January 12, 2017 to January 25, 2017. All observations were done using the same specimens i.e individuals; the resident male red hind, the intruder second male red hind and the three distended female red hind. Simultaneously, acoustic recordings of ambient sounds were made at a red hind spawning aggregation site off the west of Puerto Rico (Rowell et al. 2012), from December 2016 through May 2017. Recordings were made using a Loggerhead Instruments DSG-Ocean, with a sensitivity of  $-180 \text{ dBV}\mu\text{Pa}$ -1 frequency range 2 to 37 kHz, during 20-s every 5 min at a sample rate of 10,000 Hz and sample size of 16-bits. All the acoustic data were recorded on a memory card with header information timestamps in Coordinated Universal Time (UTC) which were converted to Atlantic Standard Time (AST, -4 UTC). From this deployment the data files (wav format) with limited acoustic interference were selected to characterize the different vocalization types identified in captivity and to compare their time series. This recording schedule was used for consistency purposes because these data are also part of ongoing acoustic monitoring programs of red hind spawning aggregations in Puerto Rico.

### Time Series Analysis

Each CAS type's occurrences for both field and captivity data sets were summed per time blocks from January 12, 2017 to January 25, 2017. A  $\chi^2$  chi square test was performed to test for independence of call type per time block. Six-time blocks composed of four hours each (0000 - 0300, 0400 - 0700, 0800 - 1100, 1200 - 1500, 1600 - 1900 and 2000 - 2300) were used to test for independence. Data from the recordings in captivity were compared with those from the wild during the same time period assuming non-normal distribution.

### Characterization of Sounds

Red hind sounds recorded in the tank and recognized at the spawning aggregation site were audibly and visually analyzed using Raven Pro 1.5 (Bioacoustics Research Program, Cornell Laboratory of Ornithology, Cornell University, Ithaca, NY, USA) 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 describe and compare the calls observed in captivity however the characterization and analyses were made only from 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, low interference with other sounds and little background noise were selected. 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 were used to test for similarity using a principal coordinate ordination (PCO). The first method, spectrogram cross correlation-PCO or SPCC-PCO (Cortopassi and Bradbury 2000, Rice and 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 (Bioacoustics Research Program, Cornell Laboratory of Ornithology, Cornell University, Ithaca, NY, USA) that creates a contingency table comparing the spectrogram of each call type pixel by pixel. The second method, parameters-based-PCO, or parameters-PCO, compared sound based on a trained observer's knowledge, and each parameter was tested for similarity.

### SPCC-PCO

Sounds were cross correlated using Raven Pro 1.5 (Bioacoustics Research Program, Cornell Laboratory of Ornithology, Cornell University, Ithaca, NY, USA) batch correlator function. Sounds were normalized, and a bandpass filter was applied between 50 and 450 Hz 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 one-way ANOSIM with a significance level of (0.1%) and 999 permutations was performed to test for significant differences among call types.

### Parameters-PCO

To characterize and quantitatively describe the sounds, seven acoustic parameters were measured using Raven Pro 1.5 (Bioacoustics Research Program, Cornell Laboratory of Ornithology, Cornell University, Ithaca, NY, USA):

- i) Peak frequency (Hz) or the frequency that has the maximum sound pressure level (SPL) value of

- the selection,
- ii) Maximum sound pressure level, SPL (dB),
  - iii) Total sound duration (s),
  - iv) 90% bandwidth (Hz) or the frequency that divides the selection into two frequency intervals containing the difference between the upper and lower frequencies,
  - v) Total number of pulses, the sum of all pulses that make up a vocalization,
  - vi) Inter-call sound interval (s) or the time between successive sounds that make up a vocalization i.e. in phonetics it would be the equivalent of rime were vowels are used to shorten or elongate a word, and
  - vii) Relative amplitude, the filtered root mean square (F-RMS) amplitude value of selected time and frequency 50 to 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 Discrete Fourier Transform (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. Data were normalized and analyzed using a resemblance matrix of Euclidian distance. These parameters were chosen to describe in time and frequency the differences in vocalization types of red hind with a baseline parameter, SPL of “natural variability” therefore, not significant in an attempt to discriminate between call types within the memory and computing limitations.

## RESULTS

Four different call types were produced by red hind and recorded during the observations made in the tank: CAS-A or ‘woot-woo’, CAS-B or Tonal, Grunt/Grunt Train and Pulse/Pulse Train. Video recordings revealed specific behavior associated with each call type during different scenarios. These call types were also in the acoustic data recorded at the spawning aggregation site, from which they were isolated and the acoustic properties quantified for further characterization (Table 1). One additional sound type, presumed to be of red hind, was recorded only in the field and it is composed of types CAS-A and CAS-B, in a repeated and overlapping continuum, which was labeled as ‘chorus’.

### CAS-A

The CAS-A, or the sound labeled as “woot-woo” (Mann et al. 2010) is a combination of a short pulse followed by a short tonal sound (Figure 4). This call type had the highest mean peak frequency value and had one of the shortest mean duration of red hind call types (Table 1). This call type was observed alone or in combination with a pulse train before or after the tonal section, it occurred with a modification in the duration of the tone, and it was also observed as a chain of multiple repeated tone sections. Associated behavior was captured on video once at dusk during scenario one, due to memory and battery limitations. As a female was swimming above the bottom of the tank,

over a simulated cave, she was slowly approached by the only male present. The female reacted to this motion by swimming towards the bottom of the tank, away from the male when the sound was produced by the male, and the female briefly swam away rapidly and remained on the bottom (see Electronic Supplementary Information, SuppInfo\_EGU\_CAS-A). There was only one male and two female red hind during this event, similar behavior is described as visual observations of courtship behavior during a red hind spawning aggregation in Shapiro et al. 1993.

### CAS-B

The CAS-B sound type is composed of a series of pulses followed by an extended tone (Figure 2.B). This call type had the highest mean max power values and highest mean number of pulses of the red hind call types. It was observed alone or in combination with a pulse train before or after the call, and also combined with CAS-A prior to or after CAS-B, and the tonal part varied in duration (Table 1). The CAS-B sound type was recorded during a specific type of interaction (see Electronic Supplementary Information, SuppInfo\_EGU\_CAS-B) when the resident male slowly approached two females that were together, and turned laterally, showing its side to one female before swimming at higher speed towards that female and swimming in circular pattern approximately 50 cm near her, after this display they swam away from each other. This event was only observed during scenario one, prior to the addition of the second male into the tank. However, CAS-B sound type was recorded throughout the recording period of all scenarios. This sound type is similar to the “patrolling sounds” described by Mann et al. 2010 where it was sometimes directed at female red hind and most cases it was not directed at particular individuals.

### Chorus

The Chorus (Figure 1) is a continuous sound type consisting of multiple, overlapping sounds that resemble the red hind vocalization types CAS-A and CAS-B. Continuous Chorus sound was detected between 50 and 300Hz throughout the recordings with this type of call. Along with the chorus, CAS-A type are distinguished and calls presumed to be from further away are observed with lower decibels. The source of this sound type is unknown as it was not recorded in the tanks but it is presumed to be the combination of different red hind vocalizations at the peak of red hind sound production during spawning events. This call type had the lowest mean F-RMS amplitude and longest mean duration (s) of all the call types studied (Table 1).

### Grunt/Grunt Train

This call can be produced as a single grunt or a grunt train, consisting of two or three successive grunts (Figure 2 C). This call type had the lowest mean peak frequency (Hz) of all call types and varied in duration (Table 1). The combination of audio and video evidence suggest female red hind are also able to produce this sound type. A female is presumed to produce this sound type during a recorded occasion during scenario one (see Electronic Supplemen-

tary Information, SuppInfo\_EGU\_FRedhind Grunt). Although not clearly visible in the video frame, it is presumed the female grunts while located in the water column below the camera (all other fish were visible in the frame and further away) after which a second female and the first male react by swimming towards the presumed source. The grunt was recorded by the video camera's audio and also by the more distant DSG, but lower sound perceived in the DSG, which was near the male at that particular moment. This was the second most recorded call during captivity.

### Pulse/Pulse Train

The pulse sound type was observed as a single pulse or as a train of pulses of consecutive short pulses resulting in a pulse train (Figure 2 D). This call type had the lowest mean max power (dB) of all call types (Table 1). Video evidence suggests that this call type is produced in combination with CAS-B and may be associated with antagonistic displays between males. Behavior observed with this sound type occurred only when the second male was placed inside the central staging area in close proximity with the first male, only during scenario three (see Electronic Supplementary Information, SuppInfo\_EGU\_PulseTrain-CAS-B). The first male exhibiting a color phase of white cranium and barred lips, was positioned laterally to the second male before swimming directly towards it and trying to bite it. This pursuit continued until the second male jumped over the fence onto the outside of the cage and swam into his artificial cave outside of the first male's territory, while exhibiting a dark barred pattern. Pulse trains were also produced by the male towards a female however, pulse trains observed during displays towards females in scenario two were not accompanied by other call types (see Electronic Supplementary Information, SuppInfo\_EGU\_Pulse Train). This was the most recorded call in captivity.

### Temporal Patterns

Frequency distributions for daily (24 hr period in AST) call type counts from Jan 12, 2017 (0 DAFM) to Jan 25, 2017 (13 DAFM) showed that chorus vocalizations in the field had maximum value on 11 DAFM (Figure 3). CAS-A type was highest on 8 DAFM both in captivity and the wild. There was an increasing trend in CAS-A until 8 DAFM that decreased to zero in captivity by 9 DAFM, but not observed in the wild. Additionally, there was a shift in the most abundant call type from CAS-B to CAS-A as the highest rate in sound production approached for both data sets. In captivity CAS-B was highest on 6 DAFM, but it exhibited a bimodal pattern in the wild with maximum values on 2 and 11 DAFM. The grunt type was highest 10 DAFM in captivity and 2 DAFM in the wild where it also showed a bimodal pattern with second maximum value 9 DAFM. In captivity pulse trains were much more common than grunts, whereas in the wild the pattern observed was reverse. Finally, pulses were highest 11 DAFM in captivity and only two calls were detected in the wild. The diel frequency distribution of red hind sound types in the wild demonstrate that three types, the chorus ( $X^2 = 545.76$ ,  $p < 0.05$ ), CAS-A ( $X^2 = 37.06$ ,  $p < 0.05$ ) and grunts ( $X^2 =$

64.79,  $p < 0.05$ ) were not randomly distributed over the time blocks selected (Figure 4). The chorus and CAS -A peaked during the 1600 - 1900 AST block, and the sum of vocalizations was highest during the 1600 - 1900 AST time block. The grunt type was detected twice at night during the 0000 - 0300 AST and 2000 - 2300 AST time blocks. CAS-B ( $X^2 = 17.58$ ,  $p < 0.05$ ) and pulse train types ( $X^2 = 3.63$ ,  $p < 0.05$ ) showed a random pattern, although CAS-B was frequently heard at night during the 0000-0300 AST and 2000 - 2300 AST time blocks. For the recordings conducted in captivity only two types, CAS-A ( $X^2 = 48.45$ ,  $p < 0.05$ ) and pulse train ( $X^2 = 87.13$ ,  $p < 0.05$ ) were not randomly distributed in time, detected highest during 1600-1900 AST and 0800- 1100 AST time blocks, respectively (Figure 4). Types CAS-A and CAS-B showed highest occurrences during the 1600 - 1900 AST time block in captivity. CAS- B ( $X^2 = 23.39$ ,  $p < 0.05$ ) and the grunt ( $X^2 = 20.07$ ,  $p < 0.05$ ) were both most commonly observed within the 1600 - 1900 AST time block.

### Comparison of Red Hind Vocalizations

Spectrographic cross correlation and PCO analysis of the different vocalization types showed three distinct groups (Figure 5 A). A posteriori one-way ANOSIM showed significant differences among all call types except Grunt/Grunt Train from Pulse/Pulse Train and CAS-B (Global test  $R = 0.542$ ,  $p = 0.001$ , Table 2). Parameter-PCO (Figure 5 B) showed a clear separation among all types, especially the chorus type, which did not overlap with any other type. Call types, CAS-A and Pulse/Pulse Train are separated over the first axis of ordination, mainly due to maximum power and F-RMS- amplitude, showing a sequential/gradual change between vocalizations CAS-A and Pulse/Pulse Train. Chorus separated from the rest over the second axis of ordination due to duration. Parameters with a correlation greater than 0.7 were, max power, F-RMS amplitude and delta time. Parameters with a correlation between 0.5 and 0.7 were; 90% bandwidth and number of pulses. The parameters peak frequency and intercall sound interval had correlations less than 0.5. However, in the third axis, which accounts for 12% of the correlation, peak frequency represented the highest correlation value. The first and second axes account for 40 % and 22 % of the correlation respectively.

### DISCUSSION

These results demonstrate the ability of red hind to combine and modify vocalization types to produce distinct sounds that can be quantitatively described. The observation of multiple call types, quantifying and characterizing the acoustic parameters for each type and describing the behavioral associations is key for the advancement of passive acoustics research. By facilitating the location of spawning aggregation and increase the chances of detection and species recognition in different habitats and context (Chérubin et al. 2018). Recordings made simultaneously in the wild suggest the reproductive behaviors observed in captivity are likely intrinsically triggered between individuals, and the comparisons of the temporal call patterns provides insight regarding the potential drivers of these patterns and their relation to density, sex ration and

environmental cues. The capability of male and female red hind to produce different types of sounds is novel, as it was previously speculated only males were sound-capable (Mann et al. 2010). All sounds recorded in captivity are presumed to have been generated by the male, except for the grunt train, only observed by a female. All call types were recorded prior the introduction of a second male into the harem. The second male remained very still near the artificial structure provided displaying darker coloration with darker bars similar to behaviors observed in the field. The females remained distended throughout the experiment, and no eggs were ever observed in the tank. Since no spawning was detected in captivity it remains unknown if sounds are associated with gamete release in this species, although this has been demonstrated for the Gulf grouper, *Mycteroperca jordani* (Rowell et al. 2019). In *Atractoscion nobilis* (white seabass) there is an association between sound production (drumrolls and thuds) and spawning (Aalbers and Drawbridge 2008). The CAS-A and Cas-B are similar to the previously described “woot-woo” (Mann et al. 2010), while the grunt/grunt train is similar to the ‘grunts’ that were recorded Fish and Mowbray (1970) during feeding times in captivity, but this is the first report confirming the sound was produced by a female. It is important to know the context of these sounds, to differentiate between complex interaction in lek like mating systems like red hind. By knowing the context of the associated behavior scientist can hone in on the best time to study FSA, complemented with other sampling methods to test for abundance or changes in sex ratio. The association of five call types of red hind associated with different behavioral displays suggests that both courtship and territoriality underlie the variability observed in the bioacoustic recordings of spawning aggregations. Vocalizations involving interactions between individuals could be important for establishing dominance in complex social structures, with obvious implications in mate choice (Donaldson 2019). For example, *Pollymirus isidori*, the African electric fish has vocalizations for distinct functions: grunts, moans, and growls are associated with courtship, but hoots and pops are associated with territory defense, all with varying intensity depending on the male-male or male-female interaction (Crawford et al. 1986). Vocalizations may serve similar functions within red hind aggregations and also provide a cue to spawn or to stimulate sex change in females that have reached their appropriate size or age (Shapiro et al. 1993). For example, CAS-A was recorded during a male-female interaction in the water column as the larger male approached the smaller female; in response, the female swam away from the male. Since red hind is thought to spawn a few meters above the seafloor, this video may represent a female that is not ready to spawn, either because eggs are not hydrated or spawning is not likely to occur yet, similar behavior prior to spawning was reported by Shapiro et al. 1993. Sounds like CAS-B were recorded during interactions between male-male and male-female, suggesting that these sounds could be associated with dominance, competition, patrolling or territorial behavior. In both instances’ males changed color phase and displayed laterally while erecting the dorsal fin and rushing towards the other individual. However, when

the interaction was male-male the sounds included pulse trains and the fish was acting more aggressive. If this call type is related to dominance, then the frequency of this interaction during the spawning aggregation could also reflect changes in the sex ratio. Likewise, the grunt or alarm call, which can be produced by both males and females, is likely related to individual interactions and could serve as defense from predators or to alert competitors, this sound were most frequently produced with the presence of humans and captivity maintenance. This call type was more abundant in the field than captivity possibly due to a higher rate of interactions with other species and differences in background sounds, since a stationary hydrophone will record vocalizations produced nearer the hydrophone. In the field, both CAS-B and grunts were randomly distributed across the diel cycle, despite a general increase in density during the aggregation. This random pattern could be explained by the fact that CAS-B had the highest values of mean max power and mean number of pulses of all call types and as video evidenced, it can be accompanied by pulse trains making it a more impressive call with a dual purpose of territory defense and courtship display. In the case of the grunts a random pattern could result because smaller individuals, presumed females, produce quieter sounds and move through the spawning aggregation site evaluating male territories (Shapiro et al. 1993, Nemeth et al. 2017). However, the hydrophone will only detect the sounds produced within a limited distance (100 meters) hence this vocalization type was recorded more in captivity than in the field (Figure 8). Overall, the acoustic patterns observed in captivity followed diel and lunar patterns as described by Mann et al. (2010), and were similar to those recorded simultaneously in the wild with maximum sound production at 1800 AST, 7-10 DAFM. These patterns were driven mainly by two call types, CAS-A and chorus. This suggests that CAS-A, and its accumulative chorus, could serve possibly as a mechanism to simulate and synchronize gametogenesis (Crews et al. 1985, 1986) and subsequent spawning rushes (Colin 1987). The rate of CAS-A call type production peaked at the same time during the lunar (8 DAFM) and diel (1800 AST) patterns for both wild and captive conditions, although with two notable difference. First, the number of individuals in the tanks was constant until the night of Jan 20 8 DAFM, whereas at the aggregation site the density was unknown, but expected to rise as spawning time approaches, particularly due to the arrival of females to the aggregation site (Nemeth et al. 2017). Secondly, there are no changes in water temperature or flow in the tank, whereas strong tidal currents have been observed at the spawning aggregation site. It has been proposed that changes in temperature, current flow and direction may be important cues determining the time of the lunar cycle in which spawning occurs (Appeldoorn et al. 2016). Other factors such as light periodicity, water temperature and hormone levels were not controlled for in captivity. The fact that the natural CAS-A call patterns were reproduced in the tank is significant because the prevailing thought (Mann et al. 2010, Rowell et al. 2012) is that red hind males increase call rates in response to increasing numbers of interactions, either by increasing their displays towards females or increasing

their territorial defense towards 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 differences in density. The tank recordings further indicate the proposed relationship between call rate and density are not sufficient to explain this variation. The pattern in CAS-A sound type may be driven by some unknown 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. 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 within a cave, which makes recording sound production of an individual feasible (Mann and Lobel 2012, Jordão et al. 2012). However, grouper spawning aggregations usually occur at shelf edges and *in situ* observations are limited by environmental conditions. Additionally, during the aggregation individuals move around the spawning aggregation making it impossible to focus on an individual. Thus, the observations of specific behaviors in captive conditions and the recognition of call types, sets a precedent for additional studies in captive conditions and simultaneous field studies to document call rate in the wild (Shapiro et al. 1993, Nemeth et al. 2017, Montie 2010). This study suggest that for red hind, that exhibits a lek-like mating system, reproductive behaviors

can be studied in captivity, which overcomes some of the limitations of being able to use inferential statistics to compare and characterize sounds produced by fish in the wild where tracking an individual's courtship and sonic behavior over long periods of time is extremely challenging.

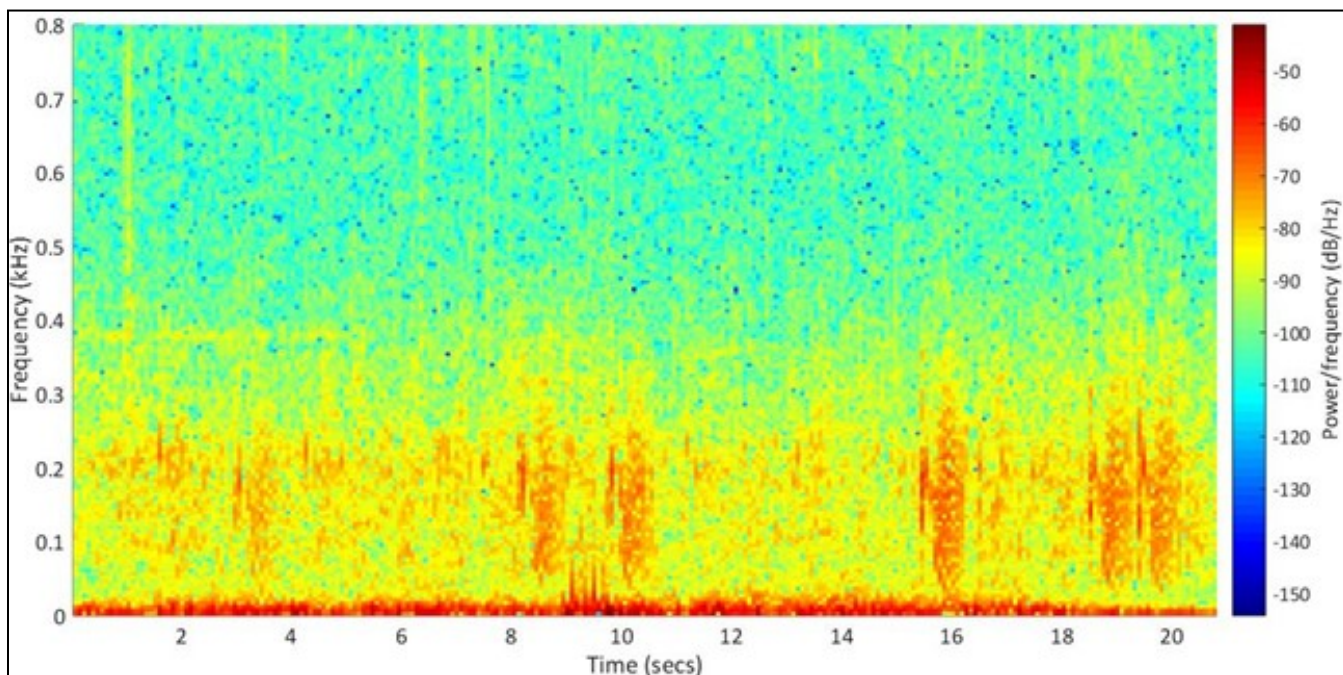
In this study we sought acoustic parameters that explained the differences among vocalizations detected quantitatively by the SPCC-PCO. Since the sound types were produced by the same species and the grouper sonic mechanism is simple compared to other species (Hazlett and Winn 1962), little variation was expected among call types. From the chosen parameters max power and F-RMS-amplitude had the highest correlations, but this is expected from field recordings where distance from the hydrophone is not known. Therefore, these are not the most adequate parameters for comparing between sounds if distance cannot be corrected for. However, call duration also showed a correlation value over 0.7, which indicates this to be an adequate parameter for comparison. Similarly, 90% bandwidth and number of pulses may be differentiating parameters as they showed correlations of over 0.5 in the first axis of ordination. Peak frequency had the highest correlation value in the third axis of ordination, which only accounted for 12% of the variation. However, this result suggests that there is variation in peak frequency and this parameter can be useful for studies of dialects and comparisons between species.

**Table 1.** Mean value  $\pm$  standard deviation of each acoustic parameter per *Epinephelus guttatus* call types recorded in the field.

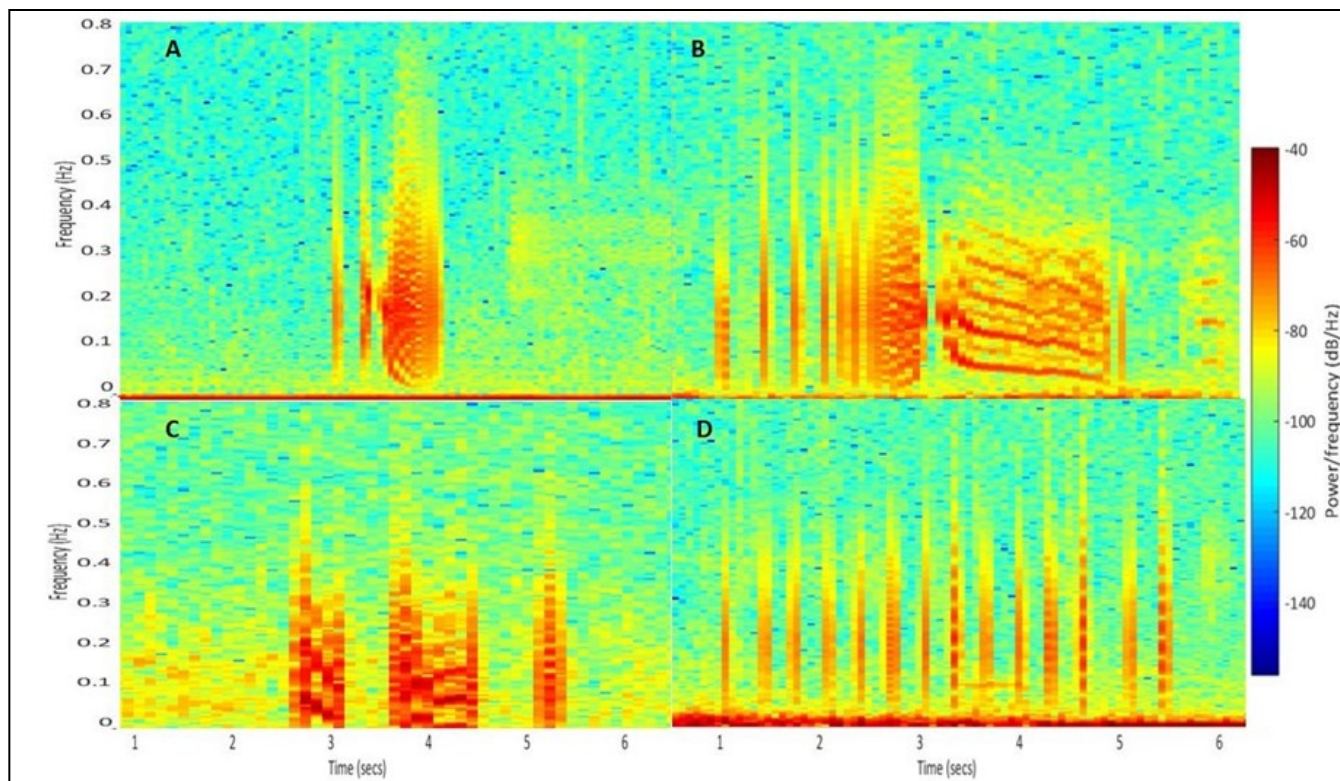
	90% Bandwidth (Hz)	Max Power (dB)	Peak Freq (Hz)	Filtered-RMS-Amplitude	Duration (s)	Intercall-interval (s)	Number of pulses
Chorus	237 $\pm$ 49	65 $\pm$ 4	174 $\pm$ 48	42 $\pm$ 17	15 $\pm$ 3	0 $\pm$ 0	1 $\pm$ 0
CAS-A	192 $\pm$ 11	73 $\pm$ 3	201 $\pm$ 18	220 $\pm$ 133	1 $\pm$ 0.2	0.1 $\pm$ 0.02	47 $\pm$ 7
CAS-B	200 $\pm$ 20	73 $\pm$ 5	172 $\pm$ 32	156 $\pm$ 135	2 $\pm$ 0.4	.18 $\pm$ .03	162 $\pm$ 34
Grunt/Grunt Train	218 $\pm$ 29	62 $\pm$ 6	147 $\pm$ 43	59 $\pm$ 54	1 $\pm$ 0.2	.54 $\pm$ .18	46 $\pm$ 16
Pulse/Pulse Train	230 $\pm$ 35	59 $\pm$ 5	151 $\pm$ 42	44. $\pm$ 28.	4. $\pm$ 1	1 $\pm$ 0	17 $\pm$ 8

**Table 2.** R values of SPCC-PCO (n = 30) showing significant difference between *Epinephelus guttatus* call types recorded in the field. \* indicates non-significant differences.

	CAS-A	CAS-B	Grunt/Grunt Train	Pulse/Pulse Train	Chorus
CAS-A	x	0.652	0.524	0.912	0.514
CAS-B	0.652	x	0.371*	0.805	0.713
Grunt/Grunt Train	0.524	0.371*	x	0.444*	0.72
Pulse/Pulse Train	0.912	0.805	0.444*	x	0.537

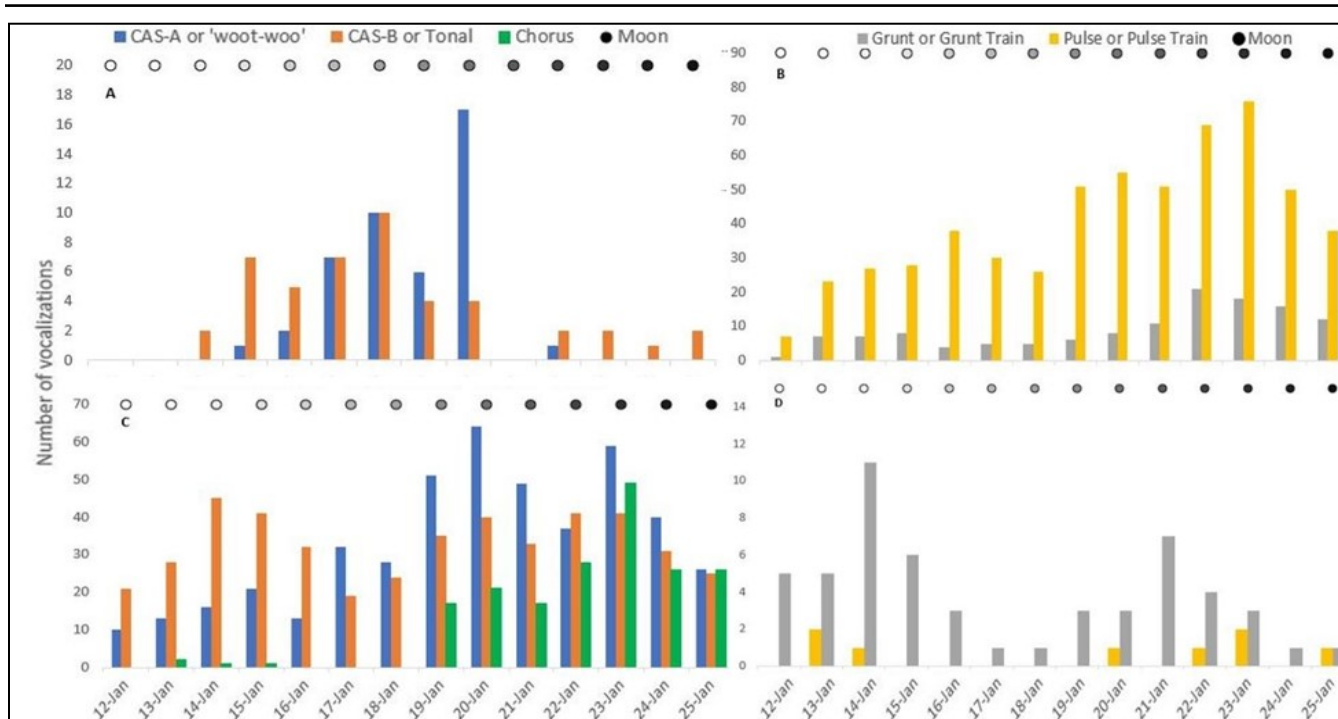


**Figure 1.** Chorus call type from top to bottom, Spectrogram (Kaiser window, fast Fourier transform (FFT) length = 1609 points, overlap = 50%), oscillogram.

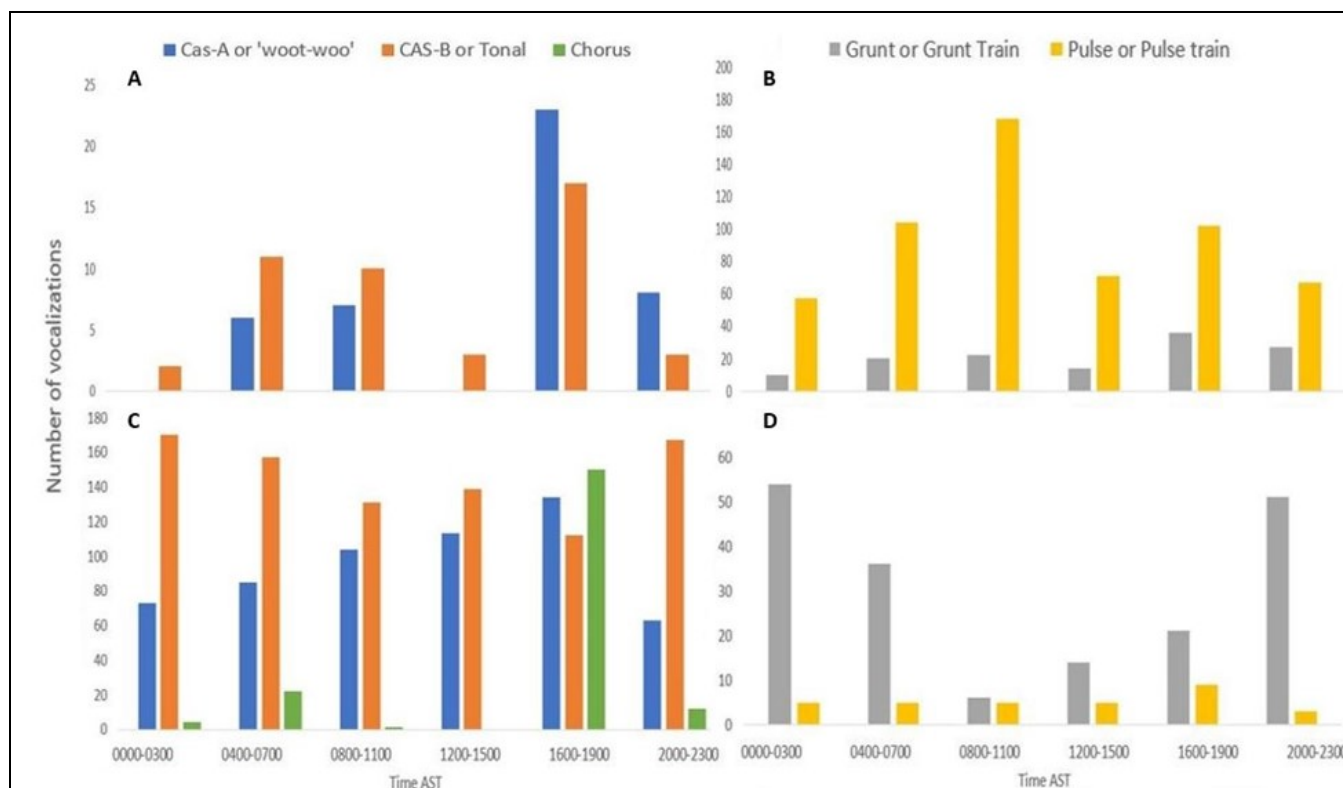


**Figure 2.** CAS-A or 'woot-woot' call type from top to bottom, Spectrogram (Kaiser window, fast Fourier transform (FFT) length = 1609 points, overlap = 50%). Figure 2.B CAS-B or Tonal, 2.C Grut/Grunt train and 2.D Pulse/Pulse Train.

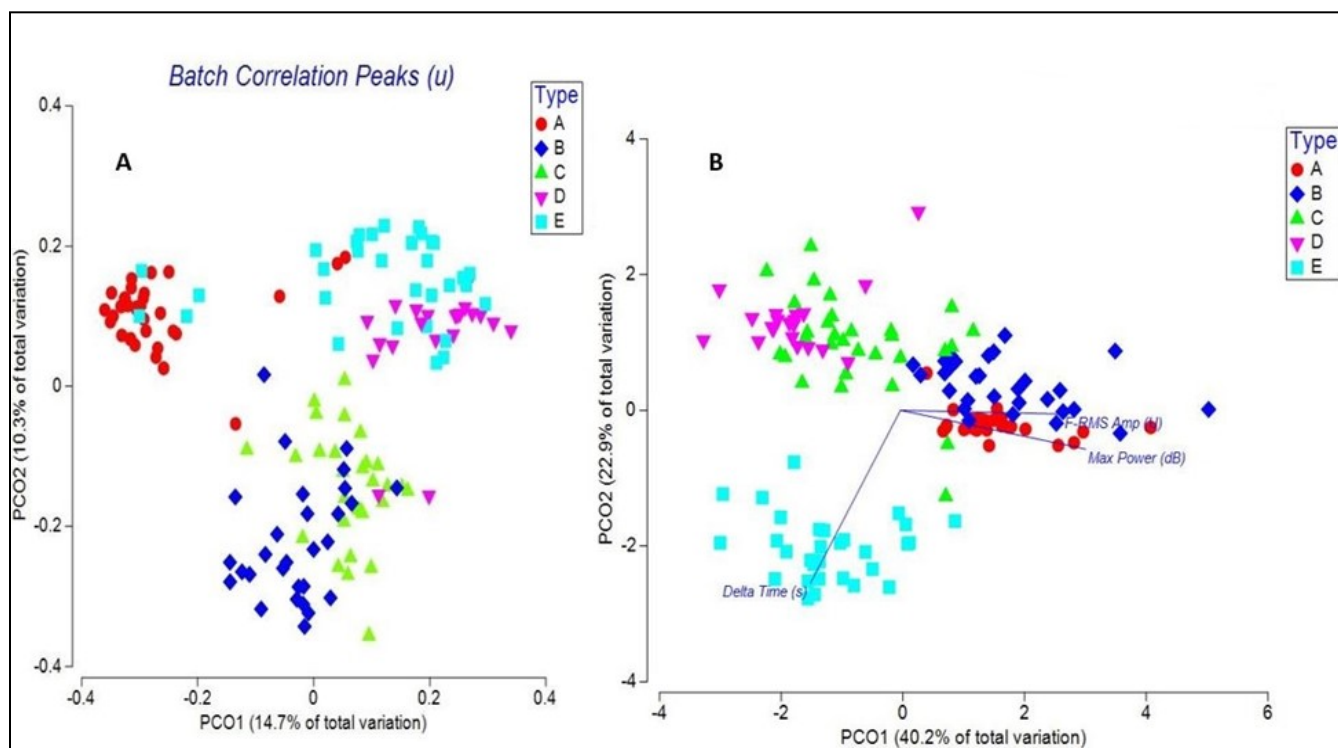




**Figure 3.** Temporal patterns of *Epinephelus guttatus* call types; 3.A Captivity recordings of CAS-A and CAS-B. 3.B Captivity recordings Grunt/Grunt Train and Pulse/Pulse Train. 3.C Field recordings of CAS- A, CAS-B and Chorus. 3.D Field recordings of Grunt/Grunt Train and Pulse/Pulse Train. The moon is represented as the circle on top white=full moon, black= 0.7% illumination.



**Figure 4.** Diel patterns of *Epinephelus guttatus* call types; 4.A Captivity recordings of CAS-A and CAS-B. 4.B Captivity recordings Grunt/Grunt Train and Pulse/Pulse Train 4.C Field recordings of CAS- A, CAS-B and Chorus 4.D Field recordings of Grunt/Grunt Train and Pulse/Pulse Train.



**Figure 5A.** Spectrogram cross correlation-PCO classification of *Epinephelus guttatus* call types. A = CAS-A or 'woot-woot', B = CAS-B or Tonal, C = Grunt/Grunt Train, D = Pulse/Pulse Train, E = Chorus. **Figure 5B.** Parameters-PCO classification of *Epinephelus guttatus* call types. A = CAS-A or 'woot-woot', B = CAS-B or Tonal, C = Grunt/Grunt Train, D = Pulse/Pulse Train, E = Chorus. Vectors which account for 0.7 or more of the correlation between call types in the first two axis are labeled.

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#### SUPPORTING INFORMATION

- SuppInfo\_EGU\_CAS-A
- SuppInfo\_EGU\_CAS-B
- SuppInfo\_EGU\_FRedhind\_Grunt
- SuppInfo\_EGU\_Pulsetrain-CAS-B
- SuppInfo\_EGU\_PulseTrain

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