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RED HIND *EPINEPHELUS GUTTATUS* VOCAL REPERTOIRE CHARACTERIZATION, BEHAVIOR AND TEMPORAL PATTERNS[§]

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ABSTRACT: Passive acoustic monitoring provides a method for studying grouper courtship associated sounds (CAS). For Red Hind (*Epinephelus guttatus*), this approach has documented spatio–temporal patterns in their spawning aggregations. This study described vocalizations produced by *E. guttatus* and their respective behavioral contexts in field and laboratory studies. Five sound types were identified, which included 4 calls recorded in captivity and one sound recorded in the wild, labeled as Chorus. Additionally, the Grunt call type recorded was presumed to be produced by a female. Call types consisted of variations and combinations of low frequency (50–450 Hz) pulses, grunts and tonal sounds in different combinations. Common call types exhibited diel and lunar oscillations during the spawning season, with both field and captive recordings peaking daily at 1800 AST and at 8 days after the full moon.

KEY WORDS: Bioacoustics, courtship associated sounds, passive acoustics, fish communication, spawning aggregation

INTRODUCTION

Red Hind (*Epinephelus guttatus*, Linnaeus, 1758) is a commercially important, mid-sized, long lived, slow growing, protogynous grouper that form transient spawning aggregations (Domeier and Colin 1997). It is one of the most common groupers in the West Indies, and inhabits shallow coral reefs 3–50 m deep from North Carolina, USA (N35°46'55.8) to the central coast of Venezuela (N10°29'16.84). After decreases in landings and overexploitation of the Nassau Grouper (*Epinephelus striatus*) fisheries, Red Hind became the most important commercial grouper landed in places like Puerto Rico (Matos–Caraballo and Sadovy 1990, Sadovy 1993, Matos–Caraballo 1997), United States Virgin Islands (USVI) (Beets and Friedlander 1998), and Bermuda (Luckhurst and Trott 2008). Red Hind females have determinate fecundity, and larger females may spawn more than once a year (Sadovy et al. 1994, Shapiro et al. 1994, Whiteman et al. 2005). Males arrive at the spawning aggregation site before females and establish territories which they defend, and exhibit courtship displays towards females (Colin et al. 1987, Shapiro et al. 1993b). Changes in body coloration occur during behavioral displays, with males exhibiting a lighter coloration anterior ventrally and darker coloration posterior ventrally with a spawning coloration of a barred pattern laterally and barred maxilla (Shapiro et al. 1993a, Ojeda–Serrano 2002). After males establish territories that range 30–40 m in diameter, small groups of 3–5 females associate with each male's patrolled territory (Colin et al. 1987). Within these harem groups spawning occurs a few meters off the bottom at twilight (Colin et al. 1987, Shapiro et al. 1993b). Red Hind males remain at the aggregation site throughout the spawning

season (December–March), while high abundances of females occur in pulses lasting 1–3 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). Migrations toward the spawning site can range up to 33 km (Nemeth et al. 2007). Red Hind exhibit site fidelity at their home range and females inhabit shallow reef areas with overlapping habitat ranges from 112–5,636 m² (Nemeth 2005). The larger Red Hind remain in deeper habitat similar to or near aggregation sites (Nemeth 2005). The number of individuals in spawning aggregations has been estimated to vary from 100 to 80,000 adults, which can occupy an area of 0.015–0.35 km² (Colin et al. 1987, Shapiro et al. 1993b, Beets and Friedlander 1998, Nemeth 2005).

During reproductive periods, Red Hind produce courtship associated sounds (CAS) associated with reproductive behaviors (Mann et al. 2010). Passive acoustics have been used to study Red Hind spawning site usage and temporal patterns of reproductive behaviors in Puerto Rico and the United States Virgin Islands (USVI), (Mann et al. 2010, Rowell et al. 2012, Appeldoorn et al. 2013). The CAS produced by Red Hind are of low frequency (50–450 Hz) and occur during the spawning aggregation, associated with reproductive behaviors and male displays towards males and gravid females (Mann et al. 2010). Visual observations, coupled with passive acoustic recordings have been used to categorize sounds associated with behavioral displays by Nassau Grouper (Schärer et al. 2012a), *Mycteroperca jordani* (Gulf Grouper; Rowell et al. 2019), *Mycteroperca bonaci* (Black Grouper; Schärer et al. 2014), *Mycteroperca venenosa* (Yel-

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lowfin Grouper; Schärer et al. 2012b), *Epinephelus polyphekadion* (Camouflage Grouper; Jublier et al. 2020), *Epinephelus marginatus* (Dusky Grouper; Bertucci et al. 2015) and *Epinephelus morio* (Red Grouper; Nelson et al. 2011), with the latter using lek-like systems of reproduction.

Passive acoustic monitoring of Red Hind has established the temporal periodicity of aggregation formation at different sites (Appeldoorn et al. 2016). Some factors known to affect CAS production and calling patterns, such as the time of day (light-levels) or lunar period could be controlled by standardizing the period (time of day) of analysis. However, other factors that may influence fish behaviors, such as hydrodynamics or number of individuals, need to be studied to determine their effects. Various studies have shown a positive relationship between sound pressure levels (SPL) and abundance estimates during fish spawning aggregations (FSA) for the following species: Red Hind (Rowell et al. 2012), Gulf Corvina (*Cynoscion othonopterus*; Rowell et al. 2017) and Weakfish (*Cynoscion regalis*; Sprague and Luczkovich 2012). However, for Red Hind, the magnitude and shape of this relationship have varied temporally (i.e., over years) and spatially (i.e., across spawning sites; Appeldoorn et al. 2013). Since there are not many characterizations of species CAS involved in aggregations (but, see Rowell et al. 2018), nor determination of whether multiple call types occur and their associated behaviors, estimations of abundance are hindered by the great variability associated with the passive acoustic data. Consequently, the objectives of this study are to: (i) identify the acoustic repertoire of Red Hind, (ii) characterize each call type, (iii) determine the temporal patterns of each call type, and (iv) describe the behavioral context of sounds produced by Red Hind. In addition, the acoustic recordings from an aggregation site in the wild were used to: (v) characterize call types and compare them between wild and captive fishes, and (vi) determine if there is a correlation between temporal patterns of wild and captive sound production.

MATERIALS AND METHODS

Aquarium facility set up and design

The acoustic and reproductive behaviors of captive Red Hind were studied during the spawning season in a land-based facility that consisted of a 5,700 L cylindrical fiberglass tank (3.5 m diameter; Figure 1). The tank was connected to a seawater circulation system at the Magueyes Island Marine Laboratory of the Department of Marine Sciences, University of Puerto Rico, Mayagüez. An open system pumped fresh seawater into the tank at a rate of 475 L/h, and the tank was covered with a 40% light-attenuating shade fabric to protect from the sun without blocking all the moonlight. Inside, the tank was partitioned with 1.5 cm plastic coated wire mesh into a 2 m diameter (3 m²) central staging area surrounded by a 1 m wide, 1.5 m² external area. The central staging area mimicked a home territory with 4 separate artificial habitat structures of coral rubble added to

FIGURE 1. Schematic diagram of the 1,500-gallon cylindrical fiberglass tank set up with a 2 m wide diameter and 1 m height mesh enclosure to observe Red Hind acoustic behaviours. The tank was covered with a shaded cloth. The hydrophone or DSG—Ocean recorder and a Go Pro video camera were located inside the mesh. Smaller fish symbols represent females, larger symbol represents a male. Artificial structure modules are represented by coral figures.

simulate their benthic habitat. The central area isolated one male (larger fish) and a group of females (small, distended abdomen) to replicate a harem, and additional larger fish were added to elicit reproductive behaviors, either inside or outside the central area.

Fish were captured from the wild on 10 January 2017 by scuba divers with hand nets at a known spawning aggregation site off the west coast of Puerto Rico. The tank originally held 3 Red Hind, one male and 2 females, the latter with distended abdomens. Subsequently, one male and one distended abdomen female were added on 20 January 2017. The female was placed inside the central area while the male was outside it. Fish were fed one California market squid (*Doryteuthis opalescens*) each at 0900 AST every 3 days.

The tank was equipped with a recording unit (DSG—Ocean Loggerhead with HTI hydrophone sensitivity of -180 dBV μ 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, to simplify data management. To record video evidence of behaviors, a Go Pro Hero 3 video camera in a waterproof housing was mounted underwater on the side of the tank to maximize the cameras' field of view. During limited periods, from 0900 to 1000 AST, the camera recorded six 10 min videos (59 FPS and audio at 16 bits 480000 Hz) every day over the period of study, as determined by the limits of battery and memory space. This recording period was chosen since higher CAS production occurs a few hours after sunrise and sunset (Mann et al. 2010), and the morning light levels were best for video recording with ambient light. Fish were recorded during sunset on only one day. Tank recordings were used to identify different call types, determine the call type's periodicity, and document behaviors associated with sounds. However, due to noisy recordings, such sounds were not analyzed for the call descriptions but allowed for the identification of the behavioral context of each of the sounds. However, recordings of video and audio files allowed the identi-

fication of the behavioral context of each of the sounds.

Red Hind were kept in captivity during the first lunar cycle of 2017, from 2 days before the full moon (10 January 2017) and 20 days after the winter solstice, to 13 days after the full moon (DAFM) on 25 January 2017. This period encompassed the expected increase, maximum peak, and decline of *E. guttatus* abundances at the spawning aggregation off the west coast of Puerto Rico (Rowell et al. 2012). Fish were acclimated during 2 days prior to the recording period. All observations were made using the same 5 fish.

Tank recordings were made under 3 different scenarios. The first, on 0–7 DAFM, consisted of the initial male and 2 females in the central area. On the 8th DAFM (20 January), a second scenario was implemented with one additional female with distended abdomen introduced into the central staging area, and a second male placed outside of the staging area where it could be sensed through the mesh. During the subsequent 13 days, a third scenario was set up beginning on 9 DAFM, when the second male was placed inside the central area at 0900 AST to simulate another male entering the first male's territory. The first male quickly chased the second male causing him to jump over the mesh boundary into the outer area. Since this happened quickly, the third scenario was repeated for 3 consecutive days to increase the potential of recording sounds produced in this interaction. The second scenario continued during the study period after 8 DAFM and was observed 5 times while the first scenario was observed 8 times.

Field recordings

Acoustic recordings of ambient sounds were made at a Red Hind spawning aggregation site at a depth of 23 m off the west of Puerto Rico (Rowell et al. 2012) from December 2016 through May 2017. Recordings were made with a hydrophone with the same specifications as in captivity except on a different recording schedule (20 s every 5 min) as part of an acoustic monitoring program of grouper spawning aggregations in Puerto Rico (Appeldoorn et al. 2016). All acoustic data were recorded on a memory card. The acoustic data (wav format) were reviewed and high signal to noise ratio (SNR) sounds were extracted matching call types identified in captivity for an in-depth characterization. These data were also used to compare the time series of Red Hind vocalizations from the tank to those recorded simultaneously in the wild.

Temporal analysis

The occurrence of each call type for both wild and captive datasets were summed per time block from 12–25 January 2017. Six 4 h time blocks (0000–0300, 0400–0700, 0800–1100, 1200–1500, 1600–1900 and 2000–2300) were chosen. A χ^2 test was performed to test for daily differences. Data from the recordings in captivity were compared with those from the wild during the same time period assuming a non-normal distribution.

Characterization of sounds

Red Hind sounds were visualized using Raven Pro 1.5 (Bioacoustics Research Program, Cornell Laboratory of Ornithology, Cornell University, Ithaca, NY, USA). Spectrogram param-

eters included a 1609 point Hann window 3 dB bandwidth = 8.9 Hz with 50% overlap and a 2048 point discrete Fourier transform (DFT) window size. Based on previous descriptions of Red Hind sounds by Mann et al. (2010), Mathews (2017), and Wilson et al. (2020), sounds were initially classified into 4 call types: RH1, RH2, Grunt/Grunt train, Pulse/Pulse train. An additional sound recorded in the field and labeled Chorus was described independently. Field recorded sounds with clear structure, low interference, and low background noise (SNR: 10 db) were selected for all analysis. Thirty representative samples of each call type were measured, except for the Pulse/Pulse train, which had 20 representative samples.

The 2 methods for testing similarity among call types are based on 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 Raven Pro 1.5 software that creates a contingency table comparing the spectrograms of each call type pixel by pixel. The second method, parameters based PCO, or parameters-PCO, compared sound based on a set of acoustic parameters, and each was tested for similarity.

Red Hind Chorus events ($n = 38$) were described separately from the other sounds and not included in the parameters-PCO and SPCC-PCO analysis for call types. To quantify chorusing events from field recordings, 3 parameters were established: (1) Sound must be composed of previously recorded CAS (Mann et al. 2010), (2) it has to be continuous in the 50–450 Hz frequency band, and (3) SPL at least 3 standard deviations (13.46 dB) above the mean background levels (30.16 dB). To calculate this threshold, daytime (1500–1700) ambient SPL were measured with Raven Pro 1.5 (same spectrogram parameters described previously) from field recordings during the spawning season. Measurements of ambient SPL excluded the fish chorusing band (50–450 Hz) and any other recording which contained whale calls or boat noise; the fish chorusing band SPL was also measured. Similar thresholds have been employed to quantify ‘fish chorusing events’ of *Cynoscion arenarius* and other fish (Locascio and Mann 2005).

SPCC-PCO

Sound types were cross correlated using Raven Pro 1.5's Batch Correlator function. Sounds were normalized to eliminate effects of amplitude (Charif et al. 2010) and bandpass filtered between 50 and 450 Hz. Thirty representative samples of each call type, except for the Pulse/Pulse train (20 samples) were used for a total of 13,225 comparisons. The resulting similarity matrix was visualized using a principal coordinate analysis (PCO; Cortopassi and Bradbury 2000). One-way analysis of similarities (ANOSIM) was used to test the null hypothesis of no difference between call types. Null hypotheses were constructed using 999 permutations of the raw data. These analyses were done using the PRIMER V7/PERMANOVA add on software (Anderson 2008, Clarke et al. 2014).

Parameters-PCO

Four acoustic parameters that are consistent with previous

TABLE 1. Mean ($\pm$ sd) of acoustic parameters of Red Hind call types and described sounds recorded in the field at a known spawning aggregation site off the west coast of Puerto Rico. SPL—sound pressure level.

Call Type	90% Bandwidth (Hz)	Peak Frequency (Hz)	Duration (s)	Number of pulses
RH1	192 $\pm$ 11	201 $\pm$ 18	1 $\pm$ 0.2	47 $\pm$ 7
RH2	200 $\pm$ 20	172 $\pm$ 32	2 $\pm$ 0.4	162 $\pm$ 34
RH1	218 $\pm$ 29	147 $\pm$ 43	1 $\pm$ 0.2	46 $\pm$ 16
RH1	230 $\pm$ 35	151 $\pm$ 42	4 $\pm$ 1	17 $\pm$ 8
Chorus	Frequency Band (Hz)	Max SPL (dB)		
	50-450	65.59 $\pm$ 5.3		

studies describing sounds produced by Red Hind (Mann et al. 2010, Mathews 2017) were measured using Raven Pro 1.5: total sound duration (s), total number of pulses that make up a call, peak frequency (Hz), and 90% frequency bandwidth (Hz) (Table 1). Frequency parameters were measured on spectrograms within 50–450 Hz frequency band in 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 parameters were measured from oscillograms. These 4 variables were normalized prior to analyses by taking the difference from the mean of each parameter and dividing by the respective standard deviation as they all had different units and ranges. The normalized data was then used to construct a resemblance matrix, which estimated the Euclidian distance among all pairs of observations, and the matrix was visualized by Principal Coordinate Analysis in Primer 7 (Anderson 2008). ANOSIM was used to test the null hypothesis of no difference between call types. Null hypotheses were constructed using 999 permutations of the raw data. These analyses were done using the PRIMER V7/PERMANOVA add on software (Anderson 2008, Clarke et al. 2014).

RESULTS

Four different call types produced by Red Hind were identified from tank recordings: 1) RH1 (courtship associated sound); 2) RH2 (courtship associated sound); 3) Grunt/Grunt Train; and 4) Pulse/Pulse train. Specific behaviors were associated with each call type, and videos presented are those where the male Red Hind was seen on frame and sound was simultaneously recorded. These call types were also recorded at the spawning aggre-

TABLE 2. *R* values resulting from spectrogram cross correlation principal coordinate ordination (SPCC-PCO) for Red Hind call types ($n = 30$ each). n.s. indicates non-significant differences. *R* significance level < 0.001 for all call types.

Call Type	RH1	RH2	Grunt	Pulse
RH2	0.567	X		
Grunt	0.596	0.396 (ns)	X	
Pulse	0.888	0.897	0.528	X

gation site offshore, from which 150 files were isolated and 4 acoustic properties quantified for each (Table 2). One additional sound event that was recorded only in the field was analyzed independently and labeled as Chorus. It is composed of multiple repetitions of call types RH1 and RH2, in an overlapping continuum.

Call types and behavioral context

The RH1 is composed of a short pulse followed by a short tonal sound (Figure 2A and 2C). This call type had the highest mean peak frequency value and had one of the shortest mean durations of all sound types (Table 1). Even though it was acoustically recorded throughout all scenarios, its associated behavior was captured on video once, during scenario 1. As a female was swimming above the bottom of the tank, over a simulated cave, she was slowly approached by the male, who exhibited spawning coloration. The female reacted to this motion by swimming away from the male downwards remaining on the bottom of the tank (see Supplemental Video 1).

The RH2 sound type is composed of a series of pulses followed by an extended tone (Figure 2B and 2D). This call type had the highest SPL values and the highest mean number of pulses (Table 1) and was acoustically recorded during all scenarios. It was video recorded once, during scenario 1 (see Supplemental Video 2) when the male oriented towards a female, exhibiting spawning coloration, slowly approached 2 females that were together, and erected its dorsal fin, turned laterally, displaying laterally and remaining still at the bottom of the tank

FIGURE 2. Spectrograms (above) and oscillograms (below) of Red Hind vocalizations recorded in the field. A. Type RH1 spectrogram. B. Type RH2 spectrogram. C. Type RH1 oscillogram. D. Type RH2 oscillogram.

FIGURE 3. Red Hind Chorus vocalizations in the field. A. Spectrogram. B. Oscillogram.

next to one of the females. The pair then swam at a higher speed in a circular pattern about 50 cm apart; after which they swam in opposite directions. This behavior was also video recorded combined with a pulse train during scenario 3 (see Supplemental Video 3) when a second male was placed inside the central area. The resident male displayed laterally to the second male, changing to a spawning coloration, erecting its dorsal fin and producing the RH2 behavior and then chased the second male into an artificial shelter inside the staging area. Pursuit continued until the second male jumped over the barrier into the outer area of the tank and into an artificial shelter, while exhibiting a dark barred pattern.

The Chorus (Figure 3) is a continuous overlap of calls between the 50 and 450 Hz frequency range, and SPL is at least 3 standard deviations above the ambient sounds (Table 1). This consisted of multiple, overlapping Red Hind RH1 and RH2 type calls. Within the continuous sound band, RH1 and RH2 type calls are sometimes distinguishable, presumably when they produced near the hydrophone. This call type was not recorded in the tank, and no video recordings are available.

Grunt calls can be produced as a single unit (Figure 4A) or a train, consisting mostly of two or three successive grunts (Figure 5A and 5C). This type had the lowest mean peak frequency of all call types and varied in duration (Table 1). This was the second most frequently recorded call in captivity

FIGURE 4. Magnified views of Red Hind single grunt and a single pulse sound types A. Grunt train oscillogram. B. Pulse train oscillogram.

and was present in all scenarios. In one video from scenario 1 (see Supplemental Video 4) and immediately after recording the Grunt call, the second female and the male reacted by swimming towards the first female. The Grunt was recorded by the video camera's audio and the more distant DSG recorder, but a lower sound was perceived in the DSG. Although the camera's audio was not calibrated, the first female was hovering next to the camera, presumably making her the sound producer of that call.

The Pulse call type was observed as a single short pulse (Figure 4B) or as a train (Figure 5B and 5D) of consecutive short pulses and combined with other calls. This call type had the highest 90% frequency bandwidth mean value (Table 1) and was the most commonly recorded call in captivity. Video evidence suggests that this call type is also produced in combination with RH2 as it was acoustically recorded in all scenarios,

FIGURE 5. Two types of Red Hind vocalizations. A. Grunt train spectrogram. B. Pulse train spectrogram. C. Grunt train oscillogram. D. Pulse train oscillogram.

FIGURE 6. Temporal patterns of the number of each Red Hind call type per day on days after the full moon (DAFM) in captivity and in the wild between 12 January 2017 (0 DAFM) and 25 January 2017 (13 DAFM). A. Types RH1 and RH2 in captivity. B. Grunt train and Pulse train in captivity. C. Types RH1, RH2 and Chorus in the wild. D. Grunt train and Pulse train in the wild.

and behaviors were recorded on video during scenarios 1 (see Supplemental Video 5) and 3 (see Supplemental Video 3), the latter accompanied with RH2 and described above. During scenario 1 the resident male approached both females, slowly turned its side to one of the females, and produced the call as the female swam under the artificial shelter. Pulse trains observed during displays towards females in scenario 1 were not accompanied by other call types (see Supplemental Video 5).

Temporal Patterns

Frequency distributions of daily sounds by call type in the wild from 0 to 13 DAFM (12–25 January) showed a peak (highest amount) in the Chorus sound on 11 DAFM (Figure 6D). Type RH1 was highest 8 DAFM both in captivity and the wild, and an increasing trend was evident prior to that day (Figure 6A and 6C). In captivity, RH1 abruptly decreased to zero by 9 DAFM (Figure 6A), while the decline was more gradual and never reached zero in the wild (Figure 6C). Additionally, there was a noticeable shift in the most abundant call type from RH2 to RH1 in both data sets. In captivity RH2 was highest on 6 DAFM, but in the wild it exhibited a bimodal pattern, with maximum values on 2 and 11 DAFM. The Grunt type was highest 10 DAFM in captivity (Figure 6B) and 2 DAFM in the

wild (Figure 6D) where it also showed a bimodal pattern, with the second maximum at 9 DAFM. In captivity Pulse trains were much more common than Grunts, whereas in the wild the reverse pattern was observed (Figure 6B and 6D). Finally, Pulses were highest 11 DAFM in captivity, but only a few such calls were detected in the wild, which precluded detecting any temporal pattern, and no Pulses were detected during the period 3–7 DAFM.

The diel frequency distributions recorded in the wild demonstrated that 2 call types RH1 ($X^2_{572} = 37.06$, $p < 0.05$), Grunts ($X^2_{182} = 64.79$, $p < 0.05$) and the Chorus ($X^2_{189} = 545.76$, $p < 0.05$), were not evenly distributed throughout a 24 h cycle (Figure 7). The Chorus and RH1 peaked during the 1600–1900 AST block (Figure 7C). The Grunt call was rare and detected only twice and always at night. The RH2 ($X^2_{876} = 17.58$, $p < 0.05$) and Pulse train ($X^2_{32} = 3.63$, $p < 0.05$) showed an unevenly distributed pattern, although RH2 was frequently heard at night (Figure 7C and 7D). For the recordings conducted in captivity (Figure 7A and 7B) only 2 types, RH1 ($X^2_{44} = 48.45$, $p < 0.05$)

FIGURE 7. Diel patterns of the number of Red Hind calls per type in 4 h time blocks (AST) as recorded in captivity (top) and in the wild (bottom) between 12–25 January 2017. A. Types RH1 and RH2 in captivity. B. Grunt train and Pulse train in captivity. C. Types RH1, RH2 and Chorus in the wild. D. Types Grunt train and Pulse train in the wild.

FIGURE 8. Two Principal coordinate ordinations for classification of 4 *Epinephelus guttatus* call types. A. A spectrogram cross correlation-principal coordinate ordination (SPCC-PCO). B. A parameter based principal coordinate ordination (Parameters-PCO)

and Pulse train ($X^2_{569} = 87.13$ $p < 0.05$) were unevenly distributed mostly during the 1600–1900 AST and 0800–1100 AST time blocks, respectively (Figure 7A and 7B).

Comparison of call types

Spectrographic cross-correlation and PCO analysis (SPCC-PCO) of the different vocalization types showed a clear separation among all types (Figure 8A). One-way ANOSIM showed significant differences among all call types except Grunt from RH2 (Global test $R = 0.575$, $p = 0.001$, Table 2). Parameter-

TABLE 3. R values resulting from Parameters-principal coordinates ordination for 4 Red Hind call types ($n = 30$ each). n.s. indicates non-significant differences. R significance level < 0.001 for all call types.

Call Type	RH1	RH2	Grunt	Pulse
RH2	0.811	X		
Grunt	0.495 (ns)	0.641	X	
Pulse	0.847	0.847	0.531	X

PCO (Figure 8B) showed 3 distinct groups. One-way ANOSIM showed significant differences among all call types (Global test $R = 0.665$, $p = 0.001$, Table 3) except Grunt from RH1. RH1 and Pulse were separated over the first axis of ordination, mainly due to 90% Frequency bandwidth and duration, showing a sequential/gradual change between RH1, Grunt and Pulse. On the second axes of ordination the parameter which separated the call types was the number of pulses. Parameters with a correlation > 0.7 , which explains most of the variability, were 90% frequency bandwidth, number of pulses and duration. Peak frequency had correlations < 0.5 in the first and second axis of ordination. However, in the third axis, which accounts for 21.8%

of the correlation, peak frequency represented the highest correlation value. The first and second axes account for 37.1% and 27.4 % of the correlation, respectively.

DISCUSSION

Our results demonstrated the ability of Red Hind to produce distinct types of sounds, and that fish sound production in simulated natural conditions is possible. The detection, quantification, and characterization of the acoustic parameters of multiple call types, and the observation of associated behaviors provided key information for the advancement of passive acoustics research in the reproduction of Red Hind. Recordings of similar sounds made simultaneously in the wild and in captivity suggest that the reproductive behaviors observed in captivity are likely triggered among individuals, showing previously unknown interactions among social groups of this species. In addition, descriptions of temporal patterns provided important observations to propose

that different Red Hind types of CAS are produced at different times during the aggregation period and at different times during the day.

This study demonstrates that Red Hind has the capability of producing at least 4 call types, of which 2, RH1 and RH2, were similar in structure to the “woot-woo” previously described by Mann et al. (2010). However, it is evident that 2 distinct call types were present, and RH2 has an extended tonal component, which was also suggested by Wilson et al. (2020). The Grunt/Grunt train is similar to the ‘grunts’ that were recorded by Fish and Mowbray (1970) during feeding times in captivity, but this is the first suggestion that this sound may be produced by a female. It was previously speculated that only males were sound-capable (Mann et al. 2010), and although sounds recorded in captivity were presumed to have been generated mostly by males, the video recording of a Grunt train is presumed to be produced by a female. All call types were recorded prior to the introduction of a second male into the tank (scenario 2). Since the second male remained very still near the artificial structure provided, displaying a darker coloration pattern, we assumed that the first male made most of the calls associated with displays towards females as he was swimming more actively than the second male. However, to confirm this observation, additional captive work is needed due to the limited observations of this study.

The females kept in captivity had abdomens that remained distended throughout the experiment, and no eggs were observed in the tank. Since no spawning was detected in captivity it remains unknown if any sounds are produced by Red Hind during gamete release, although this has been demonstrated for the Gulf Grouper (Rowell et al. 2019). In *Atractoscion nobilis* (White Seabass) there is also an association between sound production (drumrolls and thuds) and spawning (Aalbers and

Drawbridge 2008). Furthermore, calls for courtship and spawning have been differentiated for *Dascyllus albisella* (Hawaiian dascyllus) (Lobel 1992, Mann and Lobel 1995). However, video recorded observations of Red Hind spawning are non-existent, and it has only been described as observed in situ on 2 occasions. In both cases spawning occurred near sunset and about 0.5 m above the seafloor in the water column, when one female left the bottom and was joined by a male, and gametes were released without any upward rush or rapid movement (Colin et al. 1987). No acoustic signals during this behavior were reported in these studies, yet a similar behavior was observed in captivity during a male–female interaction which included the RH1 sound (see Supplemental Video1).

It is important to understand the behavioral context of these different call types in order to differentiate the complex interactions that occur in lek-like mating systems like that of Red Hind. By knowing the context of the associated behaviors, passive acoustic data can be used to detect their presence and focus sampling efforts to study FSA dynamics more efficiently. The association of 4 call types produced by Red Hind with different displays suggests that both courtship and territoriality could be responsible for the variability observed in the bioacoustic recordings of spawning aggregations. Vocalizations involving interactions among individuals could be important for establishing dominance in complex social structures, with obvious implications in mate choice and spawning success (Donaldson 2018). For example, *Pollymirus isidori*, the Elephant 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). Additionally, in *Hypoplectrus unicolor* (Butter Hamlet) sounds are produced just prior to or simultaneously with gamete release, which may facilitate synchronous gamete release (Lobel 1992), while in *Padogobius martensii* (Common Goby) and *Knipowitschia punctatissima* (Panzarolo Goby) spawning vocalizations have been shown to be modified before and during oviposition (Lugli et al. 1995). In Lusitanian Toadfish (*Halobatrachus didactylus*) call rate and effort strongly represented male size and condition (Amorim et al. 2010).

Vocalizations may serve similar functions within Red Hind aggregations and provide a cue to synchronize spawning, or to stimulate sex change in females (Shapiro et al. 1993a). Sound type RH2 were recorded during male–male and male–female interactions. 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 to male, the sounds included Pulse trains and the fish acted aggressively. If this call type is related to dominance or competition, then we can propose that the rate at which this interaction is observed during the spawning aggregation could also reflect changes in the sex ratio as females migrate through the aggregation site (Nemeth et al. 2007). Likewise, the Red Hind Grunt could be classified as an alarm call, and when produced by both males and females could be related to defensive interactions from predators or as agonistic towards competitors

for mates, similar to those described in Nassau Grouper (Rowell et al. 2018). This call type was more abundant in captivity than in the field, but in the field daily differences in call counts were larger, possibly due to a higher rate of interactions with conspecifics or other species in the wild. Additionally, differences in background sound levels may explain the differing abundances, since a stationary hydrophone will emphasize vocalizations produced nearer.

In the field, both RH2 and Pulse trains were not evenly distributed across the diel cycle, despite a general increase in density. This random pattern could be explained by the fact that RH2 can be accompanied by Pulse trains making it a call with more than one purpose (e.g., territory defense and/or courtship display), as has been shown to be the case for ‘boatwhistles’ of the Lusitanian Toadfish (Vasconcelos et al. 2010) and for the spawning vocalizations of Common Goby (Lugli et al. 1995). In the case of the Pulse train, a random pattern could result when fish produce quieter sounds as they move through the territories to set up at the aggregation site prior to spawning (Shapiro et al. 1993b, Nemeth et al. 2007). However, the hydrophone will only detect the sounds produced within a limited distance (~100 meters), hence this vocalization type was recorded more in captivity due to limited area to roam when compared to the wild.

Overall, the acoustic patterns observed in captivity followed diel and lunar patterns within spawning aggregations as those described by Mann et al. (2010) and Rowell et al. (2012) 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 2 call types, RH1 and the sound identified as Chorus. This suggests that RH1 and chorusing events are key to spawning. They could help stimulate hydration prior to gamete release or lead to synchronized spawning (Crews et al. 1985, 1986) as well as pre-spawning movements (Colin et al. 1987). The rate of RH1 production peaked at the same time during the lunar (8 DAFM) and diel (1800 AST) patterns for both wild and captive conditions, although with 2 notable differences. First, the number of individuals in the tanks was constant until 8 DAFM, whereas at the aggregation site the density was unknown, but expected to increase, particularly when females arrive at the aggregation site (Nemeth et al. 2007, Rowell et al. 2012). Secondly, there were no changes in water temperature or flow in the tank, whereas strong tidal currents and thermoclines have been observed at the spawning aggregation site. It has been proposed that changes in temperature, current flow and direction may be important factors affecting spawning (Appeldoorn et al. 2016).

The fact that the natural RH1 temporal patterns were reproduced in the tank is important as it was believed 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 and SPL could be used as an indicator of relative abundance (Mann et al. 2010, Rowell et al. 2012). However, Appeldoorn et al. (2013) found that the relationship between sound levels and abundance was not consistent across

sites or years, with peak days of calling being similar despite differences in density. The tank recordings further indicate the proposed relationship between the amount of calls per time unit and density but were not sufficient to explain this variation. In this study, chorusing activity was mostly restricted to the 1600–1900–time block as reported for CAS activity for Red Hind (Mann et al. 2010, Rowell et al. 2012, Appeldoorn et al. 2013). However, daily changes in chorusing activity and SPL within this time block (4 h) must be accounted for if SPL are to be used as indicators of abundance. Therefore, if chorusing activity corresponds to abundance or density and peak chorusing activity occurs at different times (hour) of the day within a spawning season, SPL measurement must be corrected to the time when peak chorusing activity occurs and not fixed to a specific hour. Additionally, chorusing events may mask individual call types, thus hindering their quantification.

Other studies of fish calling rates, such as for 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 1995, Jordão et al. 2012). However, grouper spawning aggregations usually occur at shelf edges and in situ observations are limited by individual fish movements and challenges inherent to environmental conditions. Thus, the observations of specific behaviors in captive conditions and the recognition of call types provides promise for studies in captive conditions and simultaneous field studies (Shapiro et al. 1993b, Montie 2010, Nemeth et al. 2007). This study suggests that for species that exhibit a lek-like mating system such as Red Hind and where tracking an individual's courtship and sonic behavior over long periods of time is extremely challenging, reproductive behaviors can be studied in captivity, thus overcoming some of the limitations of using inferential statistics to compare and characterize sounds produced by fish in the wild.

In this study we sought acoustic parameters that explained

the differences among vocalization types quantitatively. From the chosen parameters, 90% frequency bandwidth and call duration had the highest correlations (> 0.7) in the first axis of ordination which indicates this to be adequate parameters for comparison. Similarly, number of pulses and peak frequency may be differentiating parameters as they showed correlations of over 0.7 in the second and third axes of ordination, respectively. This result suggests that there is variation in peak frequency and this parameter may be used to study dialects and comparisons among species.

In comparison the SPCC–PCO was able to differentiate between the call types but did not provide information of what were possible causes for those differences. The Parameters–PCO also differentiated between call types and provided us the specific characteristics that make up those differences. However more parameters should be tested for further characterization. Parameters like interpulse interval and pulse duration could be useful to further differentiate between sounds like the Pulse train and Grunt train. Other parameters such as amplitude and power (dB) estimated levels must be corrected for sound attenuation and distance due to physical and hydrodynamic properties of spawning sites where these sounds occur. The acoustic calibration of Red Hind spawning aggregations could be especially important for the description of chorusing events and its relationship with abundance.

To summarize, the present study has demonstrated that (i) Red Hind courtship behavior can be studied in captivity, (ii) this grouper species has a richer vocal repertoire than previously known, (iii) females may be sound capable and (iv) call duration, number of pulses, 90% bandwidth and peak frequency are useful parameters to differentiate call types. With this acoustic characterization, future studies can focus on the function, the patterns of spatial and temporal variation, and the variability of call parameters among aggregation sites throughout the Red Hind's range to better understand the role sounds play in spawning aggregations.

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