Temporal Patterns Among Multiple Courtship Associated Sounds in the Red Hind *Epinephelus guttatus* Indicate Two Spawning Aggregations During a Single Lunar Cycle

Patrones Temporales Entre Múltiples Sonidos Asociados al Cortejo en el Mero Cabrilla *Epinephelus guttatus* Indican Dos Agregaciones de Desove Durante Un Ciclo Lunar

Les Schémas Temporels Parmi les Multiples sons Associés à la Parade Nuptiale dans le Mérou *Epinephelus guttatus* Indiquent Deux Regroupements de Géniteurs au Cours d'un Cycle Lunaire

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EXTENDED ABSTRACT

Introduction

Red hind (*Epinephelus guttatus)* form transient spawning aggregations that follow a lunar cycle (Colin et al. 1987, Shapiro et al. 1993, Sadovy et al. 1994). Passive acoustic monitoring has shown to be a reliable indicator of this temporal pattern in reproductive activity (Appeldoorn et al. 2015, Mann et al. 2010, Rowell et al. 2012). Long-term passive acoustic monitoring and surveys of red hind abundances at Abrir la Sierra (ALS), Puerto Rico have shown that peak aggregations consistently occur 7-10 days after full moon (DAFM). However, acoustic records revealed occasional, extended periods of increased rates of sound production prior to the expected aggregation period. Red hind calls have been previously described as a series of pulses of variable repetition rate with a minimum and maximum frequency of approximately 130Hz and 250Hz respectively (Mann et al. 2010, Rowell et al. 2012). Sounds are associated with courtship, mating or territorial behavior and are mostly produced during the aggregations associated to reproduction (Mann et al. 2010). Recently, Zayas et al. (unpublished manuscript) have characterized red hind sounds into multiple types ("A", "B", "A+B", "Other" and "Chorusing"). We analyzed calling behavior to establish temporal patterns by signal type during the lunar spawning cycle. Once this was established, we applied the same analysis to the extended periods of calling activity and used these patterns to infer behavior.

Methods

Recordings were obtained from an underwater passive acoustic recorder (LS-1 DSG, Loggerhead Instruments) scheduled to record low frequency ambient sounds during 20 sec every 5 min. The unit was deployed yearly at ALS, a well-studied *E. guttatus* spawning aggregation site located on the western shelf edge of Puerto Rico, and recorded continuously during the entire spawning season (December-March). Daily mean sound pressure levels (100Hz-200Hz band level, dB [re] 1 µPa) generated from the passive acoustic data using DSGLab for MATLAB (The Mathlab) were used to quickly identify peaks of sound levels in the frequency used by red hind. Periods of higher than background sound pressure levels have shown to be produced by red hind calling activity and directly related to population density (Rowell et al. 2012). Therefore, peaks in sound production (100 - 110 dB [re] 1 µPa) indicate when reproductive behaviors increased in occurrence. The timing of these peaks in sound levels was used to identify the lunar periodicity of red hind reproductive behavior. Manual analysis of the selected period of recordings were performed individually for each file using Audacity (ver. 2.2.2). Both auditory and visual spectrogram (Hanning window, 1024 window size) identifications were necessary to confirm the presence of a call type. Only recordings from 18:00 - 19:00 AST were analyzed as that time period that includes sunset is known to be the timing of most acoustic activity during the day, the time of observed spawning activity and less interference from vessels or divers (Colin et al. 1987, Mann et al. 2010a, Rowell et al. 2012). For each day, the presence/absence of each of four distinct call types (A, B, B-extended and A+B) was recorded for each individual 20 sec. file, for a total of 12 files per hour.

Results

From the sound levels analysis, the January and February 2015 lunar cycles were selected to quantify baseline call patterns. Similarly, the patterns of peak sound production indicated the January 2013 lunar cycle had unexpected periods of higher than background sound levels that merited further analysis. During the period of higher than background sound production for a typical lunar cycle (January & February, 2015), B-type calls were most abundant from 0-6 DAFM - presumably the onset of the aggregation. This call decreased in abundance sharply and A-type calls increased from 7 - 12 DAFM, during the presumed spawning peak. During this latter time period, chorusing, indicative of high calling activity,

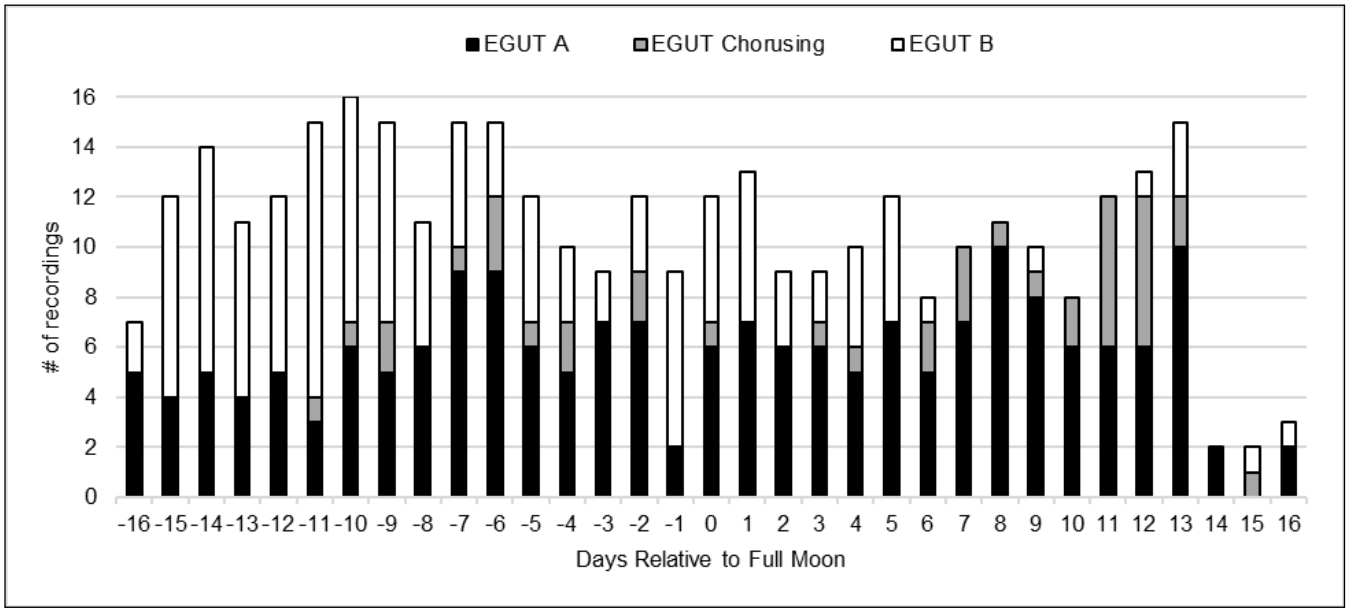

Figure 1. Presence of *E. guttatus* call types from 18:00-19:00AST (12 recordings per hour) during one lunar cycle (January 11-February 13, 2013) at ALS. Peaks in sound levels and distended females have been repeatedly observed (Rowell et al. 2012) at ALS 7-10 days after the full moon, when call A is the predominant type in our analysis. This pattern, accompanied with a higher presence of B calls, was also observed at 6-8 days before the full moon, suggesting that a similar reproductive behavior may have occurred during this time.

also increased. When the extended calling period was analyzed (Figure 1), the same pattern observed previously was repeated, with the increase in in A-type calls occurring 14 days prior to the normal expected lunar peak.

Discussion

Our results suggest that two spawning events could have occurred during one lunar cycle. This pattern of extended periods of red hind calling activity have been identified in at least four lunar cycles over 8 years of recordings at ALS (2007, 2013, 2014, 2016) indicating that this pattern may be common. Interestingly, the extended calling period in 2016 at ALS was not observed at the Buoy 4 red hind aggregation site, 9 km to the south. Underwater visual surveys during these periods are necessary to determine red hind behavior, density and indications of possible spawning. Work by Nemeth et al. (2006) in the U.S. Virgin Islands found that primary spawning months of red hind could be predicted based on the timing of the January full moon relative to the number of days after the winter solstice and their model was found to apply to the spawning aggregation at ALS using passive acoustic data (Appeldoorn et al. 2015). However, there is currently no way of predicting when periods of extended calling activity will occur. Based on these findings, further examination of the timing of periods of extended calling activity may reveal patterns related to celestial cycles. The 14-day offset further suggests that the two reproductive events could have occurred under similar conditions of current speed and direction, but that lunar light levels would be different. Previous work suggested that the most favorable day for spawning occurs when sunset (18:00AST -19:00AST) coincides with the lowest current speed and a change in the direction of flow towards off-shelf induced by tides (Shekhar & Cherubin unpublished report, Appeldoorn et al. 2015). Red hind spawning and reproductive activity in St. Thomas was also found to occur during rapid declines in current speed and water temperature (around full moon) relative to other lunar phases (Nemeth et al. 2006). Determination of the exact relationship between tides, currents and spawning activity would require the use of on-site current meters (ADCP) throughout multiple spawning seasons. Although the behavioral context of each call type produced by red hind has not been determined, our results suggest that B-type calls may be used for territorial defense, since males are first to arrive at a spawning site (Whiteman et al. 2005). As the peak of spawning approaches, females arrive and males display towards or compete for females, forming harems of typically 1 to 5 females (Colin et al. 1987). In our results, this time period coincides with the dominance of A-type calls, which suggests this call type could be involved with courtship displays, mate attraction, egg hydration or synchronization for spawning rushes (Lobel et al. 2010). Additional studies are needed to identify the specific behavior associated with each call type to further explain the temporal patterns in red hind calling behavior. This could potentially allow the examination of the behaviors and temporal dynamics of spawning aggregations to develop additional applications of passive acoustics towards the research, management and conservation of the critical habitats used for reproduction (Scharer et al. 2012).

KEYWORDS: Red Hind, *Epinephelus guttatus*, spawning aggregations

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