

**HIGH RESOLUTION TEMPORAL PATTERNS OF BLACK GROUPER,  
*MYCTEROPERCA BONACI*, COURTSHIP BIOACOUSTICS AT  
SPAWNING AGGREGATIONS IN THE GREATER CARIBBEAN**

By  
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## Abstract

Black grouper, *Mycteroperca bonaci*, are large protogynous hermaphrodites in the Family Epinephelidae. In the Caribbean, Gulf of Mexico and South Florida they form transient seasonal spawning aggregations in the winter and spring, with spawning timing influenced by lunar and diel periodicities. Two small aggregations have recently been identified off the west coast of Puerto Rico, one at Bajo de Sico, an isolated seamount, and a second off the coast of Mona Island. Passive acoustic recorders were set at the two sites and a third at Riley's Hump in the Dry Tortugas. Black grouper courtship associated call (CAS) time-series were compared to lunar and solar periodicities, water temperature and tidal stage. The populations showed similar temporal patterns at the three spawning aggregations. Spawning season is strongly correlated with water temperature. Within the spawning season, CAS production is significantly influenced by lunar and solar periodicities. Production peaked between the last quarter and new moons during evening hours. The data suggests a potential correlation with tidal stage. Temporal patterns were consistent for three consecutive years at Mona Island. Temporal patterns were consistent between the two larger and geographically isolated aggregations, Mona Island and Riley's Hump. Similar geomorphologies of these three aggregation sites indicate specific physical characteristics can be used to help in aggregation identification. Spawning aggregations were less than 150m from a promontory along the shelf edge at 25-35m depth and near deep water (>100m).

## Resumen

El mero negro, *Mycteroperca bonaci*, es considerado como un pez marino grande que pertenece a la Familia Epinephelidae. Este mismo, se caracteriza como un pez hermafrodita protógina. En el Caribe, Golfo de México, y el Sur de Florida, estos forman agregaciones transitorias de desove en el invierno y la primavera. El tiempo de desove es influenciado por las periodicidades lunares y solares. Dos agregaciones pequeñas de *M. bonaci* han sido recientemente descubiertas en las aguas al oeste de la Isla de Puerto Rico. La primera se encuentra en una montaña submarina, Bajo de Sico, y el segundo a la Isla de Mona. Grabadoras acústicas pasivas fueron posicionadas en los dos sitios y una tercera agregación en Riley's Hump en las Dry Tortugas. La temporada de desove tuvo una fuerte correlación con la temperatura del agua. Las llamadas de apareamiento (CAS por sus siglas en inglés) asociadas con el mero negro estuvieron significativamente influenciadas por los ciclos periodicidades lunares y diarios. La producción mayor fue observada durante el menguante cuarto y la nueva luna, en las últimas horas de la tarde. Los resultados sugieren una posible correlación con los niveles de la marea. En la Isla de Mona, los patrones temporales se mantuvieron consistentes por tres años consecutivos. Los patrones temporales en la Isla de Mona y Riley's Hump también se mantuvieron consistentes. La similitud geográfica entre estos tres sitios de agregación indica un patrón específico fisiológico que se pudiera usar para identificar agregaciones del mero negro. Agregaciones de desove se mantuvieron cerca de un promontorio en el borde de la plataforma a una profundidad de 25-35 m cerca de aguas más profundas aguas (> 100 m).

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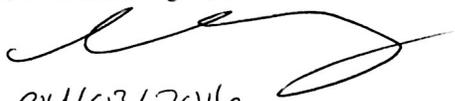
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## Introduction

Many commercially important, western Atlantic reef fishes reproduce in transient fish spawning aggregations (FSAs) (Domeier and Colin 1997). Transient FSAs form seasonally and can last anywhere from a single spawning event to a few months duration (Freitas et al., 2011). They can consist of tens to thousands of individuals (Domeier and Colin 1997). Migration distances vary by species but transient FSAs are composed of the reproductive individuals within a catchment area (Nemeth 2012). Most large, western Atlantic groupers (F. Epinephelidae) form site-specific transient FSAs. Formation is predictable in both time and space making them vulnerable to intense fishery pressure (Brule et al., 2003; Eklund et al., 2000). As a consequence a large percentage of annual catches of grouper species have historically occurred at FSAs (Claydon 2004). Due to this reproductive strategy combined with specific life history characteristics and high fishing pressure, grouper populations are decreasing throughout their ranges (Matos-Caraballo 1997).

Black groupers, *Mycteroperca bonaci*, are large, protogynous hermaphrodites. All black groupers initially mature as females. Large females undergo a sexual transition. Along the North American continental shelf, black groupers reach 50% maturity around 750-800mm TL and 50% sexual inversion around 1000-1100mm TL (Crabtree and Bullock 1998; Brule et al., 2003). By targeting the largest individuals in a population, female to male ratios become more pronounced in protogynous hermaphrodites. Black grouper sex ratios have been documented as 30:1 and higher in some populations (Crabtree and Bullock, 1998; García-Cagide and García 1996). Work on grouper populations in Brazil and Cuba identified black grouper sex inversion at much smaller sizes (García-Cagide and García 1996; Teixeira et al., 2004). This suggests potential social control on sex reversal which would not be unique to black groupers within the

family Epinephelidae (Liu and Sadovy 2004). Social cues can stimulate earlier than normal sex transitions in some reef fish (Munoz and Warner 2003; Warner and Swearer 1991).

Black groupers are reproductively active year round but the majority of their reproductive effort occurs during the winter/spring months in the Greater Caribbean. Spawning peaks occur from January to March in the northern hemisphere tropics (Brule et al., 2003; Crabtree and Bullock 1998; Koch et al., 2011; Locascio and Burton 2016; Paz and Sedberry 2008; Schärer et al., 2014), while farther north in Bermuda spawning aggregations form June through August (Luckhurst 2010). In the southern hemisphere black grouper spawning aggregations are documented from July to September (Teixeira et al., 2004), the southern hemisphere winter, when water temperatures are comparable. During these periods black grouper exhibit peak gonadal somatic indices (GSIs), oocytes in vitellogenic stages, and decreased body fat content in the body cavity (García-Cagide and García 1996; Teixeira et al., 2004).

Within the reproductive season, specific times of spawning activities correlate with lunar and diel periodicities (Luckhurst 2010; Paz and Sedberry 2008; Schärer et al., 2014). Aggregations increase in size during dusk hours when the only direct observations of gamete release have occurred (Sala et al., 2001; Paz and Sedberry 2008). Lunar periodicity shows more variation, but consistently correlates with days between the full moon and new moon (Brule et al., 2003; Luckhurst 2010; Paz and Sedberry 2008; Schärer et al., 2014). Few suggestions of controlling factors have been given for black grouper spawning timing, with the notable exception of a consistent temperature range during FSA formation (Luckhurst 2010; Paz and Sedberry 2008). However more in depth analyses have been given for other grouper species with similar timing. In the Cayman Islands at a Nassau grouper (*Epinephelus striatus*) FSA drifters were retained locally when released on the night of spawning. Drifters were not retained

in previous nights when courtship was observed prior to the night of spawning (Heppell et al., 2008). In western Puerto Rico red hind (*Epinephelus guttatus*) FSAs aggregate at different times even when located within 12 km on the same shelf edge, suggesting local conditions strongly affect FSA dynamics (Appeldoorn et al., 2016).

While UVC surveys and visual observations can be used to describe spawning behavior at an FSA, the overall potential is limited to the time that divers are in the water. Data collection is therefore subjected to sea conditions, funding for daily dive trips, and light availability (Mellinger et al., 2007). This can be problematic due to the offshore nature of most described black grouper FSAs, including Mona Island and Bajo de Sico located off the west coast of Puerto Rico. Combined with the highly skittish behavior of black grouper (Paz and Sedberry 2008) and mesophotic habitat use among Caribbean islands (author pers. obs.), diver collected data can be limiting in its scope.

Utilizing an autonomous passive acoustics recording methodology, many limitations associated with traditional fish surveys can be overcome (Luzkovitch et al., 2008). Initially applied to terrestrial and marine mammal bioacoustics, recent work has led to the development of passive acoustics monitoring among teleosts, especially serranids (Locascio and Burton, 2016; Mann et al., 2010; Rowell et al., 2011; Schärer et al., 2012; Scharer et al., 2014). Passive acoustics has the potential for long-term in situ monitoring at sites that would otherwise not be accessible. Multiple locations can be simultaneously monitored under identical recording schedules. Comparable data sets can be developed annually. When combined with density and movement data on a local scale, population dynamics (population size, density, competition, movement, etc.) of FSAs can be elucidated (Marques et al., 2011; Rowell et al., 2015). High-resolution acoustic time-series combined with current data collection methods will greatly

increase our knowledge of soniferous fish behaviors at FSAs. The information has the potential to influence more effective management policies to promote increased success of spawning events.

The purpose of this study was to develop finer resolution spatio-temporal patterns of black grouper spawning behavior than currently are available. This work expands upon the only two bioacoustics analyses of black grouper FSAs to date (Locascio and Burton 2016; Schärer et al., 2014). This is the first investigation specifically investigating black grouper behavioral patterns inter-annually at individual sites and intra-annually across multiple sites. Currently considered near threatened with a predicted 30% decrease in population over their range (Ferreira et al., 2008), black grouper FSAs are in significant need of protection to restore a productive and sustainable fishery. Effective management policies require an in depth understanding of reproductive behavior. This study provides the highest resolution courtship patterns currently available at black grouper FSAs. The objectives are as follows:

Objective 1: To utilize high-resolution acoustic time-series to study the interannual variability of temporal patterns at a single known black grouper FSA and intra-annual variability between two geographically separate FSAs.

Objective 2: To correlate these temporal patterns with water temperatures and lunar and diel periodicities.

Objective 3: To characterize the geomorphology of the known black grouper sites to test if site morphometric parameters are consistent with predictions derived from other known sites.

## **Methods**

### *Sites*

Bajo de Sico (BDS) (Figure 1) is a seamount off of the western insular shelf of Puerto Rico. Located approximately 27km west of Mayaguez, Puerto Rico, it is connected to the insular shelf via a deep, narrow corridor. BDS is a seasonal no-take zone for Caribbean Fishery Management Council (CFMC) regulated reef fishes from October 1 to March 31. While no bottom fishing or spearfishing is allowed during the closed season, fishing for highly migratory species is. Bottom jigging on top of the local FSA has been observed during closed season (author pers. obs.), suggesting the seasonal moratorium is not fully enforced. BDS is in an isolated location far removed from local enforcement sources, both on the mainland and at Isla Mona. The seamount is 3,119-hectares. The seamount supports hermatypic corals between 40 and 90m depths with large vertical drop-offs along the western and northwestern edges. The main structure runs from the northwest to southeast rising up to 30-m depth. A smaller pinnacle rises just south across a 60-m depth channel. Multiple DSGs were deployed at BDS to record acoustic behavior of the redhind and Nassau grouper. Preliminary analysis of DSG recordings indicated large increases in black grouper courtship acoustics and revealed the likely location of the aggregation. The specific DSG with the highest quantity of recorded CASs for 2013, now referred to as the Main Site, was used for analysis of temporal patterns at BDS. For the 2014 season, the DSG was redeployed at the Main Site. A second DSG location displayed strong courtship acoustic behavior beyond the distance of potential acoustics overlap. Since a DSG was only deployed one season at the location and the seamount is believed to be home to only one black grouper FSA, it's significance will need to be explored in the future. Increases in black grouper abundances were observed at both areas in drift surveys during spawning season.

Mona Island (MI) (Figure 1) is a tectonically uplifted carbonate island located roughly halfway between Puerto Rico and the Dominican Republic. Located approximately 73km west of Cabo Rojo, Puerto Rico, it is surrounded by deep water on all sides. Nearby to the northwest, there is a second, smaller uplifted carbonate island, Monito. The two islands compose the Mona and Monito Islands National Reserve. The reserve includes the entirety of the two islands and extends out to 9 nautical miles perpendicular from their coastlines. Within the National Reserve, a year round no-take marine reserve encompasses all waters from the coastlines extending to the 100 fathom (182m) depth contour. The Mona Island platform is a raised plateau rising up to 60-m elevation with continuous vertical cliffs along the north of its perimeter. The south and southwestern insular shelf is composed of a shallow lagoon bordered by fringing reefs along the seaward end. The island contains spawning aggregations for redhind and yellowfin grouper (*Mycteroperca venenosa*) at different locations than the black grouper FSA. Increases in black grouper abundances were observed at the FSA in surveys during spawning season. Black grouper courtship behavior, courtship coloration combined with ventral rubbing, has been observed (PS pers. obs.) and previously recorded at this site in conjunction with CAS production (Schärer et al., 2014). A single DSG is deployed at the black grouper FSA each spawning season.

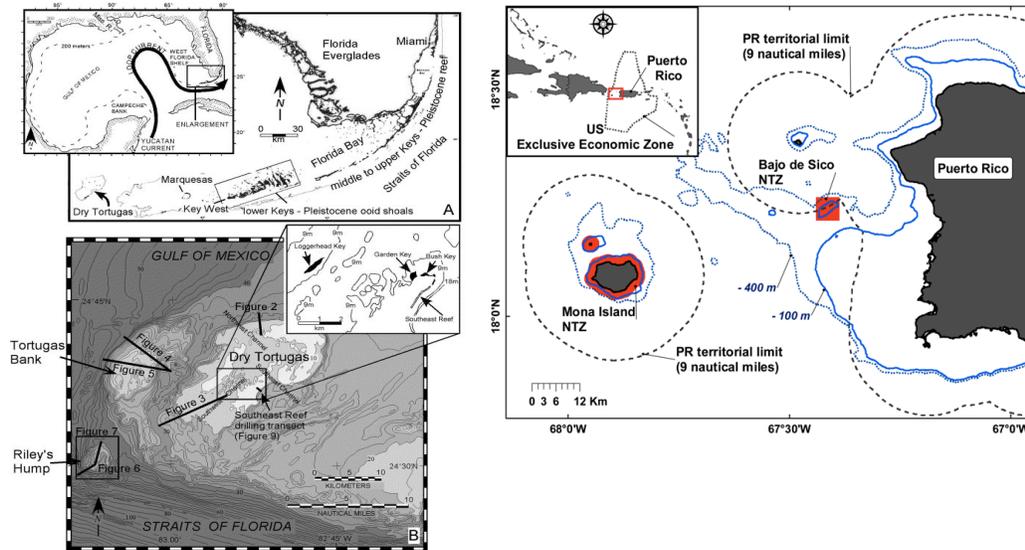


Figure 1. Maps of the three study sites. Left section shows location of Riley's Hump, borrowed from Mallinson et al, 2003. Right section shows locations of Mona Island and Bajo de Sico, borrowed from Scharer et al., 2014.

Riley's Hump (RH) (Figure 1) is an uplifted underwater carbonate plateau within the Tortuga's Ecological Reserve South (TERS). The TERS is located to the southwest of the Dry Tortugas National Park and is a part of the marine reserve network of the Dry Tortugas Islands. Riley's Hump is a multi-species FSA potentially including redhind and red grouper (*Epinephelus morio*). It is a seasonal no-take zone from May to June to protect a large mutton snapper (*Lutjanus analis*) FSA. The bank crests to 30-m depth at the shallowest point. The southern edge of the plateau consists of a vertical reef wall running east to west. The western edge is composed of a steep reef slope (Mallinson et al., 2003). To the south of the southwest corner of the plateau is a pinnacle that rises up to approximately 40m. Multiple DSGs were deployed along the southern edge of the plateau during the 2011 and 2012 spawning seasons. They were organized and analyzed to identify potential aggregations of soniferous fish at Riley's Hump (Locascio and Burton 2016). The DSG that displayed the largest increase in black grouper

CASs, during the described black grouper spawning season, was used for analysis of the black grouper FSA temporal patterns.

*Passive Acoustics Recording*

Autonomous underwater acoustic digital spectrogram recorders (DSG-Ocean, Loggerhead Instruments) were deployed during December at all sites preceding spawning seasons (Table 1). Seasons were analyzed from December 2011 through May 2012 for RH; December 2011, 2012, and 2013, through April 2012, 2013, and 2014, respectively, at MI; and December 2012 and 2013, through April 2013 and 2014, respectively, at BDS. One DSG was deployed at the know FSA location at MI. Multiple DSGs were deployed around the spawning areas at RH and BDS. The individual DSGs with the highest CAS rate at RH and BDS, respectively, were used for analysis. A DSG was redeployed the following season at the same location at BDS for comparison of temporal patterns. Call rates, sound levels, and patterns within the selected DSGs indicated strong likelihood of close proximity to an actual spawning location.

Table 1. Deployment and Recovery dates of autonomous digital spectrogram recorders (DSGs) used for analysis. Deployment and recovery specifically refer to beginning and end dates of analysis

Year	Site	Deployment	Recovery
2012	Mona Island	12/20/11	4/30/12
	Riley's Hump	12/18/11	5/29/12
2013	Mona Island	12/20/12	4/30/13
	Bajo de Sico	12/16/12	4/30/13
2014	Mona Island	12/28/13	4/30/14
	Bajo de Sico	12/20/13	4/30/14

DSGs at BDS and MI recorded audio files on a set schedule, recording for 20 seconds every 5 minutes during the entire deployment. DSGs at RH recorded audio files for 10 seconds every 10 minutes. Differences in recording schedules required different corrective factors to extrapolate total CASs from sample size to daily totals. Daily totals at RH were multiplied by 60 to calculate total CASs day<sup>-1</sup>. Daily totals at BDS and MI were multiplied by 15 to calculate total CASs day<sup>-1</sup>. After DSG recovery, recorded files were downloaded and converted to .wav format for visual detection in Ishmael V2.4 (CIMRS Bioacoustics Lab). Each individual spectrogram was analyzed visually for the presence of a black grouper CAS (Figure 2). The total number of CASs observed for any file were counted. Audio recordings are time stamped, and each recording including a black grouper CAS was transferred to an Excel sheet with the associated time and date. Every individual recorded file was analyzed for each DSG. Any questionable potential CAS was verified audibly using Windows Media Player and noise cancelling headphones. Audible detection of a black grouper specific pulse train undulation within a mean frequency band between 75 and 100hz served as verification.

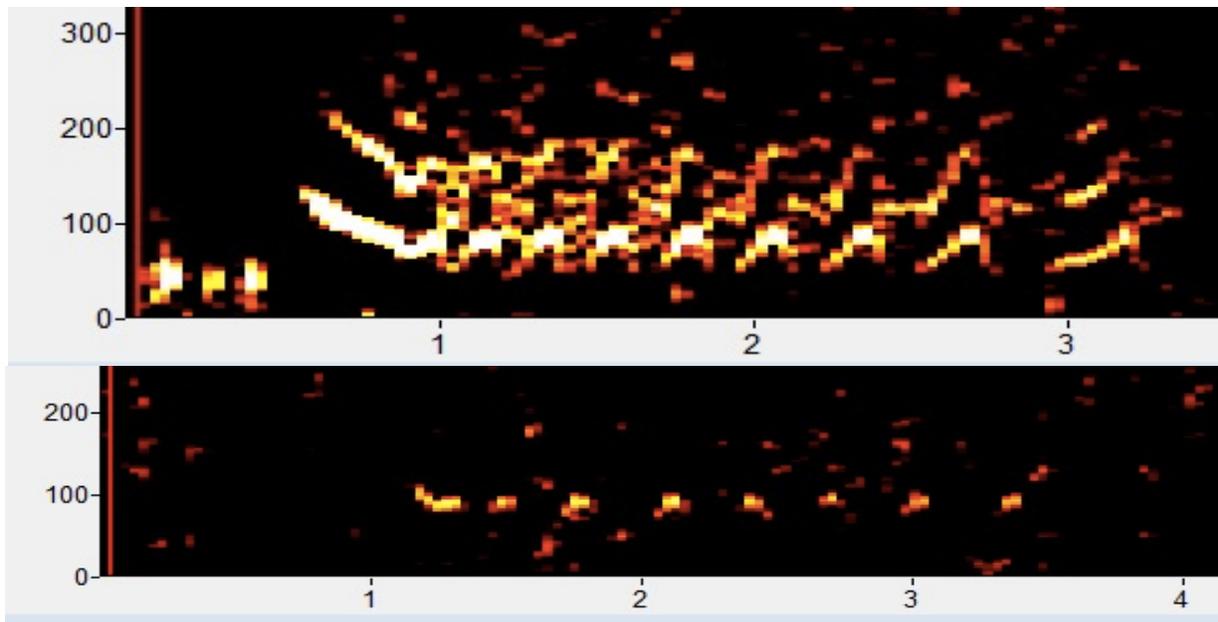


Figure 2. Examples of black grouper courtship associated calls (CASs) spectrograms recorded by a digital spectrogram recorder (DSG) at Bajo de Sico in 2013. Presence of black grouper specific pulse train undulation with mean frequency within the 75-100hz frequency band is visually detectable. Call (a) with individual pulses preceding pulse train undulation recently described as call variant BGV2 in Locascio and Burton 2016. X-axis is time in seconds. Y-axis is frequency in Hertz (Hz). Brightness of yellow color indicates increased strength of call.

### *Temporal Patterns*

Total CASs were summed for each calendar day during deployment. Average daily CASs month<sup>-1</sup> were calculated to confirm seasonality of the spawning aggregation. These totals were compared with average in-situ water temperature to determine potential temperature correlation with spawning season. After CAS totals were extrapolated to daily totals to correct for sampling schedule, daily CASs were compared to their associated day after full moon (DAFM). The calendar day of sunset on the night of the full moon is classified as day 0 and each consecutive calendar day is considered the next DAFM. On the next full moon, the numbering restarts at 0. Daily CAS totals during periods of increased activity were broken down into hour blocks. Hourly totals during these periods of increased activity were summed over entire seasons to analyze daily patterns.

At each site ANOVA tests with Fisher's Least Significance Difference (LSD) were run to determine significant differences between mean CASs month<sup>-1</sup> for each season, CASs DAFM<sup>-1</sup> during each season (January through April for BDSM/MI and January through May for RH), and CASs hour<sup>-1</sup> during periods of increased activity.

### *Temperature*

Temperature loggers (onset HOBO Water Temp Pro V2) were deployed at MI and BDS. Temperature data from Mona Island was recorded at a nearby redhind (*Epinephelus guttatus*) aggregation site at 30-m depth. Temperature data from BDS was recorded from a nearby DSG at 45-m depth.

### *Site Geomorphology*

Site geomorphology for BDS and MI was analyzed using methods from Kobara and Heyman, 2010. For a more in depth description see referenced paper. A site geomorphology analysis was not conducted at RH, just described using an existing bathymetry map borrowed from Locascio et al., (2016), originally published in Mallinson et al., (2003). Analysis was done in ArcGIS 10.3 using the Spatial Analyst and 3D toolboxes. For the morphometric parameter analysis, the specific location of the FSA is considered to be the coordinates of the deployed DSG. The associated DSGs recordings displayed strong courtship behavior and therefore are considered to be nearby to actual spawning location. The point location of the DSG was overlaid on high resolution multi-beam bathymetry maps for BDS and MI. A 1km radius buffer around the DSG was isolated from the bathymetry map. Depth was converted to slope and slope extracted into contours. Slope contours were overlaid on top of depth. The "shelf edge" was

classified as the continuous 20-degree slope contour at a steep vertical depth profile off the shallower structures. Shelf edge outline within the 1km buffer was used to visually identify promontories along the shelf edge. A promontory was considered any noticeable convex protrusion extending off the shelf edge contour within the scale of the 1-km radius buffer.

The morphometric parameters measured were the shortest distances from the FSA to (1) the shelf edge, (2) the nearest intersection with the 100m-depth contour, and (3) the inflection point of the nearest promontory feature. The depth of the shelf edge was measured at its nearest point to the FSA.

## Results

### *Temporal Patterns*

Table 2. Results of ANOVA tests comparing the dependent variable mean courtship associated calls (CAS) day<sup>-1</sup> against the independent variable Month.

SITE/YEAR	N	df	MS	F	p-value
RH 2012	149	4	2.44	1.12	0.3489
MI 2012	120	3	14.7	5.97	0.0009
MI 2013	119	3	28.85	6.21	0.0007
MI 2014	120	3	27.54	5.68	0.0013
BDS 2013	119	3	6.56	3.05	0.0328
BDS 2014	120	3	7.53	3.17	<0.0001

Table 3. Results of ANOVA tests comparing the dependent variable mean courtship associated calls (CAS) day<sup>-1</sup> against the independent variable Day After Full Moon (DAFM). N = total number of days recorded.

SITE/YEAR	N	df	MS	F	p-value
RH 2012	149	29	5.43	3.89	<0.0001
MI 2012	120	29	12.43	4.38	<0.0001
MI 2013	119	29	21.59	3.98	<0.0001
MI 2014	120	29	22.33	3.93	<0.0001
BDS 2013	119	29	4.74	2.06	<0.0001
BDS 2014	120	29	7.47	1.81	0.0178

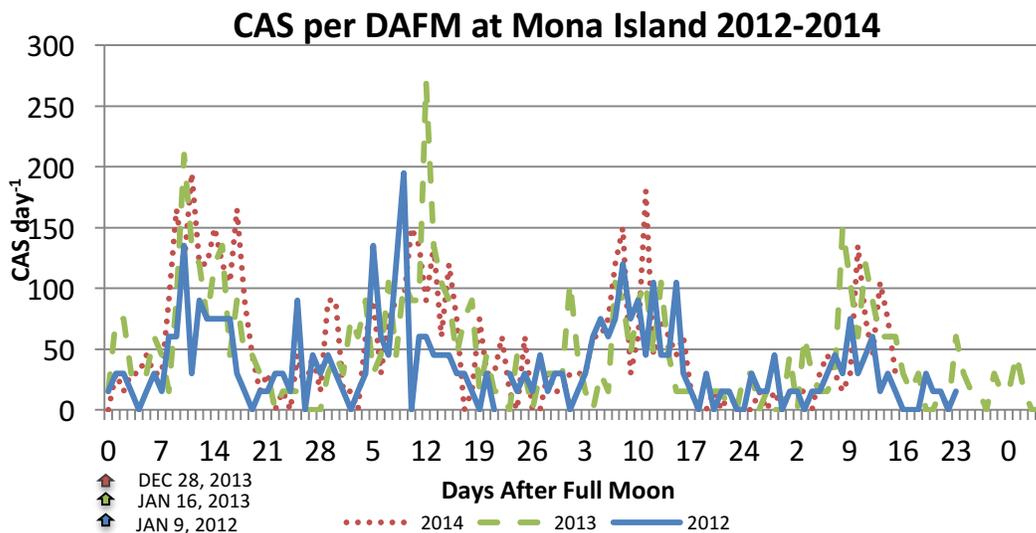
Table 4. Results of ANOVA tests comparing the dependent variable mean courtship associated calls (CAS) hour<sup>-1</sup> against the independent variable Hour of Day.

SITE/YEAR	N	df	MS	F	p-value
RH 2012	144	23	6.78	6.99	<0.0001
MI 2012	120	23	37.84	19.83	<0.0001
MI 2013	96	23	79.59	31.79	<0.0001
MI 2014	120	23	80.93	13.77	<0.0001
BDS 2013	96	23	2.78	2.34	0.0034
BDS 2014	120	23	9.21	4.68	<0.0001

### Mona Island (MI)

Mean CASs day<sup>-1</sup> peaked in February for all three seasons at MI. From January 1 to April 31, mean CASs month<sup>-1</sup> was significant for all three years ( $p < 0.05$ ). Production of CASs increased between the last quarter moon and the new moon, eight to 14 DAFM (Table 3, Figure 3). Peak days for each period of increased CAS production occurred between eight and 12 DAFM. Figure 3 shows temporal patterns in CASs DAFM<sup>-1</sup> for all three seasons at MI. CAS production was strongly correlated with time of day (Table 24). During the

Figure 3. Daily black grouper courtship associated sound (CAS) totals for three spawning seasons at Mona Island. Graph begins at the first full moon during each season, day 0, with its respective date for each year. X-axis represents the associated day after full moon (DAFM). CAS day<sup>-1</sup> are quantities calculated in recording analysis multiplied by 15 to extrapolate out recording schedule to whole day.



three seasons at MI, 54% of all recorded black grouper CASs occurred in a two-hour period between 17:00 and 19:00 local time (Table 4). The hours beginning at 16:00 and 19:00 were the next two most active hours, each containing another seven percent of the total CASs produced. These four hours, 16 percent of the day, contained 68 percent of the total CASs produced (Figure 4).

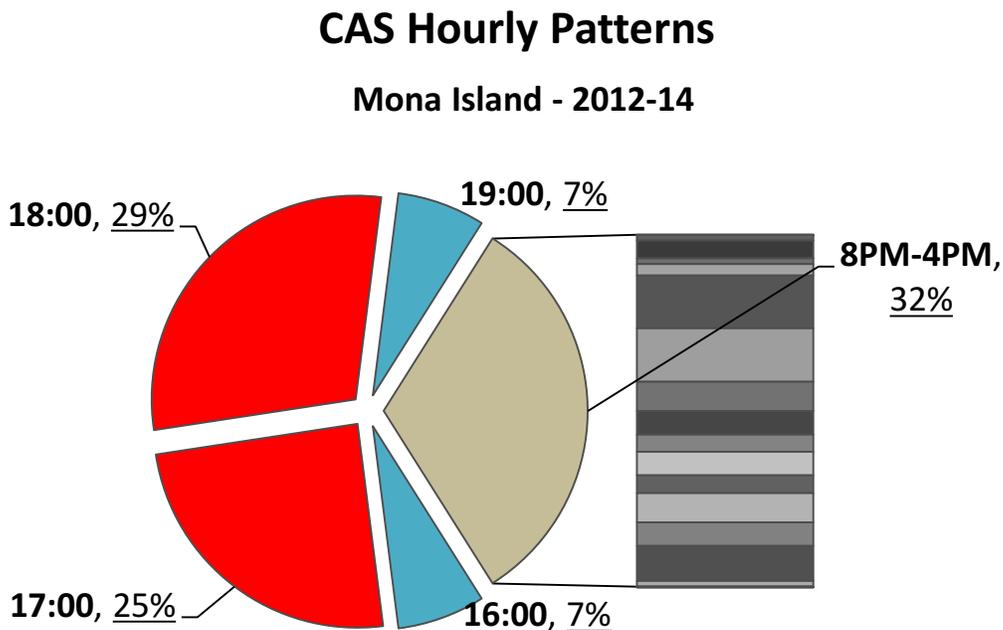


Figure 4. Percentages of courtship associated sounds (CAS) per hour block for three years of acoustics data at Mona Island. CAS data extracted only from days with increased activity surrounding peak days, and combined over all seasons. Sunset occurred between 17:45 and 18:45 for entire recording period. All times are local AST (-4GMT).

### Riley’s Hump (RH)

Mean CASs day<sup>-1</sup> peaked in April, which exhibited a two-fold increase from February, the month of lowest activity. However month was not considered significant for CAS production (Table 2). Days of increased activity occurred between seven to 11 DAFM, with peaks eight and 10 DAFM (Table 3, Figure 5). The mean number of CAS produced 10 DAFM

was significantly more than any other day (Fisher's LSD,  $p=0.05$ ,  $LSD=1.56$ ), except 29 DAFM which only occurred one time, and without a single repetition is considered an outlier.

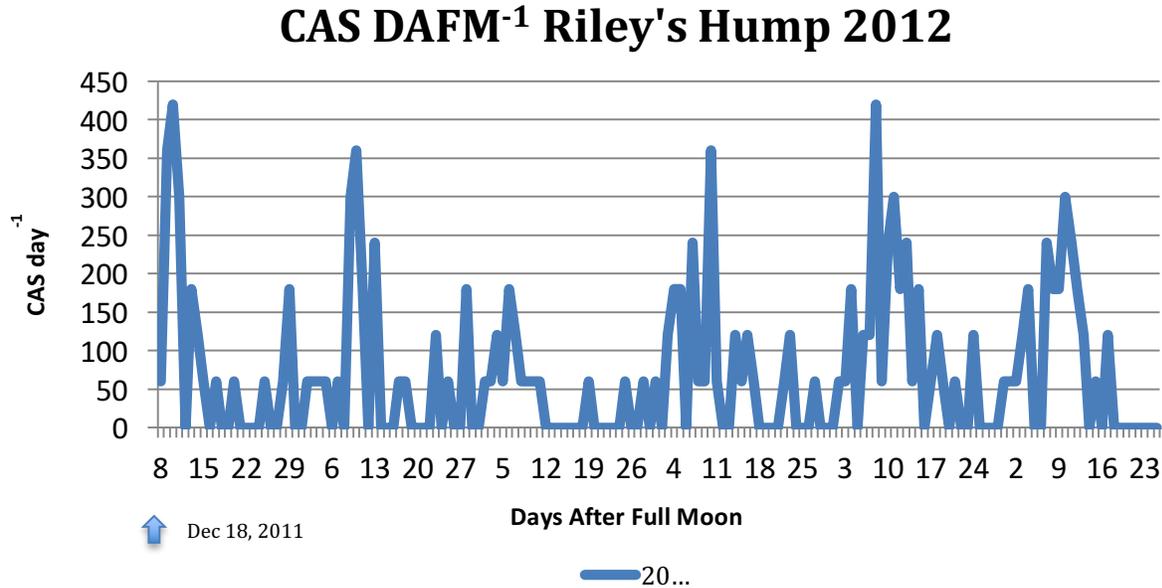


Figure 5. Daily black grouper courtship associated sound (CAS) totals for the spawning season beginning December 2011 through May 2012. The blue line represents total CASs calculated for each calendar day. The black line is the corresponding day after the full moon (DAFM) for each calendar date. Numbers in black are the associated DAFM for the peak in CAS day<sup>-1</sup> for each time period of increased activity.

Daily production of CAS is strongly correlated with hour block (Table 3). Most production occurred during late afternoon hours (Figure 6). Thirty-five percent of all calls occurred in a two-hour window from 16:00 to 18:00 local time. Including the hour before and after, 48% of all calls occurred from 15:00 to 19:00 local time.

## CAS per Hour Block at Riley's Hump 2012

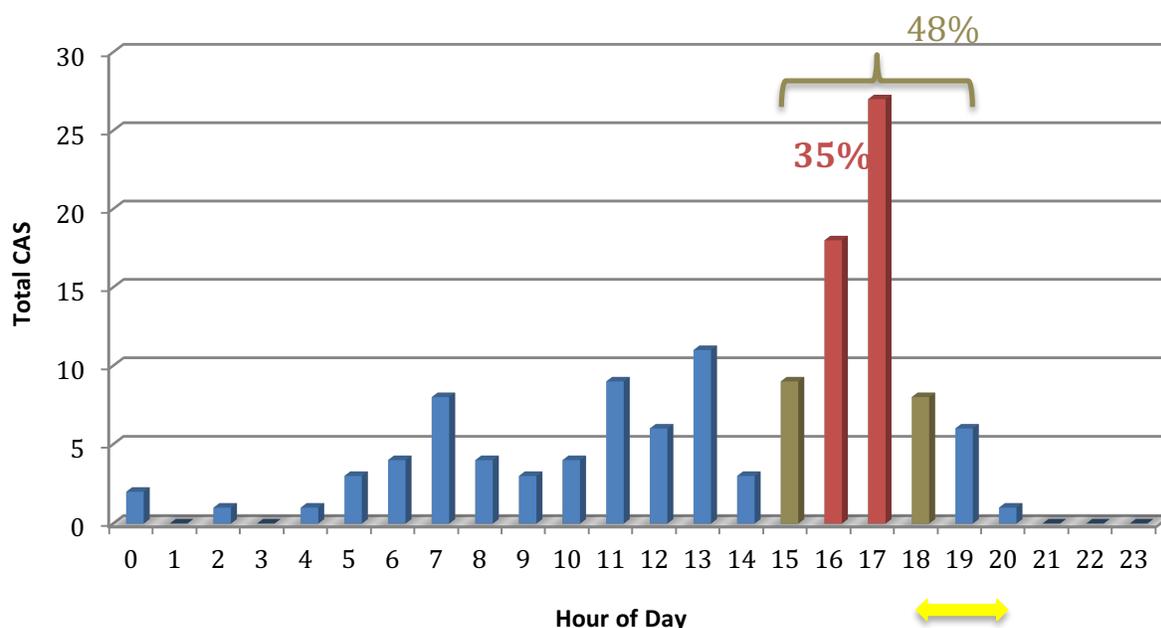


Figure 6. Total courtship associated sounds (CAS) for the respective hour of the day when they occurred. The totals are summations from days of increased CAS production. Percentages correspond color correlated hour frames. Yellow two-sided arrow indicates sunset time range during analysis, 18:45 to 20:15. All times are in Atlantic Time Zone (-4 GMT). Call totals are not corrected for sampling schedule (x15).

### Bajo de Sico (BDS)

Call rates at BDS were substantially less than recorded at the other two sites. Mean CASs day<sup>-1</sup> peaked in January and fell off precipitously from February to April for both years (Table 2). Production of CASs increased earlier in the lunar cycle than at MI, four to 10 DAFM (Table 3, Figure 7). Peak days for the two years occurred six and nine DAFM. Patterns were significant with lunar periodicity. However a defined peak period in 2013 was not found (Fisher's LSD) due to lower CAS totals overall and apparent increases between the full and new moons. Nevertheless, nine of the 11 days with the highest CAS totals were within five to 14 DAFM, from approaching the last quarter moon through the new moon. In 2014 the peaks were

much more defined. The Fisher's LSD only grouped four of the DAFM together within its highest LSD classification. These days were 6, 8, 9, and 11 DAFM. That is one day before the last quarter to four days after the last quarter.

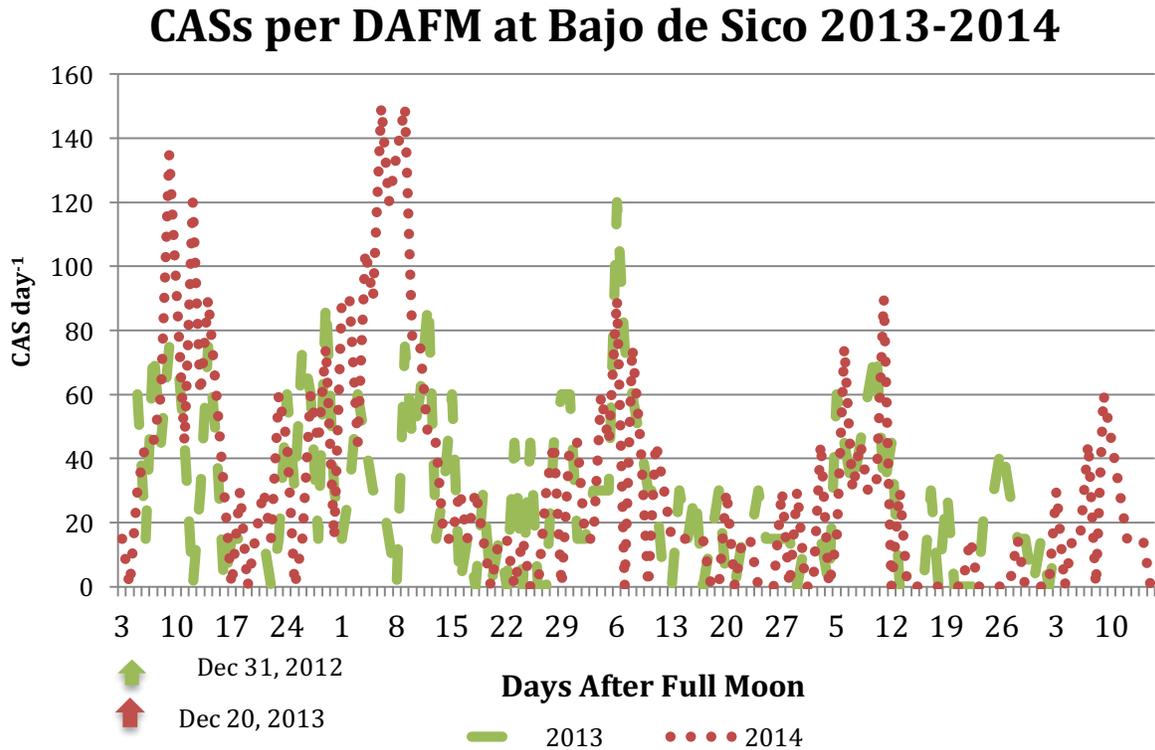


Figure 7. Daily black grouper courtship associated sound (CAS) totals for two spawning seasons at Bajo de Sico. Graph begins on the third day after the first full moon for each season, day 3, with its respective date for each year. Day 3 was chosen based on availability of data. X-axis represents the associated day after full moon (DAFM). Cass day<sup>-1</sup> are quantities calculated in recording analysis multiplied by 15 to extrapolate out recording schedule to whole day.

Similar variation was found between the two years in CASs hour<sup>-1</sup> at BDS. Hour was determined to be significant (Table 4) for both years, however patterns were more defined in 2014 (Figure 8).

## CAS Hour<sup>-1</sup> Bajo de Sico 2013 and 2014

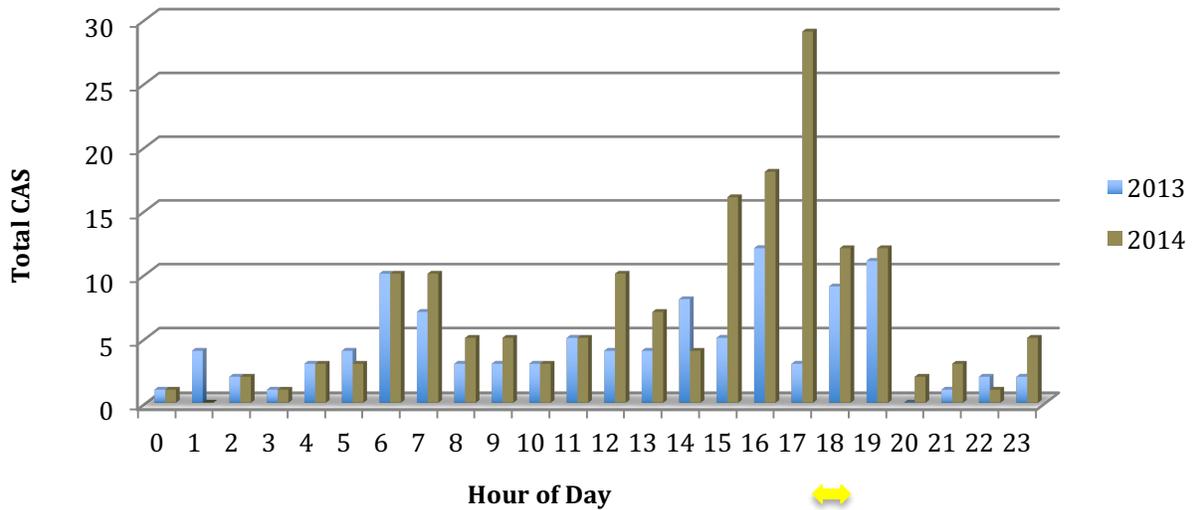


Figure 8. Total courtship associated sounds (CAS) for the respective hour of the day when they occurred. Totals are summations from days of increased CAS production. Yellow two-sided arrow indicates sunset time range during analysis, 18:05 to 18:50. All times are in Atlantic Standard Time (-4 GMT). Call totals are not corrected for sampling schedule (x15).

## Temperature

At BDS and MI, temperatures decreased from January to March. In 2013 and 2014 temperatures remained near their annual minimums most of March. Temperatures began to increase in April. For both sites temperatures dropped below 27°C from mid January to mid April (Figure 9). Water temperatures remained slightly lower at BDS than at MI, but only by tenths of a °C. In April two separate cooling events, each lasting for a week, occurred at BDS.

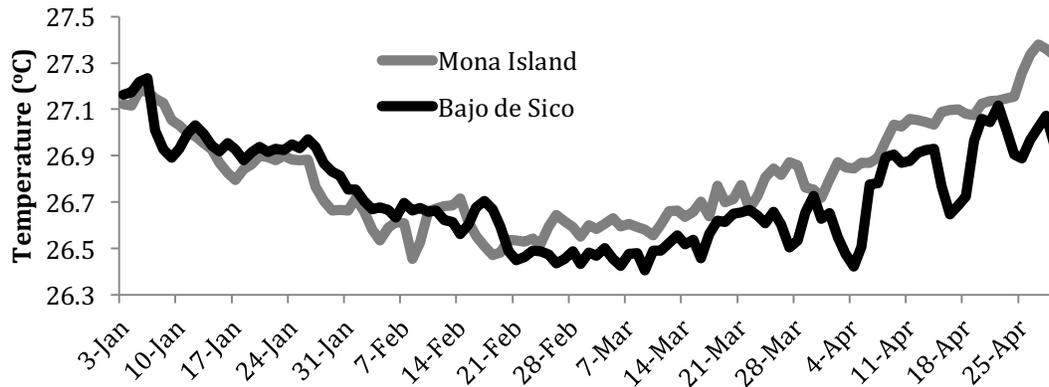


Figure 9. Temperature time-series at Mona Island (30-m depth) and Bajo de Sico (45-m depth) during the 2014 spawning season.

## Site Geomorphology

All three sites adhered to morphometric parameters described for multi-species FSAs in the Cayman Islands and Belize (Table 5), even considering that the DSG locations were used as a proxy for the actual aggregation sites. The BDS and MI sites are less than 100m from convex promontories, less than 100m from the shelf-edge, and in an area where the shelf edge is between 25-30m depth. Similar geomorphometric parameters were evident at RH, where the aggregation site likely forms on the pinnacle off the southwestern ledge of the main underwater plateau. The pinnacle at RH is approximately 35m depth and adjacent to deep water (>100m). The deep water off the southern edge of the pinnacle is the northern extent of the Florida Strait. Additionally all three sites are within 500m of a 30m vertical wall. A wall feature has not been described in previous black grouper FSA site analyses.

Table 5. Geomorphometric parameters of Mona Island and Bajo de Sico black grouper spawning aggregations in Puerto Rico. Deployment location of DSGs served as proxy for spawning aggregation site. \*Expected Mean and Standard Deviation (SD) calculated from sites that include an *M. bonaci* FSA from Table 1 in Kobara and Heyman (2010).

	Promontory Shape	Promontory Orientation	Shelf Edge Depth (m)	Distance to Shelf Edge (m)	Distance to Inflection Point (m)	Distance to 100m Depth (m)
MI	Convex	South	27	10	24	63
BDS	Convex	West	28	19	94	233
Mean*			36	25	127	59
SD*			10	25	94	35

## Discussion

Black grouper CAS recordings display consistent temporal patterns at two of the study sites, MI and RH. During winter and early spring months, black grouper CAS production increases during sunset hours between the last quarter moon and new moon. Though only one year of data is available to analyze RH at the expected FSA location, timing was very reliable through the entire period of analysis. At RH the onset of the season occurred later in the calendar year and lasted for a longer duration than at MI. At BDS, overall monthly totals increased during early winter but dropped off precipitously in late winter and early spring for both seasons analyzed. Interannual variation was very high in the CAS production patterns at BDS. Though black grouper used the site during the spawning season, the purpose of their presence is less understood based on courtship behavior alone.

General annual patterns coincided with the months of lowest annual temperature at MI and BDS, where temperature data at depth was available. For both MI and BDS, average water

temperatures dropped below 27°C during January, February, and March. Temperatures began to climb over 27°C in April for both sites. At MI, April was the first month of the calendar year to have an average water temperature above 27°C. Activity for all three years at MI peaked in February, the month of the annual water temperature minimum in 2013.

Mean monthly temperatures at MI and BDS are similar to those found at previously documented black grouper FSAs. Within the greater Caribbean and the Gulf of Mexico (Belize, Cuba, Mexico, Puerto Rico, and South Florida) black grouper FSAs form in winter months when water temperatures are at their annual minimums (Brule et al., 2003; Eklund et al., 2000; Garcia-Cagide and Garcia, 1996; Paz and Sedberry, 2008; Schärer et al., 2014). In Bermuda they form in the northern hemisphere summer when water temperatures warm up to 26-28°C (Luckhurst 2010). In Brazil the GSI index is at its highest during the southern hemisphere winter months in August and September (Teixeira et al., 2004). Average SSTs drop below 27°C in August and September (seatemperature.org).

Water temperature has been shown to have a strong effect on pelagic larval survival. In controlled studies, leopard grouper (*Mycteroperca rosacea*) and Malabar grouper (*Epinephelus malabaricus*) larvae experienced increased hatching success and survival in waters between 25°C and 28°C (Gracia-López et al., 2004; Yoseda et al., 2006). In a hatchery study of the Nassau grouper (*Epinephelus striatus*), hatching success did not vary with temperature but survival of yolksac larvae decreased substantially when increasing from 26°C, to 28°C, and finally to 30°C water (Watanabe et al., 1995). The lower temperature water delayed mortality by starvation, thereby increasing the maximum time period between hatching and first feeding (Watanabe et al., 1995). Time periods of increased courtship activity for the black grouper in

Puerto Rico occur within these temperatures. In the Caribbean these temperatures are often the annual minimums

Lunar periodicity is strongly correlated with black grouper CAS production (Table 3), a proxy for spawning in this study. During the months of increased activity, most CAS production occurred between the last quarter moon and the new moon at MI and RH. At BDS, the two years showed very different CAS production patterns. The lunar periodicity controls lunar light intensity for each evening and the timing of tidal stages. On the day of the new moon the moonrise occurs around the time of sunset during the winter months in the Caribbean and South Florida. As the lunar stage approaches the last quarter moon, moonrise approaches midnight. As the lunar stage approaches the new moon, moonrise occurs even later in the night and continues to set before the following day's sunset.

The last quarter to new moon increase in CAS production occurs during evening hours at MI and BDS, but a couple hours before sunset at RH. As light intensity decreases, CAS production increases. The possibility of a decreasing evening light level stimulus driving CAS production is supported by daily CAS production patterns. During days of increased activity, the activity peaked during sunset hours. Over half of all CAS produced at MI occurred within a two hour period surrounding sunset. At RH a two-hour period an hour before sunset contained nearly 40% of the CAS totals. Dusk timing has been suggested as a method to reduce the predation on both spawning adults and larvae (Colin and Clavijo 1988; Johannes 1978). At transient spawning aggregations, actual spawning only occurs on a few days over the entire aggregation season (Domeier et al., 1997). High predation rates on such days can lead to an unproductive spawning event. Evening spawning between the last quarter and new moons provides between six and 12 hours of darkness for fertilized eggs to disperse off of the aggregation site. Most

grouper in the western Atlantic exhibit a similar spawning timing (Colin et al., 1987; Locascio and Burton, 2016; Sala et al., 2001; Schärer et al., 2012).

Lunar periodicity controls tidal stage in addition to lunar light intensity. At MI, tidal information is available, from a NOAA tidal station, to compare with temporal patterns in CAS production. Beginning nine DAFM, the outgoing tide coincides with sunset (NOAA NOS Tides and Currents, Mona Island, Station Id 9759938). The transition from an incoming tide to slack high occurs at the beginning of the period of increased activity. The switch to an outgoing tide occurs right before the days of peak CAS production. Tidal stage is commonly hypothesized to play a large role in spawning timing of fish that produce pelagic eggs (Johannes 1978). This appears to be the case of a commercially important grouper that forms a nearby aggregation. Along the western insular shelf of Puerto Rico, tidal currents appear to play an important role in spawning timing for the redhind, *Epinephelus guttatus* (Appeldoorn et al., 2016). Tidal correlations have been suggested as either a mechanism for dispersal into open water, a method of large-scale transport, or a process to retain larvae in local nursery ecosystems (Appeldoorn et al., 1994; Cowen and Sponaugle 2009; Heppell et al., 2008). Local effects of tides have only begun to be analyzed (Appeldoorn et al., 2016).

At MI black grouper exhibit high site fidelity to a specific geological feature. It is not known whether this site is the actual spawning location or the area is part of a larger courtship arena (Nemeth 2012). Dive surveys have not been conducted during sunset hours, due to difficult local conditions, when spawning has been observed in Belize (Paz and Sedberry, 2008) and is believed to predominantly occur. Temporal patterns in CAS production support evening spawning under the assumption there is a direct relationship between CAS production and spawning.

Additionally the morphometric assessment of the FSA sites adhere to the physical parameters described for multi-species sites in the Cayman Islands and Belize (Kobara and Heyman, 2008; Kobara and Heyman 2010). Black grouper utilized 10 of the 12 multi-species FSAs analyzed in Belize in Kobara and Heyman (2010), and both of the additional two discovered. The FSAs at MI and BDS are less than 20m from the shelf edge, 100m from a convex promontory, and 250m from the 100m depth contour. The MI aggregation is much closer to deep water (>100m) than BDS, only 63m compared to 233m, respectively. At both sites the shelf edge sits in 25-35m depth of water.

Black grouper FSAs are not solely confined to these parameters throughout the Caribbean. In Belize and Bermuda black grouper FSAs were documented a few hundred meters from the shelf edge (Luckhurst 2010; Paz and Sedberry 2008). However the majority of described black grouper FSAs occur along the shelf edge near deep water (Claro and Lindeman 2003; Heyman and Kjerve 2008; Kobara and Heyman 2010; Sala et al., 2001). Reason for selection of these sites is not known, but they exhibit some general characteristics. They are high rugosity sites near deep water. Variation exists within the geomorphology of black grouper FSA locations. However, these sites exhibit similar morphometric parameters that can be used to potentially identify new spawning aggregations (Kobara and Heyman 2010).

Normally a solitary species, transient FSAs provide an opportunity to survey the density and health of fish stocks (Gannon 2008; Luczkovitch 2008). Only a handful of black grouper FSAs have been identified in US territorial waters, with the largest ones composed of only a couple hundred individuals (Eklund et al, 2000; Locascio and Burton 2016; Schärer et al., 2014). Population numbers suggest more black groupers are spawning elsewhere. Eight potential black grouper spawning aggregation sites were identified around Puerto Rico (Ojeda et al., 2007),

though none have been verified. By identifying more FSAs and elucidating their reproductive patterns, we will better understand effective reproductive population and stock sizes. Strong regularities in temporal CAS production between geographically isolated sites, MI and RH, suggests spawning timing likely follows distinct patterns for black grouper. Analysis of new sites will determine if these specific patterns are species found species wide. Local deviations, like those seen at BDS, indicates local behaviors can vary substantially from expected patterns. Finer resolution behavioral models, as developed in this study, can isolate local variations in temporal patterns and can be used to develop more effective management policies specially designed for individual populations.

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