Use of Passive Acoustic Recordings to Quantify Abundance Relationship from Courtship Associated Sounds of the Nassau Grouper (*Epinephelus striatus*) at Spawning Aggregation Sites in Puerto Rico and the US Virgin Islands

By

Kimberly A. Clouse

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Approved by:

______________________________ _______________

Richard Appeldoorn, PhD Date
Chairman, Graduate Committee

______________________________ _______________

Nikolaos Schizas, PhD Date
Member, Graduate Committee

______________________________ _______________

Edgardo Ojeda, PhD Date
Member, Graduate Committee

______________________________ _______________

Lillian Ramírez Durand, MS Date
Representative of Graduate Studies

______________________________ _______________

Ernesto Otero, PhD Date
Interim Director, Department of Marine Sciences

Abstract

The Nassau grouper, *Epinephelus striatus,* has been overfished to commercial extinction, such that many aggregations have diminished or disappeared throughout the Caribbean. Groupers produce sound, and the objective of this research was to quantify the relationship between their courtship associated sounds (CAS) to relative abundance at two spawning aggregations. Passive acoustic monitoring was conducted at Bajo de Sico, Puerto Rico (BDS) and the Grammanik Bank, USVI (GB) for 6 months during the 2013 spawning season. CAS were compared to abundance counts obtained from underwater visual census (UVC) to assess the relationship between sound and abundance at both sites. From linear regression, statistically positive correlations (0.81, 0.75; and R^2 =0.56, 0.652) for BDS-Deep and BDS-Shallow, respectively were recorded, while the relationship at GB (0.58, R^2=0.334) was more variable, however significant. Variability of site geomorphology, hydrophone placement relative to fish aggregating and fish calling behaviors may explain some variability in this relationship.

Resumen

La sobrepesca del mero chaerna, *Epinephelus striatus*, ha causado su extinción comercial y la disminución o desaparición de agregaciones en el Caribe. Esta investigación compara los sonidos asociados al cortejo (CAS) que producen los meros y la abundancia relativa entre dos agregaciones. En la temporada de agregación del 2013, se realizaron monitoreos acústicos pasivos en Bajo de Sico (BDS), Puerto Rico y Grammanik Bank (GB), USVI, durante seis meses. Se compara la relación de los CAS a las abundancias obtenidas por censos visuales (UVC), para ambos sitios. Al utilizar regresiones lineales, se obtuvieron correlaciones positivas de (0.81, 0.75; y R^2 =0.56, 0.652) para el BDS-Profundo y el BDS-Llano, respectivamente; pero la relación en GB (0.58, R^2=0.334), aunque más variable, fue significativa. Variaciones en la geomorfología del lugar, la ubicación de los hidrófonos en la agregación, y el comportamiento de los peces al vocalizar, podrían explicar alguna variabilidad en estas relaciones.

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List of symbols and abbreviations

CAS = Courtship associated sounds

DAFM = Days after the full moon

UVC = Underwater visual census

DSG = Digital spectrogram (recording unit)

BDS = Bajo de Sico

GB = Grammanik Bank

FSA = Fish spawning aggregation

PAM = Passive acoustic monitoring

Appendix List

Introduction

The purpose of this research is to monitor documented reproductive sounds of the Nassau grouper, *Epinephelus striatus*, at their spawning aggregations using passive acoustic recorders. This species is heavily overfished and sharp population declines throughout the Caribbean (as early as 1970) led to an endangered listing on the IUCN Red List in 2003 due to overexploitation of fish spawning aggregations (FSAs). Many documented aggregations have disappeared and their stocks have been greatly reduced, such that historical aggregations of 10,000 or more individuals are now dwindling in the low hundreds to the low thousands at most. Surveying these aggregations is crucial to establish a baseline for relative abundance of these depleted spawning stocks, considering they are wide-spread and solitary outside of the periods of aggregating. This species is soniferous (produces sound) and these sounds can be monitored by assessing their acoustic behavior over time. The objective of this study is to quantify the relationship between Nassau grouper courtship associated sounds (CAS) and fish abundance at two established spawning aggregations sites: Bajo de Sico, PR (BDS) and the Grammanik Bank, UVSI (GB) for the 2012-2013 spawning season. Two methods were used to assess Nassau grouper spawning dynamics: quantification of CAS from passive acoustic recordings and underwater visual census (UVC) abundance data. Statistical regression is used to determine if a relationship exists and whether any observed relationships between sound and abundance are consistent among months and locations.

Literature Review

It is often difficult to monitor protected marine species due to their low abundances and regulations prohibiting fishing that limit fishery dependent data (Appeldoorn et al. 2013). Historically considered a significant contribution to the Caribbean reef-fish fishery, Nassau grouper, *Epinephelus striatus,* populations have been exacerbated in many parts of the Caribbean by excessive fishing pressure (Sadovy 1999). Their economic importance ultimately led to declines in landings for countries such as Belize, Puerto Rico, Cuba and the USVI as early as the mid-1970s (Garcia-Moliner & Sadovy 2008, Kadison et al. 2010). During winter-spring months, susceptibility is enhanced due to the synchronous nature of annual spawning aggregations, which form at predictable times during the year, and at repeated locations in accordance with the lunar cycle (Nemeth et al. 2009, Schärer et al 2014b). The Nassau grouper is a large-bodied tropical fish, distributed throughout the Caribbean and is typically solitary and sedentary, often associated with crevices and found within rugose structures (Colin 1992, Sadovy 1999, Nemeth et al. 2006, Starr et al. 2007, Schärer-Umpierre et al. 2012a). Their life-history characteristics (slow growing, extended longevity, late maturation) also increase their vulnerability to the effects of overfishing (Sadovy 1999, Whaylen et al. 2006, Ojeda-Serrano et al. 2007, Albins et al. 2009, Archer et al. 2012). During times of reproduction, *E. striatus* is highly migratory and known to travel upwards of 100km to and from spawning sites (Bolden 2000, Colin 1992), associated with migratory corridors that are often targeted by fisherman (Kobara & Heyman 2008, Rowell et al. 2015). In response to rapid population declines, the Nassau grouper was classified as endangered on the IUCN Red List in 2003 and was proposed for US Endangered Species Act (ESA) listing in September, 2014. High fishing vulnerability of the Nassau grouper as bycatch is still problematic throughout the Caribbean when targeting other commercial species like yellowfin grouper (*Mycteroperca venenosa*) and red hind *(E. guttatus*) (Colin 1992, Nemeth et al. 2006, Kadison et al. 2010, Garcia-Moliner & Sadovy 2008, Schärer-Umpierre et al. 2012a, 2014b).

In the US Caribbean EEZ, Bajo de Sico (PR) and the Grammanik Bank (USVI) have existing spawning aggregations for various commercially important species, including the Nassau grouper. It is unknown whether these Nassau aggregations are remnant (older surviving) aggregations or whether they are newly formed aggregations from fishery closures that are designed to promote species recovery. Some management actions have targeted the Nassau grouper specifically, while the species has benefited indirectly from others (Garcia-Moliner & Sadovy, 2008). In 1990, fishing and possession of Nassau grouper was prohibited in Federal waters. These prohibitions were extended into Puerto Rico and U.S. Virgin Islands waters in 2004 and 2006, respectively (Garcia-Moliner & Sadovy 2008). Seasonal reef fishing bans were enacted at Bajo de Sico (December to February) in 1996 and Grammanik Bank (from February to April) in 2006, although these initially targeted aggregations of red hind and yellowfin groupers, respectively (Nemeth et al. 2006, Kadison et al. 2010). The closure at Bajo de Sico was extended to 6 months, October through March, in 2010. (Garcia-Sais et al. 2008).

Groupers are soniferous fishes that produce low frequency sounds attributable to behaviors including courtship (Schärer-Umpierre et al. 2012a,b). Acoustic monitoring at spawning aggregations of *E. guttatus* (Rowell et al. 2012, Appeldoorn et al. 2013) *E. itajara* (Mann et al. 2009) *E. morio* (Nelson et al. 2011) *M. venenosa* (Schärer et al. 2012b) and *M. bonaci* (Schärer et al. 2014a) have shown that an increase in sound production is associated with more frequent reproductive behavior and increases in fish density prior to spawning. The ability to identify and quantify these courtship associated sounds is useful as it may allow relative or absolute fish abundance to be assessed (Luczkovich et al. 2008a,b, Sprague and Luczkovich 2012, Rowell et al. 2012). These sites are often difficult to monitor *in situ* because of their offshore location, exposure to waves, surge and depth (Starr et al. 2007, Appeldoorn et al. 2013). The specific geomorphology of these sites may also effect sound attenuation, where benthic habitat type, rugosity and drop-offs into deeper water can subsequently impede recording potential. Despite these implications, passive acoustics have the potential to locate and map spawning aggregation sites (Rowell et al. 2011, Appeldoorn et al. 2014) and to simultaneously monitor either a single species at multiple locations or multiple species at a single location (Rowell et al. 2015). This technique is noninvasive, has multi-species monitoring potential and continuous recording that allows for high resolution assessment of spawning stocks over time.

Materials and Methods

Study sites

The study sites are within the US Caribbean EEZ. Bajo de Sico (BDS) is an offshore seamount located in the Mona Passage, west of Puerto Rico, approximately 29 km from Mayagüez (Fig. 1). The seamount covers 11 km^2, mostly at mesophotic (>30m) depths and is highly rugose with sand canals, crevices and consolidated rubble with coral, algal, sponge and large rodolith assemblages. This site is often associated with strong prevailing currents, retaining fish near the benthos (García-Sais 2008, Schärer et al. 2014b). Grammanik Bank (GB) is an insular shelf-edge site located 14km south of St. Thomas where aggregations of yellowfin grouper (*M. venenosa*) are also known to form (Nemeth et al. 2006, Kadison et al. 2010, Rowell et al. 2012, Rowell et al. 2015) from February to May. The benthic habitat of this site is dominated by stony hard coral, with the narrow bank extending 1.69km along the shelf edge and no more than 100m at its widest. The reef is highly rugose and characterized by various corals, sponges and gorgonians at 35-40m depth where an additional coral bank is located to the north and the steep drop off to the south (Nemeth et al. 2006).

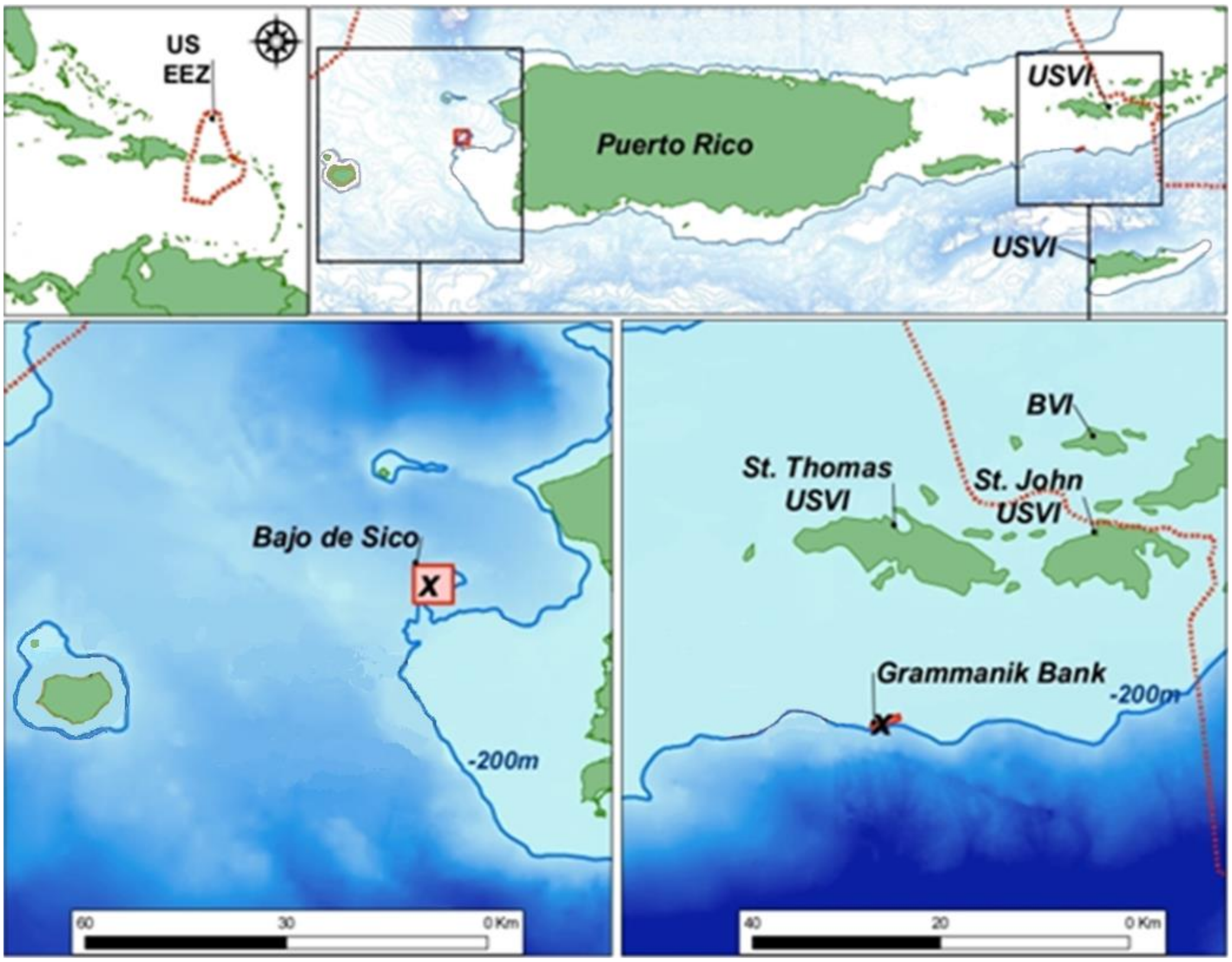

Figure 1: Spawning aggregation sites BDS and GB (X) within the US EEZ. Red boxes indicate perimeters of marine protected areas (MPAs) with seasonal closures in Puerto Rico and US Virgin Islands.

Field deployment, data collection and data management

Field deployment of passive acoustic recorders (DSG-Ocean; Loggerhead Instruments) occurred in December 2012 at both locations before the expected spawning season. Specific sites were centered where Nassau grouper were observed to aggregate during the previous year. Two recorders were deployed at BDS, one at 30m depth and a second recorder at 50m depth, while one unit recorded at the main site of GB at 50m depth, with average UVC depth at 44m at both sites. Technical Nitrox divers secured the DSGs to a mount and placed them horizontally on the seafloor. Passive acoustic monitoring (PAM) was conducted during the six months at both sites. The sample rate for all DSGs was 10 kHz and the recording schedule was 20 seconds every five minutes, with data stored to a 128GB SD-card. The DSG boards incorporated a 20dB gain and the low-power HTI hydrophone was calibrated by recording a 0.1V peak voltage signal that was analyzed in MATLAB (Mann et al. 2010, Rowell et al. 2012, Schärer et al. 2012a,b, 2014b). After the study period the units were recovered, SD card removed and digital files (.dsg) were converted into sound files (.wav) using DSG2wav software. A HOBO temperature data logger was also deployed at BDS (30m) to record minimum, maximum and average temperature (°C) during the entirety of the study period.

Estimating Nassau grouper abundance

Underwater visual census (UVC) was conducted during known aggregation times based on the lunar cycle subject to weather conditions. Surveys took place at GB usually from 13:00-17:00 and earlier at BDS from 9:00-14:00 AST. Closed-circuit rebreather was utilized at BDS to increase bottom time and reduce health risks at depth. The visual sampling methodology conducted was dependent upon the particular topography at each spawning site. The extension of GB along the insular shelf allowed for belt transects alongside the reef to estimate total population size in non-overlapping areas (Nemeth et al. 2006). The spawning aggregation site at BDS consists of a series of steep ridges which extend downwards toward a sloping plain. Surveys were conducted along the slopes of these ridges in a W-shaped transect in the direction of the prevailing current. The same target area was surveyed each time by paired divers (only one of which counted fish), between 40 and 49m depth, Nassau grouper total abundance in all directions and distances was estimated usually within 10-20m of the diver. Abundance was defined as the maximum number of Nassau grouper observed during one UVC (Schärer et al. 2014b).

Acoustic analysis

This study was based only on CAS because mean band level analysis (Mann et al. 2010) was not possible due to difficulties recording to the 128 GB SD-cards at BDS. Recording difficulties affected the process of measuring sound pressure levels due to bands of high intensity noise, occasional missing files and shortened recordings. These complications did not affect the ability to hear and count individual calls manually. Using DSGRecover software the SD cards were processed, corrupted files removed and manual assessment of time stamps, file size and dates were conducted before continued analysis. The data recovered from the Grammanik Bank had no issues and analysis was possible without use of DSGRecover. For future efforts, 32GB cards have sufficient space to comprise the spawning season and do not result in corrupted files.

Nassau grouper CAS were quantified by examining visually digital spectrograms in Ishmael 2.0 with a 1024 FFT Hann window and listened to using Abode Audition for call verification (Mann et al. 2010, Rowell et al. 2012, Schärer et al. 2012a,b, 2014b). Individual

Nassau grouper courtship associated sounds (CAS) are defined as having high signal-to-noise ratio, located within 40-350Hz (average peak tonal frequency of 103Hz) and duration of 0.8sec to 2.34sec, depending on the presence of one or two initial pulses prior to the more consistent tonal call (Figure 2) (Schärer et al. 2012a,b, 2014b). The total CAS per day were compared between sites to determine differences in peak days (day with highest CAS quantified) of sound production and to describe residence time (duration of time residing) at the spawning sites.

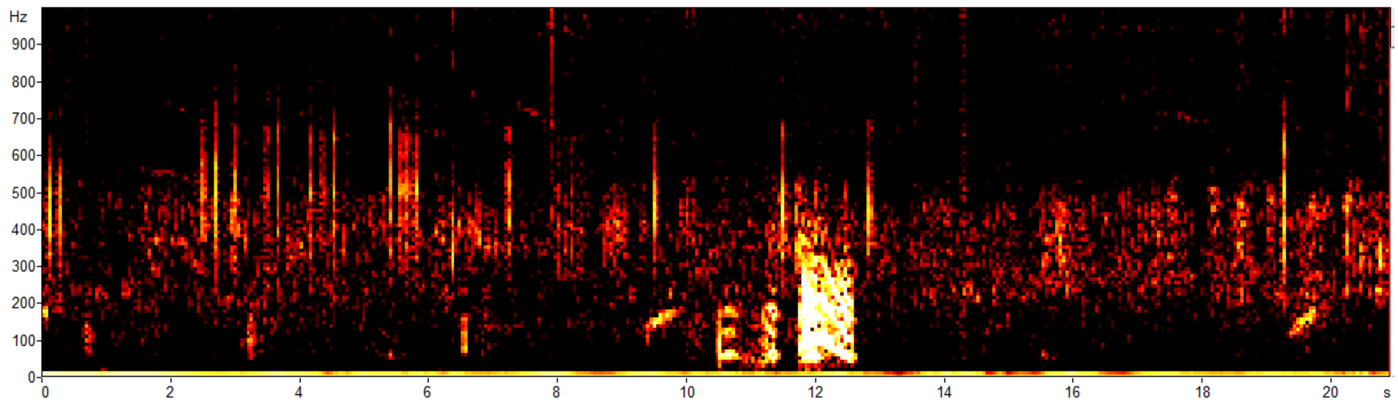

Figure 2: Digital spectrogram (BDS on 6-March-2013) with example of *E. striatus* CAS with two pulses (at 10.5 and 11.3 seconds) and tonal component (starting at 11.8 seconds) among boat noise and squirrelfish calls.

Hourly quantification of CAS/month were performed to detail Nassau grouper vocal activity throughout the day at both sites, as well as analysis of peak sound production in relation to the full moon. Lastly, the relationship between the relative abundance of Nassau grouper over time (UVC) and their courtship associated sounds (CAS) were compared using linear regression to statistically quantify the relationship and to determine the consistency of that relationship over time at each spawning site.

Results

Sound production by E. striatus at Bajo de Sico, PR & Grammanik Bank, USVI

The daily sum of CAS for all sites are shown in Figure 4, which denotes the temporal distribution of Nassau grouper CAS during the 2013 spawning season. The peak days of calling are presented in Table 1, showing elevated CAS (>70% increase in daily CAS) on days after the full moon (DAFM). Two main peak days in February and March were quantified at the BDS-Shallow site, while two minor peaks were observed in April and May. The deep site at BDS had a similar pattern, but had overall higher maximum CAS than the shallow site. For GB only a minor peak was observed in February while CAS were greatest later in the season, in March, April and May. Similar residence time was quantified between sites, despite peak days of sound production differing. From passive acoustics, residence time is defined as the amount of time from when the CAS elevates (>70% daily CAS) to a peak (maximum number of CAS quantified during elevated days) followed by the cessation of CAS that results in little to no CAS detection. These peak days of sound production exhibited a lunar cycle, (Fig. 5) where the highest CAS/day was quantified between 4 and 13 days after the full moon (DAFM) at both sites for months February, March and April; average peak sound production for the Nassau grouper was 9-10 DAFM at BDS and 8-10 DAFM at GB. At the day of peak CAS/d at BDS, the associated minimum temperatures were 25.67 and 25.19°C on Feb-5 and March-7 2013, respectively (Figure 3).

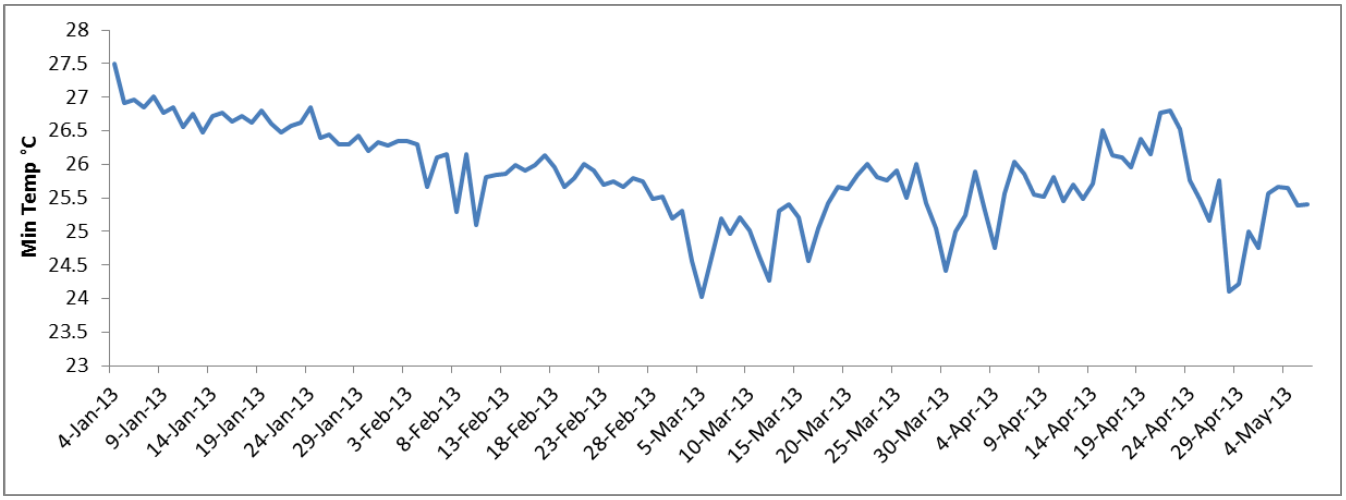

Figure 3: Minimum temperature (°C) at BDS during the 2013 spawning season with peak CAS days corresponding to drops in minimum temperature (Feb-5 and Mar-7 2013).

Table 1: Nassau grouper recorded maximum CAS/d and consecutive days of elevated activity (>70% increase in CAS until cessation of calls) by site days after full moon (DAFM) during the spawning season from Jan to May 2013

Site	Month	Peak CAS	Date of Peak CAS	DAFM	Dates of Elevated CAS	DAFM
BDS-S	Feb	34	5-Feb	9	29-Jan to 7-Feb	2 to 11
	March	36	7-Mar	10	2-Mar to 9-Mar	5 to 12
	April	9	5-Apr	9	31-Mar to 7-Apr	4 to 11
	May	10	4-May	9	30-Apr to *recording stopped*	5 to ---
BDS-D	Feb	39	5-Feb	9	1-Feb to 7-Feb	5 to 12
	March	56	7-Mar	10	2-Mar to 9-Mar	5 to 12
	April	9	6-Apr	10	1-Apr to 8-Apr	5 to 12
GB	Jan	11	27-Jan	0	24-Jan to 31-Jan	0 to 4
	Feb	12	5-Feb	9	4-Feb to 7-Feb	8 to 12
	March	46	5-Mar	7	1-Mar to 8-Mar	4 to 11
	April	26	5-Apr	9	30-Mar to 7-Apr	3 to 10
	May	23	4-May	9	29-Apr to 2-May	4 to 7

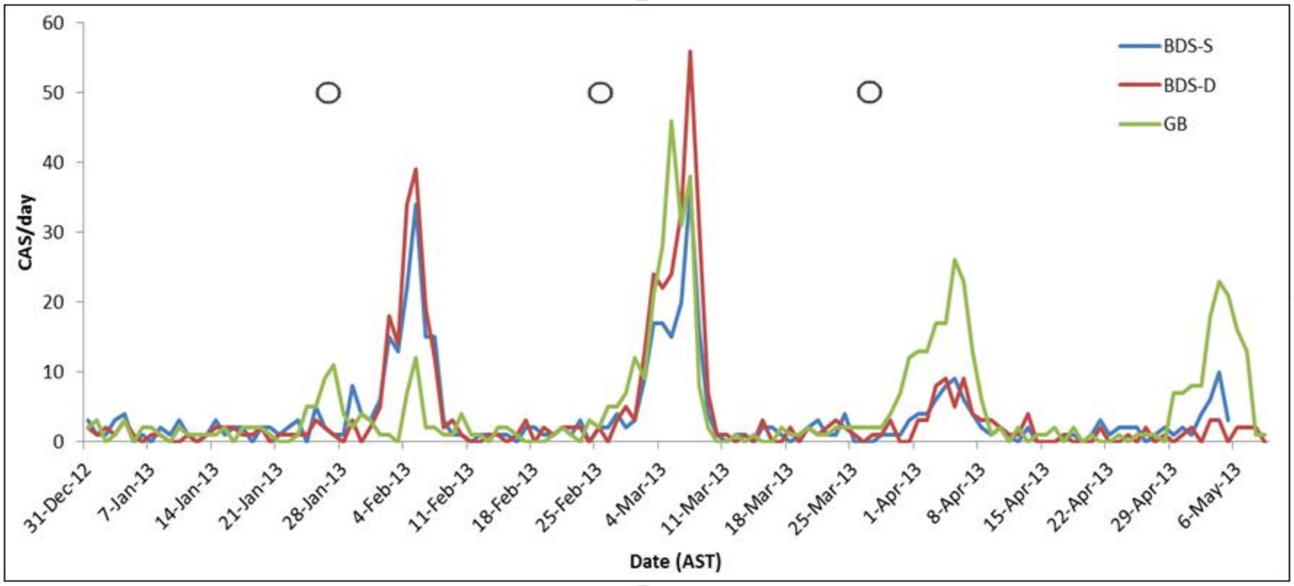

Figure 4: Temporal distribution of Nassau grouper: CAS/day at Bajo de Sico and Grammanik Bank during 2012-2013 spawning season with the circle representing the date of the full moon.

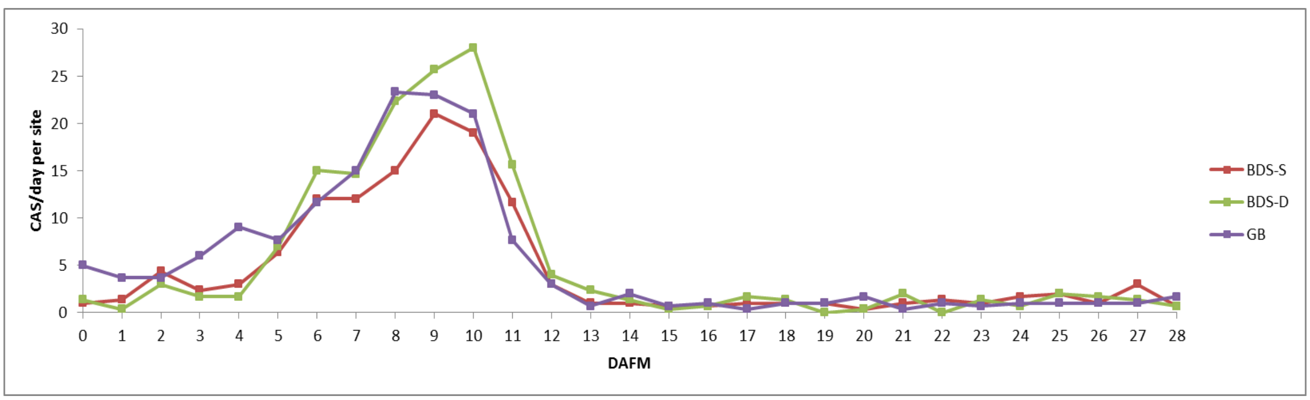

Figure 5: Nassau grouper CAS/d on days after the full moon (DAFM) at BDS-Deep, BDS-Shallow and GB peak spawning months, February, March and April pooled 2013 in AST. (BDS-D n=474; BDS-S n=391; GB n=467). Solid lines are daily CAS values averaged by site over all three months (n=1332 CAS) where zero represents the date of the full moon.

Temporal patterns of daily call frequency for E. striatus CAS

Hourly sound production for February, March and April was averaged for each site (Figure 6). Courtship associated sounds/hr were pooled by month due to variation in call frequency between sites. Results show that the highest vocal activity occurred at dawn and dusk for all sites. The five-hour period between 16:00 and 20:00 AST contained between 35 and

40% of all daily CAS activity, and peak CAS counts (11%) were observed between 18:00 and 20:00 AST. Call proportion at dawn (05:00-07:00 AST) is represented by a minor peak, where 6% of overall daily activity is observed. The sound production further increases into the night where most calls are heard at sunset (18:00-20:00 AST). The shallow unit at Bajo de Sico showed less calling activity at dawn than BDS-Deep and GB. Courtship associated sounds were recorded only at night in February at GB.

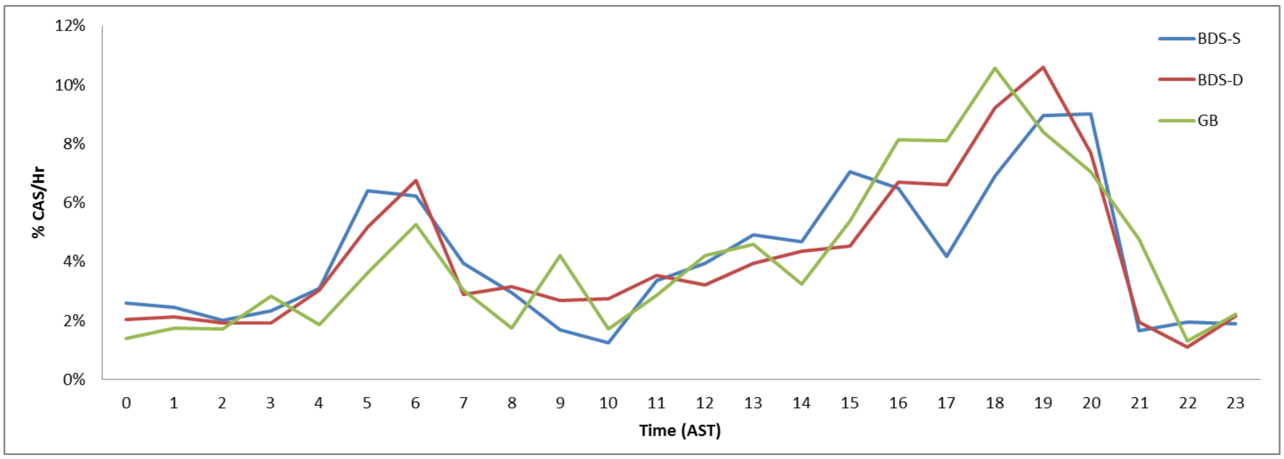

Figure 6: Nassau grouper average percent CAS/hr for Bajo de Sico and Grammanik Bank for February, March and April pooled 2013. Total number of CAS per site/month BDS-S; 151, 163, 68; BDS-D; 173, 237, 62; and GB; 63, 240, 160 for February, March and April 2013, respectively (total n= 1427).

Regression of courtship associated sounds and UVC

Daily sum of CAS from 31-Dec-2012 to 6-May-2013 showed a good correspondence with UVC maximum abundance counts (Figure 7). The highest abundance at GB was recorded during March (214) with fewer numbers recorded for late January (111), April (85) and May (97), while at BDS peak abundances were observed in February (100) and March (70). Higher CAS were consistent with higher relative abundance at both sites throughout the spawning season.

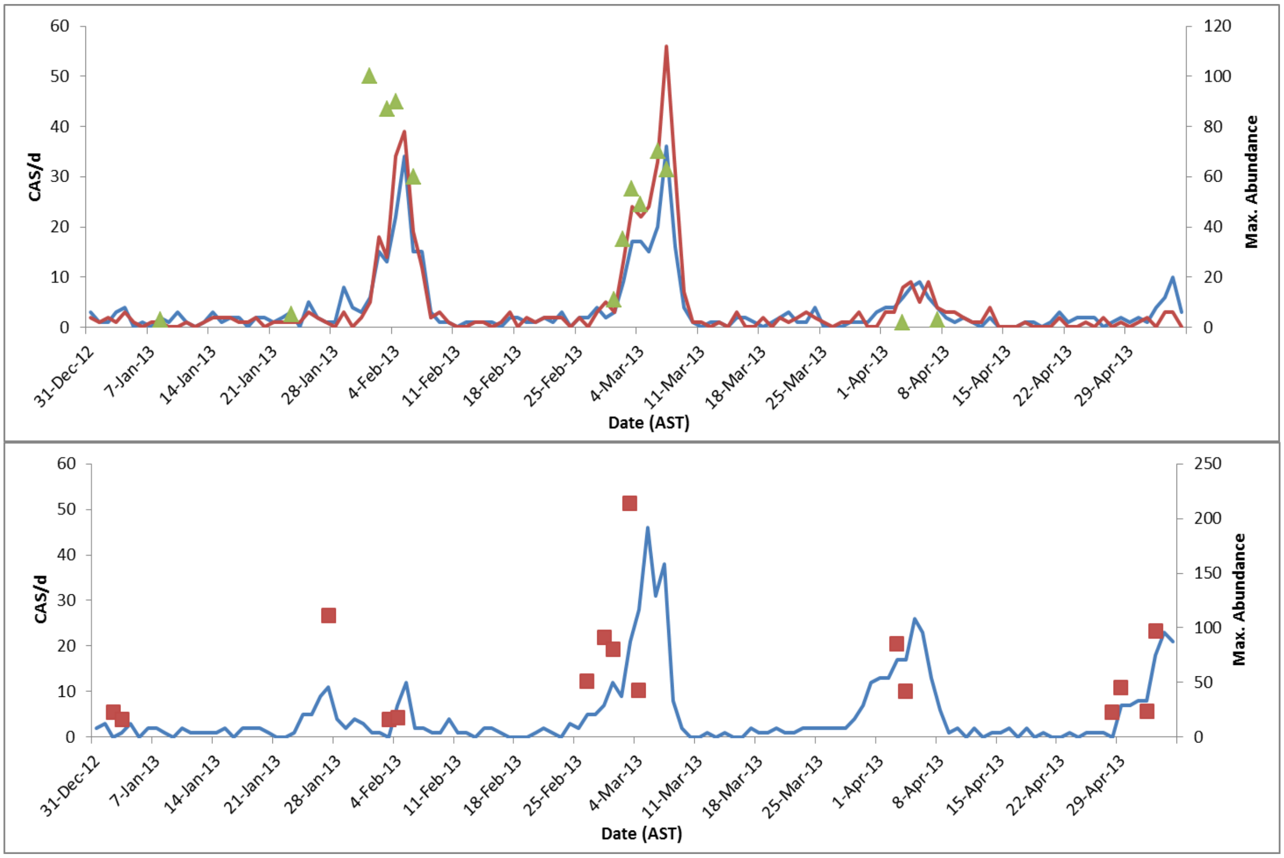

Figure 7: Maximum abundance of Nassau groupers from UVC compared to CAS/d during the 2012-2013 spawning season at Bajo de Sico (top), with blue line (BDS-S), red line (BDS-D) describing maximum CAS/d and green triangles are maximum abundance. Grammanik Bank (bottom) where the blue line is maximum CAS/d and red squares are maximum abundance of Nassau grouper.

We found a significant relationship between total CAS/d and maximum abundance of Nassau grouper for each site (3-Jan-12 to 1-May-13) (Figure 8). Data for both CAS and abundance were $\log_e$-transformed to better fit the model. There were a total of 14 *in situ* observations in both sites (two early surveys at GB outside the aggregation season were removed when fitting the regression model). CAS counts at BDS-Deep and BDS-Shallow explained 56 and 65%, respectively, of the variability observed in abundance, while at GB the corresponding level was 33% (Table 2). The intercept at BDS-S was not significantly different from 0.

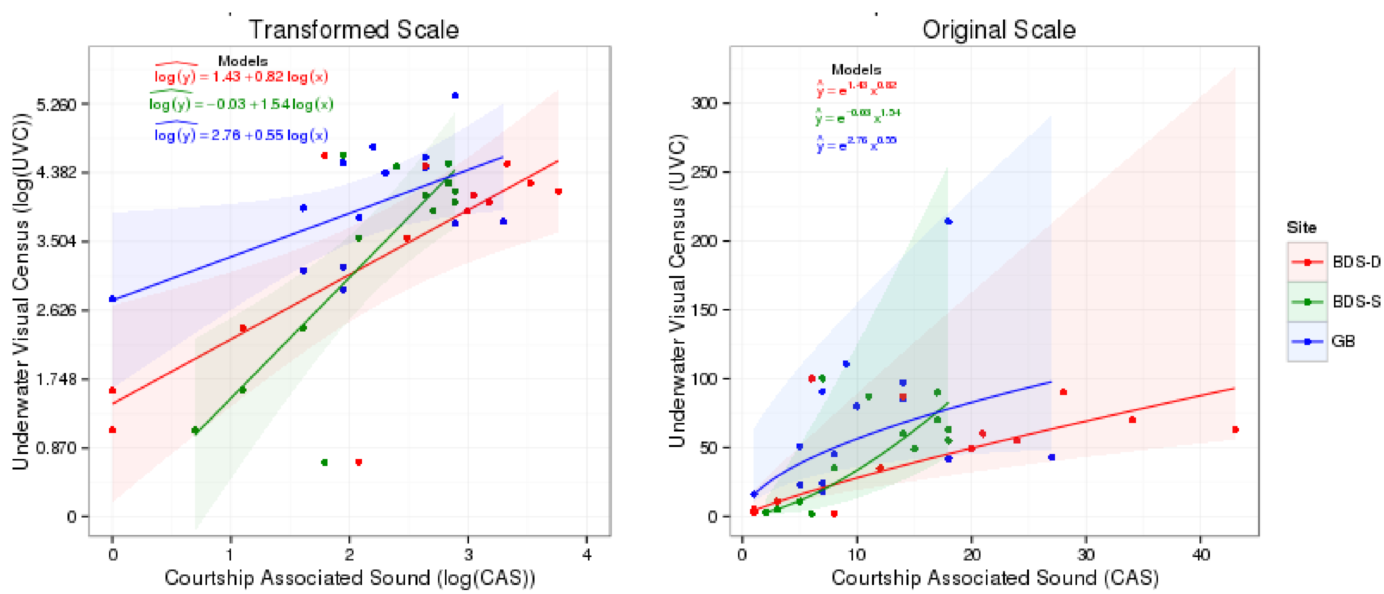

Figure 8: Linear regression of CAS vs. UVC for BDS-D (red), BDS-S (green) and GB (blue). Left: Linear regression of $\log_e$ transformed CAS and abundance data for each site for 3-Jan-13 to 1-May-13. Right: regressions back transformed into raw values. Each shaded background represents a confidence band, where the top of the band is the upper bound of the confidence interval and the bottom is the lower bound of the confidence interval for the predicted values of log (CAS) and CAS, respectively.

Table 2: Coefficients of each linear regression model of $\log_e$(CAS/day) versus $\log_e$(abundance) at each site.

Site	N	Slope	Intercept	R^2	Residual MS
BDS-D	14	0.823***	1.435**	0.561	0.957
BDS-S	14	1.539***	-0.033	0.652	0.852
GB	14	0.554**	2.755***	0.334	0.649
Note:				*p<0.1; **p<0.05; ***p<0.01	

Discussion

Bajo de Sico and GB support spawning populations of *E. striatus* which were documented to aggregate each year in a synchronous manner (Semmens et al. 2005). The average residence time was 7-8 days at both spawning sites. While the lunar cycle is a useful indicator of peak days and months of aggregating, the relationship appears to be more variable between the US and western Caribbean (Aguilar-Perera 2006, Whaylen et al. 2006, Starr et al. 2007). Nassau grouper remained at Little Cayman only 4-6 days, with aggregations peaking 1 to 5 DAFM (Whaylen et al. 2006). A trend of later aggregating is seen at BDS and GB, in which daily CAS peaks on average 9-11 DAFM at BDS and 8-10 DAFM at the GB. In 2011 and 2012, Nassau grouper CAS at GB on average peaked from 7-11 DAFM and 6-10 DAFM, respectively (Rowell et al. 2015) which is consistent with results from Schärer et al. (2013) and this study.

Both the lunar timing and duration of aggregations at BDS and GB differ from expectations based on previous studies and these may be related to the low abundances observed in this study. Olsen and La Place (1978), Colin et al. (1992) and Sadovy and Eklund (1999) described Nassau grouper aggregations consisting of 1000's of fish as occurring on or directly around the date of the full moon; in contrast, at both BDS and GB Nassau grouper aggregations seem to occur later in the lunar cycle (6-11 DAFM), an observation also noted in other species where abundance has been greatly reduced (Kadison et al. 2010, Rowell et al. 2015). Similarly, Heppell et al. (2014), studying Nassau grouper aggregations in The Cayman Islands states that smaller aggregations (<500 fish) differ in that they persist for a longer period of time potentially remaining 125 hours (5 days) longer than an aggregation containing several thousand individuals. Annual monitoring of BDS and GB therefore provides a unique opportunity to study the aggregating dynamics of two small aggregations as a baseline to test possible shifts in later spawning behavior should recovery result in a much greater abundance of spawners.

Water temperature considered optimal for spawning of Nassau grouper around 25.5-26°C (Colin 1992, Bolden 2000) and minimum temperatures were 25.67 and 25.19°C, respectively on Feb-5 and March-7 at BDS. In both cases there were significant dips in minimum temperature below 26°C, while in March a sharp drop to 24°C occurred two days before (March 5). The apparent drop in temperature at these times is thought to be associated with strong prevailing currents that induce upwelling. Although actual spawning on the days of peak calling is unknown the following days resulted in a steep drop of quantified CAS.

Within the US Caribbean, there was a significant difference in the months of aggregation and spawning potential between BDS and GB, with aggregations forming primarily in February and March at the former site, with March-May at the latter, trends also reported by Schärer et al. (2012a) and Rowell et al. (2015). This difference between BDS and the GB is unclear; however large aggregations of *M. venenosa* form at GB these months, and it has been hypothesized that Nassau grouper could be affected by reproductive behaviors from that species (Kadison et al. 2010).

There was a significant positive relationship between CAS and Nassau grouper abundance at all sites. A similar result was found by Rowell et al. (2012) for Red hind *E. guttatus* at Abrir la Sierra, a spawning aggregation site on the western insular platform of Puerto Rico. These results suggest that CAS are useful in predicting relative fish abundances during the spawning season, and hence are a direct indicator of the arrival to, residence time at (potential spawning), and departure of fish from an aggregation. This would be a powerful tool for predicting the timing of aggregation formation that could be used to refine field sampling schedules and increase fisheries management efficiency, such as minimizing closures and maximizing enforcement efforts during periods when fish are known to be vulnerable to fishing.

Furthermore, it suggests that monitoring CAS among sites and years may also be useful in assessing spawning stock status (Rowell et al. 2012, Sprague and Luczkovich 2012). However, in this study, the relationships among the three DSGs were different, both in their slopes and intercepts as well as the amount of variability explained. While differences in the former suggest that empirical relationships will need to be developed for specific spawning aggregations, low correlations are more problematic since they reduce the predictive capacity of potential CAS monitoring. Yet, further comparisons of the CAS-abundance relationships among sites suggests that much of the variability can be explained by differences in the placement of DSGs relative to the location and size of the respective aggregations and the movement of fish between courtship arenas and the sites of actual spawning.

At BDS the deep and shallow recorders are in close proximity (<100m), however some temporal differences within this site were revealed. During dawn and dusk, the daily times of peak calling activity, CAS at the deep site was higher than at the shallow location, suggesting that fish are moving off the reef closer to the deep DSG. This is supported by preliminary studies of the movements of acoustically tagged individuals (Tuohy, unpublished data). The linear regression models for BDS-Deep and BDS-Shallow are significantly different in terms of their slopes, with BDS-Deep having more calls per number of fish during the days of highest fish abundance and calling activity. These differences may result from both movement behavior and habitat use. Thus, at low abundances as the aggregation forms, more fish are associated with shelter, but as the number builds daily (and during peak calling times) they move off the reef and deeper at dusk presumably to where actual spawning occurs. Thus, the concentration of fish closer to the deep DSG increases, as does their reproductive activity and CAS as the day(s) of spawning approach, allowing the deep hydrophone to record a greater percentage of the calls. This differential movement of grouper and call activity relative to the two DSGs could then explain the higher slope observed at the BDS-Shallow site in the CAS-UVC relationship.

In future studies, later surveys from 18:00 to 20:00 AST would enhance this data; however due to the lack of light for UVC mesophotic rebreather surveys, and offshore locations of transient spawning sites this is not feasible. Tag studies offer high resolution data of full-scale movements, which would help to further describe the spatial use of the spawning area and migratory corridors by this species (Rowell et al. 2015, Tuohy et al. 2015).

The CAS-UVC relationship at GB had a noticeable higher intercept than those at BDS, indicating that less calling activity is being recorded at GB relative to the number of Nassau observed. This is opposite to expectations given the maximum number of *E. striatus* observed was over twice that at BDS, but this may be explained by the location of the DSG relative to the location of the aggregation, which as at BDS, changes position differently during the day relative to the lunar cycle. Nassau grouper are thought to move down the slope at GB as sunset approaches because the highest densities observed up on the shelf have been between 16:00 and 17:00 AST, while ROV observations of *E. striatus* at nightfall show the fish to be beyond the slope, deeper (60m) and in a cluster that moves widely (100s m) along the horizontal axis of the slope (Nemeth, unpublished). The slope at GB is slightly convex and it is likely that some of the

CAS are not being detected above the slope, both because of coral reef rugosity interference and the fish being out of range for a significant proportion of the period of high call activity. A second DSG recently placed over the slope at 70m may allow this to be tested empirically.

The CAS-UVC relationship at GB also had a markedly lower regression coefficient. In part, this is due to a smaller sample size of observations during the spawning season from periods of low fish abundance between aggregation peaks, due to the need to spread a fixed number of sampling days over an expected greater number of aggregations during the spawning season than at BDS. However, a contributing factor may again be the movement of fish relative to the location of the DSG. In addition to the movements away from the DSG at night, acoustic tagging studies (Rowell et al. 2015) have shown Nassau grouper to move more widely over the continuous expanse of shelf (relative to BDS which is an isolated seamount). This could lead to greater variation in both abundance and CAS recorded. In general, by utilizing multiple recorders simultaneously, consistency of the CAS-UVC relationship within and between sites will better describe spatial variation in sound production by *E. striatus* at GB.

Passive acoustic monitoring is increasingly useful when studying threatened species, such as the Nassau grouper. The direct relationship found between CAS and abundance at each site show that the increase CAS are, on average, indicative of an increase in numbers as the time of spawning approaches. Nevertheless, much work remains before CAS data can be used to quantitatively compare fish abundance across sites and over time. Continued studies will need to detail annual variations over the long term in the spawning dynamics between sites and years (Appeldoorn et al. 2013) to better document the factors affecting time and strength of aggregation formation affecting the CAS-abundance relationships.

In this study the regression approach used was determined the only method readily available to relate sound production to fish abundance. Sprague and Luczkovich (2012) attempted to model fish abundance by relating CAS to a known call rate of weakfish, *Cynoscion regalis.* While this approach is theoretically feasible for grouper, the average call rate of groupers is unknown and problematic given that these fish maximize peak sound production at dusk, when diver safety becomes an issue in offshore areas characterized by strong currents and large predators. Nevertheless, further research will help uncover other factors affecting sound production of spawning fish.

Historical aggregations of Nassau grouper in some parts of the Caribbean were estimated to have as many as 100,000 individuals; today these aggregations are noticeably reduced containing no more than a few thousand at most (Whaylen et al. 2006). Off St. Thomas, USVI, the historical Nassau grouper spawning aggregation was estimated to have as many as 2,000 fish (Olsen and LaPlace 1978) before its rapid decline and collapse, emphasizing how vulnerable these aggregations can be (Aguilar-Perera 2006). Continued monitoring of these spawning aggregations is of the utmost importance to recovering depleted stocks of the Nassau grouper and the use of this technology offers great potential.

Recommendations

Currently, our knowledge of the Nassau grouper and their spawning aggregations is limited, partially due to their low abundances and management regulations limiting fishery dependent data. In future endeavors, I suggest the use of multiple recorders simultaneously at spawning locations. This will provide higher resolution data of spatial preference/use and allow researchers to better assess the area of potential spawning.

Later surveys (6-8pm AST) are not an option for these offshore spawning locations, due to high risk nature of technical diving, therefore improved techniques to visually monitor *E. striatus* during peak spawning hours would be useful to shed some light on the variation between sound and fish abundance found in this project (mounted cameras are an option). These suggestions however require funding for boats, technical divers (which need to be highly trained) and additional surveying equipment. Ultimately, this research is newly developing and consecutive annual monitoring is of the utmost importance to identify the variability between sound and abundance for this species at individual spawning aggregations over time.

Conclusions

- The relationship between courtship associated sounds (CAS) and abundance (UVC) for the Nassau grouper, *Epinephelus striatus* was found statistically positive at Bajo de Sico, PR and the Grammanik Bank, USVI for the 2013 spawning season.
- The maximum number of calls for BDS occurred in February and March 2013, while GB had high vocal activity from February to May 2013.
- Nassau grouper have similar residence time (7-8 days) at both BDS and GB.
- The greatest proportion of calls are occurring at night (6-8pm, 18-20:00 AST) at both spawning sites.
- The greatest relationship between sound and abundance was at BDS-Shallow (R^2=0.652).
- Significant site variability found between BDS-Deep and BDS-Shallow:
    - Fish moving off the reef and deeper to potentially spawn (Tuohy, unpublished data, ROV observations).
    - Highest overall CAS was quantified at BDS-Deep (39 and 56 on Feb-5 and Mar-7, respectively) compared to the shallow unit.
- The relationship between sound and abundance was the lowest for Grammanik Bank (R^2=0.334).
- Variability found between sound and abundance at both sites (and within site) is possibly due to (among other factors):
    - Site / habitat geomorphology.
    - Fish calling behavior.
    - Placement of hydrophone relative to where the fish are peak calling.

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Appendix

Table 1. Results of Underwater Visual Census (UVC): Survey dates and maximum number of observed Nassau grouper in transects conducted at Grammanik Bank (GB) and Bajo de Sico (BDS).

Date	Max. Abundance (GB)	Max. Abundance (BDS)
2-Jan	23	
3-Jan	16	
8-Jan		3
23-Jan		5
27-Jan	**111**	
1-Feb		**100**
3-Feb	16	87
4-Feb	18	90
6-Feb		60
26-Feb	51	
28-Feb	91	
1-Mar	80	11
2-Mar		35
3-Mar	**214**	55
4-Mar	43	49
6-Mar		**70**
7-Mar		63
3-Apr	**85**	2
4-Apr	42	
7-Apr		3
29-Apr	23	
30-Apr	45	
2-May	24	
3-May	**97**	