Passive Acoustics as an Indicator of Red Hind, *Epinephelus guttatus*, Density at a Spawning Aggregation

By

Timothy J. Rowell

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Approved by:

Nikolaos V. Schizas, PhD — Date
Member, Graduate Committee

David A. Mann, PhD — Date
Member, Graduate Committee

Matthew T. Craig, PhD — Date
Member, Graduate Committee

Richard S. Appeldoorn, PhD — Date
President, Graduate Committee

Edgardo Ojeda Serrano, PhD — Date
Representative of Graduate Studies

John M. Kubaryk, PhD — Date
Chairperson of the Department of Marine Science

ABSTRACT

Annual spawning aggregations of red hind, *Epinephelus guttatus*, form at predictable times and locations, and have historically succumbed to overfishing. Monitoring the status and restoration of aggregations is essential for evaluating the effectiveness of fishery management measures. Passive acoustic and diver-based underwater visual census (UVC) techniques were used to develop an efficient methodology for estimating red hind density from sound production at spawning aggregations. Red hind sound production was recorded from November 2010 to April 2011 at Abrir la Sierra, Puerto Rico. UVC surveys were conducted during the spawning season to assess changes in red hind density over a fixed time and area. Sound recorded during the 18:00-19:00 Atlantic Standard Time hour was representative of total daily changes in red hind sound production, and was selected for the development of an efficient density estimation model. Pronounced daily changes in sound production and density were observed after the December 2010 and January 2011 full moons. Two hourly sound level measurements were compared to densities estimated by UVC surveys, yielding significant linear regressions, which were used to predict changes in fish density as measured on the aggregation site. Passive acoustic methods predicted changes in red hind density and habitat use at a higher temporal resolution than previously possible with traditional methods. Red hind sound production and inferred densities can be monitored and analyzed efficiently for multiple aggregation sites simultaneously, documenting short-term and long-term changes in red hind densities at spawning aggregation sites and providing important information for the support or development of management strategies.

RESUMEN

Las agregaciones anuales de desove del mero cabrilla, *Epinephelus guttatus*, se forman en lugares y tiempos predecibles, incrementando su susceptibilidad a la sobrepesca. El monitoreo del estado de las agregaciones y la restauración de las mismas son esenciales para evaluar la eficacia de las medidas de manejo pesquero. Con el fin de desarrollar una metodología eficiente para estimar la densidad del mero cabrilla, durante las agregaciones reproductivas, se utilizaron técnicas de censos visuales (UVC, por sus siglas en inglés) y técnicas acústicas pasivas para registrar el sonido producido durante el apareo. Se grabó la producción de sonido del mero cabrilla entre noviembre de 2010 a abril de 2011 en Abrir la Sierra, Puerto Rico. Durante la temporada de desove se realizaron UVC para evaluar los cambios en la densidad del mero cabrilla en un área y tiempo determinado. El sonido grabado durante las 18:00-19:00 horas (Tiempo del Atlántico), fue representativo del total de los cambios diarios del sonido producido por el mero cabrilla, por lo que fue seleccionado para desarrollar un modelo eficiente de estimación de densidad. Después de la luna llena de diciembre de 2010 y enero de 2011 se observaron cambios diarios pronunciados en la producción de sonido y densidad. Se compararon dos mediciones de nivel de sonido tomadas en periodos de una hora con los estimados de densidad obtenidos a través de UVC, produciendo regresiones lineales significativas, las cuales fueron utilizadas para predecir los cambios en la densidad de peces. Métodos acústicos pasivos predijeron cambios en la densidad y hábitat del mero cabrilla, a mayor resolución en tiempo que otros métodos tradicionales usados anteriormente. La producción de sonido y la densidad inferida del mero cabrilla pueden ser monitoreados y analizados de manera eficiente en múltiples sitios de agregación de forma simultánea, documentando los cambios de la densidad en los sitios

de agregación de desove a corto y largo plazo, y suministrando información importante para el apoyo o el desarrollo de estrategias de manejo.

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DEDICATION

To Jonathan D. Rowell

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PREFACE

The following manuscript details the methods, findings, and implications of an intensive study conducted during 2010-2011 at a red hind, *Epinephelus guttatus*, spawning aggregation site, in which the relationship between red hind sound production and aggregation density was examined. This accumulation of work was based off of a pilot study conducted during January-February 2010. The manuscript has been submitted for publication in the Marine Ecological Progress Series, and is formatted accordingly.

INTRODUCTION

Red hind, *Epinephelus guttatus*, is a commercially and recreationally important protogynous grouper (Epinephelidae) found from Bermuda to Brazil that reproduces at annual transient spawning aggregations at specific times and locations (Claydon 2004, Colin et al. 1987, Cummings et al. 1996, Domeier and Colin 1997, Sadovy et al. 1994, Shapiro et al. 1993a, Shapiro et al. 1994). On the Puerto Rico (PR) and United States Virgin Islands (USVI) shelf platform, aggregation formation and spawning occur during the week around the full moons in either December and January, January and February, or only January (Colin et al. 1987, Sadovy et al. 1994, Shapiro et al. 1993b, Whiteman et al. 2005), depending on when the full moon occurs each month (Nemeth et al. 2007). Aggregation formation typically begins with the arrival of males prior to the first full moon of the spawning season, followed by the appearance of females (Nemeth et al. 2008, Whiteman et al. 2005). Red hind densities at aggregations vary over the course of the spawning period, characterized by a build up prior to each spawning event followed by a quick departure from the site after spawning, with 5-20% of individuals remaining on location between consecutive full moons (Nemeth et al. 2007).

The predictability of grouper aggregations makes them extremely vulnerable to overfishing, which can result in a decrease in stock size, mean length, and recruitment, diminished density and biomass, female biased sex ratios, or complete disappearance of the aggregation (Aguilar-Perera 2006, Aguilar-Perera & Aguilar-Dávila 1996, Beets and Friedlander 1992, Coleman et al. 1996, Sadovy 1994, Sadovy & Domeier 2005, Sadovy and Figuerola 1992). In the USVI, protecting red hind spawning aggregations through seasonal and subsequently permanent no-take marine protected areas (MPAs) has reversed these trends and led to a substantial stock recovery (Beets and Friedlander 1999, Nemeth 2005). Similarly, in Bermuda seasonal protections at red hind spawning aggregation sites have likely resulted in increases in mean and modal sizes (Luckhurst and Trott 2008). In contrast, seasonal closures in PR have not shown positive results due to considerable increases in effort both outside the closed areas and outside the closed season (Marshak and Appeldoorn 2007) coupled with a lack of compliance by fishermen and vendors as well as minimal enforcement of regulations during the closed season (García-Sais et al. 2008, pers comm). However, if the compatible seasonal restrictions throughout PR and federal jurisdiction waters are adhered to and enforcement efforts increase, spawning stocks of red hind may start to recover (Marshak and Appeldoorn 2007).

Monitoring the effectiveness of fishery management measures is essential for determining the short-term and long-term benefits of different strategies. Traditional methods of monitoring and studying red hind aggregations include hook and line, trap fishing, spear fishing, fishermen surveys, gonad analyses, tagging studies, and diver surveys (Colin et al. 1987, Cushion et al. 2008, Nemeth 2005, Nemeth et al. 2007, Sadovy et al. 1994, Shapiro et al 1993a, Shapiro et al. 1994, Whiteman et al. 2005). These methods are often time consuming, expensive, and potentially destructive. In addition, many of red hind spawning aggregations are located along insular shelf break areas (Nemeth 2005, Sadovy et al. 1994, Shapiro et al. 1993a), where conditions are often adverse, making data collection difficult and limited.

Passive acoustics is a novel and efficient tool that can be used to study and monitor soniferous fish behavior and habitat use, and collect long-term datasets that would be difficult to acquire with traditional methods (Luczkovich and Sprague 2002, Luczkovich et al. 2008a, Mann et al. 2008, Myrberg 1997, Roundtree et al. 2006). Sound production in a number species is known to be associated with courtship, territoriality, and reproduction, warranting the use of passive acoustics to locate spawning aggregations (Luczkovich et al. 1999, Luczkovich et al.

2008b, Rowell et al. 2011, Walters et al. 2009), and determine temporal spawning behavior and habitat use by different species (Locascio and Mann 2008, Mann et al. 2009, Mann et al. 2010, Nelson et al. 2011, Schärer et al. 2012).

Red hind produce a low frequency species-specific vocalization associated with courtship and territorial behaviors at spawning aggregations (Mann and Locascio 2008, Mann et al. 2010). Daily sound levels show trends similar to the density build-up and post-spawning departure described by Nemeth et al. (2007), with maximum levels around sunset (Mann et al. 2010) when red hind have been observed spawning (Colin et al. 1987). While passive acoustic methods have been used to describe patterns of sound production at red hind spawning aggregations, the relationship between changes in red hind sound production and density has not been established.

The purposes of this study were to: examine if changes in densities are reflected by changes in sound production in a quantitative manner, determine if density can be predicted from sound measurements recorded passive acoustically, and develop an efficient passive acoustic methodology to monitor red hind densities, spawning activity, and efficacy of fishery protections at spawning aggregations.

MATERIALS AND METHODS

Study Site. Audio recordings and diver-based underwater visual census (UVC) surveys were conducted at Abrir la Sierra (ALS), a known and previously studied red hind spawning aggregation site off the west coast of Cabo Rojo, PR (Fig. 1). Benthic habitat is dominated by pavement colonized by large barrel sponges, octocorals, and small coral colonies with low (<1 m) vertical relief at depths of 20 to 30 m. As of 1996, in conjuncture with two other sites (Tourmaline and Bajo de Sico), ALS has been closed to all fishing from December 1 to the last day of February in order to protect the red hind spawning aggregation (CMFC 1996). In 2004, a fishing ban on red hind during the spawning period was established for all PR waters (< 9 nm offshore) (PR DRNA 2004), which in 2005 was expanded to include all PR and federal waters west of 67°10'W (Federal Register 2005, PR DRNA 2007).

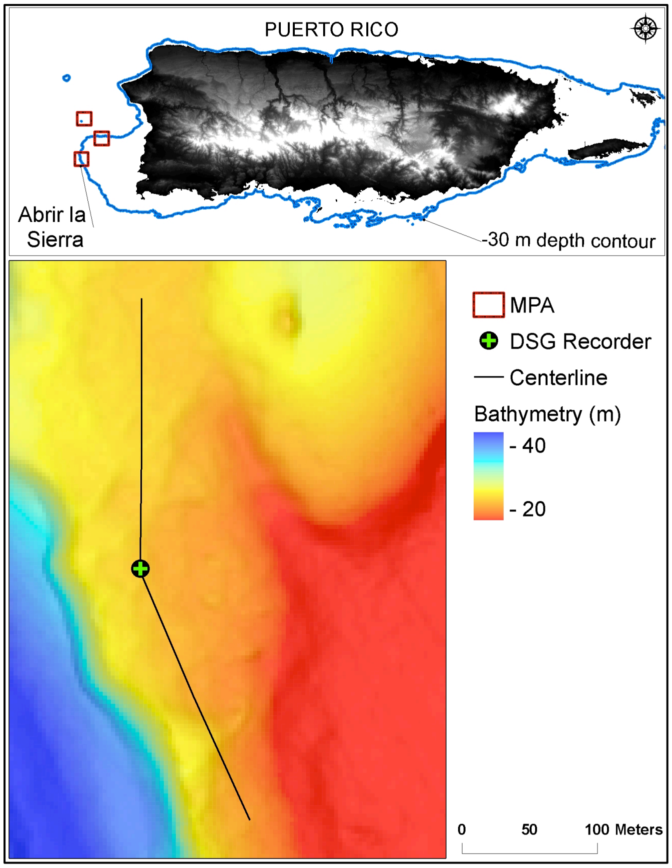


Figure 1. Location and bathymetric map of Abrir la Sierra with underwater visual census (UVC) survey centerline and Digital Spectrogram Long-Term Audio Recorder location indicated by center point. The seasonal Marine Protected Areas of Abrir la Sierra, Tourmaline, and Bajo de Sico are outlined in red.

Acoustic Recordings. A Digital Spectrogram Long-Term Acoustic Recorder (DSG; Loggerhead Instruments Inc.) was deployed at ALS from November 17, 2010 until April 30, 2011 to record passive acoustic signals. This unit consisted of a cylindrical PVC housing, single hydrophone (sensitivity = -186 dBV μPa^{-1}; frequency range 2 Hz - 37 kHz), micro-computer, circuit board, and 24 D-cell alkaline batteries. The DSG board incorporated an additional 20 dB gain and was calibrated with a 0.1 V_{peak} frequency sweep from 2 Hz to 100 kHz. Files were digitized at a sample rate of 15094 Hz for 20 seconds every 5 minutes onto a 16-gigabyte removable secure digital high capacity (SDHC) flash memory card. The DSG recorder was attached with hose clamps to rebar located within the aggregation site as estimated by previous UVC and acoustic surveys.

Underwater Visual Census Surveys. A GPS-density survey method (P. Colin, pers comm) was used for the UVC on twelve days from November 2010 to February 2011 (Table 1). Prior to the spawning season, a 400m long by 100m wide area was established with the centerline marked by subsurface buoys. The DSG was located in the middle of the 400m length of the area along the centerline. GPS-UVC surveys were conducted on each side of the centerline in order to assess red hind density over a fixed area. Surveys were conducted from north to south or reverse direction depending on the current's direction at each sampling. Red hind distributions at aggregation sites are relatively fixed with males protecting a given territory and harem (Shapiro et al. 1993a), therefore surveying the area repeatedly allowed for the documentation of daily changes in density while minimizing variance due to clustering effects.

UVC surveys began at 16:00 Atlantic Standard Time (AST) when light levels were favorable and red hind territorial, reproductive, and acoustic behaviors were known to be increasing from midday levels (Mann et al. 2010, pers comm). Two parallel belt transect surveys measuring 4 meters wide were conducted simultaneously within the survey area on all days except February 5 and February 20, when a single survey was made. Each diver towed a handheld GPS unit (Garmin GPSMAP 76Cx; Garmin Ltd.) attached to a surface buoy as a 30s interval geographic coordinate track was recorded. With the survey start and end time, the length of each transect was calculated and multiplied by 4m to determine the total area surveyed by each diver. During the survey, the number, size (total length in cm), and condition of each red hind was noted as well as the time of the observation. Red hind density (fish 100 m^{-2}) was calculated for each survey by dividing the total the number of red hind by the total area surveyed. Mean fish density day^{-1} was calculated from the two transects when available.

Analysis of Acoustic Recordings. Audio files were extracted from the SDHC cards and converted into WAV files. Red hind vocalizations from all 24 hours of recordings per day were visually and audibly identified in Ishmael 2.0 (CIMRS Bioacoustics Lab) from spectrograms generated with a Hamming window and frame size of 2048 samples. Vocalizations were summed for each day and multiplied by 15 to estimate total vocalizations day^{-1}. Following Mann et al. (2010), received sound pressure levels in the 100 to 200 Hz frequency band (band levels) were used as a second measurement of red hind sound production. Band levels were calculated in a custom MATLAB (The Mathworks, Inc.) software called DSGLab. DSGLab calculates the band level (dB re 1µPa) in the 100 to 200 Hz frequency band using 6-pole Butterworth (i.e. 36-dB/octave) high and low pass filters, a root-mean-square calculation, and a hydrophone calibration adjustment. The resulting band levels incorporated all received sound within the 100-200 Hz band into one value for each 20s file. Band levels were averaged for each day to generate a mean band level day^{-1}.

In order to determine if a single hour could be used to document daily changes in red hind sound production, in lieu of inspecting all 24 hours and thereby increasing data processing efficiency in the future, total vocalizations $hour^{-1}$ and mean band levels $hour^{-1}$ were calculated for 18:00-19:00 AST and compared to the 00:00-23:55 AST values. The 18:00-19:00 AST hour was chosen as it was the closest hour period to the diver surveys without research vessel noise interference, and coincided with the known time of red hind spawning (Colin et al. 1987) and daily peak sound production (Mann et al. 2010, pers comm). Regression statistics of total vocalizations day^{-1} (00:00-23:55 AST) vs total vocalizations $hour^{-1}$ (18:00-19:00 AST) and mean band level day^{-1} (00:00-23:55 AST) vs mean band level $hour^{-1}$ (18:00-19:00 AST) were calculated using Excel Data Analysis Tools (Microsoft Corp.).

Daily recordings from 18:00-19:00 AST were re-examined to quantify vocalizations and develop a standardized replicable methodology that limits analyst variability regarding which vocalizations should be included in the analyses, i.e. how loud a vocalization needs to be. Vocalizations were manually identified and isolated for each 20s file in Adobe Audition 3 (Adobe Systems Inc.) from spectrograms generated with a Hamming window and resolution of 1024 bands. The isolated red hind vocalizations were then verified audibly. Band levels (100-200 Hz) for each isolated vocalization were calculated in MATLAB as previously described. A frequency histogram of all vocalization band levels was generated using Excel Data Analysis Tools (Microsoft Corp.). Individual vocalizations with a band level of 105 dB re 1µPa or greater were quantified for each file. For each day, vocalizations were summed and multiplied by 15 to estimate standardized total vocalizations $hour^{-1}$ (18:00-19:00 AST).

Regression equations were generated for mean fish density day^{-1} vs standardized total vocalizations $hour^{-1}$ (18:00-19:00 AST) and mean fish density day^{-1} vs mean band level $hour^{-1}$ (18:00-19:00 AST) on days in which both survey density and passive acoustic data were collected. A regression between standardized total vocalizations $hour^{-1}$ (18:00-19:00 AST) and mean band level $hour^{-1}$ (18:00-19:00 AST) was also examined for the entire recorded period. Fish density was predicted for the entire sampling period from the standardized total vocalizations $hour^{-1}$ and mean band level $hour^{-1}$ vs mean fish density day^{-1} regression equations.

RESULTS

UVC surveys were successfully conducted throughout the preset area on all days except January 19, when divers were diverted from the centerline of the survey area by strong currents. Survey data demonstrated two periods of increased red hind density (Table 1, Fig. 2A, B). Density increased following the full moons on December 21, 2010 and January 19, 2011 and abruptly decreased in the beginning of February. Diver surveys were only conducted twice in December (vs six times in January), limiting conclusions of when density likely peaked in December. During the days surveyed in January, density peaked on January 27, 8 days after the full moon (DAFM). Fish density in between the full moons of December and January was above non-spawning season resident density, but markedly lower than peak density in January.

Table 1. ***Epinephelus guttatus*** **mean densities day^{-1} (individuals 100m^{-2}), total vocalizations hour^{-1}, and mean band levels hour^{-1} (100-200 Hz; dB re 1µPa) for 18:00-19:00 AST on days in which underwater visual census (UVC) surveys were conducted.**

Date	Days After Full Moon	Mean Density ± SD	Vocalizations	Mean Band Level ± SD
17-Nov-2010	25	0.17 ± 0.02	0	99.2 ± 0.8
20-Dec-2010	29	0.16 ± 0.05	0	99.8 ± 0.8
27-Dec-2010	6	1.04 ± 0.28	135	102.6 ± 1.4
14-Jan-2011	24	0.81 ± 0.48	NA	NA
19-Jan-2011	0	0.51 ± 0.07	300	104.9 ± 1.0
23-Jan-2011	4	1.37 ± 0.23	NA	NA
25-Jan-2011	6	2.11 ± 0.68	450	108.0 ± 1.6
27-Jan-2011	8	3.15 ± 0.16	420	108.7 ± 1.4
29-Jan-2011	10	1.68 ± 0.17	285	107.6 ± 2.4
05-Feb-2011	17	0.08	0	100.4 ± 1.1
20-Feb-2011	2	0.20	0	99.9 ± 0.9
23-Feb-2011	5	0.10 ± 0.05	0	100.9 ± 0.8

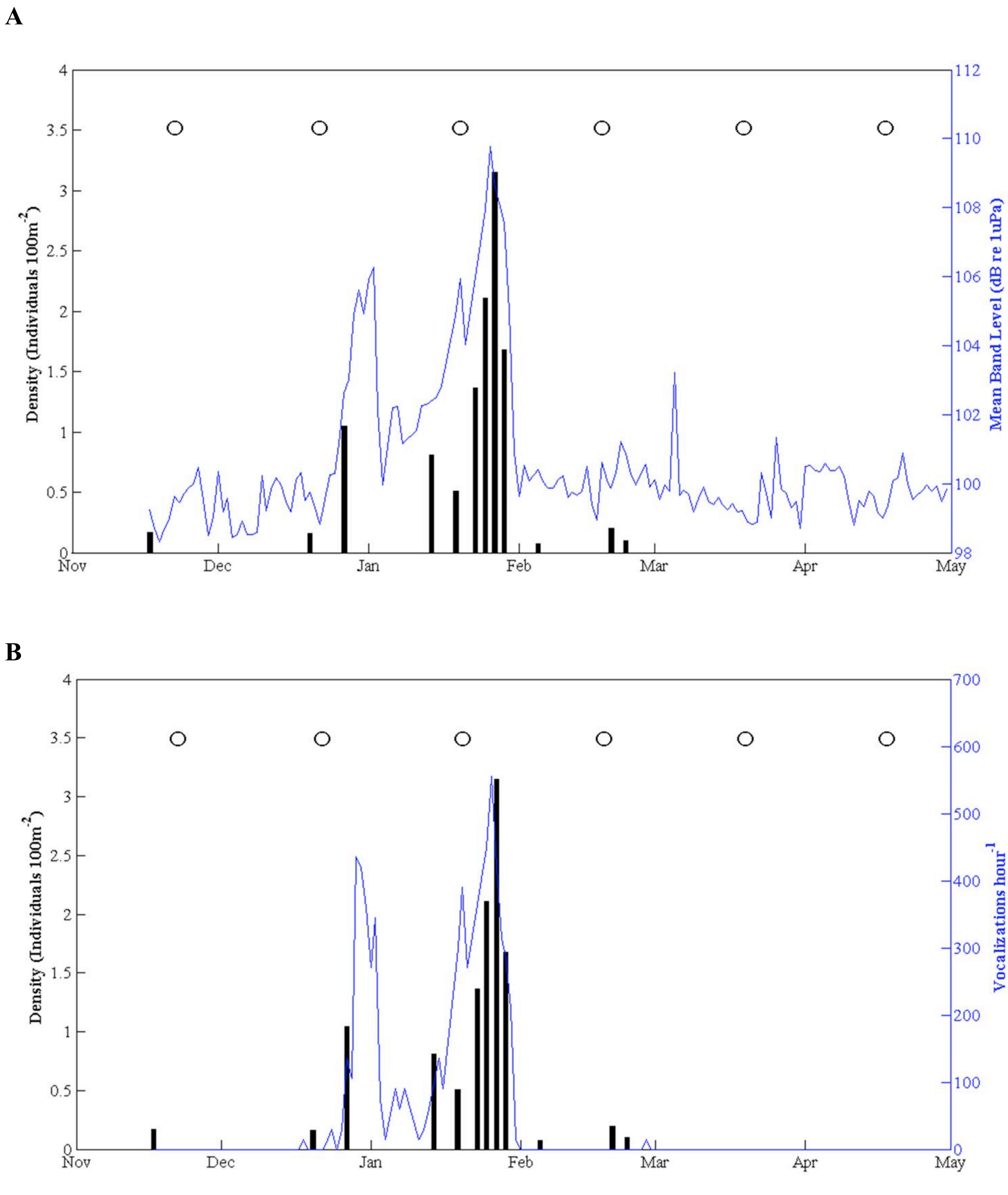


Figure 2. Surveyed *Epinephelus guttatus* densities (black bars) with (A) mean band levels hour^{-1} (100-200 Hz; dB re 1µPa; line) and (B) total vocalizations hour^{-1} (line) during 18:00-19:00 AST hour for the entire sampling period. Full moons are indicated with open circles.

The DSG audio recorder successfully recorded sound at the aggregation site from 18:00-19:00 AST on all days during the sampling period with the exception of ten days. On January 5, 9, 10, 14, 17, 18, 22, 23, 24, and February 4 there was a malfunction with the SDHC flash memory card that resulted in the truncation of select files during the extraction process. These ten days were excluded from all analyses of sound production. Linear regressions were significant for total vocalizations day^{-1} (00:00-23:55 AST) vs total vocalizations hour^{-1} (18:00-19:00 AST; r^2=0.724, $p < 0.001$) and mean band level day^{-1} (00:00-23:55 AST) vs mean band level hour^{-1} (18:00-19:00 AST; r^2=0.674, $p < 0.001$). Therefore, the 18:00-19:00 AST hour was selected as a suitable indicator of daily changes in red hind sound production for the development of an efficient replicable procedure to relate sound production to density. The frequency histogram reveals the maximum occurrence of vocalization band levels to be 56 at 104, 105, and 108 dB re 1µPa (Fig. 3). Of the 609 vocalizations isolated, 464 (76.2%) had band levels greater than 105 dB re 1µPa.

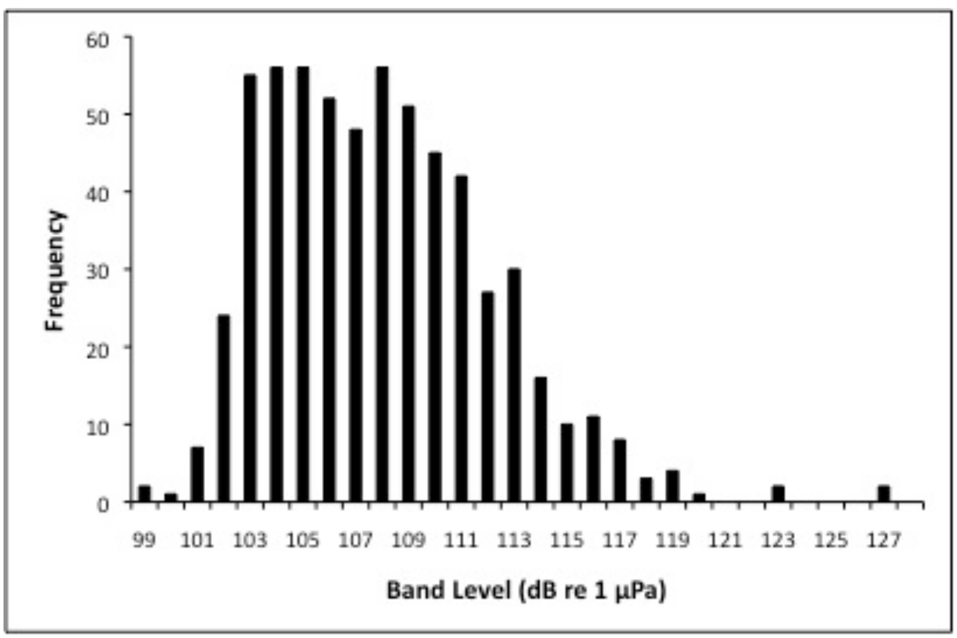
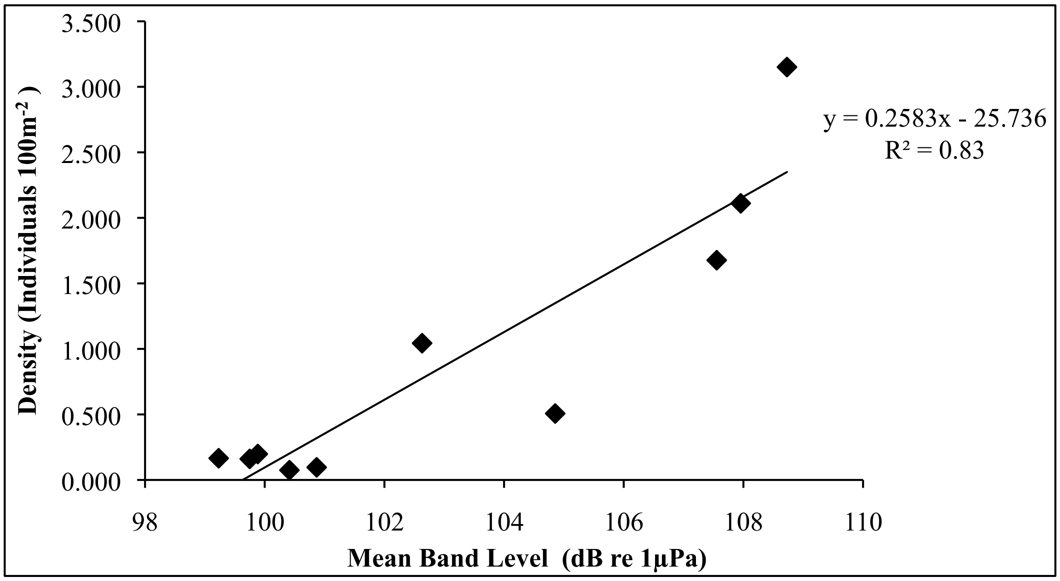


Figure 3. Frequency histogram showing the distribution of isolated vocalization band levels; n=609.

Mean band levels hour^{-1} (18:00-19:00 AST) and standardized total vocalizations hour^{-1} (18:00-19:00 AST) reveal two periods of increased sound production following the December and January full moons (Fig. 2A, B). Mean band levels and total vocalizations began to prominently increase 4 and 5 DAFM in December, respectively. Mean band levels peaked 11 DAFM (January 1) and vocalizations 8 DAFM (December 29) before both rapidly fell 13 DAFM. During the interim period between the December and January full moons, sound production was relatively low compared to peak levels, but was still above non-spawning period levels. Both sound measurements began to again increase steeply on January 19, the day of the full moon, before reaching their maxima 7 DAFM. Sound production began to fall markedly 11 DAFM in January, returning to non-spawning period sound levels 13 DAFM. The duration of elevated sound production was broader in January than December, with band levels and vocalization frequencies also higher following the January full moon. There was no increased sound production associated with the February full moon.

A



B

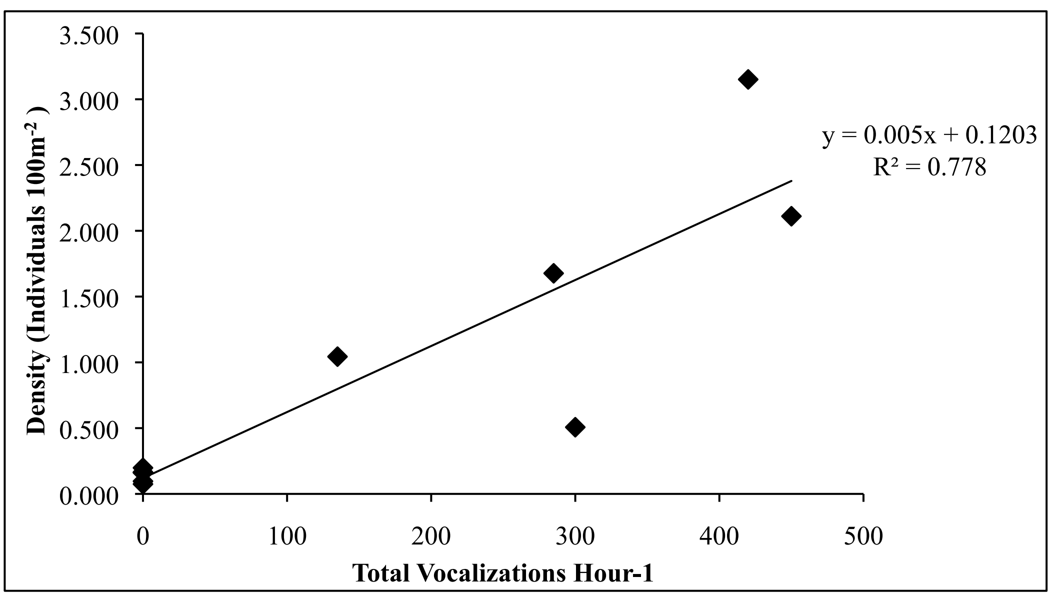


Figure 4. Regressions of surveyed *Epinephelus guttatus* density vs (A) mean band level hour^{-1} (18:00-19:00 AST; $p < 0.001$) and (B) total vocalizations hour^{-1} (18:00-19:00 AST; $p < 0.001$) on days in which both underwater visual census (UVC) density and passive acoustic data were collected.

Linear regressions were significant for mean band level hour^{-1} (18:00-19:00 AST) vs mean fish density day^{-1} (r^2=0.83, $p < 0.001$; Fig. 4A), standardized total vocalizations hour^{-1} (18:00-19:00 AST) vs mean fish density day^{-1} (r^2=0.778, $p < 0.001$; Fig. 4B), and mean band level hour^{-1} (18:00-19:00 AST) vs standardized total vocalizations hour^{-1} (18:00-19:00 AST; r^2=0.831, $p < 0.001$). The linear regressions of mean band level hour^{-1} (L) and vocalizations hour^{-1} (V) with mean fish density day^{-1} (D) yielded two equations (Equation 1, 2), respectively, which were used to predict red hind density.

$$D = 0.2583(L) - 25.736 \qquad \text{(Eq. 1)}$$

$$D = 0.005(V) + 0.1203 \qquad \text{(Eq. 2)}$$

Density predicted from both mean band level hour^{-1} and total vocalizations hour^{-1} depicts density patterns over the entire sampling period at ALS (Fig. 5).

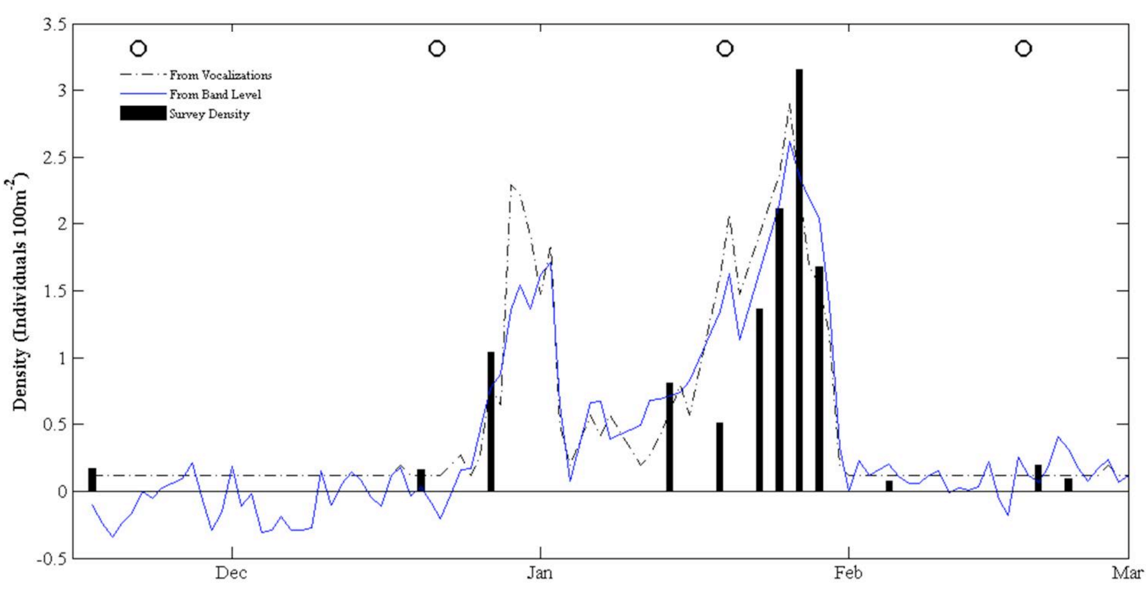


Figure 5. ***Epinephelus guttatus*** **densities (individuals 100m^{-2}) from underwater visual census (UVC) surveys (black bars), and predicted densities from total vocalizations hour^{-1} (dashed black line) and mean band levels hour^{-1} (100-200 Hz; dB re 1μPa; solid blue line). Full moons are indicated with open circles.**

DISCUSSION

The significant regressions of sound production during the 18:00-19:00 AST hour vs sound production during 00:00-23:55 AST revealed that the 18:00-19:00 AST hour reflected patterns of daily changes in vocalization totals and mean band levels. As a result, selecting the 18:00-19:00 AST hour for comparison to density accurately depicted daily changes in sound production without requiring the examination of all 24 hours. The selection of the 18:00-19:00 AST hour was based on a thorough understanding of red hind acoustic and reproductive behavior coupled with the dependent timing of diver surveys: red hind sound production has a diel periodicity, peaking around dusk (Mann et al. 2010) and with lulls at midday and midnight (pers observ). This contrasts, for example, aggregating goliath grouper, *Epinephelus itajara*, whose sound production peaks between the hours of 01:00-04:00, and the 18:00-19:00 hour is relatively quiet (Mann et al. 2009). An acoustic based density study focused on the latter time period would be less likely to portray daily changes in goliath grouper sound production and density. Therefore, prior to designing an efficient replicable long-term acoustic monitoring methodology, an understanding of reproductive and acoustic behavior over the course of the entire day and extended temporal scales is essential. Once species-specific behavioral patterns are known, processing efficiency can be increased by selecting a sampling schedule that accurately yields acoustic behavioral patterns at the desired scale.

The two measurements of sound production utilized differed in terms of processing efficiency. Calculating mean band level was quick and time efficient, while manually counting total vocalizations per file visually and audibly was more time consuming. However, it is arguable that information gathered from the manual examination of files exceeded what could have been inferred solely from band level measurements. Processing audio files manually reveals what other soniferous species are present at a given site, and may lead to new discoveries of habitat use or behavioral patterns for targeted and untargeted species.

Both mean band levels hour^{-1} and standardized vocalizations hour^{-1} revealed similar temporal patterns and amplitudes from November to April. The significant relationship between the two measurements implies that they are interchangeable given the conditions at ALS. However, this may not be true for other locations; band levels incorporate all sources of sound within the selected frequency band, such as from boats, other fishes, and marine mammals. If ALS had other aggregating soniferous species with either frequent or high-energy vocalizations within the 100-200 Hz band, such as yellowfin grouper, *Mycteroperca venenosa*, (Schärer et al. 2012) or red grouper, *Epinephelus morio*, (Nelson et al. 2011), band levels would have been influenced by the acoustic signals of these species. As a result, multi-species spawning aggregations may require the manual processing of acoustic data. However, in the case of red hind and yellowfin grouper in PR, each species typically spawns during different months, red hind in December-February, yellowfin grouper in March-May. Therefore, if the biology and reproductive behaviors of individual species are known, band levels can still be used if the investigator can confidently identify and isolate the source species. Our study area did not have this complication during the spawning period; red hind were the only species consistently producing high intensity sounds within the 100-200 Hz band from November till May, and vessel associated sound was near absent. Beginning in March, the DSG unit did record the calls of humpback whales, which accounted for the isolated high mean band levels in the month of March.

The red hind densities estimated from UVC surveys and predicted from sound measurements portray temporal patterns that have been documented at other aggregation sites

(Nemeth et al. 2007). Densities built and rapidly fell for the December and January full moons, with a percentage of individuals remaining on site between moons. The surveyed and predicted densities depict large daily changes in fish abundance over the spawning season at a resolution unattainable with traditional methods. Sound production and predicted densities suggest that the January spawning event was larger than the December event, which is corroborated by previous studies and predictions given the timing of the December and January full moons (Cushion et al. 2008, Nemeth et al. 2007).

The timing of peak predicted density and peak sound production for January was 7 DAFM, which is later in the lunar cycle than peak densities and presumed spawning at other known red hind aggregation sites in PR and the USVI. Colin et al. (1987) reported spawning 1 DAFM, Shapiro et al. (1993a) and Nemeth et al. (2007) found densities to peak on the day of the full moon, while Whiteman et al. (2005) deduced that spawning likely occurred onward from 3 days prior to the full moon. In 2007, Mann et al. (2010) also documented a delay in peak red hind sound production at ALS when compared to another aggregation site at Mona Island, PR. The timing of spawning in relation to lunar periodicity appears to differ among sites but not within sites. Passive acoustic units can be deployed at a number of aggregations with limited effort to quantify differences in sound production, density, and spawning patterns between sites and years. Long-term acoustic studies have the ability to accurately predict the months and days aggregations form and spawning likely occurs at a site specific level. Site idiosyncrasies may be a product of environmental conditions, such as temperature and ocean currents (Nemeth et al. 2007, 2008), and should be addressed in future studies.

By surveying over a fixed area, density for ALS was documented within a $0.04\ km^2$ portion of the greater spawning aggregation area, and hence these density estimations are not technically random with respect to the entire aggregation site, which was both wider and longer. Additionally, the area surveyed likely extended beyond the sound reception range of the DSG recorder. As a result, the recorded sound measurements should be considered as relative indices of density that did not directly correspond to densities over the entire aggregation or within the entire survey area, but did portray relative daily changes in density. If red hind density, cluster distribution, and behavioral patterns were assumed to be uniform over the entire aggregation site, then sound levels could be used to predict fish density for the entire site. In order to determine the exact density in which sound levels correspond to, studies on red hind sound propagation at ALS need to be conducted. With knowledge of propagation loss for red hind vocalizations, a radius of detection could be determined and be used to survey and calculate density within the range of the DSG recorder.

Male red hind are the only sex believed to produce reproductive vocalizations (Mann et al. 2010); thus, it possible that sound production only reflects male densities. Males have been found to arrive at the aggregations site prior to females (Nemeth et al. 2007, Whiteman et al. 2005) in order to establish territories (Colin et al. 1987); therefore, if male acoustic behavior was independent of female presence and was only a product of territoriality among males, sound production should appear to be roughly constant throughout the time males are located at the aggregation site. The recordings at ALS after the full moons contradict the idea that sound production is solely male density driven. Recorded vocalizations during male-male and male-female interactions suggest males vocalize as part of both courtship and territorial behaviors (Mann et al. 2010). Therefore, it is probable that the arrival of females induces increases in male acoustic territorial competition and courtship behaviors. As a result sound production likely reflects the densities of both males and females. Stimuli for red hind acoustic behaviors need to be studied in order to understand the effects of male and female densities on sound production at spawning aggregations.

Sound production and densities over the entire sampling period highlight the abrupt changes in fish densities and behaviors that occur during the red hind spawning season at ALS. These rapid increases in density have historically been targeted by fishermen, often yielding large short-term profits with limited effort. Densities during the January spawning event increased to nearly 35 times over the non-spawning season density. The prominent localization of red hind during such a short period of time leaves little doubt that fishing aggregations can have dramatic and quick felt negative impacts on a spawning stock whose home range supports an entire fishery. Unfortunately, red hind are still being landed, bought, and sold in PR during the spawning season with little resistance from regulatory enforcement, despite the current seasonal protections (pers comm), and is likely preventing the level of red hind stock recovery seen in the USVI (Nemeth 2005).

Evaluating the effects of closed areas and seasonal fishing bans is crucial for the support of current and future management strategies. Vocalization totals or received sound band levels from passive acoustic recordings may be used as a proxy for density in determining the effectiveness of management protections, collecting data at a higher temporal resolution than may be possible with traditional methods. Yearly changes in densities can be monitored by analyzing changes in levels of sound production. Red hind sound production can also be used to study the timing of migrations to and from aggregation sites and presumed spawning, providing important information on site usage and when regulatory enforcement efforts should be maximized. Passive acoustic density estimation methods can be expanded to other soniferous species, especially species known to form spawning aggregations. With many species' spawning aggregations in peril, passive acoustic methods can efficiently monitor spawning and density patterns of soniferous species throughout the world. With knowledge of the acoustic behavior of a targeted species, acoustic sampling schedules can be chosen to answer specific questions while increasing data processing efficiency. Additionally, signal processing and acoustic technologies are constantly improving and may be able to accurately carry out automated vocalization detections in the near future, enabling batch processing of data in near real-time. Sound production and inferred densities can be monitored at a number of sites simultaneously to document daily, monthly, and yearly changes, help assess the condition and productivity of stocks, and provide important information for fisheries managers and stakeholders.

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