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Michelle T. Schärer[1], Michael I. Nemeth[1], David Mann[2], James Locascio[2], Richard S. Appeldoorn[1], and Timothy J. Rowell[1]

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Reproductive behaviors and sound production of the Yellowfin grouper (*Mycteroperca venenosa*) are described from *in-situ* observations by divers as well as synchronous underwater audio-video recordings at a spawning aggregation off Mona Island, Puerto Rico. The highest abundances of *M. venenosa* estimated from underwater visual surveys were detected in March and April, five to nine days after the full moon (DAFM). Four distinct color phases were observed for *M. venenosa*; two of these were unique to the spawning aggregation and one, the white-headed phase, was exhibited during interactions with other conspecifics corresponding to courtship displays. Variations in color phases during fish interactions and group formation coupled with sounds preceded spawning, which occurred near sunset. Low-frequency (<150 Hz) sounds produced by *M. venenosa* were variable yet they were classified into two types, pulsed and tonal. Both types of sounds were associated with reproductive behaviors although not linked to spawning rushes. These sounds were most frequent between 1800 and 2100 h, peaked the eighth DAFM, and ceased between 11–13 DAFM. Temporal patterns in sound production suggest that peak reproduction occurred in April followed by a smaller aggregation in May of 2010. The association of passive acoustics with reproductive behaviors for *M. venenosa* provides a tool to help identify spawning aggregation sites and monitor spawning stock abundance to evaluate the effectiveness of management and conservation efforts for this Near Threatened grouper.

THE Yellowfin grouper, *Mycteroperca venenosa* (Serranidae), was one of the most abundant groupers in the Caribbean, but in the last few decades landings have declined significantly, and it is currently classified as Near Threatened by the World Conservation Union, IUCN (Brulé and García-Moliner, 2004). A principal factor in this decline is its reproduction in transient spawning aggregations (Beets and Friedlander, 1992; Sadovy et al., 1994; Domeier and Colin, 1997; Claro and Lindeman, 2003), which makes it (as well as other groupers) vulnerable to overfishing (Sadovy, 1994; Domeier and Colin, 1997). In Puerto Rico approximately 27 known reef fish spawning aggregations identified by fishers no longer form due to unregulated fishing (Ojeda-Serrano et al., 2007), a pattern representative of other Caribbean localities (Sadovy et al., 1994; Matos-Caraballo et al., 2006).

Spawning aggregations of *M. venenosa* have been described in association with other large groupers such as Tiger grouper (*Mycteroperca tigris*), Nassau grouper (*Epinephelus striatus*), and Black grouper (*Mycteroperca bonaci*; Beets and Friedlander, 1992; Colin, 1992; Sadovy et al., 1994; Nemeth et al., 2006; Tuz-Sulub et al., 2006; Starr et al., 2007; Heyman and Kjerfve, 2008; Kadison et al., 2010). At Mona Island, Puerto Rico, *M. venenosa* aggregate to spawn at a site that is also used by Red hind (*Epinephelus guttatus*) and *M. tigris* (Nemeth et al., 2007; Schärer et al., 2010) despite the fact that spawning aggregations were the target of commercial fisheries that significantly reduced grouper populations, most notably *E. striatus* (Colin, 1982; Sadovy, 1999). Today the remote, uninhabited islands of Mona and Monito have relatively less impacted grouper populations than other heavily fished areas of the region. Stallings (2009) found the sighting frequency of predatory fishes at Mona Island to be 2.4 times higher than other sites around Puerto Rico.

Fish emit sounds in association with various forms of behavior including aggression, courtship, spawning, threat responses, fright, and feeding (Connaughton and Taylor, 1995; Kasumyan, 2009). Some fishes like croakers and drums (Sciaenidae) form large spawning aggregations, which can produce loud, sustained sounds with temporal patterns often correlating with courtship and mating (Locascio and Mann, 2005). Sounds present a novel approach to study the dynamics of spawning aggregations in a noninvasive, cost-effective manner with potential applications for soniferous groupers such as the Goliath grouper, *Epinephelus itajara* (Mann et al., 2009), Red hind, *E. guttatus* (Mann et al., 2010), and Red grouper, *E. morio* (Nelson et al., 2011). However, the association between sound production and the reproductive behavior of *M. venenosa* is unknown; understanding this relationship could provide a valuable research and management tool.

The purpose of this study is to describe the abundance patterns, reproductive behavior, and characterize the sounds produced by *M. venenosa* during a spawning aggregation. We analyzed and quantified the abundance of this species and provide data on the sounds they produce in order to investigate temporal patterns during reproductive periods. Passive acoustic monitoring is an approach that can be used to remotely study the behavior of sound producing fishes, as long as the behavioral context is understood. The relation between sound production and reproductive behaviors may provide important insight applicable to the study and monitoring of these critical events.

MATERIALS AND METHODS

Mona Island and the smaller Monito Island are located 72 km west of Puerto Rico (18°05.0′N, 67°54.0′W; Fig. 1), and each has a separate, relatively narrow, submerged insular platform dominated by hard bottom and coral reef habitats. Because of the depths (>1000 m) in the Mona

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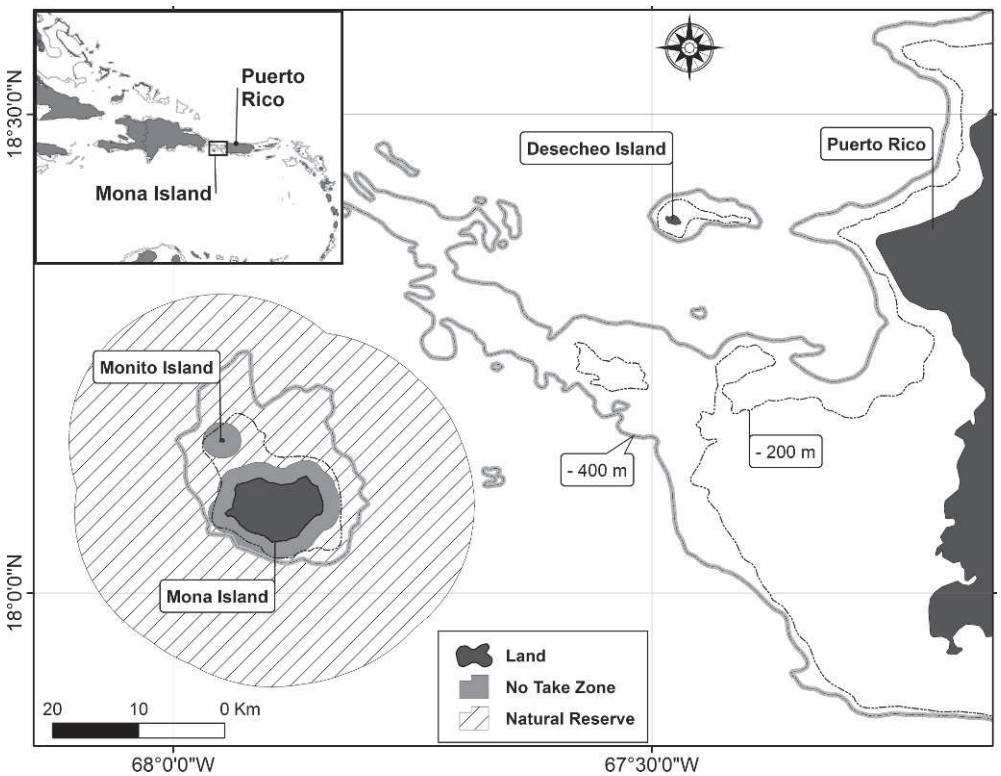

Fig. 1. Location of Mona and Monito Islands in the Mona Passage west of Puerto Rico.

Passage surrounding these islands, they may represent stepping stones for regional connectivity (Dennis et al., 2005) within a biogeographical filter that has been proposed for some coral reef species (Taylor and Hellberg, 2003; Baums et al., 2006). Waters extending out to nine nautical miles around both islands are designated as a natural reserve, which is the largest marine protected area (MPA) in Puerto Rican waters (Aguilar-Perera et al., 2006). Within the MPA there is a no-take zone, which currently extends to one nautical mile from the shore of Monito and Mona Islands, although fishing is permitted from Mona Island's camping beaches (DNER, 2010).

The aggregation site is located at the shelf break between 20 and 50 m depth, where the benthic habitat is low relief seafloor, colonized with scattered corals, octocorals, sponges, and macroalgae interspersed with sand patches in slight depressions. Currents can be strong and generally flow parallel to the shelf break. This site is frequented by other species of groupers, including *E. guttatus* and *M. tigris,* although they are separated spatially and temporally (Nemeth et al., 2007). At least ten other reef fish species have been observed spawning at this site (Schärer et al., 2010).

Divers estimated the abundance and size of groupers following the GPS-density survey method of underwater visual surveys conducted as drift dives through the aggregation site (P. Colin, pers. comm.). Groupers within 5 m of each side of a centerline were enumerated. The area covered by each survey was estimated by recording a continuous track of geographical coordinates with a handheld GPS unit, enclosed in a dry-bag, and attached to the surface dive buoy towed by divers. The length of each transect was multiplied by 10 m to estimate fish density (individuals/100 m^2). For each individual the fork length (FL) was visually estimated to the nearest cm with the aid of a PVC T-bar measuring 50 cm wide. Each individual's coloration pattern (i.e., color phase and other markings), body characteristics (i.e., distended abdomen), and behaviors were also noted. From 2005 to 2010 we conducted 82 underwater survey transects during the peak annual reproductive period reported for groupers in the Caribbean (December to May) to establish the dynamics of this spawning aggregation.

Three methods were utilized to identify and characterize behaviors and sounds produced by *M. venenosa* at the site with increasing time periods of data collection: 1) *in-situ* observations while video recording behavior and sound; 2) autonomous audio/video recorder systems deployed for period of up to 16 h; and 3) long-term audio (no video) recordings of 120 d. The observations of reproductive behaviors were made by divers using open and closed circuit SCUBA and recorded with underwater digital video cameras fitted with external hydrophones on the housing. The autonomous, audio/video recorder had a stationary video camera system coupled with hydrophones that recorded continuously throughout the day without human presence (Nelson et al., 2011). The audio/video recorder has of a low-light video camera (SSC-108WXXB.0003 Lux Low Light B/W 420 Line Board Lens) with a digital recorder (Chasecam Solid State digital recorder model PDR100, Mo-Tec USA) inside a PVC housing with a transparent acrylic lens. Two external hydrophones (High Tech, Inc., Gulfport, MS, HTI-96-MIN Series, sensitivity = -164 ± 1 dB re: 1V/μPa; flat response between 2 Hz–37 kHz) were used, one attached to the rear of the housing and the other at the end of a 1.4 m tether to help discriminate the approximate distance of sound production. Audio was recorded in two channels at a sampling rate of 44.1 kHz together with video on MPG-2 files (monochrome, dimension 640 × 480) to a digital memory card with 32-gigabyte capacity. This recording system was deployed at noon in order to capture sunset and sunrise for up to 16 hours. The system was mounted on a

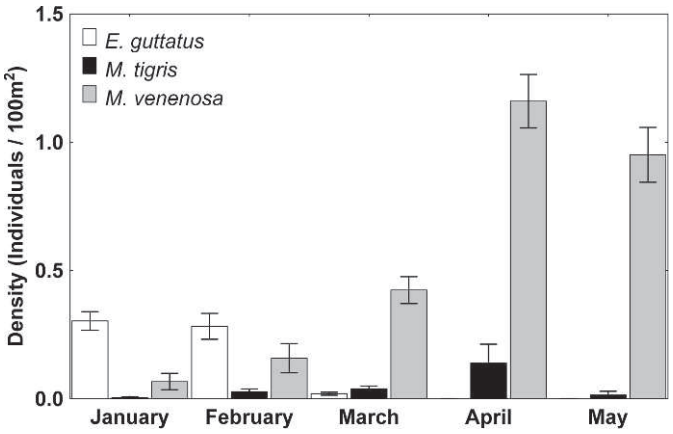

Fig. 2. Mean (SE) density (individuals/100 m^2) of *E. guttatus, M. tigris,* and *M. venenosa* by month, for years 2006–2010.

tripod set on the seafloor within the aggregation site during 2008, 2009, and 2010 on days after the full moon (DAFM) between February and May.

The long-term audio recordings at the aggregation site were made using a digital spectrogram recorder (DSG-Ocean, Loggerhead Instruments) mounted approximately 1.5 m above the bottom. Recordings were made continuously from 7 April to 14 July 2010 (127 d) with a recording interval of 20 s every 5 min. This unit consists of a cylindrical PVC housing, a single HTI-96-MIN hydrophone, micro-computer, circuit board, and was powered by D-cell alkaline batteries. Single-channel audio was recorded at a sample rate of 50 kHz, and was saved on a 16-gigabyte digital flash memory card.

Video files in which *M. venenosa* were present were used to extract and analyze the potential sounds produced by the fish. Sounds from the long-term audio files matching the physical parameters of sounds identified from the video files as probably produced by *M. venenosa* were manually isolated. These 16-bit (.wav) files were analyzed with a custom MATLAB program (HotWav), filtering signals between 30 and 1000 Hz, determined call duration, peak frequency, and sound levels with 5 Hz of resolution. To verify the suitability of these sounds as a proxy for incidence of *M. venenosa* the data from video files were analyzed using a Chi-squared contingency table. The sound and visual presence of fish were quantified from randomly selected clips (30 s intervals) during two days (5 and 6 April 2010) of continuous audio/video recordings.

We identified and defined calls of *M. venenosa* as the sound recorded by the video methods during the occurrence of displays between *M. venenosa*. The occurrence and frequency of species-specific calls was quantified by listening to 20 s audio files from the long-term recorders with headphones. The number of calls per 20 s interval was grouped by one-hour intervals, and medians were compared statistically to detect diel and lunar patterns. A Kruskal-Wallis non-parameteric ANOVA was used to compare the number of calls during the 1900 h interval (1900–1959) between the DAFM of April and May 2010. A Wilcoxon Matched Pairs Test was used to compare the number of calls on DAFM between these months. Finally a Hobo TBI32-05+37 recorder (Onset Computers) deployed at the spawning aggregation site during 2008 and 2010 provided water temperature at depth.

RESULTS

Abundances.—Density estimates from the GPS surveys identified the three most common groupers during the reproductive season as *E. guttatus, M. tigris,* and *M. venenosa,* with a few Yellowmouth grouper, *Mycteroperca interstitialis*. Due to logistical and weather constraints not all months were sampled equally; nevertheless, the mean abundance of *M. venenosa* was 9.8 and 1.7 times greater than *M. tigris* and *E. guttatus,* respectively. The relative abundance of species exhibited distinct peaks during consecutive months (Fig. 2). *Epinephelus guttatus* were found in greatest density during January and February, *M. tigris* was relatively more abundant in April, and for *M. venenosa* the highest mean density was during April and May. For the latter species the density in April and May was three times higher than in March, with peaks between five and nine DAFM (Table 1).

The areas surveyed ranged between 1,500 m^2 to 9,300 m^2 (mean = 4,972 m^2, SE = 226). Mapping of the survey GPS tracks revealed that each of the three main grouper species occupied distinct areas of the aggregation site (Nemeth et al., 2007). *Epinephelus guttatus* occupied the northern zone at depths less than 30 m, *M. tigris* was limited to a small area on the western edge of the site at the shelf break between 25 and 33 m, while *M. venenosa* aggregated on the slope from 33 to 50 m depth. Body size estimates for *M. venenosa* ranged from 25 to 100 cm FL (mean = 63.7 cm, SE = 1.05), with a few individuals smaller than 51 cm FL (Fig. 3), the average length at sexual maturity (Thompson and Munro, 1978).

Behavior.—During daytime diver surveys *M. venenosa* were distributed sparsely, with most individuals positioned near the seafloor at cleaning stations or underneath ledges, although some were observed swimming slowly, up to 5 m above the seafloor. Four distinct color phases were observed for *M. venenosa*. The most common coloration was the blotched phase, with rows of irregular shaped reticulate dark blotches overlaid on smaller gray spots on a lighter gray or white background. This phase is observed commonly during non-aggregation time. Secondly, the dark color phase is completely black although the yellow margins of the pectoral fins are still visible. The dark phase was also common outside of the aggregation when fish are under ledges or in darker backgrounds (Fig. 4A). Both of these color phases were observed during peak aggregation and non-aggregation time.

Two additional color phases were seen at the aggregation. The bicolor phase is dark gray on the dorsal half of the body that contrasts with the lighter or white ventral portion. This color phase was observed on individuals of all sizes, including fish with distended abdomens. Under artificial light the darker dorsal section is bright red. Secondly, a white-headed phase was observed on larger individuals as they interacted with conspecifics (Fig. 4B). The white-headed phase consists of lighter colored cranial markings, a darker gray (almost black) body, and highly contrasting white fins (pectoral, dorsal, anal, and caudal) with thin marginal black bands. Individuals exhibiting this color phase also had yellow patches on the maxillae, which indicates the reproductive state of males during spawning aggregations (Tuz-Sulub et al., 2006). In addition to the maxillae, yellow markings were observed over the eyes, on the anterior dorsal fin, and the dorsal portion of the caudal peduncle. These yellow markings were not ephemeral and were seen only on larger (>60 cm FL) fish of all color phases, yet consistently on the

Table 1. Data from Underwater Visual Surveys Including Year, Month, Day after the Full Moon (DAFM), Number of Transects (*n*), Mean Density (Fish/100 m^2), and Maximum Number of *M. venenosa* per Transect (Max).

Year	Month	DAFM	*n*	Density	Max
2005	January	2	1	0.02	1
		3	1	0	0
	March	4	1	0.56	30
2006	January	0	1	0.01	1
		1	2	0.06	7
	February	−4	2	0.02	2
	March	−1	2	0.25	9
		0	2	0.32	16
		1	2	0.14	8
		2	2	0.43	25
2007	January	1	4	0.02	3
		2	2	0	0
		3	1	0.05	3
		4	2	0.2	18
	February	3	2	0.13	10
		4	2	0.16	12
		5	4	0.13	23
		6	1	0.23	10
	March	2	4	0.21	21
		3	3	0.18	15
		5	1	0.85	22
2008	January	3	1	0.67	10
	February	−2	1	0.08	7
		3	1	1.11	70
		4	2	0.53	50
	March	8	2	0.77	48
2009	January	−2	2	0	0
		−1	2	0.04	4
		0	2	0.01	1
	March	6	2	0.56	18
		7	3	0.75	50
		8	2	0.79	41
2010	January	4	1	0.03	2
	February	4	5	0.01	4
		5	2	0.03	3
		24	2	0.02	2
	March	10	4	0.29	30
	April	5	1	1.02	25
		6	2	1.31	44
		7	1	0.99	53
	May	9	2	0.95	71

white-headed phase. Smaller fish displaying the other three color phases, and gravid individuals did not exhibit yellow body markings except the yellow margins of the pectoral fins. The highly contrasting white-headed phase was observable at distances greater than 5 m by divers and on videos, but yellow markings were only detected at less than 3 m from divers, and not observable on black and white video from the autonomous recorder.

Interactions between two or more individuals were observed commonly and were initiated by presumed males (based on size and coloration; Tuz-Sulub et al., 2006). These displays were directed toward presumed females (lacking yellow body markings) of smaller size and that sometimes presented a distended abdomen. Preceding the display the male accentuated the white 'sunburst' markings on the anterior portion of the head (flashing) as it approached the female. When positioned alongside the female it made a sharp 90° turn above, during which it twitched and undulated the body and head vibrating laterally. These displays occurred both near the seafloor or high in the water column (up to 10 m off the seafloor); however, no direct contact (rubbing or bumping) between fish was observed. During twitching displays divers near the fish heard a low rumbling sound that was also detected on video recordings made by divers and by stationary cameras. No interactions of this type were observed between males; instead, these were subtle and mainly consisted in one individual taking over the position of another at a cleaning station. Similar interactions initiated by male *M. tigris* toward gravid females as well as agonistic behaviors between males were observed by divers and on audio/video recordings; however, no sounds were detected.

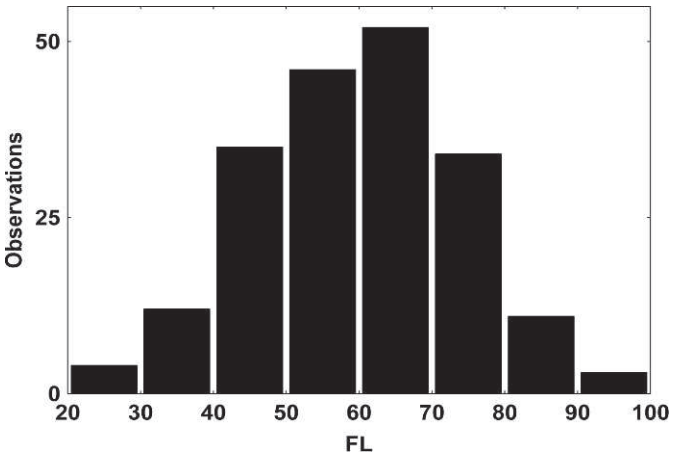

Fig. 3. Size frequency distribution of *M. venenosa* based on diver's visual estimates of fork length (cm) during underwater surveys.

Displays between *M. venenosa* were more frequent after 1400 h when fish became more active and swam two to 10 m above the seafloor along the shelf break. During peak aggregation days, after 1700 h most fish coalesced into a large group and swam in a streaming pattern with individuals following each other, rising at 45° angles up to 15 m above the seafloor. During these movement patterns fish rose slowly, away from the shelf break, toward deeper areas (>40 m), perpendicular to the depth contour later returning downward toward the shelf break. These downward swimming movements were sometimes accelerated by the presence of large reef sharks (*Carcharhinus perezii*, Carcharhinidae). Near sunset *M. venenosa* were swimming high in the water column as a large tight group. During this time reproductive displays by white-headed individuals and sound production were ongoing in the water column away from the seafloor.

Spawning.—Spawning of *M. venenosa* was recorded by the automated audio/video recorders once during each of three consecutive years. The first was on 29 March 2008 (8 DAFM) at 1835 h (sunset at 1838 h). Three individuals were seen parting from the main group; one approached from deeper water, and two from the shelf break (at 40 m depth). All three met briefly at mid-depth (at 25 m above the seafloor), and the individual approaching from off-shelf accelerated toward the surface while the other two joined into a spawning rush, to which a fourth male arrived and spawned

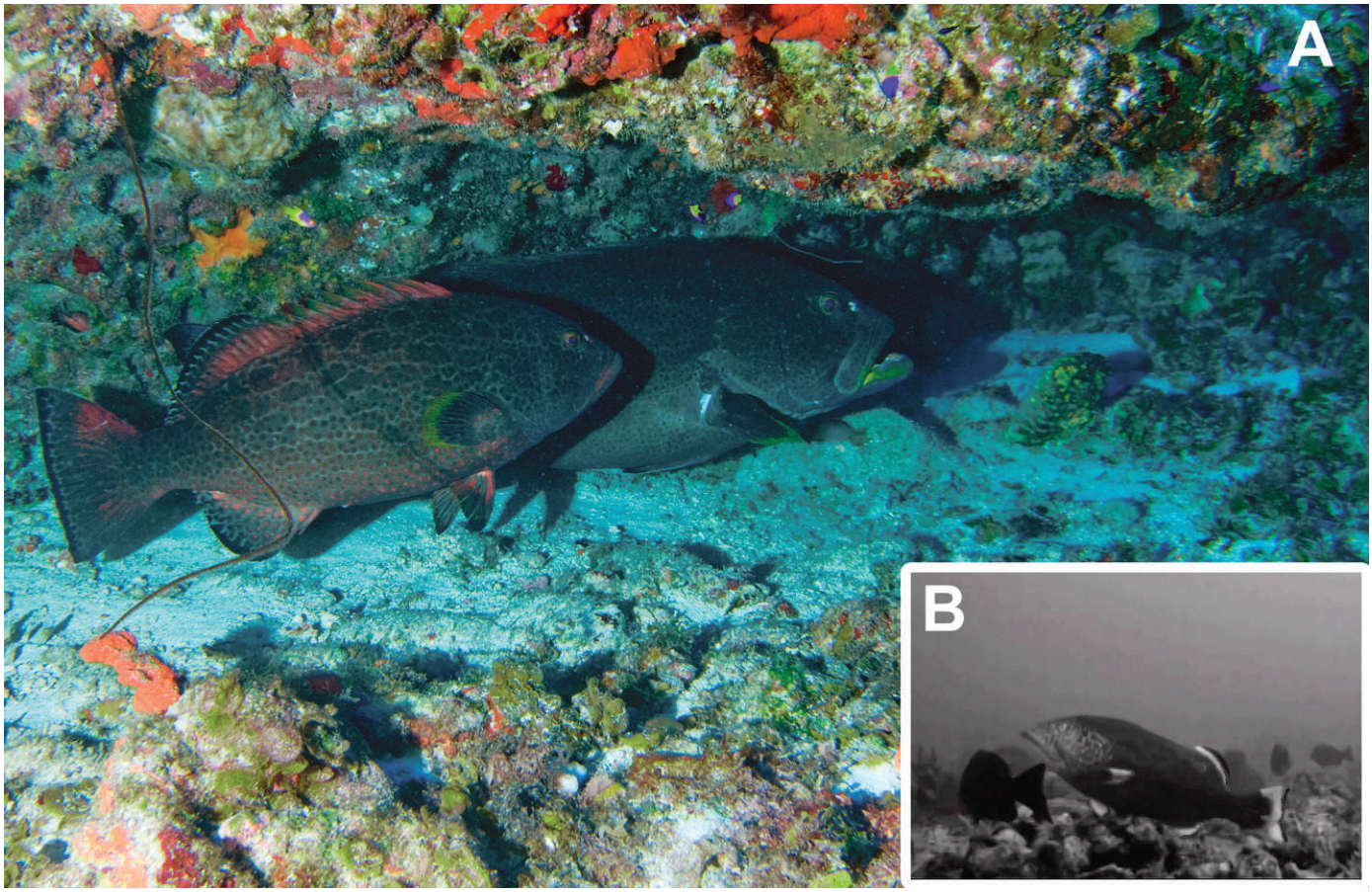

Fig. 4. (A) Sexual dimorphism in the dark color phase *M. venenosa;* the female (smaller size) exhibits red coloration and the male (larger size) yellow markings on maxilla. (B) Monochrome photo extracted from the stationary video camera of a white-headed phase *M. venenosa*.

after spawn had been released from the group. After spawning, all individuals swam slowly downward in separate directions toward deeper water. The cloud of spawn was seen drifting toward the east near the surface.

The following year, spawning was recorded on 17 April 2009 (8 DAFM) at 1858 h (sunset at 1842 h). On this occasion, four fish swam vertically from mid-depth and were joined by two more when spawn was released upward. During this event the low light did not allow us to observe the direction of the cloud of spawn. The third spawning event was recorded on 5 April 2010 (7 DAFM), when two distinct spawning rushes of five and six individuals occurred between 1838 and 1841 h (sunset at 1839 h). Water temperature during the periods around first and third spawnings were between 26.0–26.5°C, with 26.2–26.4°C at the time of actual spawning at 33 m depth. During spawning no sounds were detected, although this could be due to the distance of the spawning location from the hydrophones (over 20 m).

Sound production.—During group formation and displays by *M. venenosa* a series of sounds generated by the fish were detectable *in situ* by divers and recorded with the hand-held digital video camera with hydrophones as well as the automated audio/video recorder. Two main types of calls were identified that are different from those produced by other species such as squirrelfish (Holocentridae) and *E. guttatus* (Mann et al., 2010).

The two main types of sounds include a rather uniform pulse train (Fig. 5A) and a more variable series of faster pulses or tonal call (Fig. 5B). These were heard separately or in sequences that included both pulse and tonal calls (Fig. 5C). Descriptive statistics were generated for each of the two main types using calls with high signal to noise ratios and sound levels >100 dB, resulting in 23 pulse train calls and 34 tonal calls being analyzed. Pulse calls (Fig. 5A) had a duration of 2.96 ± 0.97 s (mean ± SD) with a range of 1.40 to 5.13 s, and peak frequency of 120.46 ± 7.54 Hz ranging from 101.4 to 132.4 Hz. Tonal calls (Fig. 5B) lasted 3.14 ± 0.95 s ranging between 1.29 and 5.69 s, and had a peak frequency of 121.04 ± 12.57 Hz (range 88.9 to 141.7 Hz). Maximum sound levels were slightly higher for the tonal call (132.6 dB re: 1 μPa) than for the pulse call (128.2 dB re: 1 μPa).

The sounds of *M. venenosa* detected in clips from the automated audio/video recordings were positively correlated with their presence in the video frame (n = 145 observations; Chi-square test P = 0.004). There were occasions, mostly during morning hours, when fish were in view, but no sounds of *M. venenosa* were detected.

Temporal patterns in sound production.—Long-term passive acoustic data recorded during April and May 2010 revealed a marked diel pattern in sound production of *M. venenosa*. The mean number of calls ranged from one to four (per 20 s interval), with significantly higher median values (Kruskal-Wallis test: H = 174.845, $P < 0.001$) after sunset (Fig. 6). Calls of *M. venenosa* were no longer detected 13 DAFM in April, and 11 DAFM in May. The median number of calls during the 1900 h interval was significantly different

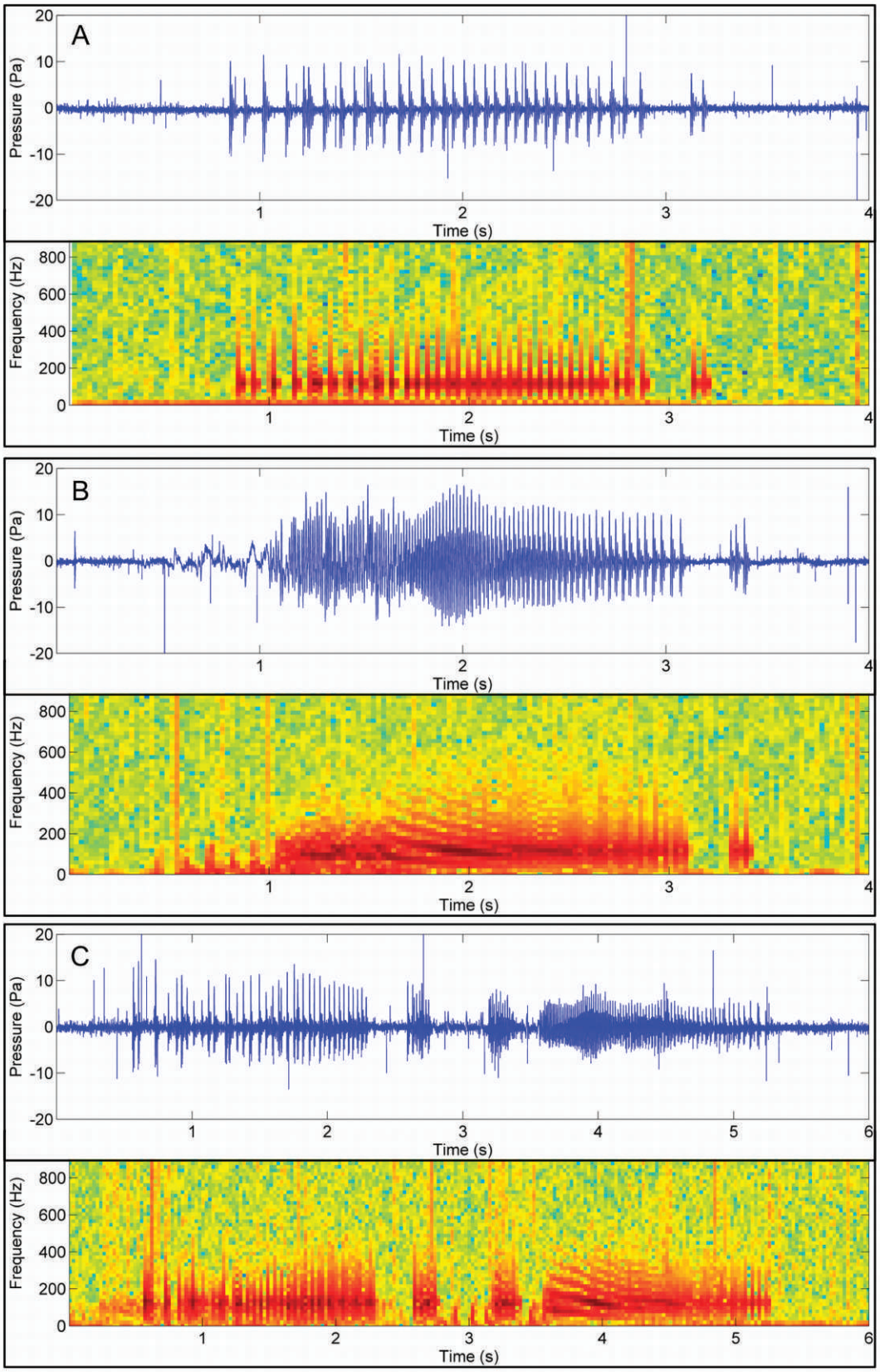

Fig. 5. Oscillograms and spectrograms of sounds produced by *M. venenosa.* (A) Pulse train call, (B) tonal call, and (C) sequential combination of the two types of calls (FFT size = 256, no overlap).

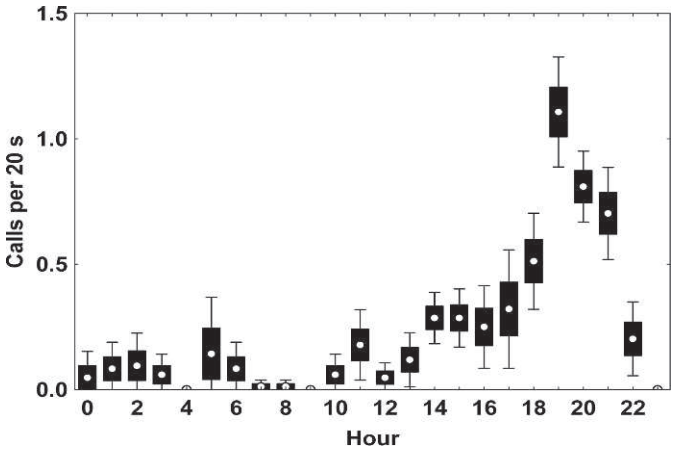

Fig. 6. Number of calls of *M. venenosa* per 20 s interval (boxes are Standard Errors and whiskers are 95% Confidence Interval), averaged per hourly interval throughout the day (from recordings between 5–11 April 2010; 7–13 DAFM).

(Kruskal-Wallis test: April: KW-H = 17.5206, $P = 0.0036$; May: KW-H = 14.554, $P = 0.0124$) among DAFM and higher in April compared to May 2010 (Fig. 7). The median number of calls during the 1900 h interval was significantly higher (Wilcoxon Matched Pairs Test; $P < 0.00001$) on the eighth DAFM in April compared to May.

DISCUSSION

Reproductive behavior.—The color phases observed for *M. venenosa* were similar to previous reports (Smith, 1971; Thompson and Munro, 1974), including spawning aggregations (Nemeth et al., 2006; Tuz-Sulub et al., 2006). Distinct color phases have been suggested to be depth or size related, associating red colored fish with deep habitats (Smith, 1971), although smaller reddish juveniles become brown as they grow (Thompson and Munro, 1974). However, we have observed the red color with artificial light on the smaller sized individuals at the spawning aggregation, which is near deep (50 m) habitats. The bi-color phase may be a result of background red coloring on the dorsum (Nemeth et al., 2006), and if so the individuals described as 'reds' captured from deep waters could have been aggregated to spawn, as the original descriptions were of nearly ripe individuals (Smith, 1971).

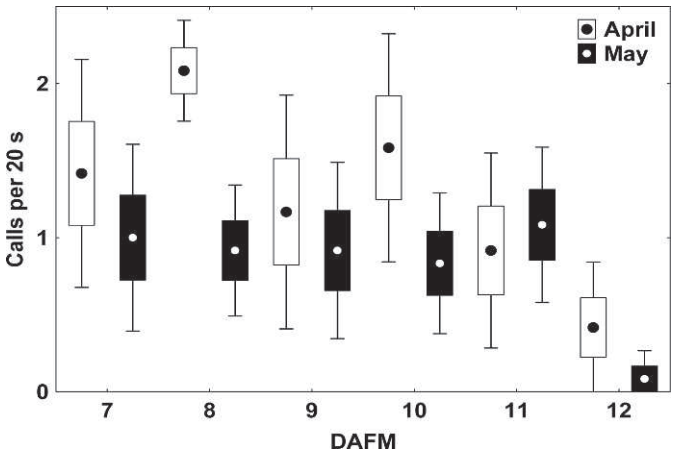

Fig. 7. Number of calls of *M. venenosa* per 20 s interval (boxes are Standard Errors and whiskers are 95% Confidence Interval), from 1900 to 2000 h, on days after the full moon (DAFM) during April and May 2010.

Some of the color phases observed for *M. venenosa* during the aggregation are common to other species in the genus. The white-headed phase in *M. venenosa* was characteristic of larger males and resembles the reproductive color phase of male *M. tigris* (Sadovy et al., 1994; Tuz-Sulub et al., 2006), the ephemeral 'sunburst,' or 'flashing' of white on the head by male *M. bonaci* (Heyman and Kjerfve, 2008; Paz and Sedberry, 2008), and the bicolor phase of male *M. microlepis* (Gilmore and Jones, 1992). Yellow markings on the maxillae of larger *M. venenosa* indicative of sexually active males, irrespective of color-phase (Tuz-Sulub et al., 2006), were observable underwater as long as color was appreciable. Sexual dichromatism and reproductive color phases may help estimate sex ratios at spawning aggregation without removing individuals from the population.

The mean sizes (TL) of females and males of *M. venenosa* at Grammanik Bank were 64.5 cm and 75.4 cm, respectively (Nemeth et al., 2006), while in Cuba the smallest reproductive sizes of individuals were 58.2 cm and 77 cm for females and males, respectively (García-Cagide and García, 1996). No individuals smaller than 50 cm TL were reported from the Grammanik Bank (Nemeth et al., 2006); however, at Mona Island many smaller (<50 cm TL), non-gravid *M. venenosa* were observed. These smaller sized fish were perhaps not reproductively active, but their presence could result from the attraction to conspecifics' sounds propagating from the spawning aggregation.

Spawning by *M. venenosa* was observed in video recordings from the automated camera and is similar to reports from other locations (Nemeth et al., 2006; Starr et al., 2007). *Mycteroperca venenosa* is a protogynous hermaphrodite with indeterminate gamete production (García-Cagide and García, 1996), and spawning has been characterized as group spawning (Domeier and Colin, 1997) similar to *E. striatus* (Colin, 1992) or *M. tigris* (Sadovy et al., 1994), in which various males follow a gravid female away from the main group rushing toward the surface to release a gamete cloud. However, *M. venenosa* are more like *E. striatus* in that they rise from the seafloor to form a large group from which spawning rushes are made. This contrasts from *M. tigris* that remain close to the seafloor during reproductive and territorial displays.

Underwater visual surveys by GPS track method were most effective during the day (before 1600 h), mainly because fish were mostly stationary, associated with the seafloor, or occupying ledges near the shelf break. During later afternoon hours *M. venenosa* coalesced away from the seafloor, rose in the water column, and moved away from the shelf as a group similar to that described at the Grammanik Bank (Nemeth et al., 2006). This behavior increases their spatial patchiness, reduces the area occupied by fish within the aggregation site, and in turn reduces the probability of encountering them. At this time a different methodology is necessary to accurately evaluate fish abundances. Our surveys and videos suggest the maximum number of *M. venenosa* at the aggregation could be between 150 and 300 individuals. However, surveys to accurately assess numbers and subsequent changes in abundance at the aggregation could be affected by small-scale changes in the spatial and temporal distribution patterns, as well as the characteristics of reproductive behaviors on a species-specific, per-site basis.

Timing of spawning.—In U.S. Caribbean waters under Federal jurisdiction the closed season for *M. venenosa*, along with other grouper species (Grouper Unit 4), extends from February through April of each year, and recently regulations have been made compatible for *M. venenosa* in the local jurisdiction that extends from shore to nine nautical miles (DNER, 2010). At Mona Island *M. venenosa* aggregated from January through May, beyond the closed season and the spawning period reported for Puerto Rico (Erdman, 1956; Matos-Caraballo and Posada, 2000) and elsewhere in the Caribbean (Munro et al., 1973; García-Cagide and García, 1996; Nemeth et al., 2006; Cushion et al., 2008; Heyman and Kjerfve, 2008). In Cuba April and May were found to be the strongest spawning months (García-Cagide and García, 1996), and although spawning was observed at Mona Island during March and April, their presence in spawning color patterns and the sound data suggest fish were conducting reproductive behaviors through May. Additional recordings will be necessary to determine the constancy of these temporal patterns, as this is the first continuous recording of passive acoustics of a spawning aggregation of *M. venenosa*. Nevertheless, sound recordings indicate that fish presence at the aggregation site is highly variable, which is an important consideration when assessing abundances and formulating or evaluating the effectiveness of management strategies.

The occurrence of the full moon during the end of the month could also explain spawning later in the season. For example, full moon was on 28 April, with peak reproductive days, eight DAFM, as inferred from the acoustic data, during May in 2010. Differences in fish density and sound recordings suggest spawning activity of *M. venenosa* is highest between six and ten DAFM, comparable to that in Belize, where peak abundances occur six to 14 DAFM (Heyman and Kjerfve, 2008). At Mona Island, spawning was observed seven and eight DAFM, although gaps in data may affect this conclusion. The lack of calls after 13 DAFM in April suggests that the reproductive behaviors ceased either because fish departed altogether, fish density decreased, or due to a lack of interactions between fish; nonetheless, calls were detected again in significant numbers between seven and 11 DAFM in May.

Timing and behavioral context of sound production.—Reproductive behavior and species-specific sound production for *M. venenosa* are described here for the first time. Although they were known to be capable of producing sound upon artificial stimuli (Fish and Mowbray, 1970) sounds produced by this species had not been documented as part of reproductive behaviors in a natural setting. Low frequency sounds of *M. venenosa* produced during interactions were associated with apparent courtship displays and during group formation prior to spawning, which provides a new consideration for studying their reproduction and behavioral ecology. Similar low frequency sounds have been described for other spawning groupers such as *Epinephelus itajara* (Mann et al., 2009), *E. guttatus* (Mann et al., 2010), and *E. morio* (Nelson et al., 2011). However, sounds of *E. itajara* are of a single, high amplitude impulse type that contrasts from *E. guttatus* and *E. morio*, which share further characteristics in the time and frequency domain with the sound produced by *M. venenosa*. Some sounds could be ascribed to particular behaviors in damselfish (Lobel, 1992; Mann and Lobel, 1997), and variations in sounds may be due to fish size, temperature, and sex. Sounds have also been related with reproductive advertisement to females or as a territorial signal in the case of *Pomacentrus partitus* (Myrberg, 1997) and with spawning events for *Dascyllus albisella* (Lobel and Mann, 1995; Mann and Lobel, 1998). Sound production reported in the weakfish (*Cynoscion regalis*) was restricted to males and induced by steroid hormones (Connaughton and Taylor, 1995). Sex change in groupers may also be caused by changes in hormonal levels that respond to external cues, and sound production may have an important function during the reproductive season.

Lateral displays and body quivering or twitching are common features in courtship or agonistic encounters of fishes (Erisman et al., 2007; Nelissen, 1991; Bleckmann, 1993). The courtship displays of *M. venenosa* are analogous to the flashing or shaking displays described for *M. bonaci* (Paz and Sedberry, 2008), *M. rosacea* (Sala et al., 2003), and *M. tigris* (Sadovy et al., 1994). The combination of reproductive displays and sound production suggests these interactions can be classified as courtship-associated sounds in which visual and acoustic stimuli are combined. These sounds could play an important role in the reproductive biology of groupers for reproductive isolation during multi-species aggregations (attracting conspecifics), as a form of mate selection (if the acoustic signal is related to fitness) or a method for opponents to evaluate the condition of competitors.

The reproductive behaviors of *M. venenosa* were similar to those described for *M. rosacea* (Sala et al., 2003) in which displays associated with courtship continued throughout the day while spawning was limited to sunset hours (Erisman et al., 2007). The temporal patterns in sound production of *M. venenosa* were similar to those of *E. guttatus* (Mann et al., 2010), which followed diel and lunar cycles. Diel patterns in sound production have been reported for other fishes (Winn et al., 1964; Connaughton and Taylor, 1995; Rowe and Hutchings, 2006), although the relationship of sounds and gamete release has not been documented. Increased sound production was also documented at night prior to spawning for a pomacentrid (Parmentier et al., 2010). Nevertheless the sound levels at a spawning aggregation site may be a function of the number of interactions between fish and is possibly density dependent. Therefore, a better understanding of the link between sound and behavior may increase our ability to examine the dynamics of spawning aggregations.

Remote video methods allowed for the observation of natural fish behavior, without interference, over longer periods than underwater surveys by divers. The association of sound with reproductive behaviors is an important piece of evidence that may help generate practical applications of passive acoustics to the study of spawning aggregations. This technology may provide an additional approach to detect the presence of a particular species at a spawning aggregation, define the geographical limits of the aggregation site, and detect temporal patterns of reproductive activity. The results from the present study may aid in establishing effective methods to assess reproductive activity and evaluate the effectiveness of management efforts. This information is essential for safeguarding the critical events that conserve threatened grouper populations in the region.

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