

Sound production by dusky grouper *Epinephelus marginatus* at spawning aggregation sites

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Sound production by the dusky grouper *Epinephelus marginatus* was monitored both in captivity and at two Mediterranean spawning sites during the summers of 2012 and 2013. The results of long-term passive acoustic recordings provide for the first time a description of the sounds produced by *E. marginatus*. Two types of sounds were mainly recorded and consisted of low-frequency booms that can be produced singly or in series with dominant frequencies below 100 Hz. Recordings in captivity validated these sounds as belonging to *E. marginatus* and suggested that they may be associated with reproductive displays usually performed during early stages of courtship behaviour. This study also allowed the identification of a third, low-frequency growl-like type of sound typically found in other grouper species. These growls were, however, not recorded in tanks and it is cautiously proposed that they are produced by *E. marginatus*. Acoustic signals attributed to *E. marginatus* were produced throughout the spawning season, with a diel pattern showing an increase before dusk, *i.e.* from 1900 to 2200 hours, before decreasing until the morning. The occurrence of sounds during the spawning season of this species suggests that they are probably involved in social activity occurring close to aggregation sites. Passive acoustics offer a helpful tool to monitor aggregation sites of this emblematic species in order to improve conservation efforts.

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Key words: animal communication; conservation; Mediterranean Sea; passive acoustics; reproductive behaviour.

INTRODUCTION

Numerous species of marine fishes are known to produce sounds in association with aggressive postures and with courtship displays (Fish & Mowbray, 1970; Ladich & Myrberg, 2006). These behaviours occur particularly within the spawning season, during which an increased vocal activity has been associated with reproductive activities in many families such as the Sciaenidae (Connaughton & Taylor, 1995; Locascio & Mann, 2008), Pomacentridae (Mann & Lobel, 1995; Parmentier *et al.*, 2010), Batrachoididae (McKibben & Bass, 1998), Percidae (Johnston & Johnson, 2000), Gadidae (Hawkins & Amorim, 2000), Gobiidae (Malavasi *et al.*, 2009; Parmentier *et al.*, 2013)

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or Ophidiidae (L. Kever & E. Parmentier, unpubl. data). Besides their role during courtship interactions (e.g. announcing male readiness to spawn, attracting gravid females and synchronizing gamete release), sounds may have other functions such as territorial defence or aggregating group members (Myrberg & Lugli, 2006). Monitoring sound production may therefore allow different aspects of the biology of fishes to be investigated.

In recent years, passive acoustics has been used as a non-invasive method to perform continuous monitoring of fishes over long periods of time by using sound production as a reliable natural acoustic signal to detect fish aggregation sites or to identify and follow seasonal activities of soniferous species in the field (Mann & Lobel, 1995; Luczkovich *et al.*, 1999, 2008). This method has thus become a useful tool in conservation studies.

Many representatives of the family Serranidae have already been documented to produce sounds (Table I). Acoustic signals of the goliath grouper *Epinephelus itajara* (Lichtenstein 1822) (Mann *et al.*, 2009), the red hind *Epinephelus guttatus* (L. 1758) (Mann *et al.*, 2010), the red grouper *Epinephelus morio* (Valenciennes 1828) (Nelson *et al.*, 2011), the yellowfin grouper *Mycteroperca venenosa* (L. 1758) (Schärer *et al.*, 2012a), the Nassau grouper *Epinephelus striatus* (Bloch 1792) (Schärer *et al.*, 2012b) and the black grouper *Mycteroperca bonaci* (Poey 1860) (Schärer *et al.*, 2014) have recently been characterized at spawning locations by means of passive acoustic recording. In these grouper species, results show that sounds are made during the spawning period and are associated with courtship behaviour, supporting the idea that the sounds are related to spawning activity.

The dusky grouper *Epinephelus marginatus* (Lowe 1834) is a serranid distributed from the British Isles (although rare) to South Africa, from the Bermuda Islands to Argentina and is widespread in the Mediterranean Sea (Heemstra & Randall, 1993; Irigoyen *et al.*, 2005). It is considered as an emblematic species, closely associated with the Mediterranean rocky and reef habitat (Pastor *et al.*, 2009). The species is considered overexploited in its entire distribution range (Reñones *et al.*, 1999; Coll *et al.*, 2004) and is classified as endangered in the IUCN red list of threatened species (Cornish & Harmelin-Vivien, 2004). *Epinephelus marginatus* is a territorial species that inhabits shelter-rich, hard substrata to depths of 250 m with maximum densities <50 m in depth (Harmelin & Harmelin-Vivien, 1999; La Mesa *et al.*, 2002). The common colour consists of a dark-brown body with a white mottled pattern. *Epinephelus marginatus*, however, shows also a variety of colour patterns that can change during the reproductive season according to the size class, sex or condition of individuals (Louisy, 1996; Zabala *et al.*, 1997a, b). As an example, male individuals present more white blotches on their body at the beginning and at the end of the spawning season. This pattern turns into a silver streaked territorial template in dominant males. In September, probably because of physical and physiological fatigue, the same individuals can display an overall light grey pattern (Zabala *et al.*, 1997b). *Epinephelus marginatus* is a monandric protogynous hermaphrodite species in which mature female size ranges between c. 40 and 100 cm total length (L_T) while males are generally larger and vary from c. 60 to 120 cm L_T (Reñones *et al.*, 2010). The largest individuals are sedentary and territorial with a high degree of site fidelity during winter and early spring when their activity and density are low (Zabala *et al.*, 1997b; Lembo *et al.*, 2002; Pastor *et al.*, 2009). Large males, however, can stay out of their shelters and swim in open water (Zabala *et al.*, 1997b).

TABLE 1. Summary of sound analysis performed in different species of groupers and referenced in the literature. Sound description is also given for *Alphesthes afer*, *Cephalopholis fulva*, *Epinephelus adscensionis*, *Epinephelus drummondhayi*, *Epinephelus nigritus*, *Mycteroperca microlepis* and *Mycteroperca* in Fish & Mowbray (1970)

Species	Sound description and time of production	Sound characteristics	Associated behaviour	References
<i>Epinephelus morio</i>	One loud boom	Call DF: 150 ± 51 Hz (mean \pm s.d.)	Solitary patrolling male	Fish & Mowbray (1970)
	Series of 1–4 pulses followed by a growl (short call)	Call dur: 1.9 ± 0.3 s	Darkening of dorsum	Nelson <i>et al.</i> (2011)
	Between sunrise and sunset	Pulses intro dur: 161 ± 33 ms	Courtship with or without body contact	
	Increase during late afternoon	Pulses intro IP: 355 ± 51 ms Growl dur: 0.7 ± 0.2 s Growl DF: 112 ± 56 Hz Intensity: 124 ± 6 dB re 1μ Pa RMS		
	Rapid 10–50 pulses train following a short call	Call DF: 131 ± 63 Hz (mean \pm s.d.)	Solitary patrolling male	
<i>Epinephelus guttatus</i>	Low level throughout day and night	Call dur: 7.7 ± 5.4 s	Darkening of dorsum	
		Pulses intro dur: 153 ± 43 ms Pulses intro IP: 370 ± 43 ms Growl dur: 1.8 ± 0.2 s Growl DF: 89 ± 55 Hz Pulse train dur: 113 ± 42 ms Pulse train IP: 201 ± 76 ms Intensity: 122 ± 4 dB re 1μ Pa RMS	Courtship with body contact	
	Series of grunts			
	Deep grunt or boom			
	Thumps			Fish & Mowbray (1970)

TABLE I. Continued

Species	Sound description and time of production	Sound characteristics	Associated behaviour	References
<i>Epinephelus itajara</i>	Single or irregular groups of short pulses (grunt-like)	FF: c. 100 Hz		Tavolga (1968)
	Series of pulses followed by tonal parts ('whooh-woo')	DF: 166 ± 36 Hz (mean ± s.e.)	Patrolling male	Mann <i>et al.</i> (2010)
	Peaked near dusk (1700 to 1900 hours)	Dur: 1.78 ± 1.02 s		
	Maximum 7–8 days after full moon	DF: 213 ± 23 Hz (mean ± s.e.)	Not directed to particular fish Males interact with females (less common)	Rowell <i>et al.</i> (2012)
	Fell 11 days after full moon	Dur: 1.04 ± 0.63 s	Not during male–male interactions Light coloured pattern on the head	
	Tremendous boom			Fish & Mowbray (1970)
	Sometimes followed by two smaller grunts			
	Several loud bursts			
	Single pulse	DF: 60.5 ± 9.2 Hz (mean ± s.d.)		Mann <i>et al.</i> (2009)
	Highest between 0100 and 0300 hours	Pulse dur: 132.0 ± 30.1 ms		
Reduced around full moon	Intensity: 124–143.8 dB re 1 µPa RMS			
Multiple pulses				
No distinctive pulses	Broad frequency range			
Deep booms				
<i>Epinephelus striatus</i>				Fish & Mowbray (1970)

TABLE I. Continued

Species	Sound description and time of production	Sound characteristics	Associated behaviour	References
<i>Mycteroperca venenosa</i>	Vibrant grunts, single or in series			
	Sustained rumbles			
	Train of 6–13 pulses	Train DF: 77.4 ± 30.3 Hz (mean \pm S.D.) Pulse dur: 0.09 ± 0.02 s DF: 99.0 ± 33.6 Hz Dur: 1.6 ± 0.3 s	Possibly alarm or warning	Schärer <i>et al.</i> (2012b)
	Tonal sound		Courtship and reproduction	
	Between 2000 and 2100 hours (1.5 h after sunset)			
	Highest 7–9 days after full moon			
	Ceased 12 days after full moon			
	Loud, deep boom			
	Uniform pulse train	DF: 120.46 ± 7.54 Hz (mean \pm S.D.) Dur: 2.96 ± 0.97 s Maximum intensity: 128.2 dB re 1 μ Pa DF: 121.04 ± 12.57 Dur: 3.14 ± 0.95 s	Swimming high as a tight group White-headed individuals	Fish & Mowbray (1970) Schärer <i>et al.</i> (2012a)
	Faster pulses, tonal call		Twitching display Presumably males, white-headed phase	
Most frequent after sunset (1800 to 2100 hours)				
Peaked 8 days after full moon				
Ceased 11–13 days after full moon				

TABLE I. Continued

Species	Sound description and time of production	Sound characteristics	Associated behaviour	References
<i>Mycteroperca bonaci</i>	Single booms Sustained thundering rolls Loud percussive sound Bursts of 5 pulses Short pulses followed by a longer tonal portion 2 h prior to sunset (1700 to 1800 hours) Maximum 10 days after full moon	Rapidly dropping pitch Tonal DF: 83.5 ± 8.6 Hz (mean \pm s.d.) Tonal dur: 4.3 ± 1.3 s Tonal intensity: 131.3 dB re 1 μ Pa RMS	Swimming sideways and twitching One fish with white-headed phase	Fish & Mowbray (1970) Tavolga (1968) Schärer <i>et al.</i> (2014)

DF, dominant frequency; FF, fundamental frequency; dur, duration; IP, interpulse; RMS, root mean square.

In the Mediterranean Sea, individuals become more active from May to July and aggregate at specific locations in order to spawn (Zabala *et al.*, 1997b). The sex ratio during the spawning season is *c.* 1 male:2 females (Bruslé & Bruslé, 1976). As non-territorial males and immature females do not take part in spawning, Zabala *et al.* (1997b) proposed a 'territorial male–adult female' ratio of 1:7. Prior to spawning, male territorial activity is the most conspicuous behaviour observed. It consists of males patrolling all day long, swimming rapidly and chasing conspecifics, especially male neighbours. During encounters, males show frontal and lateral displays with tail fluttering, sometimes tilting their bodies (Zabala *et al.*, 1997b). In the evening, males patrol a smaller zone to which females may enter in order to mate. In front of a receptive female, the aggressive behaviour switches to courtship and leads to spawning. Zabala *et al.* (1997a) described four main phases of spawning behaviour in *E. marginatus*, *i.e.* the approach of the male, a series of vertical ascents with no spawning (false rises), a final spawning rush with the release of the gametes and finally, the separation of the mates. All this happens at dusk and during the night. All the above-mentioned behaviours are associated with specific colour patterns (Zabala *et al.*, 1997a, b). Most of the reproductive activity occurs between late June and late September with spawning peaks occurring in July and August (Zabala *et al.*, 1997a, b; Marino *et al.*, 2001; Hereu *et al.*, 2006; Reñones *et al.*, 2010). During summer, divers have reported loud booming sounds near *E. marginatus* aggregation sites (pers. comm.).

The general aim of this study was to describe for the first time the sounds of *E. marginatus* and to validate the use of passive acoustics in the monitoring of this species. The two main objectives were (1) to characterize the sounds produced by *E. marginatus* during its spawning season *in situ*, as well as the behaviour associated with sound production observed in captivity and (2) to describe the temporal pattern of sound production by means of long-term passive acoustic recordings made at two locations in the Mediterranean Sea.

MATERIALS AND METHODS

RECORDINGS AT SEA

In 2012, long-term passive acoustic recordings were made off the north-western coast of Corsica (France) at La Revellata headland (42° 35' N; 08° 45' E; depth = 36 m) where *E. marginatus* have previously been observed to aggregate (P. Lejeune, pers. comm.). The sea surface temperature was monitored by means of a conductivity–temperature–depth probe routinely sampled at STARESO harbour. In 2013, passive acoustic recordings were again performed at La Revellata and also in the Marine Reserve of Cerbère-Banyuls (42° 28' N; 03° 10' E; depth = 18 m). Passive recordings were made with hydrophones (HTI96-min, High-Tech, –186 dB re 1 V μPa^{-1} , 20 dB gain; www.hightechincusa.com) connected to underwater digital spectrogram (DSG) Long-term acoustic recorders (Loggerhead Instruments; www.loggerhead.com). In 2012, the device was scheduled to record 10 min every hour from 27 June to 2 October, yielding a total of 2256 files. In 2013, two devices were scheduled to record 5 min every hour from 1 July to 30 September 2013 at La Revellata and 6 min every hour from 9 July to 30 September at Cerbère-Banyuls reserve to yield a total of 2497 and 1965 files, respectively. Acoustic signals were digitized at 20 kHz and stored on 32-GB SD cards. DSG recorders were weighted by a block of concrete, positioned on the seabed by divers and stayed in place during the entire course of the experiments. Both sites were specifically chosen because they are well known to contain territorial *E. marginatus*, and the hydrophones were placed close to the spawning sites. No other grouper species were reported at these locations (P. Lejeune & J. Payrot, pers. comm.).

RECORDINGS IN CAPTIVITY

Recordings were made in two different public aquaria. In the aquarium of La Cité de la Mer in Cherbourg (France) from 26 March to 30 March 2013, two individuals (a female of *c.* 60–65 cm and a male of *c.* 75–80 cm L_T) were housed together (water temperature: *c.* 17° C). In the aquarium of Banyuls sur Mer (France) from 5 July to 08 July 2013, two individuals (undetermined sex, *c.* 70–75 and 85–90 cm L_T) were housed in separate tanks (water temperature: *c.* 20° C). A total of 21 and 22 recordings, respectively, of 10 min each were made during opening hours of the aquaria, when public attendance was the lowest. The number of individuals recorded was quite low because of the difficulty of keeping these large animals in captivity. The purpose was to investigate the species specificity of acoustic signals in order to validate sounds heard in the wild as belonging to *E. marginatus*. All tanks contained additional species such as nursehound *Scyliorhinus stellaris* (L. 1758), small-spotted catshark *Scyliorhinus canicula* (L. 1758), red scorpionfish *Scorpaena scrofa* L. 1758, European seabass *Dicentrarchus labrax* (L. 1758) or gilthead seabream *Sparus aurata* L. 1758. A hydrophone (HTI-96-Min; sensitivity: -164.4 dB re 1 V μPa^{-1} ; flat frequency response ranges between 0.2 and 30 kHz) connected to a TASCAM DR-07 portable digital recorder (www.tascam.com/product/dr-07) was used. Acoustic activity was monitored with the recording device set to recording standby mode. When a sound was produced, the recording was started and included the two preceding seconds. The recording was set back to standby if sounds were no longer produced. Sounds were digitized at 44.1 kHz (16-bit resolution). Recording sessions were video-recorded by means of a digital video camera (Sony Handycam DCR-SR Series, Sony Corporation; www.sony.com) positioned in front of the tanks in order to link potential sound production to specific behaviour and posture. Recording sessions lasted 20 min and were distributed over the day especially during low visitation, *i.e.* from 0900 to 1000, from 1200 to 1400 and from 1700 to 1900 hours.

GENERAL ACOUSTICAL ANALYSIS

Passive acoustic recordings were down-sampled to a sampling rate of 1000 Hz, and a 10–500 Hz band-pass filter was applied. Sounds were detected by visual inspection of spectrograms [Fast Fourier Transform (FFT) 512 points Hamming window] with a 30 s time window using the Avisoft SASLab Pro 5.2.07 software (www.avisoft.com/soundanalysis). At sea and in tanks, only sounds with a good signal-to-noise ratio were used for the analysis. The following variables were measured from oscillograms: number of pulses per sound, pulse duration (ms), pulse period (peak-to-peak interval, ms) and total sound duration (from the start of the first pulse to the end of the last pulse, ms). The frequency composition of the sounds was measured from logarithmic power spectra (FFT, 512 points Hamming window). Data of acoustic features were normally distributed and met the assumptions of parametric statistics. A *t*-test and ANOVAs were therefore used to compare acoustic features of sounds from the different sites and conditions (field *v.* captivity).

Seasonal sound production series were obtained by plotting the daily sum of sounds attributed to *E. marginatus* recorded per 5 min periods. Daily sound production series were created by plotting the mean number of sounds per min of recording with a 1 h resolution. Data were not normally distributed and Kruskal–Wallis tests were used in order to compare the number of sounds recorded every hour. The proportions of the different sound types recorded in Cerbère-Banyuls reserve were compared with Friedman ANOVAs by separating the day into four 6 h phases.

RESULTS

ACOUSTIC REPERTOIRE AND CHARACTERISTICS

Two different kinds of sounds were attributed to *E. marginatus*. They consisted of single booms and serial booms, which were recorded in tanks and in the field. Growling sounds were only recorded in the field and may potentially belong to *E. marginatus*.

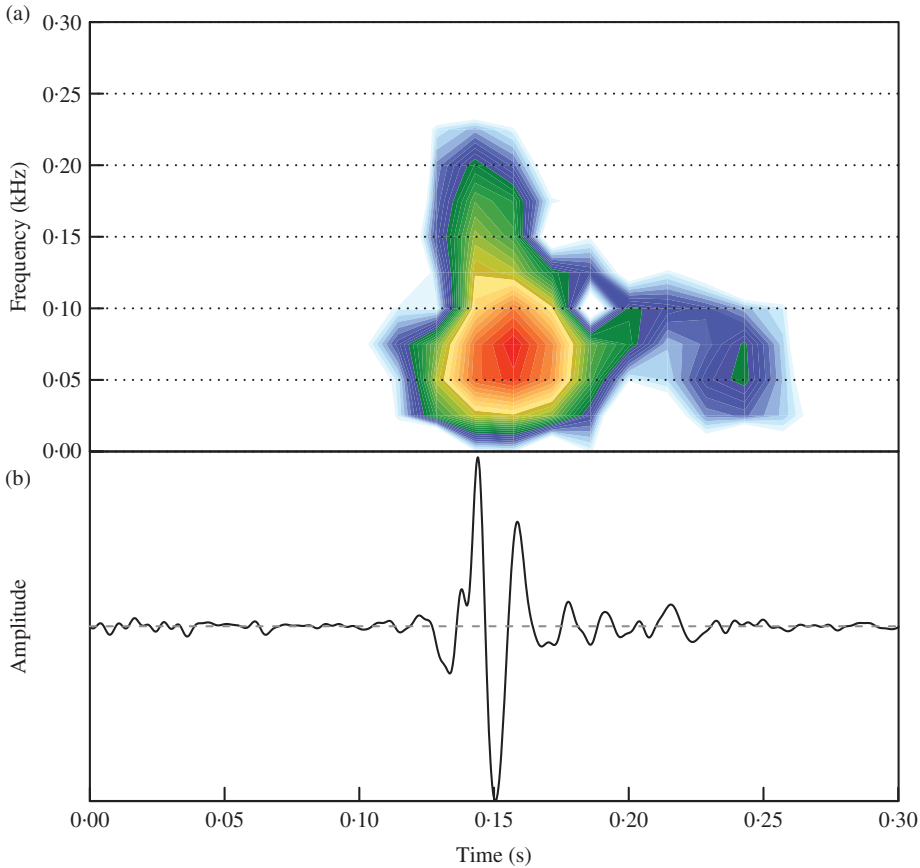


FIG. 1. (a) Spectrogram and (b) oscillogram of a single boom produced by *Epinephelus marginatus* and recorded at sea in Cerbère-Banyuls reserve. Colours indicate relative sound intensity (blue = low and red = high).

Single booms

Single booms were the most common sounds of *E. marginatus* recorded at sea (Fig. 1). Due to a high background noise and probably because the hydrophone was too far from the spawning site, only recordings from 2012 were used for quantitative analysis. Most of the sounds detected consisted mainly of frequencies below 150 Hz. At La Revellata, single booms recorded in 2013 had a duration of 76 ± 19 ms (mean \pm S.D.) and a dominant frequency of 107 ± 28 Hz ($n = 27$). In the Cerbère-Banyuls reserve, single booms ($n = 70$) had a duration of 95 ± 23 ms and a dominant frequency of 68 ± 19 Hz. Sounds recorded at Cerbère-Banyuls were significantly longer than sounds from La Revellata (t test, $t_{18,38} = 3.32$, $P < 0.001$).

Such loud single pulses with a low frequency were recorded during day time in captivity in the aquarium of Banyuls-sur-Mer. Dominant frequency was 45 ± 15 Hz ($n = 14$ sounds recorded from two individuals). Pulse and sound durations were hardly measurable due to background noise and reverberation from the walls of the tank. These single booms were produced when a heterospecific approached or entered the shelter of a grouper.

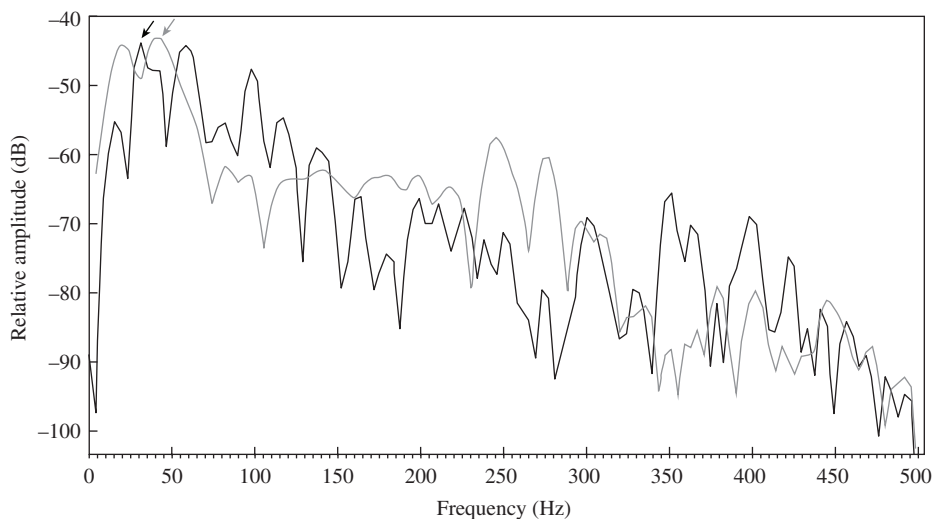


FIG. 2. Power spectra of a single boom by *Epinephelus marginatus* recorded at sea in Cerbère-Banyuls reserve (—) and at the aquarium of Banyuls sur Mer (---). →, → dominant frequencies.

All the dominant frequencies were significantly different with the lower values being recorded in the aquarium of Banyuls-sur-Mer and the higher values recorded at La Revellata (ANOVA, $F_{2,108} = 55.6$, $P < 0.001$, least significant difference (LSD) *post hoc* tests, all P values < 0.001). Despite these statistical differences, most probably due to the low number of *E. marginatus* in tanks, the comparison of oscillograms and power spectra from sounds recorded at sea and from sounds recorded in captivity showed similar patterns (Fig. 2), validating that all these booms are produced by *E. marginatus*.

Serial booms

A second type of sound consisted of serial booms. Serial booms were rarely recorded at La Revellata to be analysed. Series recorded in Cerbère-Banyuls reserve in 2013 lasted 1572 ± 671 ms, were composed of 4 ± 2 pulses with a pulse duration of 78 ± 16 ms, a pulse period of 335 ± 70 ms and their dominant frequency was 40 ± 10 Hz ($n = 120$).

From video recordings and observations performed at the aquarium of La Cité de la Mer in Cherbourg, this sound type was produced during interactions between a male and a female. The presumptive male swam towards the presumptive female until it was positioned close to it. The male subsequently leant almost horizontally on its flank and produced a series of caudal twitches with simultaneous production of sounds. While performing this behaviour, the male changed its colour pattern and briefly displayed bright silver stripes on its head and a large silver spot behind the opercular region [Fig. 3 and Video S1 (Supporting Information)]. No colour change was noticed in the female. Booms emitted in series were more discrete and quieter than single booms, reducing the degree of reverberation. These sounds ($n = 19$ sounds recorded from one individual in tank) lasted 712 ± 360 ms and were composed of 3 ± 1 pulses with a pulse duration of 52 ± 11 ms and a pulse period of 321 ± 40 ms. Their dominant frequency was 45 ± 11 Hz (Fig. 4). The same sound type was recorded on

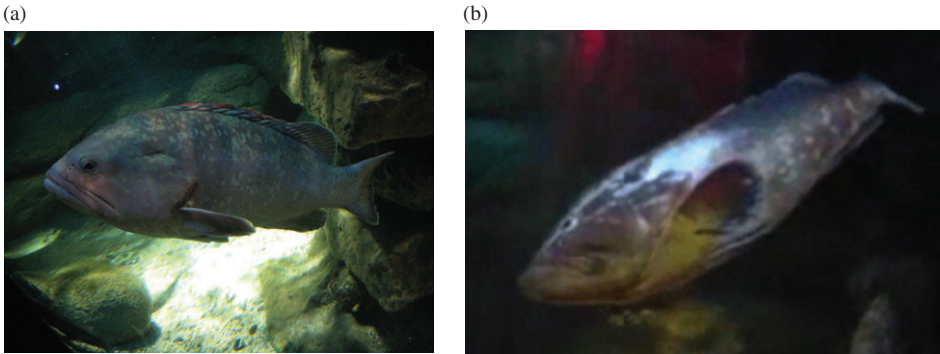


FIG. 3. Images of a captive presumptive male individual *Epinephelus marginatus* (c. 80 cm total length, L_T) in the (a) standard mottled colour pattern and (b) silver bright colour pattern displayed when interacting with a presumptive female (extracted from the video footage presented as Video S1, Supporting Information).

two occasions when an individual chased an *S. scrofa* at the Aquarium of Banyuls sur Mer, but no associated visual display or colour changes were observed.

Sounds recorded at sea had a significantly longer total duration (t -test, $t_{7,18} = 4.18$, $P < 0.001$), longer pulse duration (t -test, $t_{80,55} = 10.46$, $P < 0.001$) and lower dominant frequency (t -test, $t_{120,55} = 2.97$, $P < 0.01$) than sound recorded in captivity. Numbers of pulses and pulse periods showed no significant differences. Moreover, the power spectra showed a similar profile for serial booms recorded at sea and in captivity (Fig. 5).

Growls

A third type of sound, defined as a growl, was often recorded at sea in Cerbère-Banyuls reserve and more rarely at La Revellata (probably due to the distance of the hydrophone from the calling individuals). It consisted of short multi-harmonic calls lasting 256 ± 116 ms with a fundamental frequency (H_0) = 86 ± 13 Hz ($n = 34$), which was always the dominant frequency. The first harmonic (H_1) = 167 ± 31 Hz and the second harmonic (H_2) = 265 ± 41 Hz. Higher harmonics were weak or absent. These frequencies showed a slight downward frequency modulation (Fig. 6). Growls were not stereotyped and could be produced in series of 1–3.

TEMPORAL PATTERN OF SOUND PRODUCTION DURING THE RECORDING PERIOD

For all sound types, no significant changes were found in any of the acoustical features during the recording period. At La Revellata, vocal activity was detected throughout the recording period with an increased number of sounds recorded in July and in early September during 2012, and in August and September during 2013. Periods of increased sound production lasted 2–5 days. The pattern was different in 2013 in the Cerbère-Banyuls reserve where more sustained sonic activity was recorded throughout the recording period. Sound production did not appear to follow a lunar cycle at either site (Fig. 7).

Booming sounds (single and serial) were detected during night and day. Nevertheless, all recordings showed a marked pattern, with an increased number of sounds

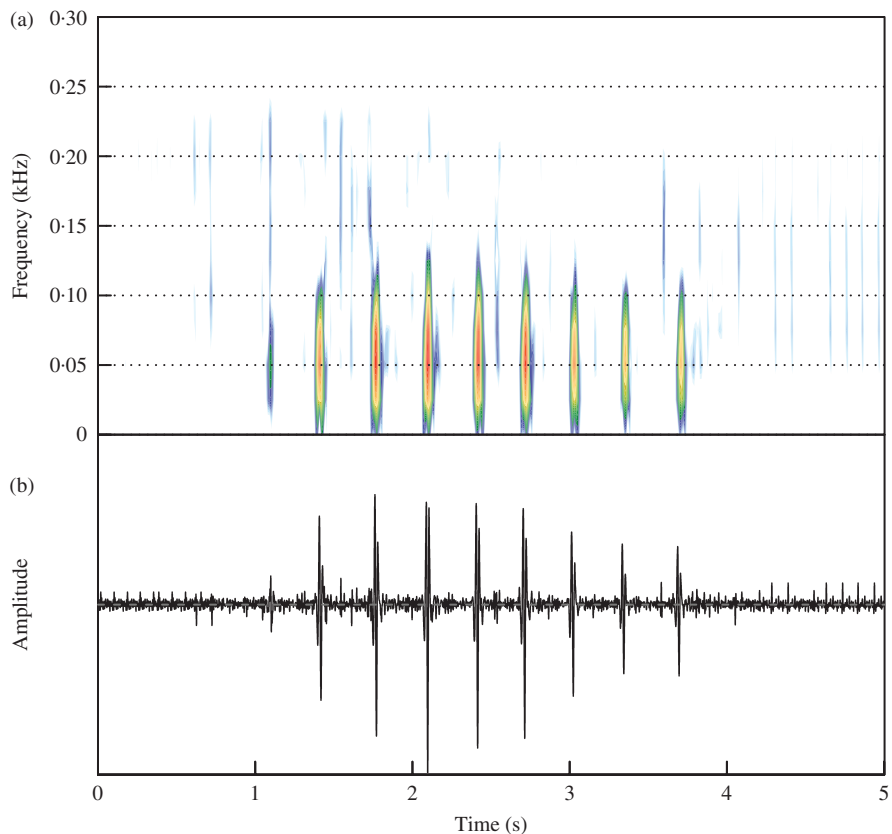


FIG. 4. (a) Spectrogram and (b) oscillogram of nine serial booms produced by *Epinephelus marginatus*. Colours indicate relative sound intensity (blue = low and red = high).

at dusk, peaking at 2200 hours before dropping off during the night until sunrise. During the day, booming sound production remained more constant, increasing slightly in the morning until 1400 to 1500 hours (Kruskal–Wallis tests, La Revellata, 2012: $H_{23,2047} = 84.86$; La Revellata, 2013: $H_{23,1184} = 110.64$; Cerbère-Banyuls: $H_{23,1729} = 173.25$, all $P < 0.001$) (Fig. 8). The high number of serial booms detected in Cerbère-Banyuls reserve in 2013 showed the same diel pattern, although the morning increase was stronger (Kruskal–Wallis test: $H_{23,1729} = 97.30$, all $P < 0.001$). Single booms and serial booms represented 60 and 35% of sounds recorded in the Cerbère-Banyuls reserve, respectively. The proportion of booms significantly increased up to 69% of sounds recorded during the 0000 to 0500 hour phase and 65% of sounds recorded during the 1800 to 2300 hour phase (Friedman ANOVA, $F_{3,20} = 14.33$, $P < 0.001$) while the percentage of serial booms significantly dropped to 22 and 30%, respectively (Friedman ANOVA, $F_{3,20} = 25.71$, $P < 0.001$; Fig. 9).

Growls detected in Cerbère-Banyuls represented 5% of the total number of sounds and were also predominantly produced at night, increasing at dusk from 1900 hours and presented three peaks of production from 2200 to 0400 hours before decreasing until sunrise (Kruskal–Wallis tests, Cerbère-Banyuls: $H_{23,1729} = 93.49$, $P < 0.001$) (Fig. 10).

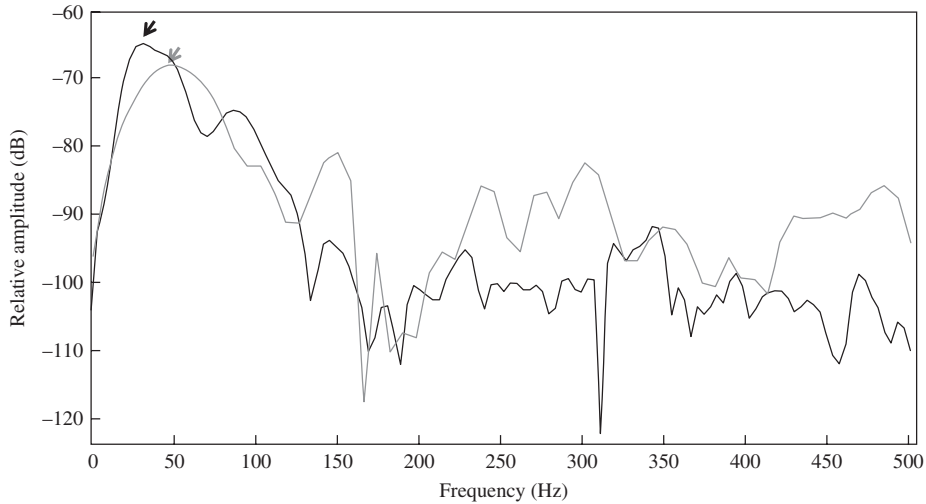


FIG. 5. Power spectra of serial booms by *Epinephelus marginatus* recorded at sea in Cerbère-Banyuls reserve (—) and at the aquarium of La Cité de la Mer in Cherbourg (---). →, → dominant frequencies.

Their proportion was significantly higher during the 0000 to 0500 hour phase (Friedman ANOVA, $F_{3,20} = 20.93$, $P < 0.001$; Fig. 9).

DISCUSSION

The results of a passive acoustic survey conducted at known spawning aggregation sites of *E. marginatus* at two locations in the Mediterranean Sea are presented. Additional recordings and behavioural observations performed in captivity revealed the potentially important social role of acoustic signals in *E. marginatus*. Two main sound types were identified, *i.e.* loud single booms and serial booms. Another sound type consisted of short growling sounds. Overall, the sounds of Mediterranean *E. marginatus* appear to have a low frequency range (<100 Hz) and to be made of single or serial booms which can be rapidly repeated to produce growls.

Observations in captivity suggest that sounds may be associated with courtship displays. The series of booms in particular were observed to be associated with a conspicuous display directed by a presumptive male towards a conspecific female while it exhibited a bright silver colour pattern. Such bright colouration usually appears in patrolling, dominant males and remains during spawning behaviours in *E. marginatus* (Louisy, 1996). The associated display has also been described in *E. marginatus* by Zabala *et al.* (1997a, b) as occurring both during patrolling by males and in the early phases of courtship. Similar colour patterns (indicating sexual dimorphism during the reproduction period and imminent spawning), sometimes associated with sound production, have been reported in other male groupers, *e.g.* *Mycteroperca tigris* (Valenciennes 1833) (Sadovy *et al.*, 1994), *M. bonaci* (Paz & Sedberry, 2007), *E. morio* (Nelson *et al.*, 2011), *E. striatus* (Archer *et al.*, 2012) and *M. venenosa* (Schärer *et al.*, 2012a). The occurrence of sounds during the spawning period and their potential association with visual displays as observed in captivity may suggest a link between

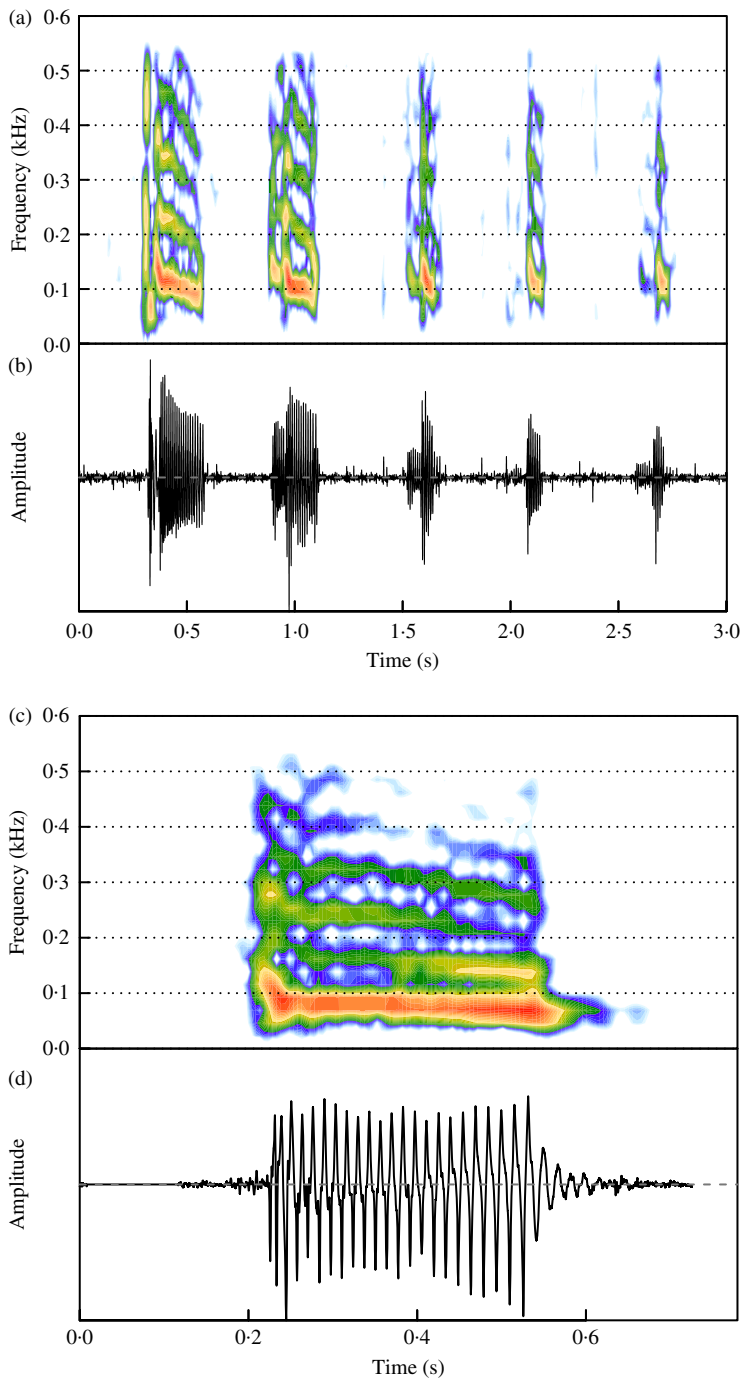


FIG. 6. (a) Spectrogram and (b) oscillogram of a series of five growls. Note that the first call is preceded by a single boom and that the second, third and fifth calls overlap with a second series of calls present in the background. (c) Spectrogram and (d) oscillogram of a growl produced by *Epinephelus marginatus*. Colours indicate relative sound intensity (blue = low and red = high).

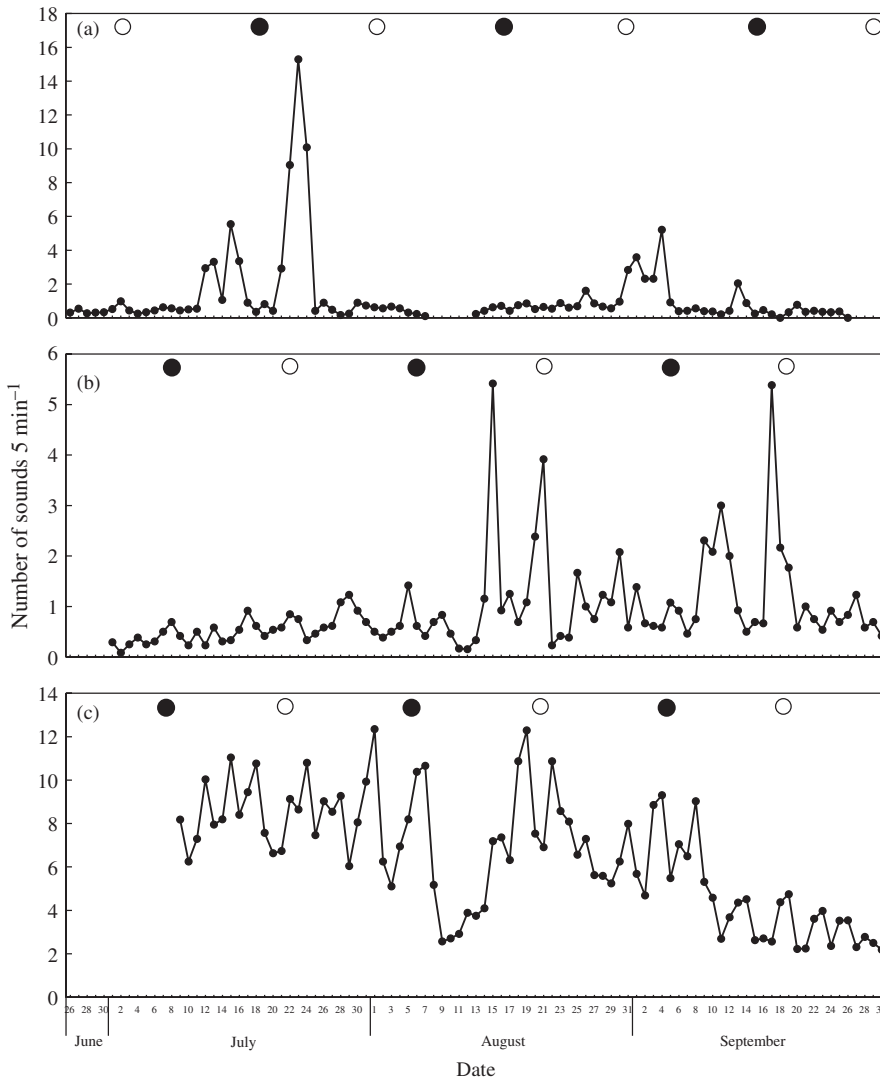


FIG. 7. Daily sound production of *Epinephelus marginatus* and moon cycle at La Revellata in (a) 2012, (b) 2013 and in Cerbère-Banyuls reserve in (c) 2013. Number of sounds is given for 5 min h⁻¹ of recording. The 8 day gap (5 to 13 August) present in 2012 was due to technical (downloading files and changing batteries) and meteorological issues before re-immersing the device. Data are for all sound types. ●, ○, moon phases.

sonic activity and reproductive behaviour in *E. marginatus*. Sounds were also produced in captivity towards heterospecifics, however, implying that booming sounds might have other functions such as antagonistic or aggressive. Further observations are needed to define more precisely the behaviour patterns associated with sound production. Even though sounds were predominantly detected at dusk and during the night when reproductive activities usually take place, no observation of direct concurrence between sound production and spawning events were made in this study or in other species of groupers. Future studies should help to understand the

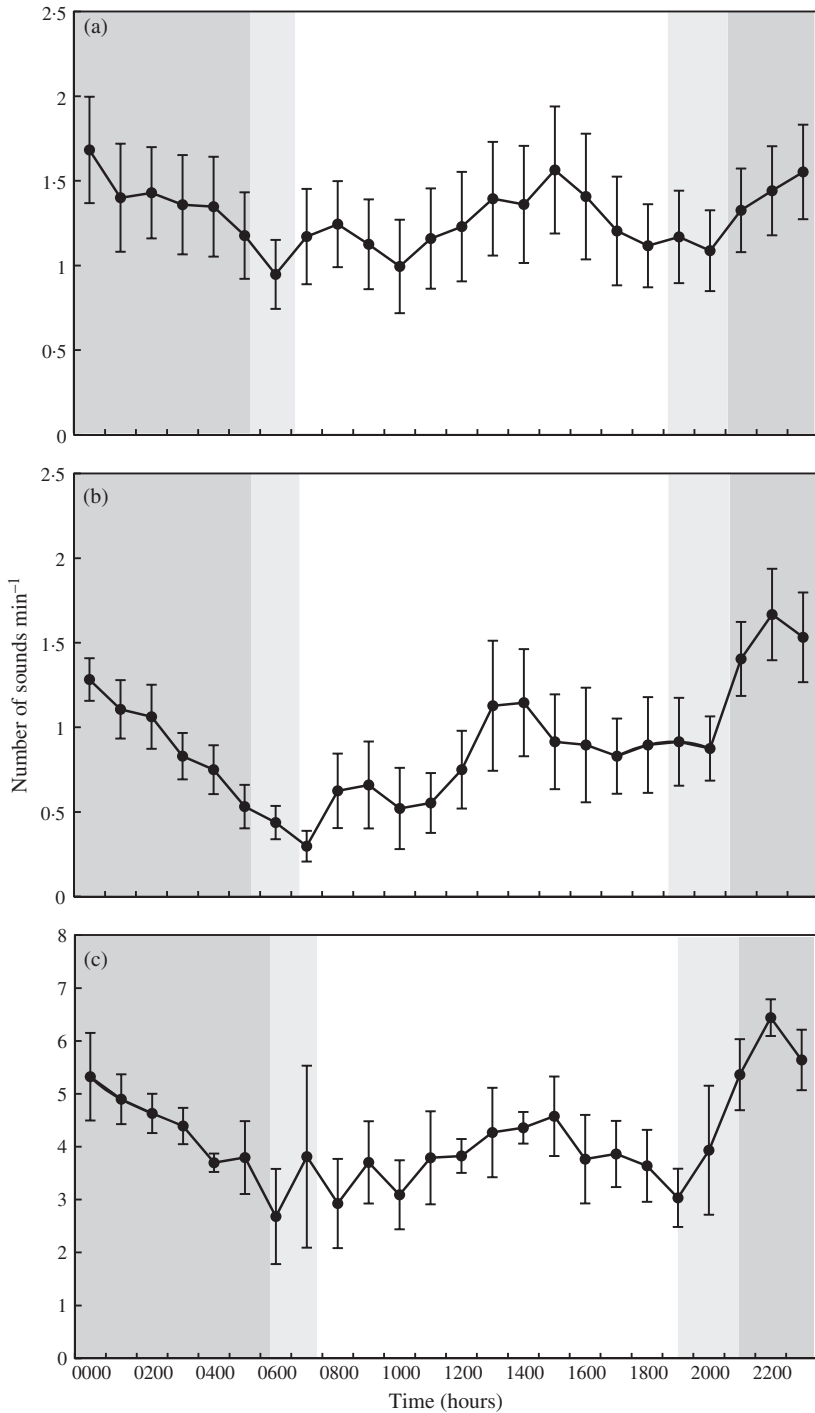


FIG. 8. Diel cycle of booming sound production of *Epinephelus marginatus* at La Revellata in (a) 2012, in (b) 2013 and in Cerbère-Banyuls reserve in (c) 2013. Values are mean \pm S.D. number of sounds min⁻¹ of recording. ■, longest nights; ■, shortest nights of the recording periods.

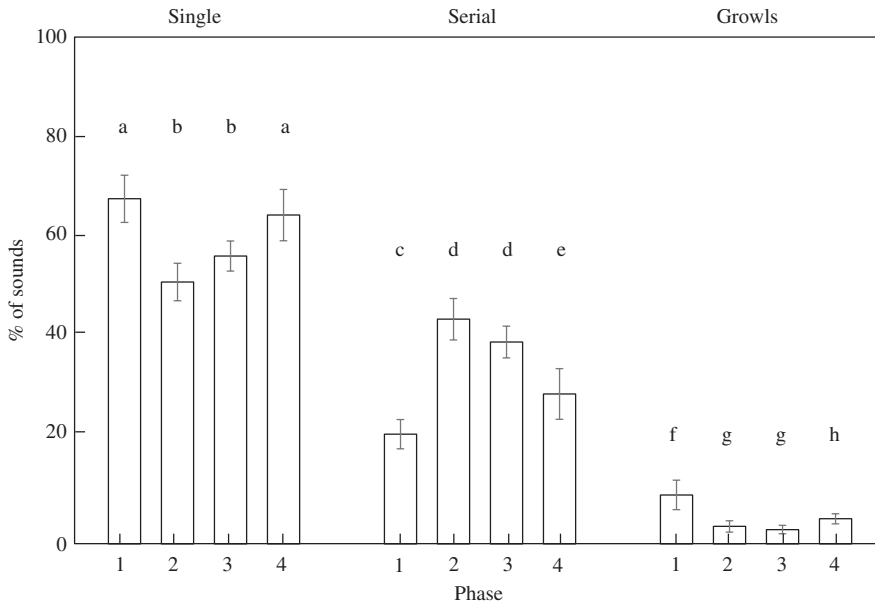


FIG. 9. Distribution of single booms, serial booms and growls of *Epinephelus marginatus* throughout the day. Phase 1: 0000 to 0500 hours, phase 2: 0600 to 1100 hours, phase 3: 1200 to 1700 hours and phase 4: 1800 to 2300 hours in Cerbère-Banyuls reserve. Values are mean \pm S.D. Different lower-case letters indicate significant differences ($P < 0.05$, Fisher *post hoc* test).

relationships between sound production and spawning events. In sciaenids, Aalbers & Drawbridge (2008) reported an increased calling activity with the use of a specific sound type during gamete release in the white seabass *Atractoscion nobilis* (Ayres 1860). As interactions during the spawning period take place in the presence of several aggregated conspecifics, the use of different sound types and the possible association of acoustic and visual modalities may provide a mechanism to synchronize spawnings. Conversely, Locascio *et al.* (2012) found a negative relationship between the levels of sound production and egg production in the black drum *Pogonias cromis* (L. 1766). Finally, Connaughton & Taylor (1996) observed the cessation of vocalizations during gamete release in the weakfish *Cynoscion regalis* (Bloch & Schneider 1801). A particular need remains to confidently attribute growling sounds to *E. marginatus* and to identify associated behaviours. The growling and low-pitched nature of these sound types, however, are comparable with similar types of sounds reported for other groupers (Table I) and are therefore probably produced by *E. marginatus*. Growl-like sounds, which might be the result of rapidly repeated overlapping pulses (Torricelli *et al.*, 1990), are often produced after a single boom and are also associated with visual courtship displays, *i.e.* twitching and white-coloured pattern, as also observed in other representatives of the family Serranidae (Table I). Overall, low-pitched booming and growling sounds appear typical of groupers. The similarity of sound types and characteristics among groupers suggest a common mechanism for sound production. This mechanism is probably similar to the mechanism described in *E. striatus* by Hazlett & Winn (1962). Further studies are however needed to better understand this mechanism.

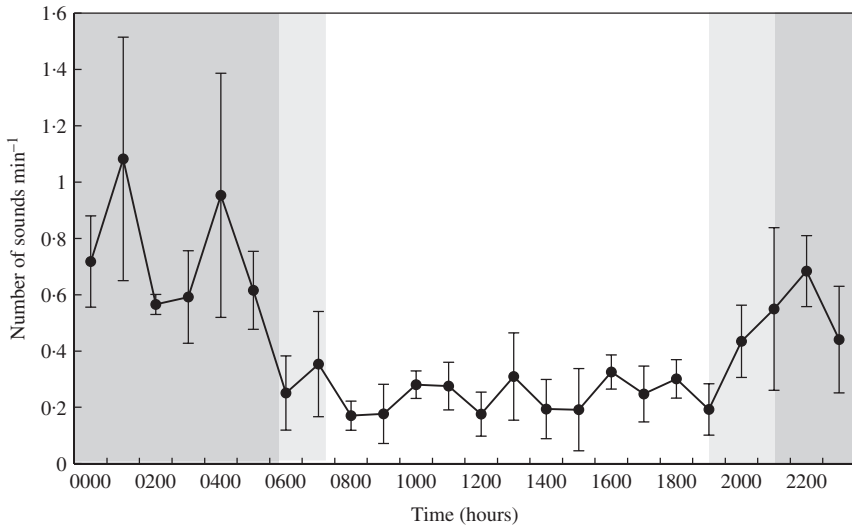


FIG. 10. Diel cycle of growl production of *Epinephelus marginatus* in Cerbère-Banyuls reserve in 2013. Values are mean \pm S.D. number of sounds min^{-1} of recording. ■, longest nights; ■, shortest nights of the recording periods.

Interspecific dissimilarities appear to exist, however, in the diel pattern of sound production. *Epinephelus itajara* appears to vocalize at night between 0100 and 0400 hours (Mann *et al.*, 2009), *E. morio* produces sounds principally during the day with call number increasing just before dawn, peaking in the late afternoon and decreasing after dusk (Nelson *et al.*, 2011) while *M. venenosus* vocalizes especially after sunset (Schärer *et al.*, 2012a). During the spawning season, *E. marginatus* exhibits a clear circadian pattern with most spawns observed at dusk and around sunset (Zabala *et al.*, 1997a; Hereu *et al.*, 2006). A consistent pattern was observed in this study for sound production with an increase in the number of sounds from 1900 to 2200 hours before decreasing during the night. From this observation, sound production may be associated with behaviour patterns preceding or associated with spawning. At the seasonal level, increases in sound production activities detected at La Revellata in 2012 and 2013 are similar to the pattern of spawning events observed in this species by Hereu *et al.* (2006) in the Medes Islands (Spain). In Spain, two peaks of spawning activity were detected each summer in August, each one lasting 2–8 days separated by 1 week. Mann & Lobel (1995) made similar observations for sonic activity in the damselfish *Dascyllus albisella* Gill 1862 in which sound production regularly varies along the spawning cycle increasing before nesting, peaking before spawning after which it decreases during brood care. In both species, the onset of spawning cannot be related to biotic or abiotic factors. The patterns detected at La Revellata and in Cerbère-Banyuls in 2013 were not the same. In Corsica, acoustic activity varied from year to year at the level of both the amount of sounds and the duration of the peaks. The level of sonic activity was higher and more constant in Cerbère-Banyuls. A similar continuous activity was observed for spawning in summer in 1999 by Hereu *et al.* (2006). In the same way, some variations in sound production cycles at the monthly level were also observed in two geographically separated populations of *E. guttatus* suggesting

different reproductive cycles (Mann *et al.*, 2010). Hereu *et al.* (2006) observed that spawning events were detected when the water surface temperature reached 21.8°C, in late May to early June. During the 2012 recording period, the sea surface temperature was above this threshold, varying from 23.5°C in June to 28.1°C in August.

Sound production also showed a slight increase during the day, *i.e.* from the morning until the beginning of the afternoon, which may suggest that sounds may be associated with other social activities not only occurring at dusk. In different sciaenid species, such as in *C. regalis*, calls have been shown to serve in the formation of spawning aggregations, rallying individuals to the same spawning site (Mok & Gilmore, 1983; Connaughton & Taylor, 1995). Here, only the results for the 3 month period during which most spawning activity is known to occur are presented. Further recordings should be made beyond this spawning season to investigate whether sounds can be produced throughout the year and may have other social roles.

Likewise, monitoring oceanographic variables (*e.g.* water temperature) at recording sites would help with understanding the periodicity of spawning events and vocal activity. In poikilotherms, water temperature is an important variable as it affects gonadal maturation and neuro-motor activity (Walker, 1975; Hutchings & Myers, 1994). Changes in neuro-motor activity would subsequently affect the characteristics of the sounds by modulating muscular activity (Connaughton *et al.*, 2000; Papes & Ladich, 2011). Further investigations at these locations would additionally provide important information on how this species responds to human-generated noise and how marine protected areas benefit this species. The use of passive acoustics and the deployment of large arrays of hydrophones may be an alternative for large-scale mapping of fish distribution based on their vocal activity (Rowell *et al.*, 2012; Wall *et al.*, 2012). While visual monitoring remains essential to fully describe reproductive behaviour, acoustic surveys appear valuable for locating spawning aggregation sites, providing information on the beginning and duration of the spawning season and for studying the behavioural responses of fishes during this critical period.

In conclusion, passive acoustic recordings made during this study have allowed, for the first time, the acoustic repertoire of this popular Mediterranean fish to be characterized and to establish the pattern of calling at different times of day during its spawning season. This type of data may be of particular interest for monitoring protected species and for planning management strategies.

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Supporting Information

Supporting Information may be found in the online version of this paper:

Video S1. Video footage of the twitching behaviour of a presumptive male *Epinephelus marginatus* produced towards a presumptive female, recorded in the aquarium of La Cité de la Mer in Cherbourg (France). Sound is turned on before the emission of the associated serial booms.

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