First evidence of underwater vocalizations in green sea turtles *Chelonia mydas*

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ABSTRACT: Marine turtles have long been considered to be silent, but few investigations have been performed to confirm such muteness. However, recent studies on the aerial and underwater hearing abilities of marine turtles have shown they have an ability to perceive sounds, suggesting the potential existence of acoustic communication among them. In the present study, audio-video recorders were deployed on 11 free-ranging juvenile green sea turtles *Chelonia mydas* at Grande Anse d'Arlet in Martinique. The recordings revealed that the turtles produced 10 different sound types that were classified into 4 main categories: pulses, low-amplitude calls (LAC), frequency-modulated sounds, and squeaks. Although other turtles were not observed in close proximity to tagged turtles during the recordings, some of the described sounds were found in most recorded individuals and their frequency characteristics ranged within the underwater hearing range of green sea turtles, suggesting that the sounds could be used for intra-specific communication. While control recordings in the study area without the presence of green sea turtles contained sounds with similar general structure (pulses, LAC), the acoustic characteristics were significantly different to those recorded for green sea turtles. The 2 types of squeaks identified for the turtles were found to be individual-specific, also suggesting they could be used for intra-species communication. Further research on sea turtles is needed to better understand the behavioral and social context of these acoustic productions, especially during the developmental period and breeding season. Thus, the vocal repertoire of green sea turtles is likely to be more diverse than that currently described.

KEY WORDS: Underwater sound · Green sea turtle · Chelonians · Individual stereotypy · Martinique

1. INTRODUCTION

The green sea turtle *Chelonia mydas* is threatened by a wide range of anthropogenic pressures and is now classified as Endangered on the IUCN Red List (https://www.iucnredlist.org). As with most marine turtles, green sea turtle populations are impacted by harvest, accidental bycatch in fishing nets, and habitat loss (Seminoff 2004, Wallace et al. 2013). The green sea turtle has a circumpolar distribution and occurs in tropical and subtropical waters. This species uses several types of habitat through

its life cycle and performs long migratory routes between feeding and nesting grounds (Baudouin et al. 2015, Chambault et al. 2015). After spending the first years offshore in pelagic waters, juveniles come to coastal waters, where they feed on seagrasses and algae (Siegwalt et al. 2020). Adult turtles only come to shore to nest (Chambault et al. 2016). The social behavior of juvenile and adult green sea turtles is not very well known, nor is their communication system. A better general understanding of this endangered species seems critical to better understand its biology, but it will also help

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in assessing the threats they are exposed to in their natural environment.

Freshwater and marine turtles belong to the order of Testudines which has long been considered as a 'silent group' (Campbell & Evans 1972), meaning there is a paucity of studies on the role of sound production in their ecology and behavior. The assumption of muteness was derived from the complementary thought that turtles were deaf. Pope (1955) noted that 'the deafness of turtles, therefore, suggests a corresponding muteness'. Such views were likely accounted for by the absence of external ears in chelonians, the difficulty in observing behavioral responses to sounds, and the relative rarity of vocalizations perceptible to human hearing (Gans & Maderson 1973, Wever 1978). However, in the last 2 decades, studies have revealed the ability of several terrestrial and freshwater turtles to emit sounds in air, as well as underwater sounds for freshwater turtles, with up to 17 categories of sounds found for *Chelonida oblonga* (Giles et al. 2009). These examples show the importance of acoustic signals in turtles' social behavior and reproduction (Galeotti et al. 2005, Giles et al. 2009). These results challenge conventional ideas about sound production in chelonians; however, very few studies have examined sound production in marine turtles.

Production of sounds in air has been noted in female nesting marine turtles, such as the leatherback sea turtle *Dermochelys coriacea* (Carr 1995, Lutcavage & Lutz 1997), and has even been precisely characterized (Mrosovsky 1972, Cook & Forrest 2005). The grunt-like sound produced by leatherbacks, on the other hand, appears to be more related to their respiration than to an acoustic communication signal. Airborne sound production has also been shown in embryos and hatchlings of different marine species, such as hawksbill sea turtles *Eretmochelys imbricata* (Monteiro et al. 2019), Kemp's ridleys *Lepidochelys kempii* (Ferrara et al. 2019), green sea turtles (Ferrara et al. 2014a), olive ridley turtles *L. olivacea* (McKenna et al. 2019) and leatherback turtles (Ferrara et al. 2014b). This may suggest acoustic communication among the embryos to synchronize hatchling and nest emergence (McKenna et al. 2019), as has been observed in freshwater turtles such as the Arrau turtle *Podocnemis expansa* (Ferrara et al. 2013), and in crocodiles and birds (Britton 2001, Vergne et al. 2007, Colombelli-Négrel et al. 2012). For freshwater turtles, Ferrara et al. (2013) also recorded 11 different types of sound, produced in air and under water, from adult females approaching and responding to hatchling sounds. While we

cannot draw direct conclusions from this on the ability of green sea turtles to produce sounds under water, these findings support the idea that this species would be equally capable of emitting sounds under water to potentially communicate with conspecifics.

Moreover, experimental studies have demonstrated that marine turtles (green sea turtle, leatherback, hawksbill, loggerhead and Kemp's ridley) can detect sounds both in air and under water (Bartol et al. 1999, Bartol & Ketten 2006, Martin et al. 2012, Piniak 2012, Piniak et al. 2016). It is now becoming evident that hearing may have a more important role in their perception of the environment than previously assumed, and this also suggests the potential existence of acoustic communication amongst marine turtles.

Underwater sound production by marine turtles has been evidenced in hatchlings (Ferrara et al. 2014a,b, 2019, McKenna et al. 2019, Monteiro et al. 2019), but it has not been investigated in juvenile or adult marine turtles, so we aimed to fill this gap. The present study examines underwater sound production in green sea turtles in their developmental area in Martinique. This population is characterized by resident juveniles that spend several years in developmental neritic habitats on the southwest coast of Martinique (Musick & Limpus 1997, Bonola et al. 2019, Siegwalt et al. 2020). The green sea turtle has long been considered to be solitary; however, social interactions between conspecifics have been observed in Martinique (Roost et al. unpubl. data), suggesting that green sea turtles could be more socially interactive and thus communicative with congeners than previously expected.

Our first aim was to describe the different sounds produced by juvenile green sea turtles, using audio-video recorders attached to free-ranging individuals. The development of novel and advanced technological instruments in the last few years, such as multi-sensor tags, has brought new opportunities and insights into the study of animal behavior (Treasure et al. 2017, Greif & Yovel 2019) and for marine turtles in particular (Jeantet et al. 2018, 2020, Siegwalt et al. 2020). In a second step, control acoustic recordings were performed (i.e. without the presence of green sea turtles) in seagrass meadows commonly used by the juveniles for feeding, to detect whether other species could also have produced sounds with the same acoustic characteristics as those recorded from the turtles. As a last step, we assessed the possible occurrence of individual acoustic signatures in the different sound types of their repertoire, and thus their potential use for intra-specific communication.

2. MATERIALS AND METHODS

2.1. Study site and animals

This study was carried out from May to October 2018 in Grande Anse d'Arlet (14° 50′ N, 61° 9′ W), Martinique island (French West Indies, France). A total of 12 juvenile green sea turtles (6 in May 2018 and 6 in October 2018) were manually captured along the coast by 3 freedivers. The relatively shallow depths of the foraging areas (between 2 and 15 m) allowed the divers to silently approach an animal in a static position at the bottom (i.e. resting or feeding). One diver caught the turtle by the nuchal shell and pygales plate, then a second diver came to hold the foreflippers and helped to lift the turtle back to the surface. A third freediver ensured the team's safety. The entire procedure is precisely described in Nivière et al. (2018). Customized Animal Tracking Solutions (CATS) cam devices were attached to the carapace on free-ranging juvenile green sea turtles using 4 suction cups, for periods ranging from several hours to several days. Air was manually released from the cups, which were held in place by the use of a galvanic timed-release system. A CATS cam device is comprised of a video-recorder (1920 × 1080 pixels at 30 frames s^{-1}) combined with a tri-axial accelerometer, a tri-axial gyroscope, magnetometer, time-depth recorder, hydrophone (HTI 96 min, frequency response: 2 Hz to 30 kHz, sensitivity: −165 dB re 1 V/mPa), thermometer, and GPS tracker. Technical issues appeared for 1 individual out of the 12 equipped turtles, causing failure of audio-video recordings.

2.2. Acoustic recordings and analyses

2.2.1. Turtles

For acoustic settings of the CATS devices, we recorded in mono at a frequency sampling rate of 24 kHz (16 bit). From these recordings, we analyzed 65 h 28 min of audio-recordings and labeled 2308 sounds that could have been produced by the tagged turtles (see Table S1 in the Supplement at www.int-res.com/articles/suppl/n048p031_supp.pdf for number of labeled calls per individual and per call type). The best-quality sounds (no noise, no overlap with other sounds) were selected for analysis, and thus 950 sounds were analyzed in both frequency and temporal domains. Labeling of sound files and acoustic analysis were performed using Avisoft SASlab Pro (Avisoft Bioacoustics). To improve visualization of the sounds on spectrograms (Hamming, Fast Fourier Transform [FFT] size 1024 pts), all sound files were down-sampled at 22 kHz, as there was no energy at frequencies above 10 kHz. An additional high-pass filter at 160 Hz was used to remove any flow noise created by the CATS cam.

For each individual, and for each sound type, we selected up to 15 replicates (and a minimum of 5 replicates) to measure their characteristics in both time and frequency domains.

For pulse-type sounds, we measured the duration (in ms, measured from the beginning of the first pulse to the end of the last pulse) of the waveform (cursor precision: 0.04 ms). For sounds composed of several pulses, the pulse rate was calculated (in Hz) using the Pulse Train Analysis function in Avisoft SASLab Pro. We also measured the period (T, in s) of pulses by measuring the peak-to-peak time interval between 2 consecutive pulses from the oscillogram (cursor precision: 0.04 ms), applying the formula F = 1/T (in Hz) to obtain the frequency of each pulse.

For non-pulse sounds, we measured the duration from the oscillogram (cursor precision: 0.1 ms) along with spectral features from the averaged energy spectrum: the frequency of the highest energy (Fmax, in Hz), the energy quartiles (Q25, Q50 and Q75, in Hz), the percentage of energy below a threshold frequency value (Eb in %, <1500 Hz for croaks, <250 Hz for rumbles and <4000 Hz for squeaks; these frequency values were determined after inspection of the spectrograms) and the frequency bandwidth in which the energy is within 6 dB of the first peak (Bdw, in Hz).

For frequency-modulated sound (FMS), unfiltered sound files were used (as frequencies could occur below 160 Hz), and minimum and maximum frequency values (Min Fo, Max Fo in Hz) of the fundamental frequency from the spectrogram (Hamming, FFT size 1024 pts, 93.75 % overlap; cursor precision = 20 Hz) were used to calculate the frequency excursion of the fundamental frequency (Exc. Freq, in Hz).

We estimated the received level (RL) of a limited number of call types in our recordings, and we used the root mean square (RMS, in V) function in Avisoft SASLab Pro on sounds that had been previously measured. To calculate RL for a given call, we used the following formula: RL (in dB re 1 μPa RMS) = $20 \log_{10}$(amplitude of the call, in V_{RMS}) − Gain (in dB) − (Hydrophone sensitivity, in dB re 1 μPa). The gain was set at +3 dB, and the sensitivity of the hydrophone was −165 dB re 1 μPa. RL (in dB re 1 μPa RMS) was calculated by selecting 1 call of each call type per tagged individual.

2.2.2. Control sessions

In November 2019, 5 acoustic control recording sessions were performed using a C54XRS Cetacean Research Technology hydrophone (frequency response: 6 Hz to 203 kHz; sensitivity: −165 dB re 1 V/µPa) connected to a digital recorder (ZOOM H4n). We were not able to use a CATS cam for these control sessions due to technical issues. The recordings were performed from a boat anchored in areas where the equipped green sea turtles had been caught previously. No green sea turtles were observed during the different control recording sessions. A total of 10 h and 40 min of audio recordings (about 2 h per session) were collected on 4 different days, and at 2 different times of day (3 mornings and 2 afternoons). Sounds that showed similarities in their temporal pattern and/or frequency characteristics to those previously described from the CATS cam deployed on green sea turtles were labeled. The same acoustic features were thus measured and their values (see Table 3) were compared to those obtained for different turtles (see Table 1) using Wilcoxon tests.

2.2.3. Individual acoustic stereotypy

To better understand the potential role or biological function of the recorded sounds, we sought to identify if the different sound types presented some individual-specific characteristics. Kruskal-Wallis tests were performed for all sound types, on each variable to assess differences among individuals. We also tested a random forest (RF) algorithm to automatically classify the produced sounds according to the individual acoustic characteristics, completing the analysis. This method is now commonly used to classify sounds into classes (individuals, call categories) (Thiebault et al. 2019, Epp et al. 2021, Indeck et al. 2021, Martin et al. 2021). This was done only on squeaks for which a sufficient number of acoustic variables and individuals to run the algorithm were available. We used the randomForest package in R (Liaw & Wiener 2002) and calculated the overall error rate (out-of-bag error, OOB). The OOB corresponds to the average error for each observation using only the predictions of trees trained without the tested observation. From the OOB, one can extract the global accuracy of prediction (1 − OOB). We also reported the importance of variables in the individual identification ranked in decreasing order using the Gini index. Gini indices relate the contribution of each variable to the classification; the variables with the highest Gini values are the ones contributing the most to the classification. Based on the RF confusion matrix, we calculated the proportion of correct predictions for each individual, giving us an indicator of 'precision' (Thiebault et al. 2019). The correct prediction rate can be compared to a prediction by chance, calculated as the number of calls for a given individual divided by the total number of calls included in the analysis. We included the 7 acoustic variables (Duration, Fmax, Q25, Q50, Q75, Eb, Bdw) that were measured for both squeak types and for individuals having 15 replicates (n = 7 individuals for short squeaks and n = 8 for long squeaks). We performed 2 RF classifications; separately on short and long squeaks, the number of variables randomly selected at each split was set at 2 (as we had only 7 variables) and the number of trees grown was set at 1000.

All statistical tests were performed in RStudio version 1.2.5042 (RStudio Team 2020).

3. RESULTS

3.1. Vocal repertoire

The underwater vocal production of 11 juvenile green sea turtles was investigated. All sounds were produced without the production of air bubbles, thus no air was exhaled. Sounds were separated into 4 main categories (Fig. 1, Tables 1 & S1): pulses, low-amplitude calls (LAC), FMS and squeaks. Within each main category, 2 to 5 sound types were defined.

For pulses, 5 types of pulsatile sounds were identified that differed in the number of pulses produced and in their general pulse rate (Fig. 1A–F). Pulses were noticeably short sounds (<3.8 ms) composed of 1 (mono), 2 (doublet) or 3 (triplet) pulses. These 3 categories of pulses had a main frequency of 1042, 1053 and 1057 Hz respectively (Table 1, Fig. 1A–C), and an average pulse rate of 26 and 27 Hz for doublets and triplets respectively, with pulses produced in series also being found. Multipulses (Fig. 1D) were composed of 5 pulses on average (range: 4 to 8 pulses), and showed an average pulse rate of 28 Hz and an average frequency of 1040 Hz, similar to those measured for doublets and triplets (Table 1). In contrast, tocs (Fig. 1E) were usually composed of a greater number of pulses than multipulses, 7 on average (range: 4 to 20 pulses), and they also showed a much lower pulse rate (11 ± 2 Hz, Table 1). The average frequency could not be measured due to their low amplitude level, but tocs were usually at frequencies below 400 Hz (Fig. 1).
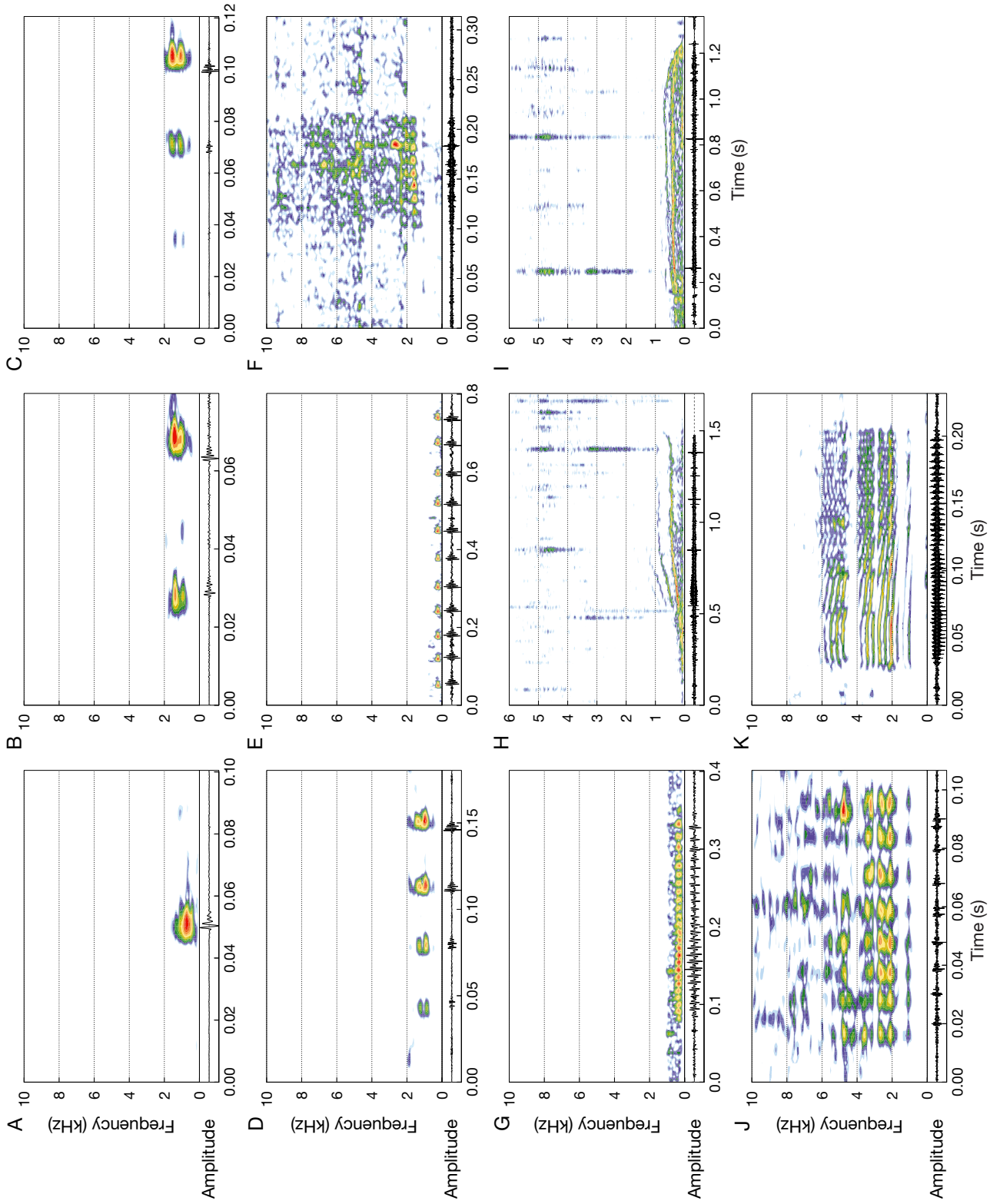
Fig. 1. Spectrograms of the different sounds detected from juvenile green sea turtle recordings. Pulses included 5 types: mono (A), doublet (B), triplet (C), multipulse (D) and toc (E); (F, G) low-amplitude calls (LAC) included croak (F) and rumble (G); (H, I) frequency-modulated sound (FMS) showed highly variable frequency modulation pattern; (J, K) squeaks included short squeak (J) and long squeak (K). Spectrograms (Hamming, FFT size: 256 pts and 1024 pts for H and I, 90 % overlap) made using Seewave (Sueur et al. 2008). For better visualization, all pulse type sounds and the rumble were low-pass filtered at 2 kHz to remove other biotic sounds

Table 1. Acoustic variables (average ± SD) in both temporal and frequency domains measured on the 10 call types described for juvenile green sea turtles from Customized Animal TrackingSolutions (CATS) cam recordings. Kruskal-Wallis tests performed on each variable to assess differences among individuals ($^*p < 0.05$). Bdw: frequency bandwidth in which energy is within 6 dB of the first peak; Eb: percentage of energy below a threshold frequency value (<1500 Hz for croaks, <250 Hz for rumbles and <4000 Hz for squeaks); Exc. Freq: frequency excursion; Fmax: frequency of the highest energy; FMS: frequency-modulated sound; ind.: individuals; LAC: low-amplitude calls; Max Fo: maximum frequency; Min Fo: minimum frequency; NA: not applicable; NM: not measurable; NS: not significant; Q25, Q50 and Q75: energy quartiles

Call type	Duration (ms)	No. of pulses	Pulse rate (Hz)	Frequency (Hz)			
Pulse							
Mono (n = 144, 10 ind.)	3.8 ± 1.8*	1	NA	1042 ± 101*			
Doublet (n = 128, 10 ind.)	43 ± 7.7*	2	26 ± 5*	1053 ± 73*			
Triplet (n = 130, 10 ind.)	79.2 ± 9.6*	3	27 ± 3*	1057 ± 74*			
Multipulse (n = 33, 5 ind.)	145.6 ± 40.6 (NS)	5 ± 1	28 ± 4 (NS)	1040 ± 67 (NS)			
Toc (n = 126, 10 ind.)	NM	7 ± 4 (NS)	11 ± 2 (NS)	NM			
	Duration (ms)	Fmax (Hz)	Q25 (Hz)	Q50 (Hz)	Q75 (Hz)	Eb1500/250 (%)	Bdw (Hz)
LAC							
Croak (n = 39, 5 ind.)	94 ± 28 (NS)	1629 ± 263*	1305 ± 124*	1606 ± 128*	1878 ± 97 (NS)	39 ± 11 (NS)	717 ± 334*
Rumble (n = 47, 9 ind.)	249.1 ± 117.6 (NS)	322 ± 96 (NS)	272 ± 40 (NS)	346 ± 53 (NS)	431 ± 54 (NS)	22 ± 12 (NS)	205 ± 98 (NS)
	Duration (ms)	Min Fo (Hz)	Max Fo (Hz)	Exc. Freq (Hz)			
FMS (n = 41, 5 ind.)	1060.7 ± 526.1*	240 ± 146 (NS)	506 ± 313 (NS)	266 ± 242 (NS)			
	Duration (ms)	Fmax (Hz)	Q25 (Hz)	Q50 (Hz)	Q75 (Hz)	Eb4000 (%)	Bdw (Hz)
Squeak							
Short squeak (n = 121, 10 ind.)	56.5 ± 18.4*	3338 ± 1295*	2772 ± 752*	3891 ± 647*	4955 ± 414*	50 ± 15*	2814 ± 1289*
Long squeak (n = 129, 10 ind.)	143.2 ± 69.1*	3816 ± 1288*	2778 ± 556*	3999 ± 626*	5014 ± 458*	49 ± 13*	2758 ± 1427*

LAC were complex sounds, i.e. composed of a fundamental frequency and its harmonic series, always recorded at a very weak amplitude. Two types of LAC were described: croaks and rumbles (Fig. 1F,G). Croaks were high-frequency calls (Fmax: 1625 ± 260 Hz) with a very short duration (9.4 ± 2.7 ms) and showed a large frequency bandwidth (725 ± 330 Hz). In contrast, rumbles were low-frequency (Fmax: 323 ± 94 Hz) and narrowband sounds (bandwidth: 207 ± 100 Hz) with a longer duration (25 ± 11.8 ms) compared to croaks.

FMS (Fig. 1H,I) were also composed of a fundamental frequency (Min Fo: 250 ± 165 Hz) and its harmonic series. They were the longest in duration (1070 ± 523 ms) and showed a frequency modulation (FM) pattern (ascending, descending or both), very variable among and within individuals. We also observed an intra-individual variability of the frequency excursion (i.e. difference between the highest and the lowest produced frequency), which ranged between 31 and 1047 Hz.

Finally, squeaks were wideband (bandwidth > 2700 Hz) and high-frequency sounds (Fmax > 3 kHz) with a harmonic structure. Two types of squeaks that differed by their total duration were described: short squeak (56 ± 19 ms, Fig. 1J) and long squeak (143 ± 69 ms, Fig. 1K).

The RLs estimated on a sub-sample of calls and measured for the 10 call types ranged from 102 to 124dB re 1 μPa RMS (Table 2). The lowest RLs were found for tocs (pulses) and LAC such as croaks and rumbles, with averaged RLs below 107 dB re 1 μPa RMS.

3.2. Comparison to control recordings

In a second step, a comparison of these 10 described sound types (Table 1) was made with the sounds collected during control recordings (Table 3). We de-

Table 2. Estimated received levels (RLs) on the acoustic tag of the 10 call types and their frequency characteristics (frequency for pulses and Fo for FMS [from our Table 1] and Fmax for other call types [from our Table 1]) (present study), and hearing thresholds extracted from audiograms measured in juvenile green sea turtles (Piniak et al. 2016, Bartol & Ketten 2006). For each call type, RL estimation was assessed using 1 call per tagged animal. For Piniak et al. (2016), sound pressure level (SPL) threshold values were directly extracted either from their Table 1 for tested frequencies (200, 300, 400, 1000 and 1600 Hz) or from their Fig. 4 on the audiogram curves for non-tested frequencies (500 Hz). For Bartol & Ketten (2006), we used threshold values from their Figs. 1 & 2 for tested frequencies (200, 300, 400 and 500 Hz). As several turtles were tested in both studies, we indicate SPL threshold ranges instead of average values. **Bold**: call types with RL falling within the hearing range of juvenile green turtles; Fmax: frequency of the highest energy; FMS: frequency-modulated sound; nt: not tested; RMS: root mean square

Call type	RL (dB re 1 µPa RMS), mean ± SD (range)	No. of calls	No. of individuals	Frequency or Fmax (Hz)	SPL threshold (dB re 1 µPa RMS) (Piniak et al. 2016)	SPL threshold (dB re 1 µPa RMS) (Bartol & Ketten 2006)
Mono	124 ± 3 (121–130)	10	10	1042	135–140 @ 1 kHz	nt
Doublet	115 ± 5 (109–120)	10	10	1053	135–140 @ 1 kHz	nt
Triplet	114 ± 3 (109–119)	10	10	1057	135–140 @ 1 kHz	nt
Multipulse	112 ± 5 (104–120)	10	10	1040	135–140 @ 1 kHz	nt
Toc	105 ± 6 (95–111)	10	10	<400	88–110 @ 400 Hz	90–110 @ 400 Hz
Croak	106 ± 4 (101–115)	9	9	1629	146–157 @ 1600 Hz	nt
Rumble	110 ± 8 (98–122)	9	9	322	85–104 @ 300 Hz	83–115 @ 300 Hz
FMS	127 ± 7 (115–133)	5	5	240–506	87–102 @ 200 Hz 100–115 @ 500 Hz	88–106 @ 200 Hz 102–115 @ 500 Hz
Short squeak	124 ± 4 (117–129)	9	9	3338	nt	nt
Long squeak	122 ± 3 (118–125)	9	9	3816	nt	nt

tected sound types similar to pulses and LAC. For pulses, the following 5 described pulse types were identified: mono, doublet, triplet, multipulse and toc; and for LAC, we found sounds similar to croaks and rumbles. Comparative analysis of temporal and frequency parameters revealed significant differences between those recorded during the control sessions (Table 3) and those obtained from the turtle-linked hydrophone (Table 1), except for the mono pulses.

3.3. Individual vocal stereotypy

Finally, to evaluate the potential for intra-specific communication, we investigated the level of individuality in the different sound types. We found that the 5 described types of pulses showed acoustic variables that were significantly different among individuals, except for toc and multipulses (Table 1, Kruskal-Wallis tests). For LAC, rumbles did not exhibit any individuality in their different variables, whereas croaks showed most of their spectral features to be significantly different among individuals. Unsurprisingly, FMS were not found to be individual-specific due to their high variability within individual recordings. In contrast, for both long and short squeaks, all acoustic variables were found to be significantly different among individuals.

Since most individuals emitted the latter two, we tested an RF algorithm to automatically classify long and short squeaks according to individual acoustic characteristics. For short squeaks, the RF classified the sounds of 7 individuals with a global accuracy of prediction of 76.2% (the overall error rate [OOB] was 23.8%). Based on the matrix of confusion, the indicator precision showed that 60 to 93% of the calls were classified to the correct individual, and these rates were always greater than those expected by chance (14.3%, Table S2). The acoustic variables showing the highest importance for classification were spectral features such as $Q25$, $Q75$ and Fmax (Gini index: 18, 14 and 12 respectively).

For long squeaks, the RF classified the sounds of 7 individuals with a global accuracy of prediction of 68.5% (the OOB was 32.5%). Based on the matrix of confusion, the individual correct classification rates ranged from 40 to 87%, and these rates were always greater than those expected by chance (12.5%,

Table 3. Acoustic characteristics of sounds detected during control recordings showing similarities with sounds described for juvenile green sea turtles from CATS cam recordings. Average values (± SD) shown, as well as range (in parentheses) for both temporal and frequency variables. *Significant difference (p < 0.05, Wilcoxon test). Fmax: frequency of the highest energy; LAC: low-amplitude calls; NS: not significant; NC: lack of comparison

Call type	No. of pulses	Pulse rate (Hz)	Frequency (Hz)
Pulse			
Mono (n = 16)	1	–	901 ± 328 (457–1281) (NS)
Doublet (n = 10)	2	19 ± 7 (9–34)*	789 ± 357 (470–1293) (NS)
Triplet (n = 19)	3	27 ± 4 (14–33) (NS)	713 ± 266 (293–1218)*
Multipulse (n = 36)	5 ± 1 (4–7) (NS)	25 ± 5 (14–34)*	650 ± 165 (350–1200)*
Toc (n = 19)	9 ± 4 (4–19)*	12 ± 3 (8–22)*	256 ± 54 (201–464) (NC)
		Duration (ms)	Fmax (Hz)
LAC			
Croak (n = 19)		55.7 ± 16.8 (29.8–96)*	463 ± 60 (361–570)*
Rumble (n = 1)		1453	478

Table S3). The acoustic variables showing the highest importance for classification were mostly spectral features such as Q25 and Q50, and also 1 temporal variable, duration (Gini index: 19, 19 and 17 respectively).

4. DISCUSSION

4.1. Underwater acoustic repertoire

This study is the first to report a wide range of underwater sounds produced by juvenile green sea turtles. We could not associate any specific behavior or activity with these vocal productions, and no sound production was observed in the presence of another turtle. However, we observed that tocs (one type of pulse sound) were mainly detected when juvenile green sea turtles were resting on the sea floor, and squeaks when animals were swimming back to the surface. Squeaks were not synchronized with flipper strokes, so perhaps they are not a by-product of their swimming movements, as might have been thought. As they are often produced when going back to sur-

face, squeaks might be associated with sea turtles preparing to breathe when reaching the surface.

The different sounds described in this study show some similarities with the underwater vocal repertoire of long-necked freshwater turtles *Chelodina oblonga* (Giles et al. 2009). Indeed, this freshwater turtle produces pulses (clacks, double clicks and staccato similar to mono, doublet, multipulses or toc respectively), but also sounds showing a clear harmonic structure, with or without an FM pattern (FMS similar to wail or high call; squeaks similar in their general structure to squawks or richly harmonic long chirps). If similarities occurred in the general acoustic structure of their vocal production (pulses, harmonic structure sound, FM pattern etc.), their durations and dominant frequencies are not similar, likely due to the clear body-size differences.

Regarding the underwater sound production evidenced in hatchlings of Kemp's ridley (Ferrara et al. 2019), some of their sounds also show similarities with sounds showing harmonic and non-harmonic structure (types I, II, III, IV, VI), some of them including an FM pattern (II, III, VI), and pulses (V). In these 3 studied species (*C. oblonga*, *Lepidochelys kempii* and *Chelonia mydas*), the sound productions ranged from pulses to much more complex sounds with harmonics and FM. These acoustic similarities gave us additional confidence that the recorded and described sounds could indeed have been produced by the juvenile green sea turtles. Our control recordings demonstrated that if some recorded sounds showed similar general structure, the frequency and/or temporal characteristics differed and thus were likely produced by other marine species such as crustaceans or fishes occurring in the green sea turtle habitat. The only uncertainty remained for mono pulses that shared similar acoustic characteristics.

The frequency bandwidth of the described sounds matched the hearing frequency range of green sea turtles measured in different electrophysiological studies (Bartol & Ketten 2006, Piniak 2012, Piniak et al. 2016). The underwater hearing sensitivity of juvenile green sea turtles, assessed by recording auditory evoked potentials, was found to be able to detect tonal sounds between 50 and 1600 Hz under water, with the highest sensitivity for frequencies ranging between 200 and 400 Hz (with an averaged threshold at 95–96 dB re 1 μPa RMS) in Piniak et al. (2016) and 600–700 Hz in Bartol & Ketten (2006). Based on these hearing abilities and the frequency bandwidth of the sounds, we can conclude that most of the described sounds in the present study can be heard by green sea turtles and thus could be used for intra-specific communication. Only 3

sounds with a frequency bandwidth outside the green sea turtle's hearing ability were found: croak, as well as both short and long squeaks. These contained frequencies both below and above 2000 Hz, suggesting that green sea turtles may only partially hear them. If we take into account the averaged RLs for the 10 described sound types, ranging from 98 to 133 dB re 1 µPa RMS (Table 2), only 3 sound types are produced at a level that could be detected by juvenile green turtles: tocs, rumbles and FMS. Most pulses, with the exception of tocs, are produced at a frequency around 1040 Hz and at an average level between 109 and 121 dB re 1 µPa RMS, so they are outside the hearing threshold described by Piniak et al. (2016). However, using audiograms performed on juvenile green sea turtles, Bartol & Ketten (2006) reported different thresholds. Similarly, Kemp's ridley hatchlings produced underwater sounds, most of which showed peak frequencies between 560 and 750 Hz (Ferrara et al. 2019), and their best hearing sensitivity is between 100 and 200 Hz (Bartol & Ketten 2006). This highlights that further investigations are needed on the hearing sense of marine turtles before making conclusions on their abilities to hear the different sounds they produce.

As we do not know the biological function of these sound productions, even if partially heard, they could still be used for intra- or inter-specific communication. Our investigations on individuality revealed that squeaks were individual-specific and thus could be used for individual recognition. In contrast, some sounds described in this study are within the best hearing sensitivity of juvenile green sea turtles, such as rumbles, tocs and FMS (Fmax ranging from 100 to 400 Hz), and these call types did not show any individuality. These sounds might be good candidates for intra-specific communication, but they would not likely be involved in individual recognition processes. Further investigations are thus needed to better understand the behavioral and social context of these acoustic productions, and how they develop throughout the long life cycle of green sea turtles (from juvenile to adult stages).

All sounds were produced without the presence of air bubbles in water, as we observed on the video recordings. This indicates that the sounds are produced with the mouth closed, and air stays within the vocal system of the turtles, as described for the long-necked freshwater turtle (Giles et al. 2009). The aerial and underwater vocal production mechanisms are still unknown in turtles, but they likely involve the larynx, the vocal cords, and 2 ventral cricoid diverticula that may act as cavity resonators (Sacchi et al. 2004, Colafrancesco & Gridi-Papp 2016). Further in-

vestigations are needed to better understand the anatomical structures involved in vocal production in turtles as well as the air flow during underwater vocal production in aquatic turtles.

4.2. Human-made noise impacts on marine turtles

This first study opens research avenues for green sea turtles and marine turtles in general, as the ability of the former to produce a wide range of sounds underwater that could be involved in acoustic communication was shown. Further knowledge is crucial to assess the impact of anthropogenic noise on both behavior and communications networks of turtles. Anthropogenic noise is dramatically increasing in all oceans (Hildebrand 2009), and is particularly impacting species inhabiting coastal waters where human activities can be very important. Green sea turtles use coastal waters for foraging, breeding, and nesting, and these areas of interest are noisy and becoming noisier. Most human activities in these areas (drilling, military sonar, airgun, vessel traffic etc.) generate low-frequency noise that overlaps with the most sensitive hearing range of green sea turtles, as well as other marine turtles such as loggerhead turtles *Caretta caretta* (Martin et al. 2012, Lavender et al. 2014), and also the range of their acoustic production (present paper).

Recent studies have shown that green and loggerhead turtles may use the soundscape of their foraging habitats to select high-quality coastal habitats by eavesdropping on low-frequency acoustic production from fishes and invertebrates (Papale et al. 2020). However, such assessment is only possible in areas with low anthropogenic acoustic pollution, as human-made noise will fully overlap these biological acoustic productions. It has already been shown that green and loggerhead turtles react to airgun sound exposure (McCauley et al. 2000) by showing an avoidance response (increased swimming speed) and stress-induced behavior (i.e. erratic behavior and agitated state). Tyson et al. (2017) deployed a multi-sensor tag (ROTAG) on 1 juvenile green sea turtle to identify activities in relation to noise sources and showed that the animal responded to noise by remaining still near the seafloor during passings of the vessel. Noise can also impact turtles physiologically, from permanent or temporary hearing loss to death (Viada et al. 2008). A recent review has clearly stated that the impact of noise on marine turtles has been neglected (Nelms et al. 2016), and further research as well as inclusion of these species in mitigation policies is urgently requested. Given the

threatened status of most marine turtle species, it is particularly critical to improve our current knowledge of marine turtles to further develop conservation and mitigation guidelines.

5. CONCLUSIONS

This analysis showed that juvenile green sea turtles were able to produce a diversity of sounds, showing diverse acoustic structure (from pulses to complex sounds). The underwater sound production described in both marine and freshwater turtles clearly suggests that acoustic communication in aquatic turtles might be more widespread than thought, and thus further investigations are needed to assess its role in crucial biological functions. Future investigations in adult green sea turtles during the breeding season, developmental period and during their long migratory routes are of major interest. Studies on other marine turtle species showing synchronized behaviors such as arribadas may also reveal the implication of acoustic signals in such mass events. In addition, the study of acoustic communication could contribute to reducing bycatch by identifying distress and/or alert sounds of marine turtles and implementing them in acoustic deterrent devices (pingers) installed on nets. In light of the potential acoustic communication occurring in these marine species, greater attention to the impact of human-made noise is crucial for the protection of these species.

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