

Preprints are preliminary reports that have not undergone peer review. They should not be considered conclusive, used to inform clinical practice, or referenced by the media as validated information.

# Using sea turtles' vocalization to reduce their bycatch?

**Damien Chevallier** 

#### damien.chevallier@cnrs.fr

Biologie des Organismes et Écosystèmes Aquatiques

#### Léo Maucourt

Biologie des Organismes et Écosystèmes Aquatiques

#### Isabelle CHARRIER

CNRS

#### **Pierre Lelong**

Biologie des Organismes et Écosystèmes Aquatiques

#### Yves Le Gall

Ifremer

#### **Eric Menut**

Ifremer

#### **Bryan Wallace**

Ecolibrium

#### **Cyrielle Delvenne**

CNRS

#### Orsolya Vincze

LIttoral, ENvironment and Societies

#### Lorène Jeantet

U. of Stellenbosch, African Institute for Mathematical Sciences

## Marc Girondot

University of Paris-Saclay

#### Jordan Martin

Biologie des Organismes et Écosystèmes Aquatiques

#### **Ouvéa Bourgeois**

Biologie des Organismes et Écosystèmes Aquatiques

#### **Muriel Lepori**

Biologie des Organismes et Écosystèmes Aquatiques

## Pascal Fournier

Groupe de Recherche et d'Etude pour la Gestion de l'Environnement

## **Christine Fournier-Chambrillon**

Groupe de Recherche et d'Etude pour la Gestion de l'Environnement

## Sidney Regis

Biologie des Organismes et Écosystèmes Aquatiques

## Nicolas Lecerf

Biologie des Organismes et Écosystèmes Aquatiques

## Fabien Lefebvre

ACWAA

## Nathalie Aubert

ACWAA

## **Mosiah Arthus**

Solda Lanmè

## Matthieu Pujol

Biologie des Organismes et Écosystèmes Aquatiques

## **Michel Anthony Nalovic**

Fishingcleaner.com

## Marie-Clémence Burg

Biologie des Organismes et Écosystèmes Aquatiques

## **Pascale Chevallier**

ANSLO-S

## Tao Chevallier

ANSLO-S

## Antony Landreau

ANSLO-S

## **Stéphane Meslier**

ANSLO-S

Eugène Larcher

Mairie des Anses d'Arlet

## **Moulanier Nicolas**

Biologie des Organismes et Écosystèmes Aquatiques

## Yvon Le Maho

Université de Strasbourg

## Article

Keywords:

Posted Date: April 2nd, 2024

License: © ④ This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.

# Using sea turtles' vocalization to reduce their bycatch?

Despite the intensive investigation of many aspects of sea turtle life-history in the wild for over four
 decades, their underwater communication capacities and behavioral responses to sound have gone
 largely overlooked. Our recent findings about sounds produced by sea turtles in the French West
 Indies island of Martinique elicit strong interest and therefore present new opportunities to reduce

6 harmful interactions between turtles and fishing gear.

7

1

Damien Chevallier<sup>1</sup>, Léo Maucourt<sup>1,2,3</sup>, Isabelle Charrier<sup>3</sup>, Pierre Lelong<sup>1,2</sup>, Yves Le Gall<sup>4</sup>, Eric Menut<sup>4</sup>,
Bryan Wallace<sup>5,6</sup>, Cyrielle Delvenne<sup>1</sup>, Orsolya Vincze<sup>7</sup>, Lorène Jeantet<sup>8</sup>, Marc Girondot<sup>9</sup>, Jordan Martin<sup>1</sup>,
Ouvéa Bourgeois<sup>1</sup>, Muriel Lepori<sup>1</sup>, Pascal Fournier<sup>10</sup>, Christine Fournier-Chambrillon<sup>10</sup>, Sidney Régis<sup>1</sup>,
Nicolas Lecerf<sup>1</sup>, Fabien Lefebvre<sup>11</sup>, Nathalie Aubert<sup>11</sup>, Mosiah Arthus<sup>12</sup>, Matthieu Pujol<sup>1</sup>, Michel
Anthony Nalovic<sup>13</sup>, Nicolas Moulanier<sup>1</sup>, Marie-Clémence Burg<sup>1</sup>, Pascale Chevallier<sup>14</sup>, Tao Chevallier<sup>14</sup>,
Antony Landreau<sup>14</sup>, Stéphane Meslier<sup>14</sup>, Eugène Larcher<sup>15</sup>, Yvon Le Maho<sup>16</sup>.

- 14
- <sup>1</sup> BOREA Research Unit, Laboratoire de Biologie des Organismes et des Ecosystèmes Aquatiques, MNHN, CNRS
   8067, SU, IRD 207, UCN, UA. Station de Recherche Marine de Martinique, Quartier Degras, Petite Anse, 97217
- 17 Les Anses d'Arlet, France.
- 18 <sup>2</sup> Université des Antilles, Campus de Schoelcher, 97275 Schoelcher Cedex, Martinique, France.
- <sup>3</sup> Institut des Neurosciences Paris-Saclay, CNRS, Université Paris-Saclay, 91400 Saclay, France.
- 20 <sup>4</sup> Ifremer. Service Acoustique Sous-marine et Traitement de l'Information. Direction de la Flotte
- 21 Océanographique. ZI de la Pointe du Diable CS 10070 29280 PLOUZANE, France.
- <sup>5</sup> Ecolibrium, Inc., 5343 Aztec Drive, Boulder, Colorado 80303, USA.
- <sup>6</sup> University of Colorado, Department of Ecology and Evolutionary Biology, 1900 Pleasant St, Boulder, CO
   80302, USA.
- <sup>7</sup> Littoral, Environnement et Sociétés (LIENSs), UMR7266, CNRS Université de La Rochelle, 2 rue Olympe de
   Gouges, 17042 La Rochelle Cedex, France.
- <sup>8</sup> African Institute for Mathematical Sciences, 7 Melrose Rd, Muizenberg, Cape Town, 7950, South Africa,
- 28 Department of Mathematical Sciences, Stellenbosch University, Victoria Street, 7602, South Africa.
- <sup>9</sup> Université Paris-Saclay, CNRS, AgroParisTech, Ecologie Systématique et Evolution, 91190, Gif-sur-Yvette,
   France.
- <sup>10</sup> Groupe de Recherche et d'Etude pour la Gestion de l'Environnement, Route de Préchac, 33730 Villandraut,
   France
- 33 <sup>11</sup>Association ACWAA, Quartier l'Etang, 97217, Les anses d'Arlet, France.
- <sup>12</sup> Solda Lanmè Caribbean Sea Soldier, 61 rue Anca Bertrand, Cité Dillon, 97200 Fort de France, France.
- <sup>13</sup> Fishingcleaner.com. 78 Rue Justin Catayee, 97300, Cayenne, Guyane Française.
- <sup>14</sup> ANSLO-S Association naturaliste de soutien logistique à la science, 7 Avenue Georges Clémenceau 49280 La
   Tessoualle, France.
- <sup>15</sup> Mairie des Anses d'Arlet, Boulevard des Arlésiens, 97217 Les Anses-d'Arlet, France.
- <sup>16</sup>Université de Strasbourg, CNRS, IPHC UMR 7178, 23 rue Becquerel, 67000 Strasbourg, France.
- 40
- 41 § Corresponding author: <u>damien.chevallier@cnrs.fr</u>
- 42

**Abstract.** Incidental capture of non-target species poses a pervasive threat to many marine 43 44 species, with sometimes devastating consequences for both fisheries and conservation efforts. Because of the well-known importance of vocalizations in cetaceans, acoustic 45 deterrents have been extensively used for these species. In contrast, acoustic communication 46 for sea turtles has been considered negligible, and this question has been largely unexplored. 47 Addressing this challenge therefore requires a comprehensive understanding of sea turtles' 48 49 responses to sensory signals. In this study, we scrutinized the avenue of auditory cues, 50 specifically the natural sounds produced by green turtles (Chelonia mydas) in Martinique, as 51 a potential tool to reduce bycatch. We recorded 10 sounds produced by green turtles and 52 identified those that appear to correspond to alerts, flight or social contact between individuals. Subsequently, these turtle sounds—as well synthetic and natural (earthquake) 53 sounds-were presented to turtles in known foraging areas to assess the behavioral response 54 55 of green turtles to these sounds. Our data highlighted that the playback of sounds produced by sea turtles was associated with alert or increased the vigilance of individuals. This therefore 56 57 suggests novel opportunities for using sea turtle sounds to deter them from fishing gear or 58 other potentially harmful areas, and highlights the potential of our research to improve sea 59 turtles populations' conservation.

Sea turtle bycatch, a major threat to many species, occurs in industrial and artisanal fisheries 60 using a variety of gear types including longlines; gill nets; trawls; traps; and pots<sup>1,2,3,4,5</sup>. Bycatch 61 threatens sea turtles globally since the areas where fisheries operate overlap with sea turtle 62 foraging habitats, breeding grounds and migratory corridors both spatially and temporally in 63 coastal and offshore ocean areas. In the French West Indies, fishing holds immense economic 64 65 significance, estimated at 20M€/year. The predominant artisanal fishing practices involve 66 small, single-person fishing companies, utilizing vessels under ten meters in length. These 67 operations encompass coastal operations, focused on demersal resources, and offshore 68 operations targeting pelagic species (Scombridae, Istiophoridae, Coryphaenidae, etc.). Coastal fishing constituted 62% of active vessels in Martinique in 2019 and 65% in Guadeloupe in 2018. 69 70 Various types of nets targeting different species (e.g., conch, lobster, reef fish) are used, including trammel nets and entangling gillnets set at the surface or ocean bottom. Although 71 72 sea turtles are known to interact with all of these (and other) fishing gears, characterizing 73 these interactions remains challenging. The prohibition of sea turtle fishing in Guadeloupe 74 (1991) and Martinique (1993) has somewhat contributed to the preservation of sea turtle 75 populations, but accidental captures of sea turtles persist. Bycatch represents a significant 76 threat and risk of direct mortality for juvenile and adult green turtles frequenting coastal 77 waters in these territories. Past studies<sup>6</sup> as well as first-hand accounts from fishermen (Topase 78 team, pers. comm.) report bycatch of sea turtles, including green (Chelonia mydas), hawksbill 79 (Eretmochelys imbricata) and loggerhead sea turtles (Caretta caretta). For fishermen, these captures not only result in diminished earnings due to the reduced catch of target species but 80 also incur additional costs (expenses and time) for repairing or replacing damaged gear. The 81 complexity of this situation makes effective communication challenging, but requires strong 82 collaboration with fishermen willing to contribute to finding solutions. The impact of bycatch 83 84 on coastal fisheries management is substantial, sometimes leading to the closure of fisheries. 85 Consequently, there is an urgent need to develop technologies to reduce bycatch, especially that of sea turtles. This is essential in order to protect sea turtles while also securing the 86 87 livelihoods of local fisheries<sup>7</sup>. Existing literature highlights diverse techniques designed to contribute to the reduction of sea turtle bycatch in gillnets, while also maintaining an 88 acceptable fishing yield<sup>8,9,10,11,12,13</sup>. The development of these technologies relies on 89

differences in sensory systems of sea turtles and those of target species of fisheries. The use 90 91 of visual deterring devices (Visual Deterrent Devices, VDD), particularly green and UV LEDs, 92 appears to reduce turtle bycatch in some fisheries. However, understanding of the specific behavioral responses of turtles to these stimuli remains limited; i.e., whether illuminating gear 93 94 alerts animals of its presence to avoid physical interactions or scares them away from the gear. Despite apparent success in reducing sea turtle bycatch, net illumination remains largely 95 96 experimental, and has not been implemented at scale in commercial fisheries for turtle 97 bycatch reduction purposes. The application of these devices presents challenges for fishers 98 ranging from entanglement in nets, concerns over the durability of LEDs, and the associated 99 financial burden of acquiring and maintaining them. Furthermore, the primary batteries 100 employed in these devices are Li-ion batteries, raising environmental concerns due to disposal 101 of spent batteries and water-intensive lithium extraction, resulting in issues such as soil 102 pollution and the depletion of water reserves. In this context, experiments designed to 103 evaluate the impact of low frequency Acoustic Deterrent Devices (ADDs) on sea turtle behavior might reveal a more efficient alternative solution to sea turtle bycatch reduction. 104 Behavioral and electrophysiological studies explored the acoustic ecology of sea turtles, 105 focusing on their auditory capabilities, their responses to acoustic stimuli and the implications 106 of this knowledge for their conservation<sup>7,14,15,16</sup>. Their research measured the underwater 107 hearing sensitivities of juvenile green, juvenile loggerhead, hatchling leatherback 108 109 (Dermochelys coriacea), and hatchling hawksbill sea turtles by recording potential responses to synthetic tonal stimuli. They concluded that sea turtles are able to perceive sound signals 110 in a range from 50 to 1600 Hz, with a maximum sensitivity between 10 and 400 Hz<sup>7,16</sup>. 111 In addition, though sea turtles have long been believed to be silent, recent studies identified 112 sound production in hatchling<sup>17,18,19,20</sup> and in juvenile green sea turtles<sup>3</sup>. Our primary objective 113 114 in the present study was therefore to explore whether turtle sound production, especially 115 those associated with alertness, escape behavior, or social contact, could provide a suitable tool for mitigating turtle bycatch. To accomplish this objective, we explored variation in 116 117 behavioral responses of foraging sea turtles to synthetic sound signals and natural sounds 118 produced by green turtles (online Methods and Fig. 1).

In a first step, we recorded the sounds produced by free-ranging juvenile green turtles and 119 their behaviors using on-board camera devices and hydrophones attached to their carapace 120 in Martinique (detailed methodology described in<sup>21,22</sup>). Overall, we recorded and described 10 121 sounds produced by green turtles and we identified four main sound categories for sounds 122 produced: Pulses, Low Amplitude Calls (LAC), Frequency Modulation Sounds (FMS), and 123 Squeaks<sup>21</sup>. In a second step, we examined the behavioral responses of green sea turtles 124 foraging in their natural environment to sounds which could potentially be associated with 125 fear, flight or social contact: Rumble (LAC category) and Squeak (Fig. 2a). Five different 126 127 recordings of the Squeak signal were presented to the turtles, varying in frequency, duration or intensity (see details in Table 2). These five recordings were presented as a single acoustic 128 129 signal in the tests. A geophonic sound (Earthquake) and three synthetic sound signals (Synth 130 FML, Synth FMA and Heavy Metal playback) were additionally tested (Fig. 2a). We used two small vessels to broadcast signals and observe behavioral responses. One vessel, referred to 131 as the "observation platform" (POBS) was employed by a diver responsible for spotting and 132 locating turtles underwater. Upon spotting an individual, the POBS informed the second 133 vessel, equipped with the acoustic platform (PACO). The PACO then positioned itself in 134 proximity of the observed turtle as the diver looked on and activated the speaker and initiated 135 136 sound playback. The POBS's diver observed and recorded (using a GoPro Hero 10 device) the

behavior of the target individual (Fig. 1). Visual observations were quantified using two 137 metrics: (i) assessing the immediate impact of sound playback on the behavior of green turtles 138 139 (referred to as "shot" hereafter), with reaction intensity rated on a scale of 0 (no reaction), 1 140 (significant reaction with alertness or watchfulness, *i.e.* turtle raises suddenly its head and look around, Fig. 1), to 2 (escaping, *i.e.* turtle swimming rapidly away from the test area); Fig. 1) 141 and (ii) assessing the change in activity by comparing the behavior recorded before and after 142 each shot. Several trials were performed, each one involved the repetition of shots of a given 143 signal on an individual turtle at variable distance (5-250 m), and the PACO moved then closer 144 to the animal, but always remained at a distance greater than five meters. Two alternative 145 versions of this protocol were used 1) to determine which sound signal triggered the turtle 146 147 behavioral responses and 2) to measure the distance and habituation effect to this sound. For 148 the first aim, if the turtle did not react within the first three shots, the trial was stopped. 149 We then tested the immediate reaction of a given individual to a defined signal within a trial. 150 For the second aim, only sounds that elicited the highest number of behavioral responses were tested with up to 13 shots per trial, on a wide range of distances (40-500m). 151

A total number of 75 initial trials to assess turtle response to each tested sound were performed on 68 individuals to assess the reaction of turtles to the different signals, with an average of 2.63±0.65 shots per trial. Secondly, 20 trials on 20 individuals were carried out to test the distance and habituation effect of particular sounds that elicited the highest level of behavioral responses, involving a mean of 5.40±2.76 shots per trial.

Reaction to signals. The three synthetic sound signals were also tested in 17 (Synth FML), five 157 158 (Synth FMA) and three (Heavy Metal playback) trials performed on 23 different feeding turtles. 159 We observed no reaction to any of these synthetic sounds. The geophonic sound (Earthquake) was tested on four feeding turtles, triggering no reaction in any of the tested individuals. 160 We presented the natural sounds produced by sea turtles, the Rumble (Fig. 2b) and the Squeak 161 162 (Fig. 2b), in playback tests to 18 and 28 feeding turtles, respectively. There was then a 163 significant difference in the proportion of turtles reacting to the sounds produced by sea 164 turtles (Fisher's Exact test, p = <0.0001), with 17 of total 18 turtles (94.4%) reacting to the Rumble and 17 of total 28 (60.7%) to the Squeak by exhibiting either a vigilance posture, 165 escaping, or a combination of the two. More precisely, Rumbles triggered only vigilance in 166 167 55.6% of observed responses, immediate escape or vigilance followed by an escape in 38.9% 168 of observations, and triggered no reaction in 5.6% of the tested individuals (Fig. 2c). Squeaks triggered vigilance in 53.6%, immediate escape or vigilance followed by an escape in 7.1%, and 169 no reaction in 39.3% of the tested turtles (Fig. 2c). The proportions of each behavioral reaction 170 varied significantly between the broadcast of Rumbles and Squeaks (Fisher's Exact test, p = 171 0.0044) with higher probability of escape behavior for Rumble and a higher frequency of no 172 reactions for Squeak (Post-hoc test for Fisher's Exact test, p = 0.0022). 173

174 Distance and habituation effect. Shots were played at different distances using the Rumble signal. When the Rumble signal was played from a distance of >300 m from the target 175 176 individual (n=17 shots), all shots resulted in no reaction. When the playback tests were performed from a distance between 200 and 300m (n=23 shots), 26.1% of turtles changed 177 their behavior, 45.9% changed their behavior from a distance between 100 and 200m (n = 37) 178 179 and 38.7% from a distance <100m (n=38 shots) from the focal individual. The distance between the focal turtle and the source of sound had thus a significant effect on the likelihood 180 of turtles to react, with an increasing probability of reaction when this distance decreased (p= 181 0.0087, Fig. 3a). Turtle's reactions occurred mainly for the first, the second and the third shots 182 183 with 70% (n=20), 60% (n=20) and 29.4% (n=17) of reactions respectively, regardless of the distance at which the shots were played. Turtles seemed to react less frequently after the fourth, fifth and sixth shots with 6.2% (n=16), 18.2% (n=11) and 12.5% (n=8) of reactions respectively. Beyond six shots, turtles stopped reacting to Rumble signal. The probability of turtles' responsiveness was inversely related to the number of shots (p < 0.0001, **Fig. 3b**).

188

The use of acoustic deterrents for cetacean bycatch reduction has been successful because of 189 the reliance of these species on acoustics for their general ecology and life history. In contrast, 190 there was a general assumption that acoustic communication is negligible in sea turtles. For 191 192 our knowledge, our study then demonstrates for the first time that sea turtles behaviorally 193 respond to the sounds they produce, and that their vocal repertoire is more functional than 194 previously thought. These findings therefore open new possibilities to reduce bycatch since 195 acoustic signals could be deployed with various fishing gears to potentially reduce sea turtle 196 interactions. The applicability of these results might extend to other sea turtle species, and 197 possibly to other marine species. Moreover, it has the potential for diverse applications beyond reducing bycatch. For example, synchronized nesting behavior, specifically the 198 massive arrivals of olive ridley sea turtles (Lepidochelys olivacea), might be coordinated in 199 some way by vocal communications among these turtles. In studies around the world that 200 201 utilize acoustic receivers to record sounds of marine species, these data could be examined to identify the sound produced by sea turtles as well. Thus, targeted studies on recording, 202 203 analyzing, and cataloging sea turtle sounds and associated visual, swimming/diving behaviors should be a research priority, particularly for researchers working on fine-scale underwater 204 turtle behavior. Once the sounds produced by sea turtles are identified, the automated 205 detection of those natural sounds emitted by each species might then enable the 206 207 establishment of an automated alert system to inform fishermen, enabling them to anticipate 208 and reduce bycatch by removing their nets before the arrival of these hundreds or thousands 209 individuals. Thus, the reduction in accidental captures could be effective not only for juveniles 210 in feeding areas, but also for adults in breeding areas.

211

#### 212 ACKNOWLEDGMENTS

This study was undertaken within Program TOPASE of the Centre National de la Recherche Scientifique (CNRS), with financial support from the Fonds Européen pour les affaires maritimes et la pêche (FEAMP), the Minister of Agriculture and FranceAgriMer. D.C was supported by the CNRS. We thank Dr. Wendy Piniak (NOAA) for her careful reading of the manuscript and valuable correction.

218

## 219 AUTHOR CONTRIBUTIONS

D.C., team leader of the TOPASE project, designed and performed the study in the field,
worked on the analyses and wrote the paper. Y.L.G., E.M. performed the acoustic test in
laboratory. Y.L.M. co-wrote the paper. P.L., L.M., I.C., worked on the analyses and added
useful modifications to the manuscript. Y.L.G. performed the physiological impact assessment.
B.W., O.V., M.G., L.J., E.L., T.C, P.C, P.F., C.F.C, A.L., S.M., M.N. added useful modifications to
the manuscript. Y.L.G., E.M, S.R., N.L., F.L., N.A, J.M., C.D., M.P., O.B., P.L., M.A., M.L., N.M.,
M-C.B. participated the study in the field.

227

#### 228 COMPETITING FINANCIAL INTERESTS

- 229 The authors declare no competing financial interests.
- 230
- 231

#### 232 DATA AVAILABILITY

- 233 The data that support the findings of this study are available from Damien CHEVALLIER but
- restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available.
- Data are however available from Damien CHEVALLIER (<u>damien.chevallier@cnrs.fr</u>) upon reasonable request and with permission of Damien CHEVALLIER.



**Figure 1** Schematic illustration of playback tests (POBS : observation platform; PACO : acoustic platform) and

illustration of immediate response types (0 = no reaction ; 1 = significant reaction).





Figure 2 (a) Frequency (Hz) and sound level (mean dB µPa @1m rms) of the presented signals tested during the study (Synthetic sounds are represented by pink rectangles), (b) Spectrograms of Squeak (top) and Rumble (bottom) recorded from wild green turtles<sup>21</sup> and (c) percent of turtles for each type of reaction to these two signals (respectively: Squeak in blue shades and Rumble in orange shades).



251

Figure 3 Probability of response to Rumbles with 95% CI obtained from generalized linear mixed model according to distance (m) (a) and shot number (b).

#### 255 ONLINE METHODS

256 Methods and any associated references are available in the online version of the paper.

Study area and permits. This study was carried out March 2023 in Anses d'Arlet (14° 50' N, 257 61° 9' W), Martinique Island (French West Indies, France). We conducted our study in five bays 258 of Les Anses d'Arlet (14°30' 9.64" N, 61°5'11.85" W): Anse Noire, Anse Dufour, Grande Anse, 259 Anse du Bourg and Anse Chaudière. The intentional disturbance of green sea turtles met 260 French ethical and legal requirements. The CNRS protocol was indeed approved by the 261 "Conseil National de la Protection de la Nature" and the "Ministère français de l'Ecologie, du 262 263 Développement Durable et de l'Energie (permit number: 971-2022-11-24-00004). The 264 fieldwork was carried out under the certification of Damien Chevallier (prefectural 265 authorizations' owner) under strict compliance of the Policy of Martinique's 266 recommendations in order to minimize animal disruption.

267 Green sea turtle sound production. We first characterized the sounds produced by green sea 268 turtles during associated behaviors in individual sea turtles equipped with underwater video cameras and hydrophones attached to their carapace, as previously described<sup>21,22</sup>. The audio 269 recordings were analyzed and the recorded sounds were categorized according to the 270 behavior associated with these (Pulse, Low Amplitude Call (LAC), Frequency Modulation 271 272 Sound (FMS), and Squeaks). Subsequently, the highest quality sound samples were selected in some sound categories (LAC and Squeak), and acoustic parameters were measured for each 273 signal (Table 2). Then, these sounds were played using a speaker in the presence of green sea 274 turtles foraging in their natural environment to examine behavioral responses to these signals. 275

276

Acoustic signals protocols. 278

279 Characteristics of the two transmission chains. Two autonomous very low frequency 280 transmission chains with internal battery have been developed:

- A so-called electrodynamic chain consisting of a prototype electrodynamic loudspeaker 281 282 resonating at 70 Hz and dedicated custom-made power supply and electronics. This chain can 283 sweep the frequency band [20-500 Hz], with a maximum peak sound level ( $SL_{pk}$ ) of 169 dB (ref. 1 μPa @ 1 m). A broadband prototype speaker [20-3000 Hz] can also be used. In this case, the 284 very low frequency energy ([20-500 Hz]) is attenuated compared to that obtained with the 285 nominal loudspeaker (emission level reduced by approximately 5 dB in the VLF band). 286

- 287 - A so-called piezoelectric chain made up of a piezoelectric transducer resonating at 180 Hz 288 (Geospectrum Bender M21-325-200) and specific custom-made power supply and electronics. 289 This chain can sweep the frequency band [100-1000 Hz], with a maximum peak sound level 290 (SL<sub>pk</sub>) of 173 dB (ref. 1 µPa @ 1 m). This piezoelectric source is less energetic in the band [20-291 150 Hz] than the electrodynamic source, but works up to 1 kHz, for an identical average emission level. The emission levels given in Table 2 come from measurements made from a 292 hydrophone (OceanSonics IcListen HF) deployed from the PACO acoustic platform during the 293
- experiment. 294

295 *Signals tested.* Table 2 summarizes the different sound signals used for the first playback tests.

Different frequency modulations (FM) were tested on the green sea turtles: linear FM (FML), 296 297 random FM (FMA) and summation of linear FM (NFML). Different frequency bands were

scanned depending on the transmission chain: [20-500 Hz], [20-3000 Hz] and [100, 1000 Hz], 298

with acoustic energies distributed differently in the band depending on the transmitter used. 299

For these FMs, the maximum peak sound level (SL<sub>pk</sub>) was of the order of 173 dB (ref. 1 µPa at 300 301 1 m). This is a very moderate emission level.

- 302 In addition to these frequency modulations, the following were tested:
- 303 A sound of the geophony, an earthquake (TT), covering the range 15-150 Hz, 304 with an energy peak at 50 Hz. The emission level of this sound sequence was 305 lower than that of the FM mode.
- 306 Sounds produced by sea turtles (Squeak and Rumble) and broadcast on one of the transmission channels (the choice of channel and HP depends on the 307 308 frequency content of these signals).

Physiological impact of the selected signals. Published literature on the physiological impact 309 thresholds of sea turtles is notably scarce. However, the US Navy has proposed threshold 310 values for sea turtles (Table 1<sup>23</sup>), in function of PTS (Permanent Threshold Shift) and TTS 311 (Temporary Threshold Shift). In an animal, exposure to sufficiently intense sound can result in 312 an elevation of hearing threshold. The duration of this threshold increase primarily hinges on 313 factors such as exposure time, signal amplitude and its frequency. This change in hearing 314 315 threshold can be either temporary (TTS) or permanent (PTS). To estimate the impact radius of 316 an acoustic source, calculations are derived from these thresholds and the levels at the source (at the reference distance of 1 m). Two metrics, "peak level" and "cumulative sound exposure 317 318 level", have been used during this study. The cumulative sound exposure level (SEL<sub>CUM</sub>) integrates all the sound sequences received by the animal, taking into account transmission 319 losses based on the distance between the sound source and the exposed turtle. 320

Group	Hearing threshold at fo	TTS threshold		PTS threshold		
	SPL (dB SPL)	SEL (weighted) (dB SEL)	peak SPL (dB SPL)	SEL (weighted) (dB SEL)	peak SPL (dB SPL)	
LF	54	168	213	183	219	
MF	54	170	224	185	230	
HF	48	140	196	155	202	
SI	61	175	220	190	226	
ow	67	188	226	203	232	
PW	53	170	212	185	218	
TU	95	189	226	204	232	
OA	11	146	170	161	176	
PA	-4	123	155	138	161	

#### 322

Table 1 Sea turtle PTS and TTS thresholds (red box)<sup>23</sup>.

323 **Peak level impact received by the animal.** The received peak level TTS threshold was 324 therefore estimated to: 226 dB ref. 1µPa. Since sound levels ( $SL_{pk}$ ) were very moderate 325 (maximum 175 dB ref. 1 µPa @ 1 m by taking 2 dB safety factor for the sound risk analysis), 326 the threshold was never reached. There was therefore no predicted deleterious impact on sea 327 turtles, even temporary, regardless of the distance.

*Impact in cumulative sound exposure level received by the animal.* The TTS threshold in 328 329 cumulative sound exposure level was therefore 189 dB ref. 1 µPa<sup>2</sup>.s. For one acoustic emission, the maximum sound exposure level (SEL<sub>1shot</sub>) was 178 dB ref. 1 µPa<sup>2</sup>.s at 1 m. For a 330 turtle at 1 m from the acoustic source, the cumulative sound exposure level threshold was 331 332 reached after 13 emissions. For a turtle at 5 m from the acoustic source, the cumulative noise 333 exposure level threshold was reached after approximately 300 emissions. For a turtle at 10 m 334 from the acoustic source, the cumulative sound exposure level threshold was reached after approximately 1300 emissions. In view of these data, we decided very cautiously not to use 335 acoustic sources within five meters of sea turtles. The levels of peak and sound exposure 336 implemented during these tests were low enough to guarantee that no physiological impact, 337 338 even temporary, could be induced for the targeted species. Importantly, this study was 339 interested in turtle reactions function of signal type (synthetic vs sound produced by turtles). 340 Thus, the reaction of turtles induce by a level of signal emissions beyond threshold could bias 341 the results, and should be avoided. In this study, we favor the particular structure of an alert signal over the amplitude of a repulsive signal. 342

Acoustic Deterrent Device Behavioral Tracking and Trials. The effectiveness tests of the
 signals used on the sea turtles were carried out using two light boats:

- A boat called "platform observation" (POBS) to tow an underwater observer responsible for
spotting turtles underwater. Once a turtle had been spotted, the POBS informed the acoustic
platform (PACO). The POBS underwater observer observed and recorded video (GoPro Hero

- 10) of any immediate changes in behavior (**Fig. 1**).
- A boat called "acoustic platform" (PACO) from which the sound sources were implemented.
- The loudspeaker was deployed as a pendulum at a depth of 1.5 (electrodynamic) to 5 meters

(piezoelectric) providing that the water depth was sufficient. The autonomous electronics box was on board. The choice of the signal and its amplitude level were controlled from an onboard PC and a Bluetooth connection with the box. Different signals could be tested on the same animal. The test of a signal on an animal is called a "trial", which is constituted of several "shots" corresponding to signal emissions. Each "shot" last between 3 and 12 seconds, depending of the signal (Table. 2). The interval between two shots was 12 seconds.

357 The visual observation of the turtles' reactions was carried out at 2 levels:

- Measurement of the immediate effect on behavior at the time of the "shot", with
   estimated intensity (0= no reaction; 1=significant reaction) (Fig. 1)
- -Analysis of the change in activity rhythm by comparison of the behavioral observationsequences before and after "shooting".

For each type of sound signal, the initial source-animal distance was approximately 100 meters and there was only one initial transmission. Depending on the behavior of the animal, several identical shots at the same distance might be emitted. PACO boat would then come closer to the animal, however remaining at a distance greater than 5 m.

This method was then used a second time using a single signal, with a wider range of distance from 40 to 500m and up to 13 repetitions, in order to obtain dataset for distance and habituation effects testing. Taking into account the specific propagation conditions in very shallow waters, it is estimated that the SPL<sub>rms</sub> (i.e. the sound level received by the turtles) from which behavioral reactions are observed on green turtles with "Rumble" type signals are of the order of 135 dB ref.1  $\mu$ Pa.

372 Statistical analyses. In order to test the behavioral reaction to the different acoustic signals, only the three first shots are kept in the analysis, since we want to test for immediate turtle's 373 reaction. First, the number of turtles that actively responded to the different signal types was 374 375 counted. A response in this sense could be alertness or escaping (as opposed to no response 376 observed), and only one reaction is taken into account per trial, even if the turtle reacted to several shots in the same trial. Secondly, for the signals to which turtles responded the most, 377 the number of turtles is counted for each type of possible response as well as for non-378 response. Inside a trial, only the strongest response behavior to a signal was retained (ordered 379 by increasing strength: 0: "no reaction", 1: "alertness", 2: "escaping"). Variations of the 380 381 proportion of turtles that responded among the different signal categories, as well as significance of difference between proportions of turtles among three possible response 382 383 behaviors (no reaction, alertness, escaping) are tested using nonparametric tests (Fisher's Exact test). The second dataset, created only with the signal eliciting the strongest behavioral 384 385 response, is used to model the distance and the habituation effect on turtle reaction. The turtle behavioral response variable is binomial (1: alertness or escaping, 0: no reaction). A 386 387 Generalized Linear Mixed Model was set up accounting for distance of the shot to the turtle and shot number as fixed effects. Trials ID, which is the ID of the series of shots on the same 388 individual, was included as a random effect. Model was fitted using package 'Ime4'<sup>24</sup> and 389 390 goodness-of-fit was assessed using package 'DHARMa'<sup>25</sup> in R v4.2.2<sup>26</sup>.

391

392

Transmission chains	Track	Signals tested	Frequency band (Hz)	Duration (s)	SPL <sub>rms</sub> (dB μPa @ 1m)	Test date (March 2023)	Number of trials
	TRACK 7	SQUEAK	100-2000	4	158	14	3
	TRACK 10	SQUEAK	100-2000	12	158	14	5
	TRACK 9	SQUEAK	150-1100	3	158	14	3
ELECTRO LB	TRACK 4	SYNTH FML	20-3000	5	160	14	1
	TRACK 11	SQUEAK	100-2000	5	158	15-16	11
	TRACK 13	HEAVY METAL	30-2000	5	160	15	3
ELECTRO V5	TRACK 1	NAT TT	15-150	11	160	16	4
	TRACK 3	SYNTH FMA	100-1000	5	168	16-17-21	4
	TRACK 1	SYNTH FML	100-1000	5	170	17-20	4
DIEZO	TRACK 2	SYNTH FML	100-1000	7	170	16-17-20	12
	TRACK 4	SYNTH FML	100-1000	5	170	17	1
	TRACK 5	RUMBLE	150-1100	3	172	17-20-21-22	38
	TRACK 6	SQUEAK	100-1300	4	169	20-21	6
						Total	95

 Table 2 Acoustic characteristics of some signals (not exhaustive) tested on green sea turtles in the wild.

#### 403 **References**

404 1. Epperly, S.P., Braun, J. & Richards, P.M. Trends in catch rates of sea turtles in North Carolina, U.S.A.
405 *Endangered Species Research* 3, 283-293 (2007).

Lewison, R.L., Crowder, L.B., Wallace, B.P, Moore, J.E., Cox, T., Zydelis, R., McDonald, S., DiMatteo,
A., Dunn, D.C., Kot, C.Y., Bjorkland, R., Kelez, S., Soykan, C., Stewart, K.R., Sims, M., Boustany, A., Read,
A.J., Halpin, P., Nichols, W.J. & Safina, C. Global patterns of marine mammal, seabird, and sea turtle
bycatch reveal taxa-specific and cumulative megafauna hotspots. *PNAS* 111 (14): 5271-5276 (2014).

- 3. Moore, J.E., Wallace, B.P., Lewison, R.L., Zydelis, R., Cox, T.M. & Crowder, L.B. A review of marine
  mammal, sea turtle, and sea bird bycatch in USA fisheries and the role of policy in shaping
  management. *Mar. Policy* 33, 435–451 (2009).
- 4. Peckham, S.H., Maldonado Diaz, D., Walli, A., Ruiz, G., Crowder, L.B., & Wallace J.N. Small-Scale
  Fisheries Bycatch Jeopardizes Endangered Pacific Loggerhead Turtles. *PLoS ONE* 2 (2007).
- 5. Wallace, B.P., Kot, C.Y., DiMatteo, A.D., Lee, T., Crowder, L.B. & Lewison R.L. Impacts of fisheries
  bycatch on marine turtle populations worldwide: toward conservation and research priorities. *Ecosphere* 4(3), 1-49 (2013).
- 6. Louis-Jean, L. Study of coastal artisanal fishing with bottom nets in the French West Indies in order
  to reduce accidental captures of sea turtles and obtain a more sustainable activity. PhD, Montpellier
  University (2015).
- 7. Piniak, W.E.D, Wang, J., Waddell, E., Barkan, J., Fisler, S., Isaac-Lowry, O.J., Cerecedo Figueroa, A.,
  Alessi, S.C. & Swimme Y. Low-Frequency Acoustic Cues Reduce Sea Turtle Bycatch in Gillnets. 148th
  Annual Meeting of the American Fisheries Society (2018).
- 424 8. Wang, J.H., Fisler, S. & Swimmer, Y. Developing visual deterrents to reduce sea turtle bycatch in 425 gillnet fisheries. *Mar. Ecol. Prog. Ser.* **408**, 241-250. (2010).
- 426 9. Wang, J., Barkan, J., Fisler, S., Godinez-Reyes, C. & Swimmer, Y. Developing ultraviolet illumination
  427 of gillnets as a method to reduce sea turtle bycatch. *Biol. Lett.* 9, (2013).
- 10. Ortiz, N., Mangel, J.C., Wang, J., Alfaro-Shigueto, J., Pingo, S., Jimenez, A., Suarez, T., Swimmer, Y.,
  Carvalho, F. & Godley, B.J. Reducing green turtle bycatch in small-scale fisheries using illuminated
  gillnets: the cost of saving a sea turtle. *Mar. Ecol. Prog. Ser.* 545, 251-259 (2016).
- 431 11. Lucchetti, A., Bargione, G., Petetta, A., Vasapollo, C., & Virgili, M. Reducing sea turtle bycatch in the
  432 mediterranean mixed demersal fisheries. *Frontiers in Marine Science* 6, 387 (2019).
- 12. Darquea, J.J., Ortiz-Alvarez, C., Córdova-Zavaleta, F., Darquea, J., Ortiz-Alvarez, C., CórdovaZavaleta, F., Medina, R., Bielli, A., Alfaro-Shigueto, J., Mangel, J. *J. Lat. Am. J. Aquat. Res.* 48, 446-455
  (2020).
- 436 13. Bielli, A., Alfaro-Shigueto, J., Doherty P.D., Godley, B.J., Ortiz, C., Pasara, A., Wang, J.H. & Mangel,
  437 J.C. An illuminating idea to reduce bycatch in the Peruvian small-scale gillnet fishery. *Biol. Conserv.* 241
  438 (2020).
- 439 14. DeRuiter, S., & Larbi Doukara, K. Loggerhead turtles dive in response to airgun sound exposure.
  440 *Endangered Species Research*, 16(1), 55–63 (2012).
- Lavender, A. L., Bartol, S. M., & Bartol, I. K. Ontogenetic investigation of underwater hearing
  capabilities in loggerhead sea turtles (Caretta caretta) using a dual testing approach. *Journal of Experimental Biology*, 217(14), 2580–2589 (2014).

- 444 16. Piniak W.E.D. Acoustic Ecology of Sea Turtles: Implications for Conservation. PhD, Duke University445 (2012).
- 446 17. Ferrara CR, Mortimer JA, Vogt RC (2014a) First evidence that hatchlings of Chelonia mydas emit 447 sounds. *Copeia* 2014: 245–247.
- 18. Ferrara CR, Vogt RC, Harfush MR, Sousa-Lima RS, Albavera E, Tavera A First evidence of leatherback
  turtle (Dermochelys coriacea) embryos and hatchlings emitting sounds. *Chelonian Conserv Biol* 13:
  110–114 (2014b).
- 451 19. McKenna LN, Paladino FV, Tomillo PS, Robinson NJ (2019) Do sea turtles vocalize to synchronize
  452 hatching or nest emergence? *Copeia* **107**: 120–123.
- 20. Monteiro CC, Carmo HMA, Santos AJB, Corso G, Sousa-Lima RS. First record of bioacoustic emission
  in embryos and hatchlings of Hawksbill Sea turtles (Eretmochelys imbricata). Chelonian Conserv. Biol.
  18: 273–278 (2019).
- 456 21. Charrier, I., Jeantet, L., Maucourt, L., Régis, R., Lecerf, N., Benhalilou, A. & Chevallier, D. First 457 evidence of underwater vocalizations in green sea turtles Chelonia mydas. *Endangered Species* 458 *Research* **48**, 31-41 (2022).
- 22. Jeantet, L., Planas-Bielsa, V., Benhamou, S., Geiger, S., Martin, J., Siegwalt, F., Lelong, P., Gresser,
  J., Etienne, D., Hiélard, G., Arque, A., Regis, S., Lecerf, N., Frouin, C., Benhalilou, A., Murgale, C., Maillet,
  T., Andreani, L., Campistron, G., Delvaux, H., Guyon, C., Richard, S., Lefebvre, F., Aubert, N., Habold, C.,
  le Maho, Y., Chevallier, D. Behavioural inference from signal processing using animal-borne multisensor loggers: a novel solution to extend the knowledge of sea turtle ecology. R. Soc. Open Sci. 7,
  200139 (2020).
- 465 23. Technical Report Criteria and Thresholds for U.S. Navy Acoustic and Explosive Effects Analysis 466 (Phase III) (2017).
- 467 24. Bates, D., Mächler, M., Bolker, B. & Walker, S. Fitting Linear Mixed-Effects Models Using Ime4. *J.*468 *Stat. Soft.* 67. 1-48 (2015).
- 469 25. Hartig, F. DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. *R* 470 package version 0.3 3.5 (2022).
- 471 26. R Core Team, R: A language and environment for statistical computing. https://www.R-project.org/472 (2022).
- 473