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Behavioral Responses of Harbor Porpoises (*Phocoena phocoena*) to Sonar Playback Sequences of Sweeps and Tones (3.5-4.1 kHz)

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Abstract

Naval sonar signals may affect the behavior of harbor porpoises (*Phocoena phocoena*). The 53C sonar system produces 1,600 ms sonar signals in the 3.5 to 4.1 kHz band, each consisting of a sweep immediately followed by two tones which are separated by a 100 ms silence. Effects of sound pressure level (SPL) and duty cycle on the behavioral responses of two harbor porpoises to these sounds were investigated. Respiration rate, distance to the transducer, swimming speed, and the number of jumps during sound exposure and baseline periods were compared. Harbor porpoises were exposed to 30-min playbacks of 53C sonar sounds at five average received SPLs (Lrecs) with a duty cycle of 2.7%, and at six Lrecs with a duty cycle of 96%, under low ambient noise conditions. They did not respond to the sounds when the duty cycle was 2.7%, even at the maximum Lrec (143 dB re 1 µPa). When the duty cycle was 96%, only Porpoise 06 increased his respiration rate when the Lrec was ≥119 dB re 1 µPa, and he moved away from the transducer only at an Lrec of 143 dB re 1 µPa. At the same Lrec and duty cycle, the effect of 53C sonar sounds on harbor porpoise behavior was weaker than that of 1 to 2 kHz, 6 to 7 kHz, and 25 kHz sonar signals observed in previous studies.

Key Words: avoidance, behavior, duty cycle, naval sonar, odontocete, respiration rate, response

Introduction

The contribution of anthropogenic noise to ambient noise in the Pacific Ocean increased steadily during the second half of the 20th century (Andrew et al., 2002, 2011; McDonald et al., 2006; Chapman & Price, 2011). Sound is particularly important for marine animals, as it is used as a means of orientation and communication, and to locate prey, conspecifics, and predators (Richardson et al., 1995). Therefore, marine animals are likely to be disturbed by noise in their environment. Noise caused by human activities, when added to the natural ambient sound in the oceans, may have negative physiological, auditory, and behavioral effects on marine fauna.

Naval sonar signals are believed to have caused strandings in some odontocete species (Balcomb & Claridge, 2001; D'Amico et al., 2009; Filadelfo et al., 2009). Several research projects have been conducted to investigate the cause of these strandings, which only occurred in certain species and in certain contexts (Azzellino et al., 2011). To elucidate the mechanisms resulting in the strandings, several behavioral response studies with naval sonar sounds have been conducted in the wild, mainly on the larger odontocetes and mysticetes (McCarthy et al., 2011; Miller et al., 2012; DeRuiter et al., 2013; Goldbogen et al., 2013; Antunes et al., 2014; Henderson et al., 2014; Isojunno et al., 2016).

The potential effects of naval sonar sounds on the harbor porpoise (*Phocoena phocoena*) are of particular interest because this small odontocete has a very wide geographical range, including the coastal waters of the North Atlantic, the North Pacific, the North Sea, the Baltic Sea, and the Black Sea. Wright et al. (2013) suggested that naval sonar was the cause of a mass stranding of harbor porpoises in Denmark. The harbor porpoise has acute hearing (its 50% hearing threshold between 100 and 140 kHz is ~33 dB re 1 µPa) and has functional hearing over a very wide frequency range (range of best hearing, defined here as within 10 dB of maximum sensitivity, is from 16 to 140 kHz; Kastelein et al., 2002, 2009, 2010). Harbor porpoises are relatively easily deterred by anthropogenic underwater noises (Amundin & Amundin, 1973; Polacheck & Thorpe, 1990; Kastelein et al., 1995, 1997, 2000, 2001, 2005, 2006; Laake et al., 1998; Culik et al., 2001; Johnston, 2002; Olesiuk et al., 2002; Koschinski et al., 2003; Teilmann et al., 2006; Tougaard et al., 2009). Behavioral response threshold levels for harbor porpoises have been determined with captive animals for noise bands and tonal sounds around 12 kHz (Kastelein et al., 2005), for a continuous 50 kHz tone (Kastelein et al., 2008a), for continuous and pulsed 70 and 120 kHz tones (Kastelein et al., 2008b), for pile driving sounds (Kastelein et al., 2013), for acoustic porpoise deterrent sounds (Kastelein et al., 2017c), and for various naval sonar sounds (Kastelein et al., 2011, 2012, 2013, 2014a, 2014b, 2015b, 2015c). In a field study with wild harbor porpoises, a response threshold level was determined for pile driving sounds (Tougaard et al., 2009). These studies found that the spectrum and the received level of an underwater sound, in combination with the duty cycle (the percentage of time in which a signal is produced) and signal type (impulsive or non-impulsive), influenced the effect that sound had on the behavior of harbor porpoises. Response thresholds and dose-behavioral response relationships need to be understood for the prediction of the effects of noise on harbor porpoises, which is required for policy development.

The U.S. Navy uses hull-mounted sonar systems that produce sounds in the ~3.5 to 4.1 kHz frequency range, known as AN/SQS-53C sonar and abbreviated here as 53C sonar. It is currently unknown how harbor porpoises respond to sonar sounds at duty cycles used in 53C sonar systems, at which received sound pressure levels (Lrecs) they begin to respond, and whether their responses change during an exposure period. Therefore, the goals of this study were to establish a dose-behavioral response relationship in harbor porpoises for 53C sonar sounds played back at two duty cycles and to see if the response changed during exposures. The effects of 53C sonar sounds are then compared with the effects of other sonar systems producing sounds in the 1 to 2 kHz, 6 to 7 kHz, and 25 kHz frequency ranges.

Methods

Study Animals

The study was conducted with two rehabilitated stranded harbor porpoises. At the time of the study, the female (identified as Porpoise 05) was 6 y old, her body mass was around 42 kg, her body length was 152 cm, and her girth at the axilla was approximately 80 cm. The male (identified as Porpoise 06) was 3 y old, his body mass was around 33 kg, his body length was 127 cm, and his girth at the axilla was approximately 80 cm.

The hearing of the harbor porpoises in the range of the sonar sounds used in the present study (*ca.* 3 to 4 kHz) had been tested with the psychophysical technique and was representative of animals of the same age and species; their 50% hearing thresholds (Kastelein et al., 2017b) were similar to those of three other young male harbor porpoises (Kastelein et al., 2002, 2009, 2010, 2015a).

Study Area

The study was conducted at the SEAMARCO Research Institute, the Netherlands. Its location is remote and quiet, and was specifically selected for acoustic research. The animals were in a pool complex built for acoustic research, which consisted of an outdoor pool (12 × 8 m, 2 m deep) connected via a channel (4 × 3 m, 1.4 m deep) with an indoor pool (8 × 7 m, 2 m deep). The study was conducted in the outdoor pool (Figure 1). The pool walls were made of plywood covered with polyester. To reduce reflections of sound in the pool, parts of the walls were covered with nets on which aquatic vegetation grew, and the bottom was covered with a 20-cm thick layer of sloping sand.

The water level was kept constant with skimmers. Sea water was pumped directly from the nearby Eastern Scheldt, a semi-enclosed tidal bay of the North Sea, into the open system; 80% recirculation through sand filters ensured year-round water clarity.

The water circulation and aeration system for the bio-filter were made as quiet as possible by choosing "whisper" pumps, mounting the pumps on rubber mats, and connecting the pumps to the circulation pipes with very flexible hoses. There was no current in the pool during the experiments, as the water circulation pump and the air pump of the bio-filter were shut off between 0800 and 1700 h. By the time a session started, no water flowed over the skimmers so that there was little or no flow noise. The average monthly water temperature varied during the year between 0 and 22°C; the salinity was around 3.4%.

The equipment used to produce and check the sound stimuli was housed out of sight of the study animals in a research cabin next to the pool (Figure 1). Great care was taken to cause no disturbances in the harbor porpoises' environment. Only the equipment operator was allowed within 15 m of the pool during test sessions, and she sat quietly in the research cabin.

Acoustics

Ambient Noise and Stimuli Measurements—Except where stated otherwise, terms and definitions follow *ISO 18405 Underwater Acoustics – Terminology* (International Organization for Standardization [ISO], 2017).

The ambient noise and 53C sonar sounds were measured by an external acoustic company (TNO)

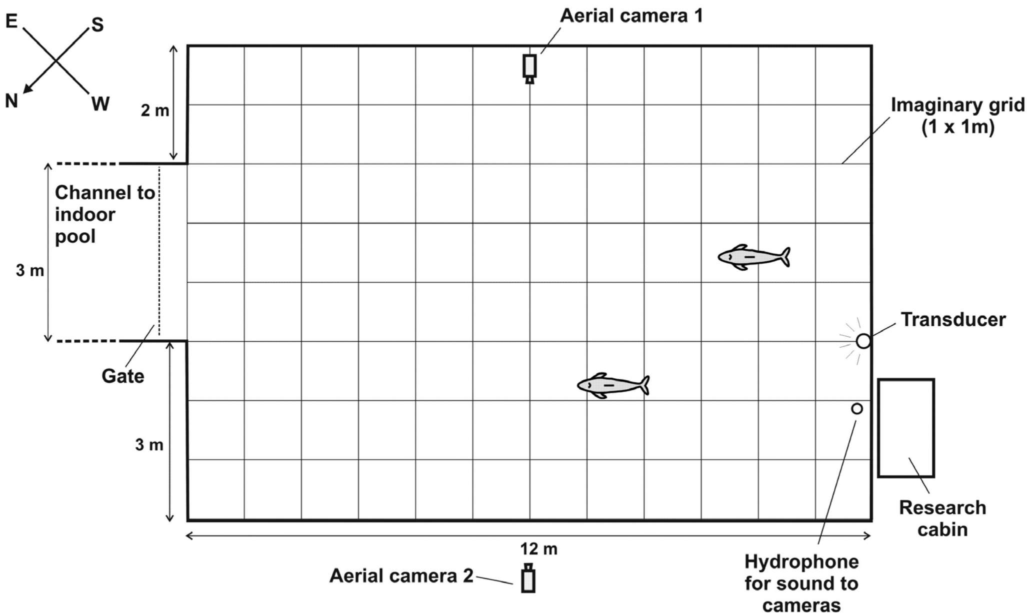

Figure 1. Top scale view of the study area (outdoor pool), showing the harbor porpoises (*Phocoena phocoena*), the location of the two aerial cameras, the underwater transducer emitting the 53C sonar sounds, and the listening hydrophone. Also shown is the research cabin which housed the audio and video equipment and the operator. The gate to the indoor pool was closed during the sessions.

at the beginning, in the middle, and at the end of the study, under conditions similar to those during the test periods. The sound measurement equipment consisted of three hydrophones (Brüel & Kjaer [B&K] – 8106), with a multichannel high frequency analyzer (B&K PULSE – Lan-xi type 3161-A-1/1) and a laptop computer with B&K PULSE software (*Labshop*, Version 20; Sample frequency: 524,288 Hz). Before analysis, the recordings were high-pass filtered (cut-off frequency, 100 Hz; third-order Butterworth filter, 18 dB/oct) to remove low-frequency sounds made by water surface movements. The system was calibrated with a pistonphone (B&K – 4229 with coupler WA 0658).

As a test stimulus, a 53C sonar sound recording supplied by the U.S. Navy was played back in the pool (see Figure 2 for a diagram of the equipment used). Each signal (see Figure 3) in the 53C sonar sound exposure comprised a frequency modulated (FM) up-sweep from 3.5 to 3.6 kHz (Component 1), a continuous wave (CW) of 3.75 kHz (Component 2), a 100 ms silence, and a CW of 4.1 kHz (Component 3). Each of the three components lasted for 500 ms, including 10 ms sigmoid on and off ramps (10 to 90% amplitude rise time of the sigmoid: 7 ms) so that the total signal duration, including the 100 ms silence, was 1,600 ms.

Multiple signals were transmitted regularly at one of two transmission cycles. Each cycle was comprised of one signal followed by a period of silence (gap) of one of two different durations. The cycle durations were 1.66 and 60 s, corresponding to gap durations of 60 ms (duty cycle 96%) and 58.4 s (duty cycle 2.7%). We define duty cycle as the percentage of time the 53C sonar signals (defined here as 1,600 ms, thus including the 100 ms pause) were produced during each session.

For the sound exposure with a duty cycle of 2.7%, the SPL was calculated by averaging over one complete 1,600 ms signal. For the 96% duty cycle, the SPL was calculated by averaging over the duration of five full transmission cycles, with a total duration of 8.3 s. For the 96% duty cycle, no distinction is made in reporting SPL values averaged over 1,600 or 8,300 ms; the difference is less than 0.2 dB and has no effect on our conclusions. One-third octave (base 10) band spectra of the SPL (Figure 4) were determined via digital filtering of the time signal. Six different amplifier settings were used for each duty cycle, from -60 to 0 dB relative to the maximum gain in steps

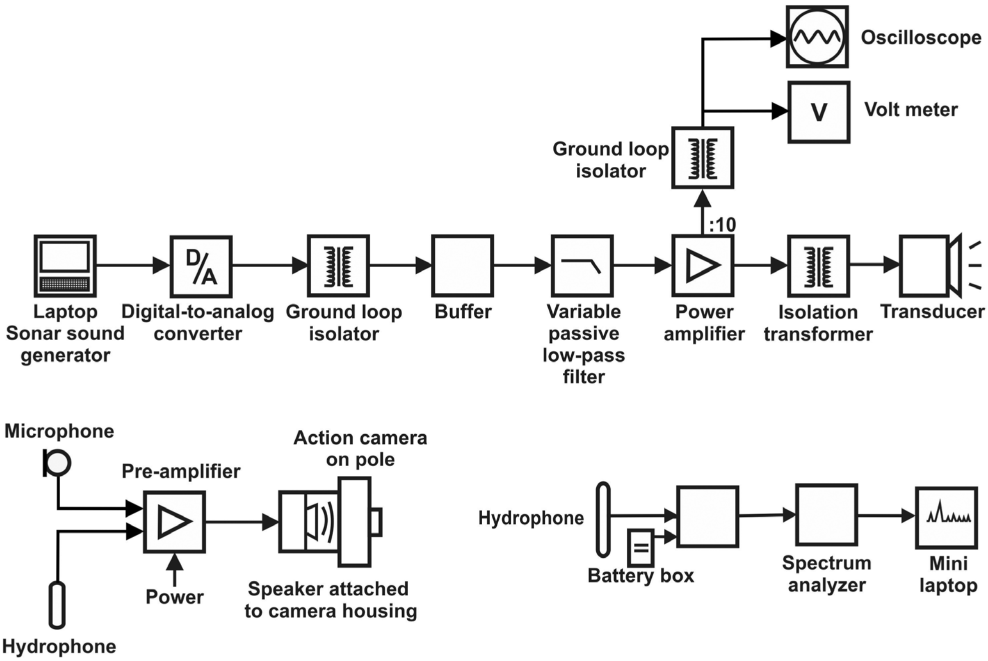

Figure 2. Block diagram of the video and audio equipment set-up, showing the equipment used to play the 53C sonar sounds, the equipment to monitor the sounds in the pool, and the video recording equipment

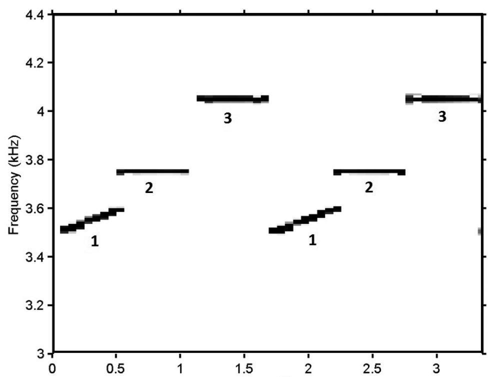

Figure 3. Spectrogram of two consecutive signals, each of 1,600 ms duration and separated by a gap of 60 ms (96% duty cycle), from the 53C sonar sound exposure. The sounds were recorded in the pool, 2 m from the sound source at 1 m depth. Components 1 (3.5 to 3.6 kHz FM), 2 (3.75 kHz CW), and 3 (4.1 kHz CW), each lasting 500 ms, are labeled.

of 12 dB. The amplitude settings of the playback equipment had good linearity (Figure 4).

The signal spectrum (Figure 5) was obtained using Welch's method (Welch, 1967) by time-averaging over three consecutive signals, each with a time window $T = 1.66$ s (block size N = T*fs; frequency resolution $\Delta f = 0.6$ Hz). Harmonics at 7.5 and 8.2 kHz had a spectral density level of *ca*. 90 dB re 1 μPa^2/Hz for the highest gain (0 dB) and *ca*. 20 dB less for a gain of -12 dB.

If an averaging time is chosen to be equal to the duration of each component, the SPL of each component can be identified separately. On average, the SPL of the 3.75 kHz CW Component 2 is the highest of the three, with a power-averaged SPL 2.0 dB higher than that of the lowest (Component 3; 4.1 kHz CW). The SPL also varies with time (with peaks up to about 6 dB higher than the troughs) within Component 1 (Figure 6). For CW Components 2 and 3, the SPL is stable and robust to changes in averaging time between 10 and 100 ms.

For the ambient noise measurements, the SPL was calculated by averaging over a recording of 10 s. One-third octave (base 10) band spectra of the SPL were determined via digital filtering of the time signal.

Played Back 53C Sonar Sound Exposure—Other sounds can be produced by the 53C sonar system, but the one used here is representative (Funnell, 2009). Sonar sounds (WAV file; Sampling: 44 kHz, 16 bit mono) were played back repeatedly by a

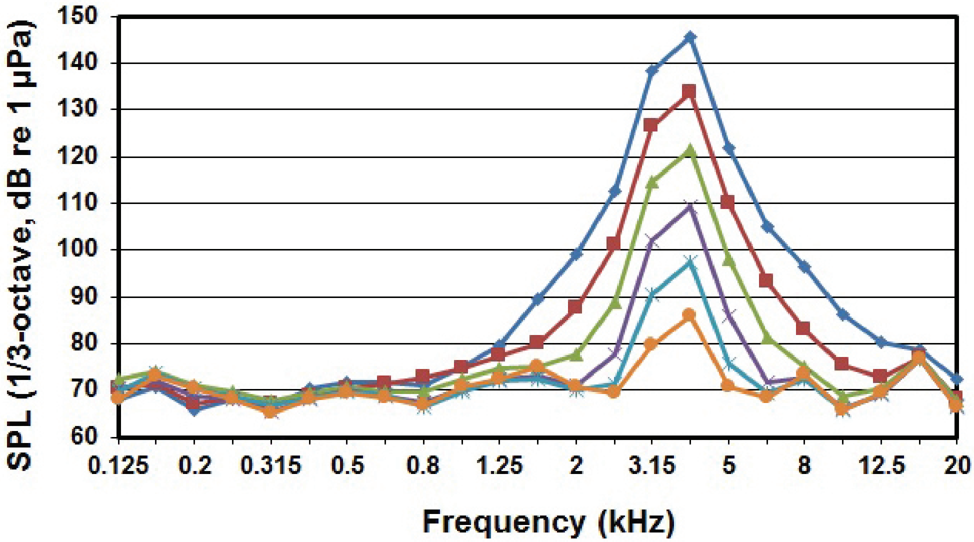

Figure 4. The spectra of the 53C sonar exposure (SPL in one-third octave [base 10] bands vs band center frequency) played back in the pool at six gain settings (in 12 dB steps from -60 to 0 dB, recorded at 2 m from the sound source at a depth of 1 m)

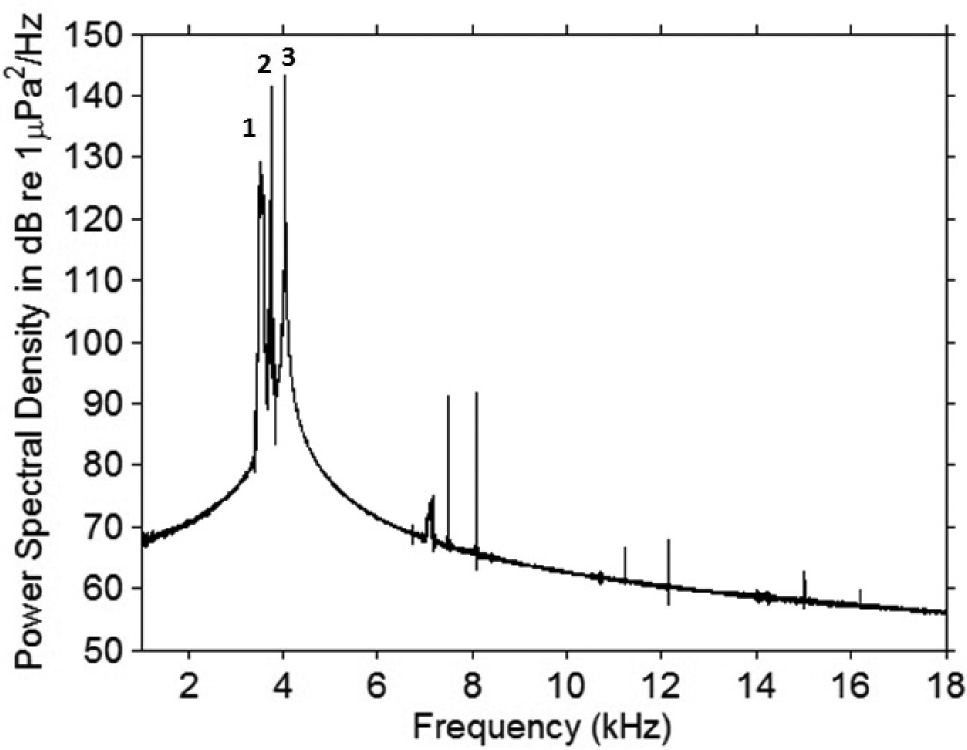

Figure 5. The power spectral density of the 53C sonar playback signal at the highest gain used in the study (0 dB), recorded at 2 m from the sound source at a depth of 1 m. Components 1 (3.5 to 3.6 kHz FM), 2 (3.75 kHz CW), and 3 (4.1 kHz CW) are labeled. Five consecutive pulses are included in the measurement for an averaging time of 8.3 s.

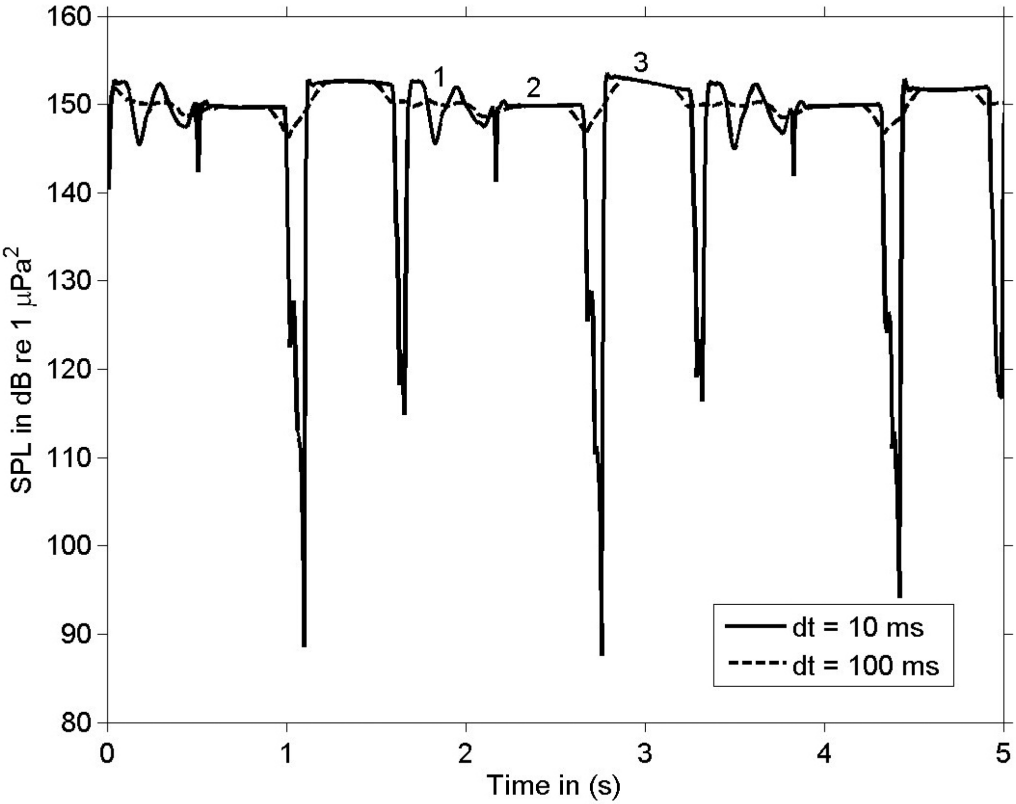

Figure 6. The SPL of the 53C sonar playback signal at gain of 0 dB, recorded at 2 m from the sound source at a depth of 1.5 m. Components 1 (3.5 to 3.6 kHz FM), 2 (3.75 kHz CW), and 3 (4.1 kHz CW) are labeled. Three consecutive pulses are included in the measurement for averaging times (dt) of 10 and 100 ms.

laptop computer (Acer Aspire 5750) with a program written in *LabVIEW* to an external data acquisition card (National Instruments – USB 6259), the output of which could be controlled in 1 dB steps with the *LabVIEW* program. The output of the card went through a ground loop isolator and custom-built buffer to a custom-built buffer/mixer, to a custom-built variable passive low-pass filter (set to 4 kHz; this caused the spectrum of the WAV file to be accurately reproduced in the pool), after which it went to a power amplifier (East & West Inc. – HS1800), which drove the transducer (Lubell – LL1424HP) through an isolation transformer (Lubell – AC1424HP). The transducer was placed at the southwestern end of the pool at 2 m depth. The linearity of the transmitter system used to play the 53C signals was checked during each calibration and was found to deviate by at most 1 dB within a 42 dB range. The output of the sound system to the transducer was checked before each test session with a digital storage oscilloscope (Tektronix 2201) and a voltmeter (Agilent 34401A) by playing a 1-kHz pure tone WAV file. The output of the underwater transducer was checked during the sessions with a custom-built hydrophone, a preamplifier (Reson-CCAS1000), and a spectrum analyzer (Velleman, PCSU1000). The weak harmonics in the signal in the pool were not present in the original signal but were due to the transmitting system.

SPL Distribution in the Pool—To determine the SPLs received by the harbor porpoise, the SPL distribution for the sonar playback sounds was measured at 77 locations in the pool (on a horizontal grid of 1 × 1 m). The SPL was measured simultaneously at three depths per location on the grid (0.5, 1.0, and 1.5 m below the water surface), using three hydrophones (B&K – 8106), resulting in 231 measurement positions.

The SPL distribution of the 53C sound exposure showed that the pool was highly reverberant at these frequencies. The reverberant field dominated the direct field at distances greater than about 4 m from the projector (Figure 7). The received level (Lrec) is calculated as the spatially averaged mean-square sound pressure in the pool, expressed as a level in decibels (power average of the 231 individual SPL measurements). The difference between the mean SPL (average over level) and Lrec (average over power) was 1 dB.

While Lrec is closely linked to the sound the harbor porpoises were exposed to and is a useful proxy, a single average value does not convey the spread of possible values, and this spread is important in behavioral studies if the animal responds to the occasional extreme value rather than to a constant sustained level. For this reason, the choice was made to report the 10, 50, and 90% exceedance levels in addition to Lrec (Figure 7). The Lrec is the SPL received by a swimming animal, averaged over a large number of sonar pulses, on the assumption that all measurement positions are equally likely to be visited by the animal. This quantity can be converted to the sound exposure level (SEL) corresponding to a total exposure duration T_{tot} (in s) using the equation SEL = Lrec + $10\log_{10}(100\, D\, T_{tot})$ dB, where D is the duty cycle (in %).

Mean SPL at 0.5, 1.0, and 1.5 m were all 131 dB re 1 µPa to the nearest decibel.

Determination of Gain Settings for Sonar Sounds—The gain settings to be used during the main study were determined during a 1-mo pilot study in which ten sessions were conducted. In the pilot study, the source level of the 53C sonar sound (96% duty cycle) was gradually increased in steps of 6 dB in the pool until a change was observed in the harbor porpoises' respiration rate, and thereafter two higher levels were tested. The maximum level used did not cause behavioral responses in the two harbor porpoises that were greater than their responses to heavy rain and was thus deemed acceptable with regards to the animals' well-being.

For the main study, the following gain settings were selected: -60 to 0 dB in five steps of 12 dB for the 96% duty cycle; and -24 to 0 dB in four steps of 6 dB for the 2.7% duty cycle. This resulted in an Lrec range for the 2.7% duty cycle of 119 to 143 dB re 1 µPa (119, 125, 131, 137, and 143 dB re 1 µPa), and for the 96% duty cycle of 83 to 143 dB re 1 µPa (83, 95, 107, 119, 131, and 143 dB re 1 µPa). At the highest gain, which caused an Lrec in the pool of 143 dB, and with 96% duty cycle, the animals never swam within 2 m of the transducer. The Lrec in the pool excluding this area was 1.0 dB lower than the overall Lrec in the pool. Thus, the Lrec experienced by

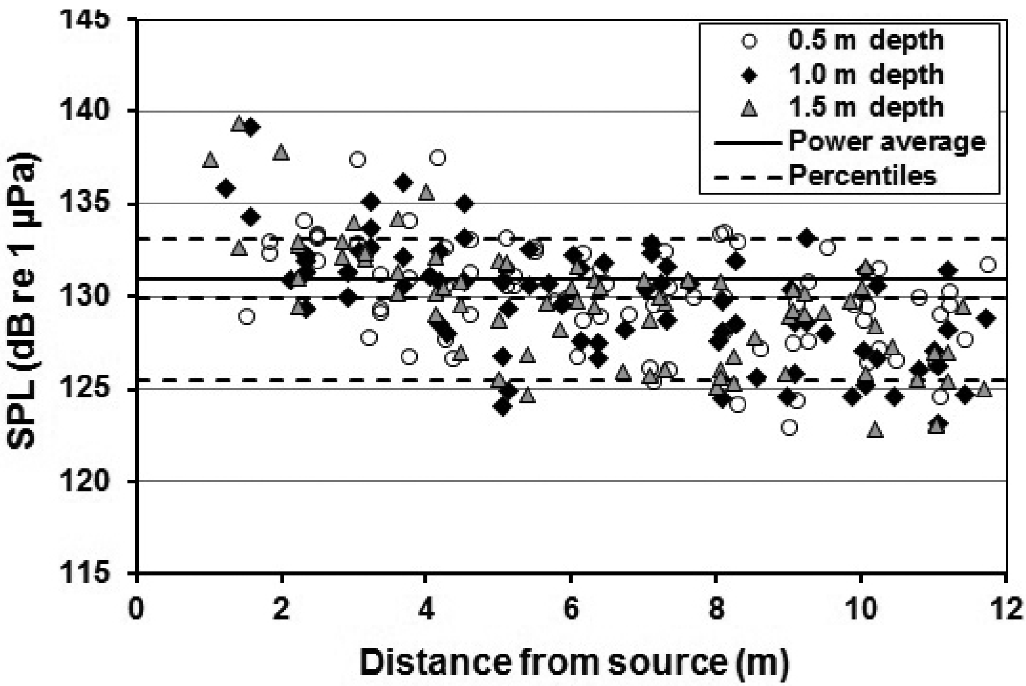

Figure 7. The SPL distribution of the 53C sonar sounds as a function of the distance to the transducer (231 measurements, 77 at each depth) for a gain of -18 dB. The power averaged SPL (horizontal solid line, 131 dB re 1 µPa) and the 90, 50, and 10% exceedance levels in this case were 126, 130, and 133 dB re 1 µPa, respectively (horizontal dashed lines).

the animal after responding to the stimulus was 1 dB lower: 142 instead of 143 dB re 1 μPa. In the results, the level the porpoise experienced before this response (143 dB re 1 μPa) is reported.

Video Monitoring

The animals' behavior was filmed from above by a waterproof action camera (GoPro 3) with a wide-angle lens. This camera was placed on a pole 6 m above the water surface on the southeastern side of the pool (aerial camera 1 in Figure 1). The entire surface of the pool was captured on the video image. As a backup, another camera was mounted on a 9 m high pole on the northwestern side of the pool (aerial camera 2 in Figure 1). The 53C sonar playback sounds were added to video recordings made by aerial camera 1 via a custom-built hydrophone and pre-amplifier, the output of which went to a small speaker that was glued to the waterproof housing of the action camera. The output was also fed to an amplified speaker so that the operator in the research cabin could monitor the ambient noise and the 53C sonar sounds during test sessions.

Experimental Procedures

At around 0800 h each day, the transducer playing the sound was positioned in the pool (Figure 1). One 30-min baseline session (without 53C sonar sound) and one 30-min test session (with 53C sonar sound; the gain setting was kept constant through each session) were conducted per day, normally 7 d/wk, beginning between 0900 and 1500 h. Baseline sessions could not be identified by the study animals; they differed from other periods of the day only in that they were quiet periods of observation. Each baseline session was conducted before the associated test session, but the period between them varied from 30 min to 5 h.

Behavioral responses to the 53C sonar sounds were quantified six times for each combination of the five or six SPLs and two duty cycles (2.7 and 96%). All SPL and duty cycle combinations were tested in random order.

Tests were not carried out during rainfall or when wind speeds were above Beaufort wind force 4 (though high wind speeds did not create as much noise in the pool as would occur at sea because the pool was in a sheltered location and the pool edge extended above the water surface by 30 cm). The study was conducted between March and September 2016.

Response Parameters and Behavioral Data Recording

To ensure that the harbor porpoises were always visible and could be distinguished from each other, they were marked with zinc ointment at the start of each day. The ointment was applied dorsally between the blowhole and the dorsal fin, with a different pattern used for each animal.

In both experiments, four parameters were quantified during baseline and test periods to describe the harbor porpoises' behavioral response: their distance from the transducer, their respiration rate (counted as the number of surfacings), the number of times they jumped out of the water, and their swimming speed (the swimming speed was derived from the total distance swum and was only analyzed for the test periods with the three highest SPLs for the 96% duty cycle and for three randomly chosen baseline periods). In response to the naval sonar sounds, the porpoises were expected to move away from the transducer, increase their respiration rate, jump more often, and swim faster. For respiration rate and distance to the transducer, the 30-min test periods were divided into 10-min sections so that potential habituation or sensitization could be assessed.

The distance between the transducer and the location where the animals surfaced was quantified to determine whether the harbor porpoises responded to the sounds by swimming away from the sound source. This was done as follows: from the action camera recordings, the locations where the porpoises surfaced during the baseline and test periods were recorded on a grid superimposed on the computer screen. The grid corresponded to an imaginary pool grid of 1 × 1 m and was made by connecting lines between 1 m markers on the pool's sides (Figure 1). The grid square in which the porpoises surfaced was determined from the video recordings, and the center point of the grid square was used to calculate the distance of the porpoises' surfacing location to the transducer via triangulation. The porpoises did not swim far away from the surfacing locations. Hence, the surfacing locations were a good indication of the porpoises' general swimming areas.

Software (*Kinovea*) was used to measure the distance each harbor porpoise swam by tracking each animal on the action camera recordings; the swimming speed was calculated from the distance swum. The 1 m markers on the sides of the pool were used for calibration. To account for the perspective in the images, calibration was done both from the side of the pool nearest the camera and from the side farthest from aerial camera 1; the mean was used for the calculations. Variations in the angle of the sun, light conditions, and weather conditions meant that this method could not be used for all sessions. Only test periods with the three highest Lrecs and 96% duty cycle (as a response was seen only in these periods) and the three randomly selected baseline periods were analyzed, with a sample

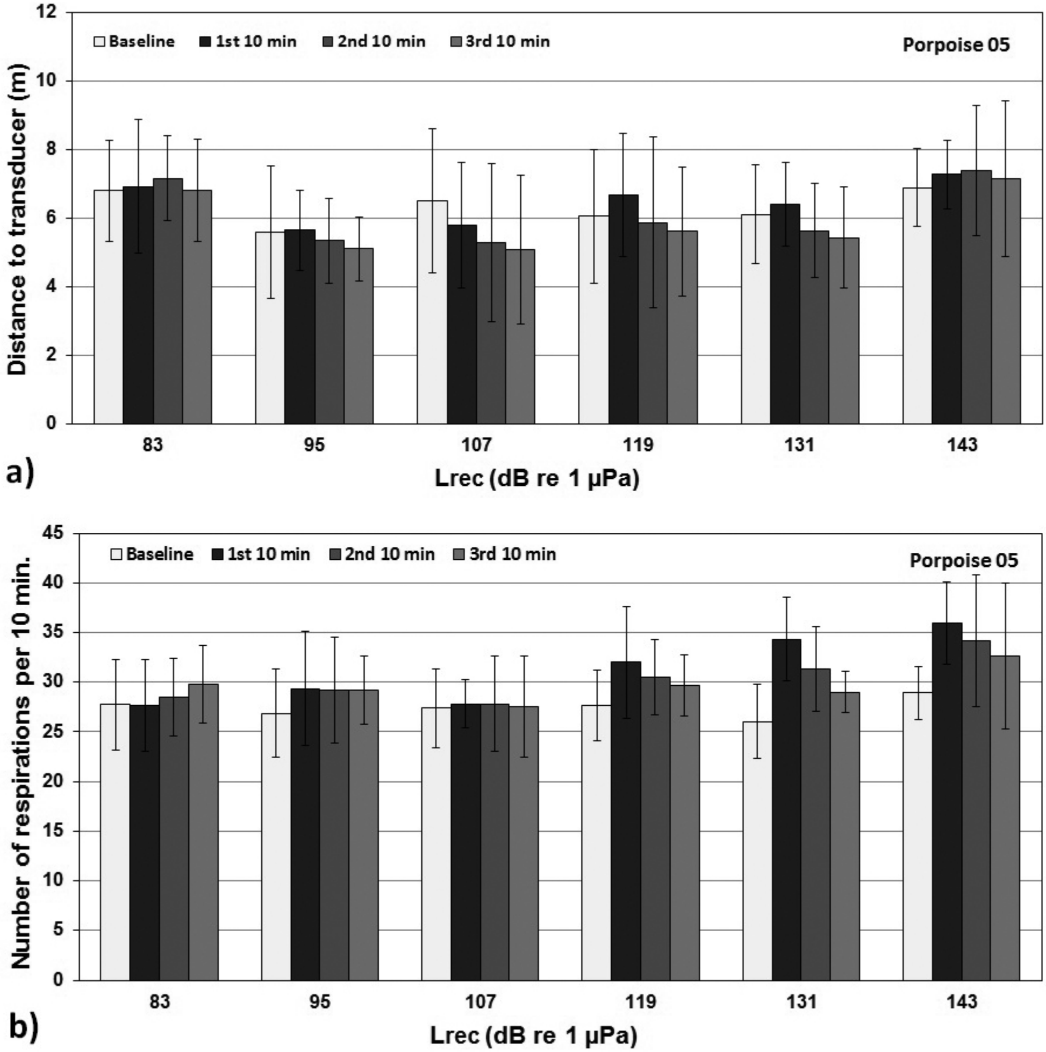

Figure 8. Mean (±SD) distances from the transducer (a) and respiration rates (b) of Porpoise 05 during the baseline periods and during the first, second, and third 10-min sections of the test periods for the 53C sonar sounds at various received levels with a duty cycle of 96%. No significant differences between the three 10-min sections were found, but a slight decrease in the respiration rate during the three test sections can be seen at Lrec of 119, 131, and 143 dB. For comparison, the values for respirations in the 30-min baseline periods have been divided by 3.

size of three for each level. As no effect on swimming speed was seen, further analysis was not conducted.

Analysis

Of the four variables used to quantify the harbor porpoises' responses to the sound, two (distance from the transducer and number of respirations) could be subjected to formal statistical analysis. Three analysts, who were unaware of the SPLs during the sessions, collected the data from the video recordings. To investigate in detail the harbor porpoises' responses to the sonar sounds, paired *t* tests were used to compare their distances from the transducer and respiration rates in baselines and associated 30-min test periods. For each animal, paired *t* tests on the same dependent variable (distance to the transducer and respiration rate) and at the same duty cycle (2.7 or 96%) were not considered to be independent, so *p* values were adjusted according to the Holm-Bonferroni method (by multiplying significant *p* values by the number of non-independent comparisons—in this case, 6 for duty cycle 96% and the comparison with the largest significant difference, then multiplying the *p* value of the next largest by 5, and the next by 4; Quinn & Keough, 2002).

To investigate potential habituation or sensitization within the 30-min test periods only, analysis of variance (ANOVA) was used to evaluate changes in the harbor porpoises' distance from the transducer and respiration rate in the 10-min sections of the test periods (without consideration of the baseline values), also taking the SPL into account (as a factor). The interaction term (10-min section × SPL) was included at first and then removed from the final analysis as it was never significant. A separate ANOVA was conducted for each parameter (distance from the transducer and respiration rate), for each animal, and for each duty cycle, so eight ANOVAs were carried out in total.

For all analyses, data conformed to the assumptions of the tests used, and the level of significance was 5% (Zar, 1999).

Results

During the 66 baseline periods (each lasting 30 min; 33 h in total), the harbor porpoises jumped out of the water only three times. In the 66 test periods, they jumped 19 times. Jumps were sporadic; however, of the 19 jumps in the test periods, 14 occurred at the highest Lrec (143 dB re 1 µPa) and when the duty cycle was 96%. Jumps were not subjected to statistical analysis.

The harbor porpoises' mean distance to the transducer and their respiration rates were similar in all baseline periods (Mean distance: Porpoise 05, 6.1 ± 1.5 m [mean ± SD], and Porpoise 06, 4.1 ± 1.1 m; Mean respiration rate per 10 min: Porpoise 05, 27.3 ± 3.9, and Porpoise 06, 26.4 ± 3.1; $N = 66$ baseline periods). They showed a regular dive pattern consisting of long dives alternated with shorter dives. The mean swimming speed was 5.2 ± 0.2 km/h for Porpoise 05 and 3.9 ± 0.7 km/h for Porpoise 06 ($n = 3$ baseline periods).

Paired *t* tests to compare the harbor porpoises' distances from the transducer and respiration rates between baseline and associated test periods showed that the porpoises did not respond behaviorally to the 53C sonar sounds when they were played back at a duty cycle of 2.7%, even at the highest Lrec (143 dB re 1 µPa); their respiration rates and distance to the transducer in test periods remained similar to those during the baseline periods. Porpoise 05 did not respond to the sounds at all (Figure 8). The paired *t* tests were significant only for Porpoise 06 when the duty cycle was 96% (Table 1).

Table 1. Results of paired *t* tests to compare Porpoise 06's distance from the transducer and respiration rate in baseline and associated test periods at each Lrec (dB re 1 µPa) for playbacks of 53C sonar sounds with duty cycle 96% and 2.7%; see also Figure 9. The sample size for each test is 6. Adjusted exact *p* values (Holm-Bonferroni method; Quinn & Keough, 2002) are shown where significant; NS = not significant. In all cases where the test was significant, the value for the test period was greater than that for the baseline period. Porpoise 06 responded to the sonar sounds by increasing his respiration rate and moving away from the transducer, but only when the duty cycle was 96%, and only when the level was sufficiently high. Results for Porpoise 05 are not shown because none of the paired *t* tests showed a significant difference between baseline and test periods after adjustment (Holm-Bonferroni method).

Lrec (dB re 1 µPa)	Duty cycle (%)	Distance from transducer (m)	Respiration rate (breaths/ 30-min period)
119	2.7	NS	NS
125	2.7	NS	NS
131	2.7	NS	NS
137	2.7	NS	NS
143	2.7	NS	NS
83	96	NS	NS
95	96	NS	NS
107	96	NS	NS
119	96	NS	0.018
131	96	NS	0.015
143	96	0.012	0.048

When the 53C sonar sounds were played back with a 96% duty cycle, only Porpoise 06 moved away from the transducer at an Lrec of 143 dB re 1 µPa (Figure 9a; Table 1). He increased his respiration rate at an Lrec of 119 dB re 1 µPa and above (Figure 9b).

Analysis of the harbor porpoises' distance from the transducer and respiration rate during the 10-min sections of the test periods showed that no habituation or sensitization took place. In all eight ANOVAs (which did not include the baseline periods), the interaction terms (section × SPL) and the factor "section" did not account for a significant portion of the variation in the dependent variables (distance from the transducer and respiration rate; Figures 8 & 9). In all four ANOVAs where the duty cycle was 96%, there was a significant effect of SPL on distance from the transducer and respiration rate. The lack of significance in the interaction term showed that the animals' pattern of response during the 10-min sections of the test period was statistically similar

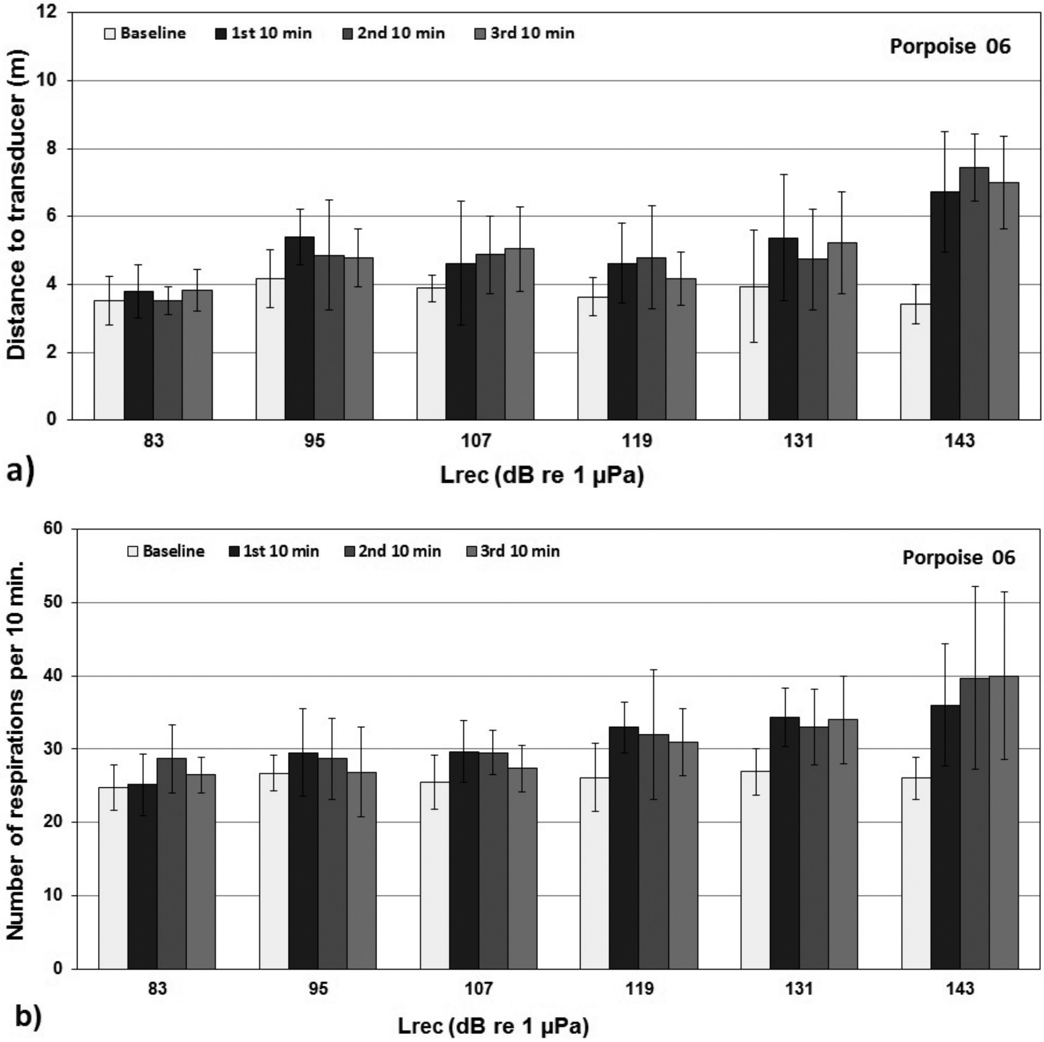

Figure 9. Mean (±SD) distances from the transducer (m) (a) and respiration rates (b) of Porpoise 06 during the baseline periods and during the first, second, and third 10-min sections of the test periods for the 53C sonar sounds at various received levels with a duty cycle of 96%. No significant differences between the three 10-min sections were found. For comparison, the values for respirations in the 30-min baseline periods have been divided by 3.

under all conditions. However, a nonsignificant slight decrease in the respiration rate during the three sections can be seen at Lrec of 119, 131, and 143 dB re 1 µPa in Porpoise 05 when the duty cycle of the sonar sounds was 96% (Figure 8b).

A very brief increase in swimming speed was seen at the two highest SPLs, but the mean swimming speeds of both harbor porpoises during the test periods with 96% duty cycle at the highest four Lrecs were similar to their mean swimming speeds during the baseline periods (Range mean speed [per SPL and control]: 5.1 to 6.0 km/h by Porpoise 05, and 3.8 to 4.2 km/h by Porpoise 06).

Discussion

Evaluation

The present study was conducted with two animals, both with hearing that was representative of that of other harbor porpoises of their age (Kastelein et al., 2017b). However, little can be said about whether their responses were within the range of responses shown by other individuals of this species. Behavioral response studies should be conducted with as many animals as possible, as responses to acoustic stimuli vary between individuals as seen for harbor porpoises in the present study and in other studies (Kastelein et al., 2000, 2001, 2008b). Behavioral responses to sounds are also context-dependent, depending on the occurrence of attractive and aversive components in the environment. The specific conditions found in the pool do not occur in the wild, though situational contexts in the wild are innumerable. However, it is unlikely to be possible in the near future to conduct a similar experiment with other harbor porpoises as the number of captive harbor porpoises is small, and most facilities are not designed for this type of behavioral response study. Therefore, despite the small sample size, the results of this study are rare and unique.

The 53C sound playbacks in the pool had very weak harmonics (Figure 5), which was the intention, as the goal was to study the response of harbor porpoises to the fundamental 53C sonar signal. Actual 53C sonar systems at sea can produce harmonics at various levels and frequencies, depending on the source level. The distance at which a harbor porpoise receives the 53C sonar sound influences not only the Lrec, but also the received spectrum, as sea water acts as a low-pass filter, reducing the higher frequencies more than the lower frequencies due to frequency-dependent absorption. The hearing sensitivity of harbor porpoises generally increases as frequency increases (up to ~130 kHz; Kastelein et al., 2017b), so the frequency and received level of the harmonics are important. Studies with 1 to 2 kHz, 6 to 7 kHz, and 25 kHz sonar systems have shown that the presence of harmonics increases the audibility of the signals for harbor porpoises (Kastelein et al., 2011), but also lowers the behavioral response threshold SPL (Kastelein et al., 2012, 2015b).

A nonsignificant slight decrease in the respiration rate during the three 10-min sections of the test period was seen at Lrec of 119, 131, and 143 dB in Porpoise 05 when the duty cycle of the 53C sonar sounds was 96%. However, day to day over the 7-mo study period, no habituation occurred; after a period of about 24 h, the harbor porpoise's behavioral sensitivity to the sounds was apparently restored. This suggests that there are short- and long-term trends in behavioral responses, and it demonstrates the importance of understanding the mechanisms involved. The slight, nonsignificant reduction in respiration rate during the 30-min test periods may have been due to the occurrence of temporary hearing threshold shift (TTS). Kastelein et al. (2017a) showed that after exposure for 30 min to the 53C sonar sound during the present study at an Lrec of 143 dB re 1 μPa (cumulative sound exposure level: 175 dB re 1 $\mu Pa^2 s$) with a 96% duty cycle, initial TTS (during the 1 to 4 min after the sound stopped) on the order of 5 dB occurred in Porpoise 06 (whose hearing was tested immediately after sound exposure stopped). The magnitude of TTS depends, among other things, on the exposure duration (Kastelein et al., 2014a). Thus, over time during a 30-min test session, the 53C sonar sounds were perceived at an increasingly lower level by the harbor porpoise, possibly reducing the animal's responses to the sounds. The same phenomenon (a decrease in behavioral response over time during test sessions) was observed when harbor porpoises were exposed to pile driving sound (Kastelein et al., 2013).

During the 96% duty cycle exposure periods, the mean swimming speeds of both harbor porpoises were similar to their mean swimming speeds during the baseline periods (~5.5 km/h for Porpoise 05 and ~3.9 km/h for Porpoise 06), and similar to the swimming speed observed in a free-ranging harbor porpoise at sea (3.3 km/h; Otani et al., 2000). This means that the slightly increased respiration rate observed in Porpoise 06 at Lrec ≥ 119 dB re 1 μPa was not due to an increased need for oxygen due to faster swimming but may have been due to an increased anxiety level. At sea, an animal can alter its course in an attempt to lower the received SPL, which was not possible in the pool, except by swimming away from very near the sound source (up to 4 m from it).

The Effect of 53C Naval Sonar

In normal navy operations, long-range sonar systems are used at various duty cycles depending on the circumstances, expected targets, and target distances. In the present study, two duty cycles from the potential range were tested: (1) 2.7% (i.e., one 1,600 ms sonar signal every 60 s), the presently most commonly used duty cycle; and (2) 96% (i.e., 60 ms silences between the 1,600 ms sonar sounds), the highest duty cycle used (resembling continuous active sonar).

The results of the present study confirm that not only should the received SPL of a signal be included in determining the acoustic dose-response relationship of a species to predict the effect of a sound on its behavior, but also the duty cycle. At a duty cycle of 2.7%, 53C sonar sounds did not elicit a response even when the Lrec was 143 dB re 1 μPa. The effect of duty cycle in harbor porpoises was also observed in exposures to 70 kHz signals (Kastelein et al., 2008b). Among other parameters, the effect of a sound is probably related to the context in which it is received and, thus, may vary with location and situation.

Even at the highest Lrec (143 dB re 1 μPa) at a duty cycle of 96%, only one of the two harbor porpoises in the present study increased its respiration rate significantly compared to the baseline periods. The increase in respiration rate was on the order of 37%. In another behavioral response study, the respiration rate of a harbor porpoise increased by 66% in response to a 1-s 1 to 2 kHz up-sweep, and by 95% in response to a 1-s 1 to 2 kHz down-sweep; both sounds were without harmonics and were played back for 30 min at a duty cycle of 19% at Lrec of 114 dB re 1 μPa (Kastelein et al., 2014b). When the animal was exposed to 1-s 6 to 7 kHz up-sweeps and down-sweeps without harmonics at a duty cycle of 19% and Lrec of 107 dB re 1 μPa, the increases in respiration rate were 200 and 210%, respectively (Kastelein et al., 2014b). Sonar sounds in the 25 kHz range also caused an increase in respiration rate in harbor porpoises at Lrecs of 118 to 125 at duty cycles between 2.4 and 8.3% (Kastelein et al., 2015b, 2015c). Although the sample sizes are small and the study animals differ, the studies suggest that at the same Lrec and duty cycle, harbor porpoises respond less to the 53C sonar sounds than to 1 to 2 kHz sweeps, 6 to 7 kHz sweeps, and sound around 25 kHz (Signal types: frequency modulation, continuous wave, and a combination of the two signals). This is probably due to a combination of a relatively low frequency, a low sweep content, and the small sweep range of the 53C sonar sounds. Behavioral responses to sound cannot be explained simply on the basis of energy and sensation level; the spectrum and temporal structure of the signal (continuous or intermittent), the duty cycle (the specific pattern of sound duration and interval durations), and the psychological interpretation by an animal (which may be individual- or species-specific) must also be taken into account.

Conclusion

In the present study, only one of the two harbor porpoises (Porpoise 06) showed a behavioral response (an increase in respiration rate) to 53C sonar sounds at a 96% duty cycle (a very high cycle that is rarely used in 53C sonar operations) and only at Lrecs ≥ 119 dB re 1 μPa. For this high duty cycle, therefore, the behavioral threshold SPL of this harbor porpoise for increasing his respiration rate is between 107 and 119 dB re 1 μPa, so approximately 113 dB re 1 μPa, and his behavioral threshold SPL for moving away from the transducer is between 131 and 143 dB re 1 μPa, so approximately 137 dB re 1 μPa, assuming the animal responded to the Lrec and not to higher SPLs that may have been present at a few locations in the pool (Figure 7). However, at the same Lrec and duty cycle, the signals produced by 53C sonar had a weaker effect than the 1 to 2 kHz sweeps, 6 to 7 kHz sweeps, and sound around 25 kHz produced by other naval sonar systems tested so far (Kastelein et al., 2014b, 2015b, 2015c).

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