Temporary hearing threshold shift in a harbor porpoise (*Phocoena phocoena*) after exposure to multiple airgun sounds

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In seismic surveys, reflected sounds from airguns are used under water to detect gas and oil below the sea floor. The airguns produce broadband high-amplitude impulsive sounds, which may cause temporary or permanent threshold shifts (TTS or PTS) in cetaceans. The magnitude of the threshold shifts and the hearing frequencies at which they occur depend on factors such as the received cumulative sound exposure level (SELcum), the number of exposures, and the frequency content of the sounds. To quantify TTS caused by airgun exposure and the subsequent hearing recovery, the hearing of a harbor porpoise was tested by means of a psychophysical technique. TTS was observed after exposure to 10 and 20 consecutive shots fired from two airguns simultaneously (SELcum: 188 and 191 dB re 1 $\mu Pa^2 s$) with mean shot intervals of around 17 s. Although most of the airgun sounds' energy was below 1 kHz, statistically significant initial $TTS_{1\text{-}4}$ (1–4 min after sound exposure stopped) of ~4.4 dB occurred only at the hearing frequency 4 kHz, and not at lower hearing frequencies tested (0.5, 1, and 2 kHz). Recovery occurred within 12 min post-exposure. The study indicates that frequency-weighted SELcum is a good predictor for the low levels of TTS observed.
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I. INTRODUCTION

During seismic surveys at sea, airgun arrays produce sequences of impulsive broadband sounds that are used to inspect the layers below the sea floor. Typical shot intervals are around 10 s. Airgun sounds may cause reductions in hearing in marine mammals, which may be temporary (TTS; temporary threshold shift) or permanent (PTS; permanent threshold shift). To prevent or reduce the impacts of seismic surveys on populations of marine mammals, some government regulators have set, or are in the process of setting, criteria for the maximum allowable levels of underwater sound that can be used [e.g., Southall *et al.*, 2007; Bundesamt für Seeschifffahrt und Hydrographie (BSH), 2013; Dekeling *et al.*, 2014; National Marine Fisheries Service (NMFS), 2016].

In order to set science-based noise criteria, research is needed on the onset and magnitude of threshold shifts for different marine mammal species and sounds. Because PTS studies in marine mammals are considered to be unethical, sound exposure levels that may lead to PTS are estimated from information on variations in TTS onset and magnitude in relation to variations in single-shot sound exposure level (SELss), exposure duration, and duty cycle (see Southall *et al.*, 2007; Finneran, 2015, for an overview). Southall *et al.* (2007) recommended criteria to avoid PTS in marine mammals based on the limited knowledge of TTS available at that time.

The harbor porpoise (*Phocoena phocoena*) is the most common marine mammal species in the North Sea, and is also common in other coastal waters in the temperate zone of the northern hemisphere. For sounds between ~4 and 150 kHz, it has the most sensitive hearing of all marine mammals that have been tested, and it is therefore of particular concern for assessing effects of underwater sound (e.g., Finneran, 2015). Since the publication of the recommendations from Southall *et al.* (2007), three more studies have been conducted on the effect of impulsive sounds on TTS in harbor porpoises; one with single sound exposures and two with longer series of impulsive sounds.

Lucke *et al.* (2009) exposed a harbor porpoise to single airgun shots, and observed TTS at 4 kHz, and no TTS at higher frequencies tested (32 and 100 kHz). In seismic surveys, airgun arrays are used that create multiple shots, typically every 10 s for hours, days or even weeks (Krail, 2010). It is still not clear how to extrapolate from the effects of exposure to one impulsive sound to exposures of sequences of impulsive sounds.

Several aspects contribute to the TTS induced by a sequence of sounds. Besides the level of the sounds and the duration of the sequence, the duty cycle (signal duration, but especially the shot interval) can affect the induced TTS. Research has shown that the duty cycle of impulsive sounds affects the magnitude of TTS (Finneran *et al.*, 2010; Kastelein *et al.*, 2014a; Kastelein *et al.*, 2015b), as hearing may recover, at least partly, between shots. Kastelein *et al.* (2014a) reported that although exposure to a single sound did not lead to measurable TTS, the cumulative effect of sequences of sounds caused TTS. Therefore, TTS can be

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induced by exposure to multiple successive sounds, such as those produced during airgun surveys. While TTS has not been demonstrated in harbor porpoises following exposure to multiple seismic sounds, it has been shown following exposure to pile driving sounds. TTS in harbor porpoises has previously been induced by exposure to long (1–6 h) sequences of playbacks of impulsive pile driving sounds (produced during the installation of wind turbines at sea; Kastelein *et al.*, 2015a; Kastelein *et al.*, 2016b).

In addition to understanding the effects of airgun shot intervals, it is important to find out which frequencies may be most affected by a fatiguing sound when measuring TTS. Sounds produced by airgun arrays typically have their dominant source energy level at low frequencies (<100 Hz); this allows the sounds to penetrate the sea floor. For narrowband fatiguing sounds, TTS may be induced at the center frequency of the fatiguing sound or above it (Finneran and Schlundt, 2013; Finneran, 2015; Kastelein *et al.*, 2012; Kastelein *et al.*, 2013; Kastelein *et al.*, 2014a). Kastelein *et al.* (2014b) showed that, in harbor porpoises, the affected hearing frequency depends not only on the spectrum of the fatiguing sound but also on the sound exposure level (SEL). Impulsive sounds are broadband, and it was not known which hearing frequencies would be most affected by exposure to playbacks of pile driving sound. Kastelein *et al.* (2015a) measured hearing thresholds in a harbor porpoise at several hearing frequencies after the animal had been exposed to playbacks of broadband pile driving sound for 1 h, and found that the hearing of the porpoise was only affected at 4 and 8 kHz and not at lower frequencies, although most energy in the pile driving sound spectrum was at 700 Hz.

Recent studies suggest that frequency-selective weighting of the SEL may improve predictions of the risk of TTS (as reviewed by Southall *et al.*, 2007; Finneran, 2015; Finneran *et al.*, 2015; Tougaard *et al.*, 2015). Finneran and Schlundt (2013) found that, in bottlenose dolphins (*Tursiops truncatus*), the relationship between exposure frequency and the exposure level required to induce TTS by tonal signals agrees closely with an auditory weighting function developed from equal-loudness contours of that species. Unlike for the bottlenose dolphin, data are not available on TTS sensitivity over the whole frequency range of harbor porpoise hearing (so far only for the 1.5–7 kHz range). Hence, little is known about the relationship between the equal-loudness contours of the harbor porpoise and its sensitivity to TTS. However, based on the audiogram and equal-latency contours of the harbor porpoise, the hearing of the harbor porpoise is assumed to be less sensitive to exposure to low-frequency sound than to exposure to high-frequency sound (Wensveen *et al.*, 2014). In a study in which harbor porpoises were exposed to sonar signals, Kastelein *et al.* (2014a) and Kastelein *et al.* (2014b) showed TTS onset (defined as occurring at ca. 6 dB TTS) at a lower cumulative sound exposure level (SELcum), and stronger increases in TTS with increasing SELcum, for 6–7 kHz up-sweeps than for 1–2 kHz up-sweeps. These results are in agreement with those of Finneran and Schlundt (2013), and suggest that the vulnerability of harbor porpoise hearing is frequency-dependent (it is more susceptible to damage by high-frequency fatiguing sounds than by low-frequency fatiguing sounds). Originally, Southall *et al.* (2007) proposed relatively flat frequency weighting curves, which only suppressed energy outside the frequency range of best hearing. More recently, other types of frequency weighting were proposed to base risk criteria on for hearing effects on marine mammals (Tougaard *et al.*, 2015; Finneran, 2016; National Marine Fisheries Service, 2016).

It has not been demonstrated that frequency selective weighting of the SEL allows for reliable prediction of TTS onset in harbor porpoises exposed to impulsive sound. Pile driving playback studies with harbor porpoises measured TTS induced by repetitive impulsive sounds (Kastelein *et al.*, 2016b), but differences in the durations, intervals and numbers of shots make it hard to extrapolate these observations to airgun exposures with longer shot intervals. Therefore, a harbor porpoise was exposed to custom-built down-scaled airguns. The onset of and recovery from TTS was tested at four hearing frequencies between 500 Hz and 8 kHz. The benefit of frequency-selective weighting for the prediction of TTS was assessed, and the potential for recovery between multiple airgun shots is discussed.

II. MATERIALS AND METHODS

A. Study animal and study area

The study was conducted with a male harbor porpoise, identified as porpoise 06, that had been rehabilitated after stranding. At the time of the study, he was 3 years old, his body mass was around 33 kg, his body length was 127 cm, and his girth at the axilla was approximately 80 cm. He was housed with a female (identified as porpoise 05) that was 6 years old. Because of time constraints porpoise 05 did not take part in the hearing tests.

The hearing of porpoise 06 for sounds in the range of the hearing test signals used in the present study (0.5–8 kHz) was tested (Kastelein *et al.*, 2017), and was found to be representative for animals of his age and species; his hearing thresholds were within a few dB of those obtained from other young male porpoises tested with behavioral methods (Kastelein *et al.*, 2002; Kastelein *et al.*, 2009; Kastelein *et al.*, 2010; Kastelein *et al.*, 2015b).

Porpoise 06 received 2–2.5 kg of thawed fish per day, divided over four meals, of which approximately 75% was given to him as rewards during the hearing sessions. Variation in his performance during the hearing tests was minimized by making weekly adjustments (usually in the order of 100 g) to his daily food ration, based on his body mass and performance during the previous week, and the expected change in water and air temperatures in the following week.

The study was conducted at the SEAMARCO Research Institute, the Netherlands. Its location is remote (no busy roads nearby that would cause the ambient noise in the pool to rise) and quiet (very few transient sounds), and was specifically selected for acoustic research. The animals were kept in a pool complex designed and built for acoustic research, consisting of an outdoor pool (12 m × 8 m; 2 m deep) in which porpoise 06 was exposed to fatiguing airgun sounds, connected via a channel (4 m × 3 m; 1.4 m deep) to an indoor pool (8 m × 7 m; 2 m deep) in which hearing tests were conducted. All pumps were switched off at the start of a working day (0800 h) and were switched on after the last hearing session of the day. Thus, by the time a hearing test started, no water flowed over the skimmers, so there was no flow noise during testing. Details of the study area are presented by Kastelein *et al.* (2012). While porpoise 06 was in the outdoor pool for sound exposure, porpoise 05 was in the indoor pool (Fig. 1). When porpoise 06 went into the indoor pool for the hearing tests, porpoise 05 swam into the outdoor pool where she was asked to perform quiet behaviors, so she would not distract porpoise 06.

B. Acoustics

Acoustical terminology follows International Organization for Standardization (ISO) (2017a) and ISO (2017b). Where symbols for non-SI units are needed, IEEE (2004) is followed.

1. Background noise and stimuli measurements

The background noise, impulsive airgun sounds, and hearing test signals (sweeps) were measured at the beginning, in the middle and at the end of the 9-month study period. The sound measurement equipment consisted of various hydrophones [Brüel & Kjaer (B&K)-8106 (sensitivity −173 dB re 1 V/μPa) for the hearing test signals and B&K-8105 for the airgun sounds (sensitivity −205 dB re 1 V/μPa)], with a multichannel high frequency analyzer (B&K Lan-XI type 3161-A-1/1), and a laptop computer with B&K PULSE software (Labshop, version 20.0.0.455; high-pass filter 22.4 H; sample frequency: 524,288 Hz for the hearing test signals and 131,072 Hz for the airgun sounds). Before analysis of the hearing test signals, the recordings were high-pass filtered (cut-off frequency 100 Hz; 3rd 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 – 4223). The broadband sound pressure level (SPL) (dB re 1 μPa) of each hearing test signal was derived from the received 90% sound exposure and the corresponding 90% energy signal duration ($t_{90\%}$); the 90% method was applied in order to minimize the influence of background noise. The output of the airguns was also checked daily with a simpler setup: a custom-built hydrophone, a pre-amplifier (Reson-CCAS1000) and a spectrum analyzer (Velleman PCSU1000).

For the hearing tests, care was taken to make the listening environment for harbor porpoise 06 as quiet as possible.

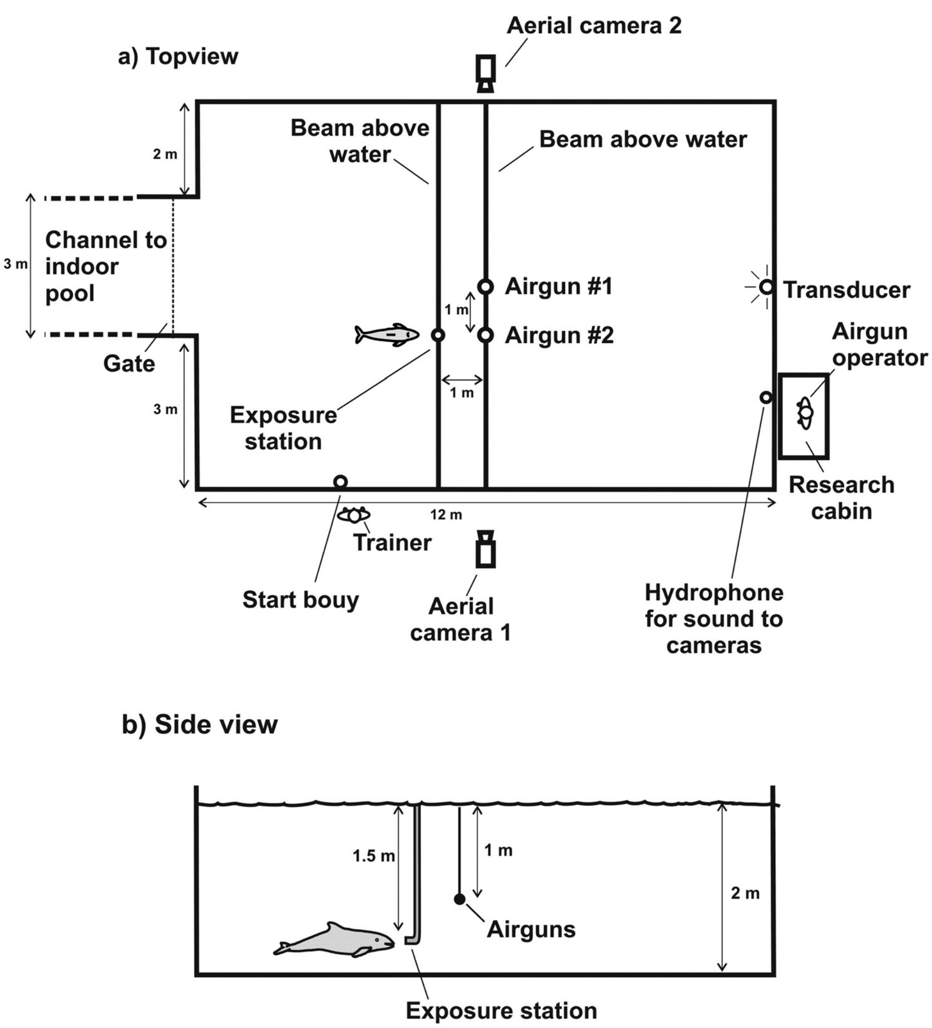

FIG. 1. The outdoor pool in which harbor porpoise 06 was exposed to airgun sounds; (a) top view, and (b) side view. This specific configuration was chosen for the tests because the highest SELcum at the animal's location was achieved in this way. For the set-up used for the hearing tests in the adjacent indoor pool, see Kastelein *et al.* (2012). The transducer was only used during the training phase of the study (Drawing by Rob Triesscheijn).

Only the three researchers involved in the hearing tests were allowed within 15 m of the pool during hearing test sessions, and they were required to stand still. Measurements of background noise conditions in the indoor pool obtained on three separate occasions during this study showed that ambient noise levels were around or below those of Sea State 0, in agreement with earlier studies (see Kastelein *et al.*, 2012). The only goal of SEAMARCO Research Institute is to conduct acoustic studies, so great attention is paid daily to the background noise level in the pools. The hearing test environment was made quiet not only to prevent masking, but also to reduce distractions for the animal.

2. Fatiguing airgun sounds

The study animal had to be willing to participate in the study, and the SEL of the airgun sounds had to be increased gradually over a long period of time so that he could habituate to the impulsive sound. Small, low-pressure airguns were needed that had a lower starting SEL than actual airguns used at sea. Therefore, the fatiguing impulsive sounds that were intended to cause TTS in this study were produced by down-scaled airguns (Reichmuth *et al.*, 2016). Two types of purpose-built down-scaled airgun were used, each with a different volume: 82 cm^3 (5 in^3, cubic inches) and 164 cm^3 (10 in^3), so that the exposure levels could be adjusted. The airgun hoses were connected to a pressurized air tank by a quick-release valve on a pressure regulator. The pressure regulator allowed the airgun operating pressure (the airgun firing pressure relative to atmospheric pressure) to be adjusted between 150 kPa (22 lbf/in^2, sometimes abbreviated "psi" for "pound-force per square inch"), the minimum pressure at which the airguns fired consistently, and 800 kPa (120 lbf/in^2). The accuracy to which the operating pressure could be adjusted was $\pm$25 kPa (3.6 lbf/in^2). The trigger required to fire the airgun was generated by a firing controller (electronic pulse generator) which controlled a solenoid valve. To increase the SEL, two 164 cm^3 (10 in^3) airguns were shot simultaneously in double airgun exposures.

The airguns were placed in the outdoor pool at mid-water depth (1 m), slightly off-center (1 m apart) to allow timely repositioning of the animal between shots (Fig. 1). While the airgun sound measurements were made in the outdoor pool, the animals were housed in the indoor pool, and a bubble wrap screen was hung across the opening of the channel connecting the two pools. This screen reduced the transmission of high-frequency sounds to such an extent that the animal in the indoor pool did not react to the airgun sounds.

As it is not yet understood how to characterize the risk posed by impulsive sounds for mammalian hearing, we report several measured characteristics of the airgun sounds to which the porpoise was exposed: sound exposure level, peak sound pressure, duration, rise time, average rising slope and kurtosis of the sound. We measured the single-shot sound exposure level (SELss; $L_{E,\mathrm{ss}}$) in dB re 1 $\mu Pa^2 s$ (ISO, 2017a). The peak sound pressure level $L_{\mathrm{pk}} = 20\ \log10(p_{\mathrm{pk}}/p0)$ dB. The rise-time τ_{rise} (ISO, 1997) is defined as the time a sound takes to rise from 10% to 90% of its maximum absolute value for sound pressure. The sound pressure kurtosis, β, (Hamernik and Qiu, 2001; ISO, 2017a) was determined over a 1 s segment in the frequency bandwidth of 22.4 Hz to 131 072 Hz. The sound's average rising slope, r_{max}, is defined as the maximum rise rate (in Pa/s) wherein the average rising slope is calculated at the slope between 10% and 90% of an unfiltered signature recorded up to 50 kHz (Hopperstadt *et al.*, 2008).

Most of the energy of the airgun sounds' spectrum was below 1 kHz; peaks occurred at 50 and 500 Hz (Fig. 2). Frequency bands higher than ~200 Hz showed greater variation (~1.4 dB) than at 50 Hz (0.5 dB) where the (unweighted) airgun one-third octave (base 10) band sound exposure level spectrum peaked. The highest SELss was measured at a distance of 1 m from one airgun and 1.4 m from the other airgun, at a depth of 1.5 m (i.e., 0.5 m above the bottom of the pool; Fig. 1). This location was selected for the exposure station of the porpoise.

Two repeated calibration measurements of each of the single airgun and double airgun set-up (separated by 2 months and 5 months respectively) were carried out to assess the stability of the sounds produced. The variation between the measurement campaigns was of the same order as the

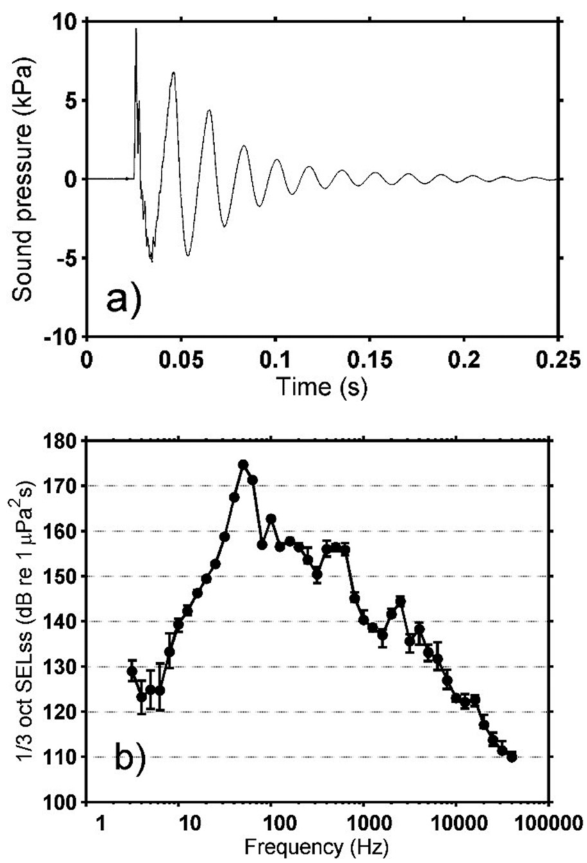

FIG. 2. Sound pressure waveform (a) and one-third octave (base 10) band SELss spectrum (median $\pm$ 1 SD) (b), for a double airgun exposure (two airguns firing simultaneously), with airgun volumes of 164 cm^3 (10 in^3) and firing pressures of 800 kPa (120 lbf/in^2). The SELss were measured in the pool using the double airgun set-up as depicted in Fig. 1 at the location of porpoise 06 at a depth of 1.5 m. Most of the energy was below 1 kHz; peaks occurred at around 50 and 500 Hz. The bars indicate $\pm$ SD (N = 10 shots).

variation observed for 10 airgun shots in a single session [Table I, and Fig. 2(b)].

3. Sound exposure strategy

In order to reduce stress and avoid potential injury to the porpoise's hearing (PTS), the SELss of the impulsive sounds had been increased slowly during the training phase for several months before the study. First, a recording of a single pile driving sound (an impulsive sound that was used as a substitute for airgun sounds during the training phase) was played back via an underwater transducer (Lubell LL1424HP; see Fig. 1 for position) up to the level of a SELss of 145 dB re 1 μPa^{2}s (see Kastelein *et al.*, 2016b).

In the second phase (the pilot study), the animal was exposed to single airgun sounds of which the received SEL was increased, by increasing the volume from 82 to 164 cm^3 (5–10 in^3) and the pressure from 150 to 800 kPa (22 to 120 lbf/in^2), and by decreasing the distance between the porpoise and the airgun from 2 to 1 m, from a SELss of 149 to 175 dB re 1 μPa^{2}s. Single shots at the highest SELss possible (175 dB re 1 μPa^{2}s) did not cause measurable TTS at 1, 2, 4, 8, 16, 32, 63, and 125 kHz. Even 10 shots from a single airgun with mean shot intervals between 12 and 20 s did not cause TTS, so the single airgun was replaced by two, fired simultaneously in a double airgun exposure (see Fig. 1). At the exposure station, this produced a SELss of 178 dB re 1 μPa^{2}s (Table I). A single shot with the double airgun set-up still did not cause TTS in the porpoise, so the number of shots he was exposed to was increased to 10. The porpoise was trained to return to the start buoy and receive a fish reward after each airgun shot. After swallowing the fish he would be sent back to the exposure station immediately. All octave hearing frequencies were tested once between 0.125 and 125 kHz after 10 shots with the double airgun set-up, and TTS appeared to be caused at certain hearing frequencies.

The third phase (the main study, of which the results are reported) consisted of replicated sessions (for statistical validation of TTS) of double-airgun series of 10 airgun shots (SELcum: 188 dB re 1 μPa^{2}s) and 20 airgun shots (this increased the sound exposure level to SELcum: 191 dB re 1 μPa^{2}s). The mean shot interval was around 17 s, but varied between 12 s and 27 s because the porpoise did not always return to the exposure station immediately after an airgun shot exposure. The highest mean peak sound pressure level obtained at the exposure station was 199 ± 0.7 dB re 1 μPa (n = 10).

4. Hearing test signals

Harbor porpoise 06 was asked to detect the hearing test signals before and after exposure to the fatiguing airgun sounds. Narrowband up-sweeps (linear frequency-modulated tones) were used as hearing test signals because they result in very stable and precise thresholds; pure tones have varying amplitudes because of surface reflections, and since the direct and reflective components are at the same frequency, they interact strongly. With sweeps, the reflected components differ slightly from the direct signals and the interaction is therefore weak (Finneran and Schlundt, 2007). The hearing test signals were generated digitally (Adobe Audition, version 3.0; sample rate 768 kHz). The sweeps started and ended at ±2.5% of the center frequency, and had pulse durations of 1 s, including linear rises and falls in amplitude of 50 ms each. The WAV files used as hearing test signals were played on a laptop computer (Micro-Star International – M5168A) with a program written in LabVIEW, to an external data acquisition card (NI – USB6251; single channel maximum sample rate 1.25 MHz), the output of which was controlled in 1 dB steps with the LabVIEW program. The output of the card went through a custom-built buffer to a custom-built variable passive low-pass filter. The sweeps were projected by a balanced tonpilz piezoelectric acoustic transducer (Lubell – LL916) through an isolation transformer (Lubell – AC202). The source level of the sound-emitting system was varied by the operator in 2 dB increments. In the pilot study, after exposures of 10 shots with shot intervals around 15 s with the double airgun set-up (SELcum of 188 dB re 1 μPa^{2}s), the hearing of the porpoise was tested at all octave hearing frequencies between 0.125 and 125 kHz. TTS appeared to occur at frequencies between 0.5 and 8 kHz. Thus, in the main study, hearing was only tested in that frequency range (at 0.5, 2, 4, and 8 kHz).

The received SPL of each hearing test signal was measured three times during the study period (at the start of the pilot study and in the middle and at the end of the main study), at the position of the harbor porpoise's head during the hearing tests. SPL measurements were conducted with

TABLE I. Exposure metrics (mean and standard deviation, N = 10 airgun shots) for the single airgun set-up and the double airgun set-up (two airguns firing simultaneously), measured at the exposure station used in experiments to generate TTS. The volume of each airgun was 164 cm^3 (10 in^3); the airguns were fired at 800 kPa (120 lbf/in^2) and positioned at 1 m depth (centrally in the water column, Fig. 1).

Metric	Symbol	Units	Single airgun exposure (mean ± SD)	Double airgun exposure (mean ± SD)
Single shot sound exposure level (SELss)	$L_{E,\,\mathrm{ss}}$	dB re 1 μPa^{2}s	175.4 ± 0.1	178.2 ± 0.6
Weighted sound exposure level (NMFS, 2016, NOAA weighting)	$L_{E,\mathrm{N}}$	dB re 1 μPa^{2}s	126.6 ± 0.7	130.4 ± 1.4
Peak sound pressure level	$L_{p,\mathrm{pk}}$	dB re 1 μPa	193.6 ± 0.1	199.1 ± 0.7
Mean peak sound pressure	p_{pk}	kPa	4.8 ± 0.03	9.1 ± 0.7
90% energy signal duration duration	$\tau_{90\%}$	ms	78.3 ± 3.6	65.2 ± 3.1
Signal rise-time	τ_{rise}	ms	59.8 ± 0.9	47.7 ± 3.1
Signal rising slope	r_{max}	Pa s^{-1}	41.3 ± 2.5	36.9 ± 7.2
Sound pressure kurtosis	β	—	23.1 ± 1.1	32.6 ± 1.7

two hydrophones, one at the location of each auditory meatus of the harbor porpoise when he was positioned at the listening station (for the method and equipment, see Kastelein *et al.*, 2012). The SPL in the two locations differed by between 0 and 2 dB, depending on the hearing test signal frequency. The mean of the two SPL measurements was calculated to describe the stimulus level during hearing threshold tests. The SPLs at the listening station were measured at levels of approximately 20 dB above the threshold levels found in the present study, because only at these levels were the signal-to-noise ratios high enough to allow accurate SPL measurements to be taken. The linearity of the transmitter system was checked during each calibration and was found to deviate by at most 1 dB within a 20 dB range.

Daily, the SPL of the hearing test signals was checked with a hydrophone (Reson TC4014) and a spectrum analyzer (Velleman PCSU1000). The hydrophone was placed 2 m from the transducer, at 1 m depth.

C. Experimental procedures

Daily, only one airgun sound exposure session was conducted, consisting of (1) pre-exposure hearing tests, (2) fatiguing airgun sound exposure, and (3) post-exposure hearing tests. Pre-exposure hearing tests started at 0830 h with porpoise 06 alone in the indoor pool to keep his environment as quiet as possible. Thereafter (at least 1 h after the pre-exposure hearing test), harbor porpoise 06 swam to a start/response buoy at the side of the outdoor pool, and the net gate between the pools was closed (keeping the other porpoise in the indoor pool). At a signal by the trainer, porpoise 06 swam from the start/response buoy to the exposure station (1.5 m deep, 1 m away from the bar above the water holding the airgun; Fig. 1). When he was stationed correctly (in the position shown in Fig. 1), the trainer signaled (unseen by the animal) to the operator, who then activated the trigger for the airguns, after which either a single gun was triggered (single exposure), or two airguns were triggered simultaneously (double airgun exposure), so that impulsive sound was produced. The animal was at the exposure station for at most 2 s. The porpoise was trained to swim back to the start/response buoy immediately after an airgun shot where he received a fish reward, after which he was sent back to the exposure station as quickly as possible. Exposure sequences of 10 and 20 impulsive sounds were conducted. The time taken by porpoise 06 to swim from the exposure station to the start/response buoy, swallow a fish and swim back to the exposure station varied between exposures. The mean inter-shot time was 17 s (range, 12–27 s).

Immediately after an airgun sound exposure sequence ended, in response to a signal from the operator, a trainer opened the gate between the indoor and outdoor pools, so that the porpoises could switch pools. Porpoise 05 swam into the outdoor pool, while porpoise 06 swam into the indoor pool and stationed at another start/response buoy, so that the post-exposure hearing test could begin immediately. Porpoise 05, who was not involved in the hearing test, was trained to be quiet while porpoise 06's hearing was being tested, so as not to distract the test animal or mask the hearing test signals. The post-exposure hearing threshold test with porpoise 06 (using the same hearing test signals used in the pre-exposure hearing test earlier that day) was conducted in the indoor pool, commencing within 1 min after the fatiguing airgun sound exposure had stopped.

To gain insight into hearing recovery (i.e., the gradual return of the hearing threshold to the pre-exposure level) after a threshold shift, porpoise 06's hearing was tested several times after airgun sound exposure ended: 1–4 min post sound exposure ($PSE_{1–4}$), 4–8 min ($PSE_{4–8}$), and 8–12 min ($PSE_{8–12}$).

Control sessions were conducted; these were the same as fatiguing sound sessions, but instead of being exposed to fatiguing airgun sound the animal was exposed to the ambient noise in the pool (a whistle was blown to instruct him to leave the exposure station, so 10 and 20 whistle sequences were conducted). Post-ambient exposure (PAE) hearing test sessions were performed with porpoise 06 1–4 min ($PAE_{1–4}$), 4–8 min ($PAE_{4–8}$), and 8–12 min ($PAE_{8–12}$) after the ambient exposure period ended. Control sessions were randomly dispersed among the fatiguing airgun sound exposure sessions, also starting at around 0830 h. The 20-shot exposure sessions were conducted after the 10-shot exposure sessions were completed. One session was conducted per day, so hearing was tested at one frequency per day. The hearing frequencies were tested in random order. The sample sizes, chosen to make optimal use of the available time, are shown in Sec. III.

Each hearing test trial began with porpoise 06 at the start/response buoy in the indoor pool. The level of the hearing test sweep used in the first trial of the session was approximately 6 dB above the hearing threshold determined during the previous sessions. When the trainer gave a hand signal, harbor porpoise 06 was trained to swim to the listening station; he then responded (by swimming from the listening station to the start-response buoy) when he heard a sound and received a reward; the methodology is described in detail by Kastelein *et al.* (2012). The hearing test signal level was varied according to the one-up one-down adaptive staircase method in 2 dB steps (Cornsweet, 1962). This conventional psychometric technique (Robinson and Watson, 1973) produces a 50% correct hearing threshold (Levitt, 1971). A change from a test signal level that the harbor porpoise responded to (a hit), to a level that he did not respond to (a miss), and *vice versa*, is called a reversal.

Each hearing test consisted of ~25 trials and at least 10 reversal pairs, and lasted for up to 12 min. However, because the porpoise's hearing recovered quickly from small TTSs, the first 12-min test session after the fatiguing airgun sound stopped was divided into three shorter test periods: 1–4, 4–8, and 8–12 min. A 4-min period was usually long enough for four reversals to be obtained; this number was considered the minimum needed for analysis. Signal-absent (catch) trials were interspersed with the signals present trials; after a whistle was blown the animal would return to the start-response buoy and receive a reward. Sessions consisted of two thirds signal-present and one third signal-absent trials offered in quasi-random order;

there were never more than three consecutive signal-present or signal-absent trials. Each session, a new random number list was used. The data for the main study were collected between June 2016 and January 2017.

D. Data analysis

The pre-exposure mean 50% hearing threshold for a hearing test sweep was determined by calculating the mean SPL of all reversal pairs in the pre-exposure hearing test. The initial TTS 1–4 min after airgun sound exposure or ambient exposure stopped (indicated as $TTS_{1–4,}$ though after control sessions there was no TTS, so that $TTS_{1–4}$ was about 0 dB) was calculated for each hearing test frequency by subtracting the pre-exposure mean 50% hearing threshold from the mean 50% hearing threshold obtained during $PSE_{1–4}$ or $PAE_{1–4}$. The same procedure was used for $TTS_{4–8}$ and $TTS_{8–12,}$ and $PAE_{4–8}$ and $PAE_{8–12.}$

An analysis of variance (ANOVA) was used to evaluate the effect of the factors control/exposure, number of shots (10/20), and hearing frequency (2, 4, or 8 kHz) on $TTS_{1–4}$. The mean $TTS_{1–4}$ for each combination of conditions was compared to zero by means of one-sample t-tests; the 8 tests were not considered to be independent, so *P*-values were adjusted according to the Holm-Bonferroni method (Quinn and Keough, 2002). Analysis was carried out on Minitab 17 for Windows with a significance level of 5%, and data conformed to the assumptions of the tests used (Zar, 1999).

III. RESULTS

The mean ($\pm$ SD; n = number of reversals the mean is based on) pre-exposure hearing thresholds of porpoise 06 were: 93 ± 1.7 dB re 1 μPa at 0.5 kHz (n = 94), 73 ± 1.6 dB re 1 μPa at 2 kHz (n = 80), 62 ± 1.8 dB re 1 μPa at 4 kHz (n = 116) and 59 ± 2.1 dB re 1 μPa at 8 kHz (n = 104).

Harbor porpoise's 06 pre-stimulus response rates (calculated as the number of responses before a test signal or whistle in a session, divided by the total number of trials in that session) were typical for him (Table II). His pre-stimulus response rates after exposure to fatiguing airgun sounds were slightly higher than after exposure to ambient noise.

The control sessions showed that TTS did not occur after exposure to low ambient noise; the mean $TTS_{1–4}$ for 2, 4, and 8 kHz was close to zero (Fig. 3 and Table III).

TABLE II. The mean pre-stimulus response rate of harbor porpoise 06 in pre-exposure hearing tests and in hearing tests conducted after airgun sound exposure (PSE; 10 and 20 shot double airgun exposures combined) and post-ambient noise exposure (PAE; control). Post-exposure hearing tests were conducted 1–4, 4–8, and 8–12 min after exposure, as indicated. Signal-present and signal-absent trials, hearing test frequencies, and the two cumulative SELs were pooled for the calculation of percentages.

Exposure	Mean pre-stimulus response rate			
Airgun sound	Pre-exposure 4.7%	$PSE_{1–4}$ 4.4%	$PSE_{4–8}$ 10.8%	$PSE_{8–12}$ 7.4%
Control	Pre-exposure 1.5%	$PAE_{1–4}$ 1.5%	$PAE_{4–8}$ 4.2%	$PAE_{8–12}$ 1.9%

The ANOVA on $TTS_{1–4}$, with crossed factors exposure/control, number of shots (10 or 20) and hearing frequency (2, 4, or 8 kHz) showed that "TTS" was significantly lower in control sessions than in exposure sessions. Tukey *post hoc* tests showed that overall (including in control sessions) TTS was significantly higher at 4 kHz than at 2 kHz; TTS at 8 kHz was statistically similar to that at both other frequencies (Table IV). The one-sample *t*-tests on TTS_{1-4} for each combination of conditions individually, comparing the means to zero, showed that significant TTS occurred only at 4 kHz (Fig. 3 and Table III).

After exposure to 10 successive double airgun shots (SELcum: 188 dB re 1 μPa^2s; mean shot interval: 17.9 $\pm$ 4.2 s), significant initial TTS occurred only at 4 kHz (Table III); mean TTS_{1-4} was 4.4 ± 1.9 dB, and recovery occurred after 12 min. After exposure to 20 successive shots (SELcum: 191 dB re 1 μPa^2s; mean shot interval: 16.6 $\pm$ 2.1 s), significant initial TTS also occurred only at 4 kHz (Table III); mean TTS_{1-4} was 3.4 ± 1.7 dB, and recovery occurred within 8 min. The TTS_{1-4} measured in each session did not show a correlation with total session duration or mean or maximum shot interval per session (during shot intervals, hearing could, at least partly, recover).

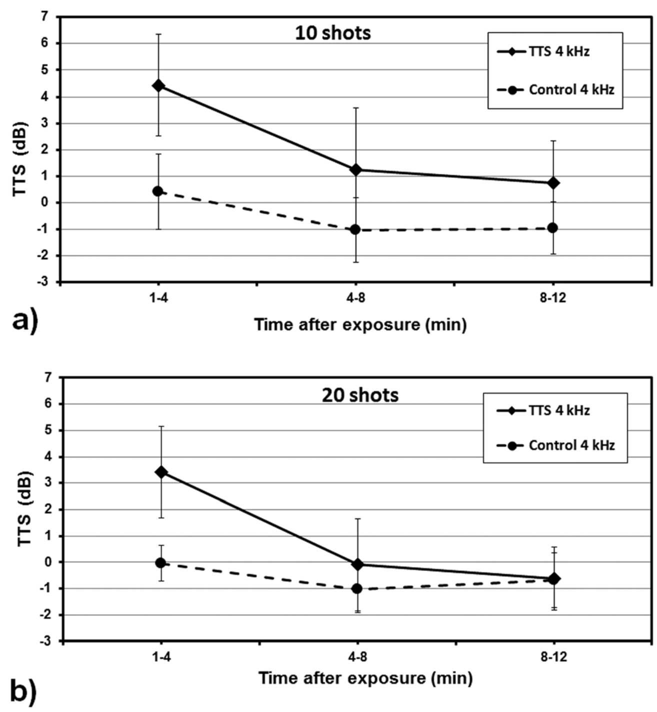

FIG. 3. Mean TTS in harbor porpoise 06 at hearing frequency 4 kHz, after exposure to a series of airgun sounds at SELss of 178.2 ± 0.6 dB re 1 μPa^2s (produced by two airguns shooting simultaneously; mean shot interval: 17.9 ± 4.2 s for 10 shots and 16.6 ± 2.1 s for 20 shots). The error bars indicate $\pm$ one standard deviation. TTS was quantified 1–4, 4–8, and 8–12 min after exposure to airgun sound stopped; (a) after 10 shots, resulting in a SELcum: 188 dB re 1 μPa^2 s, and (b) after 20 shots, resulting in a SELcum: 191 dB re 1 μPa^2 s. Also shown are the TTSs after similar duration exposures to the low ambient noise in the pool (control conditions, indicating that very little variation in hearing thresholds during the day; under these conditions, $TTS_{1–4}$ is ca. 0 dB). For sample sizes and statistics see Tables III and IV.

TABLE III. TTS_{1-4} in harbor porpoise 06, at 0.5, 2, 4, and 8 kHz after double airgun exposure to 10 and 20 successive airgun sounds (mean shot interval ~17 s); one-sample *t*-tests on TTS_{1-4} for each combination of conditions individually, comparing the mean to zero, showed that significant TTS occurred only at the hearing frequency 4 kHz. Numbers of sessions conducted per hearing frequency after double airgun exposure to the airgun sound (two airguns shot simultaneously), and after exposure to the low ambient noise in the pool (control) are shown. The sessions with 20 shots were conducted after the sessions with 10 shots, so only 4 kHz was tested, as the highest TTS occurred at this frequency after exposure to 10 shots. Only three sessions were conducted with 0.5 kHz, because no TTS occurred at this hearing frequency during those three sessions after exposure to 10 airgun shots; no control sessions were conducted for 0.5 kHz. NS = not significant, N = sample size, P = exact probability, adjusted according to the Holm-Bonferroni method (Quinn and Keough, 2002; shown only where significant).

Hearing frequency	10 shots						20 shots					
	Mean shot interval: 17.9 ± 4.2 s						Mean shot interval: 16.6 ± 2.1 s					
	SELcum 188 dB re 1μPa^{2}s						SELcum 191 dB re 1μPa^{2}s					
	After airgun sound			Control			After airgun sound			Control		
(kHz)	TTS_{1-4} (dB) Mean ± SD (range)	N	P	TTS_{1-4} (dB) Mean ± SD	N	P	TTS_{1-4} (dB) Mean ± SD (range)	N	P	TTS_{1-4} (dB) Mean ± SD	N	P
0.5	−0.9 ± 1.1 (−2.0 to 0.1)	3	—	—	0	—						
2	1.3 ± 2.1 (−1.7 to 4.2)	6	NS	−0.3 ± 1.2	6	NS						
4	4.4 ± 1.9 (1.9 to 8.4)	12	0.008	0.4 ± 1.4	9	NS	3.4 ± 1.7 (1.5–7.6)	10	0.007	0.0 ± 0.7	7	NS
8	2.0 ± 2.6 (−0.7 to 6.3)	8	NS	0.4 ± 1.2	7	NS						

IV. DISCUSSION AND CONCLUSIONS

A. Evaluation

The present study was conducted on harbor porpoise 06, of which the hearing was similar to that of five other porpoises (Kastelein *et al.*, 2010; Kastelein *et al.*, 2014b; Kastelein *et al.*, 2015c; Kastelein *et al.*, 2017). However, it is not clear how representative the TTS values found in this animal are. Studies on humans and other terrestrial mammals show large individual, genetic and population-level differences in TTS (Kylin, 1960; Kryter *et al.*, 1962; Henderson *et al.*, 1991; Henderson *et al.*, 1993; Davis *et al.*, 2003; Spankovich *et al.*, 2014). Therefore, further replication with more animals is needed to assess the generality of the results obtained.

In the present study, the airgun's volume was small and its operating pressure was low. Single airguns used during seismic surveys at sea typically have volumes between 490 cm^3 and 1300 cm^3 (30 in^3 and 800 in^3). The total volume in an airgun array is between 49 000 cm^3 and 130 000 cm^3 (3000 in^3 and 8000 in^3), and the pressure is ~13 800 kPa (2000 lbf/in^2) (Caldwell and Dragoset, 2000). Sounds recorded from conventional airguns may have high-frequency content (>1 kHz), but the characteristics of the airgun sounds that harbor porpoises are exposed to in the wild vary according to the type of airgun used, the geometry of the array, propagation effects and the distance and position of an animal relative to the airgun array (Caldwell and Dragoset, 2000; Madsen *et al.*, 2006; Lucke *et al.*, 2009; Landrø *et al.*, 2011; Sertlek and Ainslie, 2015; Thompson *et al.*, 2013; Hermannsen *et al.*, 2015; Ainslie *et al.*, 2016). The smaller volume and lower pressure of the airgun used in this study (see also Hermannsen *et al.*, 2015) resulted in a higher dominant frequency (~50 Hz rather than ~20 Hz) and a somewhat smaller bandwidth (40 Hz measured at −10 dB, rather than 100 Hz) than found in full arrays used for seismic surveying (Caldwell and Dragoset, 2000). However, the sounds produced were still similar to those used for seismic surveying (<100 Hz), and were in the frequency range for which the hearing threshold of the harbor porpoise is high (Kastelein *et al.*, 2017).

The location of an animal relative to a sound source can affect the characteristics of the sound it experiences, as propagation and reverberations alter the characteristics of sounds, such as their spectrum, rise time, pulse duration, and level. The study animal was placed at the exposure station, facing the nearest airgun. The second airgun was placed slightly off-center, which may have led to a slightly different angle of exposure. Kastelein *et al.* (2005) measured the horizontal directivity index of a harbor porpoise; it decreased with decreasing frequencies, and was only approximately 3 dB at

TABLE IV. ANOVA on TTS_{1-4}, with crossed factors exposure/control, number of shots (10 or 20) and hearing frequency (2, 4, or 8 kHz). TTS was significantly higher in exposure sessions than in control sessions. Tukey post-hoc tests showed that overall (including in control sessions) TTS was significantly higher at 4 kHz than at 2 kHz; TTS at 8 kHz was statistically similar to that at both other frequencies. DF = degrees of freedom, Adj SS = adjusted sum of squares, MS = mean squares, F = test statistic, P = calculated probability.

Source	DF	Adj SS	Adj MS	F-Value	*P*-Value
Exposure/control	1	132.8	132.8	42.14	0.000
Shots (10/20)	1	5.556	5.556	1.76	0.189
Frequency (2/4/8 kHz)	2	34.75	17.38	5.52	0.006
Error	60	189.0	3.150		
Total	64	364.8			

16 kHz (the lowest frequency tested in that study), thus the effect on the perceived exposure of the slightly off-axis exposure to the second, more distant, airgun is expected to be negligible.

B. Frequency-selective TTS and hearing frequencies most affected

In two previous studies in which harbor porpoises were exposed to impulsive sounds, the SELcum (in this case the same as SELss) leading to TTS was around 165 dB re 1 μPa^2 s for a single airgun sound [Lucke *et al.*, 2009; subtracting 10 lg (0.9) dB to convert from the reported 90% value to SELss for the entire pulse] and 183 dB re 1 μPa^{2}s for multiple pile driving playback sounds (Kastelein *et al.*, 2015a). In the present study, a measurable change in hearing threshold occurred 1–4 min after exposure to 10 shots of a downscaled double airgun set-up, with a SELcum of 188 dB re 1 μPa^{2}s.

Although both the present researchers and Lucke *et al.* (2009) exposed porpoises to airgun sounds, different experimental set-ups meant that the animals were exposed to different sounds. Lucke *et al.* (2009) used a larger airgun (20 in^3, fired at 13.8 MPa, 2000 lbf/in^2) at a greater distance (between 14 and 150 m) from the study animal, in a harbor. This shallow environment resulted in attenuation of the low-frequency component of the airgun sounds, so that the study animals experienced sounds with the highest sound pressure spectral density at around 300 Hz. The smaller airgun used in the present study closer to the study animal had its highest one-third octave (base 10) band SELss at around 50 Hz (Fig. 2). Lucke *et al.* (2009) observed TTS at 4 kHz using an auditory evoked potential (AEP) method, which is in agreement with the current findings, although they report higher TTS values. Because of differences in methods used to measure TTS (behavioral vs AEP method), magnitudes of TTS, timing of the TTS measurement (TTS_{18} vs TTS_{1-4}), spectra (lower frequency sounds were used in the present study), and potential for recovery of hearing between shots, the TTS values of these exposures cannot be compared directly.

The low background noise levels in the indoor pool and the behavioral hearing test method used allowed to test for TTS at frequencies lower than 4 kHz in unmasked conditions in the present study. TTS was not observed at frequencies of 0.5 and 2 kHz, even though the one-third octave (base 10) band SELcum values at those frequencies were higher than at 4 kHz (Table III, Fig. 5).

In a TTS study with pile driving sounds, the affected hearing frequency of harbor porpoises was also much higher than the frequency of most energy of the fatiguing sound (Kastelein *et al.*, 2015a). When a harbor porpoise was exposed to pile driving sounds for 60 min (SELss of 146 dB re 1 μPa^{2}s; SELcum: 180 dB re 1 μPa^{2}s), the largest TTS_{1-4} occurred at 8 kHz, similar TTS occurred at 4 kHz, and no TTS occurred at the other frequencies tested, which included the frequency of most energy of harbor porpoise echolocation (125 kHz). The pile driving sounds had most energy in the 500–800 Hz frequency band, but no TTS occurred at 500 Hz.

C. Frequency weighting

Apart from individual differences in vulnerability to TTS, a potential cause of the differences in SELcum required to cause a certain TTS is that the three impulsive sounds used (in the present study, by Lucke *et al.*, 2009 and by Kastelein *et al.*, 2015a), had different frequency contents, and that frequency selective sensitivity of the harbor porpoise led to different TTS SELcum thresholds.

To investigate whether frequency weighting can be used to improve predictions of the risk of TTS, and the hearing frequency range affected by the broadband airgun signal, the measured SELcum was weighted by using the NOAA (NMFS, 2016) weighting curve (Fig. 4). The resulting weighted SELcum were 130 dB re 1 μPa^{2}s for a single airgun shot (Table I), 140 dB re 1 μPa^{2}s for 10 shots and 143 dB re 1 μPa^{2}s for 20 shots (double airgun exposure). For comparison, an average SELcum spectrum was computed from the data from Kastelein *et al.* (2015a) by randomly sampling 2760 pile driving playback sounds from all measurement locations in the pool (0.5, 1, and 1.5 m depth; horizontal grid spacing of 1 m, Fig. 5), resulting in a weighted SELcum of 144 dB re 1 μPa^{2}s. In the NOAA report (NMFS, 2016), the weighted SELcum of the exposures from Lucke *et al.* (2009) is given as 140 dB re 1 μPa^{2}s. A mean TTS_{1-4} of 4.4 dB occurred in the present study after the study animal was exposed to airgun sounds with a weighted SELcum of 140.3 dB re 1 μPa^{2}s.

The frequency bands with the highest weighted SELcum (4–8 kHz) overlap with those at which TTS was observed in the pile driving study (Kastelein *et al.*, 2015a) and in the present study (Fig. 5). However, in the pile driving study, TTS was not observed around the 700 Hz peak, although the weighted SELcum exceeded the levels at which TTS at 4 kHz was induced by exposure to airgun sounds [Fig. 5(b)]. This may be an effect of uncertainty in the weighting function, or faster recovery from TTS at lower frequencies.

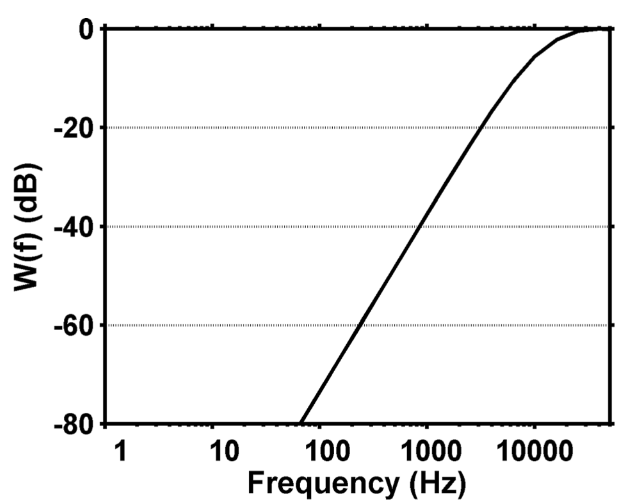

FIG. 4. The auditory weighting function $W(f)/dB = 10\log(w_{NMFS,HF})$ proposed by NOAA for weighting the SELcum to predict TTS onset in harbor porpoises (NMFS, 2016; Finneran, 2016).

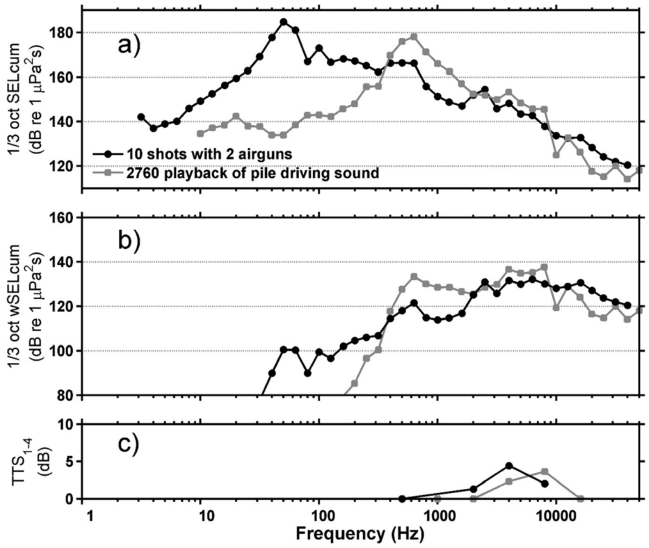

FIG. 5. (a) Unweighted one-third octave (base 10) band SELcum spectra of 10 double airgun shots (present study; shot interval: ~17 s), and of 2760 pile driving playbacks strikes/h (inter-pulse interval 1.3 s) during a 120 min exposure (Kastelein *et al.*, 2015a). (b) Measured frequency-weighted one-third octave (base 10) band SELcum from both studies, using the NOAA's (NMFS, 2016) weighting function for harbor porpoises (see Fig. 4). (c) Observed mean TTS_{1-4} for different test frequencies (0.5, 1, 2, 4, and 8 kHz). The frequency bands with maximum weighted SELcum overlap with the frequencies at which TTS occurred.

D. Effect of number of airgun exposures on TTS

In the present study, no significant difference was observed in the mean TTS after exposure of the study animal to 10 successive double airgun shots and 20 successive double airgun shots (Table IV). Kastelein *et al.* (2016b) observed only a very small increase in TTS (the ratio of TTS increase to the associated increase in SELcum was ~0.5 dB/dB) between 30 min and 6 h exposures of harbor porpoises to impulsive pile driving playback sounds (1.3 s inter-pulse intervals, duty cycle ~9.5%). The same phenomenon was observed in other TTS studies in which harbor porpoises were exposed to intermittent fatiguing sounds and continuous sounds (Kastelein *et al.*, 2014a; Kastelein *et al.*, 2016a). A possible explanation for the observed difference in mean TTS_{1-4} after 10 and 20 shots is differences in shot intervals between exposure sessions (the mean shot intervals reported in Table III are based on sessions in some of which one longer shot interval occurred). However, the TTS_{1-4} measured in each session did not show a significant correlation with total session duration, mean shot interval or maximum shot interval per session. Exposures at higher (weighted) SELss and more pulses than those achieved in this study are required to establish the TTS onset (commonly defined as a TTS of 6 dB; Southall *et al.*, 2007), and to draw conclusions on the rate of change in TTS in harbor porpoises exposed to multiple airgun sounds.

E. Effects of characteristics of airgun sounds on TTS

Current guidelines and regulations pertaining to impulsive sounds focus on the SELcum and peak sound pressure level as predictors of the onset and occurrence of TTS (Southall *et al.*, 2007; BSH, 2013; Dekeling *et al.*, 2014; NMFS, 2016). Apart from the frequency-selective weighting, other factors of the fatiguing sound may affect TTS and the frequencies that are affected. Factors, such as the SELss, peak sound pressure, duty cycle (low duty cycles may allow hearing to recover between shots), exposure duration (Southall *et al.*, 2007; Finneran, 2015), and potentially the rise time, rise rate, and kurtosis (Melnick, 1991; Hamernik and Qiu, 2001; Yost, 2007), may affect TTS. Peak-to-peak sound pressure levels as defined and reported by Lucke *et al.* (2009) were in the range of 200.2–202.1 dB re 1 μPa. The recorded sound pressure waveforms reported in Lucke *et al.* (2009) show similar amplitudes for the positive and negative peaks, hence the zero-to-peak sound pressure level $L_{p,\mathrm{pk}}$ is expected to have been approximately 6 dB lower, e.g., $L_{p,\mathrm{pk}} = 194$–196 dB re 1 μPa. The single-shot exposures with the double airgun in the present study exceeded these $L_{p,\mathrm{pk}}$ (Table I), but produced no measurable TTS_{1-4} at any of the frequencies tested. From this comparison it is concluded that the frequency-weighted SELcum was a better predictor of TTS onset than either unweighted SELcum or peak sound pressure, at least for the range of $L_{p,\mathrm{pk}}$ tested here.

Earlier TTS studies involving porpoises exposed to impulsive sound did not provide measurements of parameters such as kurtosis, rise time and rise rate, so it is not possible to assess the relative effects of these parameters on TTS. However, for future reference, the characteristics of the airgun sounds used in the present study are reported in detail in Table I.

If harbor porpoises are exposed to airgun sounds at very high SELss, or peak sound pressure, hearing frequencies higher than 4 kHz may be affected. Kastelein *et al.* (2014b) showed that, in harbor porpoises, the hearing frequency showing TTS depends on the received SPL (and consequently peak sound pressure and SELss) of the sonar sounds that they are exposed to. When the SPL of tonal sounds increases, so does the affected hearing frequency. For harbor porpoises exposed to airgun sound, it is not clear how or to what extent such a frequency shift could occur relative to the spectrum of the fatiguing noise.

F. Recovery from TTS

Recovery from the small TTSs that occurred in the present study occurred in all cases within 12 min. (Fig. 3). Other harbor porpoises suffering TTS recovered within 60 min after sound exposure stopped (in most cases probably earlier; Kastelein *et al.*, 2012, Kastelein *et al.*, 2013a, Kastelein *et al.*, 2014a, Kastelein *et al.*, 2014b, Kastelein *et al.*, 2015a, Kastelein *et al.*, 2015b). So, similar TTSs, caused by various fatiguing sound types (noise bands, tones, sweeps, and impulsive sounds) with various exposure levels and exposure times, required a similar or longer recovery time than seen in the present study. This is consistent with what is found in general for marine mammals (Finneran, 2015).

G. Ecological significance and management implications

Statistically significant TTS_{1-4}, as reported in the present study, means that hearing reduction occurred in the study animals; after exposure to fatiguing airgun sound, their hearing became temporarily less sensitive than it was before exposure, or after exposure to low ambient noise. However, in the present study the $TTS_{1–4}$ was not severe, and such small TTSs could only be measured accurately because the pools at the SEAMARCO Research Institute are very quiet, resulting in stability in threshold performance and response bias. Statistically significant TTS is not necessarily ecologically significant. During seismic surveys at sea, depending on the location of the porpoises, the sound levels they are exposed to may be higher than those used in the present study, and consequently the induced TTS may be greater and these sound levels could induce auditory damage (Kujawa and Liberman, 2006, 2009). In addition, in seismic surveys, the shot intervals may be shorter than the intervals in the present study, allowing less time for hearing to recover between airgun sounds, and thus increasing the TTS (Finneran, 2010; Kastelein *et al.*, 2016a).

It is not clear what the ecological effects of TTS are, but they are likely to depend on the magnitude of the TTS, the duration of the TTS, and the affected hearing frequency. Reduced hearing may reduce the efficiency with which harbor porpoises can carry out ecologically important activities such as navigation, orientation, communication, foraging, and predator avoidance, thus potentially reducing their fitness, reproductive output and longevity. Airgun sound is not similar in frequency to the echolocation signals of harbor porpoises (which peak at around 125 kHz; Møhl and Andersen, 1973; Verboom and Kastelein, 2003), so unless porpoises are very close to an airgun array, the airgun sounds are unlikely to interfere with echolocation during prey detection (Vater and Kössl, 2011), but may distract an animal and thus reduce foraging efficiency (Shafiei *et al.*, 2015; Chan *et al.*, 2010), or affect foraging behavior (Thompson *et al.*, 2013; Pirotta *et al.*, 2014).

The results of the present study have implications for the management and mitigation of the effects of airgun sounds on harbor porpoises. This study confirms that harbor porpoise hearing is relative to larger odontocetes less likely to be damaged by sounds of low frequency, as was already suggested from their equal latency curves (Wensveen *et al.*, 2014), and in recent reviews of effects of various sounds on porpoise hearing (Tougaard *et al.*, 2015; NMFS, 2016). It is also in agreement with data on bottlenose dolphins exposed to airgun and watergun impulses (Finneran *et al.*, 2015). A frequency weighting that accounts for lower sensitivity at low frequencies has already been adopted in new noise criteria published by NOAA in the USA (NMFS, 2016), although policy-makers in other countries rely on unweighted SELcum, or M-weighted SELcum, as a criterion for TTS and PTS onset (e.g., Southall *et al.*, 2007; BSH, 2013; Dekeling *et al.*, 2014; Houser *et al.*, 2017). Whether regulations that rely on risk thresholds based on unweighted SELcum overestimate the risk of hearing effects depends on the frequency content of sound sources considered. Although the present study was not aimed at identifying the optimal weighting function, the initial results indicate that the frequency-weighting function proposed by NOAA (NMFS, 2016) provides a reasonably robust measure of low levels of TTS occurring over a range of spectra of impulsive sound sources.

Based on hearing data alone, mitigation to reduce the risk of hearing damage in harbor porpoises should focus on reducing the high-frequency content (>100 Hz) of broadband impulsive sounds. Measures such as bubble curtains and noise mitigation screens may be more effective than previously believed, since they are typically effective at reducing the frequency elements of impulsive sounds above 100 Hz, such as those generated by airguns, detonations, and percussion pile driving (Coste *et al.*, 2014; Bellman *et al.*, 2014; Lee *et al.*, 2016).

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