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Effect of pile driving sounds' exposure duration on temporary hearing threshold shift in harbor porpoises (*Phocoena phocoena*)

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Loud underwater sounds may cause temporary hearing loss in harbor porpoises, the magnitude of which may depend on the exposure duration. After exposure to playbacks of broadband pile driving sounds, temporary hearing threshold shifts (TTS) in two porpoises were quantified at 8 kHz (highest TTS) with a psychoacoustic technique. Pile driving sounds had: pulse duration 124 ms, rate 2760 strikes per hour, inter-pulse interval 1.3 s, duty cycle ~9.5%, average received single-strike unweighted sound exposure level (SEL_{ss}) 145 dB re $1\mu Pa^2 s$, exposure durations 15, 30, 60, 120, 180, 240 and 360 min (cumulative SEL:173, 176, 179, 182, 184, 185, 187 dB re $1\mu Pa^2 s$, respectively). Control sessions under ambient noise conditions were carried out. Mean initial TTS (1-4 min after sound exposure stopped in one porpoise, and 12-16 min after exposure stopped in the other animal) increased from 0 dB after 15 min exposure to 5 dB after 360 min exposure. In both animals, recovery occurred within 60 min post-exposure. The relatively small increase in TTS between 15 and 360 min exposures is due to the relatively small amount of sound energy per unit of time to which the porpoises were exposed (average sound pressure level ~144 dB re 1μPa).

Key words: anthropogenic noise, audiogram, TTS, odontocete, offshore, hearing, hearing sensitivity, recovery.

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I. INTRODUCTION

Sequences of impulsive sounds at very high source levels are produced during offshore pile driving. Within a certain distance from the source, these sounds may cause hearing loss in marine mammals which may be temporary (TTS; temporary threshold shift) or permanent (PTS; permanent threshold shift). To prevent or reduce the impacts (i.e. behavioral disturbance or hearing reduction) of offshore pile driving activities on populations of marine mammals, many governments have set, or are in the process of setting, criteria for the maximum allowable exposure levels of underwater sound related to offshore pile driving.

The harbor porpoise (*Phocoena phocoena*) is the most common marine mammal species in the North Sea (and in many other coastal waters in the temperate zone of the northern hemisphere), and has the most sensitive hearing of all marine mammals in this area, so underwater sound criteria are often based on information derived from this species.

PTS studies are considered unethical in marine mammals. Therefore sound exposure levels that may lead to PTS are estimated using information on variations in TTS onset and magnitude in relation to variations in exposure sound pressure level (SPL) and exposure duration. Southall *et al.* (2007) recommended criteria to avoid PTS in marine mammals, based on the knowledge of TTS in marine mammals available at the time.

Information on TTS in harbor porpoises as a result of exposure to sequences of impulsive sounds was not available until recently (Kastelein *et al*., 2015a). Policy-makers therefore used data from a TTS study by Lucke et al. (2009) to estimate the PTS onset level in this species due to exposure to pile driving sounds. Lucke et al. (2009) exposed a harbor porpoise to single impulsive sounds from an airgun. Although limited data about TTS in harbor porpoises due to impulsive sounds is available, extrapolation from one impulsive sound to sequences of impulsive sounds is likely to result in large errors. Several aspects contribute to the TTS induced by a sequence of sounds. Besides the level of the sounds and the duration of exposure to the sequence, the duty cycle can have a major effect on the induced TTS. Research has shown that the duty cycle of the fatiguing sound affects the magnitude of TTS (Finneran *et al*., 2010; Kastelein *et al.*, 2014a; 2105b), as hearing may recover at least partly during inter-pulse intervals. Kastelein *et al*. (2014a) reported that although exposure to a single sound may not lead to measurable TTS, the cumulative effect of sequences of intermittent sounds may cause TTS. Therefore, it is expected that TTS might be induced by exposure to multiple successive sounds, such as those produced during pile driving, seismic airgun surveys and naval sonar signal emissions.

As well as understanding the effects of inter-pulse intervals, it is important to find out which hearing frequency is most affected by a specific fatiguing sound. For narrow-band signals this is often believed to be the center frequency of the fatiguing noise or half an octave above it (Finneran *et al*., 2013; Kastelein *et al*., 2012b; 2013b; 2014a). However, Kastelein *et al*. (2014b) showed that, in harbor porpoises, the affected hearing frequency depends not only on the spectrum of the fatiguing noise, but also on the received SEL. Because of its broadband nature, the range of frequencies most affected by exposure to pile driving sound could not be predicted. Therefore, Kastelein et al. (2015a) measured TTS in a harbor porpoise after exposure to playbacks of broadband pile driving sound at several hearing frequencies, and found that the hearing of the porpoise was only affected at 4 and 8 kHz.

Noise produced by pile driving consists of long sequences of impulsive broadband sounds that may act cumulatively on an animal's hearing: the magnitude of TTS caused by the noise is assumed to be related to total exposure duration, the inter-pulse interval between pile drive strikes, and the SPL received by the animal. Therefore, it is important to gain insight into the cumulative effect of realistic pile driving sound exposures on the hearing of the harbor porpoise. The goal of the present study was to measure the effect of exposure duration on the TTS induced in harbor porpoises due to realistic broadband pile driving sound playback exposures, and to gain insight into the process of recovery of hearing post-exposure.

II. MATERIALS AND METHODS

A. Study animals and study area

Two harbor porpoises were used in this study. One male harbor porpoise (ID no. 02) had participated in previous psychoacoustic studies (Kastelein *et al.*, 2009; 2010; 2012b, 2013b, c). During the present study he was 9 years old, his body mass was around 40 kg, his body length was 146 cm, and his girth at the axilla was approximately 75 cm. The other male harbor porpoise (ID no. 04) was 3 years old during the study, and had previously participated in a basic audiogram study and a masking study (both in preparation). His body mass was around 30 kg, his body length was 131cm, and his girth at the axilla was approximately 70 cm.

The animals received around 2 kg (no. 02) and 1.7 kg (no. 04) of thawed fish per day, divided over four or five meals. Variation in the animals' performance was minimized by making weekly adjustments (usually in the order of 100 g) to their daily food ration, based on their weight 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 and quiet, 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 x 8 m; 2 m deep) in which they were exposed to fatiguing noise, connected via a channel (4 m x 3 m; 1.4 m deep) to an indoor pool (8 m x 7 m; 2 m deep) in which hearing tests were conducted. All pumps were switched off 10 min before each test and left off during tests; 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.* (2012b).

B. Acoustics

Background noise and stimuli calibration measurements

The background noise, fatiguing pile driving noise, and hearing test signals were calibrated at the beginning and the end of the study period. The sound measurement equipment consisted of three hydrophones [Brüel & Kjaer (B&K) – 8106] with a multichannel high frequency analyzer (B&K PULSE - 3560 D), and a laptop computer with B&K PULSE software (Labshop, version 12.1; sample frequency used: 524288 Hz). Before analysis the recordings were high-pass filtered (cut-off frequency 100 Hz; 3rd order Butterworth filter; 16 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) (ANSI, 2013) of each hearing test was derived from the received 90% energy flux density and the corresponding 90% time duration (t_{90}) (Madsen, 2005).

The received sound pressure of the fatiguing noise (impulsive sounds) was analyzed in terms of unweighted sound exposure level (SEL) in dB re 1 µPa^{2}s (ANSIS1.1, 2013).

Great care was taken to make the harbor porpoises' listening environment as quiet as possible. Only 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. During test conditions the background noise in the pool was very low (see Kastelein *et al.*, 2012b).

Fatiguing noise: pile driving playback sound

The fatiguing noise, the noise intended to cause TTS, consisted of playbacks of series of offshore pile driving sounds recorded at 800 m from a 4.2 m-diameter pile being driven into the sea bed as the foundation for a wind turbine for the Dutch offshore wind farm 'Egmond aan Zee' in the North Sea. The strike rate was 2760 per hour. A WAV file was made of series of consecutive pile driving strike sounds. The recordings were sampled at 88.2 kHz and high-pass filtered at a cut-off frequency of 50 Hz. Anti-aliasing filters were applied.

A random section of five strikes from the digitized original recording of series of pile driving sounds (WAV file) was played back repeatedly by a 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 variable passive low-pass filter, after which it went to a power amplifier (East &West Inc.- LS5002), which drove the transducer (Lubell - LL1424HP) through an isolation transformer (Lubell - AC1424HP). The transducer was placed at the south-western end of the pool at 2 m depth. The linearity of the transmitter system of the fatiguing noise was checked during each calibration, and was found to be deviate at most by 1 dB within a 42 dB range.

The pile driving sounds were played back at the maximum level of the sound emitting system. This resulted in a single strike SEL (SEL_{ss}) of 155 dB re 1 μPa^2s (based on a t_{90} pulse duration of 123 ms), measured at 1 m depth, and 2 m from the source. The t_{90} pulse duration is defined as the time interval between the arrival of 5% and 95% of the total energy (Madsen, 2005). Most of the energy was in the 500-800 Hz frequency band (for the spectrum and waveform of the pile driving playback sound, see Kastelein *et al*., 2015a) ($SEL_{ss}90 = SPL\_t_{90} + 10 * \log[t_{90}/(1\ s)]$).

To determine the fatiguing noise distribution in the pool, the SEL_{ss} was measured at 77 locations (on a horizontal grid of 1 m x 1 m). The SEL_{ss} was measured at three depths per location on the grid (0.5, 1.0 and 1.5 m below the surface). The average received SEL_{ss}; (dB re 1 μPa^2s) of the played back impulsive sound (based on t_{90}), as experienced by the harbor porpoise, was calculated from the average power sum of all 231 individual measurement locations. There were only small differences in SEL_{ss} per depth per location, and hardly any gradient occurred in the SEL_{ss} in relation to the location of the transducer, so the field was fairly homogeneous (for the SEL_{ss} distribution in the pool see Kastelein *et al*., 2015a). During exposure to playbacks of pile driving sounds, the animals swam ovals throughout the entire pool, so their average received SEL_{ss} was assumed to be close to the average SEL_{ss} measured in the pool (see Results, section A. Swimming pattern; n = 231 measurements). During exposure sessions, the average received SEL_{ss} (± SD) was 145 (± 4) dB re 1μPa^2s, and the average t_{90} (± SD) was 124 (± 3.5) ms. Per exposure hour, the animals were exposed to playbacks of 2760 individual pile driving sounds with an inter-pulse interval of 1.3 s, corresponding to a duty cycle of ~9.5%. Consequently, the porpoises were exposed to an averaged SPL of 144 dB re 1μPa.

Hearing test signals

Narrow band up-sweeps (linear frequency-modulated tones) were used as hearing test signals (which the animals were asked to detect before and after exposure to the pile driving playback sounds) instead of pure tones, because sweeps lead to very stable and precise thresholds. The hearing test signals were generated digitally (Adobe Audition, version 3.0). The linear upsweeps started and ended at ± 2.5% of the center frequency, and had pulse durations of 1 s, including a linear rise and fall in amplitude of 50 ms. 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), 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 (set at 6.4 kHz for 4 kHz test signals, and at 12.8 kHz for 8 kHz signals) and another buffer (AS - 2008-3), and drove the 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.

The received SPL of each hearing test signal was measured at the position of the harbor porpoise's head during the hearing tests. Calibration measurements were conducted with two hydrophones, one at the location of each auditory meatus of the harbor porpoises when they were positioned at the listening station (for the method and equipment, see Kastelein *et al.*, 2012b). The SPL in the two locations differed by 0 to 2 dB, depending on the test frequency. The mean SPL of the two hydrophones was used to calculate the stimulus level during hearing threshold tests. The received SPLs were calibrated at levels of approximately 20 dB above the threshold levels found in the present study. The linearity of the transmitter system was checked during each calibration and was found to deviate at most by 1 dB within a 20 dB range.

C. Experimental procedures

Daily, one noise exposure test was conducted, consisting of: (1) pre-exposure hearing tests, (2) noise exposure, and (3) post-noise exposure hearing tests. Tests started at 08.30 hrs. Pre-exposure hearing tests were performed with the animals in the indoor pool. Thereafter, the harbor porpoises swam into the outdoor pool, a net gate leading to the indoor pool was closed, and the fatiguing noise exposure began. During noise exposure, the operator watched the harbor porpoises' behavior on a monitor in the outdoor research cabin, and the animals' surfacing locations and respiration rates were recorded on video.

To keep the animals' motivation and response criteria similar after all exposure durations (during both pile driving playback sound and control sessions), the porpoises were fed in the middle of the 4 h exposure and at 2 h and 4 h during the 6 h exposure. Feeding was done quickly and below the water surface, so that the porpoises continued to be exposed to the pile driving sounds during feeds. Exposure sessions shorter than 1 h started 1.5 h after the pre-exposure tests, so that there was always around 2 h between pre-exposure and initial post-exposure hearing tests (the animals were fed during hearing tests).

Five min before the fatiguing noise exposure ended, two trainers went to the gate in the channel leading to the indoor pool. In response to a signal from the operator, one trainer went to the outdoor pool and called animal 04 to the side of the pool, while the other trainer opened the gate and called animal 02 into the channel. The animal not involved in the hearing test was trained to be very quiet during the hearing tests, so not to distract the test animal or mask the test signals. When animal 02 entered the channel, the fatiguing noise ended immediately. The post-exposure hearing threshold session (using the sweep used in the pre-exposure hearing session) was conducted in the indoor pool, commencing within 1 min after the fatiguing noise had stopped. After his hearing had been tested, porpoise 02 was asked to go to the outdoor pool, and porpoise 04 was asked to come into the indoor pool where his hearing was tested, starting 12 min after the fatiguing noise stopped. Data were collected between June and December 2014.

Not only the magnitude of the initial TTS is important, but also the rate of recovery of hearing after the noise exposure stops. Therefore, animal 02's hearing was tested not only 1-4 min after exposure stopped (Post Noise Exposure $(PNE)_{1-4}$), but also 4-8 min (PNE_{4-8}), 8-12 min (PNE_{8-12}), and 48 min (PNE_{48}) min after noise exposure ended. These times were chosen because, depending on the initial TTS, hearing in this study was expected to recover after around 30 min. The 48 min period was chosen so that the animal's appetite was sufficient to ensure comparable co-operation as during the first 12 min after the exposure stopped. Animal 04's hearing was measured 12-16 min after exposure stopped (PNE_{12-16}), but also 16-20 min (PNE_{16-20}), 20-24 min (PNE_{20-24}), and 60 min (PNE_{60}) min after noise exposure ended.

In a previous study with the same set-up, the same pile driving playback sounds had caused TTS_{1-4} in animal 02 only at 4 and 8 kHz (Kastelein *et al.*, 2015a), so TTS_{1-4} was quantified in a pilot study for hearing test signals at 4 and 8 kHz in animal 02. Both frequencies and exposure durations were tested in random order (**Table 1**). The frequency which showed the highest TTS was chosen to be tested in the main experiment.

During the main experiment the porpoises were exposed to sounds for 15, 30, 60, 120, 180, 240 and 360 min, resulting in cumulative sound exposure levels (SEL_{cum}) of 173, 176, 179, 182, 184, 185, and 187 dB re $1\mu Pa^2 s$, respectively. (SEL_{cum} = SPL_T + 10lg[T/(1 s)] dB).

To gain insight into potential effects on hearing thresholds of the methodology (for instance, variation due to time between hearing tests or the time of day), control tests were conducted. Control tests were the same as fatiguing noise exposure tests, but instead of to fatiguing noise the animals were exposed to the ambient noise in the pool. The ambient noise level in the pool was very low and below sea state 0. For the ambient noise spectrum in the pool during control tests, see Kastelein *et al.* (2012b). Post-ambient exposure (PAE) hearing test sessions were then performed with animal 02 at 1-4 (PAE_{1-4}), 4-8 (PAE_{4-8}), 8-12 (PAE_{8-12}), and 48 (PAE_{48}) min after the ambient exposure period ended, and with animal 04 at 12-16 (PAE_{12-16}), 16-20 (PAE_{16-20}), 20-24 (PAE_{20-24}), and 60 min (PAE_{60}) after ambient exposure. Control sessions were randomly dispersed among the fatiguing noise exposure tests, also starting at around 08.30 hr. The goal was to conduct seven sessions for each pile driving sound exposure duration and seven for each low ambient noise exposure duration. Exposure durations were tested in random order both in fatiguing noise (pile driving sound) and ambient noise (control) sessions.

Each hearing test trial began with an animal at the start/response buoy. 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, the harbor porpoise was trained to swim to the listening station. The methodology was as described by Kastelein *et al.* (2012b). The signal level was varied according to the one-up *one-down adaptive staircase method (Cornsweet, 1962). This conventional psychometric* technique (Robinson and Watson, 1973) can produce a 50% correct hearing threshold (Levitt, 1971). 2 dB steps were used. A switch 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*, was called a reversal.

Each complete hearing session consisted of ~25 trials and at least 10 reversal pairs, and lasted for up to 12 min (the first session after the fatiguing noise stopped included the 3 test periods: 1-4, 4-8 and 8-12 min). Sessions consisted of 2/3 signal-present and 1/3 signal-absent trials offered in quasi-random order. There were never more than three consecutive signal-present or signal-absent trials.

Table I. Results of the pilot study. TTS_{1-4} in harbor porpoise 02, at 4 and 8 kHz after exposure for 60, 120 and 180 min to the pile driving playback sounds. Mean pulse duration (t_{90}) 124 ms, rate 2760 strikes/hr, inter-pulse interval 1.3 s, average received (n = 231 measurements in the pool) single-strike unweighted sound exposure level (SEL_{ss}) of 145 dB re 1µPa^{2}s.

Exposure duration (min)	4 kHz		8 kHz	
	Mean TTS_{1-4} ± SD (dB)	Sample size (n)	Mean TTS_{1-4} ± SD (dB)	Sample size (n)
60	1.6 ± 2.2	10	3.6 ± 1.0	7
120	0.6	1	4.6 ± 1.5	4
180	2.7 ± 0.2	2	5.4 ± 1.3	6

D. Data analysis

The pre-exposure mean 50% hearing threshold for a test sweep ($PE_{50\%}$) was determined by calculating the mean SPL of all reversal pairs in the pre-exposure hearing session. For the sound exposure and control conditions, TTS 1-4 min after sound exposure or ambient exposure stopped (TTS_{1-4}) was calculated for each hearing test frequency by subtracting the pre-exposure mean 50% hearing threshold ($PE_{50\%}$) from the mean 50% hearing threshold obtained during PNE_{1-4}. The same procedure was used for TTS_{4-8}, TTS_{8-12}, and TTS_{48}. A similar procedure was used for harbor porpoise 04, but at different times post-exposure.

In statistical analysis, each animal was considered independently because TTS had been measured at different times for each animal. For porpoise 02, the TTS_{1-4} after exposure to sound sequences of different durations was compared to that after the relevant control by means of t-tests. For porpoise 04, the same analysis was conducted on TTS_{12-16}. Via these analyses it was established at which sound exposure durations the initial TTS was significantly different from the control values. 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

A. Swimming pattern

The swimming pattern of porpoise 02 during exposure to the same pile driving sound playbacks at the same SEL in the same pool was described by Kastelein *et al.* (2015a). They observed that, compared to the control periods, the porpoise increased its respiration rate and moved on average 1 m further away from the transducer in response to the playback sound. However, the porpoise still used most of the pool during the test sessions, and did not specifically avoid the location of the transducer. Therefore, the average SEL ± SD of all 231 measurement locations in the pool was used as an approximation of the average received SEL experienced by the porpoises in the present study.

B. Most affected hearing frequency

The pilot study (in which hearing was tested at 4 and 8 kHz, based on Kastelein *et al.*, 2015a) showed that the highest initial TTS was at 8 kHz (**Table I**). Therefore in the main experiment, the porpoises' hearing was only tested at 8 kHz.

Effect of exposure duration on magnitude of TTS

Harbor porpoise 02

Harbor porpoise's 02 pre-stimulus response rates were typical for him (**Table II**). His pre-stimulus response rates during test and control sessions were similar.

The control sessions with low ambient noise exposure showed that, as expected, TTS did not occur after exposure to the low ambient noise conditions; the mean TTS_{1-4} for 8 kHz was close to zero (0.035 ± 1.16 dB, n= 59) after all seven ambient noise exposure durations (**Fig. 1**).

Mean TTS_{1-4} in porpoise 02 at the 8 kHz hearing frequency for all seven exposure durations increased from around 1 dB after 15 min exposure to around 5 dB after 180 min exposure. It remained at around 5 dB after longer exposures (**Fig. 1**). T-tests showed that TTS_{1-4} after pile driving sound playback exposure was significantly different from the TTS_{1-4} measured after the control sessions for all exposure durations with the exception of 15 minutes (**Table III**). The hearing of porpoise 02 recovered within 48 min post-exposure (**Fig. 2**). The slope of the recovery rate, calculated as the difference between TTS_{1-4} and TTS_{48}, was ≤ 0.1 dB/min.

Table II. The mean pre-stimulus response rate in hearing tests during the pre-exposure period, after exposure to pile driving sounds, and after exposure to ambient noise (control). All exposure durations were pooled for the calculation of percentages.

	Pre-stimulus response rate				
Porpoise 02					
Piling noise	Pre-exposure	PNE_{1-4}	PNE_{4-8}	PNE_{8-12}	PNE_{48}
	2.0 %	0.9 %	1.0 %	0.7 %	1.6 %
Control	Pre-exposure	PAE_{1-4}	PAE_{4-8}	PAE_{8-12}	PAE_{48}
	2.1 %	1.3 %	2.7 %	1.1 %	1.6 %
Porpoise 04					
Piling noise	Pre-exposure	PNE_{12-16}	PNE_{16-20}	PNE_{20-24}	PNE_{60}
	7.8 %	2.5 %	3.3 %	6.1 %	7.9 %
Control	Pre-exposure	PAE_{12-16}	PAE_{16-20}	PAE_{20-24}	PAE_{60}
	8.0 %	5.3 %	4.9 %	5.6 %	5.8 %

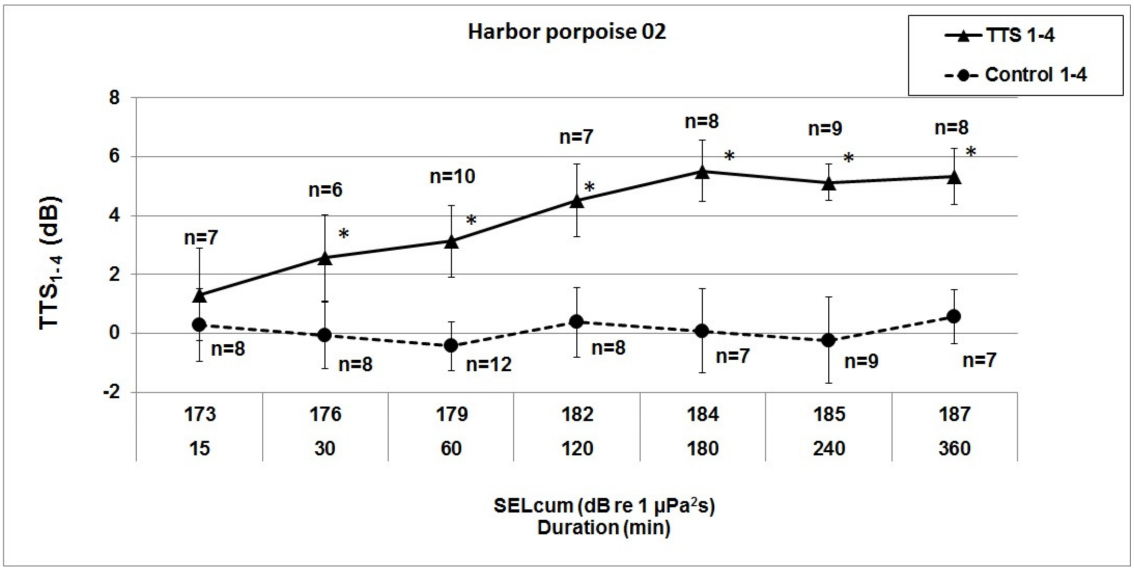

FIG. 1. Mean TTS_{1-4} in harbor porpoise 02 at 8 kHz, after exposure to playback of a series of pile driving sounds for 15, 30, 60, 120, 180, 240, and 360 min at a mean received SEL_{ss} of 145 dB re 1 $\mu Pa^2 s$ (duty cycle ~9.5%; resulting SEL_{cum}: 173, 176, 179, 182, 184, 185, and 187 dB re 1$\mu Pa^2 s$, respectively; exposure conditions), and after similar duration exposures to the low ambient noise in the pool (control conditions). n = number of sessions on which the mean was based. * indicates a significant difference between the exposure condition and the control condition (t-test; **Table III**). The error bars indicate standard deviations.

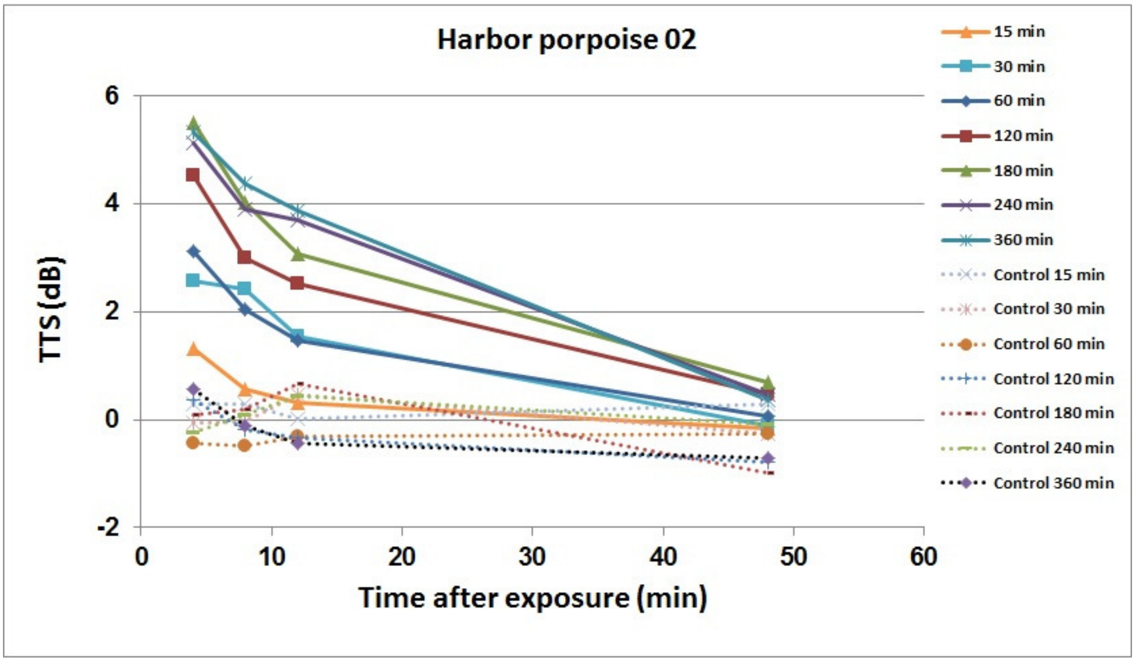

FIG. 2. The recovery of harbor porpoise 02's hearing, shown as TTS_{1-4} at 8 kHz, after exposure to series of played back pile driving sounds (solid lines) for 15, 30 60, 120, 180, 240 and 360 min at a mean received SEL_{ss} of at 145 dB re 1$\mu Pa^2 s$ (duty cycle ~9.5%; SEL_{cum}: 173, 176, 179, 182, 184, 185, and 187 dB re 1$\mu Pa^2 s$, respectively), and after similar duration exposures to the low ambient noise in the pool (control conditions, dotted lines). TTS was quantified 1-4, 4-8, 8-12, and 48 min after exposure to pile driving sounds stopped. Error bars are not shown for clarity. For sample sizes, see Figure 1.

Table III. Results of t-tests to compare TTS_{1-4} (porpoise 02) and TTS_{12-16} (porpoise 04) after exposure to sound sequences for different durations with the TTS_{1-4} and TTS_{12-16} after exposure to low ambient noise for the same durations. DF = degrees of freedom. The values in bold indicate significant differences between the mean TTS during the control and exposure conditions ($\alpha = 0.05$).

Porpoise	TTS (min after sound stopped)	Exposure duration (min)	T-value	*P*-value	DF
02	1-4	15	-1.39	0.192	11
02	1-4	30	-3.64	**0.005**	9
02	1-4	60	-7.87	**0.000**	15
02	1-4	120	-6.60	**0.000**	12
02	1-4	180	-8.35	**0.000**	10
02	1-4	240	-10.14	**0.000**	10
02	1-4	360	-9.93	**0.000**	12
04	12-16	15	-1.83	0.100	9
04	12-16	30	-2.69	**0.031**	7
04	12-16	60	-2.45	**0.044**	7
04	12-16	120	-4.65	**0.001**	12
04	12-16	180	-4.11	**0.002**	11
04	12-16	240	-3.85	**0.003**	10
04	12-16	360	-7.47	**0.000**	12

Harbor porpoise 04

Most exposure durations were tested in 7 or more sessions, but only 5 (15 min) and 3 (60 min) sessions were successfully conducted with porpoise 04, as in several cases, four reversals could not be obtained in the first 12-16 min after exposure of this animal to sounds. This was because of the time it took for animal 04 to swim from the outdoor exposure pool to the indoor hearing test pool..

Harbor porpoise's 04 pre-stimulus response rates are shown in **Table II**. These are typical pre-stimulus response rates for this animal: his rates are higher than those of porpoise 02 (probably because he had 7 years less experience with hearing tests). His pre-stimulus response rates during test and control sessions were similar.

The control sessions with low ambient noise exposure showed that, as expected, TTS did not occur after exposure to the low ambient noise conditions: the mean TTS_{12-16} for 8 kHz was close to zero (0.098 ± 1.17 dB; n = 51) after all 7 ambient noise exposure durations (**Fig. 3**).

Porpoise 04 was always tested after porpoise 02, so his hearing was tested after a longer period after the fatiguing noise had stopped. Mean TTS_{12-16} in porpoise 04 for all exposure durations increased from around 1 dB after 15 min exposure, to 3 dB after 120-240 min and to 5 dB after 360 min. T-tests showed that TTS_{12-16} after pile driving sound playback exposure was significantly different from the TTS_{12-16} measured after the control sessions for all exposure durations with the exception of 15 minutes (**Table III**). The hearing of porpoise 04 recovered well within 60 min post-exposure (**Fig. 4**). The recovery rate, calculated as the difference between TTS_{12-16} and TTS_{60}, was ≤ 0.1 dB/min.

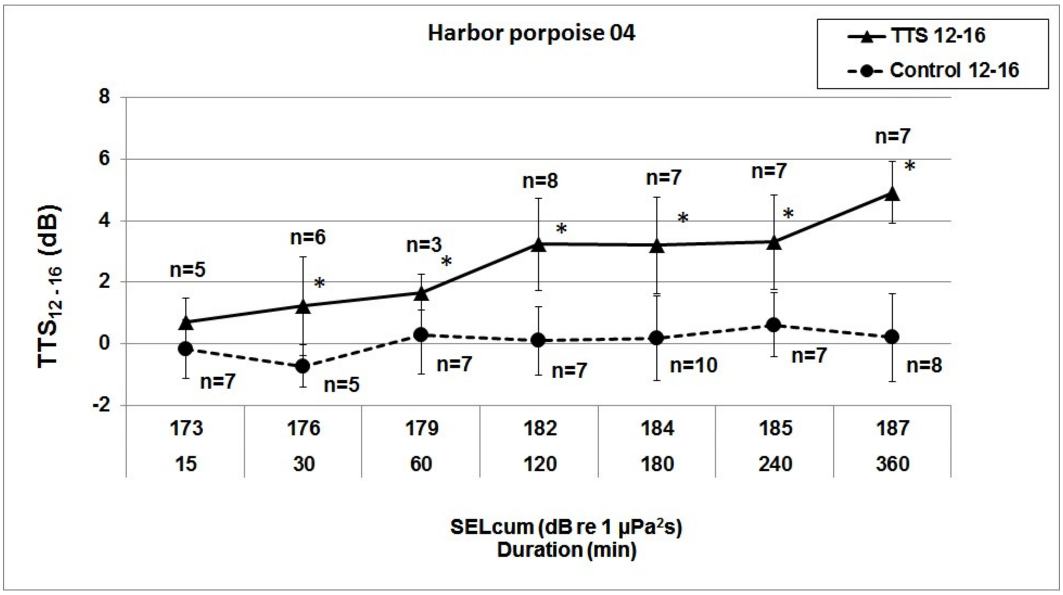

FIG. 3. Mean $TTS_{12\text{-}16}$ in harbor porpoise 04 at 8 kHz after exposure to playback of a series of pile driving sounds for 15, 30, 60 120, 180, 240, and 360 min at a mean received SEL of 145 dB re 1 μPa^2s (duty cycle ~9.5%; resulting SEL_{cum}: 173, 176, 179, 182, 184, 185, and 187 dB re 1μPa^2s, respectively; exposure conditions), and after similar duration exposures to the low ambient noise in the pool (control conditions). n = number of sessions on which the mean was based. * indicates a significant difference between the exposure condition and the control condition (t-test; **Table III**). The error bars indicate standard deviations.

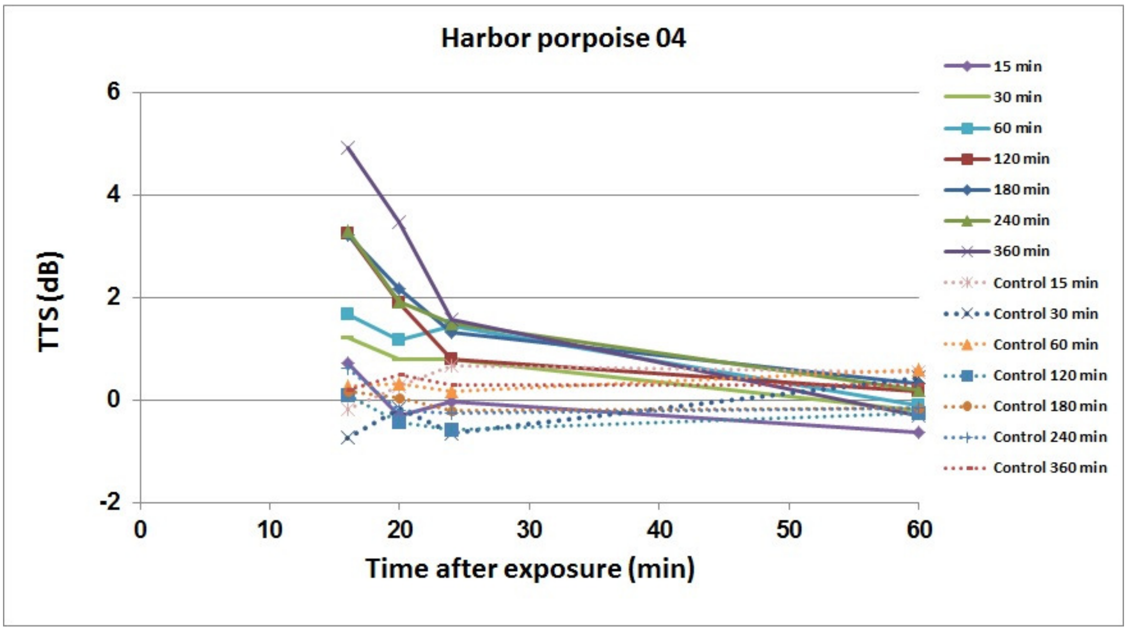

FIG. 4. The recovery of harbor porpoise 04's hearing, shown as $TTS_{12\text{-}16}$ at 8 kHz, after exposure to series of played back pile driving sounds (solid lines) for 15, 30, 60, 120, 180, 240 and 360 min at a mean received SEL of at 145 dB re 1 μPa^2s (duty cycle ~9.5%; SEL_{cum}: 173, 176, 179, 182, 184, 185, and 187 dB re 1μPa^2s, respectively), and after similar duration exposures to the low ambient noise in the pool (control conditions, dotted lines). TTS was quantified 12-16, 16-20, 20-24, and 60 min after exposure to pile driving sounds stopped. Error bars are not shown for clarity. For sample sizes see **Figure 3**.

IV. DISCUSSION AND CONCLUSIONS

A. Evaluation

The present study was conducted with two animals that had normal hearing for young male harbor porpoises (Kastelein *et al*., 2002, 2009, 2010). Therefore, the TTSs found for the specific fatiguing noise and test frequencies used in the present study are probably representative for young porpoises with good hearing. The pre-exposure hearing thresholds found in the present study (ID no. 02: 63 ± 1.6 dB at 4 kHz and 59 ± 1.0 dB at 8 kHz; ID no. 04: 61 ± 2.4 dB at 4 kHz and 55 ± 1.3 dB at 8 kHz) were similar to the hearing thresholds measured in harbor porpoise 02 for tonal signals during two previous studies (Kastelein *et al*., 2010; 2014b).

The porpoises showed behavior indicative of mild stress during the exposure periods (they increased their respiration rate and swimming speed, and jumped out of the water more). They exhibited a similar response to the highest SPL (which was the same as used as in the present study) in a behavioral response study with the same pile driving sounds; Kastelein *et al*., 2013c). However, even after 6 h of sound exposure, both porpoises immediately co-operated in the post-exposure hearing test. This suggests that the piling sounds were perceived more as annoying than as frightening.

A sound's level, spectral content, and rise time, as well as the pulse duration, affect the threshold shift it causes, and determine whether the shift is permanent or temporary (Melnick, 1991; Yost, 2007). The location of an animal relative to the source greatly affects the sound it is exposed to, as propagation and reverberations alter the characteristics of sounds, such as their spectrum, rise time and pulse duration, as well as their level. The characteristics of the pile driving playback sounds in the pool in the present study deviate from the original recording at 800 m from the piling site, but may resemble the sound at a greater distance. In modern practice, deterring devices are used in an attempt to move harbor porpoises at least 1 km away from piling sites before piling begins, in order to prevent PTS due to the first strike. Thus, it seems appropriate that the spectrum and SEL of the pile driving sounds used in the present study resembles those beyond 1 km from the piling site (depending on the conditions).

B. Cumulative effect of multiple pile driving strike sounds

The rate of increase in TTS (dB TTS/min exposure duration) was very small when sound exposure duration increased from 15 to 360 min: $TTS_{1\text{-}4}$ only increased by approximately 5 dB. Both animals showed a similar susceptibility to TTS. The same phenomenon was observed in another TTS study in which harbor porpoise 02 was exposed to intermittent fatiguing noise (Kastelein *et al*., 2014a). Exposure of this harbor porpoise to 1-second 1-2 kHz sweeps at a 10% duty cycle (an inter-pulse interval of 9 s) showed that, though there was a strong increase in TTS with increasing exposure up to around 15 min, TTS seemed to reach an asymptote for longer exposure durations. Kastelein *et al.* (2015b) reported that for 1-second 6-7 kHz sweeps, TTS was stronger after exposure to a 100% duty cycle sequence than after exposure to a 10% duty cycle sequence (with less energy per time unit).

C. Recovery of hearing

In the present study, after exposure to pile driving sound sequences with a SEL_{ss} of 145 dB re 1μPa^{2}s for 60-360 min, the $TTS_{1\text{-}4}$ in harbor porpoise 02 was around 5 dB, and $TTS_{12\text{-}16}$ in porpoise 04 was around 4 dB (both at 8 kHz). The hearing threshold had returned to the pre-exposure level within 48 min (porpoise 02) and 60 min (porpoise 04) after sound exposure stopped (probably earlier). Similar TTS_{1-4}, caused in harbor porpoise 02 after various exposures to a one-octave noise band centered around 4 kHz at mean received SPLs of 124, 136 and 148 dB re 1µPa, resulted in similar recovery rates (i.e. after TTS of around 4 dB, hearing had recovered within 48 min; Kastelein *et al.*, 2012b).

After initial TTSs of up to ~10 dB caused by a 1.5 kHz tone (Kastelein *et al.*, 2013b), 1-2 kHz sweeps (Kastelein *et al.*, 2014a), a 6.5 kHz tone (Kastelein *et al.*, 2014b), and 6-7 kHz sweeps (Kastelein *et al.*, 2015b) hearing in porpoise 02 usually recovered within 1 hour. Although little TTS was induced in the present study, the results suggest that in porpoise 02, similar TTSs, caused by different fatiguing noises (noise bands, tones, sweeps, and impulsive sounds) with different exposure levels and exposure times, required a similar recovery time.

D. Ecological significance

Significant TTS, as reported in the present study, means that TTS occurred in the study animals: after exposure to fatiguing noise, their hearing became temporarily less sensitive than it was before exposure or after exposure to low ambient noise. However, in this study (due to relatively low exposure levels) the TTS was not severe, and such small TTS could only be measured because the pools at the SEAMARCO Research Institute are very quiet. Statistically significant TTS is not necessarily ecologically significant. However, during pile driving activities at sea, the exposure levels in the vicinity of the piling site may be much higher than those used in the present study, and consequently the induced TTS may be much greater. 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 (which may include the duration of the exposure in addition to the recovery period), and the affected hearing frequency. Reduced hearing may reduce the efficiency with which harbor porpoises can carry out ecologically important activities such as navigation, communication, foraging, and predator avoidance, thus potentially reducing their fitness, reproductive output and longevity. Pile driving sound is not similar in frequency to the echolocation signals of harbor porpoises (which peaks at around 125 kHz; Møhl and Andersen, 1973; Verboom and Kastelein, 2003), so unless porpoises are very close to a piling site, the piling sounds are unlikely to interfere with echolocation during prey detection (Vater and Kössl, 2011). If porpoises are exposed to piling sounds with very high SPLs, hearing frequencies higher than 8 kHz may be affected. Kastelein *et al.* (2014b) showed that the affected hearing frequency is dependent on the received SPL: as SPL increases, the affected hearing frequency becomes higher. However, it is at present not clear up to which frequency such a shift can occur relative to the frequency spectrum of the fatiguing noise.

Harbor porpoises are likely to flee from a piling area (Tougaard *et al.*, 2009; Brandt *et al.*, 2011; Dähne *et al.*, 2013) until they experience a SEL_{ss} of around 140 dB re $1µPa^2s$ (Kastelein *et al.*, 2013c). That is about 5 dB below the SEL_{ss} used in the present study. Thus, at the behavioral threshold location (~ 30 km) and further away from the piling site, TTS is unlikely to occur (or continues to increase in case it was already elicited when the porpoise swam nearer to the piling site when piling started)..

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