Hearing frequencies of a harbor porpoise (*Phocoena phocoena*) temporarily affected by played back offshore pile driving sounds

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Hearing frequencies of a harbor porpoise (*Phocoena phocoena*) temporarily affected by played back offshore pile driving sounds

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Harbor porpoises may suffer hearing loss when exposed to intense sounds. After exposure to playbacks of broadband pile driving sounds (rate: 2760 strikes/hr, inter-pulse interval: 1.3 s) at one average received single strike (124 ms) unweighted sound exposure level (SEL) of 146 dB re 1µPa^{2}s for 60 min (cumulative SEL: 180 dB re 1µPa^{2}s), the temporary hearing threshold shift (TTS) of a porpoise was quantified at 0.5, 1, 2, 4, 8, 16, 32, 63 and 125 kHz with a psychoacoustic technique. Statistically significant TTS occurred at 4 and 8 kHz; mean TTS (1-4 min. after sound exposure stopped) was ~2.3 dB at 4 kHz, and ~3.6 dB at 8 kHz; recovery occurred within 48 min. Thus, exposure to multiple impulsive sounds can cause reduced hearing in a specific frequency band. Ecological effects of TTS depend not only on the magnitude of the TTS, its duration (which is related to the exposure duration), and the recovery time after the exposure stopped, but also on the hearing frequencies that are affected by the fatiguing noise. The hearing thresholds of harbor porpoises for the frequencies of their echolocation signals are not affected by pile driving sounds.

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

PACS numbers: 43.80.Lb; 43.80.EV; 43.80.Nd. Pages:......

I. INTRODUCTION

Offshore pile driving of large monopiles results in sequences of impulsive sounds produced at high source levels. Within a certain distance from the source, these sounds may cause hearing loss, which may be temporary (TTS; temporary threshold shift) or permanent (PTS; permanent threshold shift). A sound's level, spectral content, temporal pattern, and 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 alters the characteristics of emitted sounds.

For marine mammals, the course and speed of hearing recovery after exposure to loud sounds depend on the sound the animals were exposed to, the amount of shift incurred, and the species of animal (Finneran *et al.*, 2002; Kastelein *et al.*, 2013a). Generally, the greater the TTS, the longer the recovery period (Carder and Miller, 1972; Mills *et al.*, 1979).

The ecological effect of TTS depends not only on the magnitude of the TTS and its duration and recovery time, but also on the hearing frequency affected and the importance of that frequency for a species. Studies of TTS in marine mammals exposed to narrow band stimuli suggest that the hearing frequencies most affected by sound exposures are related to the spectral content of the stimuli (Nachtigall *et al*., 2004; Mooney *et al*., 2009; Finneran *et al*., 2005; Kastak *et al*., 1999, 2005; Kastelein *et al*., 2012a, 2012b, 2013b).

TTS due to impulsive exposures has been studied in a bottlenose dolphin (*Tursiops truncatus*) and a beluga (*Delphinapterus leucas*) (Finneran *et al*., 2002), in California sea lions (*Zalophus californianus*) (Finneran *et al*., 2003), and in harbor porpoises (*Phocoena phocoena*) (Lucke *et al*., 2009). Finneran *et al*. (2002) found that the beluga was more susceptible to a single underwater seismic impulsive sound than the bottlenose dolphin. At a sound exposure level (SEL) of 186 dB re 1 $\mu Pa^2 s$, a maximum of 7 dB TTS measured in the presence of masking noise (MTTS) was observed at 0.4 and 30 kHz in the beluga. No TTS was observed in the bottlenose dolphin at a maximum SEL of 188 dB re 1 $\mu Pa^2 s$. MTTS experiments with an arc-gap transducer resulted in no measurable TTS in California sea lions at single pulse SELs of 163 dB re 1 $\mu Pa^2 s$. Only Lucke *et al*. (2009) found high TTS due to impulsive sounds; after exposing a harbor porpoise to single seismic airgun sound (received level: ~165.8 dB dB re 1 $\mu Pa^2 s$) a 15 dB TTS was measured (with an auditory evoked potential method) at 4 kHz. In summary, only limited data on TTS due to impulsive sounds in marine mammals are available. This is partly because TTS after a single impulsive emission is difficult to measure, as very high received levels are needed to induce such a shift. Kastelein *et al*. (2013c) reported that the cumulative effect of TTS induced by intermittent exposures can be measured after a sequence of exposures, even if a single exposure does not lead to measurable TTS. Therefore, TTS might be induced after multiple successive impulsive exposures. Norro *et al*. (2013) reported that between 2114 and 3848 strikes are needed for a 5 m diameter monopile to be driven into the sediment in the Belgian part of the North Sea. If a monopile foundation is created with one sequence of pile driving strikes, it takes on average 120 min (Norro *et al*., 2013). It is assumed that a sequence of pile driving strikes has a cumulative effect on the hearing of marine mammals (i.e., TTS occurs after a certain exposure to multiple pile driving strikes for a certain time). Since the harbor porpoise, the most common marine mammal species in the North Sea, is relatively susceptible to TTS and PTS, pile driving activities might negatively affect the hearing of individuals of this species. Therefore, it is important to gain insight into the cumulative effect of pile driving on the hearing of the harbor porpoise. Marine mammals exposed to sufficiently intense underwater sounds may suffer hearing loss, resulting in temporary threshold shift (TTS) or permanent threshold shift (PTS). PTS studies are considered unethical in marine mammals, but information on variation in TTS onset and magnitude due to variations in exposure sound pressure level (SPL) and exposure time can be used to estimate the sound exposure levels that may lead to PTS. For an overview of recommended PTS criteria for marine mammals, see Southall *et al.* (2007).

The goal of the present study was to determine at which frequencies the hearing of a harbor porpoise was most affected by a sequence of broadband pile driving sound playbacks, and to gain insight into the process of recovery of hearing at the affected frequencies.

II. MATERIALS AND METHODS

A. Study animal and study area

The male harbor porpoise used in this study (ID no. 02) had participated in previous psychoacoustic studies (Kastelein *et al*., 2009; 2010; 2012b, 2013b, c). During the present study he was 7 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 animal received between 2 and 3 kg of thawed fish per day, divided over four to five meals. Variation in the animal's performance was minimized by making weekly adjustments (usually in the order of 100 g) to his daily food ration, based on his 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 animal was 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 he was 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, so that no current occurred. 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; 3^{rd} order Butterworth filter; 16 dB/octave) 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, 1994) 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 sound) was analyzed in terms of the $L_{z\text{-}p}$ (i.e., 20 times the base-10 logarithm of the maximum absolute value of the instantaneous sound pressure) and the unweighted sound exposure level (SEL) in dB re 1 μPa^2s (ANSI, 1986).

Great care was taken to make the harbor porpoise's 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 strikes/hr. 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.

The digitized original recording of series of pile driving sounds (WAV file) was played back 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 consistent to 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 SEL of 155 dB re 1 μPa^{2}s, and an $L_{z\text{-}p}$ of 180 dB re 1 μPa measured at 1 m depth, and 2 m from the source. The duration of the playback, defined as the time interval between the arrival of 5% and 95% of the total energy (t_{90}; Madsen, 2005), was 123 ms. Most of the energy was in the 500-800 Hz frequency band (**Fig. 1**).

To determine the fatiguing noise distribution in the pool, the SEL was measured at 77 locations (on a horizontal grid of 1 m x 1 m). The SEL was measured at three depths per location on the grid (0.5, 1.0 and 1.5 m below the surface). The level of the average received sound exposure ($SEL_{av.re.}$; dB re 1 μPa^{2}s) of the played back sound, as experienced by the harbor porpoise, was calculated from the average power sum of all individual measurement locations. There were only small differences in SEL per depth per location, and hardly any gradient in the SEL in relation to the location of the transducer, resulting in a fairly homogeneous field (**Fig. 2**). During exposure to the playback of pile driving sounds, the animal swam ovals throughout the entire pool, so his average received SEL was assumed to be close to the average SEL measured in the pool. During exposure sessions the $SEL_{av.re.}$ (± SD) of a single pulse was 146 (± 4) dB re 1μPa^{2}s, and the average t_{90} (± SD) was 124 (± 3.5) ms. Within each exposure session, the animal was exposed to 2760 playbacks of pile driving strikes with an inter-pulse interval of 1.3 s, resulting in a total exposure duration of 60 min [i.e., a cumulative sound exposure level (SEL_{cum}) of 180 dB re 1μPa^{2}s].

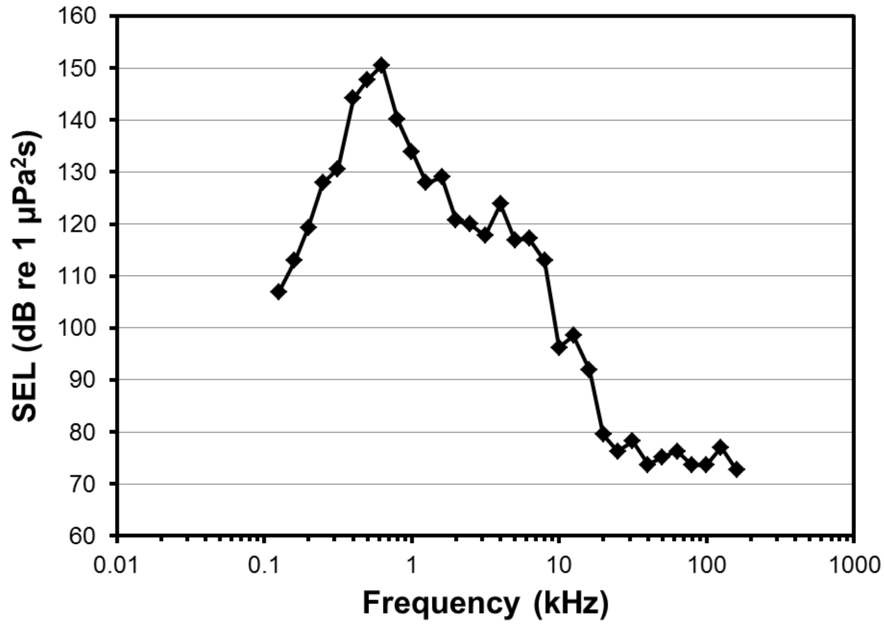

FIG. 1. The 1/3-octave band spectra of the SEL of a single played back pile driving sound in the pool. Measurements were conducted at 1 m depth, 2 m from the source. At the measurement location, the signal had a SEL of 155 dB re 1 μPa^{2}s, and an $L_{z\text{-}p}$ of 179 dB re 1 μPa. The average received sound exposure level was 146 dB re 1 μPa^{2}s, and the 1/3-octave band centered at 630 Hz contained the most energy.

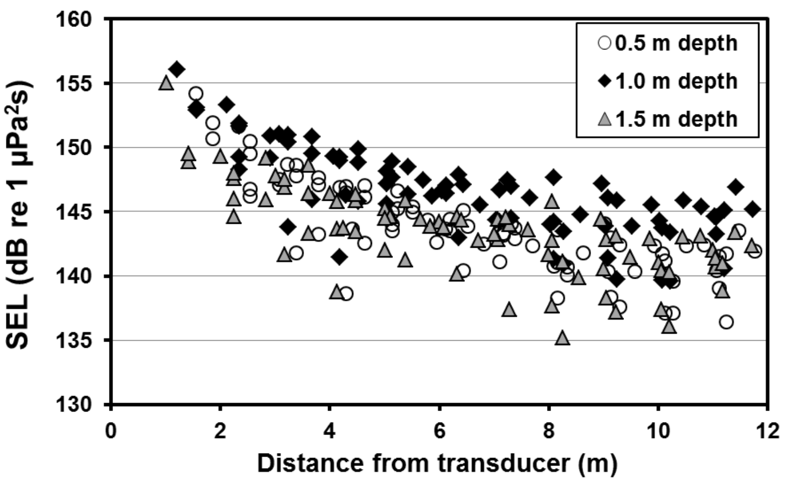

FIG. 2. The SEL distribution in the outdoor pool when the pile driving sound, as fatiguing noise, was being played back (231 measurement locations; 77 per depth). Per location, the SEL did not vary much per depth, and only a very small SEL gradient occurred in relation to the distance to the transducer. These data were used to calculate the level of the average received sound exposure (dB re 1 μPa^{2}s) that the harbor porpoise experienced during a single noise exposure: 146 ± 4 dB re 1μPa^{2}s (mean ± SD; n = 231).

Hearing test signals

Narrow band up-sweeps (linear frequency-modulated tones) were used as hearing test signals (which the animal was asked to detect before and after exposure to the fatiguing noises) instead of pure tones, because sweeps lead to very stable and precise thresholds. For TTS studies, precise hearing thresholds are very important, because harbor porpoises experiencing small threshold shifts tend to recover rapidly (Kastelein *et al*., 2012b). 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 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 and another buffer (AS - 2008-3), and drove the balanced tonpilz piezoelectric acoustic transducer (Lubell - LL916; 0.5- 8 kHz) through an isolation transformer (Lubell - AC202) and a transducer (International Transmission Company- 6084; 16-63 kHz or WAUQ7B; 125 kHz). 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 porpoise when it was 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 15 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 be consistent to 1 dB within a 20 dB range.

C. Experimental procedures

One total noise exposure test, consisting of: (1) pre-exposure hearing tests, (2) noise exposure, and (3) post-noise exposure hearing tests, was conducted each day. Tests started at 08.30 or 13.00 hrs. Pre-exposure hearing tests were performed with the animal in the indoor pool. Thereafter, the harbor porpoise 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 porpoise's behavior on a monitor in the outdoor research cabin, and the animal's surfacing locations and respiration rate were recorded on video. Five min before the fatiguing noise exposure ended, a trainer went to the gate in the channel leading to the indoor pool. In response to a signal from the operator, the trainer opened the gate and called the animal into the channel. When the animal entered the channel, the fatiguing noise ended immediately. The post-exposure hearing threshold session (using the same sweep used in the pre-exposure hearing session) was conducted in the indoor pool, commencing within 1 min after the fatiguing noise had stopped. Data were collected in April, May, and June 2013.

From an ecological viewpoint, not only the magnitude of TTS is important, but also its duration after the noise exposure stops. Therefore, not only the TTS immediately after exposure, but also the subsequent hearing recovery was recorded. The animal's hearing sensitivity was tested during up to four post-noise exposure (PNE) periods: 1-4 (PNE_{1-4}), 4-8 (PNE_{4-8}), 8-12 (PNE_{8-12}), 48 (PNE_{48}) min after noise exposure ended. These times were chosen because hearing in this study was usually (depending on the initial TTS) expected to recover after around 30 min. The 48 minute 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.

TTS was quantified for nine hearing test sweeps (centered at 0.5, 1, 2, 4, 8, 16, 32, 63 and 125 kHz), tested in random order. Each hearing frequency was tested in two sessions. Those in which a threshold shift was observed, and the ecologically important frequency 125 kHz [within the narrow (±10 kHz) frequency band of the harbor porpoise's echolocation signal; Møhl and Andersen, 1973; Kamminga and Wiersma, 1981; Verboom and Kastelein, 2003], were tested during five more sessions each, resulting in a total of seven sessions per frequency.

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 noise exposure tests, but without the fatiguing noise exposure. Each control test started with a pre-exposure hearing test session (test signals centered at 2, 4, 8, 16 or 125 kHz), but was followed by exposure to the normal (very low) ambient noise in the pool for 60 min (for the ambient noise spectrum in the pool during hearing tests, see Kastelein *et al*., 2012b). Post-ambient exposure (PAE) hearing test sessions were then performed 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. The PAE_{48} period had the same duration as a pre-exposure hearing test session. In total, seven control tests were conducted for each of the five hearing frequencies and they were randomly dispersed among the fatiguing noise exposure tests, also starting at around 08.30 or 13.00 hr.

Each hearing test trial began with the 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.

D. Observations and analysis of swimming pattern

To determine the SPL received by the harbor porpoise during tests, the area where he swam during the exposure periods was compared to the SPL distribution in the pool. To quantify the harbor porpoise's swimming pattern, videos of the noise exposure sessions and low ambient noise exposure in the pool sessions were analyzed. Each time the harbor porpoise surfaced, his location was allocated to one of 77 grid squares, each of which corresponded to a 1 m x 1 m square in the outdoor pool. In addition, his respiration rate was recorded to determine the effect of the fatiguing noise exposure on his level of exertion and/or anxiousness.

E. Data analysis

The pre-exposure mean 50% hearing threshold for a test sweep ($PE_{50\%}$) was determined by calculating per frequency the mean SPL of all reversal pairs in the pre-exposure hearing session. For the 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 mean 50% hearing threshold obtained during the $PE_{50\%}$ from the mean 50% hearing thresholds during PNE_{1-4}. The same procedure was used for TTS_{4-8}, TTS_{8-12}, and TTS_{48}.

All analysis was carried out on SPSS 20.0 for Windows with a significance level of 5%, and data conformed to the assumptions of the tests used (Zar, 1999). Independent *t*-tests (one tailed) with Bonferroni correction (corrected significance level is 1%) were used to compare TTS_{1-4} measured after the exposure and control conditions, for the frequencies 2, 4, 8, 16, and 125 kHz (i.e., n = 7 for all conditions). For each condition, one-way analysis of variance (ANOVA) (followed by Tukey HSD tests) was used to compare the TTS_{1-4} at the five hearing frequencies.

III. RESULTS

A. Swimming pattern

During the low ambient noise exposures (15 control sessions), the harbor porpoise swam on average 6.7 m (SD: ± 1.0 m) away from the transducer, surfaced 256 (SD: ± 31) times per hour and jumped only in one control session (6 times). During the 45 exposures to playbacks of pile driving sounds (all levels combined), the harbor porpoise slightly increased his mean distance to the transducer, to 7.7 m (SD: ± 0.9 m), increased his surfacing rate (i.e., respiration rate) to 273/h (SD: ± 22), and jumped on average 1.9 times/session (SD: ± 2.0). Thus, exposure to fatiguing noise had an effect on the harbor porpoise's level of exertion and anxiousness. The harbor porpoise still used most of the pool during the test sessions, and did not specifically avoid the location of the transducer. Taking into account the porpoise's movement away from the transducer, the received level (SPL) would differ from the $SPL_{av.re.}$

by at most 1.3 dB if measurements made closer than 2 m to the transducer were not used in the calculation. So the $SPL_{av.re.}$ ± SD of all 231 measurement locations in the pool was used as an approximation of the received level experienced by the porpoise.

B. Most affected hearing frequency and recovery

The harbor porpoise's pre-stimulus response rate, calculated over all hearing test frequencies, was 3.3 % for the pre-exposure tests; 2.8, 1.0, 0.8, and 1.2% respectively for PNE_{1-4}, PNE_{4-8}, PNE_{8-12}, and PNE_{48}; and 3.0, 5.0, 2.7 and 2.7% respectively for PAE_{1-4}, PAE_{4-8}, PAE_{8-12}, and PAE_{48}. These are typical pre-stimulus response rates for this animal.

The control tests with 60 min low ambient noise exposure showed that TTS did not occur: the mean TTS_{1-4} for 2, 4, 8, 16 and 125 kHz was close to zero (**Fig. 3**).

After the study animal had been exposed for 60 min to the playback series of pile driving sounds at an average received SEL of 146 dB re 1 $\mu Pa^2 s$ (SEL_{cum}: 180 dB re 1$\mu Pa^2 s$), hearing tests with the nine hearing frequencies (centered at 0.5, 1, 2, 4, 8, 16, 32, 63 and 125 kHz), during two sessions each, indicated that TTS was induced only at 4 and 8 kHz. The pre-exposure hearing thresholds for the nine test center frequencies are shown in **Fig. 4**.

To gain more insight into the TTS induced, the two frequencies of the pre-test, the neighboring octave frequencies and the ecologically important frequency (i.e., 2, 4, 8, 16, and 125 kHz) were tested multiple times (7 exposure and 7 control tests). Comparisons between the exposure conditions and control conditions showed that significant TTS_{1-4} occurred at 4 and 8 kHz. Mean TTS_{1-4} at 4 kHz was 2.3 dB [$T_{one\text{-}tailed}(12) = 2.86$, $p < 0.01$], at 8 kHz the mean TTS_{1-4} was 3.6 dB [$T_{one\text{-}tailed}(12) = 7.642$, $p < 0.001$]. No significant TTS occurred at 2, 16 and 125 kHz. One-way ANOVA showed no effect of frequency on TTS_{1-4} for the control condition. A significant effect of frequency was found for the exposure condition (i.e. frequency affected by the pile driving playback; $F(4, 30) = 8.662$, $p < 0.001$). Post hoc analysis showed that there was no significant difference between the TTS induced at 4 and 8 kHz (Tukey HSD, $p = 0.565$), and that the TTS_{1-4} measured at 8 kHz was significantly different from TTS_{1-4} measured at 2 (Tukey HSD, $p < 0.001$), 16 (Tukey HSD, $p = 0.022$) and 125 kHz (Tukey HSD, $p = 0.004$).

TTS induced at 4 and 8 kHz recovered within 48 min post exposure (**Fig. 5**). The recovery rate, calculated as the difference between TTS_{1-4} and TTS_{8-12}, was 0.17 dB/min for the 4 kHz, and 0.26 dB/min for the 8 kHz hearing frequency.

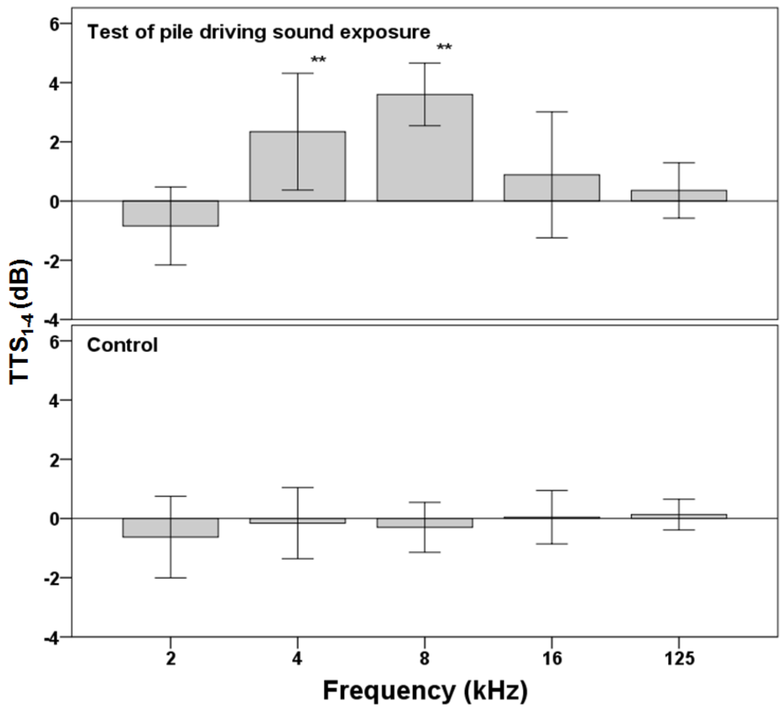

FIG. 3. Mean TTS_{1-4} in the harbor porpoise, (a) 1-4 min after exposure to playback of a series of pile driving sounds for 60 min at a mean received SEL of 146 dB re 1 µPa^2s (SEL_{cum}: 180 dB re 1µPa^2s) (exposure condition), and (b) after exposure to the low ambient noise for 60 min (control condition). n = 7 for each frequency tested per condition. ** indicates a significant difference between the exposure condition and the control condition (one-tailed *t*-test; P < 0.01).

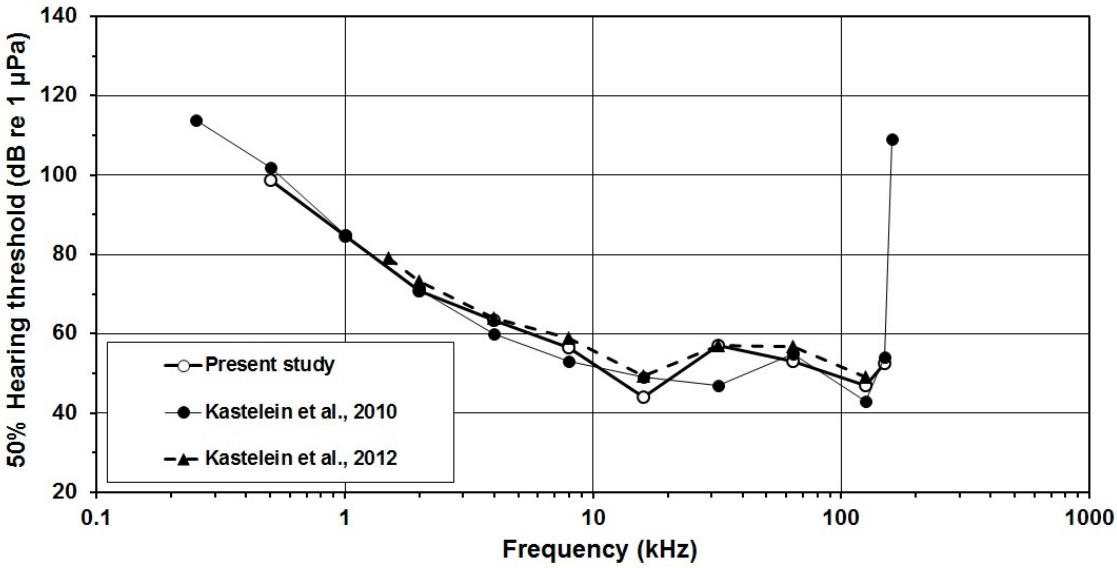

FIG. 4. Hearing thresholds of the harbor porpoise in the present study for the nine 1-s (including 50 ms rise and fall times) test signals (narrow-band up-sweeps), compared to the hearing thresholds of the same animal for the same stimuli one year prior to testing (Kastelein *et al*., 2012), and his hearing thresholds for 900 ms tonal signals (Kastelein *et al*., 2010).

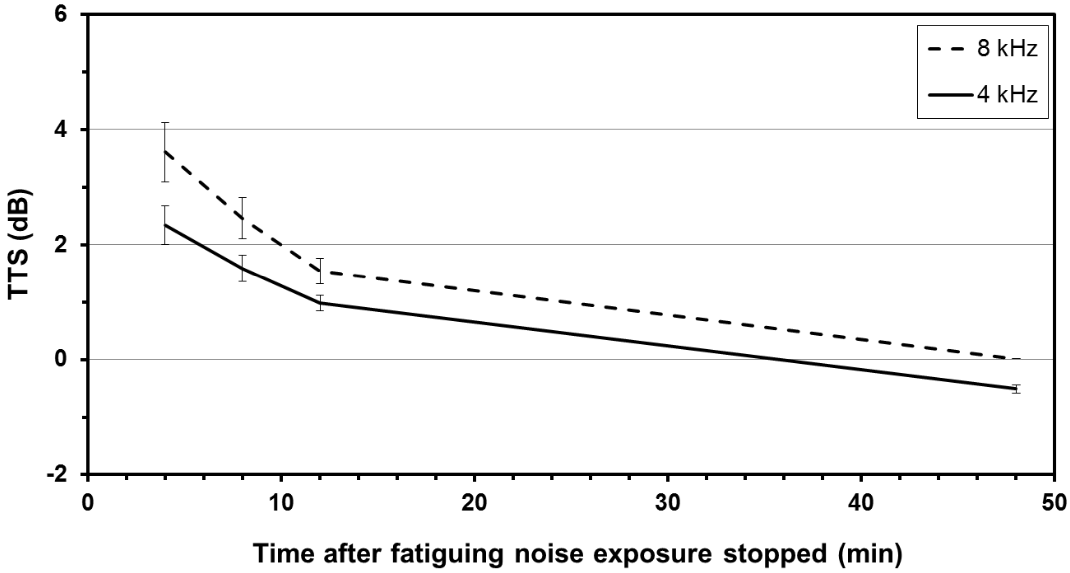

FIG. 5. The recovery of the harbor porpoise's hearing at the two frequencies (4 kHz; dashed line, and 8 kHz; solid line) where significant TTS occurred after exposure to series of played back pile driving sounds for 60 min at a mean received SEL of 146 dB re 1 µPa^{2}s (SEL_{cum}: 180 dB re 1µPa^{2}s). Recovery was measured 1-4, 4-8, 8-12, and 48 min after exposure to pile driving sounds stopped. Error bars represent the standard error (n = 7). The standard error at TTS_{48} was only 0.002 dB.

IV. DISCUSSION AND CONCLUSIONS

A. Evaluation

The present study was conducted with one animal that had normal hearing for a young male harbor porpoise (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 were similar to the hearing thresholds measured in this harbor porpoise for tonal signals during two previous studies (Kastelein *et al*., 2010; 2013c; **Fig. 4**).

Significant TTS as reported in the present study means that TTS occurred in the study animal (relative to the control sessions). Such a small TTS could only be measured because the pools at SEAMARCO are extremely quiet. Therefore this significant TTS is not the same as ecological significant TTS. It is not clear what the specific ecological effect of TTS is. The ecological effect certainly depend on the magnitude of the TTS, duration of the exposure, the duration of the recovery, and the affected hearing frequency. Reduced hearing may reduce the efficiency of ecologically important activities such as navigation, communication, foraging, predator avoidance, thus potentially reducing an animals' fitness, reproductive output and longevity.

B. Hearing frequency most affected

Comparing the test frequencies evaluated for the present study showed that when the harbor porpoise was exposed to the playback series of pile driving sound for 60 min and a SEL_{ss} of 146 dB re 1µPa^{2}s (SEL_{cum}: 180 dB re 1µPa^{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, including at the frequency of most energy in echolocation (125 kHz). The playback of the pile driving sounds had the most energy in the 500-800 Hz frequency band, but no TTS occurred at 500 Hz. TTS occurred only at the frequencies at which the porpoise's hearing is most sensitive (over the frequency range of the pile driving spectrum). Finneran and Schlundt (2013) found that in bottlenose dolphins, TTS onset for tonal signals is in agreement with the equal-loudness contours of that species. Equal-loudness contours have been established for the harbor porpoise based on equal-latency contours (Wensveen *et al*., 2014). Unlike for the bottlenose dolphin, no data are available on TTS sensitivity over the whole frequency range of harbor porpoise hearing. Hence, little is known about the relationship between the equal-loudness contours of the harbor porpoise and TTS sensitivity. However, in agreement with the equal-loudness contours of bottlenose dolphins, the hearing of the harbor porpoise is assumed to be less sensitive to low-frequency sound exposures (Wensveen *et al*., 2014). This may explain why, in the present study, statistically significant TTS was induced only at the relatively high frequencies of the pile drive spectrum (4 and 8 kHz), even though the SEL was 38 dB lower at 8 kHz than at 600 Hz (**Fig. 1**).

When exposed to very high SPLs, hearing frequencies higher that 8 kHz may be affected. Kastelein *et al*. (2014b) showed that the affected hearing frequency is dependent on the received SPL. When SPL increases, so does the affected hearing frequency. It is not clear how far such a frequency shift can occur relative to the spectrum of the fatiguing noise."

C. Cumulative effect of multiple exposures

Lucke *et al*. (2009) measured 15 dB TTS at 4 kHz after exposing a harbor porpoise to a single seismic air gun impulse with a SEL_{ss} of 166 dB re 1 µPa^2s. At a SEL_{ss} of 141 dB re 1 µPa^2s, they found no TTS. The present study and the study of Lucke *et al.* (2009) are the only published studies on TTS due to impulsive sounds in harbor porpoises. Kastelein *et al.* (2014a) showed that TTS can occur after multiple exposures even if a single exposure does not lead to measurable TTS. As it takes between 2000 and 4000 pile strikes to complete the placement of a monopile foundation (Norro *et al*., 2013), and given that pile driving strikes are audible to harbor porpoises at tens of km from pile driving sites (Kastelein *et al*., 2013b), it is almost inevitable that many harbor porpoises in the North Sea will be exposed to multiple pile driving sounds during the placement of one pile. Therefore, the effect of cumulative exposures has to be taken into account. The present study shows that when a harbor porpoise is exposed to a normal pile driving sequence with a SEL_{ss} of 146 dB re 1µPa^2s and a SEL_{cum} of 180 dB re 1µPa^2s (i.e., 2760 pile driving strikes in one hour), significant TTS occurs at the hearing frequencies 4 and 8 kHz.

D. Recovery of hearing

In the present study, after exposure to a SEL_{cum} of 180 dB re 1µPa^2s, the mean $TTS_{1\text{-}4}$ in the harbor porpoise was 2.3 dB at 4 kHz and 3.6 dB at 8 kHz. Based on the mean of seven sessions, the threshold had returned to the pre-exposure level 48 min after sound exposure stopped. Similar $TTS_{1\text{-}4}$, caused in the same harbor porpoise 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. hearing had recovered within 48 min; Kastelein *et al*., 2012b). Although little TTS was induced in the present study, the results suggest that in this animal, similar TTSs, caused by different fatiguing noises (one-octave noise band and impulsive sounds) with different levels and exposure times, required a similar recovery time.

E. Ecological significance

Temporarily reduced hearing in an animal may affect its foraging ability, interfere with its communication with conspecifics, reduce its ability to detect predators, and impede orientation by reducing its ability to detect, for example, the surf. The ecological significance of TTS in an animal depends on the magnitude of the TTS, the duration of the TTS (which depends on the duration of the fatiguing noise), the duration of the recovery period after the fatiguing noise stopped, and the ecological importance of the frequency affected by the TTS.

In the harbor porpoise, after small reductions in hearing sensitivity (threshold shifts <15 dB), recovery is relatively quick (often within ~ 60 min). As long as exposures are not cumulative or sequential, reduced hearing for such a short time period may have little effect on the ecology of a harbor porpoise. If hearing is impaired for periods of hours or days, the impact may be ecologically significant.

As well as the level of TTS, the frequency of hearing in which TTS occurs is important. The most ecologically important sound frequencies for harbor porpoises fall into a 10 kHz band around ~125 kHz: the dominant frequency band of the echolocation signals they use for foraging and navigation (Møhl and Andersen, 1973; Kamminga and Wiersma, 1981; Verboom and Kastelein, 2003). The present study shows that the harbor porpoise's hearing around 125 kHz was not influenced by the broadband playbacks of series of pile driving sounds. This was expected, as most of the energy of the pile driving sounds was between 500 and 800 Hz (**Fig. 1**). These sounds on the one hand, and echolocation signals (~125 kHz) on the other, are processed in different parts of the cochlea, due to the tonotopic organization of the basilar membrane (Vater and Kössl, 2011). Therefore, TTSs caused by pile driving sounds have little or no effect on the echolocation ability of the harbor porpoise. However, they may deter a porpoise from a wide area around the piling site (Tougaard *et al*., 2009; Brandt *et al*., 2011; Dähne *et al*., 2013; Kastelein *et al*., 2013c) and thus reduce foraging efficiency.

Nothing is known about the ecological importance of harbor porpoise hearing for frequencies in the 4-8 kHz range (where TTS occurs due to pile driving sounds), but hearing at these frequencies is likely to be used to detect larger odontocetes that may be harmful, for example killer whales (*Orcinus orca*) which may be predators, and bottlenose dolphins which may molest harbor porpoises (MacLeod *et al*., 2007).

PTS (permanent injury of the ear) has not been measured directly in marine mammals, because invasive studies are not considered ethically acceptable. Therefore, PTS onset levels are usually estimated indirectly, by adding 15-20 dB to the fatiguing noise levels which cause TTS onset (Southall *et al*., 2007). Information on TTS onset level, TTS growth due to exposure duration, received SPL and duty cycle, and recovery of hearing is needed by regulators to develop sound threshold criteria to prevent hearing damage. Therefore future research on effects of pile driving sounds should focus on TTS induced by longer exposure durations and by sounds with higher and lower received levels. Information on this is needed to gain better insight into TTS growth, and thus into the impact of offshore pile driving activities on harbor porpoise hearing.

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