*Aquatic Mammals* 2019, *45*(3), 280-292, DOI 10.1578/AM.45.3.2019.280

# Temporary Hearing Threshold Shift in Harbor Porpoises (*Phocoena phocoena*) Due to One-Sixth Octave Noise Band at 16 kHz

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## Abstract

Susceptibility to temporary threshold shift (TTS) depends on the frequency of the fatiguing sound. So far, TTS in harbor porpoises (*Phocoena phocoena*) has been tested for sounds in the 1 to 7 kHz range. To assess the impact of anthropogenic noise, TTS needs to be investigated for other frequencies within the porpoise hearing range. TTSs were quantified in two porpoises that were exposed for one hour to a continuous one-sixth octave noise band centered at 16 kHz, at average received sound pressure levels (SPLs) of 117 to 145 dB re 1 µPa, and a sound exposure level (SEL) range of 153 to 181 dB re 1 µPa$^2$s. Hearing thresholds for 16, 22.4, and 32 kHz signals were determined before and after exposure, to quantify TTS and recovery. The highest TTS, measured 1 to 4 minutes after exposure, occurred at 22.4 kHz. Statistically significant TTS occurred at 16 kHz after exposure to 159 dB SEL, at 22.4 kHz after exposure to 165 dB SEL, and at 32 kHz after exposure to 181 dB SEL. The susceptibility of the two porpoises to TTS induced by the exposures (16 kHz; 1 h) was similar. Below 6.5 kHz, it appears that susceptibility to TTS increases with increasing frequency; whereas above 6.5 kHz, it appears that susceptibility to TTS decreases with increasing frequency (for the frequency range tested so far).

**Key Words:** anthropogenic noise, audiogram, frequency weighting, hearing, hearing sensitivity, hearing damage, odontocete, TTS, temporary threshold shift

## Introduction

Anthropogenic sound sources that could affect the hearing of marine mammals and, thus, result in behavioral changes include offshore construction such as pile driving, marine exploration such as seismic surveys, underwater detonations, and sonar used during commercial and naval activities. The effects of anthropogenic sounds on harbor porpoises (*Phocoena phocoena*) are of particular interest because this odontocete species has a wide distribution area in the coastal waters of the northern hemisphere (Bjorge & Tolley, 2008), acute hearing (i.e., low hearing thresholds), and a very wide frequency range of hearing (Kastelein et al., 2017a). The harbor porpoise appears to be more susceptible to temporary threshold shift (TTS) than other odontocete species tested so far (Tougaard et al., 2016; Houser et al., 2017).

Anthropogenic sounds have various frequency spectra, and susceptibility to TTS depends on the fatiguing sound's frequency (as shown for the bottlenose dolphin [*Tursiops truncatus*] by Finneran & Schlundt, 2013), so it is important to quantify the effect of various fatiguing sound frequencies on the hearing of the harbor porpoise (National Marine Fisheries Service [NMFS], 2016; Houser et al., 2017). For regulation of underwater acoustic levels, complete equal-TTS contours are desirable, covering the entire frequency range of hearing in the harbor porpoise (0.5 to 140 kHz). Within a small frequency range (1 to 7 kHz), equal-TTS points for four frequencies have been established (Kastelein et al., 2012a, 2013, 2014a, 2014b, 2015a, 2015b, 2017b).

The goal of the present study is to increase the frequency range for which susceptibility to TTS in harbor porpoises is understood. TTS was determined in harbor porpoises after exposure to a one-sixth octave noise band centered at 16 kHz for 1 h, at several sound pressure levels (SPLs), and hearing recovery after sound exposure stopped was quantified. The ultimate goal is to generate equal-TTS susceptibility contours (see Houser et al., 2017). Once susceptibility to TTS has been quantified for the entire hearing range of the harbor porpoise, it will be possible to generate valid auditory weighting curves for cetaceans that echolocate at high frequencies.

## Methods

*Study Animals*

Two rehabilitated stranded harbor porpoises were used as study animals. The female, identified as harbor porpoise F05, was ~6.5 y old at the time of the study. Her body mass was ~42 kg, her body length was ~152 cm, and her girth at the axilla was ~84 cm. The male, identified as harbor porpoise M06, was ~3.5 y old during the study. His body mass was ~29 kg, his body length was ~130 cm, and his girth at the axilla was ~77 cm.

The hearing of the study animals in the frequency range tested in the present study (16 to 32 kHz) was representative of animals of the same age and species; the 50% hearing thresholds obtained from them were similar to those obtained from three other young male porpoises (Kastelein et al., 2017a).

The animals received 2 to 3 kg of thawed fish per day, divided over four or five meals (vitamins lost due to storage and thawing were replaced by adding a supplement). 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 body mass and performance during the previous week, and the expected change in water and air temperatures in the following week.

*Study Area*

The study was conducted at the SEAMARCO Research Institute, the Netherlands. Its location is remote and quiet, and it 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 they were exposed to fatiguing sound, which was 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 (Figure 1; for details of the pool and equipment, see Kastelein et al., 2012a). All pumps were switched off at 0800 h each day and left off during tests so that no current occurred. By the time a test started, no water flowed over the skimmers, so there was no flow noise during the hearing tests.

The equipment used to produce and monitor the stimuli for the hearing tests was housed out of sight of the study animals in an indoor research cabin ~4 m away from the underwater listening station. The equipment used to produce and monitor the fatiguing sound was placed in an outdoor research cabin to the southwest of the outdoor pool.

The operator was located in the outdoor research cabin during each sound exposure and

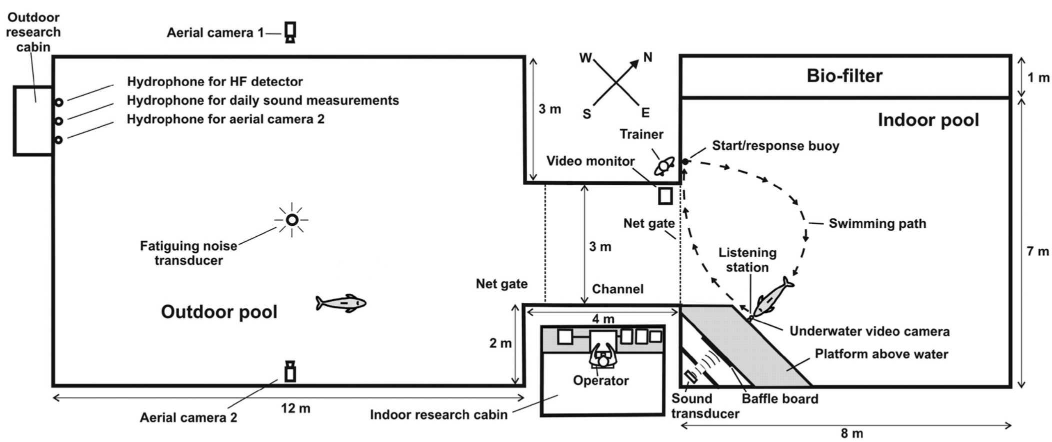


**Figure 1.** Top scale view of the outdoor pool, in which the two harbor porpoises (*Phocoena phocoena*) were exposed to fatiguing sound, and the indoor pool, in which the pre- and post-exposure hearing tests were conducted on one harbor porpoise at a time. Note the two aerial cameras, the underwater transducer emitting the fatiguing sound, and three hydrophones in the outdoor pool. The outdoor research cabin to the southwest of this pool housed the equipment producing the fatiguing sound. In the indoor pool, a harbor porpoise is shown in position for hearing tests at the listening station. The dashed line indicates the swimming path which the study animal followed during a hearing trial.

went immediately into the indoor research cabin after the fatiguing sound was switched off to start the hearing test.

*Acoustics*

*SPL Measurement Equipment*—Acoustical terminology follows *ISO 18405:2017* (2017). The background noise was measured, and the fatiguing sound and hearing test signals were calibrated every 2 mo during the study period by an acoustic measurement consultancy. The sound measurement equipment consisted of two hydrophones (Brüel & Kjaer [B&K]-8106) with a multichannel high-frequency analyzer (B&K PULSE-3560 C; sample frequency: 524288 Hz) and a laptop computer with B&K PULSE software (*Labshop*, Version 12.1). The system was calibrated with a pistonphone (B&K-4223). The SPL (in dB re 1 µPa) of each hearing test signal was derived by averaging over the 90% energy signal duration.

*Background Noise*—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 outdoor pool during sound exposure, and within 15 m of the indoor pool during hearing test sessions. They were required to stand still and make no noise. The background noise measurements took place under test conditions (water circulation system off, no rain, and Beaufort wind force 4 or below). The one-third octave band SPLs of the background noise were determined from 25 Hz to 160 kHz. Under test conditions, the background noise in the indoor pool was very low; the one-third octave level increased from 55 dB re 1 µPa at 200 Hz to 60 dB re 1 µPa at 5 kHz. The ambient noise levels in the test frequency range of the present study are similar to those reported by Kastelein et al. (2012a).

*Fatiguing Sound*—The digitized fatiguing sound in the form of a WAV file (sample rate: 768 kHz) was played by a laptop computer (Model No. 5750, Acer – Aspire) with a program written in *LabVIEW* to an external data acquisition card (Model No. USB6259; National Instruments, Austin, TX, USA; single-channel maximum sample rate: 1.25 MHz), the output of which could be controlled in 1 dB steps with the *LabVIEW* program. The output of the card went through a custom-made ground loop isolator and buffer to a custom-made passive low-pass filter (set at 20 kHz). After this, it went to a power amplifier (Model No. 2012-02, HLLY), which drove the toroidal beam transducer (Model No. 337; EDO Western, Salt Lake City, UT, USA). The transducer was placed in the middle of the outdoor pool at 1 m depth. The linearity of the transmitter system for fatiguing sound was checked during each calibration and was found to deviate at most by 1 dB within a 42 dB range.

Continuous (duty cycle 100%) one-sixth octave Gaussian white band noise centered at 16 kHz was used as the fatiguing sound (bandwidth: 15.1 to 17.0 kHz). Ideally, a 16 kHz tone would have been used, but in a pool this leads to a very inhomogeneous sound field in which some locations have very high SPLs, and others have very low SPLs. The study animals may have been able to select low SPL locations to minimize their sound exposure, and the SEL would have been overestimated. Therefore, instead of a tonal signal, a very narrow (one-sixth octave) noise band was selected. To determine the fatiguing sound's distribution in the outdoor pool, the SPL of the noise band was measured at 76 locations in the horizontal plane (on a horizontal grid of 1 m × 1 m), and at three depths per location on the grid (0.5, 1.0, and 1.5 m below the surface), resulting in 228 measurements in the pool. There were differences in mean SPL per depth (119 dB at 0.5 m, 124 dB at 1.0 m, and 125 dB at 1.5 m deep; Figures 2a, 2b & 2c).

To determine the average SPL received by the study animals, the area where they swam during the exposure periods was compared to the fatiguing sound's SPL distribution in the pool. To quantify the harbor porpoises' swimming patterns, videos of the sound exposure sessions were analyzed (see Kastelein et al., 2012a). Each time a

**0.5 m**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 7 | 119 | 116 | 120 | 118 | 122 | 122 | 122 | 118 | 120 | 116 | 119 | |
| 6 | 118 | 118 | 117 | 117 | 120 | 122 | 120 | 117 | 117 | 118 | 118 | |
| 5 | 118 | 116 | 116 | 117 | 119 | 120 | 119 | 117 | 116 | 116 | 118 | |
| 4 | 117 | 118 | 116 | 119 | 118 | | 118 | 118 | 116 | 118 | 117 | |
| 3 | 116 | 117 | 117 | 119 | 118 | 117 | 118 | 119 | 117 | 117 | 116 | |
| 2 | 119 | 120 | 118 | 117 | 119 | 120 | 119 | 117 | 118 | 120 | 119 | |
| 1 | 117 | 118 | 117 | 118 | 119 | 119 | 119 | 118 | 117 | 118 | 117 | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | |

a)

**1.0 m**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 7 | 119 | 118 | 118 | 121 | 121 | 120 | 121 | 121 | 118 | 118 | 119 | |
| 6 | 118 | 118 | 120 | 120 | 123 | 123 | 123 | 120 | 120 | 118 | 118 | |
| 5 | 119 | 121 | 123 | 125 | 123 | 126 | 123 | 125 | 123 | 121 | 119 | |
| 4 | 122 | 123 | 122 | 125 | 130 | T | 130 | 125 | 122 | 123 | 122 | |
| 3 | 121 | 122 | 123 | 127 | 130 | 133 | 130 | 127 | 123 | 122 | 121 | |
| 2 | 119 | 120 | 125 | 125 | 127 | 127 | 127 | 125 | 125 | 120 | 119 | |
| 1 | 119 | 121 | 124 | 125 | 126 | 125 | 126 | 125 | 124 | 121 | 119 | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | |

b)

**1.5 m**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 7 | 123 | 120 | 122 | 124 | 123 | 122 | 123 | 124 | 122 | 120 | 123 | |
| 6 | 121 | 123 | 124 | 125 | 127 | 125 | 127 | 125 | 124 | 123 | 121 | |
| 5 | 121 | 123 | 125 | 124 | 131 | 127 | 131 | 124 | 125 | 123 | 121 | |
| 4 | 119 | 121 | 123 | 128 | 129 | | 129 | 128 | 123 | 121 | 119 | |
| 3 | 122 | 123 | 123 | 128 | 127 | 122 | 127 | 128 | 123 | 123 | 122 | |
| 2 | 121 | 124 | 127 | 120 | 126 | 123 | 126 | 120 | 127 | 124 | 121 | |
| 1 | 123 | 122 | 125 | 122 | 124 | 123 | 124 | 122 | 125 | 122 | 123 | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | |

c)

**Figure 2.** The SPL distribution in the outdoor pool (Figure 1) of the continuous (100% duty cycle) one-sixth octave noise band centered at 16 kHz, measured at depths of (a) 0.5 m, (b) 1.0 m, and (c) 1.5 m. T = location of the transducer, which was at 1 m depth in the center of the pool. The numbers in the grey boxes indicate 1 m markings on the side of the pool. The mean SPL of all SPL measurements is based on the power sum and was, in this case, 123 dB re 1 µPa.

porpoise surfaced, its location was allocated to one of 96 grid squares (8 × 12), each of which corresponded to a 1 m × 1 m square of the outdoor pool. The animals were found to swim throughout the entire outdoor pool during exposure to the fatiguing sound, so the average fatiguing sound SPL (average of power sum of 228 measurements in the outdoor pool) was taken to be representative of the SPL received by them.

Before each test, the voltage output of the emitting system to the transducer and the voltage output of the sound-receiving system were checked with an oscilloscope (Dynatek-8300) and a voltmeter (Agilent-34401A) by producing a 16-kHz continuous signal from the laptop. The acoustic underwater signal was checked with a custom-built hydrophone, a pre-amplifier (Reson-CCAS1000), and a spectrum analyzer (Velleman-PCSU1000). If the values were the same as those obtained during the SPL calibrations by the acoustic measurement consultancy, the SPLs were assumed to be correct, and a sound exposure test could be performed.

*Hearing Test Signals*—Linear upsweeps (starting and ending at ±2.5% of the center frequency), with a duration of 1 s (including a linear rise and fall in amplitude of 50 ms each), were used as the hearing test signals that the animals were asked to detect before and after exposure to the fatiguing sound. The center frequencies tested were 16 kHz (the center frequency of the fatiguing sound), 22.4 kHz (half an octave higher than the center frequency), and 32 kHz (one octave higher than the center frequency). A sweep was used instead of a pure tone because sweeps created more stable SPLs in the area around the listening station and therefore led to little variation in threshold measurements (the standard deviation [SD] in the control thresholds was ±1 dB).

The hearing test signals were generated digitally (*Adobe Audition*, Version 3.0; sample rate: 768 kHz). The WAV files used as hearing test signals were played on a laptop computer (MSI-M5168A) with a program written in *LabVIEW* to an external data acquisition card (National Instruments-USB6251; single-channel maximum sample rate: 1.25 MHz), the output of which could be controlled in 1 dB steps with the *LabVIEW* program. The output of the card went through a custom-built buffer and a custom-built passive low-pass filter, and drove, for the 16 and 22.4 kHz signals, a balanced tonpilz piezoelectric acoustic transducer (Lubell-LL916) through an isolation transformer (Lubell-AC202), or, for the 32 kHz hearing test signal, a custom-built directional transducer (WAU-q7b) consisting of a disc of one to three composite piezoelectric materials (Material Systems Inc., Littleton, MA, USA) with an effective radiating aperture diameter of 4.5 cm. The thickness of the piezoelectric materials was 0.64 cm. The piezoelectric element was a 6.4 cm diameter disk that was encapsulated in degassed polyurethane epoxy.

The received SPL of the hearing test signal was measured every 3 mo at the position of the harbor porpoise's head during the hearing tests but in the absence of a porpoise. These calibration measurements were conducted with two hydrophones—one at each side near the position of the gape of the porpoise's mouth (the assumed location of the acoustic window in the lower jaw). The SPL in the two locations differed at most by 2 dB. The average 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 30 dB above the pre-exposure threshold levels found in the present study. The linearity of the transmitter system was checked during each calibration; the level deviated by at most 1 dB within a 24 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 (near the listening station).

*Experimental Procedures*

One total noise exposure test, consisting of (1) pre-exposure hearing tests starting at 0830 h, (2) 1-h fatiguing sound exposure in the morning or early afternoon, and (3) a number of post-noise exposure hearing tests in the afternoon, was conducted per day. Pre-exposure hearing tests were performed in the indoor pool with one animal at a time (the other animal was kept busy with quiet behaviors in the outdoor pool). The test order was always porpoise M06 first, then porpoise F05.

During the hour of fatiguing sound exposure, the animals were in the outdoor pool, and one of the net gates to the indoor pool was closed. During the sound 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. Five minutes before the fatiguing sound exposure ended, two trainers went to the net gate. In response to a signal from the operator, one trainer opened the gate and called porpoise M06 into the channel; the other trainer stepped outside and kept porpoise F05 busy with quiet behaviors. When animal M06 entered the channel, the fatiguing sound 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 (for porpoise M06) within 1 min after the fatiguing sound had stopped. After the hearing of porpoise M06 had been tested, he was directed towards the outdoor pool, and porpoise F05 entered the indoor pool so that her hearing could be tested (beginning 12 min after the fatiguing sound had stopped). Data were collected from January to July 2017.

To gain insight into hearing recovery after a threshold shift, not only the TTS immediately after exposure but also the subsequent recovery of hearing was recorded. Porpoise M06's hearing thresholds were measured during post-sound exposure (PSE) periods 1-4 min ($PSE_{1-4}$), 4-8 min ($PSE_{4-8}$), 8-12 min ($PSE_{8-12}$), 60 min ($PSE_{60}$), and (if hearing had not recovered after 60 min) 120 min ($PSE_{120}$) after the sound exposure had ended. Porpoise F05's hearing was tested 12-16 min ($PSE_{12-16}$), 16-20 min ($PSE_{16-20}$), 20-24 min ($PSE_{20-24}$), 72 ($PSE_{72}$), and (if hearing had not recovered after 72 min) 132 min ($PSE_{132}$) after the fatiguing sound had stopped. The intervals between tests were chosen so that the harbor porpoises were expected to be hungry enough to be sufficiently motivated to participate in each hearing test.

To gain insight into potential effects of the methodology on hearing thresholds, control tests were conducted in the same way as noise exposure tests but without the fatiguing sound exposure. Each control test started with a pre-exposure hearing test session and was followed by exposure to the normal (low) ambient noise (i.e., no fatiguing sound) in the outdoor pool for 1 h. Post-ambient exposure (PAE; control) hearing test sessions were then performed over three periods for porpoise M06: 1-4 ($PAE_{1-4}$), 4-8 ($PAE_{4-8}$), and 8-12 ($PAE_{8-12}$) min after the ambient noise exposure period ended. Porpoise F05 was tested at 12-16 ($PAE_{12-16}$), 16-20 ($PAE_{16-20}$), and 20-24 ($PAE_{20-24}$) min after ambient exposure. Four control tests were conducted per hearing test frequency, and they were randomly dispersed among the fatiguing sound exposure tests; on each test day, either a noise exposure test or a control test was conducted. The effects of fatiguing sounds of different average received SPLs were tested (6 SPLs for 16 and 22.4 kHz, and 2 SPLs for 32 kHz). The SPLs were tested in random order. Each average received SPL was tested at least four times, except the two lower levels, which were tested only twice each due to time constraints.

*Hearing Test Procedures*—Each hearing test trial began with an animal at the start/response buoy. The level of the hearing test signal used in the first trial of the session was approximately 6 dB above the hearing threshold determined during the previous session. Each harbor porpoise was trained to swim to the listening station in response to a hand signal from the trainer. The methodology was as described in detail by Kastelein et al. (2012a). The signal level was varied according to the one-up one-down adaptive staircase method (Cornsweet, 1962); 2 dB steps were used. During signal-present hearing test trials, the harbor porpoise stationed at the listening station and waited for a random period of between 6 and 12 s (established via a random number generator) before the signal operator produced the test signal. A switch from a test signal level that the porpoise responded to (a hit) to a level that he or she did not respond to (a miss), and vice versa, was called a reversal.

Each complete hearing test session consisted of ~25 trials and lasted for up to 12 min (subdivided into three 4-min periods in the first PSE or PAE session of each animal). Sessions consisted of two-thirds signal-present and one-third signal-absent trials, offered in quasi-random order (random but with no more than three consecutive signal-present or signal-absent trials). Only $PSE_{1-4}$, $PSE_{12-16}$, $PAE_{1-4}$, and $PAE_{12-16}$ hearing session periods with three or more reversals were used for analysis.

*Data Analysis*

The pre-exposure mean 50% hearing threshold for a hearing test sound ($PE_{50\%}$) was determined by calculating the mean SPL of all (usually 10) reversal pairs in the pre-exposure hearing session.

TTSs for porpoise M06 after the sound exposure sessions (1-4, 4-8, 8-12, 60, and 120 min) were calculated by subtracting the mean 50% hearing threshold obtained during the pre-exposure sessions from the mean 50% hearing thresholds during $PSE_{1-4}$, $PSE_{4-8}$, $PSE_{8-12}$, $PSE_{60}$, and $PSE_{120}$ periods of the same day. TTSs for porpoise F05 after the sound exposure sessions (12-16, 16-20, 20-24, 72, and 132 min) were calculated by subtracting the mean 50% hearing threshold obtained during the pre-exposure sessions from the mean 50% hearing thresholds during $PSE_{12-16}$, $PSE_{16-20}$, $PSE_{20-24}$, $PSE_{72}$, and $PSE_{132}$ periods of the same day. TTSs in the control sessions were calculated by subtracting the mean 50% hearing thresholds obtained during pre-ambient exposure periods from the mean 50% hearing thresholds obtained during the post-ambient exposure periods of the same day.

We define the onset of TTS as occurring at the lowest sound exposure level (SEL) at which a statistically significant difference could be detected between the hearing threshold shift due to the fatiguing sound exposures and the hearing threshold shift as measured after the control exposures (this shift was close to zero). The level of significance was established by conducting a one-way ANOVA on the TTS, separately for each porpoise and for each hearing test frequency, with the factor SPL (including zero as the control). When the ANOVA produced a significant value overall, the levels were compared to the control by means of Dunnett multiple comparisons. A *t* test was substituted for the ANOVA when there were only two levels. All analysis was conducted in *Minitab 17*, and data conformed to the assumptions of the tests used (Zar, 1999).

## Results

*Pre-Stimulus Response Rate*

After the 1-h noise exposure periods, the harbor porpoises were always willing to participate in the hearing tests. In a few sessions, due to a slow gating procedure, three or more reversals could not be obtained during the first 4 min after the fatiguing noise had stopped; data from these sessions were discarded. The mean pre-stimulus response rates for both signal-present and signal-absent trials (in the latter, the whistle was the stimulus) in the hearing tests for porpoise M06 varied between 1.0 and 5.3%; for porpoise F05, they varied between 6.2 and 14.5% (Table 1). The pre-stimulus response rates in the post-exposure periods did not differ much (maximum 5%) from those in the pre-exposure periods and control periods.

*Effect of SPL on TTS and Recovery Time*

The ANOVAs and *t* test showed that the $TTS_{1\text{-}4}$ was significantly affected by the fatiguing sound's SPL. Comparisons with the control revealed that the statistically significant onset of TTS varied depending on the animal and the hearing test frequency (Table 2).

*Male Porpoise M06*

With a hearing test signal of 16 kHz, statistically significant $TTS_{1\text{-}4}$ occurred in harbor porpoise M06 after exposure to an SEL of 159 dB re 1 $\mu Pa^2s$ (Table 2; Figure 3a); hearing recovered (defined as < 2 dB TTS) within 12 min (Figure 4a). With a hearing test signal of 22.4 kHz, statistically significant $TTS_{1\text{-}4}$ occurred after exposure to an SEL of 165 dB re 1 $\mu Pa^2s$ (Table 2; Figure 3a). The rate of increase in TTS with increasing SEL changed from 0.2 dB/dB fatiguing sound between 165 and 171 dB SEL, to 1.1 dB/dB fatiguing sound between 171 and 177 dB SEL, and to 1.2 dB/dB fatiguing sound between 177 and 181 dB SEL. Recovery of hearing occurred within 60 min for exposures up to an SEL of 171 dB re 1 $\mu Pa^2s$, and within 120 min for SELs 177 and 181 dB re 1 $\mu Pa^2s$ (Figure 4b). With a hearing test signal of 32 kHz, statistically significant $TTS_{1\text{-}4}$ occurred after exposure to an SEL of 181 dB re 1 $\mu Pa^2s$ (Table 2; Figure 3a), and hearing recovered within 12 min (Figure 4c). The control sessions showed that the hearing thresholds for all hearing test signals before and after 1-h exposures to the low ambient noise were very similar (Figure 4; Table 3).

*Female Porpoise F05*

With a hearing test signal of 16 kHz, statistically significant $TTS_{12\text{-}16}$ occurred in harbor porpoise F05 after exposure to an SEL of 171 dB re 1 $\mu Pa^2s$ (Table 2; Figure 3b); hearing recovered within 24 min (Figure 5a). With a hearing test signal of 22.4 kHz, statistically significant $TTS_{12\text{-}16}$

**Table 1.** The pre-stimulus response rate in hearing tests during the pre-exposure period, after exposure for 1 h to a continuous (100% duty cycle) one-sixth octave noise band centered at 16 kHz (fatiguing sound), and after exposure for 1 h to ambient noise (control). All exposure SPLs and hearing test frequencies were pooled for the calculation of percentages, and sample sizes (no. of trials within hearing tests) are shown in parentheses. Porpoise M06's hearing tests started immediately after the end of the exposure periods (fatiguing sound or control); porpoise F05's hearing test began 12 min after the end of the exposure periods.

| Porpoise M06 | | | Period | | |
|---|---|---|---|---|---|
| Fatiguing sound | Pre-exposure | $PNE_{1\text{-}4}$ | $PNE_{4\text{-}8}$ | $PNE_{8\text{-}12}$ | $PNE_{60}$ |
| | 2.4% (1,034) | 3.0% (266) | 2.6% (346) | 3.8% (345) | 1.7% (356) |
| Control | Pre-exposure | $PAE_{1\text{-}4}$ | $PAE_{4\text{-}8}$ | $PAE_{8\text{-}12}$ | $PAE_{60}$ |
| | 2.0% (302) | 5.3% (94) | 4.1% (98) | 1.0% (99) | 1.2% (86) |
| **Porpoise F05** | | | **Period** | | |
| Fatiguing sound | Pre-exposure | $PNE_{12\text{-}16}$ | $PNE_{16\text{-}20}$ | $PNE_{20\text{-}24}$ | $PNE_{72}$ |
| | 8.0% (939) | 6.2% (276) | 12.1% (307) | 11.3% (327) | 8.2% (379) |
| Control | Pre-exposure | $PAE_{12\text{-}16}$ | $PAE_{16\text{-}20}$ | $PAE_{20\text{-}24}$ | $PAE_{72}$ |
| | 9.5% (284) | 12.2% (82) | 14.5% (83) | 7.1% (85) | 7.1% (70) |

**Table 2.** Results of one-way ANOVAs on initial TTS ($TTS_{1-4}$ for porpoise M06 and $TTS_{12-16}$ for porpoise F05) after exposure for 1 h to continuous one-sixth octave band noise centered at 16 kHz, with the factor fatiguing sound level (in dB). Exact *p* values are shown alongside the results of Dunnett's multiple comparisons with the control and the statistically significant TTS onset (indicated in bold in the last column). For porpoise F05, the comparison for the hearing test frequency 32 kHz is between the control and 181 dB (only two levels), so a *t* test is used instead of a one-way ANOVA. *TTS in porpoise F05 was measured 12 to 16 min after the exposure to the fatiguing sound stopped, so if TTS occurred during the exposure, some hearing recovery had probably taken place during the first 12 min after the sound stopped.

| Porpoise | TTS (Minutes after sound stopped) | Hearing test frequency (kHz) | Results of ANOVA/ *t* test: F values/*t* value (degrees of freedom), *p* values | SELs (dB) statistically not different to control | SELs (dB) significantly different from control |
|---|---|---|---|---|---|
| M06 | 1-4 | 16 | $F_{5,20} = 15.42$ $p < 0.001$ | 153 | **159**, 165, 171, 177, 181 |
| M06 | 1-4 | 22.4 | $F_{5,16} = 109.99$ $p < 0.001$ | 153, 159 | **165**, 171, 177, 181 |
| M06 | 1-4 | 32 | $F_{2,9} = 7.87$ $p = 0.011$ | 171 | **181** |
| F05 | 12-16 | 16 | $F_{5,16} = 4.57$ $p = 0.009$ | 153, 159, 165 | **171**, 177, 181* |
| F05 | 12-16 | 22.4 | $F_{6,16} = 52.47$ $p < 0.001$ | 153, 159, 165 | **171**, 177, 181* |
| F05 | 12-16 | 32 | $t = -3.05$ $p = 0.055$; DF = 3 | 171, 181 | None; no TTS* |

occurred after exposure to an SEL of 171 dB re 1 µPa²s (Table 2; Figure 3b). Recovery of hearing occurred within 72 min for exposures up to SELs of 171 dB re 1 µPa²s, and within 132 min for SELs 177 and 181 dB re 1 µPa²s (Figure 5b). With a hearing test signal of 32 kHz, no statistically significant $TTS_{12-16}$ occurred, even at the highest SEL of 181 dB re 1 µPa²s (Table 2; Figure 3b). The control sessions showed that the hearing thresholds for all hearing test signals before and after 1-h exposures to the low ambient noise were very similar (Figure 5; Table 3).

## Discussion and Conclusions

*Evaluation*

The pre-stimulus response rates of the study animals are acceptable for psychophysical hearing tests (Dancer et al., 1976; Dancer & Conn, 1983) and fall within the range of these animals' performance in other hearing tests. The similar pre-stimulus response rates in pre-exposure and post-exposure sessions show that the performance of the harbor porpoises was not affected by the sound exposures.

In the study presented herein, the sample size per SPL was small ($n \leq 4$), but the variation in TTS per exposure level was also small (Table 3). The

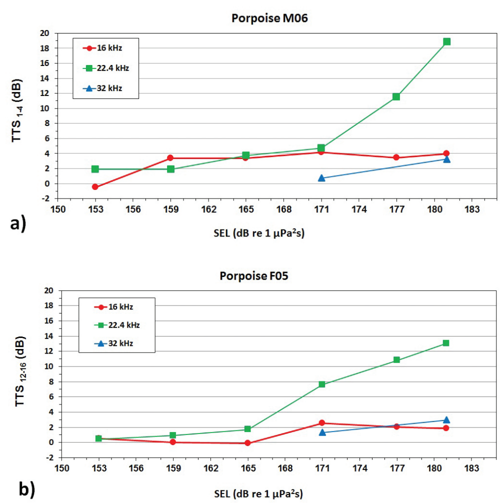


**Figure 3.** $TTS_{1-4}$ in harbor porpoise M06 (a) and $TTS_{12-16}$ in porpoise F05 (b) after exposure for 1 h to a continuous one-sixth octave noise band centered at 16 kHz at several SELs, quantified at hearing frequencies 16, 22.4, and 32 kHz (i.e., at 0, 0.5, and 1 octave above the exposure frequency). Sample size varies per data point shown (for sample sizes, ranges, and SDs, see Table 3). For SPL (dB re 1 µPa), subtract 36 dB re 1 s from the SEL values. For control values, see Figures 4 & 5.

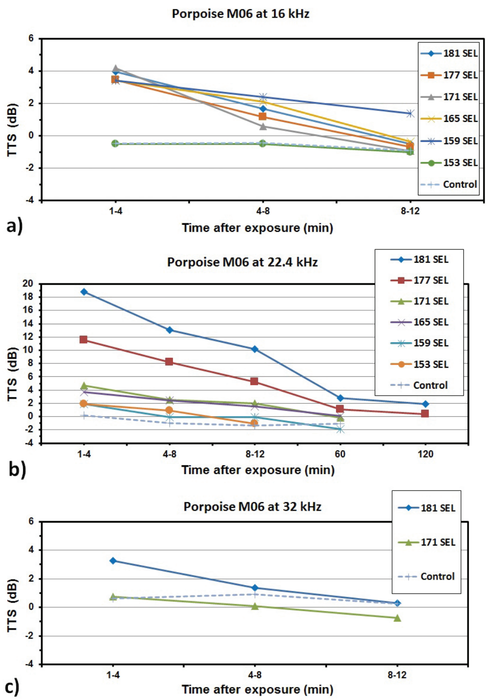


**Figure 4.** Recovery of hearing of harbor porpoise M06 at (a) 16 kHz, (b) 22.4 kHz, and (c) 32 kHz after exposure to a continuous one-sixth octave noise band centered at 16 kHz for 1 h at several SELs. For sample sizes and SDs (only for $TTS_{1-4}$), see Table 3. For average received SPLs (dB re 1 µPa), subtract 36 dB re 1 s from the SEL values.

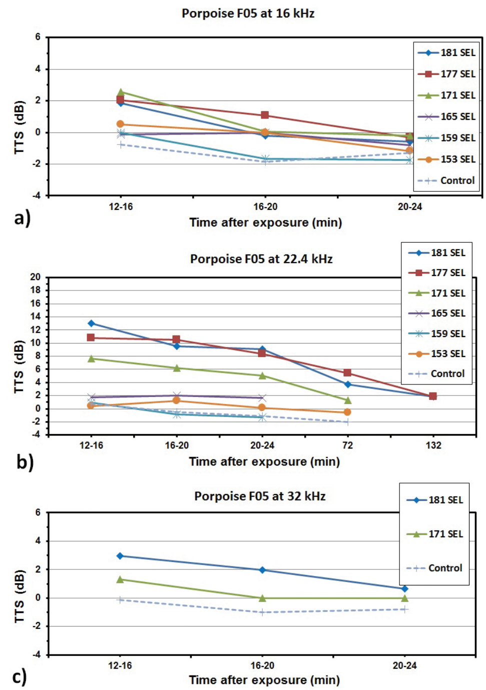


**Figure 5.** Recovery of hearing of harbor porpoise F05 at (a) 16 kHz, (b) 22.4 kHz, and (c) 32 kHz after exposure to a continuous one-sixth octave noise band centered at 16 kHz for 1 h at several SELs. For sample sizes and SDs (only for $TTS_{12-16}$), see Table 3. For average received SPLs (dB re 1 µPa), subtract 36 dB re 1 s from the SEL values.

sample sizes per SPL are similar to or larger than those in other studies of TTS in odontocetes (e.g., Mooney et al., 2009; Finneran et al., 2010). TTS research is labor-intensive; to protect the animals' hearing and to obtain consistent results, only one exposure that is likely to induce TTS can be conducted per day (or no exposure was carried out if the initial TTS of the previous day was very high and hearing still had not recovered completely the following day).

The present study was conducted with two animals. Their hearing thresholds were similar to those of three other young male harbor porpoises (Kastelein et al., 2017a), which suggests that the study animals had normal hearing for porpoises of their age. However, it is not clear how representative the TTS values found in these animals are of the mean hearing susceptibility to TTS of a population. Studies on humans and other terrestrial mammals show individual, genetic, and population-level differences in susceptibility to TTS (Kylin, 1960; Kryter et al., 1962; Henderson et al., 1991, 1993; Davis et al., 2003; Spankovich et al., 2014). Individual differences in susceptibility to TTS can only be estimated for the animals in the present study because the hearing thresholds of two study animals were always measured in the same order after the fatiguing sound stopped (first porpoise M06, followed by porpoise F05). Therefore, the $TTS_{12-16}$ measured in porpoise F05 is expected to be lower than the $TTS_{1-4}$ measured in porpoise M06, as hearing is expected to recover (fully or partly) from the fatiguing sound during the first 12 min after it stops. However, comparison of the $TTS_{8-12}$ measured in porpoise M06 at the end of his first session (8 to 12 min after the fatiguing sound stopped) with the $TTS_{12-16}$ in porpoise F05 at the beginning of her first session (12 to 16 min after the fatiguing sound stopped) shows that, for the higher SPLs, the $TTS_{12-16}$ in porpoise F05 was ~3 dB greater than $TTS_{8-12}$ in porpoise M06 and, therefore, either her TTS during the first 4 min after the sound stopped was ~2 to 3 dB greater than that of porpoise M06 or her hearing recovered more slowly than his did. It is also possible that the observed difference in TTS between the

**Table 3.** Mean (± SD) $TTS_{1-4}$ in porpoise M06 and $TTS_{12-16}$ in porpoise F05 after exposure for 1 h to a continuous one-sixth octave noise band centered at 16 kHz at several SELs, quantified at hearing frequencies 16, 22.4, and 32 kHz (i.e., at 0, 0.5, and 1 octave above the exposure frequency). Results from the control sessions are also shown (no TTS occurred). *n* = sample size; * = significant TTS relative to control sessions.

| | | | Harbor porpoise M06 | | | Harbor porpoise F05 | | |
|---|---|---|---|---|---|---|---|---|
| Hearing test frequency (kHz) | SEL (dB re 1 µPa²s) | Hearing frequency (kHz) | Mean $TTS_{1-4}$ | SD (range) | *n* | Mean $TTS_{12-16}$ | SD (range) | *n* |
| 16 | Control | 16 | -0.5 | 1.0 (-1.2-1.5) | 6 | -0.8 | 0.6 (-1.5- -0.1) | 4 |
| | 153 | 16 | -0.5 | -- | 1 | 0.5 | -- | 1 |
| | 159 | 16 | 3.4* | 0.5 (3.1-4.0) | 4 | 0.0 | 1.7 (-1.2-1.2) | 2 |
| | 165 | 16 | 3.4* | 0.8 (2.8-4.0) | 2 | -0.1 | 0.6 (-0.6-0.5) | 3 |
| | 171 | 16 | 4.2* | 1.3 (2.2-5.8) | 6 | 2.6* | 1.5 (0.8-4.3) | 5 |
| | 177 | 16 | 3.4* | 1.0 (2.5-4.9) | 4 | 2.0* | 1.4 (0.9-4.1) | 4 |
| | 181 | 16 | 4.0* | 1.1 (2.9-5.3) | 4 | 1.8* | 1.4 (0.2-3.3) | 4 |
| 22.4 | Control | 22.4 | 0.2 | 0.7 (-0.8-0.6) | 4 | 0.8 | 0.9 (0.2-2.1) | 4 |
| | 153 | 22.4 | 1.9 | 0.1 (1.8-2.0) | 2 | 0.4 | 1.3 (-0.5-1.4) | 2 |
| | 159 | 22.4 | 1.9 | 1.6 (0.8-3.0) | 2 | 0.9 | 1.6 (-0.3-2.1) | 2 |
| | 165 | 22.4 | 3.7* | 0.7 (2.8-4.4) | 4 | 1.7 | 1.1 (0.6-2.7) | 3 |
| | 171 | 22.4 | 4.7* | 1.3 (3.4-6.0) | 4 | 7.6* | 0.4 (7.1-8.0) | 4 |
| | 177 | 22.4 | 11.5* | 1.1 (10.2-12.9) | 4 | 10.8* | 0.6 (10.1-11.5) | 4 |
| | 181 | 22.4 | 18.9* | 2.3 (15.8-21.4) | 4 | 13.0* | 2.5 (10.1-15.8) | 4 |
| 32 | Control | 32 | 0.6 | 0.7 (0.1-1.6) | 4 | -0.1 | 1.0 (-1.2-1.3) | 5 |
| | 171 | 32 | 0.7 | 1.6 (-0.4-3.0) | 4 | 1.3 | -- | 1 |
| | 181 | 32 | 3.3* | 0.7 (2.6-4.0) | 4 | 3.0 | 1.6 (2.0-4.8) | 3 |

two study animals was caused, in part, by their slightly different swimming patterns (causing a difference in the SEL they experienced). Maybe the difference in age of the study animals also had an effect on the TTS susceptibility.

*Affected Hearing Frequencies*

In the present study, the hearing frequency with the highest TTS depended on the SPL of the fatiguing sound. However, at higher SPLs (above 171 dB SEL in porpoise M06), hearing was most affected at frequencies half an octave above the center frequency of the fatiguing sound. Effects of high-level sound exposure are not limited to the exposure frequency but spread to adjacent hearing frequencies, especially higher frequencies, as the exposure SPL and the resulting amount of TTS increase. Therefore, the hearing test frequency that is chosen for testing TTS influences the amount of TTS that is reported.

Data from humans and other terrestrial mammals show that, for moderate and larger shifts, the maximum TTS occurs half to one octave above the exposure frequency (Cody & Johnstone, 1981; McFadden, 1986). This has also been observed in marine mammals that were exposed to tonal and broadband noise. In bottlenose dolphins and belugas (*Delphinapterus leucas*) that were exposed to short-duration tones, Schlundt et al. (2000) measured the greatest TTS at approximately half an octave above the exposure frequency, though some TTS also occurred at the exposure frequency and at one octave above the exposure frequency. More detailed studies of the spread of TTS in bottlenose dolphins, belugas, and Yangtze finless porpoises (*Neophocaena phocaenoides asiaeorientalis*) that were exposed to tones and noise also showed that the maximum TTS occurred at approximately half an octave above the center frequencies of tones and noise bands; there was less TTS one octave above and at the exposure center frequency (Nachtigall et al., 2004; Finneran et al., 2007; Mooney et al., 2009; Popov et al., 2011, 2013). However, the maximum TTS in a harbor porpoise (identified as harbor porpoise M02), a California sea lion (*Zalophus californianus*), and harbor seals (*Phoca vitulina*) exposed to octave band noise occurred at the fatiguing sound's center frequency rather than above it (Kastak et al., 2005; Kastelein et al., 2012a, 2012b).

Kastelein et al. (2014a) showed that the hearing frequency most affected by fatiguing sound depends on the SPL of the sound: the higher the SPL, the higher the frequency showing the highest TTS. This is probably due to changes in the spread of the basilar membrane excitation pattern: as the level of the fatiguing sound increases, the affected hearing range becomes broader. This finding may explain the discrepancies reported by various authors: studies in which the maximum TTS occurred at the exposure frequency typically involved relatively small TTSs whereas studies in which the maximum TTS occurred at half an octave above the center frequency involved greater TTSs (Finneran et al., 2007; Popov et al., 2013). Studies on odontocetes in which impulsive sounds are used as the fatiguing sound show TTS occurring at frequencies above the peak frequency of the fatiguing sound (Finneran et al., 2002; Lucke et al., 2009; Kastelein et al., 2015a, 2017b). It is likely that broadband exposures produce broadband TTS with an upward frequency spread similar to that seen after exposure to tones and narrow-band noise (Finneran, 2015).

*Relationship Between the Frequency of the Fatiguing Sound and TTS*

Research by Finneran & Schlundt (2013; psychophysical technique) on bottlenose dolphins, Popov et al. (2011; physiological technique) on Yangtze finless porpoises, and Popov et al. (2013; physiological technique) on belugas shows that the magnitude of TTS induced by fatiguing sounds with the same received $SEL_{cum}$ is dependent on the frequency of the fatiguing sounds. Finneran & Schlundt (2013) exposed bottlenose dolphins to fatiguing sounds at various frequencies and found that their hearing was more susceptible to damage by high-frequency fatiguing sounds (10 to 28.3 kHz) than by low-frequency fatiguing sounds (3 kHz). Not only was the SPL required to induce TTS lower at higher frequencies, but TTS increased more with increasing SPL in the high-frequency sounds than in the low-frequency sounds. Popov et al. (2011) investigated the effect of the frequency of fatiguing sounds on the TTS they induced by exposing Yangtze finless porpoises to sounds > 11.2 kHz and found that this species is more susceptible to hearing damage by sounds in the 22.5 to 32 kHz range than by higher-frequency sounds (90 kHz). Similar results were found for belugas, which are more susceptible to hearing damage by fatiguing sounds in the 11.2 to 22.5 kHz range than by those in the 45 to 90 kHz range (Popov et al., 2013).

Susceptibility to TTS and its relationship with fatiguing sound frequency in harbor porpoises can be explored by relating equal-TTS data to fatiguing sound frequencies (NMFS, 2016; Houser et al., 2017). In the present study, $TTS_{1\text{-}4}$ at 22.4 kHz occurred at a higher SEL than that which caused TTS onset after exposure to a 6.5 kHz continuous wave in another harbor porpoise (identified as harbor porpoise M02; Kastelein et al., 2014a; psychophysical technique; Figure 6). Below 6.5 kHz, it appears that susceptibility to TTS increases with increasing frequency; but above 6.5 kHz, it appears that susceptibility to TTS decreases with increasing frequency. However, there may be individual differences in susceptibility to TTS between porpoise M02 (exposed to a one-octave noise band at 4 kHz [Kastelein et al, 2012a], a 1.5 kHz continuous wave [Kastelein et al., 2013], 1 to 2 kHz sweeps [Kastelein et al., 2014b], a 6.5 kHz continuous wave [Kastelein et al., 2014a], and 6 to 7 kHz sweeps [Kastelein et al., 2015b]) and porpoises M06 and F05 (exposed to a one-sixth octave noise band around 16 kHz; present study). These studies used the same methodology, so the results can be compared directly. Alternatively, differences in the fatiguing sound type (continuous waves, 1 kHz wide sweeps, octave noise band, and one-sixth octave noise band) may have resulted in (or contributed towards) differences in the induced TTSs. The TTS induced in porpoise M06 by 53-C sonar playback sounds in a different study, which was composed of a sweep followed by two tones in the 3.5 to 4.1 kHz range (though at a slightly lower duty cycle of 96%), was as expected from TTS studies with harbor porpoise M02 (Kastelein et al., 2017b; Figure 6).

In previous TTS studies with harbor porpoises, the fatiguing sounds used differed from those used in the present study; but although the TTS onset SELs can probably be compared, it is unclear whether the affected hearing frequency (relative to the center frequency of the fatiguing sound) that showed the highest TTS was the same for one-octave noise bands (Kastelein et al., 2012a), one-sixth octave noise bands (present study), narrow-band sweeps (Kastelein et al., 2014b, 2015b), and tonal (continuous wave) sounds (Kastelein et al., 2013, 2014a).

The results of the present study, although only representing a small part of the harbor porpoise's hearing frequency range (1.5 to 16 kHz), are in agreement with those of Finneran & Schlundt (2013) for bottlenose dolphins and suggest that the susceptibility of harbor porpoise hearing to TTS is also frequency-dependent. There are very few studies of TTS in harbor porpoises, so it is not known whether this frequency-dependence also applies to fatiguing sounds with frequencies > 16 kHz. Popov et al. (2011, 2013) showed that

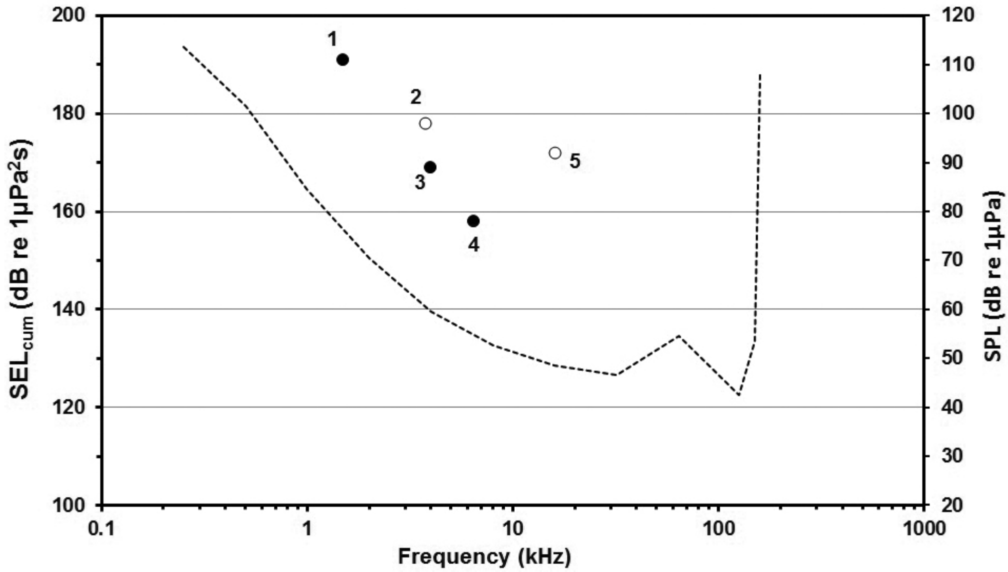


**Figure 6.** The $SEL_{cum}$ required to cause a mean $TTS_{1-4}$ of around 6 dB in harbor porpoises after 1 h exposure to (1) a 1 to 2 kHz sweep at 100% duty cycle (Kastelein et al., 2014a), (2) a 3.5 to 4.1 kHz 53-C sonar playback sound at 96% duty cycle (Kastelein et al., 2017b), (3) a one-octave noise band centered at 4 kHz at 100% duty cycle (Kastelein et al., 2012), (4) a 6.5 kHz tone at 100% duty cycle (Kastelein et al., 2014b), and (5) a one-sixth octave noise band centered at 16 kHz at 100% duty cycle (present study). The solid circles are studies with porpoise M02, and the open circles are studies with porpoise M06. Also shown is the audiogram of harbor porpoise M02, a young male (Kastelein et al., 2010; right-hand Y-axis).
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susceptibility to TTS in Yangtze finless porpoises did not increase with increasing frequency of the fatiguing sound at frequencies above 45 kHz. TTS studies in which harbor porpoises are exposed to fatiguing sounds with frequencies > 16 kHz and below 1 kHz are needed to define weighting functions to predict TTS and PTS for this species.

The present study suggests that the onset of TTS (defined as 6 dB initial TTS) in harbor porpoises that have been exposed to sounds of around 16 kHz occurs at higher SELs than was expected (Figure 6). More research is ongoing to elucidate frequency-dependent susceptibility to TTS in harbor porpoises. Once susceptibility to TTS has been quantified for the entire hearing range of harbor porpoises, it will be possible to generate valid auditory weighting curves for cetaceans that echolocate at high frequencies (Houser et al., 2017).

*Application*

Studies of TTS are often used to provide insight into sounds that may cause permanent hearing threshold shifts (PTS), though they cannot confirm the specific SELs at which PTS occurs. TTS studies are valuable in themselves, as they can provide information on TTS onset SEL, TTS growth rate, and critical levels. TTSs of various magnitudes and durations have the potential to compromise feeding, localization, communication, and predator detection in marine mammals, and may have negative effects on health and survival even if PTS does not occur.

Sounds in the frequency range that encompasses the 16 kHz range include biological sounds such as odontocete vocalizations and echolocation signals, environmental sounds such as those produced by rain, and anthropogenic sounds such as those produced by some naval sonars and fish-finding sonars. The present study gives insight into the potential effects of biological, environmental, and anthropogenic sounds on harbor porpoises, allowing safety levels to be set which may safeguard harbor porpoise hearing from damage by anthropogenic sound sources.

## Acknowledgments

We thank research assistants Shirley Van de Voorde and Léonie Huijser; students Tom Beudel, Daniek Kuipers, Tessa Kreeft, and Thaana van Dessel; and volunteers Lotte Dalmeijer, Stacey van der Linden, Céline van Putten, Stephanie de Ruijter, Irna Huisjes, Jolien Hakvoort, and Brigitte Slingerland for their help in collecting the data. We thank Rob Triesscheijn for making Figure 1, and Arie Smink for the design, construction, and maintenance of the electronic equipment. We thank Bert Meijering (Topsy Baits) for providing space for the SEAMARCO Research Institute. Erwin Jansen (TNO) conducted the acoustic measurements. We also thank Nancy Jennings (Dotmoth.co.uk), Wim Verboom (JunoBioacoustics, The Netherlands), Michael Ainslie (JASCO), and two anonymous reviewers for their valuable constructive comments on this manuscript. Funding for this project was obtained from the U.S. Navy, Living Marine Resources program (Contract No. N39430-15-C-1686). We thank Anu Kumar and Mandy Shoemaker for their guidance on behalf of the LMR program. The harbor porpoises were tested under authorization of the Netherlands Ministry of Economic Affairs with Endangered Species Permit FF/75A/2014/025. We thank Jeroen Vis (Netherlands Ministry of Economic Affairs) for his assistance in making the harbor porpoises available for this project, and the ASPRO group for providing the harbor porpoises.

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