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Temporary Hearing Threshold Shift After Exposure to a 6.5 kHz Continuous Wave in a Second Harbor Porpoise (*Phocoena phocoena*)

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Abstract Max 300 Words

So far, TTS has been measured in a few harbor porpoises for sounds in the 1–63 kHz range. To determine whether susceptibility to TTS differs between individuals, a previous study with a young adult male porpoise (M02) was partly replicated by exposing an adult female porpoise (F05) to similar fatiguing sounds and testing her hearing with similar audiometric methodology. The female porpoise was exposed for one hour to a continuous wave of 6.5 kHz (duty cycle 100%), at up to 10 average received sound pressure levels (SPLs); range of 103–148 dB re 1µPa; sound exposure level (SEL) range: 139–184 dB re 1µPa^{2}s. To quantify TTS and recovery, hearing thresholds for 6.5, 9.2 and 13 kHz signals were determined before and after exposure. Hearing was first tested 1–4 min after exposure. The lowest SELs that resulted in significant TTS_{1-4} were 145 dB re 1µPa^{2}s for 6.5 kHz (2.9 dB TTS_{1-4}), 178 dB re 1µPa^{2}s for 9.2 kHz (7.3 dB TTS_{1-4}), and 180 dB re 1µPa^{2}s for 13 kHz (6.4 dB TTS_{1-4}). At all three frequencies, the highest TTS_{1-4} occurred after exposure to an SEL of 184 dB re 1µPa^{2}s (6.4 dB TTS_{1-4} at 6.5 kHz, 14.6 dB TTS_{1-4} at 9.2 kHz, and 10.6 dB TTS_{1-4} at 13 kHz). Comparison of the TTS documented in porpoises F05 (present study) and M02 (previous study, based on n = 1 per SEL) shows that a TTS of ~6 dB occurred in both at 9.2 kHz after exposure to a SEL of around 176 dB re 1µPa^{2}s. Susceptibility to TTS after exposure to fatiguing sounds of the same frequency was similar in both individual harbor porpoises when tested using the similar methods.

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

Introduction

The harbor porpoise (*Phocoena phocoena*) appears to be more susceptible to temporary hearing threshold shift (TTS) caused by high-amplitude fatiguing sounds than the few other odontocete species that have been examined so far (Finneran, 2015; Tougaard et al., 2016; Houser et al., 2017). The effects of anthropogenic sounds on the harbor porpoise are of particular concern because of its wide distribution area in the coastal waters of the northern hemisphere where many anthropogenic offshore activities occur (Bjorge & Tolley, 2008), and because of its relatively low hearing thresholds in a wide frequency range (Kastelein et al. 2017a). Therefore, many countries have set, or are in the process of setting, acoustic criteria, such as those proposed by Southall et al. (2019), to protect the hearing of harbor porpoises. The TTS onset function proposed by Southall et al. (2019) was based on the limited published data on TTS, which span a narrow frequency range (1–6.5 kHz; Kastelein et al. 2012a; 2013; 2014a; 2014b; 2015). TTS onset and weighting functions enable the composition of important protection measures by government regulators (for example those set by the National Marine Fisheries Service in the US; NMFS, 2016). As TTS data from that and similar studies form the basis of TTS onset functions and weighting functions (Southall et al., 2007; Finneran, 2015; Tougaard et al., 2016; Houser et al., 2017; Southall et al., 2019), it is important to measure TTS due to more fatiguing sound frequencies as well as in more individuals.

Susceptibility to TTS depends not only on an animal's response to the fatiguing sound's received sound pressure level (SPL) and to the exposure duration, but to the sound's frequency (see overviews by Finneran [2015] and Houser et al. [2017]). For regulation of underwater anthropogenic sound levels to protect hearing in the harbor porpoise, complete equal-TTS contours are desirable, covering the entire frequency range (0.5–140 kHz) of this species. Since the review by Southall et al. (2019), new TTS studies have been published; the frequency range for which TTS data on continuous (non-impulsive) sound is available now spans a bandwidth of 1 to 63 kHz (Kastelein et al. 2012a; 2013; 2014a; 2014b; 2015; 2017b; 2019a; 2019b; 2019c).

The sound exposure levels (SELs; derived from SPL and exposure duration) required to cause 6 dB TTS (a marker of TTS onset used by Finneran, 2015) in harbor porpoises for the frequencies tested so far show a peculiar pattern. Below ~6.5 kHz, the SEL required to cause 6 dB TTS seems to decrease, or susceptibility to TTS seems to increase with frequency, whereas above ~6.5 kHz, susceptibility to TTS seems to decrease with frequency (see Kastelein et al., 2019c). However, the data are from only three harbor porpoises, and the observed pattern of TTS-onset SELs could be, because of the low sample size (generally only one animal has been tested for each fatiguing sound frequency), be due to individual variation in susceptibility to TTS, as is seen humans and other terrestrial mammals (Kylin, 1960; Kryter et al., 1962; Henderson et al., 1991, 1993; Davis et al., 2003; Spankovich et al., 2014).

In a previous study, two harbor porpoises that were exposed to the same sound (one-sixth octave band around 63 kHz) and had their hearing tested 1–4 min after the fatiguing sound stopped showed a similar susceptibility to TTS (Kastelein et al., 2019c). However, in that study, only one fatiguing sound frequency was tested with only two individual porpoises. Therefore, the goals of the present study are to increase the number of individual harbor porpoises in which TTS has been measured due to a particular fatiguing sound frequency and to gain insight into the individual variation in susceptibility to TTS in harbor porpoises. TTS and hearing recovery were quantified in an adult female harbor porpoise using similar fatiguing sound (a 6.5 kHz continuous wave; CW), duty cycle, exposure duration, equipment, methodology, and pool environment (very low ambient noise) as used when testing TTS in a young male harbor porpoise (Kastelein et al. 2014b). The effect of the 6.5 kHz CW was tested at three hearing frequencies (6.5, 9.2 and 13 kHz; i.e., the exposure frequency, half an octave above the exposure frequency, and one octave above the exposure frequency), because often hearing is affected at a higher frequency than the frequency of the fatiguing sound (McFadden, 1986).

Materials and Methods

Study Animal and Site

A formerly stranded and rehabilitated female harbor porpoise, identified as harbor porpoise F05, was used as the study animal. She was ~9 years old and thus an adult during the study. Her body mass was ~47 kg, her body length was ~154 cm, and her girth at the axilla was ~83 cm. The study animal's hearing for the frequencies tested in the present study (6.5, 9.2 and 13 kHz) was probably representative of that of similarly aged conspecifics (Kastelein et al., 2017a). The management of feeding was as described by Kastelein et al. (2019a).

The study was conducted at the SEAMARCO Research Institute, the Netherlands. The animal was kept in a quiet pool complex designed and built for acoustic research, consisting of an outdoor pool (12 m x 8 m; 2 m deep) in which she was exposed to the fatiguing sound, 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 the hearing tests were conducted (**Figure 1**). For details of the pool, equipment, and water flow, see Kastelein et al. (2019a).

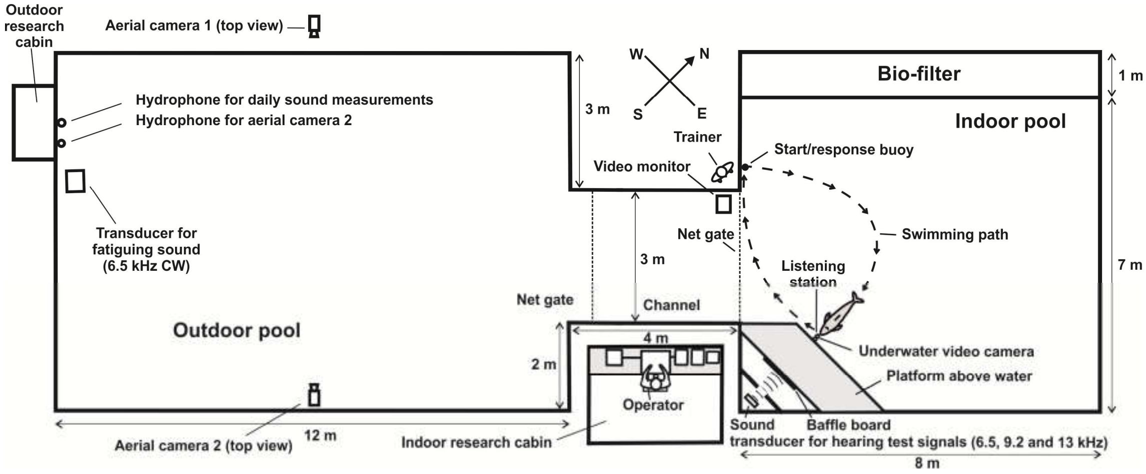


Figure 1. The pool complex in which the TTS study was conducted with the harbor porpoise. In the morning a pre-exposure hearing test was conducted in the indoor pool (one 3 3 frequencies: 6.5, 9.2 or v13 kHz), which was followed by a one-hour exposure period in the outdoor pool (6.5 kHz CW), which was followed by one of several post exposure hearing tests in the indoor pool (testing the same frequency as tested in the pre-exposure period of that day).

Acoustics

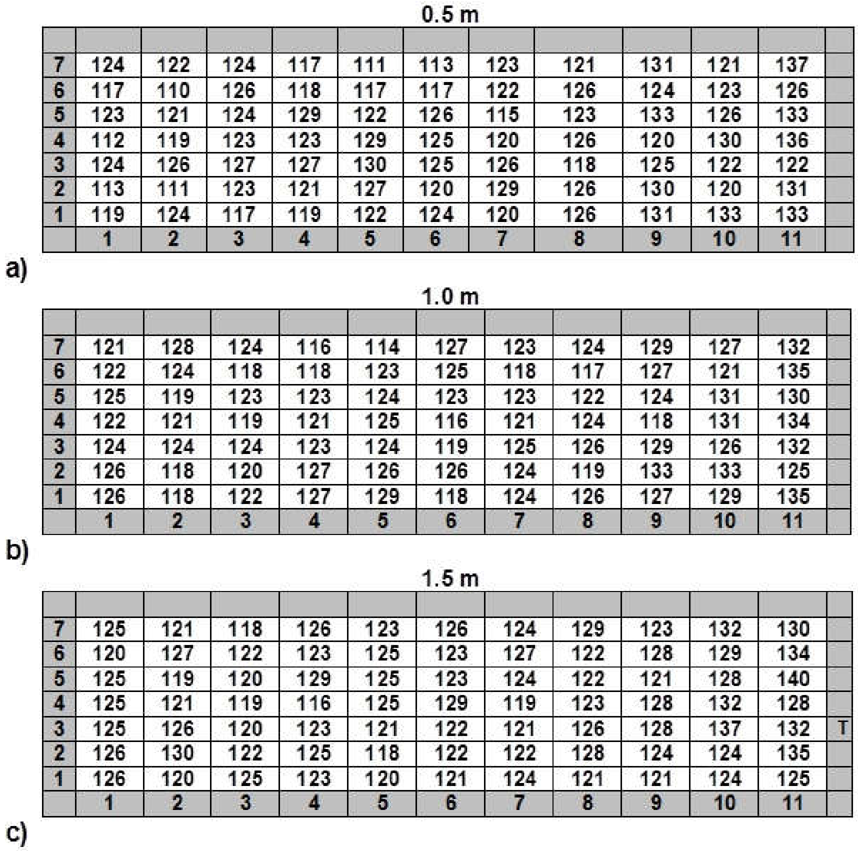
SPL Measurement Equipment and Ambient Noise - Acoustical terminology follows ISO 18405:2017. The ambient noise was measured and the fatiguing sound and hearing test signals were calibrated every two months during the study period by an external consultancy company; for details, see Kastelein et al. (2019a). Under test conditions (i.e., water circulation system off, no rain, and Beaufort wind force 4 or below), the ambient 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. This was similar to the level at which previous TTS studies had been conducted (see Kastelein et al., 2012a; 2019a).

Fatiguing Sound - The digitized fatiguing sound (WAV file; sample rate 768 kHz) was played by a laptop computer (Acer, model Aspire 5750) to an external data acquisition card (National Instruments, model USB6259; single channel maximum sample rate 1.25 MHz) using a program written in LabVIEW (National Instruments), which controlled the output of the card in 1-dB steps. This output went via a custom-built ground loop isolator, buffer, and filter to a custom-built passive low-pass filter set at 8 kHz. Finally, the output went to a power amplifier (E&W, model LS5002), which drove the transducer (Lubell, model 1224HP) through its isolation transformer (Lubell, model AC1424HP). The transducer was placed at the far side of the outdoor pool at 1.5 m depth (**Figure 1**). The linearity of the transmitter system for fatiguing sound was checked during each calibration, and was found to deviate from the expected level by at most 1 dB within a 42 dB range.

The fatiguing sound consisted of a continuous wave (CW; duty cycle 100%) of 6.5 kHz (a frequency within the range of some NATO mid-frequency active sonar systems; MFAS, 6-7 kHz sweeps). To determine the fatiguing sound's distribution in the outdoor pool, the SPL of the 6.5 kHz CW was measured at 77 (7 x 11) locations on a horizontal grid of 1 m x 1 m, and at three depths per location on the grid (0.5, 1.0, and 1.5 m below the surface), resulting in a total of 231 measurements in the pool (**Figure 2**). As expected, as the sound was a CW, the SPL varied considerably per location (the biggest difference was 27 dB re 1 μPa at 0.5 m depth), but the mean SPL per depth did not vary much (mean ± SD was: 127 ± 6 dB re 1μ at 0.5 m, 127 ± 5 dB at 1.0 m, and 128 ± 4 dB at 1.5 m). The overall mean SPL, based on the power sum of all 231 SPL measurements, was 127 dB re 1 μPa, so that exposure for one hour to that mean SPL resulted in an SEL of 163 dB re 1 μPa^2 s.

To determine the average SPL received by the study animal, the area where she swam during the exposure periods was compared to the fatiguing sound's SPL distribution in the pool. Videos of sound exposure sessions were analyzed in order to quantify the porpoise's swimming patterns. Each surfacing location of the porpoise was allocated to one of 96 grid squares (8 x 12), each of which corresponded to the middle of each 1 m x 1 m square of the outdoor pool. The animal was found to swim evenly throughout the entire outdoor pool during the low fatiguing sound SPL exposures (i.e.,103 – 115 dB re 1 μPa), so for those SPLs the average fatiguing sound SPL (average of power sum of 231 measurements in the outdoor pool; **Figure 2**) was taken to be representative of her received SPL. However, during exposure to some of the higher SPLs (i.e., 124 – 148 dB re 1 μPa), the study animal kept away from the transducer, and the SPLs of the grid areas where she swam, averaged over location and depth (**Figure 2**), were taken as her mean received SPL. Thus her mean exposure SPLs at those higher source levels were lower than the mean SPL in the pool. During the study, the study animal was exposed to mean SPLs in the range of 103–148 dB re 1 μPa, resulting in a sound exposure level (SEL) range of 139–184 dB re 1 μPa^2s due to one-hour exposures.

Before each exposure, the voltage outputs of the emitting system to the fatiguing sound transducer and of the sound-receiving system were checked with an oscilloscope (Dynatek, model 8300) and a voltmeter (Agilent, model 34401A), by producing the 6.5-kHz continuous signal from the laptop. The underwater acoustic signal was checked using a custom-built hydrophone, a pre-amplifier (Reson, model CCAS1000), and a spectrum analyzer (Velleman, model PCSU1000). If the output values corresponded to those obtained during the SPL calibrations, the SPLs were assumed to be correct and a sound exposure test was performed.



0.5 m

7	124	122	124	117	111	113	123	121	131	121	137	
6	117	110	126	118	117	117	122	126	124	123	126	
5	123	121	124	129	122	126	115	123	133	126	133	
4	112	119	123	123	129	125	120	126	120	130	136	
3	124	126	127	127	130	125	126	118	125	122	122	
2	113	111	123	121	127	120	129	126	130	120	131	
1	119	124	117	119	122	124	120	126	131	133	133	
	1	2	3	4	5	6	7	8	9	10	11	

a)

1.0 m

7	121	128	124	116	114	127	123	124	129	127	132	
6	122	124	118	118	123	125	118	117	127	121	135	
5	125	119	123	123	124	123	123	122	124	131	130	
4	122	121	119	121	125	116	121	124	118	131	134	
3	124	124	124	123	124	119	125	126	129	126	132	
2	126	118	120	127	126	126	124	119	133	133	125	
1	126	118	122	127	129	118	124	126	127	129	135	
	1	2	3	4	5	6	7	8	9	10	11	

b)

1.5 m

7	125	121	118	126	123	126	124	129	123	132	130	
6	120	127	122	123	125	123	127	122	128	129	134	
5	125	119	120	129	125	123	124	122	121	128	140	
4	125	121	119	116	125	129	119	123	128	132	128	
3	125	126	120	123	121	122	121	126	128	137	132	T
2	126	130	122	125	118	122	122	128	124	124	135	
1	126	120	125	123	120	121	124	121	121	124	125	
	1	2	3	4	5	6	7	8	9	10	11	

c)

Figure 2. The fatiguing sound's SPL (dB re 1 μPa) distribution in the outdoor pool, measured at depths of 0.5 m (a), 1.0 m (b) and 1.5 m (c). In (c), T = 0.5 m above the location of the transducer. The numbers in bold in the grey fields indicate 1 m markings on the sides of the pool. The overall mean SPL (n = 231) in the pool in this example was 127 dB re 1 μPa (at this SPL the porpoise used only part of the pool, so her received mean SPL was 124 dB re 1 μPa; Table 2). Mean (± SD) SPL per depth: 127 ± 6 dB re 1μ at 0.5 m, 127 ± 5 dB at 1.0 m and 128 ± 4 dB at 1.5 m (n = 77 per depth).

Hearing Test Signals - Linear up-sweeps (starting and ending at + and - 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 animal was asked to detect before and after exposure to the fatiguing sound. The center frequencies tested were 6.5 kHz (the center frequency of the fatiguing sound), 9.2 kHz (half an octave above the center frequency), and 13 kHz (one octave above the center frequency). The 9.2 kHz signal was used because Kastelein et al. (2014b) showed that a 6.5 kHz continuous tone caused the maximum TTS (measured within 4 min post-exposure) at 9.2 kHz when the SPLav.re. was above 148 dB re 1μPa. The hearing test signals were generated digitally, and the SPL and frequency spectrum near the listening station were checked daily (see Kastelein et al. [2019a] for details). The sounds were produced with a balanced tonpilz piezoelectric acoustic transducer (Lubell, model LL916) through an isolation transformer (Lubell, model AC202). The transducer producing the hearing test signals in the indoor pool was placed at 1 m depth, facing the porpoise's listening station.

Experimental Procedures

One sound exposure test was conducted per day. A complete test consisted of: (1) pre-exposure hearing tests starting at 08:30 h, (2) a one-hour fatiguing sound exposure in the morning or early afternoon, and (3) a number of post-sound exposure hearing tests in the afternoon. Pre-exposure hearing tests were performed in the indoor pool. Data were collected from December 2018 to July 2019, following the protocol developed and explained by Kastelein et al. (2019a).

Hearing thresholds were measured during the post-sound exposure (PSE) periods 1–4 (PSE_{1-4}), 4–8 (PSE_{4-8}), and 8–12 (PSE_{8-12}) min after the sound exposure ended. If hearing had not recovered after 12 min, it was tested again 60 min post-sound exposure (PSE_{60}), and if hearing had not recovered by then, it was tested again 120 min post-sound exposure (PSE_{120}). Hearing tests were stopped once the hearing threshold was less than 2 dB above the pre-exposure threshold level. This was defined as the hearing being fully recovered, based on fluctuations of TTS found in the control tests. The effects of fatiguing sounds of various average received SPLs were tested (**Table 2**). The SPLs were initially tested in increasing order until all SPLs had been tested once; thereafter, the SPLs were tested in random order. Sample sizes per SPL for each hearing test frequency (**Table 2**) were determined based on the swimming patterns of the animal, the magnitude of the TTS found, and the time that was available for the study.

Control tests were conducted in the same way and under the same conditions as sound exposure tests, but without the fatiguing sound exposure. Each control test started with a pre-exposure hearing test session that was followed by "exposure" to the normal, low ambient noise in the outdoor pool for one hour. Post-ambient exposure (PAE; control) hearing test sessions were then performed 1–4 (PAE_{1-4}), 4–8 (PAE_{4-8}), and 8–12 (PAE_{8-12}) min after the ambient noise exposure ended. Five control tests were conducted per hearing test frequency. Control tests were randomly dispersed among the fatiguing sound exposure tests, so that on each test day, either a sound exposure test or a control test was conducted, or, in case hearing had not recovered the previous day, a post-exposure hearing test was conducted. After very high TTSs, one or two extra days were included for safety after hearing had recovered before another sound exposure was conducted.

Hearing Test Procedures

A hearing test trial began with the porpoise stationed at a start/response buoy. In response to a hand signal by its trainer, she swam to a listening station. The porpoise stationed there for a random period of 6–12 s before the signal operator produced the test signal (in signal-present trials). The porpoise then returned to the start/response buoy to indicate that it had heard the signal. A switch from a test signal level that the porpoise responded to (a 'hit') to a level that she did not respond to (a 'miss'), and *vice versa*, was called a 'reversal'. In signal-absent trials, which were randomly dispersed among the signal-present trials, the porpoise was called back to the start/response buoy after a random period of 6–12 s by a whistle signal from its trainer. Each complete hearing test session consisted of ~25 trials (two-thirds signal-present and one-third signal-absent trials) and lasted for up to 12 min (subdivided into three 4-min periods in the first PSE or PAE test session of each animal). Only PSE_{1-4} and PAE_{1-4} test session periods with three or more reversals were used for analysis. The methodology is described in detail by Kastelein et al. (2012a, 2019a).

Data Analysis

The mean rate of pre-stimulus responses ('pre-stimuli') by the porpoise for both signal-present and signal-absent trials (in the latter, the whistle was the stimulus) was calculated as the number of pre-stimuli as a percentage of all trials conducted in each hearing test period. The pre-exposure mean 50% hearing threshold ($PE_{50\%}$) for a hearing test sound was determined by calculating the mean SPL of all (usually around 10) reversal pairs in the pre-exposure hearing session.

TTSs after the sound exposure sessions were calculated by subtracting the mean 50% hearing threshold obtained during the pre-exposure sessions from the mean 50% hearing thresholds obtained during the PSE periods of the same day. Hearing threshold 'shifts' 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 PAE periods of the same day. No TTS occurred in control sessions, so this calculation resulted in a shift that was close to zero.

We define the onset of TTS as occurring at the lowest 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 (which was close to zero). The level of significance was established by conducting a one-way ANOVA on the $TTS_{1\text{-}4}$, separately 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's multiple comparisons (Dunnett, 1964). All analyses were conducted in Minitab 18 and data conformed to the assumptions of the tests used (i.e., variances homogeneous and residuals normally distributed, Zar, 1999).

Results

Swimming Patterns

During the ambient noise level (control sessions) and the lowest four source levels (SLs) of the 6.5 kHz CW exposure (mean received SPLs: 103 – 115 dB re 1 μPa**,** Table 2), porpoise F05 swam throughout the entire pool in each session. Therefore, the mean SPL of the entire pool was calculated to represent the mean SPL that she was exposed to. At six highest SLs (mean received SPLs: 124 – 148 dB re 1 μPa), F05 avoided the area around the transducer in each session, so the mean SPL was calculated only for the part of the pool that she occupied during the exposure periods.

Performance and Pre-stimulus Response Rate

The porpoise was always willing to participate in the hearing tests, even after the one-hour sound exposure periods. However, in a few sessions (~5 %), she did not arrive at the indoor listening station within 1 min after the fatiguing sound stopped, so that the minimum of three reversals could not be obtained for $PSE_{1\text{-}4}$; data from these sessions were discarded. The mean pre-stimulus response rate for both signal-present and signal-absent trials in the hearing tests varied between 3.9% and 7.1% (**Table 1**). The pre-stimulus response rates in the post-exposure periods were of the same order of magnitude as those in the pre-exposure periods and control periods.

Effect of SEL on TTS

The ANOVAs showed that, in all cases, the $TTS_{1\text{-}4}$ was significantly affected by the fatiguing sound's SEL. Comparisons with the control revealed that the statistically significant onset of TTS varied depending on the hearing test frequency (**Table 2**).

Table 1. The pre-stimulus response rates of harbor porpoise F05 in hearing tests during the pre-exposure periods, after exposure to the fatiguing sound (CW of 6.5 kHz; post sound exposure, PSE), and after exposure to low ambient noise (control; post ambient exposure, PAE). All exposure SPLs and hearing test frequencies were pooled for the calculation of percentages. Sample sizes (i.e., total number of hearing trials per period) are shown in parentheses.

	Period					
Fatiguing sound	Pre-exposure	PSE$_{1-4}$	PSE$_{4-8}$	PSE$_{8-12}$	PSE$_{60}$	PSE$_{120}$
	6.3% (1843)	3.3% (551)	5.6% (554)	4.1% (552)	7.1% (434)	6.8% (148)
Control	Pre-exposure	PAE$_{1-4}$	PAE$_{4-8}$	PAE$_{8-12}$	--	--
	5.0% (363)	5.9% (102)	5.9% (101)	3.9% (106)	--	--

Table 2. Mean, SD, and range of TTS$_{1-4}$ in porpoise F05, after exposure for 60 min to a CW of 6.5 kHz at several SELs, quantified at hearing frequencies 6.5, 9.2, and 13 kHz (i.e., the exposure frequency, half an octave above the exposure frequency, and one octave above the exposure frequency). Results from the control sessions are also shown (no TTS occurred). * significant TTS.

Hearing test frequency (kHz)	SPL (dB re 1 μPa)	SEL (dB re 1 μPa^{2}s)	TTS$_{1-4}$ (dB)			Sample size
			Mean	SD	Range	
6.5	Ambient	Control	-0.1	0.4	-0.6 – 0.4	5
	103	139	0.5	1.0	-0.5 – 1.7	4
	109	145	2.9*	1.1	1.6 – 4.0	4
	115	151	3.5*	1.1	2.4 – 4.8	4
	121	157	3.1*	1.2	1.7 – 4.7	4
	124	160	4.4*	1.9	2.0 – 6.5	5
	130	166	3.2*	2.5	0.6 – 6.9	5
	136	172	4.6*	0.3	4.2 – 4.9	4
	142	178	3.6*	0.2	3.3 – 3.9	4
	144	180	3.6*	0.8	2.9 – 4.5	3
	148	184	6.4*	1.2	4.7 – 7.5	4
9.2	Ambient	Control	0.8	1.6	-1.6 – 2.4	5
	121	157	0.3	0.8	-0.8 – 1.1	4
	124	160	2.4	2.2	-0.7 – 4.3	4
	130	166	3.0	1.9	1.8 – 5.8	4
	136	172	2.4	3.0	-0.2 – 7.0	5
	142	178	7.3*	1.4	5.6 – 8.5	5
	144	180	12.5*	2.1	11.1 – 14.0	2
	148	184	14.6*	2.9	10.3 – 16.7	4
13	Ambient	Control	1.5	0.7	0.8 – 2.3	5
	136	172	2.7	1.4	1.0 – 4.4	4
	142	178	3.1	1.4	2.2 – 5.1	4
	144	180	6.4*	1.4	5.5 – 7.4	2
	148	184	10.6*	0.9	9.8 – 11.8	4

For hearing test signals of 6.5 kHz, statistically significant TTS$_{1\text{-}4}$ occurred in harbor porpoise F05 after exposure to SELs of ≥145 dB re 1μPa^{2}s (**Table 2; Figure 3a**). Hearing always recovered within 12 min (**Figure 4a**). For hearing test signals of 9.2 kHz, statistically significant TTS$_{1\text{-}4}$ occurred after exposure to SELs of ≥178 dB re 1μPa^{2}s (**Table 2; Figure 3a**). After exposures of up to 178 dB SEL, recovery of hearing occurred within 12 min, but after exposure to 184 dB SEL, it took around 120 min for F05's hearing to recover (**Figure 4b**). For hearing test signals of 13 kHz, statistically significant TTS$_{1\text{-}4}$ occurred after exposure to SELs of ≥180 dB re 1μPa^{2}s (**Table 2; Figure 3a**). After exposures of up to 172 dB SEL, recovery of hearing occurred within 12 min, but it took between 60 and 120 min for F05's hearing to recover after exposure to 184 dB SEL (**Figure 4c**). For each hearing test frequency the TTS varied some degree (see ranges in **Table 2**), but there was no increasing or decreasing trend during the study period. No change in susceptibility to TTS occurred over the duration of the study. The control sessions showed that the hearing thresholds for the three hearing test signals before and after 60 min exposures to the low ambient noise were very similar (**Figure 4).**

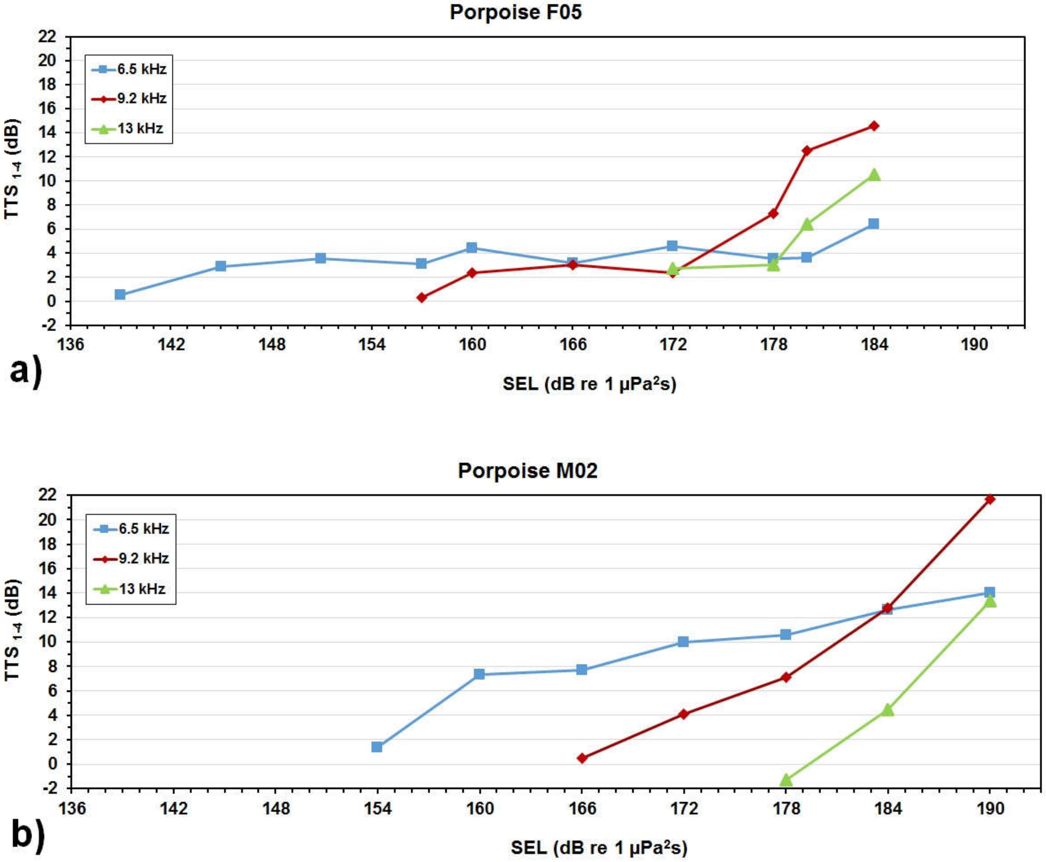


Figure 3. a) TTS$_{1\text{-}4}$ in female harbor porpoise F05 after exposure for 60 min to a CW of 6.5 kHz at several SELs, quantified at hearing frequencies 6.5, 9.2, and 13 kHz (i.e., the exposure frequency, half an octave above the exposure frequency, and one octave above the exposure frequency, respectively). For sample sizes (n = 1 for most SELs), ranges, and SDs, see **Table 2;** for control values, see **Figure 4 & Table 2.** b) TTS$_{1\text{-}4}$ in male harbor porpoise M02 after similar sound exposures in the same pool, but with a sample size of generally only 1 per SEL (adapted from Kastelein et al., 2014b). For SPL values (dB re 1 μPa), subtract 36 dB from the SEL values (Figure in color on-line).

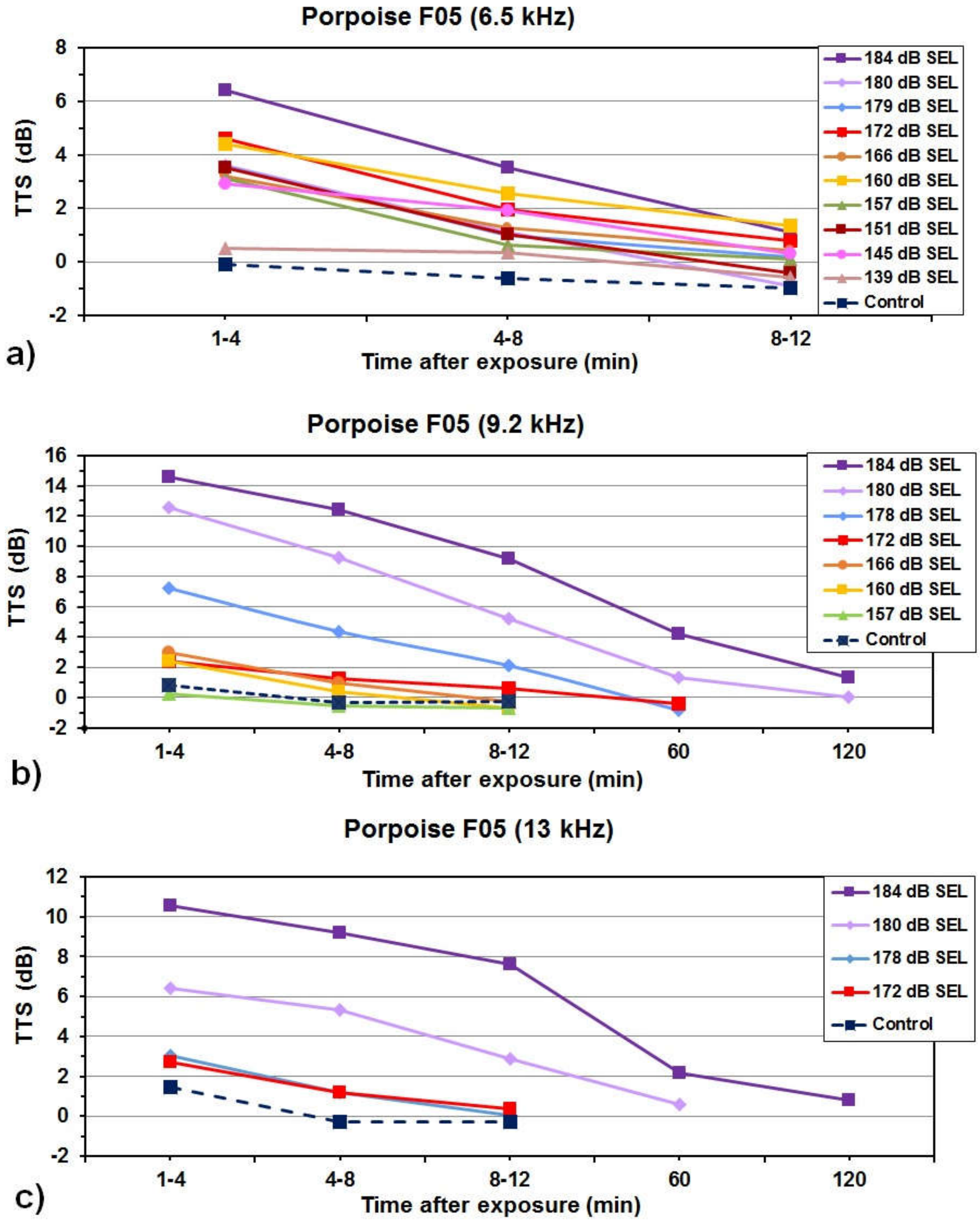


Figure 4. Changes in hearing of harbor porpoise F05 at 6.5 kHz (a), 9.2 kHz (b), and 13 kHz (c) after exposure to a CW of 6.5 kHz for 60 min at several SELs. For sample sizes and SDs, see **Table 2**. Hearing tests started within 1 min after the fatiguing sound stopped. For average received SPLs (dB re 1 μPa), subtract 36 dB re 1 s from the SEL values. (Figure in color online.)

Discussion

Variation in Susceptibility to TTS in Harbor Porpoises

The present study was conducted with only one harbor porpoise. Her hearing thresholds were similar to those of four other porpoises (young males; Kastelein et al., 2017a), which suggests that the study animal had normal hearing for porpoises of her age. However, it is not clear how representative TTS values are for the species. 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).

In the only published TTS study involving two harbor porpoises, in which both animals were tested immediately after the sound stopped, F05 and a juvenile male identified as M06 were exposed to a one-sixth octave noise band centered at 63 kHz. Their hearing was tested 1–4 min after the sound stopped, and relatively small individual differences in susceptibility to TTS were apparent (Kastelein et al., 2019c). Exposure to 180 dB SEL caused 7.3 dB $TTS_{1\text{-}4}$ in M06 and 3.2 dB in F05 at 63 kHz. Exposure to 192 dB SEL caused 5 dB $TTS_{1\text{-}4}$ in M06 and 6.6 dB in F05 at 88.4 kHz. These TTS values are in the same order of magnitude, and, according to Kastelein et al. (2019c), the differences in TTSs between the two animals may have been related to their slightly different swimming patterns (resulting in them experiencing different SELs), to their age difference, or to individual differences in susceptibility to TTS.

When comparing the TTS results of the present study (**Figure 3a**) with those reported by Kastelein et al. (2014b; **Figure 3b**), who exposed male porpoise M02 to the 6.5 kHz CW for the same duration, some similarities and differences are apparent. It should be noted that the TTS values of Kastelein et al. (2014b) were, except for the highest SEL, based on n=1 so statistical analysis to determine TTS onset was not possible due to small sample sizes, so TTS onset was defined as 2 dB (based on previous TTS studies with porpoises in the same quiet hearing test environment). For the hearing test frequency of 6.5 kHz (center frequency of the fatiguing sound), TTS started at a lower SEL in F05 (145 dB re $1\mu Pa^2s$) than in M02 (~160 dB re $1\mu Pa^2s$), but beyond M02's TTS-onset SEL, $TTS_{1\text{-}4}$ was higher in M02 for the same SELs than in F05. Both study animals experienced low TTS at this hearing test frequency. For the hearing test frequency of 9.2 kHz (half an octave above the frequency of the fatiguing sound), the TTS started at a higher SEL in F05 (178 dB re $1\mu Pa^2s$) than in M02 (~172 dB re $1\mu Pa^2s$), and beyond M02's TTS-onset SEL, $TTS_{1\text{-}4}$ values were similar and both animals experienced high TTS. The SEL which caused ~6 dB TTS at this frequency (9.2 kHz) was the same for both animals (176 dB re $1\mu Pa^2s$). For the hearing test frequency of 13 kHz (one octave above the frequency of the fatiguing sound), the TTS-onset SEL was the same in both animals (~180 dB re $1\mu Pa^2s$). The TTS in both animals at this hearing test frequency was similar to that at 9.2 kHz. Despite some differences, the overall TTS patterns of the two animals appear to be similar.

The present study and the study by Kastelein et al. (2014b) raise important questions about conducting and comparing TTS studies. For example, at which hearing frequencies should TTS be measured? Should one focus on the hearing frequency which reaches 6 dB $TTS_{1\text{-}4}$ at the lowest SEL (which is often the center frequency), or on the hearing frequency which shows the highest TTS? Since 6 dB TTS is a small shift from which hearing usually recovers within 12 min after the fatiguing sounds stops (and probably does not have a great ecological impact if it does not occur often), it seems more logical to focus on the hearing frequency that is most likely to be reduced permanently when an animal is exposed to higher SELs. In most cases, this is higher than the center frequency of the fatiguing sound, and often half an octave above the center frequency is chosen. However, both the present study and the study by Kastelein et al. (2014b) suggest that, after very high SELs, the most vulnerable hearing frequency may be as much as one octave above the center frequency of the fatiguing sound. Such levels are not usually generated in noise-induced hearing loss studies with captive marine mammals because of potential hearing damage, so future studies, in which we will test narrow-band continuous sounds, will be focused on the hearing frequency half an octave above the center frequency of the fatiguing sound. These studies will allow the comparison of susceptibility to TTS caused by different frequencies in individual conspecifics and in different marine mammal species.

Susceptibility to TTS in Relation to SPL and Frequency

Data from humans and other terrestrial mammals show that, for moderate and large hearing shifts, the maximum TTS occurs half an octave to one octave above the exposure frequency (Cody & Johnstone, 1981; McFadden, 1986). This has also been observed in several odontocete species that were exposed to tonal and broadband noise (Schlundt et al. 2000, Nachtigall et al., 2004; Finneran et al., 2007; Mooney et al., 2009; Popov et al., 2011; Popov et al., 2013). However, the maximum TTS in a harbor porpoise (M02; Kastelein et al. 2012a), a California sea lion (*Zalophus californianus*; Kastak et al. 2005), and harbor seals (*Phoca vitulina*; Kastak et al. 2005; Kastelein et al. 2012b) that were exposed to octave-band noise occurred at the fatiguing sound's center frequency rather than above it. The relationship between the fatiguing sound's SEL and the hearing frequency showing most TTS is probably related 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 also explain the discrepancies reported by various authors of TTS studies with marine mammals: studies in which the maximum TTS occurred at the exposure frequency typically involved relatively small TTSs, whereas studies in which the maximum TTS occurred half an octave above the center frequency typically involved greater TTSs (Finneran et al., 2007; Popov et al., 2013).

If only those SELs are used which cause 6 dB TTS at half an octave above the center frequency of the fatiguing sound, and all the available (and validly comparable) TTS information on harbor porpoises are combined (**Figure 5**), it appears that, for fatiguing sounds with frequencies below ~6.5 kHz, susceptibility to TTS increases with increasing frequency (Kastelein et al., 2012, 2014a, 2017b) . However, above ~6.5 kHz, it appears that susceptibility to TTS decreases with increasing frequency (Kastelein et al, 2019a;b; present study).

The results of the present study and previous studies, although representing only part of the harbor porpoise's hearing frequency range (1.5–63 kHz), are in agreement with the general conclusions of Finneran & Schlundt (2013) and Popov et al. (2011; 2013): all studies suggest that the susceptibility of odontocete hearing to TTS is frequency-dependent. The pattern of susceptibility is most similar to that found in bottlenose dolphins (*Tursiops truncatus*) by Finneran & Schlundt (2013). However, 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 >63 kHz. Popov et al. (2011; 2013) showed that susceptibility to TTS in Yangtze finless porpoises (*Neophocaena phocaenoides asiaeorientalis*), a species more closely related to the harbor porpoise than the bottlenose dolphin, did not increase with increasing frequency of the fatiguing sound at frequencies >45 kHz. TTS studies in which harbor porpoises are exposed to fatiguing sounds with frequencies >63 kHz and <1 kHz are needed in order to define weighting functions for TTS in this species.

Conclusions

The present study, which partly replicated the study by Kastelein et al. (2014b) using a different harbor porpoise, suggests that susceptibility to TTS due to 6.5 kHz CW exposure is similar in porpoises, with slight individual differences in TTS-onset SELs and TTS magnitudes (no's 8 and 4b in **Figure 5**). Both studies suggest that harbor porpoises that are exposed to sounds of ~6.5 kHz experience TTS onset (defined as 6 dB TTS) at ~ 177 dB re $1\mu Pa^2s$. This suggest that the data from the limited number of animals may be seen as representative for harbor porpoises. However, it is too early to say anything in general about individual differences in TTS susceptibility, as susceptibility to TTS has been measured in only two individual porpoises 1–4 min after exposure to fatiguing sound of only two frequencies (6.5 kHz CW, and one-sixth octave noise band centered at 63 kHz; Kastelein et al., 2019c,. in review, no's 7a & b in **Figure 5**). Therefore we recommend further tests of susceptibility to TTS in more individual harbor porpoises, using fatiguing sounds within the frequency range tested so far (1-63 kHz).

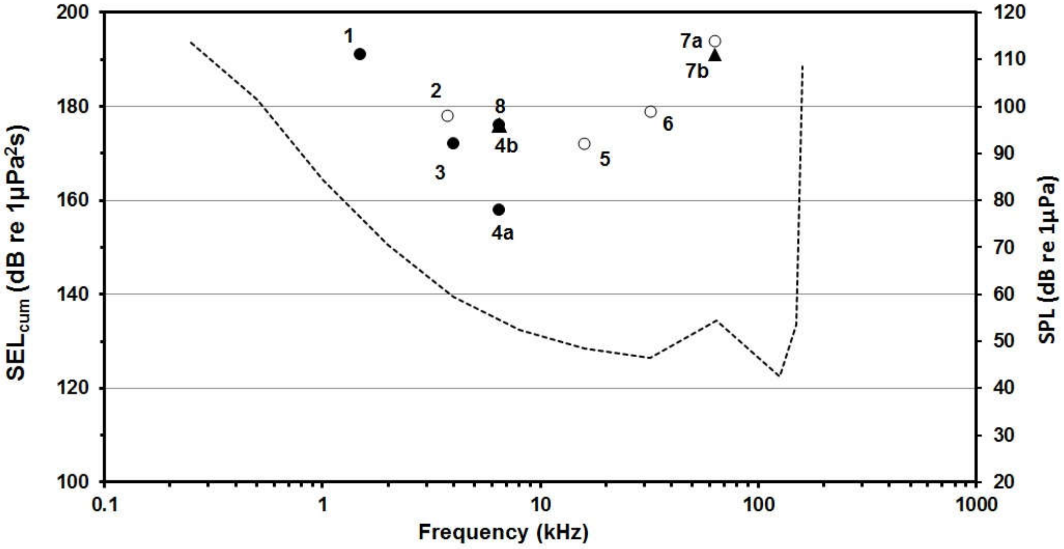


Figure 5. The cumulative SEL (SEL_{cum}) required to cause a mean $\text{TTS}_{1\text{-}4}$ of around 6 dB in harbor porpoises after exposure to: 1) a 1–2 kHz sweep at 100% duty cycle for 60 min (Kastelein et al., 2014a), 2) a 3.5–4.1 kHz 53-C sonar playback sound at 96% duty cycle (Kastelein et al., 2017b), 3) a one-octave noise band centered at 4 kHz at 100% duty cycle (Kastelein et al., 2012), 4a &b) a 6.5 kHz tone at 100% duty cycle (Kastelein et al., 2014b), 5) a one-sixth-octave noise band centered at 16 kHz at 100% duty cycle (Kastelein et al., 2019a), 6) a one-sixth-octave noise band centered at 32 kHz at 100% duty cycle (Kastelein et al., 2019b), 7) a one-sixth-octave noise band centered at 63 kHz at 100% duty cycle (7a: M06 at 88.4 kHz extrapolated and 7b: F05 at 88.4 kHz), and 8) a 6.5 kHz tone at 100% duty cycle (present study). ● = studies with porpoise M02, ○ = studies with porpoise M06, and ▲ = studies with porpoise F05. Also shown as a dashed line is the audiogram of porpoise M02 (Kastelein et al., 2010; right-hand y-axis). Note that numbers 1 – 4a were measured at the center frequency of the fatiguing sound, and numbers 2, 4b, 5, 6, 7a, 7b and 8 were measured half an octave above the center frequency.

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