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Temporary Threshold Shift in a Second Harbor Porpoise (*Phocoena phocoena*) After Exposure to a One-Sixth-Octave Noise Band at 1.5 kHz and a 6.5 kHz Continuous Wave

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Abstract

To determine whether susceptibility to noise-induced temporary hearing threshold shift (TTS) differs between individual harbor porpoises (*Phocoena phocoena*), studies with an 8-year-old male (M02) were repeated by exposing a 9-year-old female (F05) to similar fatiguing sounds. F05 was exposed for one hour to a continuous one-sixth-octave noise band (NB) centered at 1.5 kHz at six sound pressure levels (SPLs; resulting sound exposure level [SEL] range: 180 to 201 dB re 1 µPa^{2}s), and to a 6.5 kHz continuous wave (CW) at 4 to 10 SPLs (resulting SEL range: 139 to 184 dB re 1 µPa^{2}s). To quantify TTS, hearing thresholds for 1.5, 2.1, 3, 6.5, 9.2, and 13 kHz signals were determined before and after exposures. After exposure to the NB at 1.5 kHz, the lowest SELs resulting in significant TTS_{1-4} were 186 dB re 1 µPa^{2}s for 1.5 kHz (1.0 dB), 194 dB re 1 µPa^{2}s for 2.1 kHz (4.7 dB), and 190 dB re 1 µPa^{2}s for 3 kHz (1.5 dB). The highest TTS_{1-4} was 9.3 dB, measured at 2.1 kHz after exposure to SEL 201 dB re 1 µPa^{2}s. After exposure to the 6.5 kHz CW, the lowest SELs resulting in significant TTS_{1-4} were 145 dB re 1 µPa^{2}s for 6.5 kHz (2.9 dB), 178 dB re 1 µPa^{2}s for 9.2 kHz (7.3 dB), and 180 dB re 1 µPa^{2}s for 13 kHz (6.4 dB). Six dB TTS was elicited in F05 at 2.1 kHz after exposure to the NB at 1.5 kHz at SEL 198 dB re 1 µPa^{2}s, and in M02 at 1.5 kHz after exposure to 1 to 2 kHz downsweeps at SEL ~190 dB re 1 µPa^{2}s. The difference in susceptibility to TTS may be due to individual differences in TTS susceptibility and/or differences in the fatiguing sounds (i.e., sweeps, CWs, and NBs). Susceptibility to TTS was similar in both porpoises after exposure to a 6.5 kHz CW: 6 dB TTS was elicited at 9.2 kHz in both animals after exposure to SEL ~176 dB re 1 µPa^{2}s.

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 the species' wide distribution 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 over a wide frequency range (Kastelein et al., 2017b). Therefore, many governments have set, or are in the process of setting, acoustic criteria for high-amplitude sounds produced, for example, by naval sonar systems, seismic surveys, and during construction of offshore wind parks (especially percussion pile driving) 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 to 6.5 kHz; Kastelein et al., 2012a, 2013, 2014a, 2014b, 2015). TTS onset and weighting functions allow important protection measures to be set by government regulators (e.g., by the National Marine Fisheries Service in the United States; NMFS, 2016). As empirical TTS data form the basis of TTS onset functions and weighting functions (Southall et al., 2007, 2019; Finneran, 2015; Tougaard et al., 2016; Houser et al., 2017), it is important to quantify TTS elicited by a wider range of fatiguing sound frequencies as well as in more individual harbor porpoises.

An animal's susceptibility to TTS depends not only on the received sound pressure level (SPL) and the duration of exposure to the fatiguing sound, but also on the sound's frequency (see Finneran, 2015; Houser et al., 2017). For the regulation of underwater anthropogenic sound levels to protect hearing in the harbor porpoise, complete equal-TTS contours are desirable, covering the entire hearing frequency range (~0.5 to 140 kHz) of this species. Since the review by Southall et al. (2019), new TTS studies have been published; for the harbor porpoise, the frequency range for which TTS data on continuous (non-impulsive) sounds are available now spans a bandwidth of 1 to 63 kHz (Kastelein et al., 2012a, 2013, 2014a, 2014b, 2015, 2017a, 2019a, 2019b, 2020).

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 increasing frequency, whereas above ~6.5 kHz, susceptibility to TTS seems to decrease with increasing frequency (see Kastelein et al., 2020). The data are from only three harbor porpoises, however. The observed pattern of TTS-onset SELs could result from the low sample size (generally only one individual has been tested for each fatiguing sound frequency), or be related to individual variation in susceptibility to TTS, as is seen in 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 (a one-sixth-octave noise band [NB] centered at 63 kHz) and had their hearing tested 1 to 4 min after the fatiguing sound stopped showed a similar susceptibility to TTS (Kastelein et al., 2020). However, only this one fatiguing sound frequency has been tested with two individual porpoises; all other frequencies have been tested on single individual animals. Therefore, the goals of the present study are to increase the number of individual harbor porpoises in which TTS due to a particular fatiguing sound frequency has been measured and to gain insight into individual variation in susceptibility to TTS in harbor porpoises. TTS and hearing recovery were quantified in an adult female harbor porpoise using similar fatiguing sounds (a one-sixth-octave NB centered at 1.5 kHz, and 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 an 8-y-old male harbor porpoise (M02; Kastelein et al., 2013, 2014a, 2014b). The effects of the NB at 1.5 kHz and the 6.5 kHz CW were tested at three hearing frequencies per fatiguing sound (1.5, 2.1, and 3 kHz, and 6.5, 9.2, and 13 kHz, respectively; i.e., the center frequency of the fatiguing sound, half an octave above the center frequency, and one octave above the center frequency) because hearing is often affected at frequencies higher than the center frequency of the fatiguing sound (McFadden, 1986).

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 y old (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 (1.5, 2.1, 3, 6.5, 9.2, and 13 kHz) was probably representative of that of similarly aged conspecifics (Kastelein et al., 2017b). Her feeding regime was described by Kastelein et al. (2019b).

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

Acoustics

SPL Measurement Equipment and Ambient Noise—Acoustical terminology follows ISO 18405:2017 (International Organization for Standardization [ISO], 2017). The ambient noise was measured, and the fatiguing sound and hearing test signals were calibrated every 3 mo during the study period by an independent consultancy (for details, see Kastelein et al., 2019b). Under test conditions (i.e., water circulation system off, no rain, and Beaufort wind force 4 or below), the ambient noise level 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, 2013, 2014a, 2014b, 2015, 2017a, 2019a, 2019b, 2020).

Fatiguing Sound—The fatiguing sounds consisted of (1) a continuous (duty cycle 100%) one-sixth-octave NB centered at 1.5 kHz, and (2) a 6.5 kHz CW (duty cycle 100%). Both sounds were within the frequency range of NATO active sonar systems. The digitized fatiguing sounds (WAV files; sample rate 768 kHz) were played by a laptop computer (Model No. 5750; Acer – Aspire, New Taipei City, Taiwan) to an external data acquisition card (Model USB6259; National Instruments, Austin, TX, USA; 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 2 kHz (for the NB at 1.5 kHz) or 8 kHz (for the 6.5 kHz CW). Finally, the output went to a power amplifier (Model LS5002; East & West Sound, Seoul, Korea), which drove the transducer (Model 1424HP; Lubell Labs, Columbus, OH, USA) through its isolation transformer (Model AC1424HP, Lubell Labs). The fatiguing sound transducer was placed in the outdoor pool at 1.5 m depth, either at one end of the pool (for the 6.5 kHz CW and for the lower SPLs of the NB at 1.5 kHz) or in the middle of the side of the pool (for the higher SPLs of the NB at 1.5 kHz; 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.

To determine the fatiguing sounds' distributions in the outdoor pool, one SPL of the NB at 1.5 kHz and one of the 6.5 kHz CW were measured at 77 (7 × 11) locations 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 a total of 231 measurements for each fatiguing sound in the pool (Figure 2). At one location on the grid (2 m from the sound source at 0.5, 1.0, and 1.5 m depth), all SPLs used in the study were measured; the linearity was within 1 dB. As expected for a CW, with the 6.5 kHz CW, the SPL varied considerably per location on the horizontal plane (the largest variation 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 1 µPa at 0.5 m, 127 ± 5 dB at 1.0 m, and 128 ± 4 dB at 1.5 m). The overall mean (based on the power sum of all 231 SPL measurements) SPL of the examples shown in Figure 2 was 170 dB re 1 µPa for the NB at 1.5 kHz (source level: 180 dB re 1 µPa) and 127 dB re 1 µPa for the 6.5 kHz CW (source level: 138 dB re 1 µPa).

To determine the average SPL received by the study animal, the area where she swam during the exposure periods was recorded by a person in the outdoor research cabin next to the pool and was compared to the fatiguing sounds' SPL distributions in the outdoor pool. The study animal was found to swim evenly throughout the entire outdoor pool during exposure to the fatiguing sound at low SPLs (mean 144, 150, 154, and 158 dB re 1 µPa for the NB at 1.5 kHz, and mean 103, 109, and 115 dB re 1 µPa for the 6.5 kHz CW), so for those SPLs, the average fatiguing sound SPL (mean power sum of all 231 measurements in the outdoor pool) was

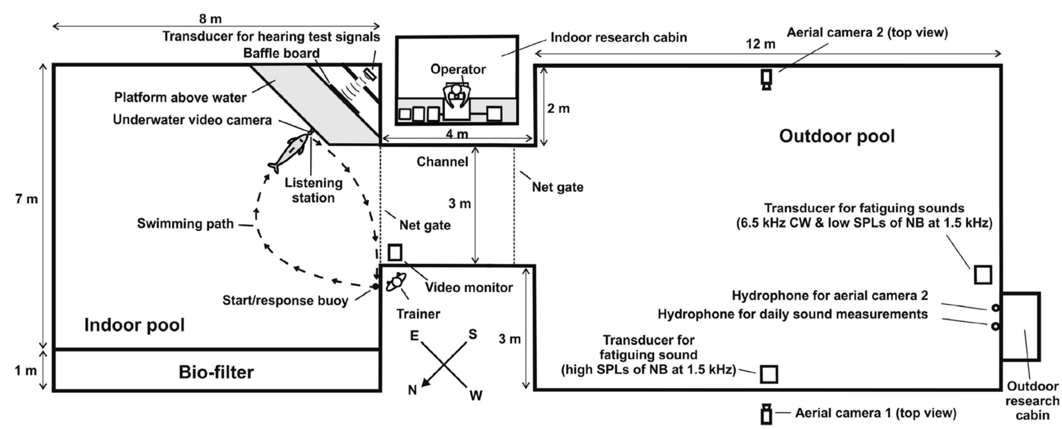

Figure 1. The pool complex in which both the present TTS study with harbor porpoise F05 and the previous TTS studies with harbor porpoise M02 were conducted (Kastelein et al., 2013, 2014a, 2014b). Every day during the present study period, a pre-exposure hearing test was conducted in the indoor pool (testing one of six frequencies: 1.5, 2.1, or 3 kHz before exposure to the noise band [NB] centered at 1.5 kHz; or 6.5, 9.2, or 13 kHz before exposure to the 6.5 kHz continuous wave [CW]). This was followed by a 1-h exposure period in the outdoor pool, then by one or several post-exposure hearing tests in the indoor pool (testing the same frequency as used in the pre-exposure period of that day).

NB at 1.5 kHz (0.5 m)

7	169	169	170	169	168	166	167	167	164	161	162
6	167	172	168	170	170	170	168	167	167	170	167
5	171	172	172	171	171	173	171	168	171	167	169
4	171	170	171	173	172	173	170	173	170	170	166
3	171	169	171	173	173	173	175	171	171	169	165
2	169	172	174	174	171	173	175	173	170	168	163
1	165	172	172	172	172	172	172	170	168	164	161
	1	2	3	4	5	6	7	8	9	10	11

a)

NB at 1.5 kHz (1.0 m)

7	169	165	165	160	161	163	162	165	168	169	171
6	170	168	163	167	163	165	165	165	165	166	171
5	167	165	165	167	166	167	168	170	166	166	170
4	168	168	169	169	168	168	168	165	168	165	162
3	168	168	169	165	170	168	168	171	170	165	162
2	168	167	169	175	178	178	174	161	166	162	162
1	161	165	173	178	178	179	175	165	162	164	159
	1	2	3	4	5	6	7	8	9	10	11

b)

NB at 1.5 kHz (1.5 m, depth of transducer)

7	167	169	169	167	164	167	166	167	167	169	170
6	167	169	172	170	168	169	170	172	169	163	168
5	171	170	170	173	168	173	170	169	162	166	166
4	168	171	169	172	169	172	168	165	170	170	165
3	169	168	174	173	173	174	171	173	169	170	165
2	171	168	170	172	164	169	172	171	170	163	160
1	169	167	164	173	180	180	163	167	164	162	159
	1	2	3	4	5	6 (T)	7	8	9	10	11

c)

6.5 kHz CW (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

d)

6.5 kHz CW (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

e)

6.5 kHz CW (1.5 m, depth of transducer)

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	

f)

Figure 2. Examples of the fatiguing sounds' sound pressure level (SPL; dB re 1 µPa) distributions in the outdoor pool for the NB at 1.5 kHz (a, b & c) and for the 6.5 kHz CW (d, e & f), measured at depths of 0.5, 1.0, and 1.5 m. At the bottom in (c) and on the right in (f), T indicates the location of the fatiguing sound transducer. The numbers in bold in the grey fields indicate 1 m markings on the sides of the pool. The overall mean SPLs (n = 231) in the pool in these examples were 170 dB re 1 µPa for the NB at 1.5 kHz (a, b & c) and 127 dB re 1 µPa for the 6.5 kHz CW (d, e & f; at this SPL, the harbor porpoise used only part of the pool, so her average received SPL was 124 dB re 1 µPa; see "Results"). Mean (± SD) SPL per depth for the NB at 1.5 kHz: 170 ± 3 dB re 1 µPa at 0.5 m, 167 ± 4 dB re 1 µPa at 1.0 m, and 169 ± 4 dB re 1 µPa at 1.5 m; for the 6.5 kHz CW: 127 ± 6 dB re 1 µPa at 0.5 m, 127 ± 5 dB re 1 µPa at 1.0 m, and 128 ± 4 dB re 1 µPa at 1.5 m (n = 77 per depth).

taken to be representative of her received SPL. However, during exposure to the fatiguing sound at all other (higher) SPLs, the harbor porpoise kept away from the transducer, so the SPLs of the grid areas where she swam or floated, averaged over location and depth, were taken as her average received SPL. For the NB at 1.5 kHz, received SPLs were 161 (n = 21) and 165 (n = 5) dB re 1 µPa; for the 6.5 kHz CW, received SPLs were 121 to 144 (n = 126) and 148 (n = 5) dB re 1 µPa. At these higher source levels, the study animal's average received SPLs were lower than the mean SPLs in the pool. Therefore, during the study, the overall mean SPLs received by the study animal were in the range of 144 to 165 dB re 1 µPa, resulting in an SEL range of 180 to 201 dB re 1 µPa^{2}s during 1-h exposures to the NB at 1.5 kHz, and were 103 to 148 dB re 1 µPa, resulting in an SEL range of 139 to 184 dB re 1 µPa^{2}s during 1-h exposures to the 6.5 kHz CW.

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 (Model 2201; Tektronix, Beaverton, OR, USA) and a voltmeter (Model 34401A; Agilent, Santa Clara, CA, USA) by producing the fatiguing sounds from the laptop. The underwater acoustic signal was checked using a custom-built hydrophone, a pre-amplifier (Model CCAS1000; Reson, Slangerup, Denmark), and a spectrum analyzer (Model PCSU1000; Velleman, Gavere, Belgium). 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.

Hearing Test Signals—Linear upsweeps (starting at -2.5% 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 study animal was asked to detect before and after exposure to the fatiguing sound. The center frequencies used for the hearing test signals were the center frequency of the fatiguing sound, half an octave above the center frequency, and one octave above the center frequency. For the NB at 1.5 kHz, these frequencies were 1.5, 2.1, and 3 kHz; for the 6.5 kHz CW, they were 6.5, 9.2, and 13 kHz. Hearing test signals were generated digitally, and the SPL and frequency spectrum near the harbor porpoise's listening station (Figure 1) were checked daily (for details, see Kastelein et al., 2019b). The sounds were produced with a balanced tonpilz piezoelectric acoustic transducer (Model LL916, Lubell Labs) via an isolation transformer (Model AC202, Lubell Labs). The transducer producing the hearing test signals in the indoor pool was placed at 1 m depth, facing the harbor porpoise's listening station.

Experimental Procedures

One sound exposure test was conducted per day. A complete test consisted of (1) a pre-exposure hearing test starting at ~0830 h, (2) a 1-h fatiguing sound exposure in the morning or early afternoon, and (3) a number of post-exposure hearing tests in the afternoon. Hearing tests were performed in the indoor pool. Data were collected from September 2019 to April 2020 for the NB at 1.5 kHz, and from December 2018 to July 2019 for the 6.5 kHz CW, following the protocol developed and explained by Kastelein et al. (2019b). Hearing was defined as being fully recovered once the post-exposure threshold had returned to within 2 dB of the pre-exposure threshold level.

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}). The effects of fatiguing sounds of 6 (NB at 1.5 kHz) and 10 (6.5 kHz CW) average received SPLs were tested (see "Results"). For each fatiguing sound, the SPLs were tested in increasing order until all SPLs had been tested once (for safety reasons); thereafter, the SPLs were tested in random order. Sample sizes per SPL for each hearing test frequency 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 (see "Results" for sample sizes).

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 1 h. 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. Seven control tests were conducted per hearing test frequency for the NB at 1.5 kHz; five control tests were conducted per hearing test frequency for the 6.5 kHz CW. Control tests were randomly dispersed among the exposure tests for each fatiguing sound. On each test day, either a sound exposure test or a control test was conducted.

Hearing Test Procedures

A hearing test trial began with the harbor porpoise stationed at a start/response buoy. In response to a hand signal from her trainer, she swam to the listening station (Figure 1). The porpoise stationed there for a random period of 6 to 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 she had heard the signal. A switch from a test signal level to which the porpoise responded (a "hit") to a level to which she did not respond (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 between 6 to 12 s by a whistle signal from her trainer. Each complete hearing test session consisted of ~25 trials (two-thirds of trials were signal-present and one-third were signal-absent trials) and lasted for up to 12 min (subdivided into three 4-min periods in the first PSE or PAE test session). 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 more detail by Kastelein et al. (2012a, 2019b).

Data Analysis

The mean incidence of pre-stimulus responses, or "pre-stimuli," by the harbor 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 frequency was determined by calculating the mean SPL of all (usually around 10) reversal pairs in the pre-exposure hearing test session.

TTSs after the sound exposure sessions were calculated by subtracting the $PE_{50\%}$ 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 $PE_{50\%}$ 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_{1-4} separately for each fatiguing sound and for each hearing test frequency with the factor SEL (including the control). When the ANOVA produced a significant value overall, the levels were compared to the control by means of Dunnett's (1964) multiple comparisons. All analyses were conducted in *Minitab 18* (Minitab LLC, State College, PA, USA), and data conformed to the assumptions of the tests used (i.e., variances homogeneous and residuals normally distributed; Zar, 1999).

Results

Performance, Pre-stimulus Response Rate, and Variation in TTS

The harbor porpoise was always willing to participate in the hearing tests, both in the morning and after the 1-h sound exposure periods. In a few sessions (~6%), however, she did not arrive at the indoor listening station within 1 min after the fatiguing sound stopped; therefore, the minimum of three reversals could not be obtained for PSE_{1-4}, and 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 0.0 and 6.1% for the NB at 1.5 kHz, and between 3.3 and 7.1% for the 6.5 kHz CW. The pre-stimulus response rates in the post-exposure periods were of the same order of magnitude as those in the pre-exposure and control periods (Table 1).

For each hearing test frequency, the TTS varied to some degree, 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 all hearing test signals before and after 60-min exposures to the low ambient noise were very similar; no TTS occurred in the absence of a fatiguing sound (Table 2).

Effect of SEL on TTS

The ANOVAs showed that, in all cases, the TTS_{1-4} 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).

With the NB at 1.5 kHz as the fatiguing sound, hearing test signals of 1.5 kHz revealed that statistically significant TTS_{1-4} occurred after exposure to SELs of 186 and ≥ 194 dB re 1 μPa^2s (Table 2; Figure 3a). Hearing usually recovered within 12 min, and it always recovered within 60 min (Figure 4a). For hearing test signals of 2.1 kHz, statistically significant TTS_{1-4} occurred after exposure to SELs of ≥ 194 dB re 1 μPa^2s (Table 2; Figure 3a). After exposures to SELs of up to 194 dB, hearing recovered within 12 min; and after 197 and 201 dB, hearing recovered within 60 min (Figure 4b). For hearing test signals of 3 kHz, statistically significant TTS_{1-4} occurred after exposure to SELs of ≥ 190 dB re 1 μPa^2s (Table 2; Figure 3a). After exposures to SELs of 190 to 197 dB, hearing recovered within 8 min, but it took around 60 min for hearing to recover after exposure to an SEL of 201 dB (Figure 4c). For comparison, the $TTSs_{1-4}$ of M02 after exposure for 1 h to continuous 1 to 2 kHz downsweeps (at 1.5 kHz) and a 1.5 kHz CW (at 1.5 and 2 kHz) are shown in Figure 3b.

With the 6.5 kHz CW as fatiguing sound, hearing test signals of 6.5 kHz revealed that statistically significant TTS_{1-4} occurred after exposure to SELs of ≥ 145 dB re 1 μPa^2s (Table 2; Figure 3c). Hearing always recovered within 12 min (Figure 4d). For hearing test signals of 9.2 kHz, statistically significant TTS_{1-4} occurred after exposure to SELs of ≥ 178 dB re 1 μPa^2s (Table 2; Figure 3c). After exposure to an SEL of 178 dB, hearing recovery took 12 min; but after exposure to an SEL of 180 dB, recovery took around 60 min; and after exposure to an SEL of 184 dB, recovery took around 120 min (Figure 4e). For hearing test

Table 1. The pre-stimulus response rates of harbor porpoise F05 in hearing tests during pre-exposure periods, after exposure to the fatiguing sounds (one-sixth-octave noise band [NB] centered at 1.5 kHz or 6.5 kHz continuous wave [CW]; post-sound exposure [PSE]), and after exposure to low ambient noise (control; post-ambient exposure [PAE]). All exposure levels and hearing test frequencies were pooled for the calculation of percentages. Sample sizes (total number of hearing trials per period) are shown in parentheses; subscript numbers indicate the time periods (in minutes after fatiguing sound or ambient noise exposure) during which the hearing tests were conducted.

	Period					
Fatiguing sound (NB at 1.5 kHz)	Pre-exposure 3.8% (1,408)	PSE_{1-4} 5.9% (455)	PSE_{4-8} 4.9% (485)	PSE_{8-12} 4.1% (508)	PSE_{60} 3.0% (202)	PSE_{120} 0.0% (34)
Control	Pre-exposure 3.4% (447)	PAE_{1-4} 3.3% (153)	PAE_{4-8} 5.4% (149)	PAE_{8-12} 6.1% (164)	-- --	-- --
Fatiguing sound (6.5 kHz CW)	Pre-exposure 6.3% (1,843)	PSE_{1-4} 3.3% (551)	PSE_{4-8} 5.6% (554)	PSE_{8-12} 4.1% (552)	PSE_{60} 7.1% (434)	PSE_{120} 6.8% (148)
Control	Pre-exposure 5.0% (363)	PAE_{1-4} 5.9% (102)	PAE_{4-8} 5.9% (101)	PAE_{8-12} 3.9% (106)	-- --	-- --

Table 2. Mean, SD, and ranges of TTS_{1-4} in F05 after exposure for 60 min to fatiguing sounds (a one-sixth-octave NB centered at 1.5 kHz or a 6.5 kHz CW) at several SELs, quantified at hearing frequencies corresponding to the center frequency of the fatiguing sound, half an octave above the center frequency, and one octave above the center frequency. Mean SPLs and SELs shown are those experienced by the harbor porpoise as she swam freely in the pool. Results from the control sessions (exposure to the low ambient noise level) are also shown (no TTS occurred). * = statistically significant TTS.

Fatiguing sound	Hearing test frequency (kHz)	Mean SPL (dB re 1 µPa)	Mean SEL (dB re 1 µPa²s)	TTS_{1-4} (dB) Mean	SD	Range	Sample size
NB at 1.5 kHz	1.5	Ambient	Ambient	-0.2	0.5	-0.9-0.3	7
		144	180	0.6	1.0	-0.7-1.7	4
		150	186	1.0*	0.9	-0.2-1.8	4
		154	190	0.2	0.5	-0.4-0.8	4
		158	194	1.3*	0.4	1.0-1.9	4
		161	197	2.9*	0.2	2.6-3.2	4
		165	201	7.6*	0.2	7.5-7.8	2
NB at 1.5 kHz	2.1	Ambient	Ambient	0.2	1.0	-1.5-1.4	7
		144	180	0.0	2.1	-2.3-2.7	4
		150	186	1.0	0.9	0.0-2.1	4
		154	190	1.4	0.9	0.3-2.3	4
		158	194	4.7*	1.0	4.1-6.2	4
		161	197	5.4*	1.5	3.9-8.0	5
		165	201	9.3*	0.4	9.0-9.8	4
NB at 1.5 kHz	3.0	Ambient	Ambient	-0.2	0.6	-0.8-1.0	7
		144	180	-0.4	--	--	1
		150	186	0.4	0.8	-0.6-1.3	4
		154	190	1.5*	1.4	-0.4-2.8	4
		158	194	2.2*	0.2	2.0-2.5	4
		161	197	3.5*	0.7	2.5-4.2	4
		165	201	6.2*	1.0	5.1-7.0	3
6.5 kHz CW	6.5	Ambient	Ambient	-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
6.5 kHz CW	9.2	Ambient	Ambient	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
6.5 kHz CW	13	Ambient	Ambient	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

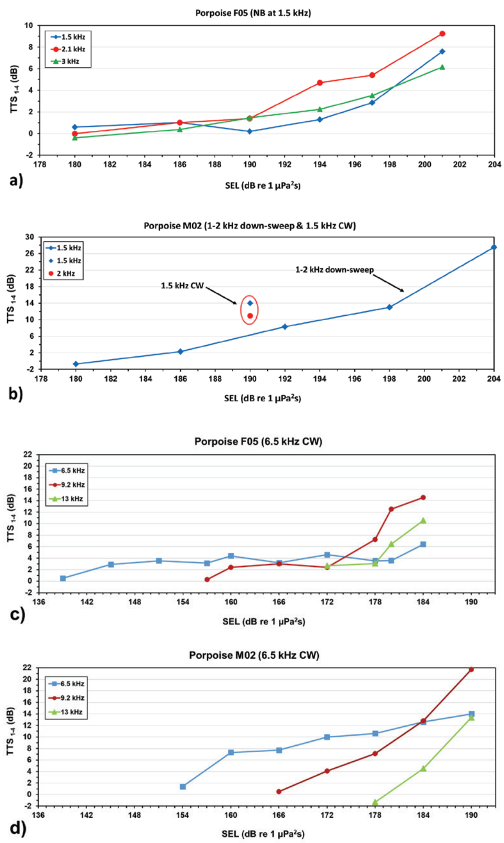

Figure 3. (a) Mean $TTS_{1\text{-}4}$ in F05 after exposure for 60 min to a continuous one-sixth-octave NB centered at 1.5 kHz at several SELs, quantified at hearing frequencies 1.5, 2.1, and 3 kHz (the center frequency of the fatiguing sound, half an octave above the center frequency, and one octave above the center frequency). For sample sizes, ranges, and SDs, see Table 2; for control values, see Figure 4 & Table 2. (b) Mean $TTS_{1\text{-}4}$ in M02 in the same pool after exposure to a 1.5 kHz CW (only tested at 190 dB SEL; *n* = 7 per hearing frequency; data from Kastelein et al., 2013), and continuous 1 to 2 kHz downsweeps (*n* = 1 per SEL; TTS only measured at 1.5 kHz; data from Kastelein et al., 2014b). Note the different scales on the y-axes in (a) and (b). (c) Mean $TTS_{1\text{-}4}$ in F05 after exposure for 60 min to a 6.5 kHz CW at several SELs, quantified at hearing frequencies 6.5, 9.2, and 13 kHz (the center frequency of the fatiguing sound, half an octave above the center frequency, and one octave above the center frequency). For sample sizes (*n* = 4 or *n* = 5 for most SELs), ranges, and SDs, see Table 2; for control values, see Figure 4 & Table 2. (d) Mean $TTS_{1\text{-}4}$ in M02 after similar sound exposures in the same pool but with a sample size of generally only one per SEL (data from Kastelein et al., 2014a). For SPL values (dB re 1 µPa), subtract 36 dB re 1 s from the SEL values.

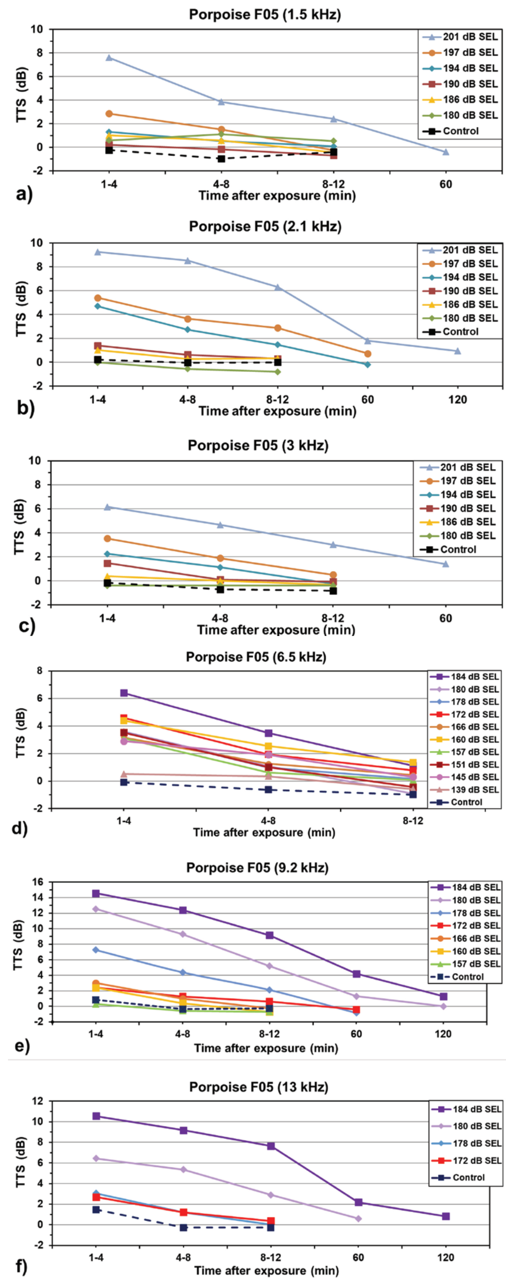

Figure 4. Changes over time in mean threshold shift of F05 at 1.5 kHz (a), 2.1 kHz (b), and 3 kHz (c) after exposure to a continuous one-sixth-octave NB centered at 1.5 kHz for 60 min at several SELs, and at 6.5 kHz (d), 9.2 kHz (e), and 13 kHz (f) after exposure to a 6.5 kHz CW for 60 min at several 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.

signals of 13 kHz, statistically significant TTS_{1-4} occurred after exposure to SELs of ≥ 180 dB re 1 µPa²s (Table 2; Figure 3c). It took between 60 and 120 min for F05's hearing to recover after exposure to SELs ≥ 180 dB (Figure 4f). For comparison, the TTS_{1-4} at 6.5, 9.2, and 13 kHz of M02 after exposure for 1 h to the same 6.5 kHz CW is shown in Figure 3d.

Discussion

Individual Variation in Susceptibility to TTS in Harbor Porpoises

The present study was conducted with only one harbor porpoise, identified as F05. Her hearing thresholds were similar to those of four other harbor porpoises (young and adult males, including M02; Kastelein et al., 2017b), which suggests that F05 had normal hearing for porpoises of her age and was representative of the species, and that results between studies are comparable as far as hearing sensitivity is concerned. In the present study, F05 was exposed to a one-sixth-octave NB centered at 1.5 kHz and to a 6.5 kHz CW. These fatiguing sounds were in the same frequency bands of the sounds to which M02 was exposed: a 1.5 kHz CW (Kastelein et al., 2013), 1 to 2 kHz downsweeps (Kastelein et al., 2014b), and a 6.5 kHz CW (Kastelein et al., 2014a). In all studies, the animals were exposed to continuous sound for at least 60 min in the same quiet test environment, using the same audiometric technique.

For the fatiguing sounds centered at ~1.5 kHz, with hearing test frequency 1.5 kHz, statistically significant TTS started at a similar SEL in F05 as in M02 (186 dB re 1 µPa²s with 1 to 2 kHz downsweeps; Kastelein et al., 2014b); but at SELs above 186 dB, TTS_{1-4} was greater in M02 for the same SELs than in F05 (Figures 3a & b). The SEL that caused ~6 dB TTS (a marker of TTS onset used by Finneran, 2015) at 1.5 kHz was ~190 dB re 1 µPa²s in M02 (Kastelein et al., 2014b) and 200 dB re 1 µPa²s in F05 (present study). Thus, the SEL required to elicit 6 dB TTS using a fatiguing sound in the 1.5 kHz frequency range was at least 10 dB higher in F05 than in M02 (when measured at 1.5 kHz, the center frequency of the fatiguing sound). However, in the present study, with the one-sixth-octave NB centered at 1.5, the sample size for each SEL was generally four, whereas Kastelein et al. (2014b) had a sample size of one per SEL with 1 to 2 kHz downsweeps as the fatiguing sounds. The higher frequencies in the 1 to 2 kHz downsweeps may have resulted in the greater susceptibility to TTS found by Kastelein et al. (2014b), as in the ~1 to 6 kHz frequency region, the higher the fatiguing sound's frequency, the greater the susceptibility to TTS (Kastelein et al., 2020).

In the present study, hearing was tested at three frequencies, which revealed that the greatest TTS occurred not at 1.5 kHz but at half an octave above this frequency (at 2.1 kHz). After exposure to continuous 1 to 2 kHz downsweeps, Kastelein et al. (2014b) only tested the hearing of M02 at 1.5 kHz, so the frequency at which the highest TTS would have occurred is unknown (Figure 3b). Kastelein et al. (2013) exposed M02 to a 1.5 kHz CW but only at one SEL (190 dB re 1 µPa²s). TTS_{1-4} was 14 dB at 1.5 kHz and 11 dB at 2 kHz, and no TTS occurred at higher frequencies (Figure 3b). In the present study with F05, 190 dB SEL elicited 0.2 dB TTS at 1.5 kHz and only 1.4 dB TTS_{1-4} at 2.1 kHz. Thus, after exposure to an SEL of 190 dB re 1 µPa²s, more TTS_{1-4} was elicited in M02 by the 1.5 kHz CW (Kastelein et al., 2013) than by the 1 to 2 kHz downsweeps (Kastelein et al., 2014b) or by the one-sixth-octave NB centered at 1.5 kHz (present study with F05). It is unclear whether the observed differences in TTS are due to individual differences in TTS susceptibility or differences in the fatiguing sound types (i.e., downsweep, CW, and NB) centered at 1.5 kHz.

For the fatiguing sounds centered at ~6.5 kHz, better comparisons are possible, as the only differences are the study animal and the sample sizes per SEL (Kastelein et al., 2014a). For the hearing test frequency of 6.5 kHz (the center frequency of the fatiguing sound), statistically significant TTS occurred at a lower SEL in F05 (145 dB re 1 µPa²s; present study) than in M02 (~160 dB re 1 µPa²s; Kastelein et al., 2014a); however, at SELs above 160 dB, TTS_{1-4} was greater 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), TTS started at a higher SEL in F05 (178 dB re 1 µPa²s) than in M02 (~172 dB re 1 µPa²s); while at SELs above 172 dB, TTS_{1-4} values were similar, and both animals experienced high TTS (Figures 3c & d; Kastelein et al., 2014a; present study). The SEL that caused ~6 dB TTS at 9.2 kHz was the same for both study animals (~176 dB re 1 µPa²s). For the hearing test frequency of 13 kHz (one octave above the frequency of the fatiguing sound), the SEL that caused TTS at ~6 dB was also similar in both study animals (~180 dB re 1 µPa²s). The severity of TTS in both animals at this hearing test frequency was similar to that at 9.2 kHz. Thus, the overall pattern of TTS onset, magnitude, and recovery in both animals was similar after exposure to a 6.5 kHz CW. The similarities in study methodology suggest that the differences found, especially at 6.5 kHz, are due to individual variation in susceptibility to TTS. In the only published study in which TTS in two harbor porpoises was quantified immediately after the fatiguing sound stopped, F05 and a juvenile male, M06, were exposed to a one-sixth-octave NB centered at 63 kHz (Kastelein et al., 2020). The small (1.6 dB at 88.4 kHz) 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 their susceptibility to TTS (Kastelein et al., 2020). Individual variation shows that replication is important in hearing studies, as variation in the hearing of healthy harbor porpoises is unquantified.

Susceptibility to TTS in Relation to SEL 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 frequency of the fatiguing sound (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, 2013). However, when 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) were exposed to octave-band noise, the maximum TTS 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 SPL of the fatiguing sound increases, the affected hearing range becomes broader. This relationship may also explain variation in TTS in marine mammals reported by various researchers; studies in which the maximum TTS occurred at the fatiguing sound's center 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 the center frequency and half an octave above the center frequency of the fatiguing sound, so that all the available directly comparable TTS information on harbor porpoises is presented (Figure 5), it appears that, for fatiguing sounds with frequencies

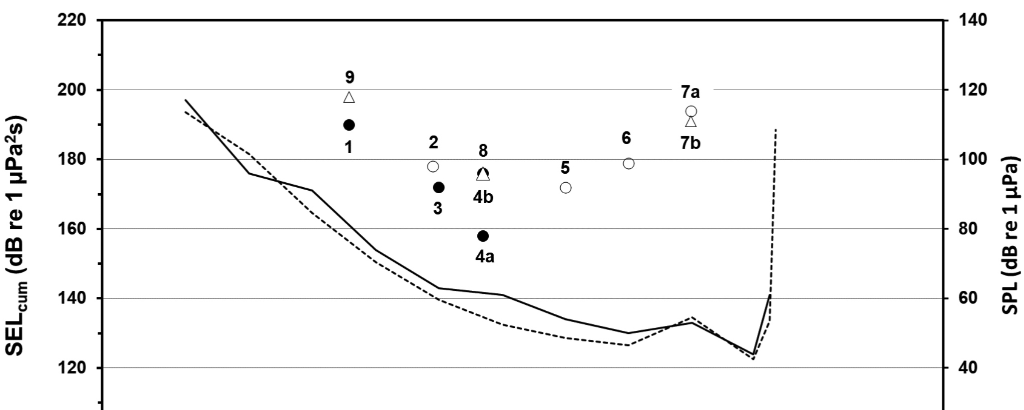

Figure 5. The cumulative SEL (SEL_{cum}) required to cause a mean TTS_{1-4} of around 6 dB in harbor porpoises after 60 min exposure to (1) a 1 to 2 kHz downsweep (Kastelein et al., 2014b), (2) a 3.5 to 4.1 kHz 53-C sonar playback sound (Kastelein et al., 2017a), (3) a one-octave NB centered at 4 kHz (Kastelein et al., 2012a), (4a & 4b) a 6.5 kHz CW (Kastelein et al., 2014a), (5) a one-sixth-octave NB centered at 16 kHz (Kastelein et al., 2019b), (6) a one-sixth-octave NB centered at 32 kHz (Kastelein et al., 2019a), (7a & 7b) a one-sixth-octave NB centered at 63 kHz (7a: M06 at 88.4 kHz extrapolated; 7b: F05 at 88.4 kHz; Kastelein et al., 2020), (8) a 6.5 kHz CW (present study), and (9) a one-sixth-octave NB centered at 1.5 kHz (present study). ● = studies with M02, ○ = studies with M06, and △ = studies with F05. In studies 1, 3, and 4a, hearing was quantified at the center frequency of the fatiguing sound; in studies 2, 4b, 5, 6, 7a, 7b, 8, and 9, it was quantified half an octave above the center frequency. The duty cycle was 100% in all studies except study 2, in which it was 96% (this data point may have been situated slightly lower if 100% duty cycle had been used). Also shown are the audiograms of M02 (dashed line) and F05 (solid line; Kastelein et al., 2010, 2017b; right-hand Y axis).

below ~6.5 kHz, susceptibility to TTS increases with increasing frequency (Kastelein et al., 2012a, 2014b, 2017a, present study). However, above ~6.5 kHz, it appears that susceptibility to TTS decreases with increasing frequency (Kastelein et al., 2019a, 2019b, 2020).

The results of the present study and previous studies represent only part of the harbor porpoise's hearing frequency range (1.5 to 63 kHz of the ~0.5 to 140 kHz hearing range), but they support the conclusions of Popov et al. (2011, 2013) and Finneran & Schlundt (2013) that the susceptibility of odontocete hearing to TTS is frequency dependent. In harbor porpoises, the pattern of susceptibility is most similar to that found in bottlenose dolphins (*Tursiops truncatus*) by Finneran & Schlundt (2013). However, there are only a small number of TTS studies in harbor porpoises, and it is not known whether this frequency dependence also applies to fatiguing sounds with frequencies < 1.5 kHz or > 63 kHz. Popov et al. (2011, 2013) showed that susceptibility to TTS in the Yangtze finless porpoise (*Neophocaena phocaenoides asiaeorientalis*), an odontocete 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.

Directions for Future Studies

The study by Kastelein et al. (2014a) and the present study raise important questions about conducting and comparing TTS studies. For example, at which hearing frequencies should TTS be measured? Should research be focused on the hearing frequency at which ~6 dB TTS_{1-4} is reached at the lowest fatiguing sound's SEL (often the center frequency), on the hearing frequency at which statistically significant TTS (relative to the control) occurs at the lowest SEL, or on the hearing frequency which shows the highest TTS regardless of the SEL? Since 6 dB TTS is a small shift from which harbor porpoise hearing usually recovers within 12 min after the fatiguing sound stops, and which probably has limited ecological impact unless it occurs repeatedly, it seems more logical to focus on the hearing frequency that is most likely to show permanent damage if a porpoise is exposed to higher SELs. In most cases, this hearing frequency is above the center frequency of the fatiguing sound; half an octave above the center frequency is often chosen in studies. However, the study by Kastelein et al. (2014a) and the present study show that, at one octave above the center frequency of the fatiguing sound, TTS started to occur at higher SELs; and at higher SELs, it increased more with increasing SELs than at half an octave above the center frequency of the fatiguing sound (after exposure to both the NB at 1.5 kHz and the 6.5 kHz CW). After exposure to very high SELs, the hearing frequency most susceptible to TTS in harbor porpoises may be one octave, rather than half an octave, above the center frequency of the fatiguing sound. Such high SELs are not usually generated in noise-induced hearing loss studies of captive marine mammals because of the risk of permanent hearing damage. Future studies in which narrow-band continuous sounds will be tested on F05 should therefore be focused on the hearing frequency half an octave above the center frequency of the fatiguing sound. These future studies will allow the comparison of susceptibility to TTS caused by fatiguing sounds of different frequencies between individual conspecifics as well as between different marine mammal species.

It is recommended to test the effects of different sound types (i.e., sweeps, CWs, and NBs) on the susceptibility of harbor porpoise hearing and to determine the affected hearing frequencies relative to the frequency (band) of the sound type to which the animals were exposed. TTS studies in which harbor porpoises are exposed to fatiguing sounds with frequencies > 63 kHz and < 1.5 kHz are also needed to define weighting functions for TTS in this species based on data for its entire hearing range.

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