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Temporary Hearing Threshold Shift at Ecologically Relevant Frequencies in a Harbor Porpoise (*Phocoena phocoena*) Due to Exposure to a Noise Band Centered at 88.4 kHz

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Abstract

Susceptibility to temporary hearing threshold shift (TTS) in harbor porpoises (*Phocoena phocoena*) depends on the frequency of the fatiguing sound causing the shift. TTS in harbor porpoises has been tested for sounds within the 1 to 63 kHz frequency range. Susceptibility to TTS caused by sounds of ~88 kHz is ecologically relevant since these sounds are expected to affect hearing in the frequency range of harbor porpoise echolocation signals. TTS was quantified in a female porpoise after exposure for 1 h to a continuous one-sixth-octave noise band centered at 88.4 kHz, at average received sound pressure levels of 137 to 161 dB re 1 µPa (resulting sound exposure levels [SELs]: 173 to 197 dB re 1 $\mu Pa^2 s$). To quantify TTS and recovery, hearing thresholds for 88.4, 100, and 125 kHz tonal signals were determined before and after exposure. Control trials were used as a baseline and to determine which exposure levels resulted in statistically significant TTS in the 4 min after the fatiguing sound stopped (TTS_{1-4}). At 88.4 kHz, the lowest SEL that resulted in significant TTS_{1-4} (3.6 dB) was 185 dB re 1$\mu Pa^2 s$; at 100 kHz, the lowest SEL that resulted in significant TTS_{1-4} (5.2 dB) was 191 dB re 1 $\mu Pa^2 s$; and at 125 kHz, the lowest SEL that resulted in significant TTS_{1-4} (5.4 dB) was 191 dB re 1 $\mu Pa^2 s$. At higher SELs, the TTS at this frequency remained similar. The highest TTS_{1-4} (13.1 dB) occurred at 100 kHz after exposure to an SEL of 197 dB re 1 $\mu Pa^2 s$. In most cases, hearing recovered within 12 min after the fatiguing sound stopped; in the remaining cases, recovery took less than 1 h. TTS onset (defined as 6 dB TTS; Southall et al., 2019) occurred after exposures to SELs of ~191 dB re 1 $\mu Pa^2 s$ (when hearing was measured at 100 kHz, one third of an octave above the center frequency of the fatiguing sound).

Key Words: anthropogenic noise, audiogram, frequency weighting, harbor porpoise, *Phocoena phocoena*, hearing, hearing damage, hearing loss, hearing sensitivity, odontocete, temporary threshold shift, TTS

Introduction

The harbor porpoise (*Phocoena phocoena*) is of particular interest when studying the effects of anthropogenic underwater sound on marine mammals, as this odontocete species not only has a wide distribution area in the coastal waters of the northern hemisphere (Bjorge & Tolley, 2008) but also possesses acute hearing (i.e., low hearing thresholds) in a wide frequency range (Kastelein et al., 2017b). The harbor porpoise appears to be particularly susceptible to temporary hearing threshold shifts (TTSs) caused by fatiguing sounds (e.g., from vessel traffic, pile driving, seismic surveys, detonations, and sonar), as TTS onset occurs at lower sound exposure levels (SELs) in the harbor porpoise than in the other odontocete species that have been tested (Finneran, 2015; Tougaard et al., 2016; Houser et al., 2017).

Depending on exposure parameters, sound-induced TTSs vary in magnitude and duration, and have the potential to compromise feeding, orientation, communication, and predator detection in wild harbor porpoises and other marine mammals that mainly rely on acoustics for these life functions (e.g., Au, 1993). Therefore, TTSs may negatively impact individual health, reproduction, and survival, even if permanent hearing threshold shift does not occur. In the long term, TTSs could have adverse population effects.

As susceptibility to TTS depends not only on the fatiguing sound's received sound pressure level (SPL) and the exposure duration but also on the sound's frequency (see Finneran, 2015), 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 the regulation of underwater acoustic levels in areas where harbor porpoises occur, complete equal-TTS susceptibility contours are desirable, covering the entire frequency range of hearing in the harbor porpoise (i.e., 0.5 to 140 kHz). Within the 1 to 63 kHz range, an equal-TTS susceptibility contour for the following nine frequencies has been established: (1) 1.5 kHz, (2) 1 to 2 kHz, (3) 4 kHz, (4) 3.5 to 4.1 kHz, (5) 6 to 7 kHz, (6) 6.5 kHz, (7) 16, (8) 32, and (9) 63 kHz (Kastelein et al., 2012, 2013, 2014a, 2014b, 2015b, 2017a, 2019a, 2019b, 2020a, 2020b). However, susceptibility to TTS in the harbor porpoise has not been assessed in the frequency range that includes the peak frequency of its echolocation (~125 kHz; Møhl & Andersen, 1973). Once quantification of susceptibility to TTS over the entire hearing range is complete, it will be possible to model a research-based auditory weighting curve for harbor porpoises and other odontocetes that echolocate at high frequencies (Southall et al., 2019). Weighting is applied to measured sound levels in an attempt to account for the relative susceptibility to TTS of an animal, as the ear's susceptibility to TTS varies with frequency within the hearing range.

The present study builds upon our previous TTS research by investigating the susceptibility to TTS of harbor porpoises after exposure to fatiguing sounds centered at 88.4 kHz. In odontocetes, TTS usually occurs half an octave above the frequency of the narrow-band fatiguing sound (Popov et al., 2011, 2013; Finneran, 2015; Kastelein et al., 2014b, 2019a, 2019b, 2020a, 2020b), so fatiguing sounds of 88.4 kHz are expected to affect hearing at the peak frequency of echolocation clicks produced by the harbor porpoise (~125 kHz; Møhl & Andersen, 1973). Sounds with frequencies in the 88.4 kHz range include biological sounds such as echolocation signals of bottlenose dolphins (*Tursiops truncatus*; Au, 1993) and anthropogenic sounds such as some types of fish-finding sonars (range: 20 to 200 kHz; Discovery of Sound In The Sea [DOSITS], 2019). TTS and the rate of hearing recovery were quantified as functions of SEL and hearing test frequency in a female harbor porpoise after exposure to a one-sixth-octave noise band (NB) centered at 88.4 kHz. The goal was to increase the frequency range for which an equal-TTS susceptibility contour for harbor porpoises can be generated (see Houser et al., 2017) to improve their regulatory protection.

Methods

Study Animal and Site

The study animal, a previously stranded and rehabilitated adult female harbor porpoise (identified as F05; age: ~8 y old, body mass: ~41 kg, body length: 154 cm, and girth at axilla: ~79 cm), had participated in previous studies of TTS induced by sounds of 3.5 to 4.1, 16, 32, and 63 kHz (Kastelein et al., 2017a, 2019a, 2019b, 2020a). These previous studies did not compromise her auditory ability, and her hearing thresholds in the frequency range tested in the present study (88.4 to 125 kHz) are representative of those of similar-aged harbor porpoises (Kastelein et al., 2017b).

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, consisting of an outdoor pool (12 m × 8 m; 2 m deep) connected via a channel (4 m × 3 m; 1.4 m deep) to an indoor pool (8 m × 7 m; 2 m deep). 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 (for calibration methods, 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 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 with harbor porpoises had been conducted (see Kastelein et al., 2012, 2013, 2014a, 2014b, 2015a, 2015b, 2017a, 2019a, 2019b, 2020a, 2020b).

Fatiguing Sound—The digitized fatiguing sound was produced, transmitted, calibrated, and checked before each exposure session, as described by Kastelein et al. (2019b). The fatiguing sound consisted of a continuous (duty cycle 100%) one-sixth-octave Gaussian white noise band (NB), centered at 88.4 kHz (bandwidth: 78.8 to 99.2 kHz). Ideally, a 88.4 kHz tone would have been used, but in a pool, a pure tone can lead to a very heterogeneous sound field due to reverberation. Therefore, instead of a tonal signal, a very narrow NB was used. The center frequency was selected based on previous TTS studies (e.g., Popov et al., 2011, 2013; Finneran, 2015; Kastelein et al., 2014b, 2019a, 2019b, 2020a, 2020b) in which the highest TTS occurred half an octave above the center frequency of the fatiguing sound. Half an octave above 88.4 kHz is ~125 kHz, which, for harbor porpoises, is in the range of most sensitive hearing (Kastelein et al., 2017b); it also is the peak frequency of their echolocation (Møhl & Andersen, 1973).

To determine the fatiguing sound's distribution in the outdoor pool, the SPL of the NB 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 a total of 228 measurements in the pool (Figure 1). Differences in mean SPL per depth (based on the power sum) were minimal (e.g., 144 ± 3 dB at 0.5 m, 144 ± 2 dB at 1.0 m, and 143 ± 1 dB at 1.5 m deep; Figures 1a, b & c, respectively). In the example shown in Figure 1, the average SPL of all 228 measurements based on the power sum was 144 ± 2 dB re 1 µPa.

0.5 m

7	**141**	**142**	**143**	**144**	**144**	**143**	**144**	**144**	**143**	**142**	**141**
6	**141**	**143**	**142**	**145**	**146**	**145**	**146**	**145**	**142**	**143**	**141**
5	**142**	**142**	**143**	**146**	148	149	148	**146**	**143**	**142**	**142**
4	**142**	**144**	**144**	**147**	152		152	**147**	**144**	**144**	**142**
3	**139**	**141**	**142**	**146**	143	148	143	**146**	**142**	**141**	**139**
2	**140**	**142**	**144**	**143**	**143**	**144**	**143**	**143**	**144**	**142**	**140**
1	**139**	**140**	**142**	**143**	**143**	**141**	**143**	**143**	**142**	**140**	**139**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**

a)

1.0 m

7	**141**	**142**	**143**	**144**	**145**	**144**	**145**	**144**	**143**	**142**	**141**
6	**141**	**143**	**142**	**145**	**146**	**145**	**146**	**145**	**142**	**143**	**141**
5	**141**	**143**	**141**	**144**	148	149	148	**144**	**141**	**143**	**141**
4	**143**	**142**	**145**	**142**	145	**T**	145	**142**	**145**	**142**	**143**
3	**142**	**142**	**143**	**145**	145	148	145	**145**	**143**	**142**	**142**
2	**142**	**143**	**142**	**143**	**144**	**143**	**144**	**143**	**142**	**143**	**142**
1	**141**	**142**	**142**	**143**	**144**	**143**	**144**	**143**	**142**	**142**	**141**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**

b)

1.5 m

7	**140**	**142**	**142**	**143**	**143**	**144**	**143**	**143**	**142**	**142**	**140**
6	**142**	**143**	**143**	**144**	**144**	**144**	**144**	**144**	**143**	**143**	**142**
5	**142**	**143**	**142**	**142**	143	143	143	**142**	**142**	**143**	**142**
4	**142**	**143**	**144**	**142**	140		140	**142**	**144**	**143**	**142**
3	**142**	**142**	**143**	**144**	143	143	143	**144**	**143**	**142**	**142**
2	**141**	**143**	**143**	**142**	**143**	**145**	**143**	**142**	**143**	**143**	**141**
1	**141**	**141**	**142**	**143**	**142**	**144**	**142**	**143**	**142**	**141**	**141**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**

c)

Figure 1. An example of the SPL distribution in the outdoor pool used by the harbor porpoise (*Phocoena phocoena*) during exposure to the continuous (100% duty cycle) one-sixth-octave noise band (NB) centered at 88.4 kHz (the fatiguing sound), measured at depths of 0.5 m (a), 1.0 m (b), and 1.5 m (c). T = location of the transducer, which was placed at 1 m depth in the center of the pool (source level: 152 dB re 1 µPa). The numbers in the grey fields indicate 1 m markings on the side of the pool. In this example, the mean SPL for the entire pool is 144 ± 2 dB re 1 µPa (*n* = 228). During the exposure sessions, the porpoise avoided the area adjacent to the transducer in the center of the pool. Therefore, the mean SPL in the area used by the porpoise during sound exposure (bold numbers) is 143 ± 2 dB re 1 µPa (*n* = 204).

To determine the average SPL received by the harbor porpoise, the area where she swam during exposure periods was quantified following the methods of Kastelein et al. (2019b). During all exposure periods, the porpoise avoided the area within ~1.5 m of the transducer, which was in the center of the pool at 1 m depth. Therefore, measurements from this area were excluded, and the SPL she was exposed to was calculated as the mean of the SPL measurements in the area where she swam (mean exposure was 143 dB ± 2 dB re 1 µPa based on 204 measurements; Figure 1). Thus, exposure for 1 h resulted in an SEL of 179 dB re 1 µPa^{2}s.

Hearing Test Signals—Linear upsweep tonal sounds with a duration of 1 s (and 50 ms on and off ramps) were used as the psychophysical hearing test signals that the harbor porpoise was asked to detect before and after exposure to the fatiguing sound (see Kastelein et al., 2019b). The center frequencies of the sweeps tested were 88.4 kHz (the center frequency of the fatiguing sound), 100 kHz (an arbitrary intermediate frequency), and 125 kHz (half an octave higher than the center frequency). We did not test at higher frequencies, as harbor porpoise hearing sensitivity declines rapidly above 130 kHz (Kastelein et al., 2017b). The hearing test signals were generated digitally, and were calibrated and checked daily, as explained by Kastelein et al. (2019b).

Experimental Procedures

One total sound exposure test, consisting of (1) a pre-exposure hearing test starting at ~0830 h, (2) fatiguing sound exposure (or control period) for 1 h (although expressed in hours in this manuscript, the exposure periods were exactly 60 min, timed with stopwatches) in the morning or early afternoon, and (3) a number of post-sound exposure hearing tests in the afternoon, was conducted per day. All hearing tests were performed in the indoor pool. During the 1-h fatiguing sound exposure, the harbor porpoise was in the outdoor pool. Data were collected from February to October 2018, following the protocol developed and explained by Kastelein et al. (2019b).

The harbor porpoise was always tested immediately after the fatiguing sound stopped. Her 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}), and, if hearing had not recovered within 12 min, 60 min (PSE_{60}) after the sound exposure had ended. Hearing was considered to have recovered when the hearing threshold was less than 2 dB above the pre-exposure threshold level. The SPLs of the fatiguing sound were tested in random order (six fatiguing sound SPLs for hearing test sound frequency 88.4 kHz, three SPLs for 100 kHz, and six SPLs for 125 kHz with a resulting SEL range of 173 to 197 dB re 1 µPa^{2}s). Each SEL was tested at least four times per hearing frequency. Exceptions to this were the lowest SELs, which caused less than 2 dB TTS, and the highest SEL (197 dB), testing of which was limited due to animal welfare considerations (for those SELs, only two tests were conducted per frequency compared to four or five tests for most other frequency-SPL combinations; for sample sizes, see the "Results" section).

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 and was followed by exposure to the normal ambient noise in the outdoor pool for 1 h with all the equipment installed. The transducer was placed in the pool as usual but did not emit sound. Post-ambient exposure (PAE; control) hearing test sessions were then performed 1-4 min (PAE_{1-4}), 4-8 min (PAE_{4-8}), and 8-12 min (PAE_{8-12}) after the ambient noise exposure period ended. At least 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 sound exposure test or a control test was conducted.

Hearing Test Procedures

A hearing test trial began with the harbor porpoise at the start/response buoy. Following a hand signal by her trainer, she swam to the listening station. The porpoise stationed there for a random period of between 6 and 12 s before the signal operator produced the test signal (in signal-present trials). She then either swam back to the start/response buoy to indicate that she had heard the signal or stayed at the listening station if she had not heard the signal. About two thirds of each session consisted of signal-present trials and about one third consisted of signal-absent (catch) trials (during which the trainer used a whistle after between 6 and 12 s to instruct the porpoise to return to the start/response buoy where she received a food reward). After a correct response to a signal-present trial, the porpoise went to the start/response buoy and received a food reward. After an incorrect response to a signal-present trial, the porpoise was asked to return to the start/response buoy, and no food reward was given. A switch from a test signal level to which the porpoise responded to a level to which she did not respond, or 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). During pre-exposure and PSE_{60} hearing test sessions, the goal was to obtain 10 reversals. During each of the 4-min periods within the first PSE and PAE sessions, the goal was to obtain a minimum of three reversals. If this goal was not met, the session was not used for analysis. The methodology is described in more detail by Kastelein et al. (2019b).

Data Analysis

When the harbor porpoise returned to the start/response buoy before receiving a test signal (in signal-present trials) or hearing the trainer's whistle (in signal-absent trials), her response was called a "pre-stimulus." The mean pre-stimulus response rate for both signal-present and signal-absent trials was calculated as the number of pre-stimuli as a percentage of all trials 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 reversal pairs obtained during the pre-exposure hearing session. TTSs after the sound exposure sessions (TTS_{1-4}, TTS_{4-8}, TTS_{8-12}, and TTS_{60}) were calculated by subtracting $PE_{50\%}$ from the mean 50% hearing thresholds during PSE_{1-4}, PSE_{4-8}, PSE_{8-12}, and PSE_{60} periods of the same day (see Kastelein et al., 2019b). Similarly, the hearing thresholds measured on a control session day were compared by subtracting $PE_{50\%}$ from the mean 50% hearing thresholds obtained during the PAE periods of the same day.

The onset of TTS is commonly defined as occurring at 6 dB (Houser et al., 2017; Southall et al., 2019). We use this definition in the "Discussion," but define the onset of statistically significant TTS as occurring at the lowest SEL at which a statistically significant difference could be detected between the TTS due to the fatiguing sound exposures and the "TTS" as measured after the control exposures (this "shift" was close to zero). The level of significance was established by conducting a separate one-way ANOVA on the mean TTS_{1-4} for each hearing test frequency with the factor SEL (including zero as the control), followed by Dunnett multiple comparisons between the control and the other levels of the factor (Dunnett, 1964). All analyses were conducted using the software *Minitab 18* (Minitab LLC, State College, PA, USA), and data conformed to the underlying assumptions of the tests applied (i.e., homogeneity of variances and normal distribution of residuals; Zar, 1999).

Results

Pre-Stimulus Response Rate

Before and after the 1-h sound exposure periods, the harbor porpoise was always willing to participate in the hearing tests. In ~5% of the sessions, she moved slowly from the outdoor (exposure) pool to the indoor (testing) pool, so the minimum of three reversals could not be obtained in the first time period after the fatiguing sound had stopped (i.e., PSE_{1-4}); data from these sessions were therefore discarded. The mean pre-stimulus response rate for both signal-present and signal-absent trials in the hearing tests varied between 3.8 and 7.7% (Table 1). The pre-stimulus response rates in the pre-exposure, post sound-exposure, and post-ambient exposure (control) periods were similar.

Effect of SEL on TTS

The ANOVAs showed that the TTS_{1-4} was significantly affected by the fatiguing sound's SEL at all three hearing test frequencies. Post-hoc Dunnett multiple comparisons with the controls revealed that the onset of statistically significant TTS occurred at SELs of either 185 or 191 dB re 1 $\mu Pa^2 s$, depending on the hearing test frequency (Table 2; Figure 2).

Table 1. The pre-stimulus response rate by harbor porpoise F05 in hearing tests during the pre-exposure periods, after exposure to the fatiguing sound (a continuous one-sixth-octave noise band centered at 88.4 kHz), and after exposure to ambient noise (control). All exposure levels and the three hearing test frequencies were pooled for the calculation of percentages. Sample sizes (total numbers of hearing trials in all sessions per period) are shown in parentheses.

	Period				
Fatiguing sound	Pre-exposure	PSE_{1-4}	PSE_{4-8}	PSE_{8-12}	PSE_{60}
	6.7% (1,108)	3.8% (339)	6.8% (355)	7.7% (378)	5.8% (138)
Control	Pre-exposure	PAE_{1-4}	PAE_{4-8}	PAE_{8-12}	--
	5.2% (557)	5.7% (193)	6.2% (193)	5.8% (206)	--

Table 2. Results of one-way ANOVAs of mean $TTS_{1\text{-}4}$ (in dB) in F05 after exposure for 1 h to the fatiguing sound (a continuous one-sixth-octave noise band centered at 88.4 kHz) with the factor "fatiguing sound exposure level" (SEL). Df = degrees of freedom. Standard deviation (SD) is shown for each mean $TTS_{1\text{-}4}$, as well as the range and sample size (*n*). Mean initial TTSs that were significantly different from the control according to Dunnett multiple comparisons are indicated with an asterisk, and the SELs of the onset of statistically significant TTS are indicated in bold.

Hearing test frequency (kHz)	ANOVA results ($F_{df\,1,\,df\,2}$, *p* value)	SPL (dB re 1 µPa)	SEL (dB re 1 µPa²s)	$TTS_{1\text{-}4}$ (dB)			
				Mean	SD	Range	*n*
88.4	$F_{6,26} = 8.40$ $p < 0.001$	Control	Control	0.8	0.7	-0.1-1.8	9
		137	173	0.7	1.0	-0.4-1.9	4
		143	179	1.8	0.9	0.6-2.7	4
		149	**185**	3.6*	0.5	3.1-4.1	4
		155	191	2.9*	1.1	1.8-4.5	5
		158	194	2.9*	1.3	1.9-4.7	5
		161	197	0.1	1.0	-0.6-0.9	2
100	$F_{3,10} = 129.1$ $p < 0.001$	Control	Control	-0.1	0.7	-1.0-0.6	4
		149	185	-0.1	0.3	-0.4-0.4	4
		155	**191**	5.2*	1.3	3.9-6.9	4
		161	197	13.1*	0.7	12.6-13.6	2
125	$F_{6,24} = 39.14$ $p < 0.001$	Control	Control	0.3	0.8	-1.4-1.3	14
		137	173	-0.3	0.8	-0.8-0.3	2
		143	179	1.8	--	--	1
		149	185	1.6	0.6	1.0-2.4	4
		155	**191**	5.4*	1.2	4.2-6.7	4
		158	194	6.1*	1.5	4.8-7.9	4
		161	197	5.9*	0.2	5.8-6.1	2

For hearing test signals of 88.4 kHz, statistically significant $TTS_{1\text{-}4}$ occurred in the harbor porpoise after exposure to an SEL of 185 dB re 1 µPa²s (Table 2; Figure 2). Hearing recovered within 60 min even after the greatest $TTS_{1\text{-}4}$ measured (3.6 dB; Figure 3a). For hearing test signals of 100 kHz, statistically significant $TTS_{1\text{-}4}$ occurred after exposure to an SEL of 191 dB re 1 µPa²s (Table 2; Figure 2), and hearing recovered within 60 min even after the greatest $TTS_{1\text{-}4}$ (13.1 dB; Figure 3b). For hearing test signals of 125 kHz, statistically significant $TTS_{1\text{-}4}$ occurred after exposure to an SEL of 191 dB re 1 µPa²s (Figure 2), and hearing recovered within 12 min, even after the greatest $TTS_{1\text{-}4}$ (6.1 dB; Figure 3c). As expected, the control sessions showed that the hearing thresholds for all three hearing test signal frequencies before and after 1-h exposures to the low ambient noise were very similar (Table 2; Figure 3).

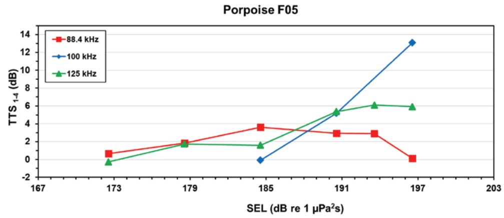

Figure 2. $TTS_{1\text{-}4}$ in harbor porpoise F05 after exposure for 1 h to the fatiguing sound (a continuous one-sixth-octave NB centered at 88.4 kHz) at several SELs, quantified at hearing frequencies 88.4, 100, and 125 kHz (i.e., the center frequency of the fatiguing sound, an arbitrary intermediate frequency, and half an octave above the center frequency). Sample size varies between 1 and 5 per data point (see Table 2). For average received SPLs (dB re 1 µPa), subtract 36 dB re 1 s from the SEL values. For control values, see Table 2 & Figure 3.

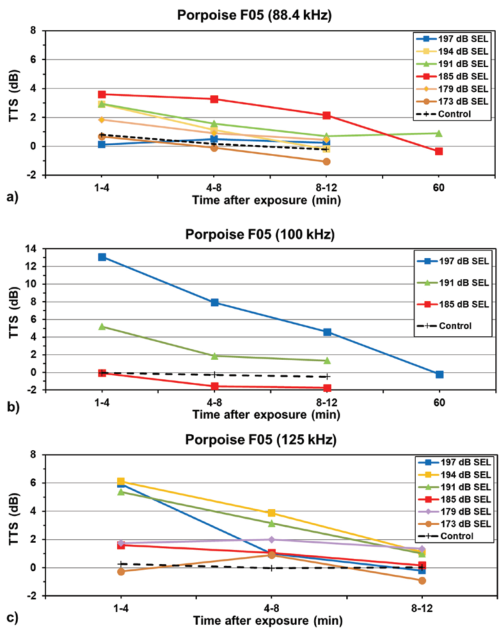

Figure 3. Changes over time, including recovery, in the hearing of F05 at 88.4 kHz (a), 100 kHz (b), and 125 kHz (c) after exposure to the fatiguing sound (a continuous one-sixth-octave NB centered at 88.4 kHz) at several SELs. For sample sizes and SDs at $TTS_{1\text{-}4}$, see Table 2. For average received SPLs (dB re 1 µPa), subtract 36 dB re 1 s from the SEL values. Note the different X and Y axis scales in a, b, and c.

Discussion

Affected Hearing Frequencies

Most TTS studies in odontocetes suggest that the greatest TTS occurs half an octave above the center frequency of the fatiguing sound (e.g., Popov et al., 2011, 2013; Kastelein et al., 2014b, 2019a, 2019b, 2020a, 2020b; Finneran, 2015). The present study showed that the hearing frequency showing the greatest TTS depends on the SPL and related SEL of the fatiguing sound. This phenomenon has been observed in previous TTS studies with harbor porpoises (Kastelein et al., 2019a, 2019b, 2020a, 2020b), demonstrating that the hearing frequency of greatest TTS is difficult to identify (Figure 2). At the highest tested SEL (197 dB re 1 µPa²s), the greatest TTS occurred at the hearing frequency approximately one third of an octave higher than the fatiguing sound frequency, not at half an octave higher as is often reported in harbor porpoises for frequencies < 88.4 kHz (Kastelein et al., 2014b, 2019a, 2019b, 2020a, 2020b). However, in most previous research, only the center frequency and half an octave above the center frequency were tested. This complicates comparison between TTS onset due to different fatiguing sound frequencies, as the TTS onset SEL usually varies per hearing frequency.

Studies with odontocetes in which the fatiguing sound was broadband and impulsive also show TTS occurring at hearing frequencies above the peak frequency of the fatiguing sound (Finneran et al., 2002; Lucke et al., 2009; Kastelein et al., 2015a, 2017a). It is likely that broadband exposures at high levels produce broadband TTS with an upward frequency spread, similar to that seen after exposure to pure tones and narrow-band noise (Finneran, 2015).

Relationship Between the Frequency of the Fatiguing Sound and Susceptibility to TTS

Susceptibility to TTS and its relationship with fatiguing sound frequency can be explored by relating equal-TTS susceptibility data to fatiguing sound frequencies (NMFS, 2016; Houser et al., 2017). Research suggests that susceptibility to TTS is frequency dependent in harbor porpoises. In the present study with the 88.4-kHz fatiguing sound, significant $TTS_{1\text{-}4}$ at 100 kHz occurred at a higher SEL than that which caused TTS after the same (F05) and other (M02 and M06) harbor porpoises were exposed to sounds of 6-7, 16, 32, and 63 kHz using the same methods (Kastelein et al., 2014b, 2019a, 2019b, 2020a; Figure 4).

There may be individual differences in susceptibility to TTS between harbor porpoises M02 (exposed to a one-octave NB at 4 kHz: Kastelein et al., 2012; 1 to 2 kHz sweeps: Kastelein et al., 2014a; and 6 to 7 kHz upsweeps: Kastelein et al., 2015b), M06 (exposed to 3.5 to 4.1 kHz tonal sounds: Kastelein et al., 2017a; and NBs around 16, 32, and 63 kHz: Kastelein et al., 2019a, 2019b, 2020a), and F05 (exposed to NBs around 1.5, 16, 32, 63, and 88.4 kHz: Kastelein et al., 2019a, 2019b, 2020a, 2020b, present study; and a 6.5-kHz

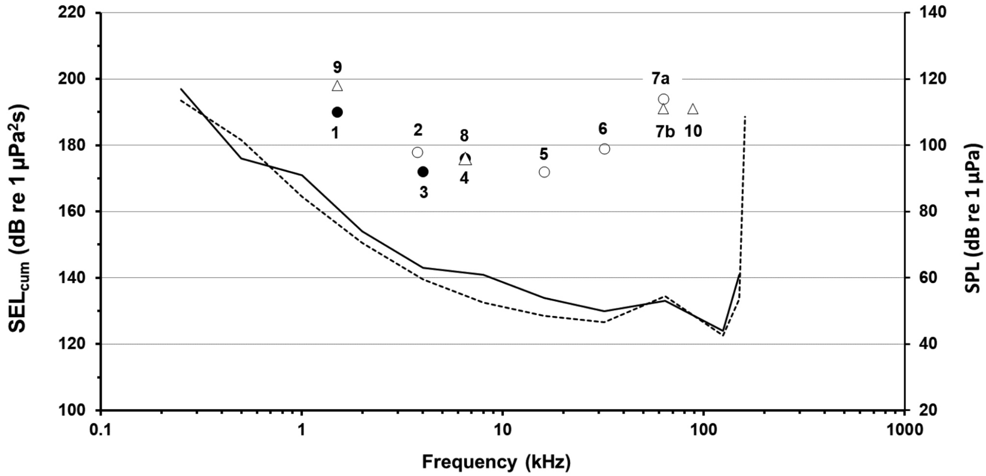

Figure 4. Audiograms of F05 (present study animal; solid line) and M02 (dashed line; SPL on right-hand Y axis vs frequency on X axis; Kastelein et al., 2017b), and the cumulative sound exposure level (SEL_{cum} on left-hand Y axis) required to cause a mean TTS_{1-4} of around 6 dB (a marker of TTS onset in marine mammals; Houser et al., 2017) in harbor porpoises after exposure for 1 h 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., 2017a), (3) a one-octave NB 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), (5) a one-sixth-octave NB centered at 16 kHz at 100% duty cycle (Kastelein et al., 2019b), (6) a one-sixth octave NB centered at 32 kHz at 100% duty cycle (Kastelein et al., 2019a), (7) a one-sixth-octave NB centered at 63 kHz at 100% duty cycle in M06 (7a) and in F05 (7b) (Kastelein et al., 2020a), (8) a 6.5 kHz tone at 100% duty cycle (Kastelein et al., 2020b), (9) a one-sixth octave NB centered at 1.5 kHz at 100% duty cycle (Kastelein et al., 2020b), and (10) a one-sixth-octave NB centered at 88.4 kHz at 100% duty cycle (present study). The solid circles represent M02, the open circles represent M06, and the open triangles represent F05 (present study animal). Note that in numbers 1 through 3, TTS was measured at the center frequency of the fatiguing sound; in 4 through 9, it was measured half an octave above the center frequency; in 10 (present study), it was measured at one third of an octave above the center frequency of the fatiguing sound (100 kHz).

continuous wave [CW]). 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), and susceptibility to TTS in harbor porpoises may vary within and between populations as well. The present study was conducted with only one harbor porpoise, which limits population-level inferences about susceptibility to TTS, but varying fatiguing sound frequency within one study animal allows frequency-dependent effects on susceptibility to TTS to be recognized, as they are not obscured by individual differences (Finneran & Schlundt, 2013). It is important to test more individuals over large frequency ranges to see if they have the same TTS susceptibility patterns.

Differences between the fatiguing sounds used in the present study and in some previous TTS studies with harbor porpoises may have resulted in (or contributed towards) differences in the induced TTSs. It is unclear whether the hearing frequency (relative to the center frequency of the fatiguing sound) at which the greatest TTS was experienced was similar across all the sound types tested, including one-octave NBs (Kastelein et al., 2012), one-sixth-octave NBs (Kastelein et al., 2019a, 2019b, 2020a, 2020b, present study), narrow-band sweeps (Kastelein et al., 2014a, 2015b), a composite of a sweep followed by two tones (Kastelein et al., 2017a), and tonal (CW) sounds (Kastelein et al., 2013, 2014b, 2020b). However, the TTS induced in M06 when he was exposed in another study to 3.5 to 4.1 kHz 53-C sonar playback sounds (at a slightly lower duty cycle of 96%; Kastelein et al., 2017a) was as expected from TTS studies with M02 (Figure 4). Thus, in the 1.5 to 6.5 kHz range, the susceptibility to TTS of M06 appears to be similar to that of M02.

The results of the present and previous TTS studies with harbor porpoises, although representing only part of their total hearing frequency range (1.5 to 88.4 kHz; Kastelein et al., 2012, 2013, 2014a, 2014b, 2015a, 2015b, 2017a, 2019a, 2019b, 2020a, 2020b, present study), suggest that the susceptibility of harbor porpoise hearing to TTS is frequency dependent. This has also been shown for bottlenose dolphins (*Tursiops truncatus*; Finneran & Schlundt, 2013), Yangtze finless porpoises (*Neophocaena phocaenoides asiaeorientalis*; Popov et al., 2011), and belugas (*Delphinapterus leucas*; Popov et al., 2013). Finneran & Schlundt (2013) found greater susceptibility to TTS in bottlenose dolphins for fatiguing sound frequencies between 10 and 30 kHz than for 80 kHz. Popov et al. (2011) showed that susceptibility to TTS in the Yangtze finless porpoise, a species more closely related to the harbor porpoise, is also frequency dependent: susceptibility decreased with increasing fatiguing sound frequency (i.e., 32, 45, 64, and 128 kHz). A similar effect was found for belugas, which are more susceptible to TTS for fatiguing sound frequencies 11.2 and 22.5 kHz than for 45 and 90 kHz (Popov et al., 2013). Corresponding to the trend observed in these previous studies, the present study suggests that the onset of TTS (defined as 6 dB TTS) in harbor porpoises that have been exposed to sounds of 88.4 kHz occurs at higher SELs (191 dB re 1 μPa^2s; hearing measured at 100 kHz, which is one third of an octave above the center frequency of the fatiguing sound) than after exposure to sounds of 4, 6.5, 16, and 32 kHz (Kastelein et al., 2012, 2014a, 2019a, 2019b, 2020b, present study; Figure 4). The next step in quantifying susceptibility to TTS over the entire hearing range will be to test one more fatiguing sound frequency: 0.5 kHz. Completion of this step will allow an auditory weighting curve to be modeled for the harbor porpoise.

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