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Temporary Hearing Threshold Shift in Harbor Porpoises (*Phocoena phocoena*) Due to One-Sixth-Octave Noise Bands at 63 kHz

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Abstract

Susceptibility to temporary hearing threshold shift (TTS) depends on the frequency of the fatiguing sound. So far, TTS in harbor porpoises (*Phocoena phocoena*) has been tested for sounds in the 1 to 32 kHz range. It is, however, unclear if TTS growth functions differ across frequencies. To assess impacts of anthropogenic sound on porpoise hearing, full insight into the TTS growth functions across all ecologically relevant hearing frequencies for the harbor porpoise is required. In the present study, which is part of a series studying the susceptibility of harbor porpoises to TTS over their entire hearing range, TTS growth functions were quantified after two harbor porpoises (M06 and F05) were exposed for one hour to a continuous one-sixth-octave noise band centered at 63 kHz, at average received sound pressure levels (SPLs) of 106 to 156 dB re 1 µPa, resulting in a sound exposure level (SEL) range of 142 to 192 dB re 1 µPa^{2}s. Hearing thresholds for 63, 88.4, 100, and 125 kHz signals were determined before and after exposure to quantify TTS and recovery. Porpoise M06's hearing was tested 1 to 4 minutes after exposure. At 63 kHz, the lowest SEL which resulted in significant TTS_{1-4} (6.5 dB) was 154 dB re 1 µPa^{2}s, and the highest TTS_{1-4} (7.8 dB) occurred at 175 dB SEL. Porpoise F05's hearing was tested both 1 to 4 and 12 to 16 minutes after exposure. At 63 kHz, the only SEL which resulted in significant TTS_{1-4} (3.2 dB) was 181 dB re 1 µPa^{2}s; no TTS_{12-16} occurred. At 88.4 kHz, the lowest SEL which resulted in significant TTS_{1-4} (6.6 dB) and TTS_{12-16} (3.2 dB) was 192 dB re 1 µPa^{2}s. No evidence was found for TTS at 100 or 125 kHz in either animal, but sample sizes were very small for those frequencies. Based on the seven available TTS studies conducted with harbor porpoises and 60-minute continuous sound, it appears that susceptibility to TTS increases with increasing frequency of the fatiguing sound below ~6.5 kHz but decreases with increasing frequency of the sound above ~6.5 kHz. For the frequency used in the present study (63 kHz), harbor porpoises are less susceptible to TTS than formerly assumed.

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

Introduction

The effects of anthropogenic sounds on harbor porpoises (*Phocoena phocoena*) are of particular interest because this odontocete species has a wide distribution area in the coastal waters of the Northern Hemisphere (Bjorge & Tolley, 2008) and acute hearing (i.e., low hearing thresholds) in a wide frequency range (Kastelein et al., 2017b). The harbor porpoise appears to be more susceptible to temporary threshold shift (TTS) caused by sounds than the few other odontocete species—bottlenose dolphin (*Tursiops truncatus*), beluga whale (*Delphinapterus leucas*), and Yangtze finless porpoise (*Neophocaena phocaenoides asiaeorientalis*)—that have been examined so far (Finneran, 2015; Tougaard et al., 2016; Houser et al., 2017).

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 (Finneran, 2015). For regulation of underwater anthropogenic sound levels, complete equal-TTS contours are desirable, covering the entire frequency range of hearing in the harbor porpoise (0.5 to 140 kHz; Kastelein et al., 2017b). Within the 1 to 32 kHz bandwidth, equal-TTS points for six frequencies have been established (Kastelein et al., 2012a, 2013, 2014a, 2014b, 2015b, 2017a, 2019a, 2019b).

As part of a series of TTS studies on the harbor porpoise, the goal of the present study is to increase the frequency range for which susceptibility to TTS in harbor porpoises is known (therefore this "Introduction" is deliberately kept short). TTS and recovery of hearing were quantified in two harbor porpoises after exposure to a noise band centered at 63 kHz. Once susceptibility to TTS has been quantified for the entire hearing range of the harbor porpoise, it will be possible to generate research-based auditory weighting curves for harbor porpoises as well as other cetaceans that echolocate at high frequencies (as proposed by Southall et al., 2019).

Methods

Study Animals and Site

Two formerly stranded and rehabilitated harbor porpoises were used as study animals. The female, identified as Porpoise F05, was ~8 years old during the study, her body mass was ~46 kg, her body length was ~154 cm, and her girth at the axilla was ~82 cm. The male, identified as Porpoise M06, was ~5 years old during the study, his body mass was ~33 kg, his body length was ~130 cm, and his girth at the axilla was ~80 cm. The hearing of the study animals in the frequency range tested in the present study (63 to 125 kHz) was probably representative of the hearing range of similarly aged conspecifics, as all five individuals tested so far with the same behavioral audiometric technique had very similar hearing thresholds (Kastelein et al., 2017b). The management of the food rations was described by Kastelein et al. (2019a). Near the end of the study (December 2017), Porpoise M06 suddenly became ill. Despite intensive medical treatment, he died of pneumonia a few days later. After M06's death, the study was continued with just Porpoise F05.

The study was conducted at the SEAMARCO Research Institute, the Netherlands. The animals were kept in a quiet pool complex (i.e., in a remote area, no pumps on at the entire institute during hearing tests, all other activities at the facility focused on being quiet or stopped during hearing tests, walls covered with aquatic vegetation, and a sandy bottom in the pool) designed and built for acoustic research, consisting of an outdoor pool (12 m × 8 m; 2 m deep) in which the study animals were 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. 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 2 mo during the study period (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, in the form of a WAV file (sample rate: 768 kHz), was played by a laptop computer (Acer – Aspire, Model No. 5750) with a program written in *LabVIEW* to an external data acquisition card (Model No. USB6259, single channel maximum sample rate 1.25 MHz; National Instruments, Austin, TX, USA), the output of which could be controlled in 1-dB steps with the *LabVIEW* program. The output of the card went through a custom-made ground loop isolator and buffer to a custom-made passive low-pass digital filter (set at 70 kHz). After this, it went to a custom-built high-frequency power amplifier, which drove the ball transducer (Model 4033; RESON, Slangerup, Denmark). The transducer was placed in the middle of the outdoor pool at 1 m depth in a 1-m diameter cage to prevent the harbor porpoises from approaching the transducer too closely. The linearity of the transmitter system for fatiguing sound was checked during each calibration and was found to only deviate from the expected level by at most 1 dB within a 42 dB range.

The fatiguing sound consisted of a continuous (duty cycle 100%) one-sixth-octave Gaussian white band noise centered at 63 kHz (bandwidth: 59.5 to 66.7 kHz). A narrow noise band was selected to cause a homogeneous sound field. To determine the fatiguing sound's distribution in the outdoor pool, the SPL of the noise band was measured at 76 (7 x 11, minus one blocked off location in the middle of the pool due to the cage surrounding the transducer) 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 228 measurements in the pool. There were small differences in mean SPL per depth (Figure 1).

To determine the average SPL received by the study animals, the area where they swam during the exposure periods was compared to the fatiguing sound's SPL distribution in the pool. To quantify the harbor porpoises' swimming patterns, videos of the sound exposure sessions were analyzed (see Kastelein et al., 2012a). Each time a porpoise surfaced, its location was allocated to one of 96 grid squares (8 × 12), each of which corresponded to a 1 m × 1 m square of the outdoor pool. The animals were found to swim evenly throughout the entire outdoor pool during the low SPL exposure to fatiguing sounds, so for those SPLs, the average fatiguing sound SPL (average of power sum of 228 measurements in the outdoor pool; an example of one source level is shown in Figure 1) was taken to be representative of the SPL received by them. However, during exposure to some of the higher SPLs, the animals' swimming patterns seemed to be influenced by the sound (the animals swam on average further away from the transducer), and the mean SPLs of the areas where they swam was taken as the mean SPL they received.

Before each exposure, the voltage output of the emitting system to the transducer and the voltage output of the sound-receiving system were checked with an oscilloscope (Model 2201; Tektronix, Beaverton, OR, USA) and a voltmeter

0.5 m

7	143	141	143	144	147	148	147	144	143	141	143
6	142	144	146	145	146	149	146	145	146	144	142
5	143	143	144	147	150	152	150	147	144	143	143
4	142	144	145	148	150		150	148	145	144	142
3	139	142	140	147	142	151	142	147	140	142	139
2	140	141	138	141	141	145	141	141	138	141	140
1	141	141	138	145	141	141	141	145	138	141	141
	1	2	3	4	5	6	7	8	9	10	11

a)

1.0 m

7	144	145	145	145	145	146	145	145	145	145	144
6	144	143	146	147	148	147	148	147	146	143	144
5	143	144	144	147	152	150	152	147	144	144	143
4	144	144	146	146	147	T	147	146	146	144	144
3	144	145	146	145	149	150	149	145	146	145	144
2	144	144	143	146	148	149	148	146	143	144	144
1	143	145	145	145	146	146	146	145	145	145	143
	1	2	3	4	5	6	7	8	9	10	11

b)

1.5 m

7	142	143	144	144	144	144	144	144	144	143	142
6	142	143	144	145	145	145	145	145	144	143	142
5	142	142	144	144	146	146	146	144	144	142	142
4	144	144	145	145	147		147	145	145	144	144
3	141	143	145	145	146	147	146	145	145	143	141
2	142	142	143	144	144	145	144	144	143	142	142
1	142	143	143	143	144	144	143	143	143	143	142
	1	2	3	4	5	6	7	8	9	10	11

c)

Figure 1. The SPL (dB re 1 µPa) distribution in the harbor porpoises' (*Phocoena phocoena*) outdoor pool of the continuous (100% duty cycle) one-sixth-octave noise band centered at 63 kHz (the fatiguing sound), measured at depths of 0.5 m (a), 1.0 m (b), and 1.5 m (c). In (b), T= location of the transducer, which was placed at 1 m depth in a 1-m diameter cage in the center of the pool. The numbers in the grey fields indicate 1-m markings on the sides of the pool. In this example, the mean SPL based on the power sum of all 228 SPL measurements was 145 dB re 1 µPa so that exposure for 1 h resulted in an SEL of 181 dB. Mean (± SD) SPL per depth: 144 ± 4 dB re 1 µ at 0.5 m, 146 ± 2 dB at 1.0 m, and 144 ± 1 dB at 1.5 m deep.

(Model 34401A; Agilent, Santa Clara, CA, USA) by producing a 63-kHz continuous signal from the laptop. The acoustic underwater signal was checked with a custom-built hydrophone, a pre-amplifier (Model CCAS1000, RESON), and a spectrum analyzer (Model PCSU1000; Velleman, Gavere, Belgium). If the values corresponded to those obtained during the SPL calibrations, the SPLs were assumed to be correct, and a sound exposure test could be performed.

Hearing Test Signals—Linear upsweeps (starting and ending at ± 2.5% of the center frequency), with a duration of 1 s (including a linear rise and fall in amplitude of 50 ms each), were used as the hearing test signals that the animals were asked to detect before and after exposure to the fatiguing sound. The center frequencies of the hearing test signals were 63 kHz (the center frequency of the fatiguing sound), 88.4 kHz (half an octave above the center frequency), and 125 kHz (one octave above the center frequency). In addition, 100 kHz, a frequency approximately between 88.4 and 125 kHz, was tested once to verify the frequency showing maximum TTS was not missed accidentally. The hearing test signals were generated digitally, and the SPL and spectrum near the listening station were checked daily (see Kastelein et al., 2019a). The sounds were produced with a custom-built directional transducer (WAU-q7b) consisting of a disk of composite piezoelectric materials (Material Systems Inc., Littleton, MA, USA) with an effective radiating aperture diameter of 4.5 cm. The thickness of the piezoelectric materials was 0.64 cm. The piezoelectric element was a 6.4-cm diameter disk that was encapsulated in degassed polyurethane epoxy. The transducer was placed at 1 m depth, facing the harbor porpoise's listening station.

Experimental Procedures

A maximum of one noise exposure test was conducted per day. A complete test consisted of (1) pre-exposure hearing tests starting at 0830 h, (2) 1-h fatiguing sound exposure in the morning or early afternoon, and (3) one or multiple post-noise exposure hearing tests in the afternoon. Pre-exposure hearing tests were performed in the indoor pool with one animal at a time. When one harbor porpoise was being tested, the other remained in the outdoor pool and was quietly kept occupied by its trainer using behavioral husbandry. Data were collected from October 2017 to February 2018 following the protocol developed and explained by Kastelein et al. (2019a).

Porpoise M06 was initially always tested first and Porpoise F05 second. After Porpoise M06's death, Porpoise F05 was tested immediately after the fatiguing sound stopped. Hearing thresholds of Porpoises M06 and F05 were measured during post-sound exposure (PSE) periods 1 to 4 min (PSE_{1-4}), 4 to 8 min (PSE_{4-8}), 8 to 12 min (PSE_{8-12}), sometimes at 60 min (PSE_{60}), and, if hearing had not recovered after 60 min, at 120 min (PSE_{120}) after the sound exposure had ended. Porpoise F05's hearing was also tested at 12 to 16 min (PSE_{12-16}), 16 to 20 min (PSE_{16-20}), 20 to 24 min (PSE_{20-24}), 72 min (PSE_{72}), and, if hearing had not recovered after 72 min, at 132 min (PSE_{132}) after the sound exposure had ended.

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 the fluctuations of the TTS during control sessions in the present and several previous studies with these harbor porpoises: Kastelein et al., 2017a, 2019a, 2019b). The effects of fatiguing sounds of various average received SPLs were tested (see "Results" for sample sizes and SPLs). 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 were determined based on the TTS found and the available time.

Control tests were conducted in the same way and under the same conditions as noise exposure tests but without the fatiguing sound exposure. Each control test started with a pre-exposure hearing test session and was followed by exposure to the normal (low) ambient noise (i.e., no fatiguing sound) in the outdoor pool for at least 1 h. Post-ambient exposure (PAE; control) hearing test sessions were then performed for Porpoise M06 1 to 4 (PAE_{1-4}), 4 to 8 (PAE_{4-8}), and 8 to 12 (PAE_{8-12}) min after the ambient noise exposure period ended. Porpoise F05 was tested 1 to 4 (PAE_{1-4}), 4 to 8 (PAE_{4-8}), and 8 to 12 (PAE_{8-12}) min, as well as 12 to 16 (PAE_{12-16}), 16 to 20 (PAE_{16-20}), and 20 to 24 (PAE_{20-24}) min after ambient noise exposure ended. Four control tests were conducted per hearing test frequency, except for Porpoise M06 at 100 kHz for which no control test could be conducted. Control tests were randomly dispersed among the fatiguing sound exposure tests. On each test day, either a noise exposure test or a control test was conducted.

Hearing Test Procedures

A hearing test trial began with the tested harbor porpoise stationed at a start/response buoy. In response to a hand signal by its trainer, it swam to a listening station. 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 it had heard the signal. A switch from a test signal level that the porpoise responded to (a "hit") to a level that he or she did not respond to (a "miss"), and vice versa, was called a "reversal." 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 to 12 s by a whistle signal from the 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 session of each animal). Only PSE_{1-4}, PSE_{12-16}, PAE_{1-4}, and PAE_{12-16} hearing session periods with three or more reversals were used for analysis. 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 harbor porpoises 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 in each hearing test period. The pre-exposure mean 50% hearing threshold for a hearing test sound (PE50%) was determined by calculating the mean SPL of all (usually 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 during PSE_{1-4}, PSE_{4-8}, PSE_{8-12}, PSE_{60}, and PSE_{120} periods of the same day for both harbor porpoises, as well as during PSE_{12-16}, PSE_{16-20}, PSE_{20-24}, PSE_{72}, and PSE_{132} periods of the same day for Porpoise F05.

TTSs in the control sessions were calculated by subtracting the mean 50% hearing thresholds obtained during pre-ambient exposure periods from the mean 50% hearing thresholds obtained during the post-ambient exposure periods of the same day. No TTS occurred in control sessions, so this calculation was always close to zero (for values, see "Effect of SPL on TTS" in the "Results" section).

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, separately for each harbor porpoise and for each hearing test frequency with the factor SPL (including zero as the control). When the ANOVA produced a significant value overall, the levels were compared to the control by means of Dunnett's (1964) multiple comparisons or to each other by means of Tukey pairwise comparisons. All analysis was 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 lowest seven source levels (SLs), Porpoise M06 swam throughout the entire pool. Therefore, the mean SPL of the entire pool was used to calculate the exposure level. Only at the highest SL did he avoid the area around the transducer, and the exposure was based only on SPLs in the periphery of the pool; in addition, the harbor porpoises never lifted their heads out of the water in an attempt to reduce the received SPL. During the lowest five SLs, Porpoise F05 swam throughout the entire pool. Therefore, the mean SPL of the entire pool was used to calculate the exposure level. Only at the three highest SLs did she avoid the area around the transducer, and the exposure was based only on SPLs in the periphery of the pool.

Pre-Stimulus Response Rate

The harbor porpoises were always willing to participate in the hearing tests after the 1-h noise exposure periods. In a few sessions, the test porpoise moved from the outdoor (exposure) pool to the indoor (testing) pool too slowly (possibly by being distracted either by the other animal or by personnel) so that the minimum of three reversals could not be obtained for PSE_{1-4}; 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 0.7 and 6.7% for Porpoise M06, and between 3.7 and 14.8% for Porpoise F05 (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 and control periods.

Effect of SPL on TTS

The ANOVAs showed that, in most cases, TTS_{1-4} and TTS_{12-16} were significantly affected by the fatiguing sound's SEL. Where possible, comparisons with the control revealed that the statistically significant onset of TTS varied depending on the animal and the hearing test frequency (Table 2).

Porpoise M06

For hearing test signals of 63 kHz, statistically significant TTS_{1-4} (of 6.5 dB) occurred in Porpoise M06 after exposure to an SEL of 154 dB re 1 µPa^{2}s (Table 2; Figure 2a). Hearing recovered within

Table 1. The pre-stimulus response rates (those in the signal-present and signal-absent combined; in the signal-absent, the whistle of the trainer was the stimulus) of the harbor porpoises (*Phocoena phocoena*) in hearing tests during the pre-exposure periods, after exposure to the fatiguing sound (a continuous one-sixth-octave noise band centered at 63 kHz), and after exposure to ambient noise (control). All exposure SPLs and hearing test frequencies were pooled for the calculation of percentages. Sample sizes (= total number of hearing trials per period) are shown in parentheses.

Porpoise M06	Period				
Fatiguing sound	Pre-exposure	PNE_{1-4}	PNE_{4-8}	PNE_{8-12}	PNE_{60}
	2.8% (719)	0.7% (281)	3.4% (297)	2.1% (291)	3.5% (317)
Control	Pre-exposure	PAE_{1-4}	PAE_{4-8}	PAE_{8-12}	
	0.6% (154)	6.7% (60)	3.2% (63)	1.6% (64)	
Porpoise F05	**Period**				
Fatiguing sound	Pre-exposure	PNE_{12-16}	PNE_{16-20}	PNE_{20-24}	PNE_{72}
	8.8% (1,357)	5.4% (298)	11.8% (321)	8.4% (346)	13.9% (79)
Control	Pre-exposure	PAE_{12-16}	PAE_{16-20}	PAE_{20-24}	
	8.8% (285)	12.1% (58)	4.7% (64)	7.4% (81)	
Porpoise F05	**Period**				
Fatiguing sound	Pre-exposure	PNE_{1-4}	PNE_{4-8}	PNE_{8-12}	
	8.7% (1,294)	7.9% (215)	7.3% (220)	10.4% (231)	
Control	Pre-exposure	PAE_{1-4}	PAE_{4-8}	PAE_{8-12}	
	8.8% (285)	14.8% (27)	3.7% (27)	7.1% (28)	

60 min, even after exposure to the highest fatiguing sound level tested (Figure 3a). TTS appeared to reach an asymptote for SELs of 166 dB re 1 µPa²s and higher. For hearing test signals of 88.4 kHz, TTS_{1-4} occurred after exposure to an SEL of 187 dB re 1 µPa²s (Table 2; Figure 2a). Recovery of hearing occurred within 60 min for exposures up to an SEL of 192 dB re 1 µPa²s (Figure 3b). For hearing test signals of 100 and 125 kHz, no TTS_{1-4} occurred, even after exposure to an SEL of 192 dB re 1 µPa²s (Table 2; Figure 2a). No change in TTS susceptibility was observed over the duration of the study.

The control sessions showed that the hearing thresholds for all four hearing test signals before and after 60-min exposures to the low ambient noise were very similar (Figure 3; Table 2).

Porpoise F05 (1 to 4 min)
The TTS results collected 1 to 4 min after the exposure to the fatiguing sound stopped were collected after Porpoise M06 died. For hearing test signals of 63 kHz, statistically significant TTS_{1-4} occurred in Porpoise F05 only after exposure to an SEL of 180 dB re 1 µPa²s, and not after the two higher SELs that were tested (Table 2; Figure 2b); hearing recovered within 12 min (Figure 4a). For hearing test signals of 88.4 kHz, statistically significant TTS_{1-4} occurred only after exposure to an SEL of 192 dB re 1 µPa²s (Table 2; Figure 2b). Recovery of hearing probably occurred within 30 min (TTS was not measured after 12 min when TTS was still only 2.5 dB; Figure 4b). No change in TTS susceptibility was observed over the duration of the study. The control sessions showed that the hearing thresholds for both hearing test signals before and after 60-min exposures to the low ambient noise were very similar (Figure 4; Table 2).

Porpoise F05 (12 to 16 min)
The TTS results of Porpoise F05 12 to 16 min after the fatiguing sound stopped were collected after the same exposures after which Porpoise M06 was tested first (Figure 2a). For hearing test signals of 63 kHz, no statistically significant TTS_{12-16} occurred in Porpoise F05 even after exposure to an SEL of 192 dB re 1 µPa²s (Table 2; Figures 2c & 5a). For hearing test signals of 88.4 kHz, statistically significant TTS_{12-16} (of 3.2 dB) occurred only after exposure to an SEL of 192 dB re 1 µPa²s (Table 2; Figure 2c). Recovery of hearing

Table 2. Mean (± SD) TTS$_{1-4}$ in Porpoise M06 and TTS$_{1-4}$ and TTS$_{12-16}$ in Porpoise F05 after exposure for 60 min to a continuous one-sixth-octave noise band centered at 63 kHz at several SELs (which differed slightly between animals depending on the swimming patterns), quantified at hearing frequencies 63, 88.4, 100, and 125 kHz (i.e., the exposure frequency, half an octave above the exposure frequency, an intermediate frequency, and one octave above the exposure frequency, respectively). Results from the control sessions are also shown (no TTS occurred). *N* = sample size, * = significant TTS, and † = TTS assumed but not statistically evidenced due to small sample size.

	Porpoise M06				Porpoise F05						
Hearing test frequency (kHz)	SEL: dB re 1 µPa^{2}s	Mean TTS$_{1-4}$	SD (range)	*N*	SEL: dB re 1 µPa^{2}s	Mean TTS$_{1-4}$	SD (range)	*N*	Mean TTS$_{12-16}$	SD (range)	*N*
63	Control	-0.3	0.6 (-1.3–0.0)	4	Control	0.8	--	1	1.0	1.4 (-0.4–2.3)	4
	142	2.1	0.5 (1.5–2.7)	4	142				1.0	0.7 (0.1–1.8)	4
	154	6.5*	1.1 (5.7–7.3)	2	154				2.0	0.7 (1.5–2.5)	2
	166	7.5†	--	1	166				0.6	--	1
	172	7.2*	2.2 (5.0–10.0)	4	172				1.4	1.6 (-0.2–3.6)	4
	175	7.8*	1.5 (6.2–9.6)	4	175	1.2	0.5 (0.5–1.5)	4	1.3	0.9 (0.0–1.9)	4
	181	7.3*	2.2 (4.1–9.1)	4	180	3.2*	0.5 (2.9–4.0)	4	1.8	0.7 (0.9–2.4)	4
	187	6.7†	--	1	186	1.9	1.0 (0.9–3.0)	4	0.3	1.7 (-0.9–1.5)	2
	192	7.6†	--	1	192	0.4	1.2 (-0.8–2.2)	4	0.9	0.3 (0.7–1.1)	2
88.4	Control	0.9	1.7 (-0.3–2.1)	2	Control	-0.5	0.3 (-0.8– -0.2)	3	0.5	1.5 (-0.5–2.2)	3
	166	0.9	--	1	166				-0.4	--	1
	175	0.2	1.4 (-0.7–1.8)	3	175				0.1	1.4 (-1.5–1.1)	3
	181	2.9	--	1	180	0.2	0.7 (-0.7–1.1)	4	-0.8	0.5 (-1.3– -0.3)	3
	187	4.4*	3.6 (1.8–6.9)	2	186	1.8	1.3 (0.3–3.3)	4	0.8	1.8 (-1.2–2.3)	3
					189	3.1	1.6 (2.0–4.3)	2			
	192	5.0*	1.5 (3.2–7.4)	5	192	6.6*	1.6 (4.3–7.9)	4	3.2*	0.8 (2.0–3.9)	5
100	192	1.8	--	1	192				-1.1	--	1
125	Control	-0.2	1.9 (-1.6–1.1)	2	Control				0.0	1.1 (-0.8–0.8)	2
	175	0.0	--	1	175				0.5	--	1
	192	0.4	0.7 (-0.1–0.9)	2	192				-0.1	0.6 (-0.6–0.4)	2

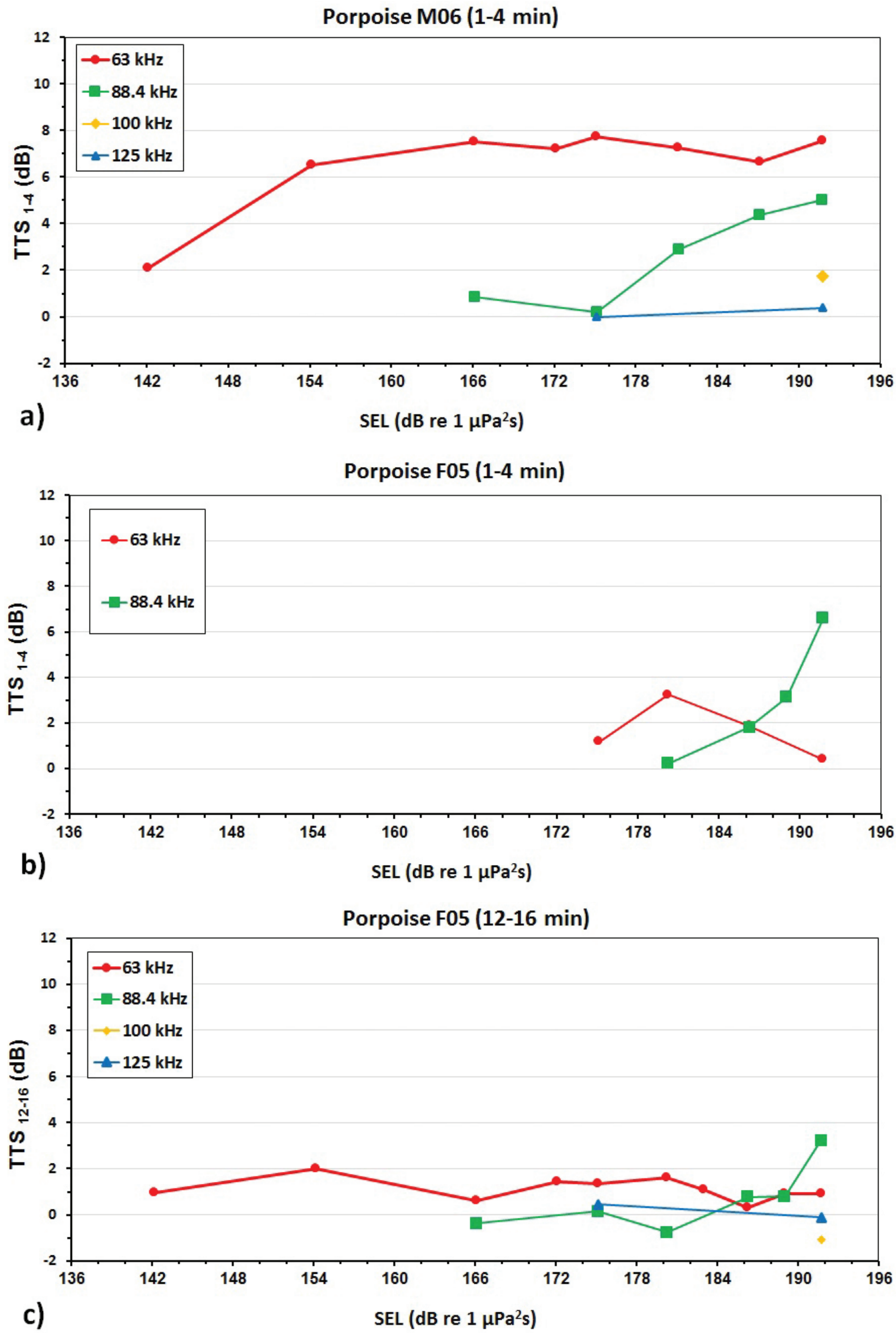

Figure 2. TTS$_{1\text{-}4}$ in Porpoise M06 (a) and TTS$_{1\text{-}4}$ (b) and TTS$_{12\text{-}16}$ (c) in Porpoise F05 after exposure for 60 min to a continuous one-sixth-octave noise band centered at 63 kHz at several SELs, quantified at hearing frequencies 63, 88.4, 100, and 125 kHz (i.e., the exposure frequency, half an octave above the exposure frequency, an intermediate frequency, and one octave above the exposure frequency, respectively). Sample size varies per data point shown (for sample sizes, ranges, and SDs, see Table 2). For SPLs (dB re 1 μPa), subtract 36 dB re 1 s from the SEL values. For control values, see Figures 3 & 4; Table 2.

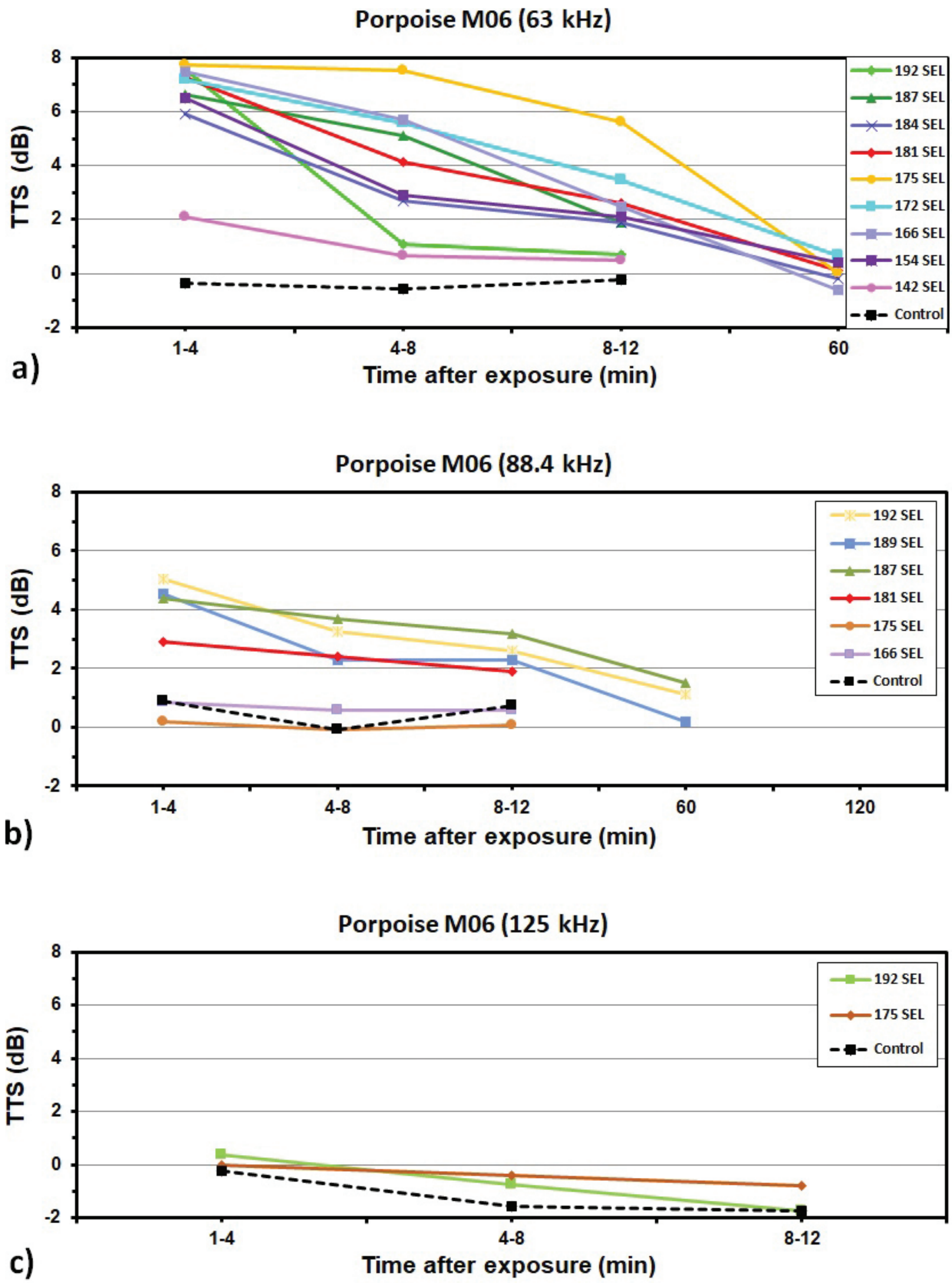

Figure 3. Changes in Porpoise M06's hearing at 63 kHz (a), 88.4 kHz (b), and 125 kHz (c) after exposure to a continuous one-sixth-octave noise band centered at 63 kHz for 60 min at several SELs. Sample sizes and SDs for TTS_{1-4} are shown in Table 2. Hearing tests started within 1 min after the fatiguing sound stopped. For average received SPLs (dB re 1 µPa), subtract 36 dB re 1 s from the SEL values.

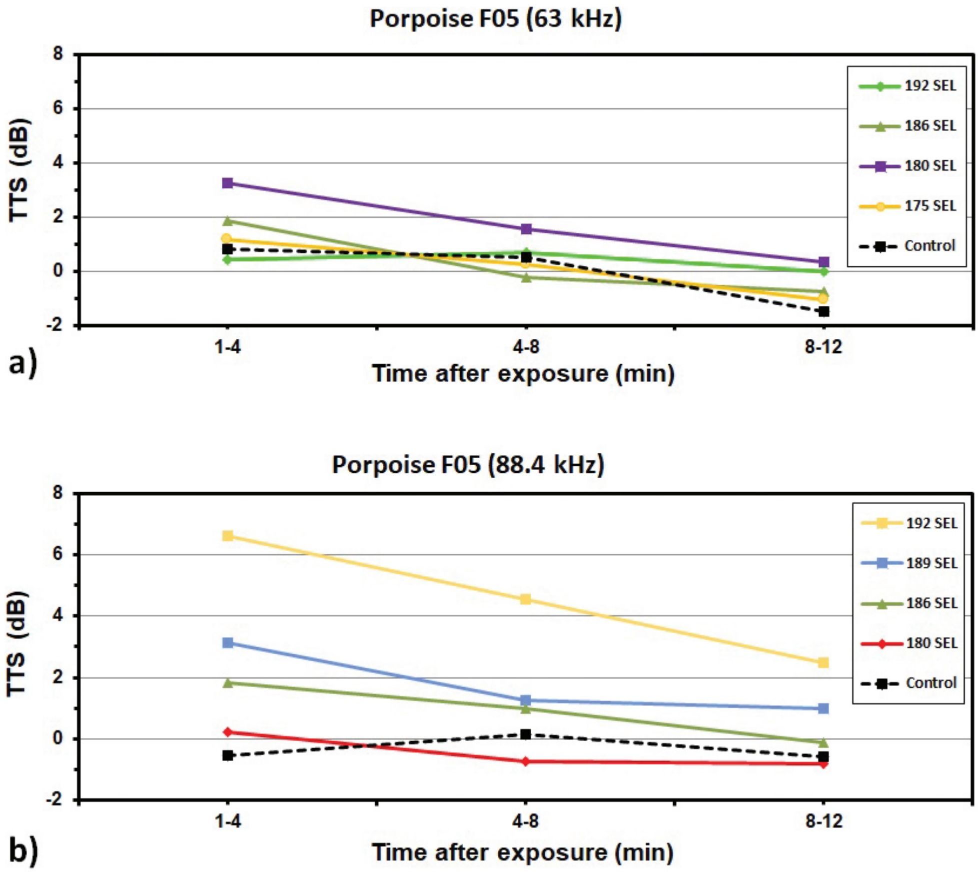

Figure 4. Changes in Porpoise F05's hearing at 63 kHz (a) and 88.4 kHz (b) after exposure to a continuous one-sixth-octave noise band centered at 63 kHz for 60 min at several SELs. Sample sizes and SDs for TTS_{1-4} are shown in Table 2. Hearing tests started within 1 min after the fatiguing sound stopped. For average received SPLs (dB re 1 µPa), subtract 36 dB re 1 s from the SEL values.

probably occurred within 72 min when TTS was 1.2 dB (Figure 5b). For hearing test signals of 100 and 125 kHz, no TTS_{12-16} occurred, even at the highest SEL of 192 dB re 1 µPa^{2}s (Table 2; Figure 2c). No change in TTS susceptibility was observed over the duration of the study. The control sessions showed that the hearing thresholds for all four hearing test signals before and after 60-min exposures to the low ambient noise were very similar (Figure 5; Table 2).

Discussion

Evaluation

In the present study, we exposed the animals to the fatiguing sound for 1 h. Relative to particular high-amplitude sound sources, such as sonars, percussion pile driving, and seismic surveys, this is a long time (some may last long but generally produce short-duration intermittent sounds). This duration was selected so lower SPLs could be used to cause TTS. It is very important in TTS research to have clean signals without high-SPL harmonics, as the harmonics may affect other hearing frequencies than the expected target hearing frequencies in the range between the center frequency of the fatiguing sound and one octave above this frequency. It is very difficult to produce high SPLs without harmonics; therefore, we did not push our transducer to the maximum, which would have produced harmonics, but chose slightly lower SPLs and longer exposures. The equal energy hypothesis assumes that sound exposures with the same energy (expressed in SEL_{cum}) lead to the same TTS (Southall et al., 2007; i.e., an SEL

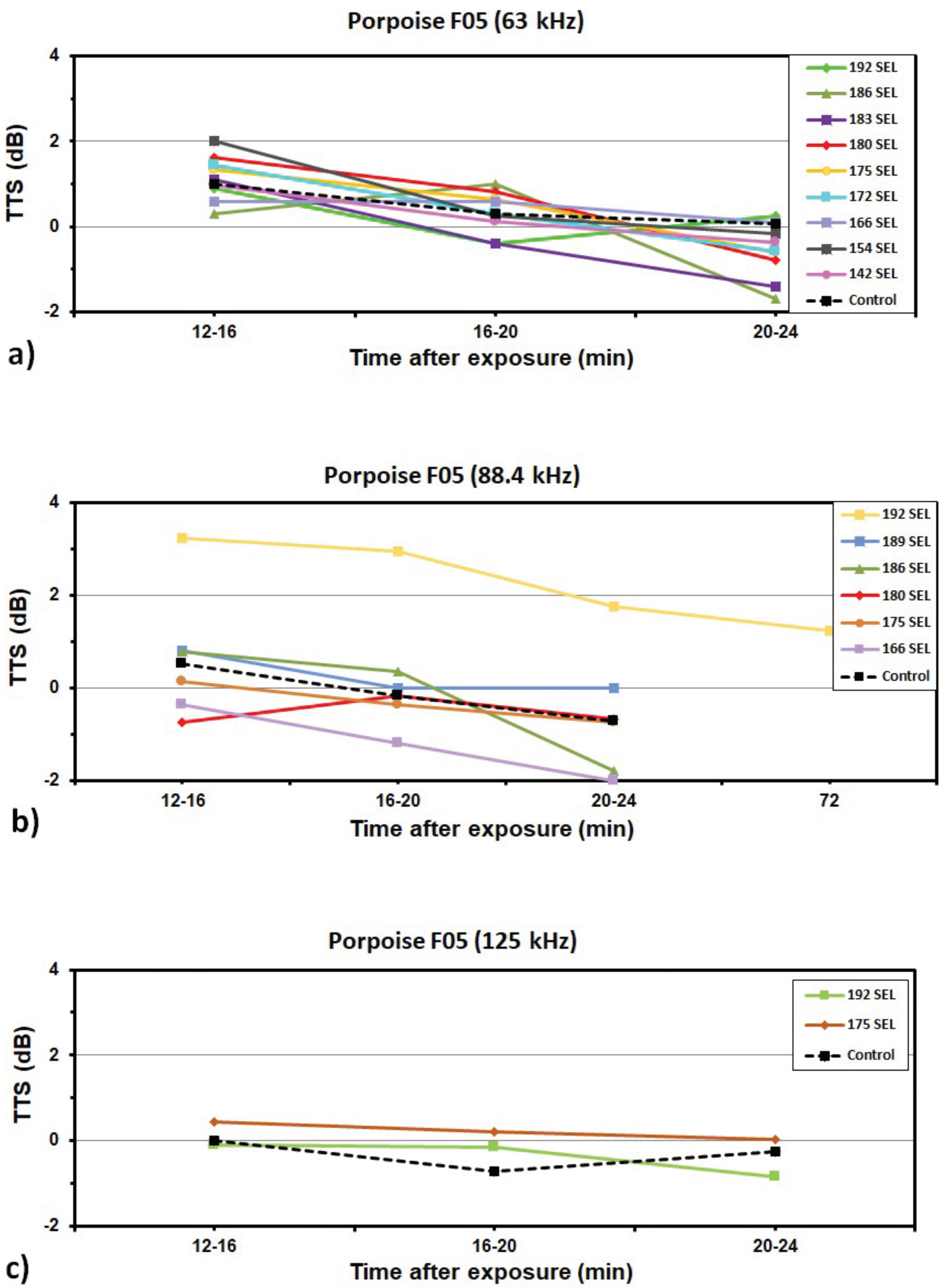

Figure 5. Changes of Porpoise F05's hearing at 63 kHz (a), 88.4 kHz (b), and 125 kHz (c) after exposure to a continuous one-sixth-octave noise band centered at 63 kHz for 60 min at several SELs. Sample sizes and SDs for $TTS_{12\text{-}16}$ are shown in Table 2. Hearing tests started 12 min after the fatiguing sound stopped. For average received SPLs (dB re 1 µPa), subtract 36 dB re 1 s from the SEL values.

composed of a high SPL and a short exposure duration causes the same TTS as the same SEL composed of a low SPL and long exposure duration). However, Kastelein et al. (2012a) showed that this assumption is not met for TTS resulting from low amplitude, long duration noise exposures in a harbor porpoise. The assumption also fails for bottlenose dolphins (Mooney et al., 2009; Finneran & Schlundt, 2010) and harbor seals (*Phoca vitulina*; Kastelein et al., 2012b) in which an increase in SEL due to an increase in exposure duration has a different effect on the induced TTS than the same increase in SEL due to an increase in SPL. So extrapolation of the results to exposures with SELs composed of higher SPLs and shorter durations may not be straightforward.

Comparison of TTS Between the Study Animals

The present study was conducted with two animals. Their hearing thresholds were similar to those of three other harbor porpoises (young males; Kastelein et al., 2017b), which suggests that the study animals had normal hearing for porpoises of their age. However, it is not clear how representative the TTS values found in these animals are. 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). Relatively small individual differences in susceptibility to TTS were apparent in the animals in the present study. Exposure to 180 dB SEL caused 7.3 dB TTS_{1-4} in Porpoise M06 and 3.2 dB in Porpoise F05 at 63 kHz. Exposure to 192 dB SEL caused 5 dB TTS_{1-4} in Porpoise M06 and 6.6 dB in Porpoise F05 at 88.4 kHz. These TTS values are in the same order of magnitude, and the observed difference in measured TTSs between the two study animals may be related to their slightly different swimming patterns (resulting in them experiencing different SELs), to their age difference, or to individual differences in TTS susceptibility.

Relationship Between SEL and TTS

In previous TTS studies with harbor porpoises, the hearing frequency with the highest TTS depended on the SPL of the fatiguing sound (Kastelein et al., 2014a, 2019a, 2019b). However, for the SEL range tested in the present study, Porpoise M06 experienced most TTS at the center frequency of the fatiguing sound. At the higher levels, TTS growth increased more strongly half an octave above the center frequency of the fatiguing sound (88.4 kHz; Figure 3a). Maybe if even higher SELs could have been produced, the TTS measured at the half an octave higher frequency than the center frequency of the fatiguing sound would have been larger in magnitude than the TTS measured at the center frequency. The pattern in Porpoise F05 was more in line with expectations: at low SELs, most TTS was elicited at the center frequency of the fatiguing sound; but when the SEL increased, the highest TTS_{1-4} occurred half an octave above the center frequency of the fatiguing sound. The "switch" was around 186 dB SEL (Figure 2b & c).

Data from humans and other terrestrial mammals show that, for moderate and large hearing shifts, maximum TTS occurs half 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, 2013). However, the maximum TTS in a harbor porpoise (identified as Porpoise M02), a California sea lion (*Zalophus californianus*), and harbor seals exposed to octave-band noise occurred at the fatiguing sound's center frequency rather than above it (Kastak et al., 2005; Kastelein et al., 2012a, 2012b), possibly because the SELs the animals were exposed to were low.

The relationship between fatiguing sound SPL and hearing frequency showing most TTS is probably due to changes in the spread of the basilar membrane excitation pattern: as the level of the fatiguing sound increases, the affected hearing range becomes broader. This finding may 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). Studies on odontocetes in which impulsive sounds are used as the fatiguing sound show that TTS occurs at frequencies above the peak frequency of the fatiguing broadband sound (Finneran et al., 2002; Lucke et al., 2009; Kastelein et al., 2015a, 2017c). It is likely that broadband exposures produce broadband TTSs with an upward frequency spread, similar to that seen after exposure to tones and narrow-band noise (Finneran, 2015).

Relationship Between Fatiguing Sound Frequency and TTS

Susceptibility to TTS and its relationship with fatiguing sound frequency can be explored by relating equal TTS data to fatiguing sound frequencies (Finneran, 2015; Houser et al., 2017; Southall et al., 2019). Research on bottlenose dolphins (Finneran & Schlundt, 2013), Yangtze finless porpoises

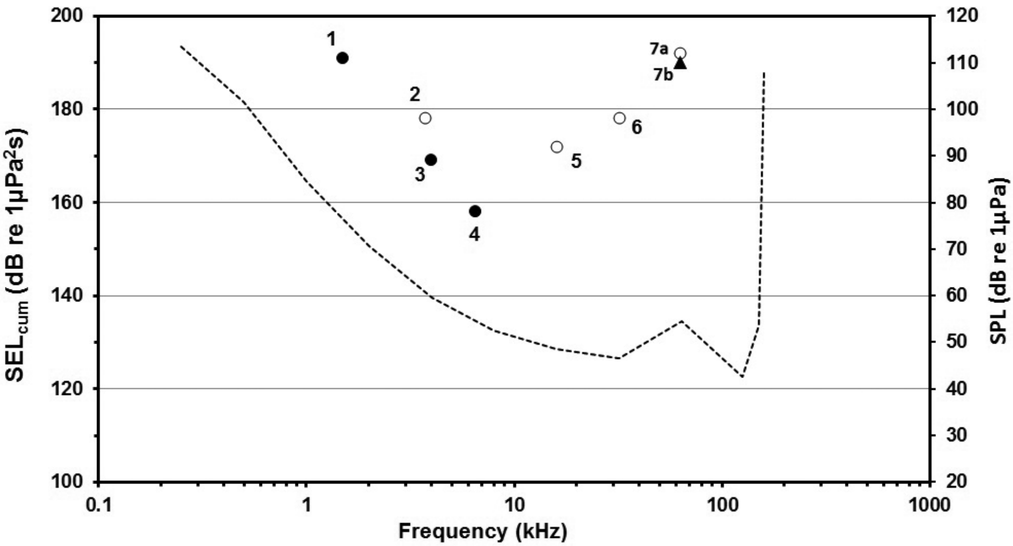

Figure 6. The cumulative SEL (SEL_{cum}) required to cause a mean TTS_{1-4} of around 6 dB in harbor porpoises after exposure to (1) a 1 to 2 kHz sweep at 100% duty cycle for 60 min (Kastelein et al., 2014b), (2) a 3.5 to 4.1 kHz 53-C sonar playback sound at 96% duty cycle (Kastelein et al., 2017a), (3) a one-octave noise band centered at 4 kHz at 100% duty cycle (Kastelein et al., 2012a), (4) a 6.5 kHz tone at 100% duty cycle (Kastelein et al., 2014a), (5) a one-sixth-octave noise band centered at 16 kHz at 100% duty cycle (Kastelein et al., 2019b), (6) a one-sixth-octave noise band centered at 32 kHz at 100% duty cycle (Kastelein et al., 2019a), and (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, present study). The solid circles are studies with young male Porpoise M02, the open circles are studies with Porpoise M06, and the solid triangle is Porpoise F05. The audiogram of Porpoise M02 (Kastelein et al., 2010; right-hand y-axis) is shown as a dashed line. Note that numbers 1, 3, and 4 were measured at the center frequency of the fatiguing sound, and numbers 2, 5, 6, 7a, and 7b were measured half an octave above the center frequency.

(Popov et al., 2011), and belugas (Popov et al., 2013) showed that the magnitude of TTS induced by fatiguing sounds with the same received SEL is dependent on the frequency of the fatiguing sounds, and that this frequency-dependent TTS susceptibility is species specific.

In the present study, TTS_{1-4} at 88.4 kHz occurred at a higher SEL than that which caused TTS onset after exposure to sounds of 16 and 32 kHz in the same harbor porpoise (Porpoise M06; Kastelein et al., 2019a, 2019b), and 6 to 7 kHz in Porpoise M02 (Kastelein et al., 2014a, using the same psychophysical technique; Figure 6). For fatiguing sounds with frequencies of ~6.5 kHz, it appears that susceptibility to TTS increases with increasing frequency; but above ~6.5 kHz, it appears that susceptibility to TTS decreases with increasing frequency (based on the studies by Kastelein et al., 2019a, 2019b, and the present study).

There may be individual differences in susceptibility to TTS between Porpoise M02 (exposed to 6 to 7 kHz sweeps; Kastelein et al., 2014a) and Porpoises M06 and F05 (exposed to a one-sixth-octave noise band around 16, 32, and 63 kHz; Kastelein et al., 2019a, 2019b, and the present study), however. Alternatively, differences in fatiguing sound type (sweeps vs noise bands) may have resulted in (or contributed to) differences in the induced TTSs. Although the TTS onset SELs from previous TTS studies with harbor porpoises can probably be compared (Figure 6), it is unclear whether the affected hearing frequency (relative to the center frequency of the fatiguing sound) that showed the highest TTS was the same for one-octave noise bands (Kastelein et al., 2012a), one-sixth-octave noise bands (Kastelein et al., 2019a, 2019b, and the present study), narrow-band sweeps (Kastelein et al., 2014b, 2015b), tonal (continuous wave) sounds (Kastelein et al., 2013, 2014a), and broadband impulsive sound (Kastelein et al., 2015a, 2017c). Note that the SEL causing 6 dB TTS in the aforementioned studies was only measured, or occurred in some cases, at the center frequency of the fatiguing sound, and sometimes half an octave above the center frequency. This may have depended on the SELs used in the studies, as the frequency at which the highest TTS occurs after a sound exposure is not only influenced by the frequency of the fatiguing sound but also by the SEL (Kastelein et al., 2014a).

The results of the present study as well as previous studies, although only representing part of the harbor porpoise's hearing frequency range (1.5 to 63 kHz), are in agreement with those of Finneran & Schlundt (2013) and Popov et al. (2011, 2013) in other odontocete species, and suggest that the susceptibility of harbor porpoise hearing to TTS is also frequency-dependent. The pattern of susceptibility is most similar to that found for bottlenose dolphins 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, a species more closely related to the harbor porpoise, 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 below 1 kHz are needed to define weighting functions for improved TTS prediction in this species.

Conclusions and Application of Results

The present study suggests that harbor porpoises that are exposed to sounds of around 63 kHz experience TTS only if the sounds have relatively high SELs (Figure 6). Research is ongoing to elucidate frequency-dependent susceptibility to TTS in harbor porpoises. Once susceptibility to TTS has been quantified for the entire hearing range of harbor porpoises, it will be possible to generate valid auditory weighting curves for cetaceans that echolocate at high frequencies using harbor porpoises as a model (Houser et al., 2017).

For fatiguing sounds around 63 kHz, we advise using the TTS_{1-4} onset (as generally defined as 6 dB) SEL at 88.4 kHz hearing test frequency for TTS susceptibility comparison with other fatiguing sound frequencies (data points 7a and 7b in Figure 6) because (1) the TTS_{1-4} in Porpoise M06 at 63 kHz hearing test frequency showed an unusual pattern (in that relatively high TTS_{1-4} occurred at lower SELs at 63 kHz) compared to previous TTS studies with this species; (2) Porpoise M06's TTS at 63 kHz hearing test frequency appeared to asymptote; (3) at the three highest tested SELs, the TTS growth at hearing test frequency 88.4 kHz was strong suggesting that if even higher SELs had been tested, the highest TTS_{1-4} would occur at 88.4 kHz hearing test frequency; and (4) Porpoise F05 showed the more usual TTS pattern as seen in all previous continuous sound TTS studies with this species (i.e., that only small or no TTS is generated at the center frequency of the fatiguing sound and that strong TTS growth occurred at half an octave above the center frequency at higher SELs).

The present study gives insight into the potential effects of some biological sounds (snapping shrimp; Au & Banks, 1998) and anthropogenic underwater sounds (some naval and fish-finding sonars which are in the frequency range of 20 to 200 kHz; and data communication and positioning systems consisting of modems and transponders which are in the frequency range of 10 to 70 kHz; see the Discovery of Sound in the Sea [DOSITS] website: https://dosits.org) on harbor porpoises. The results of the present study, previous studies, and future TTS studies with harbor porpoises allow safety levels to be set which may safeguard harbor porpoise hearing from damage by anthropogenic sound sources.

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