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Temporary Hearing Threshold Shift and Testing the Equal-Energy Hypothesis in a Harbor Porpoise (*Phocoena phocoena*) After Exposure to a Continuous Noise Band at 8 kHz, and a Revised TTS-Onset Function

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Abstract

Susceptibility to temporary hearing threshold shifts (TTS) in harbor porpoises (*Phocoena phocoena*) depends in part on the frequency of the fatiguing sound (the sound causing the shift). The TTS induced and the pattern of recovery were documented in a female porpoise after exposure for one hour to a continuous one-sixth-octave noise band centered at 8 kHz. This fatiguing sound was emitted at average received sound pressure levels (SPLs) between 126 and 144 dB re 1 µPa, resulting in average sound exposure levels (SELs) of 162 to 180 dB re 1 µPa^{2}s. Hearing thresholds for narrow-band sweeps centered at 8, 11.3, and 16 kHz were determined before and after exposure. Control sessions were used to determine which SELs resulted in statistically significant TTS in the first four minutes after the fatiguing sound stopped (TTS_{1-4}). At 8 kHz, the lowest SEL that resulted in significant TTS_{1-4} (4.4 dB) was 174 dB re 1 µPa^{2}s; at 11.3 kHz, the lowest SEL that resulted in significant TTS_{1-4} (4.9 dB) was 168 dB re 1 µPa^{2}s; and at 16 kHz, the lowest SEL that resulted in significant TTS_{1-4} (1.3 dB) was 174 dB re 1 µPa^{2}s. The hearing frequency that was most affected was 11.3 kHz, half an octave above the fatiguing sound's center frequency. The equal-energy hypothesis was tested by exposing the porpoise to the same noise band with SPLs of 137 to 153 dB re 1 µPa and exposure durations between two and 80 minutes; all seven combinations resulted in the same fatiguing SEL of 174 dB re 1 µPa^{2}s; and for these combinations, the equal-energy hypothesis was upheld. The results add to the body of data on TTS-onset SELs that were used to generate a revised auditory weighting function and, thus, enhance regulatory protection of wild harbor porpoises that are exposed to anthropogenic noise at sea.

Key Words: anthropogenic noise, audiogram, EIA, frequency weighting, harbor porpoise, hearing loss, hearing sensitivity, low frequency, odontocete, temporary threshold shift, TTS, TTS-onset function

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 has a large geographic range in the coastal waters of the northern hemisphere (Bjorge & Tolley, 2008) and possesses hearing over a wide frequency range (~0.5 to 140 kHz; Kastelein et al., 2017b). The harbor porpoise seems to be particularly susceptible to temporary hearing threshold shifts (TTS) after exposure to loud fatiguing sounds (Lucke et al., 2009; Finneran, 2015; Tougaard et al., 2016; Houser et al., 2017). Depending on the exposure parameters, sound-induced TTS varies in magnitude and duration, and it may compromise feeding, orientation, communication, and predator detection in wild harbor porpoises and other marine mammals that rely strongly on acoustics for these life functions (e.g., Au, 1993). The harbor porpoise has a high metabolism and feeding rate (Wisniewska et al., 2016; Kastelein et al., 2018a, 2018b) so that even minor acoustic exposures, resulting in small TTS from which recovery is rapid, may impact harbor porpoises, especially if the exposures are frequent. The cumulative time lost for feeding during both exposure and recovery, for example, may have health repercussions. Therefore, TTS may negatively impact a harbor porpoise's health, reproduction, and survival, even if permanent hearing threshold shifts do not occur. As a result, TTS of a certain magnitude and occurring frequently may have adverse population effects in the long term.

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), so it is important to quantify the effect of fatiguing sounds of various frequencies on the hearing of the harbor porpoise (Houser et al., 2017; National Marine Fisheries Service [NMFS], 2018). 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 of the harbor porpoise (i.e., ~0.5 to 140 kHz). Susceptibility to TTS for the following fatiguing sound frequencies has been established in harbor porpoises: 0.5, 1.5, 1-2, 4, 3.5-4.1, 6-7, 6.5, 16, 32, 63, and 88.4 kHz (Kastelein et al., 2012a, 2013, 2014a, 2014b, 2015b, 2017a, 2019a, 2019b, 2020a, 2020b, 2020c, 2021a). Fatiguing sound frequencies higher than 88.4 kHz have not been tested, as fatiguing sounds around 88.4 kHz cause shifts at the upper frequency limit of harbor porpoises' hearing. Exposure to fatiguing sounds of 88.4 kHz is likely to affect hearing between 88.4 and 125 kHz (Kastelein et al., 2020c; maximal TTS usually occurs half an octave above the center frequency of the fatiguing sound; McFadden, 1986), and harbor porpoise hearing sensitivity decreases by ~60 dB between 125 and 140 kHz (Kastelein et al., 2017b). Therefore, fatiguing sounds of > 88.4 kHz are unlikely to cause TTS, unless they are particularly high-amplitude exposures.

The present study was designed to provide data to improve the regulatory protection of harbor porpoise hearing at sea. It adds to previous research by quantifying susceptibility to TTS in a harbor porpoise after exposure to fatiguing sound centered at 8 kHz.

The first aim was to add a data point to the frequency range (8 kHz, a fatiguing sound frequency between the already tested 6.5 and 16 kHz) on which an equal-TTS susceptibility function for harbor porpoises can be based (see Houser et al., 2017) to facilitate improved modelling of the auditory weighting function. Recovery times from TTS due to the noise band centered at 8 kHz were also measured.

The second aim of the present study was to test the equal-energy hypothesis (i.e., that different combinations of SPL and exposure duration resulting in the same sound exposure levels [SELs] elicit similar TTSs; Ward et al., 1981). In most studies of TTS in harbor porpoises, exposure to the fatiguing sounds lasted for 1 h and a limited range of SPLs was used, so understanding of the effects of other SPLs and durations is limited. If the equal-energy hypothesis is upheld, it will be possible to extrapolate the results of the present study and others to sound exposures with different SPL and duration combinations. This will increase the practical value of all previous and future TTS studies with harbor porpoises, and will mean that the results can be used with more confidence in Environmental Impact Assessments (EIAs).

The third aim of the present study was to establish a revised TTS-onset function based on TTS-onset SELs from Kastelein et al. (2012a, 2014a, 2014b, 2017a, 2019a, 2019b, 2020a, 2020b, 2020c) and from the present study.

Methods

Study Subject and Site

The study subject, a previously stranded and rehabilitated adult female harbor porpoise (identified as F05; age: ~11 y, body mass: ~49 kg, body length: 155 cm, girth at axilla: ~83 cm), had participated in previous studies of TTS induced by sounds of 0.5, 1.5, 3.5-4.1, 6.5, 16, 32, 63, and 88.4 kHz (Kastelein et al., 2017a, 2019a, 2019b, 2020a, 2020b, 2020c, 2021a). These previous studies did not compromise her auditory ability, and her hearing thresholds in the frequency range tested in the present study (8 to 16 kHz) are believed to be 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 (Figure 1) designed and built for acoustic research, consisting of an indoor pool (8 m × 7 m; 2 m deep) in which the study was conducted, connected via a channel (4 m × 3 m; 1.4 m deep) to an outdoor pool (12 m × 8 m; 2 m deep) which the porpoise had access to when the study was not being conducted. For details of the pool, equipment, and water flow, see Kastelein et al. (2019b).

Equipment Calibration

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 by an independent research organization (TNO) just before and at the end of the study period (for calibration methods, see Kastelein et al., 2019b). Under test conditions

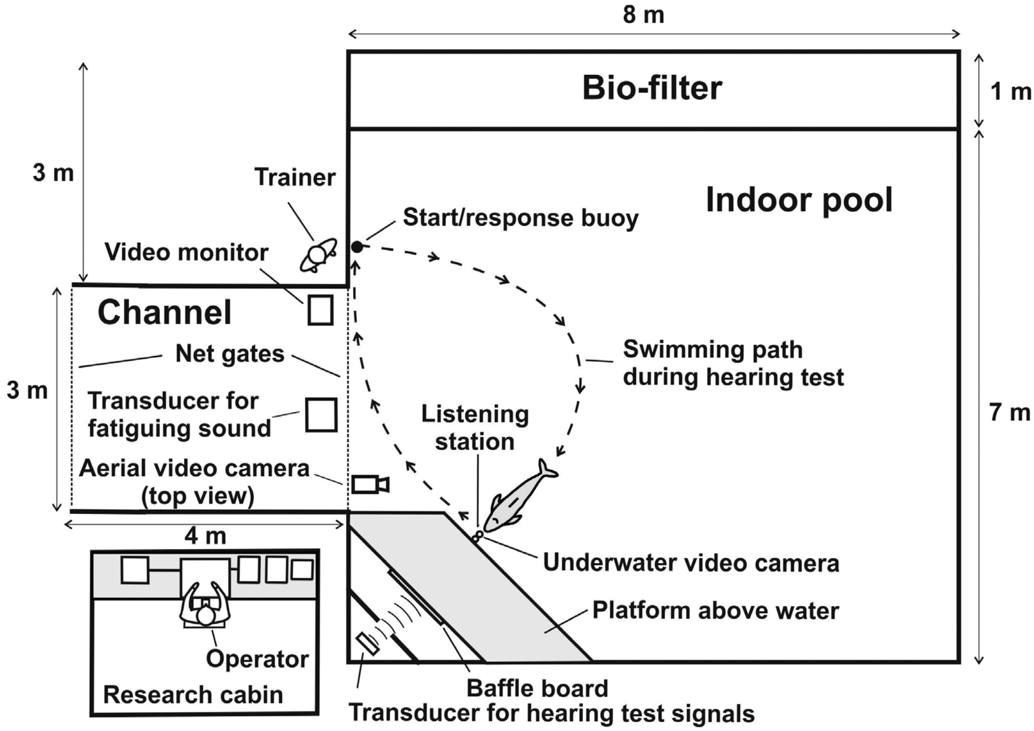

Figure 1. The indoor pool in which the temporary hearing threshold shift (TTS) study with harbor porpoise F05 was conducted. On each test day, a pre-exposure hearing test was conducted to test one of three hearing frequencies (8, 11.3, or 16 kHz). This was followed by between 2 and 80 min of exposure to the one-sixth-octave noise band centered at 8 kHz (or to 1 h of low ambient noise in control sessions), then by one or several post-exposure hearing tests (to test the same hearing frequency as used in the pre-exposure hearing test of that day).

(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 between 5 and 25 kHz. This was similar to the background noise level at which previous TTS studies with harbor porpoises had been conducted (see Kastelein et al., 2012a, 2013, 2014a, 2014b, 2015a, 2015b, 2017a, 2019a, 2019b, 2020a, 2020b, 2020c, 2021a).

Hearing Test Signals

The hearing thresholds were tested at the frequency of the fatiguing sound, half an octave higher, and one octave higher (8, 11.3, and 16 kHz). The linear upsweeps used as hearing test signals started and ended at ± 2.5% of the center frequency and had durations of 1,000 ms, including a linear rise and fall in amplitude of 50 ms. The hearing test signals were generated digitally and were calibrated and checked daily, as explained by Kastelein et al. (2019b).

Fatiguing Sound

The digitized fatiguing sound was produced, transmitted, calibrated, and checked before each exposure session, as described by Kastelein et al. (2019b); the sound was transmitted into the pool by an underwater transducer (Model LL1424HP; Lubell, Columbus, OH, USA) through an isolation transformer (Model AC1424HP, Lubell). The transducer was placed at 1 m depth in the channel adjoining the pool (Figure 1). The fatiguing sound consisted of a continuous (duty cycle 100%) one-sixth-octave Gaussian white noise band, centered at 8 kHz (bandwidth: 7.6 to 8.5 kHz; thus, a narrow bandwidth). Ideally, an 8 kHz tone would have been used, but a pure tone can lead to a very heterogeneous sound field in a pool due to reverberation leading to standing waves. Therefore, instead of a tonal signal, a very narrow noise band was selected.

To determine the fatiguing sound's pattern of distribution in the indoor pool, the SPL of the noise band was measured at 40 locations in the horizontal plane (on a horizontal grid of 1 m × 1 m), and

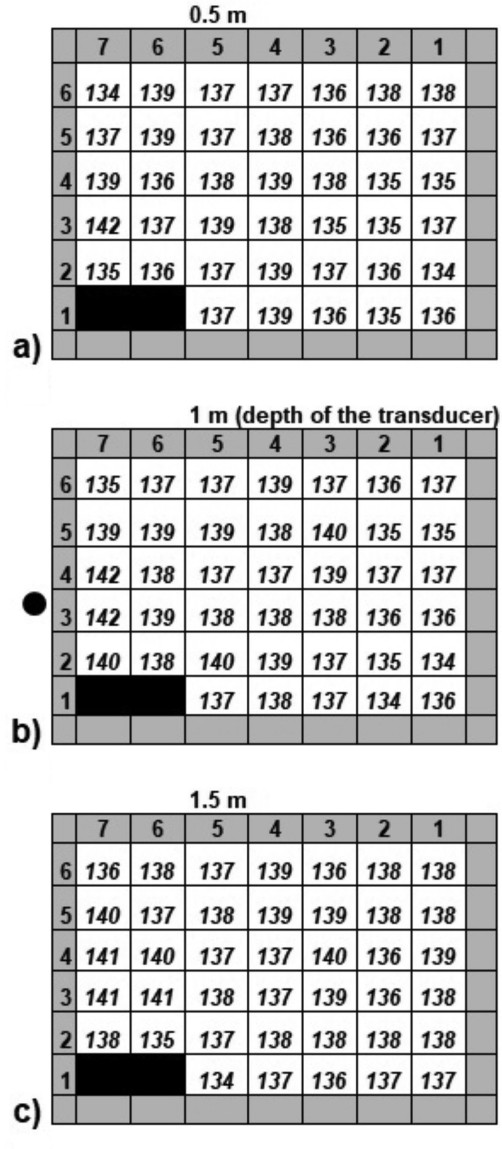

Figure 2. An example of the sound pressure level (SPL) distribution in the harbor porpoise's (*Phocoena phocoena*) indoor pool during exposure to the continuous (100% duty cycle) one-sixth-octave noise band centered at 8 kHz (the fatiguing sound), measured at depths of 0.5 m (a), 1.0 m (b), and 1.5 m (c). The black dot in (b) indicates the location of the transducer, which was placed at 1 m depth in the channel adjoining the pool. The numbers in the grey fields indicate 1-m markings on the side of the pool. The mean SPL in this example is 138 dB re 1 µPa (range: 134 to 142 dB re 1 µPa; standard deviation: 2; $n = 120$). The mean sound exposure level (SEL) over the 1 h exposure to this mean SPL is 174 dB re 1 $\mu Pa^2 s$.

at three depths per location on the grid (0.5, 1.0, and 1.5 m below the surface), resulting in a total of 120 measurements in the pool (Figure 2). Apart from just around the transducer, the differences in mean SPL at different depths (based on the power sum) were minimal. 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 sound exposure sessions, the one-sixth-octave noise band centered at 8 kHz was projected for 1 h, or for time periods between 2 and 80 min when testing the equal-energy hypothesis, at various source levels, resulting in mean SPLs in the pool (assumed to be the mean received SPL by the harbor porpoise in the present study) ranging in the TTS growth study from 126 to 153 dB re 1 µPa (SEL range: 162 to 180 dB re 1 $\mu Pa^2 s$), and from 137 to 153 dB re 1 µPa in the equal-energy study (all seven combinations resulting in an SEL of 174 dB re 1 $\mu Pa^2 s$). The TTS after exposure to 126 dB re 1 µPa SPL was only tested with 11.3 kHz to determine TTS-onset SEL. (See further on in the "Methods" section for more details about the equal-energy hypothesis, and see the "Results" section for sample sizes of each hearing test frequency.)

Experimental Procedures

On each test day, one total sound exposure test was conducted, consisting of (1) a pre-exposure hearing test starting at ~0830 h, (2) a fatiguing sound exposure (for 60 min, or for between 2 and 80 min when testing the equal-energy hypothesis; replaced by exposure for 60 min to ambient noise during control sessions; all exposures were timed precisely), and (3) a number of post-exposure hearing tests. Both the hearing tests and fatiguing sound (or ambient noise) exposures took place in the indoor pool (Figure 1).

Post-exposure hearing tests started within 1 min after the fatiguing sound stopped. The harbor porpoise's hearing thresholds were measured during post-sound exposure (PSE) periods 1-4 min ($PSE_{1\text{-}4}$), 4-8 min ($PSE_{4\text{-}8}$), 8-12 min ($PSE_{8\text{-}12}$), and, if hearing had not recovered then, 60 min (PSE_{60}), 120 min (PSE_{120}), 240 min (PSE_{240}), and 1,440 min (24 h; $PSE_{1,440}$) after sound exposure had ended. Hearing was considered to have recovered when the hearing threshold was ≤ 2 dB above the pre-exposure threshold level, as fluctuations of ≤ 2 dB occurred after control sessions in the quiet conditions of the present study (see "Results") and in other, similar studies (Kastelein et al., 2012a, 2013, 2014a, 2014b, 2015b, 2017a, 2019a, 2019b, 2020a, 2020b, 2020c, 2021a). The SELs of the fatiguing sound were tested in random order. Each SEL was tested at least four times per hearing frequency, except 144 dB SEL, which was tested only three times at 8 and 16 kHz. Once it was clear that the highest TTS was at 11.3 kHz, we did not want to fatigue the animal's ear more than necessary with this high SEL.

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 indoor pool for 1 h; the transducer was placed in the pool as usual but did not emit sound. Control sessions lasting 1 h were also conducted while testing the equal-energy hypothesis. The control periods did not match the specific exposure periods (2 to 80 min), as the animal was exposed to the ambient noise most of the day. Post-ambient exposure (PAE; control) hearing test sessions were 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. Seven or eight control tests were conducted per hearing test frequency. The control tests 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 (Figure 1). The porpoise stationed there for a random period of between 6 and 12 s before the operator produced the hearing test signal (in signal-present trials). Upon hearing the signal, the porpoise swam back to the start/response buoy, where she received a food reward. If she did not hear the signal, she stayed at the listening station until she was asked to return to the start/response buoy (by the trainer tapping three times on the side of pool); no food reward was given. About two thirds of each session consisted of signal-present trials and about one third consisted of signal-absent trials (also called "catch" trials). During signal-absent trials, the trainer blew a whistle after the porpoise had spent 6 to 12 s at the listening station to instruct her to return to the start/response buoy, where she received a food reward. Signal-absent trials were included to maintain the porpoise's attention and motivation, and to allow quantification of any response bias. The up-down staircase hearing test method was used. A switch from a test signal level to which the porpoise responded to a level (2-dB steps) to which she did not respond, or vice versa, was called a "reversal." Each complete hearing test session consisted of ~25 trials and lasted up to 12 min. However, the first PSE or PAE sessions were subdivided into three 4-min periods. 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 (the threshold calculation for each 4-min period was based on the mean of these 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 were collected in October, November, and December 2021.

Testing the Equal-Energy Hypothesis

To test the equal-energy hypothesis (Ward et al., 1981), which states that all combinations of SPL and exposure duration that result in the same SEL elicit similar initial TTSs, the harbor porpoise was exposed to seven SPL and exposure duration combinations, all of which resulted in an SEL of 174 dB re 1 μPa^{2}s: SPL 153 dB re 1 μPa for 2 min, SPL 149 dB re 1 μPa for 5 min, SPL 146 dB re 1 μPa for 10 min, SPL 143 dB re 1 μPa for 20 min, SPL 140 dB re 1 μPa for 40 min, SPL 138 dB re 1 μPa for 60 min, and SPL 137 dB re 1 μPa for 80 min. The duty cycle was always 100%, and hearing was tested (see above) at 11.3 kHz, as the highest TTS_{1-4} occurred at this hearing frequency (see "Results"). Each combination was tested four times in random order, and control tests were also conducted.

Data to test the equal-energy hypothesis were collected between December 2021 and September 2022, following the protocol developed and explained in Kastelein et al. (2022) for a similar study with California sea lions (*Zalophus californianus*).

Data Analysis

When the harbor porpoise returned to the start/response buoy before being presented with a test signal (in signal-present trials) or before hearing the trainer's whistle (in signal-absent trials), her response was called a "pre-stimulus." The mean incidence of pre-stimuli is shown as a percentage (calculated as the number of pre-stimuli in both signal-present and signal-absent trials divided by all trials in each hearing test period × 100).

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

The TTS after the sound exposure sessions (TTS_{1-4}, TTS_{4-8}, TTS_{8-12}, TTS_{60}, TTS_{120}, TTS_{240}, and $TTS_{1,440}$) were calculated by subtracting $PE_{50\%}$ from the mean 50% hearing thresholds during the PSE_{1-4}, PSE_{4-8}, PSE_{8-12}, PSE_{60}, PSE_{120}, PSE_{240}, and

$PSE_{1,440}$ periods of the same day (see Kastelein et al., 2019b). Similarly, "TTS" (no actual shifts occurred) measured on control test days were calculated by subtracting $PE_{50\%}$ from the mean 50% hearing thresholds obtained during the PAE periods of the same day.

Researchers have classified increases in hearing thresholds of ≥ 6 dB as TTS; smaller increases could not be distinguished from random fluctuations in threshold measurements (Finneran, 2016; Houser et al., 2017; Southall et al., 2019). This definition is used here for comparison with other studies (see "Discussion"). However, the low background noise levels at the SEAMARCO Research Institute allow hearing threshold increases < 6 dB to be distinguished from random fluctuations. We define TTS onset as occurring at the lowest SEL at which a statistically significant difference could be detected between the TTS after fatiguing sound exposures and the "TTS" measured after the control exposures (this "shift" was close to zero, though some natural variation in hearing thresholds occurred). The level of significance was established by conducting a separate one-way ANOVA on mean TTS_{1-4} for each hearing test frequency with the factor SEL (including the control). ANOVAs that produced significant values overall were followed by Dunnett multiple comparisons between the control and the other levels of the factor to identify where significant differences occurred (Dunnett, 1964). The equal-energy hypothesis was tested by conducting a one-way ANOVA on TTS_{1-4} with the factor SPL (including the control), followed by Tukey multiple comparisons. All analyses were conducted using the software *Minitab 18* (Minitab LLC, USA), and data conformed to the underlying assumptions of the test applied (i.e., homogeneity of variances and normal distribution of residuals; Zar, 1999).

Results

Motivation and Pre-Stimuli

Before and after the sound exposure periods, the harbor porpoise was always willing to participate in the hearing tests. If 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 discarded. The mean incidence of pre-stimuli for both signal-present and signal-absent trials in the hearing tests varied between 1.1 and 6.3% (Table 1). The incidence of pre-stimuli in the pre-exposure, post-exposure, and post-ambient exposure (control) periods was also within this range.

Temporary Hearing Threshold Shifts and Recovery After Exposure to the Noise Band at 8 kHz

The ANOVAs showed that 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 174 dB re 1 μPa^2s for the hearing test frequencies 8 and 16 kHz, and at an SEL of 168 dB re 1 μPa^2s for the hearing test frequency of 11.3 kHz (Table 2; Figure 3).

For hearing test signals of 8 kHz, statistically significant TTS_{1-4} (≥ 4.4 dB) occurred in the harbor porpoise after exposure to SELs ≥ 174 dB re 1 μPa^2s (Table 2; Figure 3). Hearing recovered within 8 min after exposure to an SEL of 174 dB re 1 μPa^2s and within 12 min after exposure to an SEL of 180 dB re 1 μPa^2s (Table 2; Figure 4a).

For hearing test signals of 11.3 kHz, statistically significant TTS_{1-4} (≥ 4.9 dB) occurred after exposure to SELs ≥ 168 dB re 1 μPa^2s (Table 2; Figure 3), and hearing recovered within 12 min after exposure to an SEL of 168 dB re 1 μPa^2s, within 120 min after exposure to an SEL of

Table 1. The incidence of pre-stimuli (number of pre-stimuli as a percentage of all trials in each hearing test period) by harbor porpoise F05 in hearing tests during pre-exposure periods, in seven PSE periods (i.e., after exposure to a continuous one-sixth-octave noise band centered at 8 kHz), and in three PAE periods (i.e., after exposure to ambient noise in control sessions). All sound exposure levels (SELs) 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.

	Hearing test period							
Fatiguing sound	Pre-exposure	PSE_{1-4}	PSE_{4-8}	PSE_{8-12}	PSE_{60}	PSE_{120}	PSE_{240}	$PSE_{1,440}$
	4.7% (723)	1.4% (293)	1.5% (275)	2.2% (276)	3.1% (162)	3.8% (106)	1.1% (90)	2.3% (43)
Control	Pre-exposure	PAE_{1-4}	PAE_{4-8}	PAE_{8-12}	--	--	--	--
	3.8% (422)	1.1% (180)	6.1% (180)	6.3% (189)	--	--	--	--

Table 2. Results of one-way ANOVAs of mean $TTS_{1\text{-}4}$ (temporary hearing threshold shift 1 to 4 min after exposure, in dB) in harbor porpoise F05 after exposure for 1 h to the fatiguing sound (a continuous one-sixth-octave noise band centered at 8 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* = number of exposure tests). Mean values for $TTS_{1\text{-}4}$ that were significantly different from the control according to Dunnett multiple comparisons (Dunnett, 1964) are indicated with an asterisk, and SELs that mark the onset of statistically significant TTS are indicated in bold. Approximate hearing recovery times after significant TTS are also shown.

Hearing test frequency (kHz)	ANOVA results ($F_{factor\text{-}df,\ error\text{-}df}$ *p* value)	Mean SPL (dB re 1 µPa)	SEL (dB re 1 µPa²s)	$TTS_{1\text{-}4}$ (dB)				Recovery time (Min)
				Mean	SD	Range	*n*	
8	$F_{3, 14} = 69.57$ $p < 0.001$	Control	Control	-0.1	0.4	-0.5-0.6	7	--
		132	168	0.0	0.8	-1.2-0.8	4	--
		138	**174**	4.4*	0.8	4.1-5.6	4	8
		144	180	6.9*	1.5	5.2-8.2	3	12
11.3	$F_{4, 19} = 343.53$ $p < 0.001$	Control	Control	0.3	1.0	-1.6-1.2	8	--
		126	162	0.0	1.0	-0.8-1.5	4	--
		132	**168**	4.9*	1.5	3.1-6.7	4	12
		138	174	13.0*	1.2	11.9-14.8	4	120
		144	180	22.3*	0.6	21.4-22.9	4	1,440
16	$F_{2, 11} = 128.01$ $p < 0.001$	Control	Control	-0.6	0.8	-1.4-0.7	7	--
		138	**174**	1.3*	0.3	1.0-1.5	4	4
		144	180	6.1*	0.4	5.9-6.6	3	8

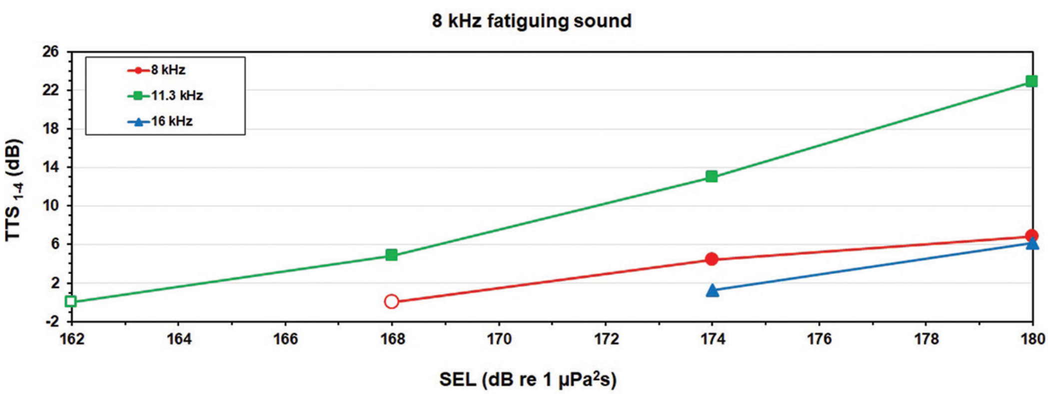

Figure 3. Mean $TTS_{1\text{-}4}$ (temporary hearing threshold shift 1 to 4 min after exposure, in dB) in harbor porpoise F05 after exposure for 1 h to the fatiguing sound (a continuous one-sixth-octave noise band centered at 8 kHz) at several sound exposure levels (SELs), quantified at hearing frequencies 8, 11.3, and 16 kHz (i.e., the center frequency of the fatiguing sound, half an octave above the center frequency, and one octave above the center frequency). Solid symbols indicate statistically significant $TTS_{1\text{-}4}$ relative to the control values; and open symbols indicate $TTS_{1\text{-}4}$ statistically similar to the control values. Sample sizes were three or four for each level (see Table 2). For control values, see Table 2 and Figure 4.

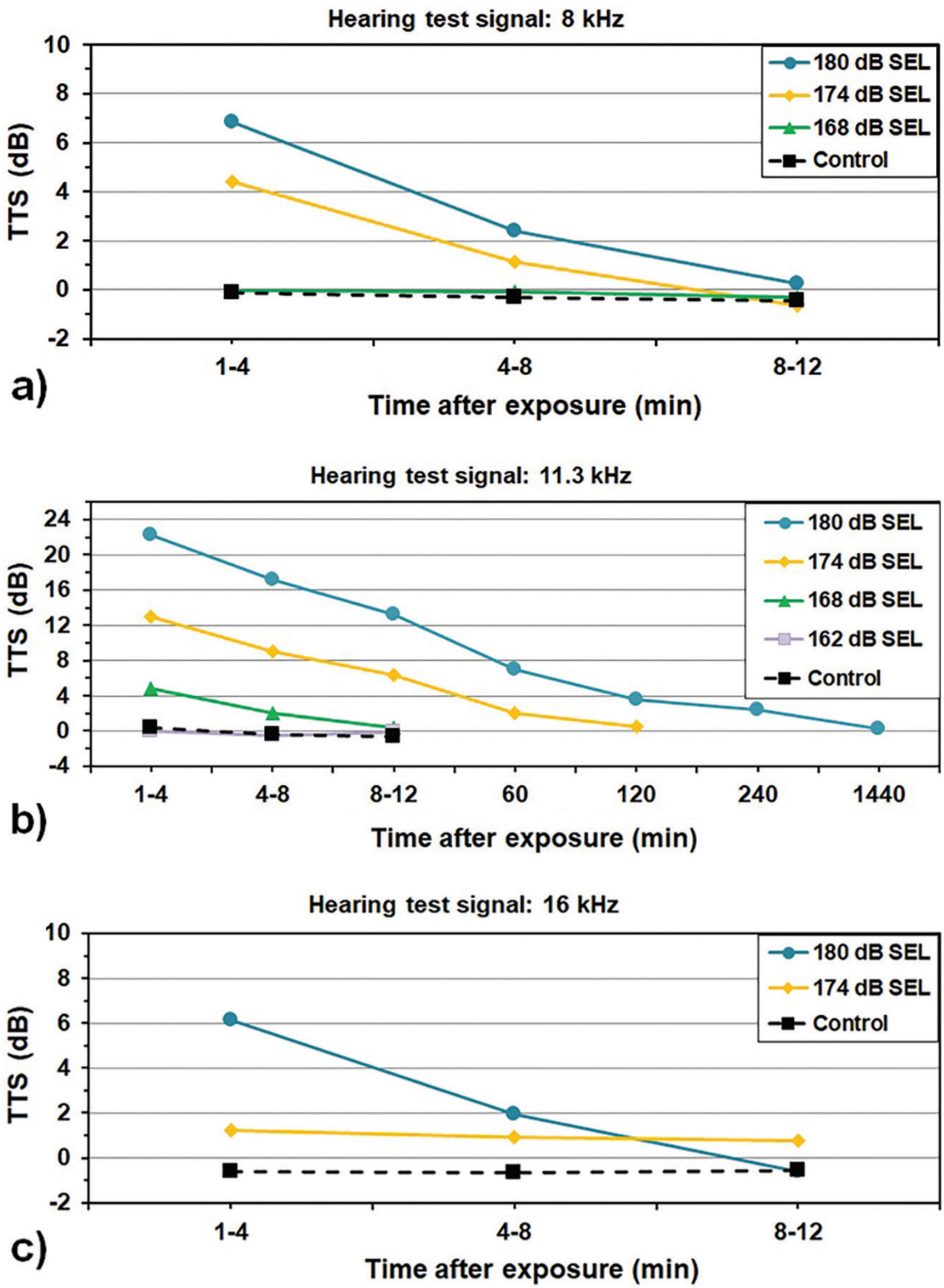

Figure 4. Changes over time, including recovery, in the hearing (TTS_{1-4}, temporary hearing threshold shift 1 to 4 min after exposure, in dB) of harbor porpoise F05 at 8 kHz (a), 11.3 kHz (b), and 16 kHz (c), after 1 h exposure to the fatiguing sound (a continuous one-sixth-octave noise band centered at 8 kHz) at several sound exposure levels (SELs). For sample sizes and standard deviations of mean TTS_{1-4}, see Table 2. Note that the x- and y-axes of (b) differ from those of (a) and (c).

174 dB re 1 µPa²s, and within 1,440 min (24 h) after exposure to an SEL of 180 dB re 1 µPa²s (Table 2; Figure 4b).

For hearing test signals of 16 kHz, a small, but statistically significant, TTS_{1-4} (1.3 dB—below the level used to define hearing recovery) occurred after exposure to an SEL of 174 dB re 1 µPa²s. A larger TTS_{1-4} (6.1 dB) occurred after exposure to an SEL of 180 dB re 1 µPa²s (Table 2; Figure 3), and hearing recovered within 8 min (Table 2; Figure 4c). As expected, 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 4).

Testing the Equal-Energy Hypothesis

After exposure to a one-sixth-octave noise band at 8 kHz, the equal-energy hypothesis held true for the harbor porpoise. After exposure to seven combinations of SPL and exposure duration that resulted in the same underwater SEL (174 dB re 1 µPa²s), hearing was tested at 11.3 kHz, four times for each combination. The one-way ANOVA was

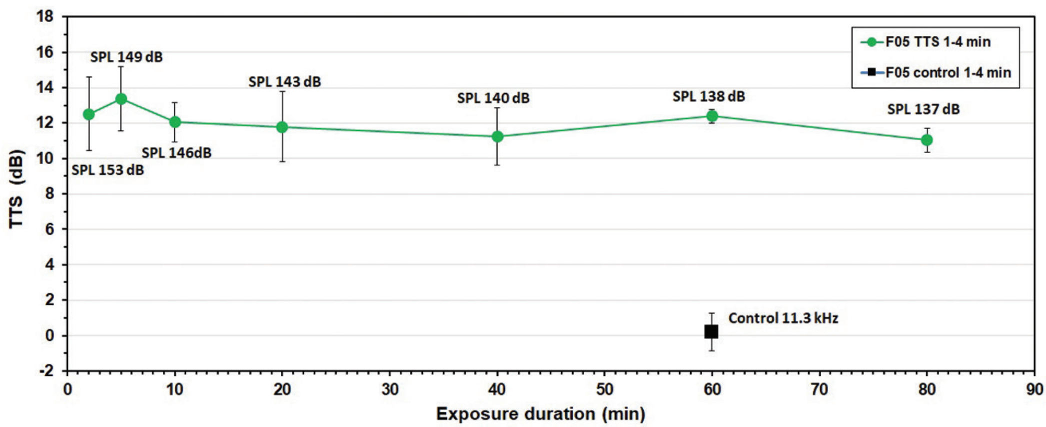

Figure 5. Testing the equal-energy hypothesis in the harbor porpoise. The mean TTS_{1-4} (temporary hearing threshold shift 1 to 4 min after exposure, in dB ± standard deviation; $n = 4$) of harbor porpoise F05 at 11.3 kHz after exposure to a one-sixth-octave noise band centered at 8 kHz for 2 to 80 min, at SPLs of 137 to 153 dB re 1 µPa. All seven combinations resulted in the same sound exposure level (SEL; 174 dB re 1 µPa²s). The mean "TTS" (and standard deviation) after 1 h control sessions is also shown.

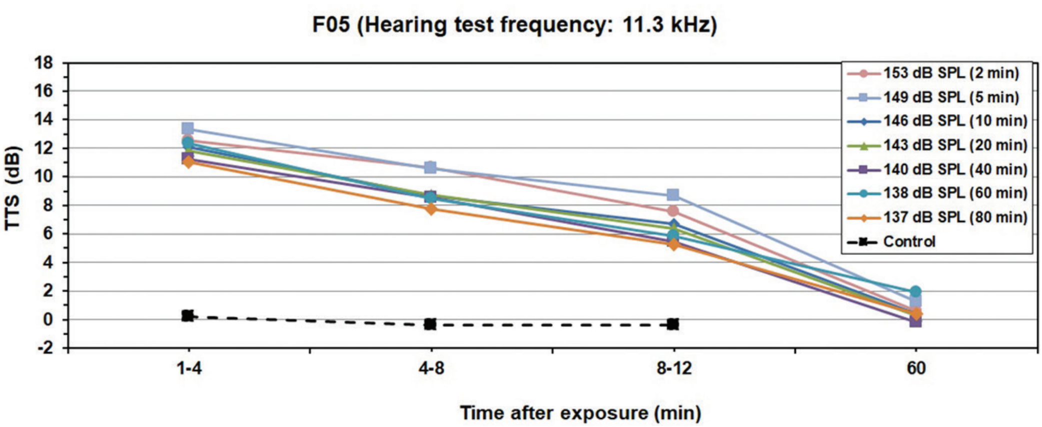

Figure 6. Changes in hearing over time, including recovery, while testing the equal-energy hypothesis. Mean TTS (temporary hearing threshold shift, in dB; $n = 4$) at 11.3 kHz of harbor porpoise F05, measured 1 to 12 and 60 min after exposure to the noise band at 8 kHz. The sound exposure level (SEL) of 174 dB re 1 µPa²s was composed of seven different combinations of sound pressure level (SPL; 137 to 153 dB re 1 µPa) and exposure durations (2 to 80 min). The mean "TTS" values during control sessions (no shifts occurred) are also shown (black dashed line).

significant ($p < 0.001$), and Tukey multiple comparisons showed that similar TTS occurred after all exposure combinations, since they all differed significantly from the control and not from one another (Figure 5). Recovery patterns were similar after exposure to all combinations of SPL and exposure duration (Figure 6).

Proposed Revised TTS-Onset Function

For fatiguing sound around 8 kHz, the SEL required to cause 6 dB TTS_{1-4} (a marker of the onset of TTS; Finneran, 2016; Houser et al., 2017; Southall et al., 2019) was 169 dB re 1 µPa²s. A proposed revised TTS-onset function for harbor porpoises was fitted to the 13 data points shown in Figure 7 (TTS-onset SELs derived from Kastelein et al., 2012a, 2014a, 2014b, 2017a, 2019a, 2019b, 2020a, 2020b, 2020c, present study; Table 3), based on Equation 3 of Southall et al. (2019), with the package 'nls' in *R*, Version 4.1.1 (R Core Team, 2021). The equation is

SEL = 164.59 - 10*log10((F/7.0)^(2*1.8) / (((1+(F/7.0)^2)^1.8)*((1+(F/21.16)^2)^2.47)))

The values for the fitting constants, as per Equation 3 in Southall et al. (2019), are

K = 164.59, f1 = 7.0, f2 = 21.16, a = 1.8, b = 2.47

where F is the frequency in kHz. This equation is a good fit to the data: the Pearson product-moment correlation is $R^2 = 0.889$, $t = 9.41$, $n = 13$, and $p = 0.000001$.

Discussion

Temporary Hearing Threshold Shifts and Recovery After Exposure to the Noise Band at 8 kHz

In the present study, a noise band was used instead of a pure tone to make the sound field as homogenous as possible (i.e., by preventing standing waves). A very narrow noise band was used to make the difference in bandwidth between a tone and the noise band small. It is not clear if the TTS results would have been different if a pure tone had been used as the fatiguing sound. More research is needed to evaluate the effect of fatiguing sound type on the magnitude of TTS, affected hearing frequencies, and recovery.

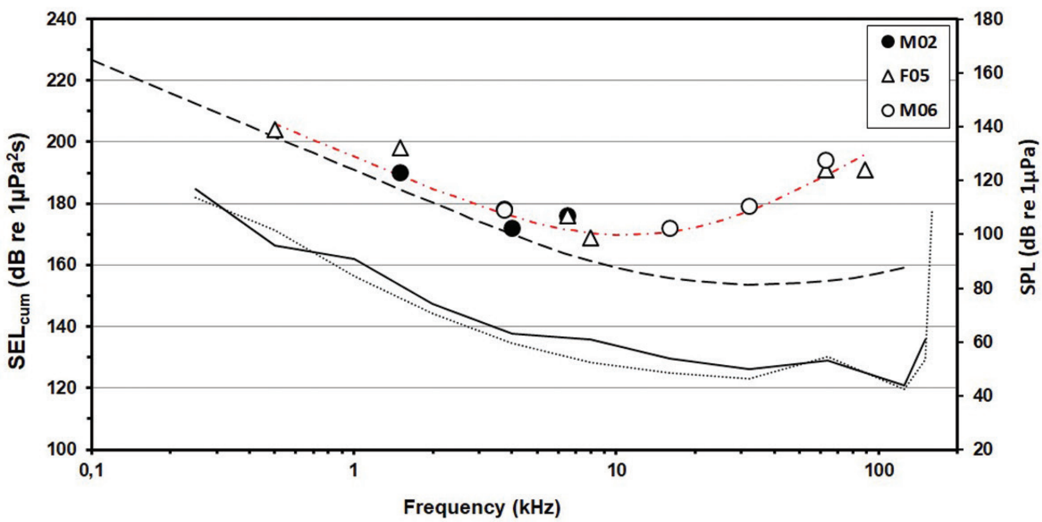

Figure 7. Hearing reduction in relation to hearing acuity in the harbor porpoise. Audiograms (sound pressure level [SPL] on the right-hand y-axis vs frequency on the x-axis) of F05 (present study subject; solid line) and M02 (dotted line; both reported by Kastelein et al., 2017b), and the cumulative sound exposure level (SEL_{cum}; left-hand y-axis) required to cause a mean initial temporary hearing threshold shift (TTS_{1-4}) of around 6 dB (a marker of TTS onset used in marine mammal hearing tests; Houser et al., 2017; Southall et al., 2019) in three harbor porpoises (M02, M06, and F05) after exposures to 11 different fatiguing sound frequencies (on the x-axis; see Table 3 for the references and details). Except for 0.5 kHz (4 h exposure), all results are from 1 h exposures to continuous sound (100% duty cycle, except for the data point at 3.5 to 4.1 kHz, which was 96%). The black dashed line represents the TTS-onset function for cetaceans echolocating at high frequencies proposed by Southall et al. (2019), based on the three available data at the time (which are part of this dataset). The red dashed/dotted line indicates the proposed revised TTS-onset function based on the TTS-onset data shown here for harbor porpoises (Table 3).

Table 3. Published comparable research on temporary hearing threshold shifts (TTS) in the harbor porpoise: the study subjects and symbols used in Figure 7, fatiguing sound center frequency (or frequency range), sound type, duty cycle, and hearing test frequency relative to the center frequency of the fatiguing sound at which ≥ 6 dB TTS_{1-4} was measured for the data points shown in Figure 7. All exposure durations were 1 h, except for 0.5 kHz which was 4 h. NB = noise band, CW = continuous wave, M = male, and F = female.

Subject, symbol in Figure 7	Fatiguing sound frequency (kHz)	Sound type	Duty cycle (%)	Hearing test frequency relative to fatiguing sound	Reference
F05 △	0.5	One-sixth-octave NB	100	Center	Kastelein et al., 2021a
F05 △	1.5	One-sixth-octave NB	100	Half an octave higher	Kastelein et al., 2020b
M02 ●	1-2	Sweep	100	Equal	Kastelein et al., 2014b
M06 ○	3.5-4.1	Composite	96	Equal	Kastelein et al., 2017a
M02 ●	4	One octave NB	100	Equal	Kastelein et al., 2012a
M02 ●	6.5	CW	100	Half an octave higher	Kastelein et al., 2014a
F05 △	6.5	CW	100	Half an octave higher	Kastelein et al., 2020b
F05 △	8	One-sixth-octave NB	100	Half an octave higher	Present study
M06 ○	16	One-sixth-octave NB	100	Half an octave higher	Kastelein et al., 2019b
M06 ○	32	One-sixth-octave NB	100	Half an octave higher	Kastelein et al., 2019a
M06 ○	63	One-sixth-octave NB	100	Half an octave higher	Kastelein et al., 2020a
F05 △	63	One-sixth-octave NB	100	Half an octave higher	Kastelein et al., 2020a
F05 △	88.4	One-sixth-octave NB	100	One third of an octave higher	Kastelein et al., 2020c

The mean SPL measurements in the pool were used as the mean SPL received by the animal. This method could potentially overestimate the received level due to the directionality of hearing, body shadowing, and ears being at surface or above water during breathing. However, due to reflections in the pool, the directionality of hearing and body shadowing probably did not play a major role. The SPL near the surface was only a few dB below the mean SPL of the pool and, thus, is also not expected to have played a major role. During surfacings (generally coinciding with respirations), the ears of harbor porpoise remained below the water surface.

TTSs up to ~8 dB recovered usually within 12 min. TTSs of ~13 dB took 2 h to recover, and TTSs of ~23 dB took 3 h to recover. This implies that TTSs above ~10 dB may start to have an impact on harbor porpoises as the recovery time is much longer than for TTS below 10 dB; reducing the sensitivity of hearing on the order of hours could impact the ecology of a harbor porpoise. It is not clear which frequencies around 8 kHz could be of ecological relevance to porpoises, but social vocalizations of killer whales (*Orcinus orca*), a predator of harbor porpoises, come to mind (Andriolo et al., 2015).

Most TTS studies in terrestrial and marine mammals (including odontocetes) suggest that the greatest TTS generally occurs at the center frequency, or half an octave above the center frequency, of the fatiguing sound (e.g., McFadden, 1986; Popov et al., 2011, 2013; Finneran, 2015; Finneran et al., 2023; Kastelein et al., 2014a, 2019a, 2019b, 2020a, 2020b). In the present study, in common with others, the greatest TTS_{1-4} occurred at 11.3 kHz, half an octave above the center frequency of the fatiguing sound. Previous TTS studies with harbor porpoises indicate that the hearing frequency showing the greatest TTS due to fatiguing sound of a particular frequency also depends on the SPL (and related SEL) of the fatiguing sound (Kastelein et al., 2019a, 2019b, 2020a, 2020b, 2020c). Therefore, the results of the present study agree with previous findings.

Susceptibility to TTS and its relationship with fatiguing sound frequency can be explored by relating equal-TTS susceptibility data to fatiguing sound frequencies (Houser et al., 2017; NMFS, 2018). The present study shows that, for a fatiguing sound around 8 kHz, the SEL required to cause 6 dB TTS_{1-4} (a marker of the onset of TTS; Finneran, 2016; Houser et al., 2017; Southall et al.,

2019) was 169 dB re 1 μPa²s, the lowest TTS-onset SEL of all fatiguing sound frequencies that have been tested. This suggests that harbor porpoise hearing is most vulnerable to injury (TTS and permanent hearing threshold shifts) by sounds at around this frequency (see Figure 7). The present study, combined with TTS studies using the same methodology, cover the effects of fatiguing sounds in the 0.5 to 88.4 kHz frequency range on hearing frequencies over the harbor porpoise's entire hearing range (~0.5 to 140 kHz; Kastelein et al., 2012b, 2013, 2014a, 2014b, 2015a, 2015b, 2017a, 2019a, 2019b, 2020a, 2020b, 2020c). The results confirm that the susceptibility of harbor porpoise hearing to TTS depends on the frequency of the fatiguing sound. Harbor porpoise hearing appears to be most vulnerable to sound between ~4 and 32 kHz. Below ~4 kHz and above 32 kHz, their hearing appears to be less vulnerable to sound. The data in Figure 7 show that the TTS-onset function proposed by Southall et al. (2019; based on the very few available data at the time) is close to the values found in recent studies for fatiguing sounds between 0.5 and 4 kHz (Figure 7). However, harbor porpoise hearing is much less susceptible to TTSs and permanent hearing threshold shifts above 4 kHz than was assumed by Southall et al. (2019).

Frequency-dependency of TTS vulnerability has also been shown for bottlenose dolphins (*Tursiops truncatus*; Finneran & Schlundt, 2013; Finneran et al., 2023), Yangtze finless porpoises (*Neophocaena phocaenoides asiaeorientalis*; Popov et al., 2011), and beluga whales (*Delphinapterus leucas*; Popov et al., 2013). Finneran & Schlundt (2013) found greater susceptibility to TTS in bottlenose dolphins for fatiguing sound frequencies of between 10 and 30 kHz than of 80 kHz. A similar effect was found for belugas, which are more susceptible to TTS for fatiguing sound frequencies of 11.2 and 22.5 kHz than of 45 and 90 kHz (Popov et al., 2013). In the Yangtze finless porpoise, a species more closely related to the harbor porpoise than to the bottlenose dolphin (McGowen et al., 2020), susceptibility to TTS decreases with increasing fatiguing sound frequency (at 32, 45, 64, and 128 kHz; Popov et al., 2011).

Testing the Equal-Energy Hypothesis

The equal-energy hypothesis states that exposure to continuous (100% duty cycle) fatiguing sounds with the same energy, expressed in SEL, results in similar TTS (Southall et al., 2007). The present study showed that the equal-energy hypothesis is supported in harbor porpoises for a noise band at 8 kHz in the SPL (137 to 153 dB re 1 μPa) and duration (2 to 80 min) ranges tested.

Other TTS studies with marine mammals have both supported the equal-energy hypothesis (for certain SPL and duration combinations, in California sea lions: Kastak et al., 2007; Kastelein et al., 2021b, 2022; and in harbor porpoises: Kastelein et al., 2012a, 2014b) and refuted it (for certain SPL and duration combinations, in harbor seals [*Phoca vitulina*]: Kastelein et al., 2012b; in bottlenose dolphins: Mooney et al., 2009; Finneran & Schlundt, 2010; in harbor porpoises: Lucke et al., 2009; Kastelein et al., 2012b; and in belugas: Popov et al., 2013).

In belugas, when the fatiguing SEL was equal, greater TTS occurred at higher SPLs with shorter exposures than at lower SPLs with longer exposures (Popov et al., 2013); however, within a certain range, the SEL of a fatiguing sound can be used to predict the initial TTS it induces. Data obtained from high-SPL, short-duration exposures might result in overestimation of the TTS induced as a function of the exposure duration, particularly if they are extrapolated to low-SPL, long-duration exposures (Popov et al., 2013).

This suggests that, in general, support for the equal-energy hypothesis is limited to certain combinations of fatiguing sound level and duration (and maybe particular frequencies). Therefore, caution should be applied when predicting the effects of sounds far outside the range (i.e., fatiguing sound's frequency, SPL, and duration) that has been tested on any species of cetacean. However, in the absence of more specific data, the equal-energy hypothesis still provides a useful tool for forecasting noise-induced threshold shift in Environmental Impact Assessment—even slightly outside the tested ranges of SPL and duration.

Conclusion

Depending on the frequency of the hearing test sound, significant TTS onset in the harbor porpoise occurred at SELs of 168 (11.3 kHz) or 174 (8 and 16 kHz) dB re 1 μPa²s. The hearing frequency that was most affected was half an octave above the fatiguing sound's center frequency. The observed frequency-dependent susceptibility to TTS in harbor porpoises in the present and previous TTS studies with harbor porpoises demonstrates the importance of investigating TTS susceptibility over a species' entire hearing range. The equal-energy hypothesis was found to apply for the combinations that were tested; further testing of the equal-energy hypothesis (for other fatiguing sound frequencies and SPL and duration ranges) is needed for Environmental Impact Assessments and to improve the prediction of TTS in marine mammals that are exposed to fatiguing sounds. The final step in the larger research project on TTS in harbor porpoises, of which this study is a part, involved modeling an auditory weighting function for the harbor porpoise. This weighting function may be valid for other species of porpoises echolocating at high frequencies (VHF group; Southall et al., 2019). The function, in combination with data on the equal-energy hypothesis, will facilitate the implementation of specific acoustic protection measures in areas of overlap between harbor porpoises and human activity, thus benefiting the conservation of the harbor porpoise and possibly other cetaceans echolocating at high frequencies.

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