Aquatic Mammals 2021, *47*(2), 135-145, DOI 10.1578/AM.47.2.2021.135

Temporary Hearing Threshold Shift in a Harbor Porpoise (*Phocoena phocoena*) Due to Exposure to a Continuous One-Sixth-Octave Noise Band Centered at 0.5 kHz

Ronald A. Kastelein,[1] Lean Helder-Hoek,[1] Suzanne A. Cornelisse,[1]
Linde N. Defillet,[1] Léonie A. E. Huijser,[1] and Robin Gransier[2]

[1]Sea Mammal Research Company (SEAMARCO), Julianalaan 46, 3843 CC Harderwijk, The Netherlands
E-mail: researchteam@zonnet.nl
[2]KU Leuven – University of Leuven, Department of Neurosciences, ExpORL,
Herestraat 49 bus 721, B-3000 Leuven, Belgium

Abstract

Susceptibility to temporary hearing threshold shift (TTS) in harbor porpoises (*Phocoena phocoena*) depends on the frequency of the fatiguing sound causing the shift. This study is part of a larger project in which TTS in harbor porpoises was tested after exposure to fatiguing sounds within the 0.5 to 88.4 kHz frequency range. Herein, we report on the TTS induced and hearing recovery in a female harbor porpoise after exposure to 1, 2, and 4 h of continuous one-sixth-octave noise band centered at 0.5 kHz, which is within the frequency range of many high-amplitude anthropogenic sounds. This fatiguing sound was emitted at an average received sound pressure level of 163 dB re 1 µPa, resulting in sound exposure levels (SELs) of 199 to 206 dB re 1 µPa^{2}s. Hearing thresholds for 0.5, 0.71, and 1 kHz tonal signals were determined before and after exposure. Control sessions were used as a baseline and to determine which SELs resulted in statistically significant TTS in the first 4 min after the fatiguing sound stopped (TTS$_{1-4}$). At 0.5 kHz, the lowest SEL that resulted in significant TTS$_{1-4}$ (4.3 dB) was 202 dB re 1 µPa^{2}s. At 0.71 and 1 kHz, the lowest SEL that resulted in significant TTS$_{1-4}$ (5.8 dB and 3.8 dB, respectively) was 205 dB re 1 µPa^{2}s. Hearing always recovered within 60 min after the fatiguing sound stopped. Within the SEL range that was tested, the greatest mean TTS$_{1-4}$ (7.6 dB) occurred at 0.5 kHz, the center frequency of the fatiguing sound, after exposure to an SEL of 205 dB re 1 µPa^{2}s (4-h exposure); 0.5 kHz is close to the lower bound of porpoise hearing, and ecological impacts of reduced hearing at this frequency are unclear. Results will be used in a future study of this series to generate an auditory weighting curve and to enhance regulatory protection of the harbor porpoise.

Key Words: anthropogenic noise, audiogram, frequency weighting, harbor porpoise, *Phocoena phocoena*, hearing, hearing damage, hearing loss, hearing sensitivity, low frequency, odontocete, temporary hearing 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 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 (TTSs). Significant TTS relative to control sessions occurs at lower sound exposure levels (SELs) in the harbor porpoise than in any other odontocete species that has been tested so far (Lucke et al., 2009; Finneran, 2015; Tougaard et al., 2016; Houser et al., 2017).

TTS in free-ranging marine mammals may be caused by fatiguing sounds (i.e., sounds that could potentially cause TTS if the SEL is high enough) such as those from vessel traffic, pile driving, seismic surveys, detonations, and sonar. 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 mainly 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), meaning that seemingly minor disturbances (e.g., short and/or small TTSs) could impact them disproportionately, especially if they occur frequently. The cumulative time lost for feeding, for example, could have health repercussions. Therefore, TTS may negatively impact a porpoise's health, reproduction, and survival, even if permanent hearing threshold shift does not occur. As a result, TTS could have adverse population effects in the long term.

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 88.4-kHz fatiguing sound range, the susceptibility to TTS for each of the following hearing frequencies has been established: 1.5, 1 to 2, 4, 3.5 to 4.1, 6 to 7, 6.5, 16, 32, 63, and 88.4 kHz (Kastelein et al., 2012, 2013, 2014a, 2014b, 2015b, 2017a, 2019a, 2019b, 2020a, 2020b, 2020c). Fatiguing sound frequencies higher than 88.4 kHz have not been tested, as fatiguing sounds around 88.4 kHz reach the upper frequency limit of the porpoises' hearing. The 88.4 kHz exposures affect hearing between 88.4 and 125 kHz (Kastelein et al., 2020c) due to the half-octave upward frequency shift at which TTS often occurs at higher exposure levels, and harbor porpoise hearing sensitivity decreases by ~60 dB between 125 and 140 kHz (Kastelein et al., 2017b). Susceptibility to TTS in the harbor porpoise has not yet been assessed for low-frequency sounds (< 1 kHz), but much of the energy of anthropogenic sound is below 1 kHz, so this frequency range is of great interest.

The present study adds to our previous TTS research in which higher frequency fatiguing sounds have been used, by investigating the susceptibility to TTS of harbor porpoises after exposure to fatiguing sounds centered at 0.5 kHz. Once quantification of susceptibility to TTS over the entire hearing range is completed with the present study, it will be possible to model a research-based auditory weighting curve for harbor porpoises and other odontocetes that echolocate at high frequencies. A new weighting function for such "high-frequency cetaceans," a classification made by Southall et al. (2019), will be proposed in the next and final contribution in this series of TTS studies with harbor porpoises. Weighting is applied to sound levels to account for the frequency-dependent susceptibility to TTS of a species. The aim of the present study was to increase the frequency range for which equal-TTS susceptibility contours for harbor porpoises can be generated (see Houser et al., 2017), to allow the modeling of the auditory weighting curve to improve the regulatory protection of harbor porpoise hearing.

Methods

Study Animal and Site

The study animal, a previously stranded and rehabilitated adult female harbor porpoise (identified as F05; age: ~9 y, body mass: ~44 kg, body length: 154 cm, girth at axilla: ~80 cm), had participated in previous studies of TTS induced by sounds of 1.5, 3.5 to 4.1, 6.5, 16, 32, 63, and 88.4 kHz (Kastelein et al., 2017a, 2019a, 2019b, 2020a, 2020b, 2020c). These previous studies did not compromise her auditory ability, and her hearing thresholds in the frequency range tested in the present study (0.5 to 1 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. F05 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) connected via a channel (4 m × 3 m; 1.4 m deep) to an outdoor pool (12 m × 8 m; 2 m deep). For details of the pool, equipment, and water flow, see Kastelein et al. (2019b).

Acoustics

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 external sound measurement company (TNO), just before and at the end of 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, 2020c).

Fatiguing Sound—The digitized fatiguing sound was produced, transmitted, calibrated, and checked before each exposure session, as described by Kastelein et al. (2019b), with the exception that the sound was transmitted into the pool by a low-frequency transducer with its accompanying power amplifier (Hydrosounder, Model No. 350; Data Physics Corporation, San Jose, CA, USA). The transducer was placed at 1 m depth on one side of the pool (Figures 1

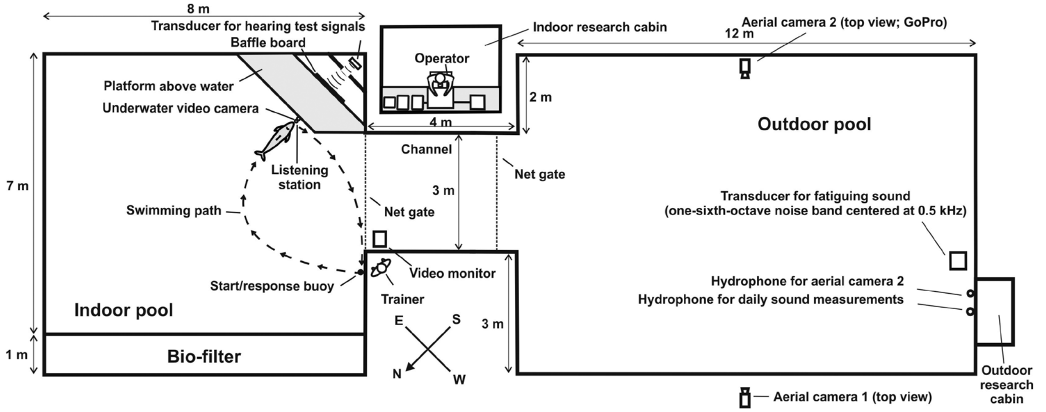


Figure 1. The pool complex in which the TTS study with harbor porpoise F05 was conducted. On each test day, a pre-exposure hearing test was conducted in the indoor pool to test one of three hearing frequencies (0.5, 0.71, or 1 kHz). This was followed by a 1 to 4 h period of exposure to the one-sixth-octave noise band centered at 0.5 kHz (or to 1 h of low ambient noise in control sessions) in the outdoor pool, then by one or several post-exposure hearing tests in the indoor pool (to test the same hearing frequency as used in the pre-exposure hearing test of that day).

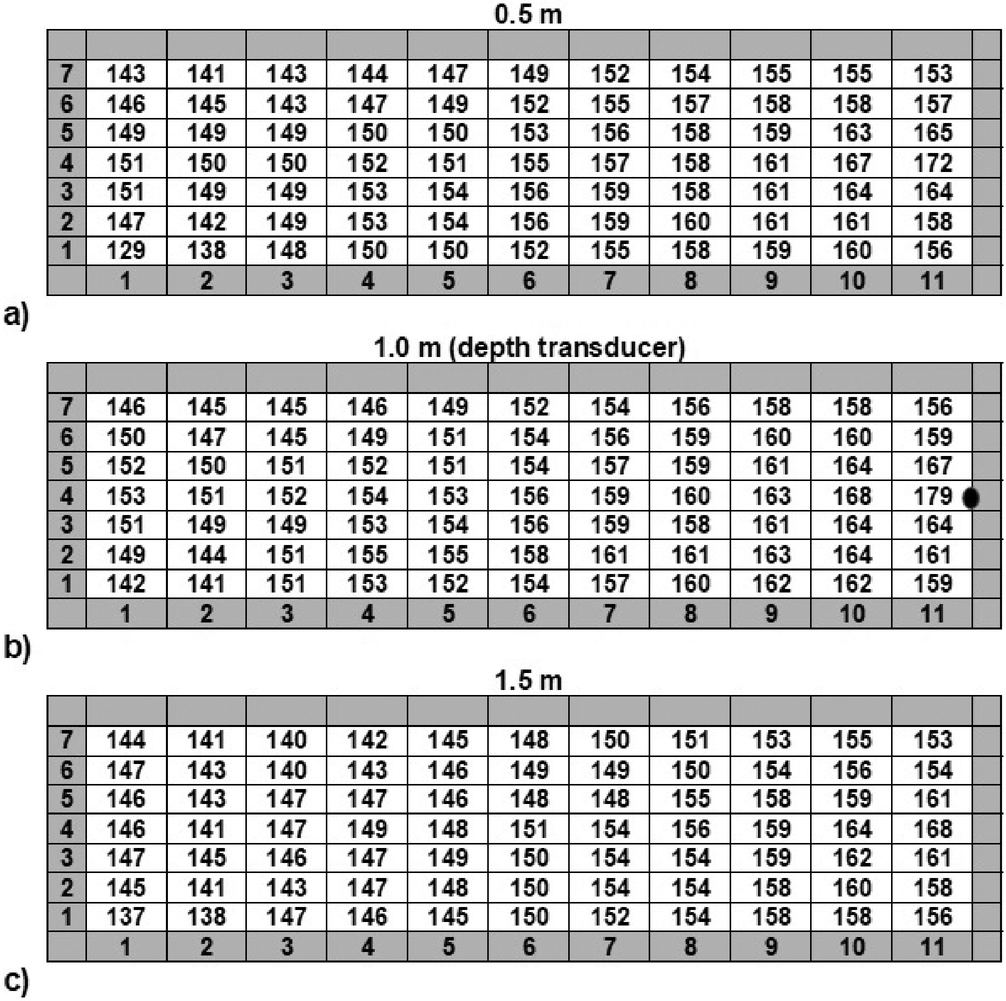
& 2). The fatiguing sound consisted of a continuous (duty cycle 100%) one-sixth-octave Gaussian white noise band, centered at 0.5 kHz (bandwidth: 472 to 550 Hz). Ideally, a 0.5 kHz tone would have been used; however, in a pool, a pure tone can lead to a very heterogeneous sound field due to reverberation. Therefore, instead of a tonal signal, a very narrow noise band was selected.

To determine the fatiguing sound's distribution in the outdoor pool, the SPL of the noise band was measured at 77 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 231 measurements in the pool (Figure 2). Apart from just around the transducer, the differences in mean SPL per depth (based on the power sum) were minimal. However, an SPL gradient occurred from the transducer towards the rest of the pool. To determine the average SPL received by the porpoise, the area where she swam during exposure periods was quantified following the methods of Kastelein et al. (2019b). During 52 of the 56 exposure periods, the porpoise used the entire pool, so the mean SPL ($n = 231$) of the pool was used to calculate the mean SPL to which she was exposed. In four of the six 2-h exposures, the porpoise spent most of the exposure period within 1 m of the transducer; for those exposures, the mean SPL to which she was exposed was calculated from the SPL measurements within 2 m around the transducer. With the exception of these four exposures, the mean received SPL was constant, as it could not be increased without introducing harmonics; variation in SEL resulted from different exposure durations. The mean received SPL of the fatiguing sound was 163 dB re 1 µPa, resulting in SELs of 199, 202, and 205 dB re 1 µPa^{2}s due to exposures for 1, 2, and 4 h, respectively. For the four deviant exposures when the porpoise was near the transducer, the mean received SPL was 167 dB re 1 µPa, resulting in an SEL of 206 dB re 1 µPa^{2}s after 2-h exposures.

Hearing Test Signals—Linear upsweep tonal sounds with a duration of 1 s (including linear on- and off-ramps of 50 ms) 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 frequencies tested were 0.5 kHz (the center frequency of the fatiguing sound), 0.71 kHz (half an octave above the center frequency), and 1 kHz (one octave above the center frequency). The hearing test signals were generated digitally and were calibrated and checked daily, as explained by Kastelein et al. (2019b).

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 1, 2, or 4 h (timed with minute precision using stopwatches) in the morning and/or early afternoon (or exposure to ambient noise for 1 h during control sessions), and (3) a number of post-exposure hearing tests in the afternoon. All hearing tests took place in the indoor pool; fatiguing sound (or ambient noise) exposures took place in the outdoor pool (Figure 1). Data were collected from July to September 2019, following the protocol developed and explained in Kastelein et al. (2019b).



0.5 m

7	143	141	143	144	147	149	152	154	155	155	153	
6	146	145	143	147	149	152	155	157	158	158	157	
5	149	149	149	150	150	153	156	158	159	163	165	
4	151	150	150	152	151	155	157	158	161	167	172	
3	151	149	149	153	154	156	159	158	161	164	164	
2	147	142	149	153	154	156	159	160	161	161	158	
1	129	138	148	150	150	152	155	158	159	160	156	
	1	2	3	4	5	6	7	8	9	10	11	

1.0 m (depth transducer)

7	146	145	145	146	149	152	154	156	158	158	156	
6	150	147	145	149	151	154	156	159	160	160	159	
5	152	150	151	152	151	154	157	159	161	164	167	
4	153	151	152	154	153	156	159	160	163	168	179	●
3	151	149	149	153	154	156	159	158	161	164	164	
2	149	144	151	155	155	158	161	161	163	164	161	
1	142	141	151	153	152	154	157	160	162	162	159	
	1	2	3	4	5	6	7	8	9	10	11	

1.5 m

7	144	141	140	142	145	148	150	151	153	155	153	
6	147	143	140	143	146	149	149	150	154	156	154	
5	146	143	147	147	146	148	148	155	158	159	161	
4	146	141	147	149	148	151	154	156	159	164	168	
3	147	145	146	147	149	150	154	154	159	162	161	
2	145	141	143	147	148	150	154	154	158	160	158	
1	137	138	147	146	145	150	152	154	158	158	156	
	1	2	3	4	5	6	7	8	9	10	11	

Figure 2. An example of the SPL distribution in the harbor porpoise's outdoor pool during exposure to the continuous (100% duty cycle) one-sixth-octave noise band centered at 0.5 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 at the side of the pool (source level: 179 dB re 1 µPa). The numbers in the grey fields indicate 1-m markings on the side of the pool.

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$_{1-4}$), 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 ≤ 2 dB above the pre-exposure threshold level, as fluctuations of ≤ 2 dB occurred after control sessions in the quiet background noise conditions in the present study (see "Results") and in previous studies of this series. The SELs of the fatiguing sound were tested in random order. Each SEL was tested four times per hearing frequency except for the exposure combination of 202 dB SEL and 1 kHz, which was tested only twice. As it became clear after two 2-h sessions that with this combination no major TTS occurred, we deemed further testing redundant and switched to longer exposures.

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 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. Six control tests (four, plus two extra on days when unforeseen circumstances did not allow full exposures) 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.

Harbor porpoises have a high metabolism, so the study animal was fed once half-way through the 4-h exposure periods. This was done within 2 min and in such a way that she kept her head below the water surface except when taking a breath.

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 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 for this. 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 the latter, the trainer used a whistle after 6 to 12 s to instruct the porpoise 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 to participate throughout the session. 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. 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. 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 the PSE_{1-4}, PSE_{4-8}, PSE_{8-12}, and PSE_{60} periods of the same day (see Kastelein et al., 2019b). Similarly, "TTSs" 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.

Previous research often used the value of 6 dB to define TTS onset, as lower amounts of TTS could not be distinguished from fluctuations in threshold measurements that typically occur across test sessions (Finneran, 2016; Houser et al., 2017; Southall et al., 2019). However, due to the low background noise levels at the SEAMARCO study site, TTSs below 6 dB could be distinguished from control values. Therefore, for comparison between studies, we use the definition of TTS onset at 6 dB in the "Discussion," but in the "Results," we define TTS onset 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," due to the natural variation of the hearing thresholds, was close to zero). The level of significance was established by conducting a separate one-way ANOVA on the mean initial TTS (TTS_{1-4}) for each hearing test frequency with the factor SEL (including the control). When the ANOVA produced a significant value overall, it was followed by Dunnett multiple comparisons between the control and the other levels of the factor to identify which was/were significantly different (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-, 2-, and 4-h sound exposure periods, the harbor porpoise was always willing to participate in the hearing tests. In ~5% of the post-exposure sessions, she moved too 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 discarded. The mean pre-stimulus response rate for both signal-present and signal-absent trials in the hearing tests varied between 4.4 and 10.1% (Table 1). The pre-stimulus response rates in the pre-exposure, post-exposure, and post-ambient exposure (control) periods were of the same order of magnitude.

Effect of SEL on TTS

The ANOVAs showed that the initial TTS (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 an SEL of 202 dB re 1 μPa^2s for the hearing test frequency of 0.5 kHz and at an SEL of 205 dB re 1 μPa^2s for the hearing test frequencies of 0.71 and 1 kHz (Table 2; Figure 3).

Table 1. The pre-stimulus response rate (number of pre-stimuli as a percentage of all trials in each hearing test period) by harbor porpoise F05 in hearing tests during the pre-exposure periods, during four PSE periods (i.e., after exposure to a continuous one-sixth-octave noise band centered at 0.5 kHz), and during 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}
	8.1% (841)	9.6% (272)	10.1% (274)	5.2% (282)	4.4% (225)
Control	Pre-exposure	PAE_{1-4}	PAE_{4-8}	PAE_{8-12}	--
	8.7% (391)	6.8% (132)	9.3% (129)	9.3% (129)	--

Table 2. Results of one-way ANOVAs of mean initial TTS (TTS_{1-4}, in dB) in F05 after exposure for 1, 2, and 4 h to the fatiguing sound (a continuous one-sixth-octave noise band centered at 0.5 kHz) with the factor fatiguing sound SEL. *df* = degrees of freedom. Standard deviation (SD) is shown for each mean TTS_{1-4}, as well as the range and sample size (*n* = number of exposure tests). Mean initial TTSs that were significantly different from the control according to Dunnett multiple comparisons are indicated with an asterisk (*), and the SELs that mark the onset of statistically significant TTSs are indicated in bold. The variation in SEL results from different exposure durations; mean sound pressure level (SPL) was constant, except for a high SPL experienced by the porpoise in four sessions when she remained close to the transducer (indicated by #). Approximate recovery times from significant TTSs are also shown.

Hearing test frequency (kHz)	ANOVA results ($F_{\text{factor-}df,\ \text{error-}df}$ *p* value)	Mean SPL (dB re 1 µPa)	Exposure duration (h)	SEL (dB re 1 μPa^2s)	TTS_{1-4} (dB)				Recovery (min)
					Mean	SD	Range	*n*	
0.5	$F_{3,14} = 28.35$ $p < 0.001$	Control	1	Control	0.2	0.9	-1.2–1.7	6	--
		163	1	199	2.1	1.9	-0.6–3.7	4	--
		163	2	**202**	4.3*	1.2	2.4–5.0	4	4-8
		163	4	205	7.6*	1.1	6.3–8.9	4	60
0.71	$F_{3,14} = 52.16$ $p < 0.001$	Control	1	Control	0.2	0.6	-0.7–0.9	6	--
		163	1	199	0.9	0.8	-0.3–1.6	4	--
		163	2	202	1.4	0.9	0.2–2.4	4	--
		163	4	**205**	5.8*	0.5	5.3–6.5	4	60
1	$F_{4,15} = 52.61$ $p < 0.001$	Control	1	Control	0.3	0.4	-0.3–0.8	6	--
		163	1	199	0.9	0.8	0.1–1.9	4	--
		163	2	202	-0.8	0.6	-1.3–0.4	2	--
		163	4	**205**	3.8*	1.1	2.3–4.8	4	8-12
		167#	2	206	5.7*	0.5	5.1–6.3	4	60

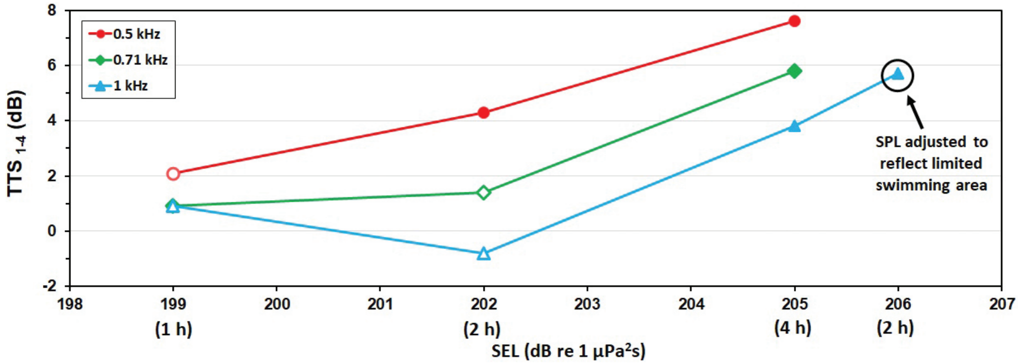


Figure 3. Mean TTS_{1-4} in F05 after exposure for 1, 2, or 4 h to the fatiguing sound (a continuous one-sixth-octave noise band centered at 0.5 kHz) at several sound exposure levels (SELs), quantified at hearing frequencies 0.5, 0.71, and 1 kHz (i.e., the center frequency of the fatiguing sound, half an octave above the center frequency, and one octave above the center frequency). The sound pressure level (SPL) experienced by the study animal was calculated based on where she swam. In all but one SEL (206 dB re 1 µPa^{2}s; indicated), she swam throughout most of the pool. Solid symbols indicate statistically significant TTS_{1-4} relative to the control values; open symbols indicate TTS_{1-4} statistically similar to the control values. Sample sizes were six for control sessions and four for all but one test combination (see Table 2). For control values, see Table 2 and Figure 4.

For hearing test signals of 0.5 kHz, statistically significant TTS_{1-4} occurred in the harbor porpoise after exposure to SELs of 202 and 205 dB re 1 µPa^{2}s (Table 2; Figure 3). Hearing always recovered within 60 min, even after the greatest TTS_{1-4} measured (7.6 dB; Table 2; Figure 4a). For hearing test signals of 0.71 kHz, statistically significant TTS_{1-4} (5.8 dB) occurred after exposure to an SEL of 205 dB re 1 µPa^{2}s (Table 2; Figure 3), and hearing recovered within 60 min (Table 2; Figure 4b). For hearing test signals of 1 kHz, statistically significant TTS_{1-4} occurred after exposure to SELs of 205 and 206 dB re 1 µPa^{2}s (Table 2; Figure 3). Hearing always recovered within 60 min, even after the greatest TTS_{1-4} (5.7 dB; 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).

Discussion

Affected Hearing Frequencies

Most TTS studies in terrestrial and marine mammals (including odontocetes) suggest that the greatest TTS occurs half an octave above the center frequency of the fatiguing sound (e.g., McFadden, 1986; Popov et al., 2011, 2013; Finneran, 2015; Kastelein et al., 2014a, 2019a, 2019b, 2020a, 2020b). In the present study, the greatest TTS_{1-4} occurred at the center frequency of the fatiguing sound (i.e., 0.5 kHz) rather than half an octave above that frequency (i.e., 0.71 kHz). However, harbor porpoise hearing is very insensitive to low-frequency sounds (Figure 5; Kastelein et al., 2017b), which may have contributed to this discrepancy. In addition, the hearing frequency most susceptible to TTS may have been higher if it had been possible to generate fatiguing sounds at higher SPLs without introducing harmonics. Previous TTS studies with harbor porpoises indicate that the hearing frequency showing the greatest TTS 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 do not necessarily disagree with previous findings. 0.5 kHz is close to the lowest frequency of harbor porpoise hearing (Kastelein et al., 2017b), and ecological impacts of reduced hearing at this frequency are unclear.

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). The present study shows that, for fatiguing sound around 0.5 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 higher than the SEL required for fatiguing sounds of 1 to 88.4 kHz to cause 6 dB TTS_{1-4} in the study animal and in two other harbor porpoises (Figure 5; Table 3; Kastelein et al., 2012, 2014a, 2014b, 2017a, 2019a, 2019b, 2020a, 2020b, 2020c).

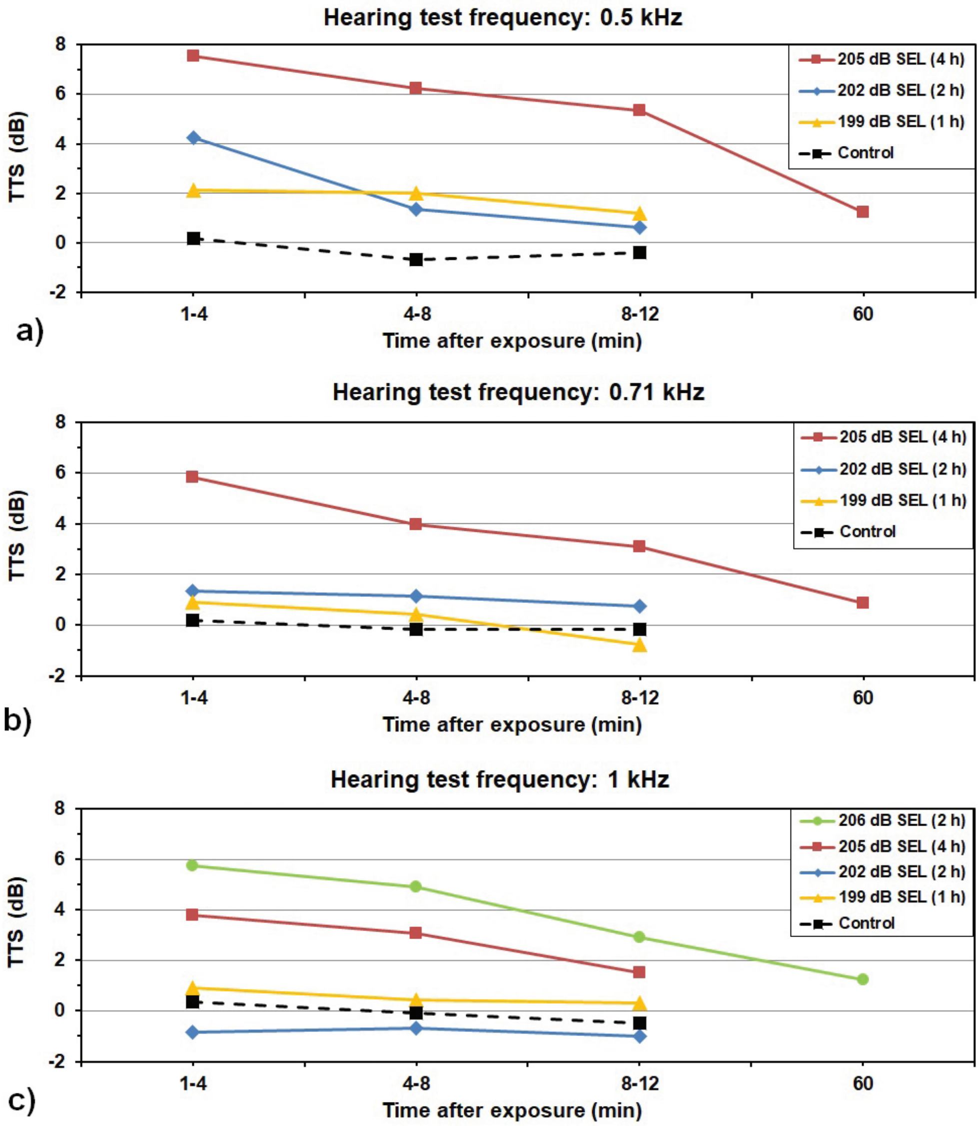


Figure 4. Changes over time, including recovery, in the hearing of F05 at 0.5 kHz (a), 0.71 kHz (b), and 1 kHz (c) after 1, 2, or 4 h of exposure to the fatiguing sound (a continuous one-sixth-octave noise band centered at 0.5 kHz) at several SELs. For sample sizes and standard deviations of mean $TTS_{1\text{-}4}$, see Table 2.

The present study and previous TTS studies from this series combined cover the effects of fatiguing sounds in the frequency range of 0.5 to 88.4 kHz on hearing frequencies across the harbor porpoise's entire hearing range (~0.5 to 140 kHz; Kastelein et al., 2012, 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. Frequency dependency of TTS has also been shown for common 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. In the Yangtze finless porpoise,

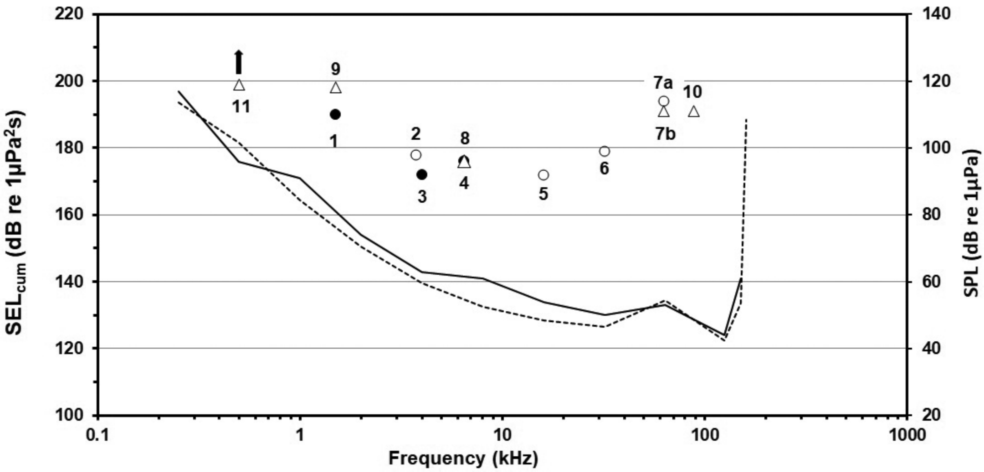


Figure 5. Audiograms (SPL on right-hand y-axis vs frequency on x-axis) of F05 (present study animal; solid line) and M02 (dashed line; both reported by Kastelein et al., 2017b), and the cumulative sound exposure level (SEL_{cum}; on left-hand y-axis) required to cause a mean TTS_{1-4} of around 6 dB (a marker of TTS onset used in marine mammal hearing tests; Houser et al., 2017; Southall et al., 2019) in three different harbor porpoises (M02, F05, and M06) after exposures for 1 h to 10 different sounds (see Table 3 for the references and details). In the present study, 6 dB TTS was not reached with 1-h exposures (199 dB SEL caused only 2.1 dB TTS_{1-4} at 0.5 kHz; Figure 3). The arrow indicates that in order to cause 6 dB TTS with a continuous sound around 0.5 kHz for 1 h, the SEL needs to be higher than 199 dB SEL. The solid circles represent M02, the open circles represent M06, and the open triangles represent F05. Only results from 1-h exposures to continuous sound (100% duty cycle; except in study number 2, 96%) are included in this figure to allow valid comparison. Therefore, exposures > 1 h in the present study, which generated higher SELs, are not shown here.

Table 3. The study subjects, 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 5. All exposure durations were 1 h. NB = noise band; CW = continuous wave.

Data point #	Subject and symbol	Frequency (kHz)	Sound type	Duty cycle (%)	Hearing test freq. relative to fatiguing noise	Reference
1	M02 (●)	1-2	Sweep	100	Center	Kastelein et al., 2014b
2	M06 (○)	3.5-4.1	Composite	96	Center	Kastelein et al., 2017a
3	M02 (●)	4	1-octave NB	100	Center	Kastelein et al., 2012
4	M02 (●)	6.5	CW	100	+ 1/2 octave	Kastelein et al., 2014a
5	M06 (○)	16	1/6-octave NB	100	+ 1/2 octave	Kastelein et al., 2019b
6	M06 (○)	32	1/6-octave NB	100	+ 1/2 octave	Kastelein et al., 2019a
7a	M06 (○)	63	1/6-octave NB	100	+ 1/2 octave	Kastelein et al., 2020a
7b	F05 (△)	63	1/6-octave NB	100	+ 1/2 octave	Kastelein et al., 2020a
8	F05 (△)	6.5	CW	100	+ 1/2 octave	Kastelein et al., 2020b
9	F05 (△)	1.5	1/6-octave NB	100	+ 1/2 octave	Kastelein et al., 2020b
10	F05 (△)	88.4	1/6-octave NB	100	+ 1/3 octave	Kastelein et al., 2020c
11	F05 (△)	0.5	1/6-octave NB	100	Center	Present study

a species more closely related to the harbor porpoise than the bottlenose dolphin, susceptibility to TTS decreases with increasing fatiguing sound frequency (at 32, 45, 64, and 128 kHz; Popov et al., 2011). 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). In line with these observations from other odontocetes, harbor porpoise hearing appears to be most susceptible to TTS for fatiguing sounds between ~4 and 32 kHz. Below 4 kHz and above 32 kHz, their hearing appears to be less susceptible to TTS.

The observed frequency-dependent susceptibility to TTS in harbor porpoises in the present and previous studies demonstrates the importance of investigating TTS susceptibility over the species' entire hearing range; this has now been completed. The final step in the larger project on TTS in harbor porpoises, of which this study is a part, will be the modeling of an auditory weighting curve for the harbor porpoise, which may be valid for other cetaceans echolocating at high frequencies (Southall et al., 2019). The curve will facilitate the implementation of specific acoustic protection measures in areas of overlap between harbor porpoise and human activity, thus benefiting the conservation of the harbor porpoise and other high-frequency echolocating cetaceans.

Acknowledgments

We thank research assistants Stacey van der Linden and Kimberly Biemond; students Femke Kuiphof, Luna Korsuize, Eline Theuws, and Amber Verhoef; and volunteers Emmy Post, Stephanie de Ruijter, and Brigitte Slingerland for their help in collecting the data. We thank Arie Smink for the design, construction, and maintenance of the electronic equipment; MARIN in Wageningen for making the Hydrosounder 350 transducer available to SEAMARCO; Bert Meijering (Topsy Baits) for providing space for the SEAMARCO Research Institute; and Erwin Jansen (TNO) for conducting the acoustic measurements. We also thank Nancy Jennings (Dotmoth.co.uk) and two anonymous reviewers for their valuable constructive comments on this manuscript. Nancy Jennings also conducted the statistical analysis. Funding for this study was obtained from the U.S. Navy, Living Marine Resources program (Contract No. N39430-15-C-1686). We thank Anu Kumar and Mandy Shoemaker for their guidance on behalf of the LMR program. The harbor porpoise was tested under authorization of the Netherlands Ministry of Economic Affairs with Endangered Species Permit FF/75A/2014/025. We thank the ASPRO group for providing the porpoise.

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