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Temporary Hearing Threshold Shift and Testing the Equal-Energy Hypothesis in Harbor Seals (*Phoca vitulina*) After Exposure to a One-Sixth-Octave Noise Band Centered at 8 kHz

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Abstract

Susceptibility to temporary hearing threshold shift (TTS) in harbor seals (*Phoca vitulina*) depends, in part, on the frequency of the fatiguing sound (the sound causing the shift). The TTS induced and the pattern of hearing recovery were documented in two female harbor seals after exposure for one hour to a continuous, constant-amplitude one-sixth-octave noise band (NB) at 8 kHz. This fatiguing sound was emitted at average received sound pressure levels (SPLs) estimated at between 138 and 156 dB re 1 µPa, resulting in sound exposure levels (SELs) of 174 to 192 dB re 1 µPa^{2}s. Hearing thresholds for narrow-band sweeps were determined at 8, 11.3, and 16 kHz. The hearing frequency most affected was 11.3 kHz, half an octave above the fatiguing sound's center frequency. Higher SELs were more likely to result in TTS than lower SELs. At hearing frequencies 8 and 16 kHz, initial TTS (1 to 4 min after the sound stopped) only occurred after exposure to the highest SEL (192 dB re 1 µPa^{2}s). Recovery of hearing took longer after large TTSs than after small TTSs. The equal-energy hypothesis was tested by exposing the seals to the same continuous fatiguing sound with SPLs between 149 and 165 dB re 1 µPa, and exposure durations between two and 80 min; all seven combinations had the same SEL of 186 dB re 1 µPa^{2}s. The equal-energy hypothesis was supported in both seals for the frequency, SPL, and duration ranges that were tested; thus, SEL can be used to predict the TTS elicited in harbor seals by continuous, constant-amplitude sound around 8 kHz. The TTS-onset SEL for the NB at 8 kHz, taken together with the TTS-onset SELs for fatiguing sound frequencies tested in previous studies, can form the basis for a revised TTS-onset function for harbor seals.

Key Words: anthropogenic sound, equal-energy hypothesis, equal-energy rule, mitigation, phocid, hearing sensitivity, recovery of hearing, sound safety levels, temporary threshold shift (TTS)

Introduction

Marine mammals that are exposed to high-amplitude continuous and impulsive sounds generated by anthropogenic offshore activities, such as dredging, shipping, offshore windfarms, pile driving, seismic surveys, sonar, and detonations, may suffer temporary or permanent hearing threshold shifts (TTS or PTS, respectively; Melnick, 1991; Yost, 2007). For the regulation and management of such activities, it is important to know at what sound exposure levels (SELs) hearing may be reduced in marine mammals (see overview by Finneran, 2015). SELs are combinations of received sound pressure level (SPL) and exposure duration; thus, SELs are used to quantify the energy of exposure. Safety criteria for underwater sound to protect marine mammal hearing were proposed by Southall et al. (2007, 2019) based on the limited TTS data available for each of the "marine mammal hearing groups" into which the authors divided the marine mammal species.

One such group, the "phocid carnivores" (Southall et al., 2019, p. 131), includes the harbor seal (*Phoca vitulina*). The harbor seal occurs in temperate and Arctic coastal areas of the Northern Hemisphere (Burns, 2009), where high levels of human activity can produce underwater sound with high enough SELs to cause TTS or PTS (Ainslie et al., 2009; Mannerla et al., 2013; Merchant et al., 2016).

So far, nine studies on TTS in harbor seals due to continuous underwater sound have been published (Kastak et al., 1999, 2005; Kastelein et al., 2012, 2013, 2019a, 2019b, 2020a, 2020b, 2020c). The present study adds to this research and is in line with the need identified by Southall et al. (2019) for a larger dataset on which to base auditory weighting functions for marine mammal species. Progress is necessarily slow because limited trained subjects are available, and because it is important to avoid accidentally causing PTS. SEL can only be increased slowly, and TTS must be tested for at all frequencies at which PTS could occur, including those between the center frequency of the fatiguing sound and one octave higher.

In the present study, harbor seals were exposed to a fatiguing sound frequency that had not yet been tested in this species (a continuous, constant-amplitude one-sixth-octave noise band centered at 8 kHz). The first goal was to quantify TTS as a function of the fatiguing sound SEL at three hearing test frequencies, and to describe the recovery of hearing after the exposure stopped. The second goal was to test the equal-energy hypothesis (also called equal-energy rule; Roberto et al., 1985). The hypothesis states that different combinations of SPL and exposure duration resulting in the same SEL elicit similar TTS. In most studies of TTS in harbor seals, exposure to the fatiguing sounds lasted for 1 h, and a limited range of SPLs was used; therefore, an understanding of the effects of other SPLs and exposure durations is limited. If the equal-energy hypothesis is upheld, it will be possible to extrapolate the results of the present study and previous TTS studies with continuous fatiguing sounds to predict the effects of sound exposures with different SPL and duration combinations. This will increase the practical value of all previous and future TTS studies with harbor seals (and, until TTS data for other species in this family become available, for Phocidae in general). Evidence supporting predictions and extrapolations will mean that results can be used with more confidence in environmental impact assessments (EIAs), with the restriction that the equal-energy hypothesis is only valid for TTS predictions due to exposure to continuous, constant-amplitude sound.

Methods

Study Animals and Study Area

The study animals were two healthy adult female harbor seals, identified as F01 and F02. They were 15 y old during data collection. The two seals had very similar girths, and body weights that increased from ~51 kg at the start of the study to 55 kg by the study's end. Details on their husbandry and food rations are provided by Kastelein et al. (2019b).

The study was conducted at the SEAMARCO Research Institute, the Netherlands, in an outdoor pool (measuring 8 × 7 m and 2 m deep; see Kastelein et al., 2019a, for details). The pool walls were covered with aquatic vegetation, and the bottom had a 20-cm-thick sloping sand layer. The pool had haul-out areas, but they were barred during sound exposure sessions so that the harbor seals could not leave the water. During hearing tests, the harbor seal not being tested was kept in the water next to the main haul-out area and was rewarded with food while performing quiet husbandry behaviors.

Acoustics

Terminology and Ambient Noise—Acoustical terminology follows ISO 18405 (International Organization for Standardization [ISO], 2017). Ambient noise was measured, and the fatiguing sound and hearing test signals were calibrated once every 3 mo during the study period by an acoustic consultancy company (TNO; see Kastelein et al., 2019a, 2019b). Under test conditions (i.e., only researchers involved in the study allowed within 15 m of the pool, water circulation system off, no rain, and wind force Beaufort 4 or below), ambient noise in the pool was very low (Figure 1; see Kastelein et al., 2019a, 2019b).

Fatiguing Sound—The fatiguing sound consisted of a continuous (100% duty cycle for 1 h), constant-amplitude one-sixth-octave noise band (NB) centered at 8 kHz. A NB was used to avoid standing waves, to create a homogenous sound field, and because it elicits little behavioral response in the harbor seals. A one-sixth octave NB was chosen as it is narrow-band (close to a tone in bandwidth). For details of the emitting and receiving equipment, see Kastelein et al. (2019a, 2019b). The NB was played by an underwater

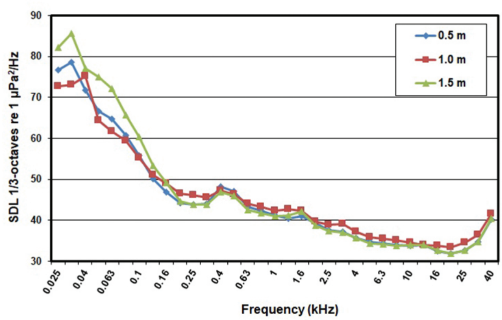

Figure 1. Low underwater ambient noise under test conditions, at three depths (0.5, 1.0, and 1.5 m) in the 2 m deep pool, at the harbor seal (*Phoca vitulina*) listening station. The recordings were analyzed in one-third-octave bands and converted to spectral density levels (SDLs).

transducer (Lubell Model 1424HP; Lubell Labs Inc., Columbus, OH, USA) at 1.5 m depth. Before each sound exposure test, the voltage output of the emitting system to the transducer and the voltage output of the sound-receiving system were checked (see Kastelein et al., 2019a, 2019b). The voltage meter and the underwater sounds were monitored by the operator throughout the exposure sessions.

To determine distribution of the fatiguing sound in the pool, the SPL was measured on a horizontal grid of 1.2 × 1.3 m, at three depths per location on the grid (0.5, 1.0, and 1.5 m below the surface). The sound field was mostly homogeneous: no gradient existed in the SPL in relation to the distance to the transducer, but the SPL was relatively high within 1 to 2 m of the transducer (see Figure 2 for an SPL distribution example). The highest SPL that was attainable without harmonics was the highest used in the study (165 dB re 1 µPa), as harmonics could have affected the results. The fatiguing sound was projected at the following mean SPLs in the pool: 138, 144, 150, and 156 dB re 1 µPa, which, for exposures of 1 h, resulted in SELs of 174, 180, 186, and 192 dB re 1 µPa^{2}s. To test the equal-energy hypothesis, various combinations of mean SPLs (149 to 165 dB re 1 µPa) and exposure durations (2 to 80 min) were used, all of which resulted in an SEL of 186 dB re 1 µPa^{2}s.

The harbor seals generally swam throughout the entire pool during each exposure. The mean SPL of all measurement locations was used to indicate the mean SPL to which they were exposed.

Hearing Test Signals—The hearing test signals that the harbor seals were asked to detect before and after the fatiguing sound or ambient noise exposures were generated digitally with *Adobe Audition*, Version 3.0 (Adobe, Sunrise, FL, USA). Hearing thresholds were tested at the frequency of the fatiguing sound, half an octave above that frequency, and one octave above it (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 calibrated and checked daily, as explained by Kastelein et al. (2019a).

All hearing test signals were transmitted with an underwater transducer (ITC Model No. 6084; International Transducer Corporation, Santa Barbara, CA, USA) that was 1.5 m away from the listening station and at the same depth (1 m). The listening station was an L-shaped polyvinyl-chloride 3-cm diameter water-filled tube with an end cap on which the harbor seal positioned its nose during hearing tests. The underwater SPL at the location of the seal's head at the listening station

0.5 m depth

137	137	136	136	139	144
141	137	133	135	X	144
136	136	134	135	X	140
135	136	133	136	133	140
137	136	137	134	138	X
137	137	139	137	X	X

1.0 m depth

135	136	134	138	136	136
137	135	135	138	X	143
133	134	134	139	X	140
135	135	136	135	134	141
136	137	133	136	138	X
136	139	137	138	X	X

1.5 m depth (depth of transducer)

135	136	134	138	136	136	
137	135	135	138	X	143	T
133	134	134	139	X	140	
135	135	136	135	134	141	
136	137	133	136	138	X	
136	139	137	138	X	X	

Figure 2. An example of the sound pressure level (SPL) distribution in the harbor seal pool when the continuous, constant-amplitude one-sixth-octave noise band centered at 8 kHz, used as the fatiguing sound, was being played at mean SPL of 138 dB re 1 µPa (standard deviation: 3 dB; $n = 93$). The mean sound exposure level for 1 h exposure for this example was 174 dB re 1 µPa^{2}s. Per location, the SPL did not vary systematically with depth. "T" indicates the location of the transducer; "X" indicates the locations of the floating and fixed platforms beneath which SPL measurements were not made. The seals had no access to the area in the lower right-hand corner of the pool. Figure not to scale; each rectangle was 1.3 × 1.2 m and the pool was 2 m deep.

was varied by the operator in 2 dB increments (for details of calibration, see Kastelein et al., 2019a, 2019b). During free-field calibrations before hearing tests, two hydrophones, one where each of the seals' ears would be when the seals were positioned at the listening station, were used to measure the received SPL during hearing tests. The SPL at the two locations differed by 0 to 2 dB, depending on hearing test frequency; the mean SPL of the two measurement locations per hearing test frequency was used to calculate the stimulus SPL during hearing threshold tests.

Experimental Procedures

One sound exposure or control test was conducted per day, consisting of (1) a pre-exposure hearing test session in which the baseline hearing threshold of each harbor seal for one hearing frequency was quantified; (2) fatiguing sound exposure for 1 h (or 2 to 80 min while testing the equal-energy hypothesis), or ambient noise exposure for 1 h (control; 1 h control periods were also used while testing the equal-energy hypothesis); and (3) post-sound exposure (PSE) or post-ambient noise exposure (PAE) hearing test session(s) in which the hearing threshold was quantified for comparison to the baseline threshold (using the same test frequency as in the pre-exposure hearing test). The exposure durations were carefully timed to the second using stopwatches.

Each pre- and post-exposure hearing test session consisted of ~25 trials and lasted for up to 12 min per harbor seal. For each seal, the first PSE session was divided into three 4-min periods: 1-4 (PSE_{1-4}), 4-8 (PSE_{4-8}), and 8-12 (PSE_{8-12}) min after exposure for harbor seal F02, and 12-16 (PSE_{12-16}), 16-20 (PSE_{16-20}), and 20-24 (PSE_{20-24}) min after exposure for harbor seal F01. F02 was always tested first in the post-exposure hearing tests as she was more eager to participate than F01, and because her swimming behavior was influenced less by the exposure noise than that of F01. Testing the two seals in the same order also ensured a consistently quick and efficient start once sound exposure stopped. Sessions were comprised of two thirds signal-present trials and one third signal-absent trials, offered in quasi-random order (see Kastelein et al., 2019a, 2019b). Effective training and good control over the seals' behavior allowed the PSE hearing test to commence within 1 min after the fatiguing sound had stopped for F02, and at 12 min after the sound had stopped for F01. The audiometric method used (go/no go, behavioral method, operant conditioning) is described in detail by Kastelein et al. (2019a, 2019b). The PAE sessions were conducted in the same way as these PSE sessions, but after exposure for 1 h to low level ambient noise instead of to fatiguing sound.

Besides the magnitude of the initial TTS (defined as TTS_{1-4} in F02 and TTS_{12-16} in F01), subsequent changes in hearing were recorded over time. The hearing sensitivity of F02 was always tested during PSE_{1-4}, PSE_{4-8}, and PSE_{8-12}. If F02's hearing had not recovered during PSE_{8-12}, it was also tested 60 (PSE_{60}) min after exposure. If TTS had not recovered during PSE_{60}, testing continued until TTS recovered at 120 (PSE_{120}), 240 (PSE_{240}), or 1,440 ($PSE_{1,440}$) min after the fatiguing sound exposure ended. The hearing sensitivity of F01 was always tested during PSE_{12-16}, PSE_{16-20}, and PSE_{20-24}. If F01's hearing had not recovered during PSE_{20-24}, it was also tested 72 (PSE_{72}) and, if necessary, 132 (PSE_{132}) min after the fatiguing sound exposure ended. Hearing recovery was defined for this purpose as a return to within ~2 dB of the pre-exposure hearing threshold (see "Data Collection and Analysis" below).

Control tests, conducted in the same way as sound exposure tests but without fatiguing sound exposure, were randomly dispersed among the fatiguing sound exposure tests. Sample sizes were chosen to maximize the study time available for testing SELs in which TTS seemed to occur, and to avoid repeated testing of SELs for which TTS obviously did not occur. To avoid damaging their hearing, the harbor seals were exposed to a fatiguing sound at most once per day. The SELs they were exposed to were relatively low, and the number of exposures to the highest SEL was kept as low as possible. No discomfort was observed during the exposures, and the magnitude of the TTS was monitored to determine the maximum SEL to which the subjects were exposed. Randomizing the order in which the seals were tested while maintaining equal sample sizes was considered, but not implemented, as it would have doubled the length of the study period. The TTS growth study was conducted between October 2021 and March 2022.

To test the equal-energy hypothesis, which states that all combinations of SPL and exposure duration that result in the same SEL elicit similar initial TTSs (Roberto et al., 1985), the harbor seals were exposed to continuous, constant-amplitude fatiguing sounds at seven SPL and exposure duration combinations, all of which resulted in an SEL of 186 dB re 1 $\mu Pa^2 s$: 165 dB re 1 µPa for 2 min, 161 dB re 1 µPa for 5 min, 158 dB re 1 µPa for 10 min, 155 dB re 1 µPa for 20 min, 152 dB re 1 µPa for 40 min, 150 dB re 1 µPa for 60 min, and 149 dB re 1 µPa for 80 min. Hearing was always tested at 11.3 kHz, as the highest initial TTS occurred at this hearing frequency (see "Results" below). Each combination was tested four or five times in random order, and eight 60-min control tests were also conducted, following the protocol developed and explained in a similar study with California sea lions (*Zalophus californianus*; Kastelein et al., 2021).

While the equal-energy hypothesis was being tested (April-May 2022), both during fatiguing sound exposure and at other times when no research was conducted, F01 began "bottling" (Riedman, 1990): surface bobbing with her head fully above water and the rest of the body submerged vertically (see "Data Collection and Analysis" below; Figure 3). In this position, her inner, middle, and outer ears were above the water surface. We assumed that this behavior resulted in reduced sound exposure, evidenced by a drop in her initial TTS (TTS_{12-16}), so data collected after exposures during which the seal had been bottling were discarded. To encourage her to keep her ears under water, a 40-cm-long remote-controlled boat was slowly moved around the pool in an irregular pattern. With the boat in the pool, the seal always showed normal swimming behavior; signs of stress (e.g., increased swimming speed or respiration rate) were not observed. The highest received 8 kHz one-third-octave levels of the boat detected at hydrophones placed 0.5, 1.0, and 1.5 m below the surface ranged from 79 to 86 dB re 1 µPa when the boat was at the surface 1 to 4 m from the hydrophones.

Data Collection and Analysis

Occasionally during hearing tests, the harbor seal responded before the stimulus occurred, resulting in a pre-stimulus response. The mean incidence of pre-stimuli by the seals for both signal-present and signal-absent trials was calculated as the number of pre-stimuli as a percentage of all trials in the hearing test. In signal-absent trials, the feedback stimulus indicating a correct lack of response was a whistle (see Kastelein et al., 2019a, 2019b). Pre-stimuli were noted both when quantifying TTS after exposure to the NB at 8 kHz and when testing the equal-energy hypothesis.

To investigate behavioral responses and determine the mean received SELs during fatiguing sound exposure, the harbor seals were monitored by a researcher who was out of their sight in a research cabin next to the pool (see Figure 1 in

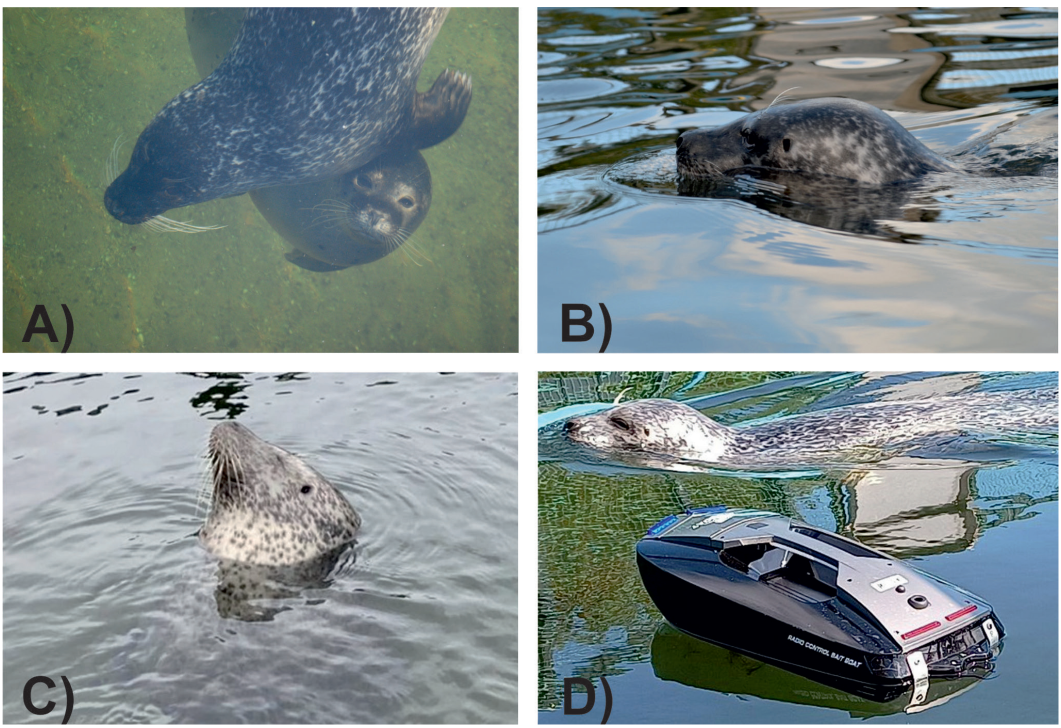

Figure 3. Harbor seal head positions observed during the study: (a) head completely submerged; (b) head at water surface with nose in air and mandible and lower part of skull (containing inner, middle, and most of outer ears) under water; normal breathing position; (c) head completely out of water (including inner, middle, and outer ears), with body in a stationary vertical position in water, referred to as "bottling"; and (d) head at water surface in normal breathing position, with body horizontal, in response to remote-controlled boat that was successfully used to discourage bottling. The boat induced regular swimming, both completely submerged (a) and at the water surface (b).

Kastelein et al., 2012), and recordings were made by a video camera mounted on a pole to provide a complete top view of the pool. From the video recordings, the seals' locations in the pool were monitored, as was the position of their heads, which could be (a) completely submerged; (b) at the water surface with their nose in the air and mandible and lower part of the skull (containing the inner, middle, and most of the outer ear) under water; or (c) completely out of the water (including the inner, middle, and outer ear), while their body was held in a vertical position in the water (referred to as bottling when it continued for longer than a few seconds; see Figure 3). When the seals surfaced to breathe, they were typically at the water surface with their ears mostly submerged. Sound exposure is similar in head positions a and b (Kastelein et al., 2018a). For details of the method used for the analysis of video recordings, see Kastelein et al. (2019a). Due to poor lighting conditions, the recordings of 7% of the sessions could not be analyzed.

In the hearing tests, a switch from a test signal level to which a harbor seal responded (a "hit"), to a level to which she did not respond (a "miss"), and vice versa, was called a "reversal." The pre-exposure mean 50% hearing threshold ($PE_{50\%}$) for each hearing test session was determined by calculating the mean SPL of all reversals in the pre-exposure hearing session. Only pre-exposure sessions with at least 10 reversals were included in the analysis (the maximum was 12 reversals per session). The TTS in F02 during PSE_{1-4} (TTS_{1-4}) for each hearing test frequency was calculated by subtracting the $PE_{50\%}$ from the mean 50% hearing threshold measured during PSE_{1-4} (using only sessions with at least four reversals during each 4-min period). The same method was used to calculate the other TTSs in F02 (TTS_{4-8}, TTS_{8-12}, etc.) and the TTSs in F01 (TTS_{12-16}, TTS_{16-20}, TTS_{20-24}, etc.).

Before the study started, recovery of hearing was defined so that hearing tests could stop once recovery had taken place. Based on the threshold fluctuations observed in previous TTS studies with the same harbor seals (Kastelein et al., 2012, 2018b, 2019a, 2019b, 2020a, 2020c), recovery was defined for this purpose as a return to within 2 dB of the pre-exposure hearing threshold (TTS ≤ 2 dB).

"TTS onset" has been defined as occurring at the lowest SEL causing ≥ 6 dB initial TTS (Schlundt et al., 2000; Southall et al., 2007, 2019). This definition is used in the present study where sample sizes were too small to allow statistical analysis, and for comparison with other studies (see "Quantifying Temporary Hearing Threshold Shift" in the "Discussion" section). For the hearing frequency 11.3 kHz, our sample sizes were large enough to use a more robust definition: we define the "inception 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 (i.e., a TTS) and the hearing threshold shift as measured after the control exposures (this shift was close to zero). The level of significance was established by conducting a one-way ANOVA on the TTS separately for each harbor seal, with the factor SEL (including the control). When the ANOVA produced a significant value overall ($p \leq 0.05$), the levels were compared to the control by means of Dunnett multiple comparisons. Similar one-way ANOVAs were conducted to test the equal-energy hypothesis by comparing the TTSs at different combinations of SPL and duration with each other, and with a control.

Percentages of time spent at the water surface during sound exposures to test the equal-energy hypothesis were compared to exposure SPL by using Spearman's rho correlations. All analyses were conducted in *Minitab 18* (Minitab LLC, State College, PA, USA), and data conformed to the assumptions of the tests used (Zar, 1999).

Results

Quantifying Temporary Hearing Threshold Shift

The pre-stimulus response rates of both harbor seals for all trials in the pre-exposure, post-exposure, and control hearing tests were low and of the same order of magnitude (Table 1).

The control sessions for both harbor seals showed that hearing thresholds for all three hearing test signals before and after 1 h exposure to low ambient noise were very similar (Table 2). After sound exposure sessions, both seals were always willing to participate in the hearing tests, and no change in susceptibility to TTS was observed over the duration of the study.

After exposure to the NB centered at 8 kHz, TTS occurred in both harbor seals at the hearing frequency 11.3 kHz (half an octave above the center frequency of the fatiguing sound). At this frequency in F02, inception of TTS_{1-4} occurred at an SEL of 180 dB re 1 µPa^2s (Table 2; Figure 4); using the definition of Southall et al. (2019), the ≥ 6 dB onset of TTS_{1-4} occurred at an SEL of 181 dB re 1 µPa^2s. In F01, inception of TTS_{12-16} (thus measured after some recovery of hearing) occurred at an SEL of 186 dB re 1 µPa^2s (Table 2; Figure 4). In F02, the ≥ 6 dB onset level of TTS_{1-4} at both of the other hearing frequencies (8 and 16 kHz) was at 192 dB re 1 µPa^2s; in F01, TTS_{12-16} did not occur at 8 and 16 kHz (Table 2). Therefore, TTS was greatest at the hearing frequency half an octave above the center frequency of the fatiguing sound, and after exposure to the fatiguing sound with relatively high SELs.

Table 1. The harbor seals' (*Phoca vitulina*) mean pre-stimulus response rates in pre-exposure hearing tests and after exposure for 1 h either to low-amplitude ambient noise (control) or to the fatiguing sound: a continuous (100% duty cycle), constant-amplitude one-sixth-octave noise band (NB) centered at 8 kHz. Pre-stimulus response rates were similar for all sound exposure levels (SELs), so they were pooled for the calculation of percentages. Sample sizes (in parentheses) are the number of trials within hearing tests. PSE = post-sound exposure; PAE = post-ambient noise exposure (control). The subscript numbers indicate the time of hearing tests in minutes after the exposure ended.

Exposure type	Pre-stimulus response rates – Harbor seal F02							
	Pre-exposure	$PAE_{1\text{-}4}$/ $PSE_{1\text{-}4}$	$PAE_{4\text{-}8}$/ $PSE_{4\text{-}8}$	$PAE_{8\text{-}12}$/ $PSE_{8\text{-}12}$	PSE_{60}	PSE_{120}	PSE_{240}	$PSE_{1,440}$
Control	8.3% (156)	7.6% (92)	5.3% (94)	3.4% (87)	--	--	--	--
NB at 8 kHz	2.9% (383)	6.5% (232)	3.3% (244)	6.3% (223)	4.5% (117)	2.6% (39)	6.5% (46)	0.0% (17)
	Pre-stimulus response rates – Harbor seal F01							
	Pre-exposure	$PAE_{12\text{-}16}$ / $PSE_{12\text{-}16}$	$PAE_{16\text{-}20}$/ $PSE_{16\text{-}20}$	$PAE_{20\text{-}24}$/ $PSE_{20\text{-}24}$	PSE_{72}	PSE_{132}	--	--
Control	3.3% (180)	3.4% (89)	3.6% (84)	2.3% (87)	--	--	--	--
NB at 8 kHz	3.3% (459)	4.2% (262)	4.7% (276)	3.2% (280)	1.3% (79)	0.0% (20)	--	--

Table 2. Mean initial temporary hearing threshold shift (TTS; in dB, $TTS_{1\text{-}4}$ in harbor seal F02 and $TTS_{12\text{-}16}$ in harbor seal F01) after exposure for 1 h to a continuous, constant-amplitude one-sixth-octave noise band centered at 8 kHz at several sound pressure levels (SPLs) and equivalent sound exposure levels (SELs), quantified at hearing frequencies 8, 11.3, and 16 kHz (SD = standard deviation; *n* = sample size). No TTS occurred during the control sessions. One-way ANOVAs were conducted for the hearing frequency 11.3 kHz; * indicates TTSs that differ significantly ($p \leq 0.05$) from the control according to Dunnett multiple comparisons following our definition of "inception of TTS." † indicates TTSs that were assumed to differ from controls based on the definition of "TTS onset" as the lowest SEL causing ≥ 6 dB TTS (Schlundt et al., 2000; Southall et al., 2007, 2019). In these cases, formal statistical analysis was not conducted because the sample sizes were too small ($n = 2$).

Hearing frequency (kHz)	Fatiguing sound		Harbor seal F02				Harbor seal F01			
			$TTS_{1\text{-}4}$ (dB)				$TTS_{12\text{-}16}$ (dB)			
	SPL (dB re 1 µPa)	SEL (dB re 1 µPa²s)	Mean	SD	Range	*n*	Mean	SD	Range	*n*
8 (center)	--	Control	-0.6	1.3	-1.6 to 0.3	2	-0.8	0.1	-0.8 to -0.9	2
	150	186	0.7	1.7	-0.5 to 1.9	2	0.7	1.1	-0.4 to 1.7	3
	156	192	6.6†	0.7	6.1 to 7.1	2	1.9	1.5	0.9 to 2.9	2
11.3 (+ half an octave)	--	Control	-0.4	1.3	-2.3 to 0.9	5	0.4	1.2	-1.3 to 1.7	5
	138	174	1.0	1.0	-0.2 to 2.3	4	0.9	1.7	-1.5 to 2.8	5
	144	180	4.4*	0.3	3.9 to 4.7	4	1.6	0.4	1.2 to 2.2	5
	150	186	10.1*	1.2	9.1 to 11.7	4	4.2*	0.5	3.7 to 5.0	5
	156	192	21.5*	0.2	21.4 to 21.7	2	9.2*	1.7	8.0 to 10.5	2
16 (+ one octave)	--	Control	0.8	1.6	0.0 to 1.7	2	-0.6	0.7	-1.2 to -0.1	2
	150	186	0.1	0.1	0.0 to 0.1	2	0.0	0.7	-0.5 to 0.5	2
	156	192	6.9†	0.4	6.7 to 7.2	2	0.2	1.7	-0.8 to 2.2	3

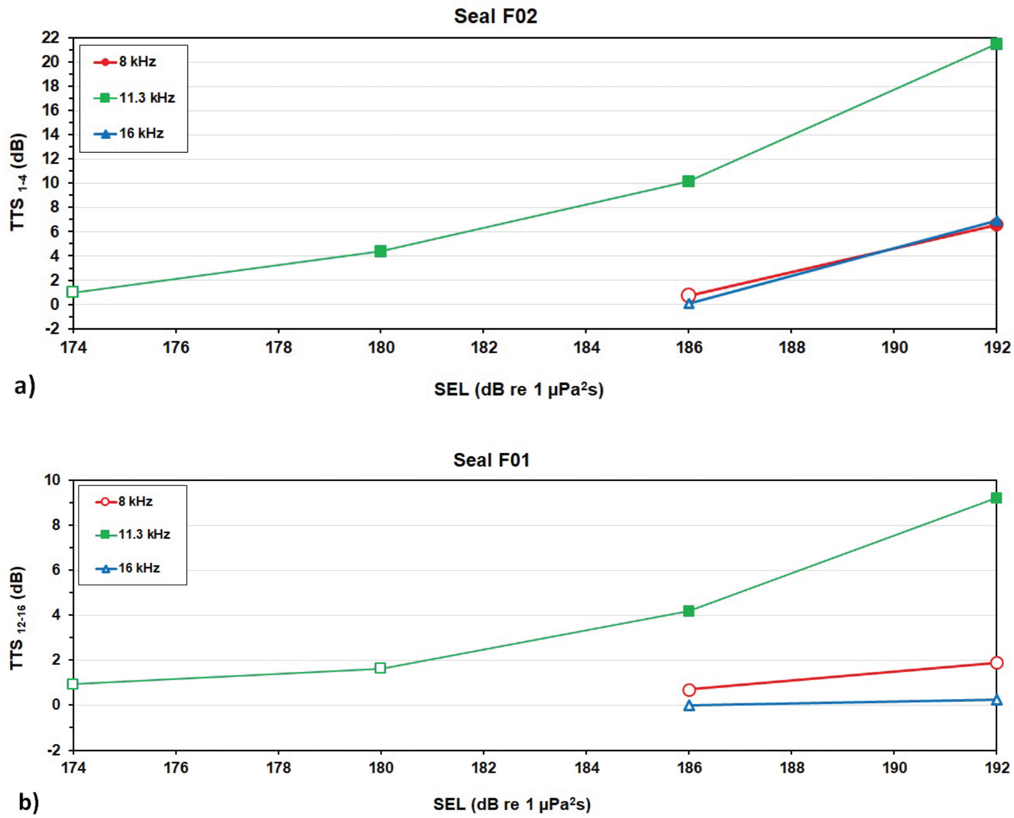

Figure 4. Temporary hearing threshold shifts: initial $TTS_{1\text{-}4}$ in harbor seal F02 (a) and $TTS_{12\text{-}16}$ in harbor seal F01 (b) after exposure to a continuous, constant-amplitude one-sixth-octave noise band centered at 8 kHz for several sound exposure levels (SELs) for 1 h, quantified at hearing frequencies 8, 11.3, and 16 kHz (center frequency of the fatiguing sound and half an octave and one octave above that frequency). For sample sizes, standard deviations, definitions, and control values, see Table 2. Solid symbols indicate significant TTS. For sound pressure levels, subtract 36 dB from the SEL values. The y-axes differ in (a) and (b).

Hearing recovery was variable (Figures 5 & 6). The hearing of F02 recovered within 12 min when $TTS_{1\text{-}4}$ was 4.4 and 6.6 dB, and within 60 min when $TTS_{1\text{-}4}$ was 6.9 and 10.1 dB. When $TTS_{1\text{-}4}$ was at its highest (21.5 dB), recovery occurred between 240 min (4 h) and 1,440 min (24 h) after the fatiguing sound stopped. The hearing of F01 recovered within 24 min when $TTS_{12\text{-}16}$ was 4.2 dB, and within 132 min (2 h 12 min) when it was 9.2 dB. In general, the recovery of hearing took longer after large TTSs than after small TTSs.

Testing the Equal-Energy Hypothesis

The first 12 exposures to the continuous one-sixth-octave NB centered at 8 kHz while testing the equal-energy hypothesis were discarded because F01 was bottling; in the following sessions, the harbor seal was successfully discouraged from bottling by using the remote-controlled boat (Figure 3). Apart from the bottling, no changes were observed in the swimming patterns over time. Spearman's rho correlations showed that the seals spent increasing amounts of time at the water surface with increasing SPLs (Table 3). The pre-stimulus response rates of both seals for all trials in the pre-exposure, post-exposure, and control hearing tests were low and of the same order of magnitude (Table 4).

Both one-way ANOVAs examining initial TTS levels in relation to fatiguing SPLs and including the control were significant ($p < 0.001$). Dunnett multiple comparisons showed that similar levels of TTS occurred after all seven exposure combinations since they all differed significantly from the control and not from one another (Figure 7). For this fatiguing sound frequency (8 kHz; hearing tested at 11.3 kHz) and for these combinations of SPL and exposure durations, the equal-energy hypothesis held true for the harbor seals. Recovery patterns (Figure 8) were similar after exposure to all combinations of SPL

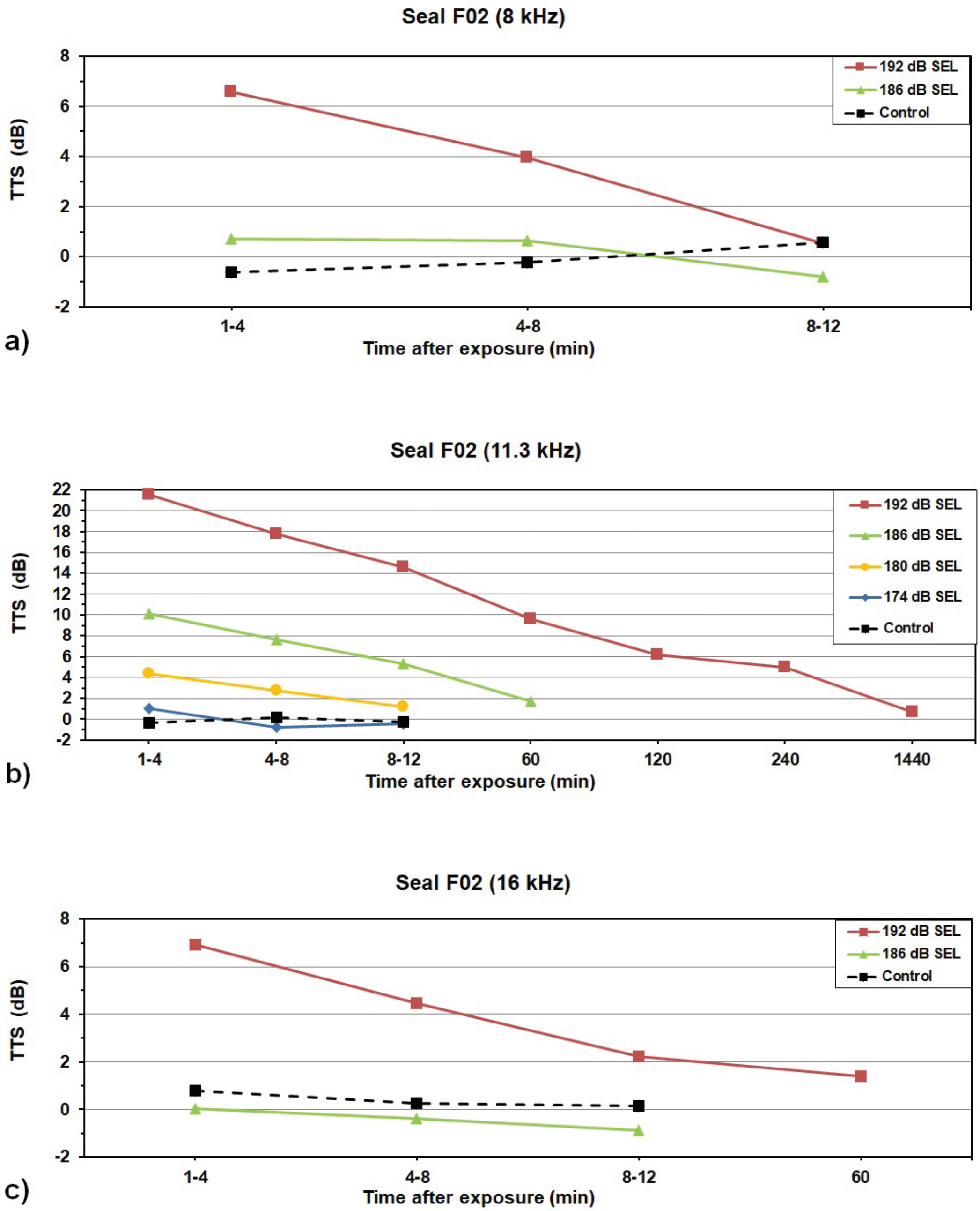

Figure 5. Changes over time, including recovery, of the hearing of harbor seal F02 at 8 kHz (a), 11.3 kHz (b), and 16 kHz (c) after exposure for 1 h to a continuous, constant-amplitude one-sixth-octave noise band centered at 8 kHz at several sound exposure levels (SELs). Note that the axes differ in the three graphs. Mean temporary hearing threshold shifts (TTSs) are shown. For sample sizes and standard deviations (only for TTS_{1-4}), see Table 2. Also shown are the "TTS" values during control sessions; no TTS occurred. Recovery is defined as a return to ≤ 2 dB of the pre-exposure hearing threshold.

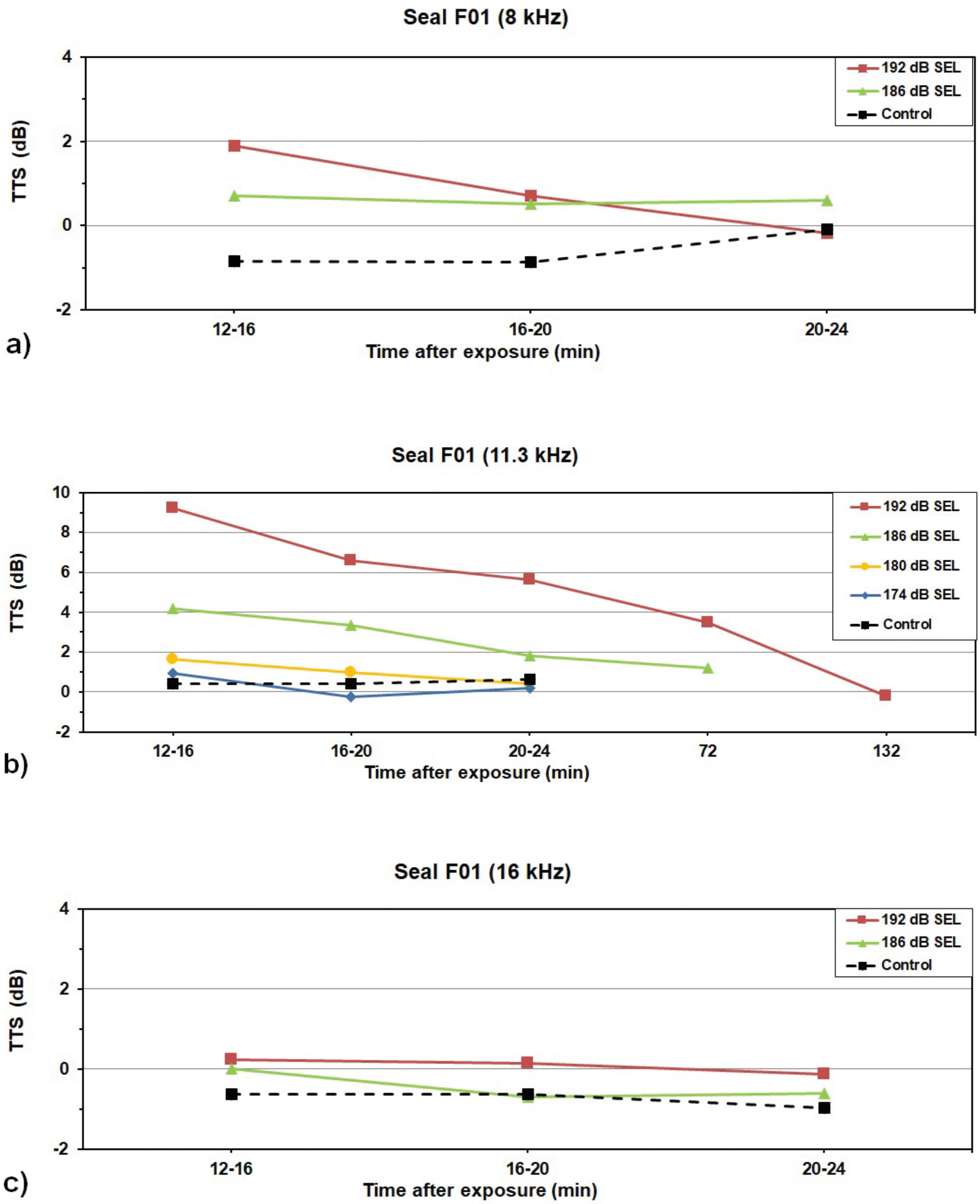

Figure 6. Changes over time, including recovery, in the hearing of harbor seal F01 at 8 kHz (a), 11.3 kHz (b), and 16 kHz (c) after exposure for 1 h to a continuous, constant-amplitude one-sixth-octave noise band centered at 8 kHz at several sound exposure levels (SELs). Note that both axes differ in the three graphs. Mean temporary hearing threshold shifts (TTSs) are shown. For sample sizes and standard deviations (only for $TTS_{12\text{-}16}$), see Table 2. Also shown are the "TTS" values during control sessions; no TTS occurred. Recovery is defined as a return to ≤ 2 dB of the pre-exposure hearing threshold. Testing of the hearing of F01 always started 12 min after the fatiguing sound stopped.

Table 3. The mean percentage of time that harbor seals F02 and F01 spent with their heads at the water surface, during control sessions (with ambient noise only), and during exposure to a continuous, constant-amplitude one-sixth-octave noise band centered at 8 kHz, while testing the equal-energy hypothesis. The harbor seals were exposed to seven SPLs and seven exposure durations in random order, all of which resulted in an SEL of 186 dB re 1 µPa²s. SD = standard deviation; *n* = number of sessions for which video recordings were viewed. The seals spent the remainder of the time with their heads completely submerged; sessions in which bottling was observed are not included here. Spearman correlations between the percentages of time spent at the water surface by the seals during the noise exposure and the SPLs were rho = 0.929, $p = 0.003$ for F02 and rho = 0.750, $p = 0.052$ for F01.

Fatiguing sound exposure		Harbor seal F02			Harbor seal F01		
Duration (Min)	SPL (dB re 1 µPa)	Mean % of time with head at water surface	SD	*n*	Mean % of time with head at water surface	SD	*n*
Control	Ambient	18	8	8	18	9	8
2	165	97	5	5	86	23	5
5	161	64	32	5	79	14	4
10	158	74	29	5	84	24	4
20	155	55	25	5	50	15	4
40	152	48	15	4	48	15	4
60	150	29	4	4	42	22	6
80	149	47	19	4	62	14	5

Table 4. The harbor seals' mean pre-stimulus response rates while testing the equal-energy hypothesis in pre-exposure hearing tests and after exposure to low-amplitude ambient noise (control), or to the fatiguing sound: a continuous (100% duty cycle), constant-amplitude one-sixth-octave noise band (NB) centered at 8 kHz at a sound exposure level of 186 dB re 1 µPa²s. Pre-stimulus response rates were similar for all sound pressure levels, so they were pooled for the calculation of percentages. Sample sizes (in parentheses) are the number of trials within hearing tests. PSE = post-sound exposure; PAE = post-ambient noise exposure (control). The subscript numbers indicate the time of the hearing test in minutes after the end of the sound exposure.

Exposure type	Pre-stimulus response rates – Harbor seal F02				
	Pre-exposure	PAE_{1-4}/ PSE_{1-4}	PAE_{4-8}/ PSE_{4-8}	PAE_{8-12}/ PSE_{8-12}	PSE_{60}
Control	2.7% (188)	3.3% (90)	6.2% (97)	3.4% (87)	--
NB at 8 kHz	3.0% (639)	5.6% (339)	5.4% (367)	3.0% (336)	2.6% (543)
	Pre-stimulus response rates – Harbor seal F01				
	Pre-exposure	PAE_{12-16}/ PSE_{12-16}	PAE_{16-20}/ PSE_{16-20}	PAE_{20-24}/ PSE_{20-24}	
Control	2.3% (171)	3.3% (92)	1.2% (85)	2.1% (95)	--
NB at 8 kHz	2.7% (626)	4.1% (320)	3.1% (327)	2.8% (317)	--

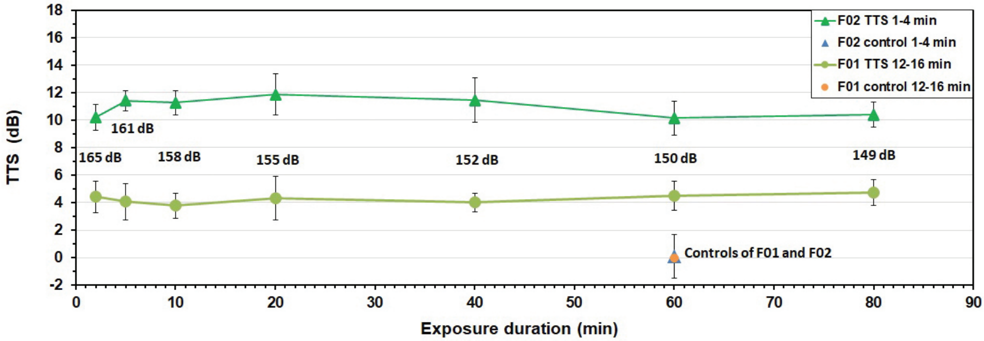

Figure 7. Testing the equal-energy hypothesis in harbor seals with initial temporary hearing threshold shift (TTS). The graph shows the mean (± standard deviation; n = 4 to 6) TTS_{1-4} of harbor seal F02 and TTS_{12-16} of harbor seal F01 at hearing test frequency 11.3 kHz after exposure to a continuous, constant-amplitude one-sixth-octave noise band centered at 8 kHz for 2 to 80 min at sound pressure levels (SPLs) ranging from 149 to 165 dB re 1 µPa; all combinations resulted in an identical SEL (186 dB re 1 μPa^2s). Control sessions (n = 9) were also conducted with 11.3 kHz hearing test signals.

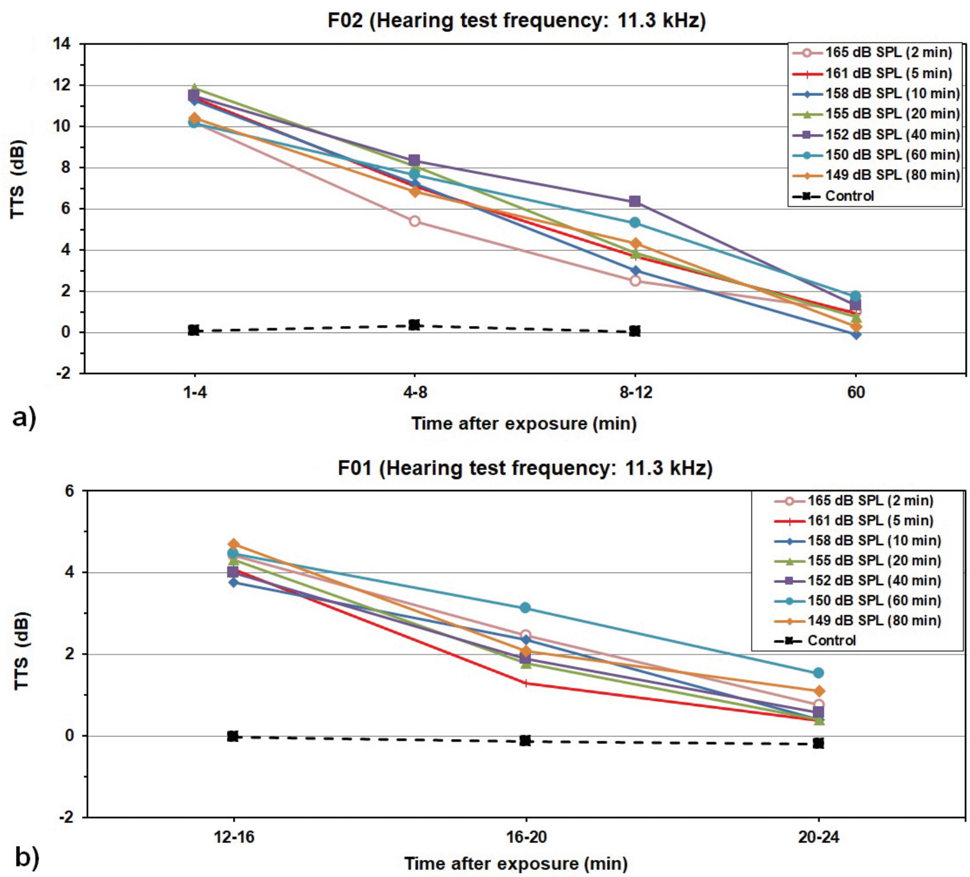

Figure 8. Changes in hearing over time, including recovery, while testing the equal-energy hypothesis. Mean temporary hearing threshold shift (TTS; n = 4 to 6) at 11.3 kHz of harbor seal F02, measured 1 to 12 and 60 min after exposure to the noise band at 8 kHz (a); and of harbor seal F01 measured 12 to 24 min after exposure (b). The sound exposure level (SEL) of 186 dB re 1 μPa^2s was composed of seven different combinations of sound pressure level (SPL; 149 to 165 dB re 1 µPa) and exposure durations (2 to 80 min). The mean "TTS" values during control sessions (no shifts occurred) are also shown.

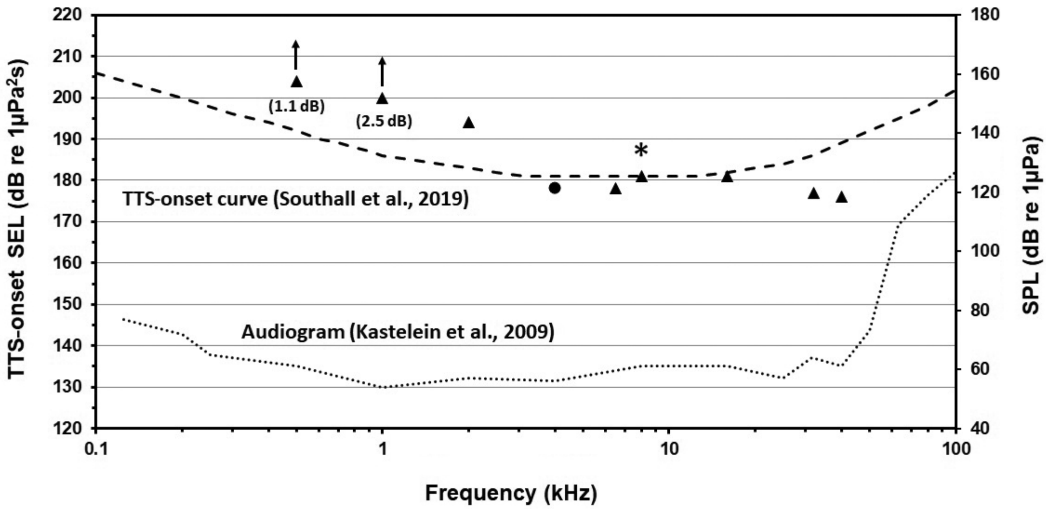

Figure 9. The temporary hearing threshold shift (TTS) onset function (dashed line) for underwater sounds in "phocid carnivores," as proposed by Southall et al. (2019), is defined as the sound exposure level (SEL) required to cause 6 dB TTS_{1-4} (initial TTS). The data points are TTSs of ~6 dB for harbor seals after exposure for 1 h to various continuous, constant-amplitude fatiguing sounds. In two cases, 6 dB TTS_{1-4} could not be reached (0.5 and 1 kHz). Data points from individual studies are indicated as ▲ for harbor seal F02 and ● for harbor seal F01. From left to right, data points are a noise band (NB) at 0.5 kHz (Kastelein et al., 2020b) with an SEL of 204 dB re 1 µPa²s which elicited only 1.1 dB TTS_{1-4}; and NB at 1 kHz (Kastelein et al., 2020b) with an SEL of 200 dB re 1 µPa²s which elicited only 2.5 dB TTS_{1-4}. The arrows pointing upwards indicate that the SELs required to cause 6 dB TTS_{1-4} must be higher than these two data points. The remaining data points indicate fatiguing sounds that elicited 6 dB TTS: NB at 2 kHz (Kastelein et al., 2020b); NB at 4 kHz (Kastelein et al., 2012); continuous wave at 6.5 kHz (Kastelein et al., 2019a); NB at 8 kHz (present study, indicated by an * above the data point); NB at 16 kHz (Kastelein et al., 2019b); NB at 32 kHz (Kastelein et al., 2020a); and NB at 40 kHz (Kastelein et al., 2020c). Also shown is the mean audiogram of F01 and F02 for tonal signals (right-hand y-axis, dotted line; Kastelein et al., 2009a; mean threshold difference between seals 2 ± 1.2 [standard deviation] dB; n = 16 frequencies). In most cases, 6 dB TTS_{1-4} onset was detected at a hearing frequency half an octave above the center frequency of the fatiguing sound; in one case (40 kHz; Kastelein et al., 2020c), it was detected at one third of an octave above the center frequency of the fatiguing sound.

and exposure duration and were similar to recoveries from similar TTS_{1-4} and TTS_{8-12} (Figures 5 & 6, respectively). Hearing recovered within 60 min for F02 and within 24 min for F01.

Discussion

Evaluation

The pre-exposure hearing thresholds found in the present study for hearing test frequencies 8, 11.3, and 16 kHz were within ~2 dB of the hearing thresholds measured in these same harbor seals for tonal signals approximately 15 y before the present study (Kastelein et al., 2009a, 2009b, 2010). This shows that these seals' hearing sensitivity for these frequencies has been stable over time and suggests that defining recovery as a return to within ~2 dB of the pre-exposure hearing threshold was appropriate. The similar pre-stimulus response rates in hearing tests before and after the sound exposures in both seals show that their decision-making criteria in the post-exposure hearing tests were not influenced by the sound exposure.

The critical ratios of harbor seals at 8, 11.3, and 16 kHz (hearing test frequencies used) are around 21 to 25 dB (Renouf, 1980; Turnbull & Terhune, 1990; Kastelein et al., unpub. data: 22 to 25 dB), indicating that these hearing test signals were not masked by ambient noise in this study. In the equal-energy hypothesis study, the one-third octave band noise at 8 kHz from the boat was at least 63 dB below the lowest SPL used (80-min exposure) and would not have influenced the resulting TTS levels.

When testing the equal-energy hypothesis, the harbor seals (in particular F02) spent increasing amounts of time swimming at the water surface with increasing SPLs (Table 3). When harbor seals swim at the water surface, sound energy reaches their ears as if they are completely submerged (Kastelein et al., 2018a). However, it seems likely that the seals were attempting to reduce their sound exposure by swimming in this way.

Most Affected Hearing Frequency

The results of the present study show that, after exposure to a one-sixth-octave NB centered at 8 kHz, the harbor seals showed the highest TTSs at 11.3 kHz, half an octave above the center frequency of the fatiguing sound. In previous TTS studies with harbor seals (Kastelein et al., 2019a, 2019b, 2020a, 2020b, 2020c; all involving 60-min sound exposures), the hearing frequency at which the highest TTS was measured depended on the SEL (as in humans; McFadden & Plattsmier, 1983). However, the hearing frequency that was most affected after exposure to high SELs was usually half an octave higher than the center frequency of the fatiguing sound. Only after exposure to a NB at 40 kHz, near the upper end of the underwater hearing range of harbor seals, was the highest TTS at one third of an octave above the center frequency of the fatiguing sound (Kastelein et al., 2020c). TTS research in other marine and terrestrial mammals also indicates that, after high-amplitude exposures, the maximum TTS is generally induced half an octave above the fatiguing sound's frequency (Cody & Johnstone, 1981; McFadden, 1986; Finneran, 2015).

Quantifying Temporary Hearing Threshold Shift

The frequency-dependent susceptibility of harbor seals to TTS caused by underwater sound, as indicated by TTS onset (the lowest SEL required to elicit ≥ 6 dB TTS_{1-4}) in the fatiguing sound frequency range, which was tested in previous studies with the same harbor seals (0.5 to 40 kHz; Kastelein et al., 2012, 2018b, 2019a, 2019b, 2020a, 2020b, 2020c), is compared to results from the present study (see Figure 9). Most data points are for F02, as she was tested mostly during the first 4 min after the fatiguing sound stopped; and one data point (at 4 kHz) is for F01 (Figure 9). The 6 dB TTS_{1-4}-onset SEL of F02 in the present study (181 dB re 1 μPa^2s) fits well with TTS-onset data for other fatiguing sound frequencies. The present study provides additional data on TTS-onset SELs that can be used to improve or generate auditory weighting functions and, thus, enhance the regulatory protection of harbor seals. For example, growth rates of TTS levels with increasing SELs have been used to predict the onset SELs of PTS (Southall et al., 2019). To be conservative, it has been assumed that PTS in marine mammals may occur when the SEL is 20 dB above the "onset" (6 dB level) of TTS (Southall et al., 2019).

The theoretical TTS-onset function for underwater sounds in "phocid carnivores," as proposed by Southall et al. (2019), was based on the few data available at the time. The function is a good fit to subsequently published data between 4 and 16 kHz, but is too low at lower frequencies and too high at higher frequencies (Figure 9). Just below their upper frequency discrimination limit of ~64 kHz (Møhl, 1967), harbor seals seem to be more susceptible to TTS from high-frequency continuous noise than predicted from the theoretical TTS-onset function proposed by Southall et al. (2019). This was also noted by Tougaard et al. (2022). A similar situation was found in California sea lions (Kastelein et al., 2024). The lower ≥ 6 dB TTS levels at 32 and 40 kHz of harbor seals (Kastelein et al., 2020a, 2020c) indicate that the pattern deviates from that of the unmasked audiogram, which shows reduced hearing sensitivity at these higher frequencies (Figure 9; Kastelein et al., 2009a, 2009b, 2010). The theoretical TTS-onset function was based on the concept that susceptibility to TTS is high at frequencies with the most acute hearing (Houser et al., 2017; Southall et al., 2019). In Figure 9, in harbor seals, the susceptibility to TTS (i.e., the TTS-onset SEL) is similar for continuous, constant-amplitude sounds between 4 and 40 kHz.

The TTS data from harbor seals for 0.5 and 1 kHz (Kastelein et al., 2020b) indicate that low-frequency noise (e.g., from large ships; Duarte et al., 2021) is unlikely to be of high enough amplitude to induce TTS (unless a seal is very close to a sound source for a long period of time), but data on TTS caused by fatiguing sounds at lower frequencies (< 0.5 kHz) are still lacking, as these frequencies are difficult to generate in a pool. Also, it is difficult to generate low-frequency sounds at high SPLs without harmonics.

Testing the Equal-Energy Hypothesis

The equal-energy hypothesis states that exposure to sounds with similar energy results in similar TTSs, independent of the SPL and exposure duration combination (Southall et al., 2007). In the present study, the equal-energy hypothesis held true in harbor seals, at least for conditions tested here (in terms of signal type [continuous, constant-amplitude noise], fatiguing sound frequency [NB centered at 8 kHz], SEL [186 dB re 1 μPa^2s], durations [2 to 80 min], and SPLs [149 to 165 dB re 1 µPa]). With a few exceptions (Kastelein et al., 2012), the equal-energy hypothesis appears to apply to harbor seals, so, with caution, SEL can be used to predict TTS caused by continuous, constant-amplitude sound in this species (Finneran, 2015). However, TTS predictions based on continuous fatiguing sound SELs are likely to be conservative for intermittent sound because intervals without sound and reductions in the amplitude of the sound may result in reductions in TTS due to recovery of hearing in the intervals, as seen in California sea lions (Kastelein et al., 2021, 2022). More information about effective quiet (Ward et al., 1976) and recovery of hearing during intermittent sounds is needed to make better predictions of TTS due to real-world sound exposures. With these caveats, the SEL of a continuous, constant-amplitude fatiguing sound can be used to predict initial TTS in harbor seals, and this principle should continue to be used to guide policies.

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