Frequency of greatest temporary hearing threshold shift in harbor seals (*Phoca vitulina*) depends on fatiguing sound level

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Harbor seals may suffer hearing loss due to intense sounds. After exposure for 60 min to a continuous 6.5 kHz tone at sound pressure levels of 123–159 dB re 1 μ Pa, resulting in sound exposure levels (SELs) of 159–195 dB re 1 μ Pa²s, temporary threshold shifts (TTSs) in two harbor seals were quantified at the center frequency of the fatiguing sound (6.5 kHz) and at 0.5 and 1.0 octaves above that frequency (9.2 and 13.0 kHz) by means of a psychoacoustic technique. Taking into account the different timing of post-exposure hearing tests, susceptibility to TTS was similar in both animals. The higher the SEL, the higher the TTS induced at frequencies above the fatiguing sound's center frequency. Below $\sim$ 179 dB re 1 μ Pa²s, the maximum TTS was at the center frequency (6.5 kHz); above $\sim$ 179 dB re 1 μ Pa²s, the maximum TTS was at half an octave above the center frequency (9.2 kHz). These results should be considered when interpreting previous TTS studies, and when estimating ecological impacts of anthropogenic sound on the hearing and ecology of harbor seals. Based on the results of the present study and previous studies, harbor seal hearing, in the frequency range 2.5–6.5 kHz, appears to be approximately equally susceptible to TTS.

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I. INTRODUCTION

Marine mammals exposed to sufficiently intense sounds may suffer hearing loss, resulting in threshold shifts, which may be temporary (TTS; temporary threshold shift) or permanent (PTS; permanent threshold shift). A fatiguing sound's level, spectral content, temporal pattern, and duration may influence the threshold shift it causes and determine whether the shift is permanent or temporary (Melnick, 1991; Yost, 2007). The course and speed of recovery from a threshold shift depend on the sound to which an animal was exposed, the amount of shift incurred, and the species (e.g., Kastelein *et al.*, 2013a; Houser *et al.*, 2017). Generally, the greater the threshold shift, the longer the recovery period (Carder and Miller, 1972; Mills *et al.*, 1979); if the shift is too large, complete recovery may not occur and the hearing ability of the animal may be reduced permanently (PTS). For the regulation and management of anthropogenic offshore activities that generate high levels of underwater sound (i.e., percussion pile driving, seismic surveys, sonars, detonations) it is important to know at what exposure sound pressure level (SPL) and durations PTS may be induced in different marine mammal species and for different sounds that are produced in areas in which the species occur. As it is not considered ethical to induce PTS in marine mammals, research on the onset and development of TTS is used to estimate the cumulative sound exposure level (SEL_{cum}) that may lead to PTS.

Studies of TTS do not indicate the specific SELs at which PTS occurs, but are valuable in themselves. They give insight into effects of less harmful exposures, by providing TTS onset SEL, TTS growth rate, and critical levels. TTSs of various magnitudes and durations have the potential to compromise feeding, localization, communication, and predator detection, and may have negative effects on health and survival even if PTS does not occur. Pinnipeds are often active in murky water, during the night, and at depths where light hardly penetrates; they depend therefore to a large degree on sound and mechano-reception (via their whiskers), rather than on vision, for orientation and communication (Supin *et al.*, 2001). Hearing damage may reduce the efficiency of these activities, thus reducing the animals' fitness, reproductive output and longevity. The ecological effect of reduced hearing depends not only on the magnitude of the threshold shift, its duration (which depends on the exposure duration plus the recovery time after exposure stops), but also on the hearing frequency range affected and the ecological importance of this frequency range for the animal.

Generally TTS is observed at frequencies higher than the center frequency of the fatiguing sound. It often occurs half an octave above the center frequency (McFadden, 1986), but at higher exposure levels it may affect a much wider bandwidth of hearing (Wang *et al.*, 2002). This half-octave phenomenon has been observed in marine mammals (Finneran, 2015). In terrestrial mammals, the hearing frequencies affected by fatiguing sound depend not only on the center frequency of the fatiguing sound, but also on the received SPL, as shown for guinea pigs (*Cavia porcellus*) by

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Cody and Johnstone (1981) and for humans by McFadden and Plattsmier (1983). Although several mechanisms have been hypothesized, the psychophysical origin of this frequency shift is not fully understood (Ramamoorthy and Nuttall, 2012). In marine mammals, the “received SPL-affected frequency” phenomenon in noise induced hearing loss studies has only been studied in a small odontocete species (the harbor porpoise, *Phocoena phocoena*) that was exposed to a continuous wave of 6.5 kHz (Kastelein *et al.*, 2014b).

It is important to know whether hearing frequencies affected by fatiguing sound also depend on the received SPL in pinnipeds. In this study, harbor seals (*Phoca vitulina*) were used. The harbor seal’s wide distribution area in the coastal waters of the northern hemisphere (Burns, 2002) overlaps with areas in which noisy anthropogenic activities occur. The underwater sounds produced may cause TTS or PTS in harbor seals if the received SPL and/or exposure duration are high/long enough. So far, only three studies have been conducted in which TTS due to underwater sounds was studied in harbor seals. After exposing a harbor seal to octave-band noise centered at 2.5 kHz, Kastak *et al.* (2005) tested its hearing at two frequencies (2500 and 3530 Hz) and found that the highest TTS had occurred at the center frequency. After exposing two harbor seals to octave-band noise centered at 4 kHz, Kastelein *et al.* (2012) tested their hearing only at 4 kHz. After exposing two harbor seals to playbacks of pile-driving sound (the one third octave band centered at 630 Hz contained the most energy), Kastelein *et al.* (2018b) tested their hearing at 0.25, 0.5, 1, 2, 4, 8, 16, and 32 kHz, and only found TTS at 4 and 8 kHz (the highest TTS occurred at 4 kHz).

The limited information on TTS in harbor seals does not allow prediction of which hearing frequency needs to be measured after sound exposure, in order to detect the greatest sound-induced TTS. This information is required for setting safety criteria for underwater sounds, in order to prevent PTS in wild harbor seals. Underwater sound exposure limits should be based on changes across all hearing frequencies, and not just at the frequency at which the maximum TTS is detected. Hearing frequencies required for localization, prey detection, or communication may be important for health or survival, though they may not be the frequencies at which maximum TTS occurs. The duration of the TTS (i.e., the speed of recovery of hearing) is also an important factor to consider when evaluating the impact of a sound. Therefore, as a step towards testing susceptibility to TTS in the harbor seal’s entire hearing range, the goals of the present study were to expose harbor seals to a 6.5 kHz continuous pure tone and to quantify the TTS induced by the received SPL in the affected hearing frequencies. This will allow us to determine whether or not the hearing frequency that is most affected depends on the received SPL, and to gain insight into the process of hearing recovery in harbor seals that have been exposed to loud underwater sounds.

II. MATERIALS AND METHODS

A. Study animals and study area

The study animals were two female harbor seals, identified as harbor seals F01 and F02. During the study they were

8–10 yr old and the body mass of each was around 40 kg in summer and 65 kg in winter. The seals received most of their daily fish ration during hearing test sessions. They were healthy throughout the study, and had participated in eight previous hearing studies, which had shown that they had sensitive and very similar hearing (Kastelein *et al.*, 2009a; Kastelein *et al.*, 2009b; Kastelein *et al.*, 2010a; Kastelein *et al.*, 2012; Kastelein *et al.*, 2013b; Kastelein *et al.*, 2015b; Kastelein *et al.*, 2018a; Kastelein *et al.*, 2018b). Variation in the animals’ performance was minimized by making weekly adjustments (usually in the order of 100 g) to their daily food ration, based on their weight and performance during the previous week and the expected change in water and air temperatures in the following week.

The study was conducted at the SEAMARCO Research Institute, The Netherlands, in a remote and quiet location. The animals were kept, and the study was conducted, in an outdoor pool (8 m × 7 m, 2 m deep, with haul-out areas). The pool walls and the sandy bottom were covered with aquatic vegetation. Skimmers kept the water level constant so that sound conditions were stable. Seawater was pumped directly from the nearby Oosterschelde, a lagoon of the North Sea, into the water circulation system. Recirculation through biological and sand filters ensured year-round water clarity. The average monthly temperature varied during the study period between 0°C and 22°C and the salinity was around 3.4‰. The water circulation system and the aeration system for the bio-filter were made as quiet as possible. During the sound exposure sessions, barriers were placed so that the seals could not leave the water. During the hearing tests, the seal not being tested was kept in the water next to the haul-out area and was asked to perform quiet behaviors to keep her occupied.

B. Acoustics

1. SPL measurement equipment

The ambient noise was measured, and the fatiguing sound and hearing test signals were calibrated, once every two months during the study period by an acoustic consulting agency (TNO). The sound measurement equipment consisted of three hydrophones [Brüel & Kjaer (B&K), Model No. 8106] with a multichannel high-frequency analyzer (B&K PULSE, Model No. 3560 D), and a laptop computer with B&K PULSE software (Labshop, version 12.1). The system was calibrated with a pistonphone (B&K, Model No. 4223). The broadband sound pressure level (SPL; dB re 1 µPa, ANSI, 2013) of each hearing test signal was derived from the 90% received energy flux density and the corresponding 90% time duration (t_{90}) (Madsen, 2005). The SPL of the fatiguing sound was determined over a period of 10 s.

2. Ambient noise

Great care was taken to make the harbor seals’ listening environment as quiet as possible. Only researchers involved in the hearing tests were allowed within 15 m of the pool during hearing test sessions, and they were required to stand still. Under test conditions (water circulation system off, no rain, and wind force Beaufort 4 or below) the ambient noise

in the pool was very low and fairly constant in amplitude (Fig. 1).

3. Fatiguing sound

A 6.5 kHz continuous (i.e., 100% duty cycle) sinusoidal wave (CW) without harmonics was used as the fatiguing sound (i.e., the sound intended to cause TTS). This sound was selected because it is within the frequency range of some naval active sonar systems. This digitally generated sound (WAV file, sample rate: 768 kHz) was played back by a laptop computer (Acer Aspire, Model No. 5750) with a program written in LabVIEW, to an external data acquisition card (National Instruments, Model No. USB 6229), the output of which could be controlled in 1 dB steps with the LabVIEW program. The output of the card went through a ground loop isolator and custom-built buffer to a custom-built passive low-pass filter set to 8 kHz, after which it went to a power amplifier (East & West, Model No. LS5002) which drove the transducer (Lubell, Model No. LL1424HP) through an isolation transformer (Lubell, Model No. AC1424HP). The transducer was placed at the north-eastern end of the pool at 1.5 m depth. The linearity of the transmitter system producing the fatiguing sound was checked during each calibration, and was found to be consistent to 1 dB within a 42 dB range (overlapping the SPL range used in the study).

To determine the fatiguing sound’s distribution in the pool, the SPL was measured at 34 locations (on a horizontal grid of 1.2 m × 1.2 m), at three depths per location on the grid (0.5, 1.0, and 1.5 m below the surface). To determine their acoustic dose, the seals were monitored during the fatiguing sound exposure periods, in terms of both their location in the pool and the position of their head (at the water surface or completely submerged). During exposure to the 6.5 kHz tone, it was observed that the animals swam throughout the entire pool, at all depths. Therefore, the average received SPL (SPL_{av.re.}) as experienced by the harbor seals was calculated as the energetic average of the SPL at all 102 individual measurement locations. There were only small differences in SPL per depth and per location, and no gradient existed in the

FIG. 1. Mean underwater ambient noise (n = 3) under test conditions (only researchers involved in the study within 15 m of the pool, water circulation system off, no rain, and wind force Beaufort 4 or below). Measurements were analyzed in one-third octave (base 10) bands. Above ~10 kHz the ambient noise was so low that it was dominated by the electronic noise of the measurement system.

SPL in relation to the distance to the transducer, resulting in a fairly homogeneous field (Fig. 2). The 6.5 kHz CW fatiguing sound was projected at various source levels, resulting in SPL_{s.av.re.} ranging from 123 to 159 dB re 1 μPa. For the 60 min exposure, the SEL_{cum.} calculated as: SPL_{av.re.} + 10log₁₀ (60 min/1 s), ranged from 159 to 195 dB re 1 μPa²s.

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 with an oscilloscope (Dynatek-8300) and a voltmeter (Agilent-34401A), by producing a 6.5 kHz continuous tone from the laptop. The acoustic underwater signal was checked with a hydrophone (B&K 8101), a pre-amplifier (B&K 2365) and a spectrum analyzer (Velleman-PCSU1000). If the values obtained were the same as those obtained during the SPL calibrations by the acoustic consulting agency, the SPLs were assumed to be correct, and a sound exposure test could be performed.

4. Hearing test signals

The harbor seals were trained to detect signals presented during hearing tests before and after exposure to the

0.5 m depth					
153	154	146	146	149	160
146	147	156	X	X	155
147	153	147	149	153	162
150	144	149	153	149	151
146	147	153	155	149	157
153	153	152	148	149	155
1.0 m depth					
144	146	151	152	156	151
147	156	154	X	X	158
149	149	152	149	147	150
151	146	147	151	149	153
139	145	146	149	149	153
152	149	148	148	149	152
1.5 m depth (depth of transducer)					
147	153	159	158	158	159
153	152	157	X	X	158
148	144	146	154	154	152
147	144	156	150	154	161
147	152	152	155	151	158
153	148	158	155	151	154

FIG. 2. The SPL distribution in the outdoor pool when the 6.5 kHz CW, used as the fatiguing sound, was being played (102 measurement locations: 34 locations, three per depth). Per location, the SPL did not vary systematically with depth. These data were used to calculate the average received SPL (SPL_{av.re.}) that the harbor seals experienced during sound exposures (SPL_{av.re.} shown: 153 dB re 1 μPa; SD: ±5 dB; n = 102). The two boxes with an X represent the location of the floating haul-out area; the black dot represents the location of the transducer at 1.5 m depth.

fatiguing sound. Narrow band up-sweeps (linear frequency-modulated tones) were used as hearing test signals instead of pure tones, because sweeps lead to more stable received SPLs at the listening station (Finneran and Schlundt, 2007) and thus to more stable thresholds. For TTS studies, precise hearing thresholds are very important, as sometimes only small threshold shifts occur and study animals recover rapidly (Kastelein *et al.*, 2012). The hearing test signals were generated digitally (Adobe Audition, version 3.0). The linear up-sweeps started and ended at $\pm 2.5\%$ of the center frequency, and had durations of 1 s, including a linear rise and fall in amplitude of 50 ms. The WAV files used as hearing test signals were played on a laptop computer (Acer Aspire 5750 Model P5WEO) with a program written in LabVIEW, to an external data acquisition card (National Instruments, Model No. USB6229); the output was controlled in 1 dB steps with the LabVIEW program. The output of the card went through a ground-loop isolator and a custom-built passive low-pass filter and drove a balanced tonpilz piezoelectric acoustic transducer (Lubell, Model No. LL916) through an isolation transformer (Lubell, Model No. AC202). The SPL in the water at the location of a harbor seal's head while it was at the listening station was varied by the operator in 2 dB increments.

The hearing thresholds were tested at the frequency of the fatiguing sound (6.5 kHz), half an octave above the frequency of the fatiguing sound (9.2 kHz), and one octave above the frequency of the fatiguing sound (13 kHz; limited sample size).

The received SPL of each hearing test signal was measured at the position of the harbor seal's head during the hearing tests. The calibration measurements were conducted with two hydrophones, one at the location of each auditory meatus of the harbor seal when it was positioned at the listening station. The linearity of the transmitter system was checked during each calibration and was found to be consistent to 1 dB within a 30 dB range (from 10 dB above the hearing threshold). The SPL at the two locations differed by 0–2 dB, depending on the test frequency. The mean SPL of the two hydrophones was used to calculate the stimulus level during hearing threshold tests.

C. Experimental procedures

Each hearing test trial began with one of the harbor seals at the start/response buoy. The level of the hearing test sweep used in the first trial of the session was approximately 6 dB above the hearing threshold determined during the previous pre-exposure or post-exposure session. The harbor seals were trained to swim from the start/response buoy to the listening station in response to a hand signal from the trainer. The methodology was as described by Kastelein *et al.* (2012). The signal level was varied according to the one-up one-down adaptive staircase method (Cornsweet, 1962); 2 dB steps were used. This conventional psychometric technique (Robinson and Watson, 1973) produces a 50% correct detection threshold (Levitt, 1971). A switch from a test signal level that the harbor seal responded to (a hit), to a level that she did not respond to (a miss), and vice versa,

was called a reversal. Signals were produced at a random time 4–12 s after a seal stationed at the start/response buoy.

Each hearing test session consisted of ~ 25 trials and lasted for up to 12 min per animal (the first session after the fatiguing sound stopped was divided into three periods: 1–4, 4–8, and 8–12 min). Sessions consisted of two thirds signal-present and one third signal-absent trials, offered in quasi-random order (never more than three consecutive signal-present or signal-absent trials).

One total sound exposure test, consisting of (1) pre-exposure hearing test, (2) fatiguing sound exposure, and (3) post-sound exposure (PSE) hearing test or tests, was conducted per day, starting at 0830 h. The SPL of the sound during the fatiguing sound exposure period was increased slowly during the first 60 s, to avoid startle responses, which may have led to large changes in the seals' swimming pattern. To see whether the seals selected particular locations in the pool during sound exposures, their surfacing locations were recorded on video and the operator watched their behavior on a monitor in the research cabin. The PSE hearing test (using the same sweep as used in the pre-exposure hearing test) commenced within 1 min after the fatiguing sound had stopped for F02, and 12 min after the sound had stopped for F01.

The two seals were always tested in the same order (to ensure a quick and efficient start after sound exposure stopped): first seal F02, then seal F01. In order to protect their hearing, the study animals could only be exposed to fatiguing sounds once per day, and randomizing the order in which they were tested, while maintaining the sample sizes, would have doubled the study period.

To gain insight into the duration of the TTS, not only the magnitude of TTS immediately after the exposure was recorded, but also the subsequent hearing recovery. The hearing sensitivity of seal F02 was tested during up to six PSE periods: 1–4 (PSE₁₋₄), 4–8 (PSE₄₋₈), 8–12 (PSE₈₋₁₂), 60 (PSE₆₀), 120 (PSE₁₂₀), and 1440 (PSE₁₄₄₀) min after the fatiguing sound exposure ended. The hearing of seal F01 was tested 12–16 (PSE₁₂₋₁₆), 16–20 (PSE₁₆₋₂₀), 20–24 (PSE₂₀₋₂₄), 72 (PSE₇₂), 132 (PSE₁₃₂), 252 (PSE₂₅₂), and 1200 (PSE₁₂₀₀) min after the fatiguing sound exposure ended.

TTS was quantified for three hearing test sweeps (centered at 6.5, 9.2, 13 kHz). Sample sizes were chosen to maximize the time available for testing SPLs in which TTS seemed to occur, while minimizing the risk of hearing damage (from repeated exposure to the loudest sounds) and avoiding repeated testing of SPLs for which it was clear without analysis that TTS did not occur. As a precaution, sound exposure tests were not conducted on days after the seals had been exposed to the three highest SPLs.

Control tests were conducted in the same way as sound exposure tests, but without fatiguing sound exposure. Each control test started with a pre-exposure hearing test session (test signals centered at 6.5, 9.2, 13 kHz), but was followed by exposure to the normal (very low) ambient noise in the outdoor pool for 60 min (Fig. 1). The post-ambient exposure (PAE) hearing test session was divided into four periods: 1–4 (PAE₁₋₄), 4–8 (PAE₄₋₈), 8–12 (PAE₈₋₁₂), and 60 (PAE₆₀) min after ambient noise exposure for seal F02, and 12–16 (PAE₁₂₋₁₆), 16–20 (PAE₁₆₋₂₀), 20–24 (PAE₂₀₋₂₄), and

72 (PAE₇₂) min after ambient noise exposure for seal F01. The PAE test sessions had the same durations as pre-exposure hearing test sessions. Control tests were randomly dispersed during the study period among the fatiguing sound exposure tests, and also started at around 0830 h. Data were collected between July 2014 and March 2017.

D. Data collection and analysis

The percentage of time that the seals spent with their heads at the water surface (nose in the air, mandible and lower part of skull under water) and with their head completely submerged was quantified from the video recordings, by using stopwatches. Because this analysis was very time-consuming it was conducted only on a sample of the sessions; for each SPL_{av.re}, one to nine 60 min fatiguing sound exposures were randomly selected for analysis (the number varied, as the number of times each SPL_{av.re} was tested varied). The seals could not always be distinguished from each other from the video recordings, so the data were pooled. Via a grid on the monitor screen, the pool was divided into 34 approximately 1.2 × 1.3 m rectangles. The grid location of the seals was recorded every 5 s.

The pre-exposure mean 50% hearing threshold for each hearing test session (PE_{50%}) was determined by calculating the mean SPL of all reversal pairs in the pre-exposure hearing session. Only pre-exposure sessions with at least 10 reversals were included in the analysis. The TTS quantified in seal F02 1–4 min after sound exposure stopped (TTS_{1–4}), for each hearing test frequency, was calculated by subtracting the mean 50% hearing threshold obtained during the PE_{50%} from the mean 50% hearing thresholds during PSE_{1–4}. A similar method was used to calculate TTS_{12–16} for seal F01.

We define the onset of TTS as occurring at the lowest SEL_{cum} at which a statistically significant difference could be detected between the hearing threshold shift due to the fatiguing sound exposures and the hearing threshold shift as measured after the control exposures (this shift was close to zero). When the sample size was 1 or 2, we compared the hearing threshold to the control level without statistical analysis in order to judge whether TTS occurred or not. For larger sample sizes, the level of significance was established by conducting a one-way analysis of variance (ANOVA) on the TTS, separately for each seal and for each hearing test frequency, with the factor SPL (including zero as the control). When the ANOVA produced a significant value overall, the levels were compared to the control by means of Dunnett multiple comparisons. All analysis was conducted in Minitab 18, and data conformed to the assumptions of the tests used (Zar, 1999).

III. RESULTS

A. Swimming pattern

During the 60 min control sessions (only ambient noise), the seals spent ~20% of their time with their heads at the water surface, and thus ~80% of their time with their heads completely submerged. On a few occasions per session they raised their heads fully above the water surface for up to 2 s. During 60 min exposures to the continuous 6.5 kHz tone, the

TABLE I. The mean percentage of time the two harbor seals spent with their heads at the water surface during control sessions with ambient noise only, and during exposure to a continuous 6.5 kHz tone at 7 SELs; SD = standard deviation. The remainder of the time was spent with their heads completely submerged.

Exposure SEL (dB re 1 $\mu\text{Pa}^2\text{s}$)	Mean % of time with head at the water surface	SD	n
0 (control)	20	11	9
159	28	—	1
165	28	4	4
171	27	6	9
177	28	5	8
183	31	6	9
189	42	8	9
195	39	11	7

time the seals spent with their heads at the water surface depended on the SEL (Table I). They spent ~30% of their time with their heads at the water surface when the SEL = 159–183 dB re 1 $\mu\text{Pa}^2\text{s}$. At and above 189 dB re 1 $\mu\text{Pa}^2\text{s}$, they spent ~40% of the time with their heads at the water surface. During the control and exposure sessions, the seals used most of the pool, and did not specifically avoid the location of the transducer.

B. Pre-stimulus response rate

After the 60 min sound exposure periods, the seals were always willing to participate in the hearing tests. The pre-stimulus response rates for both signal-present and signal-absent trials (in the latter, the whistle was the stimulus, indicating

TABLE II. The harbor seals' pre-stimulus response rate in hearing test sessions (calculated from responses before the hearing test signal in signal-present trials and responses before the whistle was blown in signal-absent trials) during the pre-exposure period, after exposure for 60 min to a continuous (100% duty cycle) 6.5 kHz tone (the fatiguing sound), and after exposure for 60 min to low-amplitude ambient noise (control). All exposure SPLs and hearing test frequencies were pooled for the calculation of percentages. Seal F02's hearing tests began immediately after the end of the exposure periods (fatiguing sound or control), whereas seal F01's hearing tests began 12 min after the end of the exposure periods. Sample size = the number of trials within hearing tests. PSE = post sound exposure. PAE = post ambient noise exposure.

Pre-stimulus response rate					
Seal F02					
Fatiguing sound	Pre-exposure	PSE _{1–4}	PSE _{4–8}	PSE _{8–12}	PSE ₆₀
	6.6%	6.3%	8.4%	6.1%	5.3%
Sample size	1763	727	742	686	1628
Control	Pre-exposure	PAE _{1–4}	PAE _{4–8}	PAE _{8–12}	PAE ₆₀
	6.5%	10.3%	7.4%	13.5%	5.6%
Sample size	492	224	216	200	497
Seal F01					
Fatiguing sound	Pre-exposure	PSE _{12–16}	PSE _{16–20}	PSE _{20–24}	PSE ₇₂
	4.7%	4.2%	5.4%	5.0%	3.5%
Sample size	1630	734	687	700	1371
Control	Pre-exposure	PAE _{12–16}	PAE _{16–20}	PAE _{20–24}	PAE ₇₂
	4.7%	6.9%	5.7%	5.2%	3.4%
Sample size	473	202	194	194	435

TABLE III. Mean ($\pm$ SD) initial TTS (in dB; TTS₁₋₄ in harbor seal F02 and TTS₁₂₋₁₆ in seal F01) after exposure to a continuous 6.5 kHz tone at several SELs for 60 min, quantified at hearing frequencies 6.5, 9.2, and 13 kHz (i.e., at 0, 0.5, and 1 octave above the frequency of the fatiguing sound). Results from the control sessions are shown in bold (no TTS occurred); n = sample size, * TTS significantly different from control value, ^{NS} TTS not significantly different from control value, ^{AS} (assumed similar): no analysis carried out, but TTS assumed to be similar to control value, and ^{AD} (assumed different): no analysis carried out, but TTS assumed to be different to control value.

SEL	Hearing frequency	Seal F02, TTS ₁₋₄ (in dB)				Seal F01, TTS ₁₂₋₁₆ (in dB)			
		Mean	$\pm$ SD	Range	n	Mean	$\pm$ SD	Range	n
dB re 1 μ Pa ² s	kHz	TTS ₁₋₄				TTS ₁₂₋₁₆			
Control	6.5	-0.6	1.5	-2.3-1.6	6	0.3	1.8	-2.0-2.9	7
159	6.5	1.5 ^{AS}	1.1	0.7-2.3	2	0.5 ^{AS}	—	0	1
165	6.5	1.9 ^{NS}	1.9	0.2-4.2	4	4.0*	1.3	2.4-5.6	4
171	6.5	1.6 ^{NS}	1.9	-1.3-3.8	8	4.5*	1.8	2.4-6.7	7
177	6.5	4.3*	1.3	2.4-5.8	7	4.7*	1.3	3.1-6.2	5
183	6.5	3.9*	0.8	2.5-4.7	6	5.7*	2.4	2.4-9.2	7
189	6.5	5.6*	1.3	3.8-7.3	7	4.7*	0.7	3.8-5.6	6
195	6.5	6.5*	2.7	2.9-10.4	5	8.9*	2.3	4.7-11.7	6
Control	9.2	0.4	1.3	-1.9-2.4	8	0.5	1.5	-2.4-1.9	8
171	9.2	1.6 ^{NS}	1.9	-1.4-3.6	7	0.4 ^{NS}	2.0	-2.6-3.7	7
177	9.2	2.3 ^{NS}	0.9	1.0-3.4	7	1.6 ^{NS}	3.0	-3.1-5.8	7
183	9.2	9.8*	3.3	5.9-15.4	6	4.2*	1.9	1.8-7.4	7
189	9.2	18.4*	4.6	10.8-23.7	6	8.1*	2.7	3.0-11.4	7
195	9.2	13.9 ^{AD}	1.2	13.0-14.8	2	14.6*	0.0	14.6-14.6	2
Control	13	0.2	1.3	-1.4-2.4	6	-0.1	0.3	-0.5-0.2	4
177	13	0.1 ^{AS}	—	0	1	0.0 ^{AS}	—	0	1
189	13	1.5 ^{AS}	—	0	1	4.4 ^{AD}	—	0	1

a correct signal-absence response) in the pre-exposure, post-exposure, and control hearing tests were similar (Table II).

C. Effect of SEL on TTS and recovery time

The ANOVAs showed that both TTS₁₋₄ (seal F02) and TTS₁₂₋₁₆ (seal F01) were significantly affected by the fatiguing sound's SEL. Comparisons with the control revealed that the statistically significant onset of TTS varied depending on the animal and the hearing test frequency (Table III).

1. Seal F02

With a hearing test signal of 6.5 kHz, statistically significant TTS₁₋₄ occurred in seal F02 after exposure to an SEL of 177 dB re 1 μ Pa²s [Table III; Fig. 3(a)]; hearing recovered within 60 min [Fig. 4(a)]. With a hearing test signal of 9.2 kHz, statistically significant TTS₁₋₄ occurred after exposure to an SEL of 183 dB re 1 μ Pa²s [Table III; Fig. 3(a)]. The rate of increase in TTS with increasing SEL (hearing test signal of 9.2 kHz) was 1.3 dB/dB fatiguing sound between 177 and 189 dB SEL; mean TTS after exposure to 195 dB re 1 μ Pa²s was 4.5 dB lower than after exposure to 189 dB re 1 μ Pa²s (Table III). Recovery of hearing occurred within 60 min for exposures up to an SEL of 177 dB re 1 μ Pa²s, and within 24 h (1440 min) for SELs of 183 and 195 dB re 1 μ Pa²s [Fig. 4(b)]. Hearing took more than 24 h (1440 min) to recover after exposure to 189 dB re 1 μ Pa²s. The exact recovery time is not known, as hearing was not measured between 24 and 48 h after exposure, but 48 h after exposure, hearing had recovered [not shown in Fig. 4(b)]. Hearing recovery rates at 9.2 kHz varied between 0.05 and 0.13 dB/min. With a hearing test signal of 13 kHz, no TTS₁₋₄

FIG. 3. (Color online) TTS₁₋₄ in seal F02 (a) and TTS₁₂₋₁₆ in seal F01 (b) after exposure for 60 min to a continuous tone of 6.5 kHz at several SELs, quantified at hearing frequencies 6.5, 9.2, and 13 kHz (i.e., at 0, 0.5, and 1 octave above the frequency of the fatiguing sound). Sample size (n) varies per data point shown. For SPL (dB re 1 μ Pa), subtract 36 dB re 1 s from the SEL values. For control values, see Figs. 4 and 5.

FIG. 4. (Color online) Recovery of the hearing of seal F02 at 6.5 kHz (a), 9.2 kHz (b), and 13 kHz (c), after exposure to a continuous 6.5 kHz tone for 60 min at several SELs. Mean TTSs are shown. For sample sizes and SDs (only for TTS₁₋₄), see Table III and Fig. 3. For average received SPLs (dB re 1 μ Pa), subtract 36 dB re 1 s from the SEL values. Also shown are the TTS values during control sessions.

appeared to occur even after exposure to an SEL of 189 dB re 1 μ Pa²s [Table III; Fig. 3(a)]. The control sessions showed that the hearing thresholds for all three hearing test signals before and after 60 min exposure to low ambient noise were very similar (Fig. 4, Table III).

2. Seal F01

With a hearing test signal of 6.5 kHz, statistically significant TTS₁₂₋₁₆ occurred in seal F01 after exposure to an SEL of 165 dB re 1 μ Pa²s [Table III; Fig. 3(b)]; hearing recovered within 2 h [Fig. 5(a)]. With a hearing test signal of 9.2 kHz, statistically significant TTS₁₂₋₁₆ occurred after exposure to an SEL of 183 dB re 1 μ Pa²s [Table III; Fig. 3(b)]. Recovery of hearing occurred within 120 min for exposures up to SELs of 183 dB re 1 μ Pa²s, within 240 min for an SEL of 189 dB re 1 μ Pa²s and within 24 h (1440 min) for an SEL of 195 dB re 1 μ Pa²s [Fig. 5(b)]. Her hearing recovery rate at 9.2 kHz was around 0.3 dB/min. With a hearing test signal of 13 kHz, TTS₁₂₋₁₆ appeared to occur at an SEL of 189 dB re 1 μ Pa²s [Table II; Fig. 3(b)]. Recovery of hearing was within 12 min. The control sessions showed that the hearing thresholds for all three hearing test signals before and after 60 min exposure to low ambient noise were very similar (Fig. 5, Table III).

FIG. 5. (Color online) Recovery of the hearing of seal F01 at 6.5 kHz (a), 9.2 kHz (b), and 13 kHz (c), after exposure to a continuous 6.5 kHz tone for 60 min at several SELs. Mean TTSs are shown. For sample sizes and SDs (TTS₁₂₋₁₆ only), see Table III and Fig. 3(b). For average received SPLs (dB re 1 μ Pa), subtract 36 dB re 1 s from the SEL values. Also shown are the TTS values during control sessions.

IV. DISCUSSION AND CONCLUSIONS

A. Evaluation

The animals in the present study had normal hearing for young female harbor seals (Kastelein *et al.*, 2018a). The pre-exposure hearing thresholds found in the present study for the three hearing test signals were within a few dB of the hearing thresholds measured in these harbor seals for tonal signals during two previous studies (Kastelein *et al.*, 2009a; Kastelein *et al.*, 2010b).

Although the hearing of seal F02 was always tested before the hearing of seal F01, the sound-induced TTS can

TABLE IV. Comparison of mean TTS measured at 9.2 kHz in seal F02 8–12 min after the fatiguing sound stopped, and in seal F01 12–16 min after the fatiguing sound stopped.

SEL dB re 1 μ Pa ² s	TTS ₈₋₁₂ in seal F02 dB	TTS ₁₂₋₁₆ in seal F01 dB
183	8	4
189	13	8
195	8	15

be compared. The closest possible comparison is of TTS in seal F02 8–12 min and in seal F01 12–16 min after the fatiguing sound stopped. Comparing the recovery of both animals with hearing test signals of 9.2 kHz after exposure to the three highest SELs [Figs. 4(b) and 5(b) and Table IV] shows that the TTS was lower in seal F01 than in seal F02 in two cases (as expected, as seal F02's hearing had had more time to recover). However, at the highest exposure level, TTS was 7 dB higher in seal F01 than in seal F02. Studies on humans and other terrestrial mammals show large individual, genetic and population-level differences in susceptibility to TTS (Kylin, 1960; Kryter *et al.*, 1962; Henderson *et al.*, 1993; Davis *et al.*, 2003; Spankovich *et al.*, 2014). Therefore, further replication with more harbor seals is needed to assess the generality of the results obtained.

The mean pre-stimulus response rates in hearing tests before and after the sound exposures and during the control sessions were of similar magnitudes in both animals (Table II). This indicates that the seals used the same decision criteria in all conditions, and that their decision-making process in the post exposure hearing tests was not influenced by the sound exposure.

No change in the seals' susceptibility to TTS was observed over time during the present study. The TTSs were less severe than those elicited in studies on terrestrial mammals in which susceptibility to TTS did change due to conditioning (Kujawa and Liberman, 1997; Mannström *et al.*, 2015).

No aerial sound was projected during exposure sessions, based on results from a noise-band study in which the same TTS₁₋₄ was found in harbor seals with and without aerial fatiguing sound in addition to the same underwater fatiguing sound (Kastelein *et al.*, 2012). Harbor seals' hearing thresholds for underwater sounds are the same when they are swimming with their heads at the water surface (nose in the air, mandible and lower part of skull under water) as when they are fully submerged (Kastelein *et al.*, 2018a). However, the seals in the present study spent more time swimming at the water surface during exposure to the 6.5 kHz continuous tone than during the control periods. In addition, the percentage of time swimming at the water surface increased at the two highest SPLs (Table I). A similar phenomenon was seen in the same seals in response to pile driving sounds (Kastelein *et al.*, 2018b). The animals in the present study may have spent time with their heads at the water surface in an attempt to reduce the received level.

Significant TTS as reported in the present study means that statistically significant TTS occurred in the hearing of the study animals (relative to the control sessions). This is not the same as ecologically significant TTS, and it is unclear what the specific ecological effect of TTS is. The ecological effect certainly depends on the magnitude of the TTS, the duration of the exposure (because TTS can begin to occur during exposure), the duration of the recovery of hearing, and the affected hearing frequency. Reduced hearing in seals may reduce the efficiency of ecologically important activities such as navigation, communication, foraging, and predator avoidance, thus potentially reducing their fitness, reproductive output and longevity.

B. Recovery

There were differences in recovery rates of hearing between the two seals. Seal F02 showed hearing recovery rates varying between 0.05 and 0.13 dB/min. Seal F01 showed a much stronger initial recovery (0.3 dB/min) than seal F02. All TTSs up to 6 dB recovered within 2 h, most within 1 h. In seal F02, TTS of around 19 dB recovered within 24 h. The results of the present study and studies by Kastelein *et al.* (2012) and Kastelein *et al.* (2018b) suggest that in harbor seals, similar TTSs, caused by different fatiguing sounds (one octave continuous noise band, intermittent impulsive sounds, and a CW) with different exposure levels and exposure times, require similar recovery times.

C. Hearing frequency most affected

The results from the present study show that, after they were exposed to a 6.5 kHz CW, the harbor seals suffered TTSs at 6.5, 9.2, and 13 kHz, and that the hearing frequency at which the greatest TTS occurred depended on the SPL, and the most affected frequency at high SPLs was half an octave higher (9.2 kHz) than the center frequency of the fatiguing sound (6.5 kHz). TTS research in terrestrial mammals also suggests that the maximum TTS is induced half an octave above the fatiguing sound's frequency (Cody and Johnstone, 1981; McFadden and Plattsmier, 1983; McFadden, 1986), and that the magnitude of TTS induced at frequencies higher than the fatiguing sound's frequency is related to the SPL of the fatiguing sound. Cody and Johnstone (1981) found TTS/SPL curves for guinea pigs to be much steeper for frequencies ≥ 0.5 octave above the fatiguing sound's frequency than for frequencies < 0.5 octave above that of the fatiguing sound.

In marine mammals, the relationship between received SPL and affected hearing frequency has so far only been studied in one odontocete species (Kastelein *et al.*, 2015a); after exposure for 60 min to a 6.5 kHz continuous tone (the same fatiguing sound used in the present study) at SPL_{av.re.} ranging from 118 to 154 dB re 1 μ Pa, the TTSs of a harbor porpoise were quantified at the center frequency (6.5 kHz), at 0.5 (9.2 kHz), 1.0 (13 kHz), and 1.3 octaves (16 kHz) above the center frequency of the fatiguing sound. As in the harbor seals, the hearing frequency at which the maximum TTS occurred in the harbor porpoise depended on the SPL_{av.re.} The higher the SPL_{av.re.}, the higher the TTS induced at frequencies higher than the frequency of the fatiguing sound; below 148 dB re 1 μ Pa, the maximum TTS was at 6.5 kHz, whereas above 148 dB re 1 μ Pa, the maximum TTS occurred at 9.2 kHz.

The results from the previous studies should be taken into consideration when interpreting studies of TTS in pinnipeds (Finneran *et al.*, 2003; Kastak *et al.*, 1999; Kastak *et al.*, 2005, 2007; Kastak and Schusterman, 1996; Kastelein *et al.*, 2012; Kastelein *et al.*, 2013a; Kastelein *et al.*, 2015b). In some studies, hearing may have been quantified at a frequency that did not show the maximum TTS elicited by the fatiguing sound. When TTS data from previous studies are used for regulatory purposes, the received SPL should be

taken into account to determine the affected hearing frequency, to evaluate the potential effect of anthropogenic sounds on pinniped hearing, and to assess the ecological impact of the sounds.

D. Susceptibility to TTS in relation to the frequency of the fatiguing sound

Based on the presently available data on TTS caused by underwater sound in harbor seals (Kastak *et al.*, 2005, Kastelein *et al.*, 2012, and the present study), their susceptibility to TTS in the fatiguing sound frequency range tested (2.5–6.5 kHz) varies little with hearing frequency (Fig. 6). Kastak *et al.* (2005) did not specify how soon after the sound (octave-band noise centered at 2500 Hz) stopped, or for how long, they tested the hearing (at 2500 Hz) of the seal, so recovery before and during the post-exposure hearing tests cannot be evaluated, but in the study by Kastelein *et al.* (2012) and in the present study (using the same seals), the hearing measurement moments and periods were precisely specified. In the present study, the SEL_{cum} required to elicit 6 dB TTS 1–4 min after the sound exposure stopped at 9.2 kHz was ~180 dB re 1 $\mu\text{Pa}^2\text{s}$ in seal F02 [Fig. 3(a)]; for 6 dB TTS 12–16 min after the sound exposure stopped (thus after eight more minutes of recovery) it was ~186 dB re 1 $\mu\text{Pa}^2\text{s}$ in seal F01 [Fig. 3(b)]. Thus, the two harbor seals in the present study seem to have similar susceptibility to TTS in the frequency range within which their hearing was tested, and the seal studied by Kastak *et al.* (2005) also appears to have similar susceptibility to TTS in this range. In the frequency range in which TTS has been tested in harbor seals, the hearing thresholds of the seals are very similar (Kastelein *et al.*, 2009a, 2009b; Fig. 6). More fatiguing sound frequencies need to be tested in harbor seals in order to produce equal TTS curves, on which weighting functions can be based that can be used to set safety criteria for broadband sounds in the marine environment (Houser *et al.*, 2017).

FIG. 6. The SEL_{cum} required to cause 6 dB TTS in harbor seals after exposure to octave-band noise (OBN) centered at 2.5 kHz (Kastak *et al.*, 2005, ■ no. 1, male), OBN centered at 4 kHz (Kastelein *et al.*, 2012, ○ no. 2: F01 measured 1–4 min after sound stopped, ▲ no. 3: F02 measured 12–16 min after the sound stopped), and the CW of 6.5 kHz (present study, no. 4: F02 measured 1–4 min after the sound stopped, no. 5: F01 measured 12–16 min after the sound stopped). Also shown is the mean audiogram of the seals of the present study for tonal signals (right-hand y-axis, dashed line; Kastelein *et al.*, 2009a).

E. Comparison of susceptibility to TTS in phocids and odontocetes

In a study on TTS in a harbor porpoise, Kastelein *et al.* (2014b) used the same sound (6.5 kHz CW) and methodology as used in the present study, so these studies can be compared directly. At 6.5 kHz, the porpoise showed TTS_{1–4} onset (defined as 6 dB TTS) at 158 dB SEL, and harbor seal F02 at 189 dB SEL. At 9.2 kHz, the porpoise showed TTS_{1–4} onset at 176 dB SEL and seal 02 at 180 dB. Thus it appears (based on very small sample sizes) that the harbor porpoise is more susceptible to TTS (i.e., TTS occurs at lower SELs) due to a 6.5 kHz CW than the harbor seal. A similar difference in susceptibility to TTS between odontocetes and pinnipeds was shown in a review of TTS and PTS literature by Finneran (2015). The SEL at which the most affected hearing frequency changed from the center frequency of the fatiguing sound to half an octave above it was 184 dB SEL in the harbor porpoise and 179 dB SEL in harbor seal F02. This is a small difference, taking into account individual differences in susceptibility to TTS that are likely to occur in both species.

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