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Temporary Hearing Threshold Shift in California Sea Lions (*Zalophus californianus*) Due to One-Sixth-Octave Noise Bands Centered at 0.6 and 1 kHz

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Abstract

To determine the frequency-dependent susceptibility of California sea lions (*Zalophus californianus*) to noise-induced temporary hearing threshold shift (TTS), one of two subjects were exposed for 60 minutes to two continuous one-sixth-octave noise bands (NBs) as fatiguing sounds: one centered at 0.6 kHz, at sound pressure levels (SPLs) of 168 to 174 dB re 1 µPa (sound exposure levels [SELs] of 204 to 210 dB re 1 µPa^{2}s), or one centered at 1 kHz, at SPLs of 144 to 159 dB re 1 µPa (SELs of 180 to 195 dB re 1 µPa^{2}s). Using a psychoacoustic technique, TTSs were quantified at 0.6, 0.85, 1, 1.2, 1.4, and 2 kHz (at the center frequency of each NB, half an octave higher, and one octave higher). When significant TTS occurred, higher SELs resulted in greater TTSs. In the sea lion that was tested 1 to 4 minutes after exposure to the fatiguing sounds, the largest TTSs occurred when the hearing test frequency was half an octave higher than the center frequency of the two fatiguing sounds. The highest TTS levels elicited were 8.7 dB at 0.85 kHz and 9.6 dB at 1.4 kHz. When their hearing was tested at the same time after the fatiguing sounds stopped, initial TTSs and hearing recovery patterns were similar in both sea lions. These findings will contribute to the protection of hearing of species in the Otariidae family from anthropogenic noise by facilitating the development of an evidence-based underwater sound weighting function.

Key Words: anthropogenic noise, audiogram, auditory weighting, fatiguing sound, hearing damage, hearing recovery, hearing sensitivity, Otariidae, pinniped, TTS

Introduction

Underwater anthropogenic noise in the oceans has had a wide variety of adverse effects on marine animals (Duarte et al., 2021). Among these, loud sounds of sufficient duration can result in short-term reduced hearing sensitivity (temporary threshold shift [TTS]) in the listener. Reduced hearing sensitivity could result in an inability to detect biologically important sounds and may have population-level consequences for animals that are regularly exposed to loud sounds.

The California sea lion (*Zalophus californianus*) is a species of the Otariidae family (eared seals) that occurs year-round along the west coast of North America (Melin et al., 2018). In some of these coastal waters, California sea lions are subjected to significant levels of noise resulting from anthropogenic activities. Understanding the parameters of sounds that cause TTS and the consequences for California sea lions experiencing TTS will help regulatory agencies determine safe and acceptable noise exposure levels for this species and perhaps for other species of the Otariidae family (as suggested by Houser et al., 2017, and Southall et al., 2019). TTS in California sea lions has previously been studied by Kastak et al. (1999, 2005), Finneran et al. (2003), and Kastelein et al. (2021b, 2022). Comparative studies on various taxa will help elucidate general principles associated with TTS in marine mammals (e.g., harbor seals [*Phoca vitulina*] and harbor porpoises [*Phocoena phocoena*]; Kastelein et al., 2012a, 2012b).

Both within and between species, the causes and effects of TTS, and recovery times, are diverse. Fatiguing sounds of different sound pressure levels (SPLs) and durations often result in a mix of levels of reduced hearing sensitivity at different frequencies, and recovery times also vary (Popov et al., 2014; Finneran, 2015; Kastelein et al., 2020, 2021a, 2021b). The present study is one of four in a comprehensive research project on TTS in California sea lions. Each of the four studies reports on TTS caused by two fatiguing sound frequencies as follows: 0.6 and 1 kHz (present study), 2 and 4 kHz (Kastelein et al., 2021b), 8 and 16 kHz (Kastelein et al., 2022), and 32 and 40 kHz (ongoing study).

The goals of the present study are the following: (1) to quantify TTS in two California sea lions and determine the TTS-onset sound exposure level (SEL) after exposure to fatiguing sounds with center frequencies of 0.6 and 1 kHz at several SELs; (2) to determine how three different hearing frequencies (corresponding to the center frequency of each NB, half an octave higher, and one octave higher) are affected by exposure to the fatiguing sounds at each SEL; (3) to describe the pattern of hearing recovery after the fatiguing sounds stop; and (4) to assess differences in susceptibility to TTS between the two California sea lions.

Methods

A condensed version of the methods is presented herein. The subjects, study area, acoustics, experimental procedures, and data analyses are described in more detail by Kastelein et al. (2021b, 2022).

Subjects and Study Area

The subjects were an adult female California sea lion (hereafter F01) and her juvenile male offspring (hereafter M02). Both sea lions were healthy throughout the study. The subjects had hearing thresholds similar to each other and to three of the five other California sea lions for which the hearing has been tested (Schusterman et al., 1972; Kastak & Schusterman, 1998; Southall et al., 2005; Mulsow et al., 2012; Reichmuth & Southall, 2012; Reichmuth et al., 2013).

The study was conducted at the SEAMARCO Research Institute, the Netherlands, in a remote and quiet location. The sea lions were kept, and the study was conducted, in a pool complex consisting of an outdoor pool (7 × 4 m, 2 m deep) with a haul-out area above part of the pool, connected via two channels (each 2 × 2 m, 1 m deep) to an indoor pool. The indoor pool consisted of a deep part (6 × 4 m, 2 m deep) where the sea lions were kept during the sound exposures and where the hearing tests were conducted, and a shallow part (6 × 3 m, 1 m deep) where the transducers for the fatiguing sounds were placed (three for the 0.6 kHz fatiguing sound and one for the 1 kHz fatiguing sound; see Kastelein et al., 2021b, for a top view of the pool complex). During sound exposure and control sessions, both sea lions were confined to the deep part of the indoor pool and could not leave the water. During the hearing tests, the sea lion not being tested was kept in the outdoor pool.

Acoustics

Sound Pressure Level Measurement—The ambient noise was measured, and the fatiguing sound (in air and underwater) and hearing test signals were calibrated once every 3 months during the study period by an acoustic consulting agency (TNO, the Hague, the Netherlands).

Background Noise—The sea lions' listening environment was kept as quiet as possible while their hearing thresholds were being measured. The ambient noise in the indoor pool was very low and fairly constant in SPL under test conditions (Figure 1). Test conditions were as follows: (1) water circulation system turned off at least half an hour before the first hearing test was conducted; (2) no rain; (3) generally wind force Beaufort ≤ 4, depending on the wind direction; and (4) only researchers involved in the hearing tests within 15 m of the pool complex, and those researchers standing still.

Fatiguing Sounds—Digitally generated continuous (i.e., 100% duty cycle) one-sixth-octave noise bands (NBs) centered at 0.6 or 1 kHz, without harmonics, were used as fatiguing sounds (i.e., sounds intended to cause TTS; see Kastelein et al., 2021b, for details of equipment and settings). The sound-generating equipment could not produce the fatiguing sound at a suitable SPL at

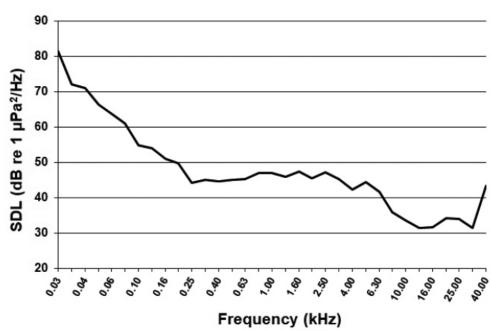


Figure 1. The underwater ambient noise level in the indoor pool used for California sea lion (*Zalophus californianus*) hearing tests under test conditions. Measurements were recorded as one-third-octave bands and converted to spectrum density levels (SDLs).

0.5 kHz (one octave below 1 kHz), so 0.6 kHz was used as the base frequency for the lower frequency measures.

To produce the NB at 0.6 kHz at a sufficient SPL to elicit at least 6 dB TTS in the sea lions, the sound from the sound-generating chain was split to three power amplifiers (a SYNQ amplifier, Model DIGIT 3K6, SYNQ Audio Research India Pvt. Ltd, Telangana, India; an HQ amplifier, Model VPA2900MB, Velleman, Gavere, Belgium; and an HQ amplifier, Model VPA2200MBN, Velleman). Each power amplifier drove one of three transducers (Model 1424 HP; Lubell Labs, Columbus, OH, USA) each via an isolation transformer (Model AC1424HP; Lubell Labs).

The NB at 1 kHz was projected via one power amplifier (HQ amplifier, Model VPA2200MBN; Velleman) which drove one transducer (Model LL1424HP; Lubell Labs) through an isolation transformer (Model AC1424HP; Lubell Labs). The transducers were suspended in the shallow part of the indoor pool at 1 m depth, 10 cm above the pool floor (see Figure 2 for the approximate transducer locations relative to the deep part of the pool). The linearity of the transmitter system producing the fatiguing sound was checked during each calibration and was consistent to 1 dB within a 42 dB range (overlapping the SPL range used in this study).

To quantify the distribution of the fatiguing sounds in the deep part of the indoor pool (where the sea lions were during exposure and control sessions), the SPL was measured at 42 points (Figure 2). The sea lions swam throughout the entire deep part of the indoor pool at all depths when the fatiguing sound was being projected. Therefore, the average fatiguing SPL experienced by the sea lions was calculated as the energetic average of the SPL at all 42 individual measurement points. SPL varied little with depth or location, resulting in a very homogeneous sound field for both fatiguing sounds (Figure 2).

During sound exposure sessions, the one-sixth-octave NB centered at 0.6 kHz was projected for 60 min at three source levels, resulting in mean SPLs ranging from 168 to 174 dB re 1 µPa (SEL range: 204 to 210 dB re 1 µPa^{2}s). The one-sixth-octave NB centered at 1 kHz was projected for 60 min at four source levels, resulting in mean SPLs ranging from 144 to 159 dB re 1 µPa (SEL range: 180 to 195 dB re 1 µPa^{2}s). These were the highest SPLs that could be generated without distortion or harmonics.

When the fatiguing sounds were being generated, the sea lions mostly took single, short breaths while lifting only their noses out of the water. On a few occasions when the sea lions jumped, their heads were completely out of the water for < 1 s

a) 0.5 m depth (0.6 kHz)

	1	2	3	4	5
1	*178*	*177*	*175*	*175*	*172*
2	*174*	*175*	*174*	*164*	*168*
3	*170*	*175*	*179*	*174*	

b) 1.0 m depth

	1	2	3	4	5
	T			TT	
1	*178*	*178*	*176*	*175*	*172*
2	*176*	*177*	*175*	*165*	*171*
3	*172*	*176*	*180*	*176*	

c) 1.5 m depth

	1	2	3	4	5
1	*172*	*172*	*170*	*169*	*166*
2	*172*	*173*	*170*	*162*	*167*
3	*167*	*171*	*175*	*171*	

d) 0.5 m depth (1 kHz)

	1	2	3	4	5
1	*153*	*150*	*151*	*156*	*156*
2	*154*	*150*	*148*	*147*	*151*
3	*149*	*150*	*150*	*149*	

e) 1.0 m depth

	1	2	3	4	5
				T	
1	*154*	*151*	*155*	*156*	*156*
2	*154*	*154*	*156*	*155*	*155*
3	*151*	*151*	*155*	*155*	

f) 1.5 m depth

	1	2	3	4	5
1	*151*	*145*	*152*	*151*	*152*
2	*150*	*151*	*154*	*153*	*154*
3	*145*	*147*	*151*	*152*	

Figure 2. Examples of the sound pressure level (SPL) distribution (values in dB re 1 µPa) in the deep part of the indoor pool (6 × 4 m, 2 m deep; not to scale) when the fatiguing sounds were being projected. These were continuous one-sixth-octave noise bands (NBs) centered at 0.6 kHz (a-c) and 1 kHz (d-f). Measurements were taken at 14 locations on a horizontal grid with cells of 1 × 1 m (the outer hydrophone locations were 1 m from the pool wall), at three depths per grid cell. Per location, the SPL did not vary systematically with depth, and there was no sound gradient in the pool. These data were used to calculate the average received SPL that the sea lions experienced during sound exposures. In this example, the mean (± standard deviation [SD]) SPL for 0.6 kHz (a-c) was 174 ± 4 dB re 1 µPa (n = 42); for 1 kHz (d-f), it was 153 ± 2.9 dB re 1 µPa (n = 42). A letter **T** above a box in (b) and (e) indicates the approximate location of a transducer (at 1 m depth) in the adjacent shallow part of the indoor pool (three transducers were used to generate the fatiguing sound at 0.6 kHz; see [b]). The grey area indicates the location of the hearing test signal transducer and baffleboard; this part of the pool could not be accessed by the sea lions (see Kastelein et al., 2021b, for a drawing of the pool to scale).

during sound exposure sessions. The aerial SPL was measured in two locations, while the NBs at 0.6 and 1 kHz were being projected underwater at each of the levels used. Aerial SPL varied by at most 1 dB between the two measurement locations, so the mean of the two measurements was used to represent the aerial SPL that the sea lions were exposed to while their heads were completely out of the water (see Tables 1 & 2 in the "Results" section).

Before each sound exposure test (see "Experimental Procedures"), the voltage output of the emitting system to the transducer and the voltage output of the sound-receiving (monitoring) system were checked for consistency. If the values were the same as those obtained during

SPL calibrations, the sound exposure test was performed.

Hearing Test Signals—The sea lions were trained to detect signals presented during hearing tests before and after exposure to the fatiguing sound. Narrowband upsweeps (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 & Schlundt, 2007).

The hearing thresholds (staircase method; 50% correct detection levels) were tested at the frequency of each fatiguing sound, half an octave higher, and one octave higher. Thus, for the NB at 0.6 kHz, the hearing test frequencies were 0.6, 0.85, and 1.2 kHz; for the NB at 1 kHz, the hearing test frequencies were 1, 1.4, and 2 kHz. The hearing test signals were generated digitally using the software *Adobe Audition*, Version 3.0 (Adobe Inc., San Jose, CA, USA). The linear upsweeps started and ended at ± 2.5% of the center frequency and had durations of 1,000 ms, including a linear rise and fall in SPL of 50 ms.

The WAV files used as hearing test signals were projected into the pool using equipment described by Kastelein et al. (2021b). The output drove, for the 0.6, 0.85, and 1.2 kHz hearing test signals, a balanced tonpilz piezoelectric acoustic transducer (Model LL916; Lubell Labs) through an isolation transformer (Model AC202; Lubell Labs). The 1, 1.4, and 2 kHz hearing test signals were produced by a cylindrical hydrophone (EDO Western, Model 337; EDO Corporation, Salt Lake City, UT, USA).

The free-field received SPL of each hearing test signal was measured at the position of the sea lion's head during the hearing tests. The calibration measurements were conducted with two hydrophones—one at the location of each auditory meatus of a sea lion positioned at the listening station. The linearity of the transmitter system 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 to 3 dB, depending on the test frequency. The mean SPL of the two hydrophones was used to calculate the stimulus level during hearing tests.

Experimental Procedures

A go/no-go, one-up/one-down staircase method (Cornsweet, 1962) was applied with 2 dB steps, producing a 50% correct detection threshold (Levitt, 1971). A switch between a test level and a subsequent level that had been increased or decreased by 2 dB (depending on the sea lion's response) was called a "reversal." For each hearing trial, the signal was produced at a random time 4 to 12 s after a sea lion stationed properly at the listening station, and ~25 trials were conducted in hearing test sessions which lasted up to 12 min.

One total sound exposure test was conducted per day, starting at around 0900 h. A total sound exposure test consisted of (1) a pre-exposure hearing test session, (2) a fatiguing sound exposure, and (3) one or more post-sound exposure (PSE) hearing test sessions. The first PSE hearing test (using the same hearing test signal as used in the pre-exposure hearing test) commenced within 1 min after the fatiguing sound had stopped for the first sea lion to be tested (usually F01), and 12 min after the sound had stopped for the second sea lion to be tested (usually M02). It took less than 1 min for the sea lions to swap places by moving between the indoor and outdoor pools, so testing of the second sea lion could begin without delay.

In addition to the magnitude of TTS immediately after sound exposure, subsequent recovery times were recorded. The numbers associated with the PSE periods are the minutes following the cessation of the fatiguing sound, starting with three consecutive 4-min periods (in the first PSE hearing test). The hearing sensitivity of F01 was tested mostly during up to four PSE periods: 1-4 min (PSE_{1-4}), 4-8 min (PSE_{4-8}), 8-12 min (PSE_{8-12}), and 60 min (PSE_{60}) after the fatiguing sound exposure ended. The hearing sensitivity of M02 was tested mostly 12-16 min (PSE_{12-16}), 16-20 min (PSE_{16-20}), and 20-24 min (PSE_{20-24}) after the fatiguing sound exposure ended. Testing was planned to continue until hearing recovery had taken place. Recovery was defined as a return to < 2 dB TTS.

Control tests were randomly dispersed among the fatiguing sound exposure tests and were conducted in the same way as sound exposure tests but with exposure to low ambient noise instead of fatiguing sound. The post-ambient exposure (PAE) hearing test session was divided into the same three 4-min periods per subject as with the fatiguing sound exposure tests; no PAE tests were conducted beyond those periods.

To investigate individual differences in susceptibility to TTS, the order in which the sea lions were tested was reversed in four sessions for each fatiguing sound. In these sessions, M02 was tested first at 210 dB re 1 μPa^2s dB for the NB at 0.6 kHz (measured with a 0.85 kHz hearing test signal), and then at 195 dB re 1 μPa^2s for the NB at 1 kHz (measured with a 1.4-kHz hearing test signal).

Hearing was tested at three frequencies for each fatiguing sound. If no TTS was found at a certain frequency after exposure to a fatiguing sound with a particular SPL, this frequency was not tested after exposure to lower SPLs. The sample size was generally four (see "Results") for each combination of test parameters (NB, SPL, and hearing frequency). Data for the NB centered at 1 kHz were collected between May and July 2021, and data for the NB centered at 0.6 kHz were collected between August and September 2021.

Data Analysis
To investigate false positives in the data, the mean rate of pre-stimulus responses by the sea lions was calculated as a percentage of the trials in each hearing test period. Both signal-present and signal-absent trials were included in the calculations (in the latter, a whistle indicating the end of the test period was the stimulus; see Kastelein et al., 2021b).

The pre-exposure mean 50% hearing threshold ($PE_{50\%}$) for each test was determined by calculating the mean SPL of all reversal pairs in the pre-exposure hearing test session. TTS_{1-4} (mostly for F01) was calculated by subtracting the $PE_{50\%}$ from the mean 50% hearing threshold during PSE_{1-4}. A similar method was used to calculate TTS_{12-16} (mostly for M02).

We define the onset of TTS as occurring at the lowest SEL at which a statistically significant difference could be detected between the hearing thresholds of the PSE_{1-4} or PSE_{12-16} time periods and the hearing thresholds measured after the control tests (PAE_{1-4} or PAE_{12-16}), both relative to the pre-exposure thresholds. The statistical significance ($p < 0.05$) was established by conducting a one-way ANOVA (or *t* test if there was only one SEL to compare with the control) on the initial TTS, separately for each sea lion and for each hearing test frequency, with the factor SEL (including the control). When the ANOVA produced a significant value overall, the levels were compared to the control by means of Dunnett multiple comparisons. All analyses were conducted in *Minitab 18*, and data conformed to the assumptions of the tests used (for ANOVA, equal variances, normal distribution of data, and residuals; for *t* tests, normal distribution of the data; Zar, 1999). Recovery of hearing and individual differences in susceptibility to TTS are described without formal statistical analysis.

Results

Pre-Stimulus Response Rate
The California sea lions always participated in the hearing tests before and after the 60-min sound exposure and control sessions, and they produced few false positives overall. The pre-stimulus response rates of F01 during the pre- and post-exposure hearing test periods and control tests with NBs at 0.6 kHz varied between 4.3 and 8.7%; with NBs at 1 kHz, they varied between 3.4 and 8.7%. The pre-stimulus response rates by M02 during the pre- and post-exposure hearing test periods and control tests with NBs at 0.6 kHz varied between 0.0 and 7.8%; with NBs at 1 kHz, they varied between 3.2 and 8.1%.

Effect of SEL of the NB at 0.6 kHz on TTS Levels
The one-way ANOVAs and *t* tests to investigate onset of TTS showed that only TTS_{1-4} (F01) with the hearing test frequency at 0.85 kHz was significantly affected by the 0.6-kHz fatiguing sound's SEL (Table 1). No significant TTS_{12-16} occurred in M02 at the TTS-onset SEL for F01 (i.e., 207 dB re 1 µPa²s; Table 1), so M02 was not tested at the lower SEL (204 dB).

Table 1. The mean, standard deviation (SD), and range of initial TTS (TTS_{1-4} in California sea lion [*Zalophus californianus*] F01 and TTS_{12-16} in M02), after 60-min exposures to ambient noise (control) and to a continuous one-sixth-octave noise band centered at 0.6 kHz at several SELs, quantified at hearing frequencies 0.6, 0.85, and 1.2 kHz. Mean underwater SELs (calculated from mean underwater SPLs) and mean aerial SPLs are shown for each underwater SPL. TTS levels were calculated as the differences between pre- and post-exposure hearing thresholds. No TTS occurred during control tests. *n* = sample size; *TTS = significantly different from control value ($p < 0.05$).

Hearing test frequency (kHz)	SPL in water (dB re 1 µPa)	SEL in water (dB re 1 µPa²s)	SPL in air (dB re 20 µPa)	F01 TTS_{1-4} (dB)				M02 TTS_{12-16} (dB)			
				Mean	SD	Range	*n*	Mean	SD	Range	*n*
0.6	Ambient	Control	32	0.6	1.0	-0.5-1.8	4	0.2	0.2	-0.1-0.4	4
	174	210	92	0.4	1.9	-2.0-2.5	4	0.8	0.7	-0.2-1.5	4
0.85	Ambient	Control	32	0.1	1.0	-0.8-1.7	5	-0.2	1.3	-2.1-1.5	5
	168	204	86	0.1	0.9	-0.7-1.4	4	--	--	--	0
	171	207	89	5.3*	1.2	4.3-6.9	4	0.1	1.1	-1.2-1.3	4
	174	210	92	6.7*	0.8	6.0-7.5	4	0.3	0.8	-0.8-1.5	6
1.2	Ambient	Control	32	0.0	1.3	-1.0-1.8	4	0.7	1.5	-1.2-2.3	4
	174	210	92	1.1	1.2	-0.4-2.6	4	0.5	1.4	-0.4-2.6	4

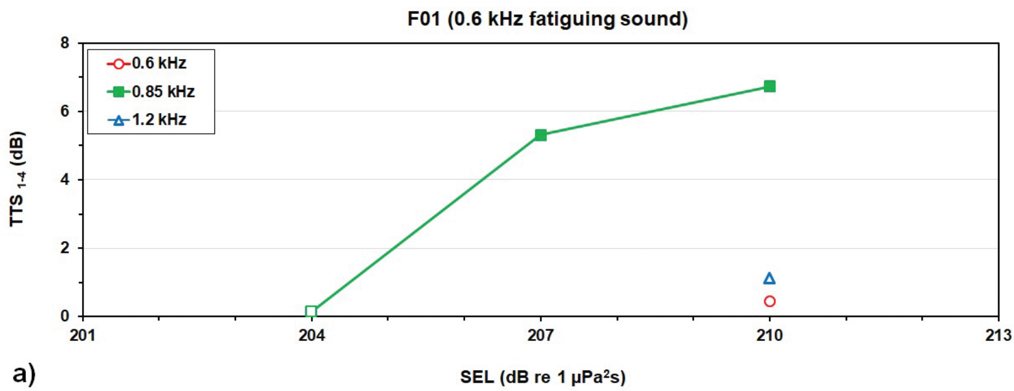
No change in susceptibility to TTS was observed during the study. As expected, the control tests showed that the hearing thresholds for all three hearing test signals before and after 60-min exposure to low ambient noise were very similar (Table 1).

TTS and Recovery After Exposure to the NB at 0.6 kHz

Statistically significant $TTS_{1\text{-}4}$ could not be elicited in F01 with a hearing test signal of 0.6 kHz (Table 1; Figures 3a & 4a). With a hearing test signal of 0.85 kHz, statistically significant $TTS_{1\text{-}4}$ occurred after exposure to SELs of ≥ 207 dB (re 1 µPa²s; Table 1; Figure 3a). Recovery of hearing occurred within 8 min after exposure to an SEL of 207 dB and within 12 min after exposure to an SEL of 210 dB (Figure 4b). With a hearing test signal of 1.2 kHz, no significant $TTS_{1\text{-}4}$ could be elicited (Table 1; Figures 3a & 4c). With all three hearing test signals (0.6, 0.85, and 1.2 kHz), no statistically significant $TTS_{12\text{-}16}$ could be elicited in M02, even after exposure to an SEL of 210 dB (Table 1; Figures 3b & 5).

Individual Differences in TTS After Exposure to the NB at 0.6 kHz

During four sessions, the order in which the sea lions were tested at hearing frequency 0.85 kHz after exposure to the NB at 0.6 kHz (SEL 210 dB re 1 µPa²s) was reversed. The mean $TTS_{1\text{-}4}$ in M02 (8.7 dB, SD = 1.4 dB, n = 4) was 2 dB higher than the mean $TTS_{1\text{-}4}$ in F01 (6.7 dB, SD = 0.8 dB, n = 4) after exposure to the same SEL. The recovery patterns were similar (Figure 6a). The mean $TTS_{12\text{-}16}$



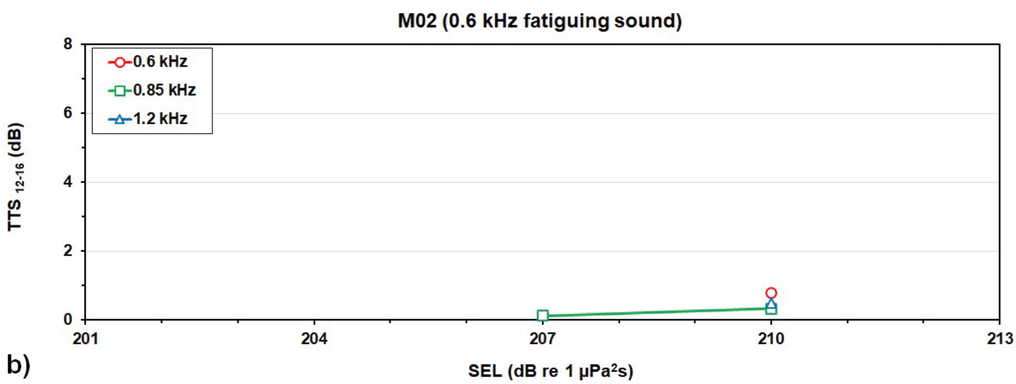


Figure 3. Mean $TTS_{1\text{-}4}$ in F01 (a) and mean $TTS_{12\text{-}16}$ in M02 (b) after 60-min exposure to a continuous one-sixth-octave NB centered at 0.6 kHz, at several SELs, quantified at hearing frequencies 0.6, 0.85, and 1.2 kHz (i.e., at the center frequency of the fatiguing sound, half an octave above it, and one octave above it). Open symbols indicate thresholds similar to those in control tests (no TTS); solid symbols indicate statistically significant TTS relative to the control tests. Sample size varies per data point (see Table 1). For average received SPLs (dB re 1 µPa), subtract 36 dB from the SEL values. For SDs and mean control values, see Table 1 and Figures 4 & 5.

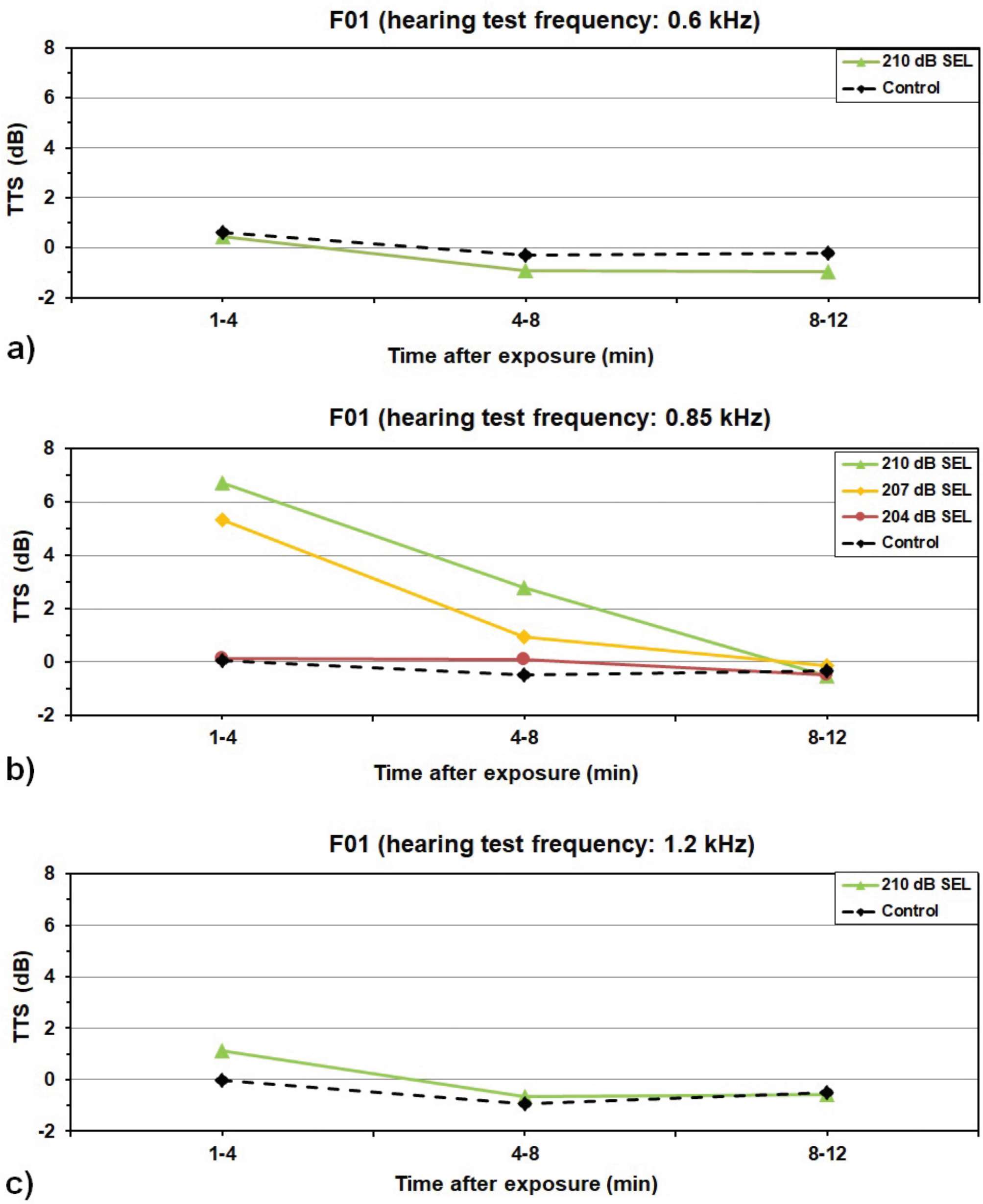


Figure 4. Changes in the mean TTS of F01, including recovery, tested at 0.6 (a), 0.85 (b), and 1.2 kHz (c), after 60-min exposure to a continuous one-sixth-octave NB centered at 0.6 kHz, at several SELs. Hearing was considered recovered once TTS was < 2 dB. For sample sizes and SDs (only for TTS_{1-4}), see Table 1. For average received SPLs (dB re 1 µPa), subtract 36 dB from the SEL values. The mean "TTS" values during control tests (no shifts occurred) are also shown.

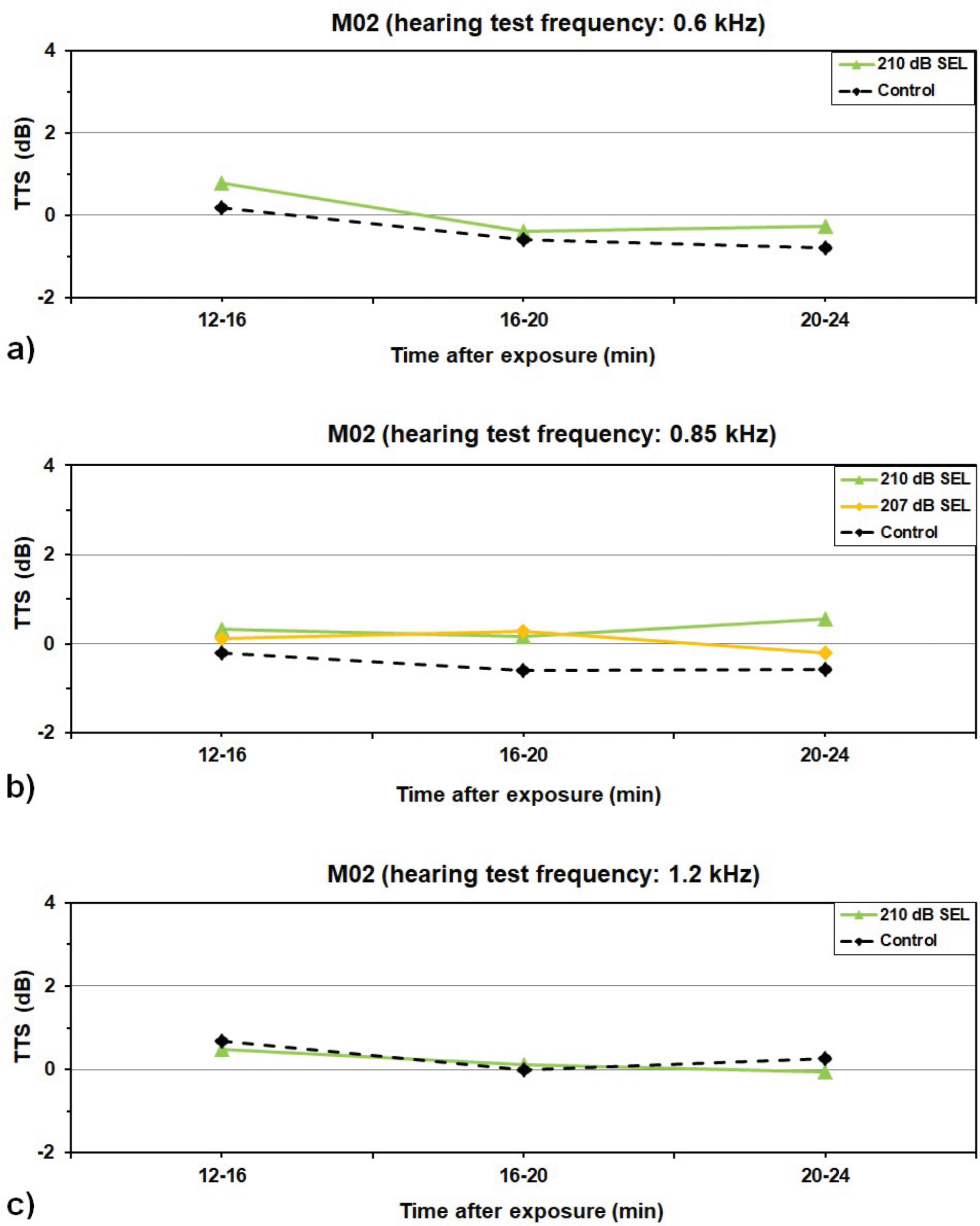


Figure 5. Changes in mean TTS of M02, including recovery, tested at 0.6 (a), 0.85 (b), and 1.2 kHz (c), after 60-min exposure to a continuous one-sixth-octave NB centered at 0.6 kHz, at several SELs. No significant TTS was detected. For sample sizes and SDs (only for $TTS_{12\text{-}16}$), see Table 1. For average received SPLs (dB re 1 µPa), subtract 36 dB from the SEL values. The mean "TTS" values during control tests (no shifts occurred) are also shown.

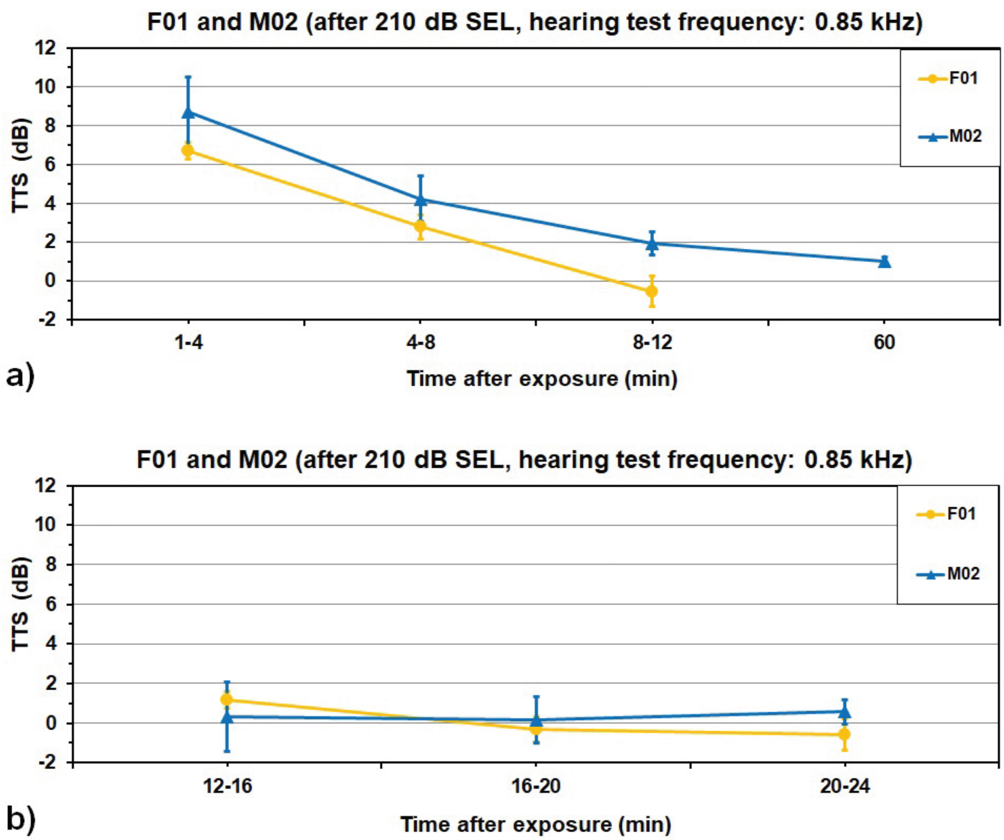


Figure 6. Testing individual differences in susceptibility to TTS. Mean TTS (± SD; n = 4-6) at 0.85 kHz in F01 and M02, measured 1 to 12 and 60 min (a) and 12 to 24 min (b) after 60-min exposure to the continuous NB at 0.6 kHz, at an SEL of 210 dB re 1 μPa^2s.

in F01 (1.2 dB, SD = 0.6 dB, n = 4) was similar to the mean $TTS_{12\text{-}16}$ in M02 (0.3 dB, SD = 0.8 dB, n = 6) after exposure to the same SEL. The recovery patterns were also similar (Figure 6b).

Effect of SEL of the NB at 1 kHz on TTS Levels

The one-way ANOVAs and t test to investigate onset of TTS showed that $TTS_{1\text{-}4}$ (F01) was affected by the SEL of the NB at 1 kHz at all three hearing test frequencies, and $TTS_{12\text{-}16}$ (M02) was affected by the SEL only at 1 and 1.4 kHz, not at 2 kHz. At the TTS-onset SELs for F01 at hearing test frequencies 1.4 and 2 kHz (i.e., 183 and 195 dB re 1 μPa^2s, respectively; Table 2), significant $TTS_{12\text{-}16}$ in M02 did not occur and was therefore not tested at lower SELs. Significant $TTS_{12\text{-}16}$ in M02 was also not detected at hearing test frequency 1 kHz at F01's TTS-onset SEL (190 dB), but TTS was slightly higher than the control value, and was therefore tested at a lower SEL (Table 2). Comparisons with the control revealed that the statistically significant onset of TTS varied depending on the sea lion and the hearing test frequency (Table 2).

No change in susceptibility to TTS was observed during the study. As expected, the control tests showed that the hearing thresholds for all three hearing test signals before and after 60-min exposure to low ambient noise were similar (Table 2).

TTS and Recovery After Exposure to the NB at 1 kHz

With a hearing test signal of 1 kHz, statistically significant $TTS_{1\text{-}4}$ occurred in F01 after exposure to SELs of ≥ 189 dB (re 1 μPa^2s; Table 2; Figure 7a). Recovery of hearing occurred within 8 min after exposure to an SEL of ≤ 189 dB and within 60 min after exposure to an SEL of 195 dB (Figure 8a).

With a hearing test signal of 1.4 kHz, statistically significant $TTS_{1\text{-}4}$ occurred after exposure to SELs of ≥ 183 dB (Table 2; Figure 7a). Recovery

Table 2. The mean, SD, and range of initial TTS ($TTS_{1\text{-}4}$ in F01 and $TTS_{12\text{-}16}$ in M02) after 60-min exposures to ambient noise (control) and a continuous one-sixth-octave noise band centered at 1 kHz at several SELs, quantified at hearing frequencies 1, 1.4, and 2 kHz. Mean underwater SELs (calculated from mean underwater SPLs) and mean aerial SPLs are shown. TTS levels were calculated as the differences between pre- and post-exposure hearing thresholds. No TTS occurred during control tests. *n* = sample size; * = TTS significantly different from control value ($p < 0.05$).

Hearing test frequency (kHz)	SPL in water (dB re 1 µPa)	SEL in water (dB re 1 µPa²s)	SPL in air (dB re 20 µPa)	F01 $TTS_{1\text{-}4}$ (dB)				M02 $TTS_{12\text{-}16}$ (dB)			
				Mean	SD	Range	*n*	Mean	SD	Range	*n*
1	Ambient	Control	39	0.1	1.0	-1.0-1.6	5	-0.1	1.1	-1.6-1.1	5
	147	183	80	0.5	1.0	-0.4-1.7	4	0.0	0.4	-0.4-0.5	4
	153	189	86	5.5*	0.7	4.5-6.1	4	1.2	0.8	0.1-1.8	4
	159	195	92	8.0*	0.6	7.2-8.5	4	5.0*	0.4	4.6-5.3	4
1.4	Ambient	Control	39	-0.7	0.6	-1.4-0.2	6	0.3	0.9	-1.1-1.2	6
	144	180	77	0.7	0.7	-0.3-1.1	4	--	--	--	0
	147	183	80	3.3*	1.2	1.9-4.7	4	0.4	2.0	-1.8-2.8	5
	153	189	86	5.5*	1.3	4.6-7.3	4	1.4	1.2	-0.2-2.3	4
	159	195	92	9.6*	1.1	8.1-10.6	4	4.0*	1.4	3.0-5.9	4
2	Ambient	Control	39	0.7	0.4	0.4-1.2	4	0.9	0.6	0.5-1.8	4
	153	189	86	0.8	1.1	-0.4-2.0	4	--	--	--	0
	159	195	92	4.5*	0.4	4.0-5.1	4	0.3	0.7	-0.6-0.7	4

of hearing occurred within 8 min after exposure to an SEL of 183 dB, within 12 min after 189 dB, and within 60 min after 195 dB (Figure 8b).

With a hearing test signal of 2 kHz, significant $TTS_{1\text{-}4}$ only occurred after exposure to an SEL of 195 dB (Table 2; Figure 7a), and recovery of hearing occurred within 8 min (Figure 8c).

With hearing test signals of 1 and 1.4 kHz, statistically significant $TTS_{12\text{-}16}$ occurred in M02 only after exposure to SELs of 195 dB (re 1 µPa²s; Table 2; Figure 7b), and recovery of hearing occurred within 24 min (Figure 9a & b). With a hearing test signal of 2 kHz, no significant $TTS_{12\text{-}16}$ occurred, even after exposure to an SEL of 195 dB (re 1 µPa²s; Table 2; Figures 7b & 9c).

Individual Differences in Susceptibility to TTS After Exposure to the NB at 1 kHz

During four sessions, the order in which the sea lions were tested at hearing frequency 1.4 kHz after exposure to the NB at 1 kHz (SEL 195 dB re 1 µPa²s) was reversed. The mean $TTS_{1\text{-}4}$ of M02, measured at 1.4 kHz (8.7 dB, SD = 1.0 dB, *n* = 4), was only 0.9 dB lower than the mean $TTS_{1\text{-}4}$ of F01 (9.6 dB, SD = 1.1 dB, *n* = 4) after exposure to the same SEL. The recovery patterns were similar (Figure 10a). The mean $TTS_{12\text{-}16}$ of F01 measured at 1.4 kHz (5.4 dB, SD = 0.4 dB, *n* = 4) was 1.4 dB higher than the mean $TTS_{12\text{-}16}$ of M02 (4.0 dB, SD = 1.4 dB, *n* = 4) after exposure to the same SEL. The recovery patterns were also similar (Figure 10b).

Discussion

Baseline Hearing Thresholds, Performance, and Aerial Sound Exposure

During pre-exposure periods, the hearing thresholds of the two California sea lions for hearing test signals between 0.6 and 32 kHz differed by only a few dB from each other (Kastelein et al., 2021b, 2022, present study) and were similar to the threshold reported by Reichmuth et al. (2013) for similar hearing test frequencies (Figure 11). This suggests that the hearing of the sea lions in the present study was representative of their species.

The performance of both sea lions was consistent throughout the study period. Most mean TTS values had standard deviations of ≤ 2 dB (Tables 1 & 2). In part, this consistency in TTS was due to the low ambient noise level and the ample time taken to allow the sea lions to habituate to each new hearing test frequency. The time required varied depending on the individual sea lion and the hearing test frequency. Mean pre-stimulus response rates (i.e., false positives) by both

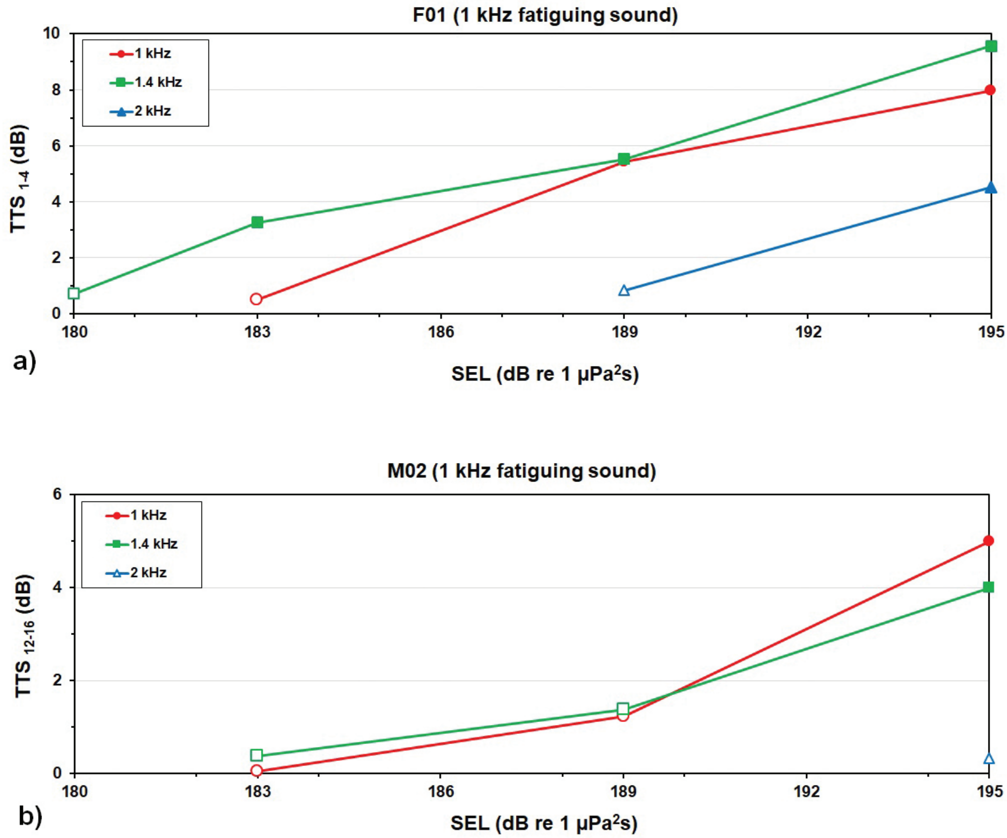


Figure 7. Mean $TTS_{1\text{-}4}$ in F01 (a) and mean $TTS_{12\text{-}16}$ in M02 (b) after 60-min exposure to a continuous one-sixth-octave NB centered at 1 kHz, at several SELs (dB re 1 μPa^2s), quantified at hearing frequencies 1, 1.4, and 2 kHz (i.e., at the center frequency of the fatiguing sound, half an octave above it, and one octave above it). Open symbols indicate thresholds similar to those in control tests (no TTS); solid symbols indicate statistically significant TTS relative to the control tests. Sample size varies per data point (see Table 2). Note that the y-axis scales differ. For average received SPLs (dB re 1 µPa), subtract 36 dB from the SEL values. For SDs and mean control values, see Table 2 and Figures 8 & 9.

sea lions were low and similar in all pre-exposure hearing tests, control tests, and hearing tests after exposure to the NBs at 0.6 and 1 kHz, suggesting that performance was similar in all test periods.

Both sea lions exhibited consistent response patterns in terms of both initial TTS and recovery. The susceptibility of terrestrial mammals to TTS may change over time (Kujawa & Liberman, 1997; Mannström et al., 2015), but changes were not observed in the present study. Susceptibility to TTS may have been stable throughout the study period due to the relatively short exposure periods and relatively low TTSs elicited in the present study compared to those in the studies of Kujawa & Liberman (1997) and Mannström et al. (2015), as discussed by Houser (2021).

Short breaks in the fatiguing sound are known to result in significantly lower levels of TTS (Kastelein et al., 2022). Such breaks could theoretically occur when sea lions lift their heads out of the water during exposure. However, it was assumed that as long as the lower jaw (and thus part of the skull) remained below the water surface, acoustic energy reached the ears as if the entire head was below the water surface (as occurs in harbor seals; Kastelein et al., 2018). Even when their heads were completely out of the water during occasional jumps, the subjects were exposed to the fatiguing sound at high SPLs just above the water surface, as demonstrated by the SPLs measured in air during exposure periods (Tables 1 & 2). In addition, the building around

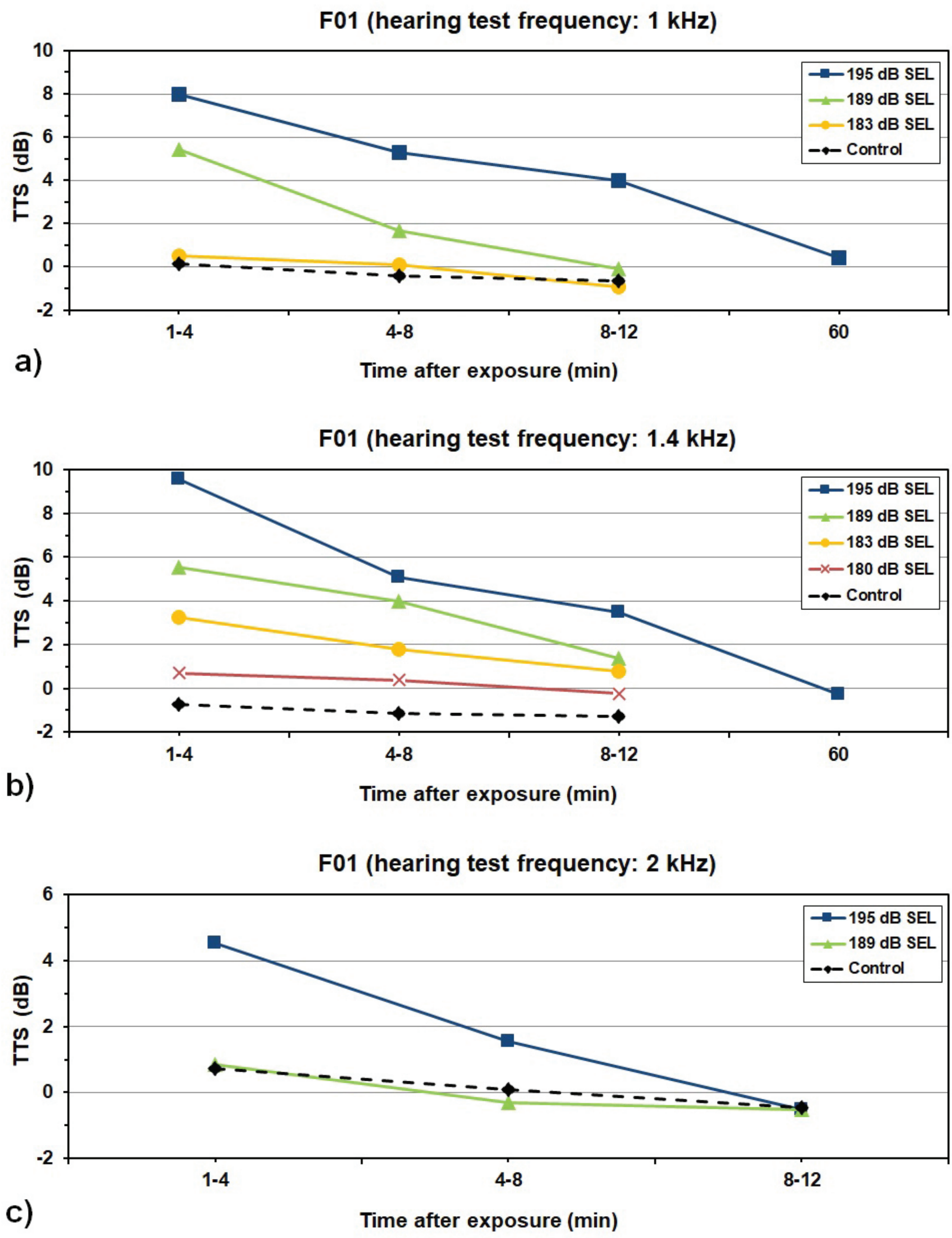


Figure 8. Changes in the mean TTS of F01, including recovery, tested at 1 (a), 1.4 (b), and 2 kHz (c), after 60-min exposure to a continuous one-sixth-octave NB centered at 1 kHz, at several SELs (dB re 1 µPa^{2}s). Hearing was considered recovered once TTS was < 2 dB. For sample sizes and SDs (only for TTS_{1-4}), see Table 2. Note that the x- and y-axis scales in (c) differ from those in (a) and (b). For average received SPLs (dB re 1 µPa), subtract 36 dB from the SEL values. The mean "TTS" values during control tests (no shifts occurred) are also shown.

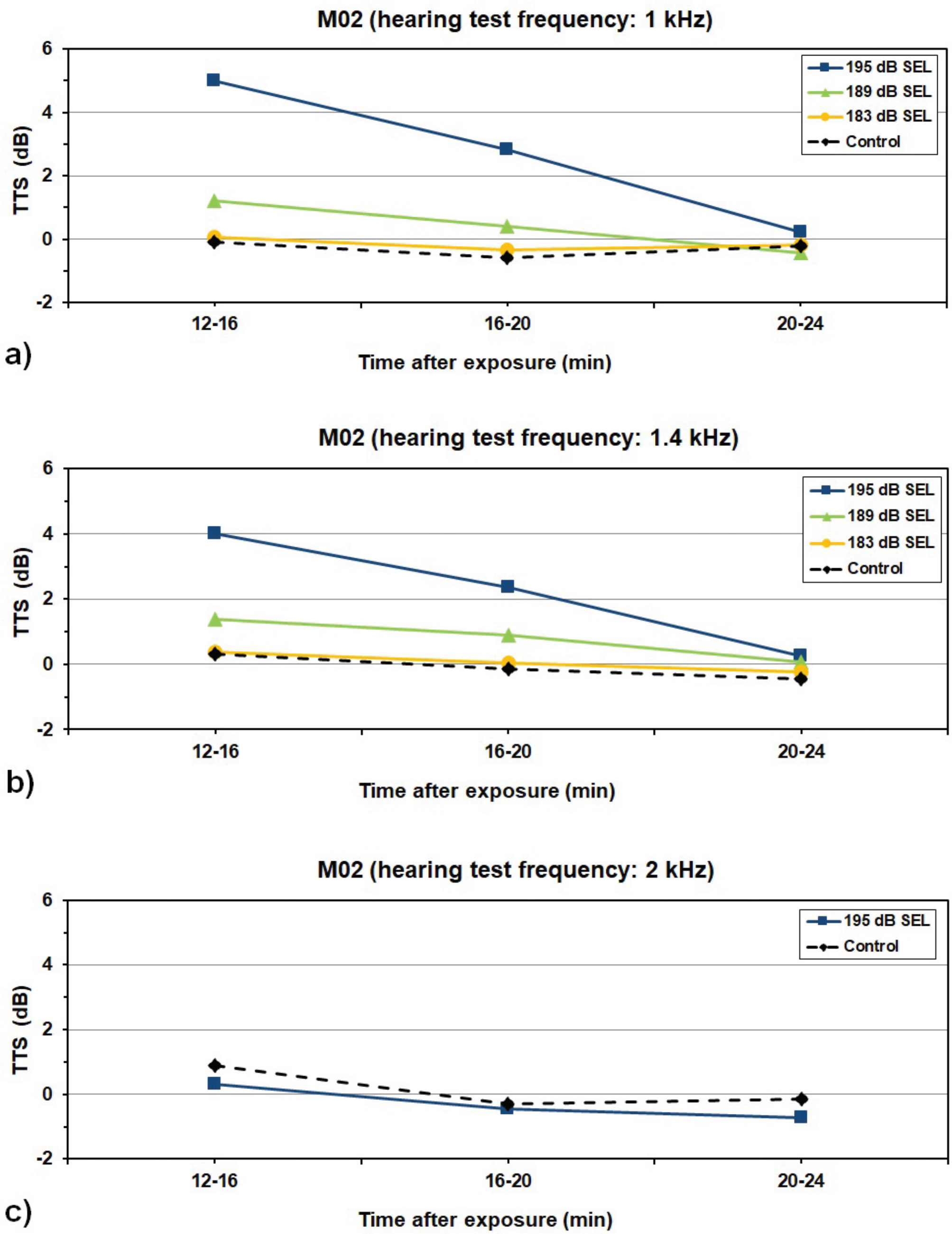


Figure 9. Changes in the mean TTS of M02, including recovery, tested at 1 (a), 1.4 (b), and 2 kHz (c), after 60-min exposure to a continuous one-sixth-octave NB centered at 1 kHz, at several SELs (dB re 1 µPa²s). Hearing was considered recovered once TTS was < 2 dB. For sample sizes and SDs (only for TTS_{12-16}), see Table 2. For average received SPLs (dB re 1 µPa), subtract 36 dB from the SEL values. The mean "TTS" values during control tests (no shifts occurred) are also shown.

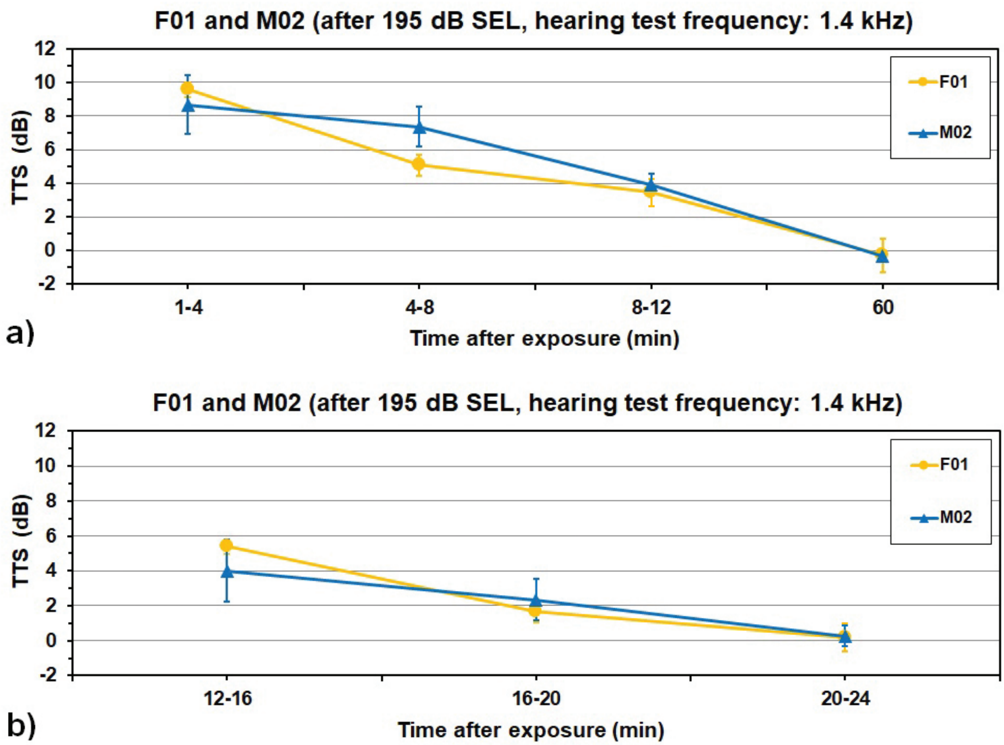


Figure 10. Testing individual differences in susceptibility to TTS. Mean TTS (± SD; *n* = 4) at 1.4 kHz in F01 and M02, measured 1 to 12 and 60 min (a) and 12 to 24 min (b) after 60-min exposure to the continuous NB at 1 kHz, at an SEL of 195 dB re 1 µPa^{2}s.

the pool had hard inner surfaces, which caused the SPL in air to be fairly homogeneous due to reflections. Therefore, it was considered unnecessary to project additional fatiguing sound with aerial loudspeakers during exposure sessions, and our underwater SELs and TTS measurements are assumed to be accurate.

Three sound projectors with substantial power amplifiers were required to create undistorted sound fields at 0.6 kHz; lower frequencies with sufficient amplitude to elicit TTS could not be produced in the pool. The technical difficulties of creating narrow-bandwidth fatiguing sounds below 0.6 kHz that have sufficient SPL to cause TTS in the sea lions precludes testing at lower frequencies. This is unfortunate because many anthropogenic sounds have the most energy at low frequencies (Duarte et al., 2021). It is likely that TTS-onset values at low frequencies will have to be extrapolated based on unmasked hearing thresholds and TTS-onset patterns at higher frequencies, such as those presented by Southall et al. (2019).

Hearing Frequencies Most Affected, Magnitude of TTS, and Recovery

When F01 was exposed to the NB at 0.6 kHz, TTS_{1-4} only occurred when the hearing test frequency was half an octave higher than the center frequency of the fatiguing sound (i.e., 0.85 kHz). M02 showed no TTS_{12-16} after exposure to the NB at 0.6 kHz, which was expected given the recovery pattern of F01; her TTS_{8-12} was similar to control values.

When F01 was exposed to the NB at 1 kHz, TTS_{1-4} occurred at all three hearing test frequencies, but was largest when the hearing test frequency was half an octave higher than the center frequency of the fatiguing sound (i.e., 1.4 kHz). In contrast, M02 showed the largest mean TTS_{12-16} at 1 kHz—the center frequency of the NB. The difference in mean TTS_{12-16} between 1 and 1.4 kHz, however, was only 1 dB, and ranges were 4.6 to 5.3 dB and 3.0 to 5.9 dB, respectively. Therefore, this difference in largest TTS_{12-16} may be an artifact from low sample sizes (i.e., four) rather than a difference in most affected hearing frequency between F01 and M02, and M02's TTS_{12-16} may actually be very similar for

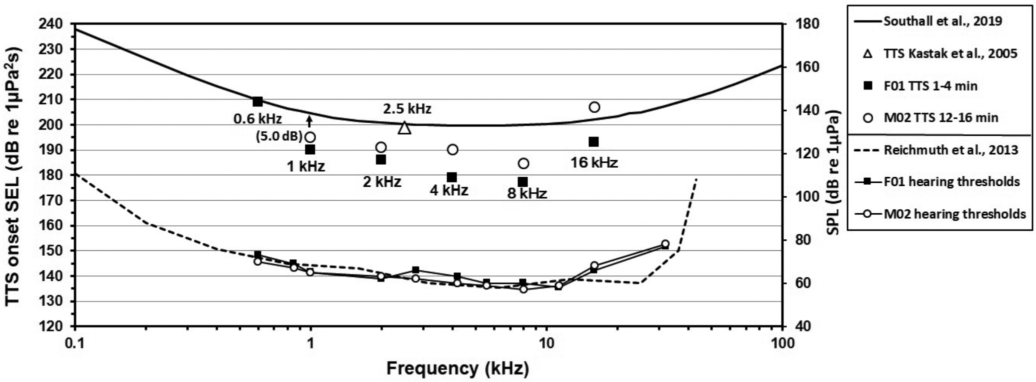


Figure 11. The SELs (left-hand y-axis) of one-sixth-octave NBs centered at 0.6 and 1 kHz (present study), 2 and 4 kHz (Kastelein et al., 2021b), and 8 and 16 kHz (Kastelein et al., 2022), which would cause 6 dB TTS$_{1-4}$ in F01 (■) and 6 dB TTS$_{12-16}$ in M02 (O). The maximum SEL at which the NB at 1 kHz could be produced only elicited 5.0 dB TTS$_{12-16}$ in M02, so to cause 6 dB TTS, the SEL would have to be slightly higher (as indicated with the arrow above the open circle symbol). No TTS$_{12-16}$ could be elicited in M02 after exposure to the NB at 0.6 kHz. In this figure, the lowest SEL required to cause 6 dB TTS is defined as a marker of TTS onset (following Southall et al., 2019). The published TTS-onset curve for California sea lions (upper solid line; Southall et al., 2019) was based on a study by Kastak et al. (2005; △) in which a California sea lion was exposed to a continuous one-octave NB centered at 2.5 kHz. The audiogram of a California sea lion (dashed line; Reichmuth et al., 2013) and the mean pre-exposure hearing thresholds of the two California sea lions used in the present study between 0.6 and 32 kHz (Kastelein et al. 2021b, 2022, present study) are also shown (right-hand y-axis, showing SPLs).

both hearing frequencies. In addition, the hearing frequency most affected depends on the SEL of the fatiguing sound (Kastelein et al., 2019). If higher SELs were tested in the present study, the most affected hearing frequency of M02 would probably have been 1.4 kHz.

Furthermore, when after exposure to the NB at 1 kHz the sea lions were tested in opposite order (see "Individual Differences in Susceptibility to TTS"), F01 showed a mean TTS$_{12-16}$ of 5.4 dB at hearing frequency 1.4 kHz, whereas her mean TTS$_{8-12}$ in normal-order sessions averaged 3.5 dB. For M02, mean TTS$_{8-12}$ and his normal mean TTS$_{12-16}$ at 1.4 kHz were quite similar: 3.9 and 4.0 dB, respectively. These findings may partially be attributed to the sea lions needing a brief period of time to adjust to "testing mode," resulting in slightly lower TTS values at PSE$_{8-12}$ (i.e., the end of the first test session) compared to PSE$_{12-16}$ (i.e., the start of the second test session). This could (partially) explain the relatively large discrepancy for F01, as she did not usually have to wait for 12 min after the fatiguing sound stopped before being tested. In addition, recovery from TTS does not seem to occur at a constant rate, but to decelerate slightly over time, making the TTS values measured during PSE$_{8-12}$ and PSE$_{12-16}$ more similar than those measured during PSE$_{1-4}$ and PSE$_{4-8}$, for instance.

TTS-Onset SEL

To indicate the onset of TTS, we used statistically significant differences with control values, though Southall et al. (2019) proposed the lowest SEL required to elicit 6 dB TTS as a marker of TTS onset. Hearing frequency was not specified. By this definition, and considering all hearing test frequencies, the 6 dB onset of TTS$_{1-4}$ in F01 after exposure to the NB at 0.6 kHz occurred at an SEL of 209 dB re 1 µPa^{2}s (at 0.85 kHz); after exposure to the NB at 1 kHz, onset occurred at 190 dB re 1 µPa^{2}s (at 1.4 kHz). The 6 dB onset of TTS$_{12-16}$ in M02 after exposure to the NB at 1 kHz (followed by some recovery of hearing) was not reached, but a 5 dB TTS occurred after exposure to the maximum SEL (195 dB re 1 µPa^{2}s at 1.4 kHz; Figure 11). These results, combined with data on fatiguing sounds centered at 2, 4, 8, and 16 kHz (Kastelein et al., 2021b, 2022), suggest that susceptibility to TTS is frequency-dependent in California sea lions, as it is in other marine mammals in which TTS has been tested: bottlenose dolphins (*Tursiops truncatus*; Finneran & Schlundt, 2013), harbor porpoises (Kastelein et al., 2021a), Yangtze finless porpoises (*Neophocaena phocaenoides asiaeorientalis*; Popov et al., 2011), and harbor seals (Kastelein et al., 2020).

Individual Differences in Susceptibility to TTS
Testing the hearing of both sea lions at the same times after the fatiguing sound stopped showed that TTSs and recovery patterns were similar (Figures 6 & 10). Susceptibility to TTS in both subjects was also similar for NBs at 2, 4, 8, and 16 kHz (Kastelein et al., 2021b, 2022). However, the sample size (two individuals) is too small to draw general conclusions about variability in susceptibility to TTS within the species. In addition, F01 and M02 are genetically related (mother and son). Studies on humans and other terrestrial mammals show individual, genetic, and population-level differences in susceptibility to TTS (Kylin, 1960; Kryter et al., 1962; Henderson et al., 1993; Davis et al., 2003; Spankovich et al., 2014). Therefore, further replication with more California sea lions, using the same methodology, is needed to assess the generality of the results obtained in the present study.

Towards Improved Protection of Otariidae from Underwater Anthropogenic Noise
Hearing damage, whether permanent or temporary (but repeated), may compromise an individual sea lion's fitness and/or survival. All SELs at which the (6 dB) onset of TTS_{1-4} has been observed in F01 so far (Kastelein et al., 2021b, 2022, present study) are below the (6 dB) TTS-onset levels modeled and predicted by Southall et al. (2019), as are most of the TTS_{12-16} onset SELs of M02 (Figure 11). This means that California sea lions are more susceptible to TTS than predicted and that they experience TTS as a result of exposure to sounds of lower SELs than predicted. Therefore, we suggest that the TTS-onset SEL function for Otariidae (marine mammal group OCW; Southall et al., 2019) should be lowered and changed in shape (see Figure 11). Assessment of TTS onset based on statistical significance also suggests greater susceptibility to TTS than is indicated by the 6-dB onset definition; 6 dB is a useful, simple definition, but it is arbitrary. Once the TTS-onset threshold SELs have been measured for NBs at 32 and 40 kHz (in the last study in the current research project), a new, more accurate TTS-onset function can be established.

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Literature Cited

Cornsweet, T. N. (1962). The staircase-method in psychophysics. *American Journal of Psychology*, *75*(3), 485-491. https://doi.org/10.2307/1419876

Davis, R. R., Kozel, P., & Erway, L. C. (2003). Genetic influences in individual susceptibility to noise: A review. *Noise Health*, *5*, 19-28.

Duarte, C. M., Chapuis, L., Collin, S. P., Costa, D. P., Devassy, R. P., Eguiluz, V. M., Erbe, C., Gordon, T. A. C., Halpern, B. S., Harding, H. R., Havlik, M. N., Meekan, M., Merchant, N. D., Miksis-Olds, J. L., Parsons, M., Predragovic, M., Radford, A. N., Radford, C. A., Simpson, S. D., Slabbekoorn, H., . . . Juanes, F. (2021). The soundscape of the anthropocene ocean. *Science*, *371*, eaba4658. https://doi.org/10.1126/science.aba4658

Finneran, J. J. (2015). Noise-induced hearing loss in marine mammals: A review of temporary threshold shift studies from 1996-2015. *The Journal of the Acoustical Society of America*, *138*(3), 1702-1726. https://doi.org/10.1121/1.4927418

Finneran, J. J., & Schlundt, C. E. (2007). Underwater sound pressure variation and bottlenose dolphin (*Tursiops truncatus*) hearing thresholds in a small pool. *The Journal of the Acoustical Society of America*, *122*(1), 606-614. https://doi.org/10.1121/1.2743158

Finneran, J. J., & Schlundt, C. E. (2013). Effects of fatiguing tone frequency on temporary threshold shift in bottlenose dolphins (*Tursiops truncatus*). *The Journal of the Acoustical Society of America*, *133*(3), 1819-1826. https://doi.org/10.1121/1.4776211

Finneran, J. J., Dear, R., Carder, D. A., & Ridgway, S. H. (2003). Auditory and behavioral responses of California sea lions (*Zalophus californianus*) to single underwater impulses from an arc-gap transducer. *The Journal of the Acoustical Society of America*, *114*(3), 1667-1677. https://doi.org/10.1121/1.1598194

Henderson, D., Subramaniam, M., & Boettcher, F. A. (1993). Individual susceptibility to noise-induced hearing loss:

An old topic revisited. *Ear and Hearing*, *14*(3), 152-168. https://doi.org/10.1097/00003446-199306000-00002

Houser, D. S. (2021). When is temporary threshold shift injurious to marine mammals? *Journal of Marine Science and Engineering*, *9*, 757. https://doi.org/10.3390/jmse9070757

Houser, D. S., Yost, W., Burkard, R., Finneran, J. J., Reichmuth, C., & Mulsow, J. (2017). A review of the history, development and application of auditory weighting functions in humans and marine mammals. *The Journal of the Acoustical Society of America*, *141*(3), 1371-1413. https://doi.org/10.1121/1.4976086

Kastak, D., & Schusterman, R. J. (1998). Low-frequency amphibious hearing in pinnipeds: Methods, measurements, noise, and ecology. *The Journal of the Acoustical Society of America*, *103*(4), 2216-2228. https://doi.org/10.1121/1.421367

Kastak, D., Schusterman, R. J., Southall, B. L., & Reichmuth, C. J. (1999). Underwater temporary threshold shift induced by octave-band noise in three species of pinniped. *The Journal of the Acoustical Society of America*, *106*(2), 1142-1148. https://doi.org/10.1121/1.427122

Kastak, D., Southall, B. L., Schusterman, R. J., Reichmuth, C. J., & Kastak, C. (2005). Underwater temporary threshold shift in pinnipeds: Effects of noise level and duration. *The Journal of the Acoustical Society of America*, *118*(5), 3154-3163. https://doi.org/10.1121/1.2047128

Kastelein, R. A., Helder-Hoek, L., & Gransier, R. (2019). Frequency of greatest temporary threshold shift in harbor seals (*Phoca vitulina*) depends on the fatiguing sound level. *The Journal of the Acoustical Society of America*, *145*(3), 1353-1362. https://doi.org/10.1121/1.5092608

Kastelein, R. A., Helder-Hoek, L., & Terhune, J. M. (2018). Hearing thresholds, for underwater sounds, of harbor seals (*Phoca vitulina*) at the water surface. *The Journal of the Acoustical Society of America*, *143*(4), 2554-2563. https://doi.org/10.1121/1.5034173

Kastelein, R. A., Gransier, R., Hoek, L., & Olthuis, J. (2012a). Temporary threshold shifts and recovery in a harbor porpoise (*Phocoena phocoena*) after octave-band noise at 4 kHz. *The Journal of the Acoustical Society of America*, *132*(5), 3525-3537. https://doi.org/10.1121/1.4757641

Kastelein, R. A., Gransier, R., Hoek, L., Macleod, A., & Terhune, J. M. (2012b). Hearing threshold shifts and recovery in harbor seals (*Phoca vitulina*) after octave-band noise exposure at 4 kHz. *The Journal of the Acoustical Society of America*, *132*(4), 2745-2761. https://doi.org/10.1121/1.4747013

Kastelein, R. A., Helder-Hoek, L., Cornelisse, S. A., Defillet, L. N., Huijser, L. A. E., & Gransier, R. (2021a). Temporary hearing threshold shift in a harbor porpoise (*Phocoena phocoena*) due to exposure to a continuous one-sixth-octave noise band centered at 0.5 kHz. *Aquatic Mammals*, *47*(2), 135-145. https://doi.org/10.1578/AM.47.2.2021.135

Kastelein, R. A., Helder-Hoek, L., Cornelisse, S. A., Defillet, L. N., Huijser, L. A. E., & Terhune, J. M. (2020). Temporary hearing threshold shift in harbor seals (*Phoca vitulina*) due to one-sixth-octave noise bands centered at 0.5, 1, and 2 kHz. *The Journal of the Acoustical Society of America*, *148*(6), 3873-3885. https://doi.org/10.1121/10.0002781

Kastelein, R. A., Helder-Hoek, L., Defillet, L. N., Huijser, L. A. E., Terhune, J. M., & Gransier, R. (2021b). Temporary hearing threshold shift in California sea lions (*Zalophus californianus*) due to one-sixth-octave noise bands centered at 2 and 4 kHz: Effect of duty cycle and testing the equal-energy hypothesis. *Aquatic Mammals*, *47*(4), 394-418. https://doi.org/10.1578/AM.47.4.2021.394

Kastelein, R. A., Helder-Hoek, L., Defillet, L. N., Kuiphof, F., Huijser, L. A. E., & Terhune, J. M. (2022). Temporary hearing threshold shift in California sea lions (*Zalophus californianus*) due to one-sixth-octave noise bands centered at 8 and 16 kHz: Effect of duty cycle and testing the equal-energy hypothesis. *Aquatic Mammals*, *48*(1), 36-58. https://doi.org/10.1578/AM.48.1.2022.36

Kryter, K. D., Weisz, A. Z., & Wiener, F. M. (1962). Auditory fatigue from audio analgesia. *The Journal of the Acoustical Society of America*, *34*(6), 383-391. https://doi.org/10.1121/1.1918138

Kujawa, S. G., & Liberman, M. C. (1997). Conditioning-related protection from acoustic injury: Effects of chronic differentiation and sham surgery. *Journal of Neurophysiology*, *78*(6), 3095-3106. https://doi.org/10.1152/jn.1997.78.6.3095

Kylin, B. (1960). Temporary threshold shift and auditory trauma following exposure to steady-state noise. *Acta Oto-Laryngology*, *51-56*(Supp. 152), 1-94.

Levitt, H. (1971). Transformed up-down methods in psychoacoustics. *The Journal of the Acoustical Society of America*, *49*(2B), 467-477. https://doi.org/10.1121/1.1912375

Mannström, P., Kirkegaard, M., & Ulfendahl, M. (2015). Repeated moderate noise exposure in the rat—An early adulthood noise exposure model. *Journal of the Association of Research on Otolaryngology*, *16*(6), 763-772. https://doi.org/10.1007/s10162-015-0537-5

Melin, S. R., Trillmich, F., & Aurioles-Gamboa, D. (2018). California, Galápagos, and Japanese sea lions—*Zalophus californianus*, *Z. wollebaeki*, and *Z. japonicus*. In B. Würsig, J. G. M. Thewissen, & K. M. Kovacs (Eds.), *Encyclopedia of marine mammals* (3rd ed., pp. 153-157). Academic Press. https://doi.org/10.1016/B978-0-12-804327-1.00003-0

Mulsow, J., Houser, D. S., & Finneran, J. J. (2012). Underwater psychophysical audiogram of a young male California sea lion (*Zalophus californianus*). *The Journal of the Acoustical Society of America*, *131*(5), 4182-4187. https://doi.org/10.1121/1.3699195

Popov, V. V., Supin, A. Ya., Rozhnov, V. V., Nechaev, D. I., & Sysueva, E. V. (2014). The limits of applicability of the sound exposure level (SEL) metric to temporal threshold shifts (TTS) in beluga whales, *Delphinapterus leucas*. *Journal of Experimental Biology*, *217*(10), 1804-1810. https://doi.org/10.1242/jeb.098814

Popov, V. V., Supin, A. Ya., Wang, D., Wang, K., Dong, L., & Wang, S. (2011). Noise-induced temporary threshold shift and recovery in Yangtze finless porpoise *Neophocaena phocaenoides asiaeorientalis*. *The Journal of the Acoustical Society of America*, *130*(1), 574-584. https://doi.org/10.1121/1.3596470

Reichmuth, C., & Southall, B. L. (2012). Underwater hearing in California sea lions (*Zalophus californianus*): Expansion and interpretation of existing data. *Marine Mammal Science*, *28*(2), 358-363. https://doi.org/10.1111/j.1748-7692.2011.00473.x

Reichmuth, C., Holt, M. M., Mulsow, J., Sills, J. M., & Southall, B. L. (2013). Comparative assessment of amphibious hearing in pinnipeds. *Journal of Comparative Physiology A*, *199*(6), 491-507. https://doi.org/10.1007/s00359-013-0813-y

Schusterman, R. J., Balliet, R. F., & Nixon, J. (1972). Underwater audiogram of the California sea lion by the conditioned vocalization technique. *Journal of Experimental Animal Behavior*, *17*(3), 339-350. https://doi.org/10.1901/jeab.1972.17-339

Southall, B. L., Schusterman, R. J., Kastak, D., & Reichmuth Kastak, C. (2005). Reliability of underwater hearing thresholds in pinnipeds. *Acoustics Research Letters Online*, *6*(4), 243-249. https://doi.org/10.1121/1.1985956

Southall, B. L., Finneran, J. J., Reichmuth, C., Nachtigall, P. E., Ketten, D. R., Bowles, A. E., Ellison, W. T., Nowacek, D. P., & Tyack, P. L. (2019). Marine mammal noise exposure criteria: Updated scientific recommendations for residual hearing effects. *Aquatic Mammals*, *45*(2), 125-232. https://doi.org/10.1578/AM.45.2.2019.125

Spankovich, C., Griffiths, S. K., Lobariñas, E., Morgenstein, K. E., de la Calle, S., Ledon, V., Guercio, D., & Le Prell, C. G. (2014). Temporary threshold shift after impulse-noise during video game play: Laboratory data. *International Journal of Audiology*, *53*(Supp. 2), S53-S65. https://doi.org/10.3109/14992027.2013.865844

Zar, J. H. (1999). *Biostatistical analysis*. Prentice-Hall.