Aquatic Mammals 2021, *47*(4), 394-418, DOI 10.1578/AM.47.4.2021.394

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

Ronald A. Kastelein,[1] Lean Helder-Hoek,[1] Linde N. Defillet,[1]
Léonie A. E. Huijser,[2] John M. Terhune,[3] and Robin Gransier[4]

[1]Sea Mammal Research Company (SEAMARCO), Julianalaan 46, 3843 CC Harderwijk, The Netherlands
E-mail: researchteam@zonnet.nl
[2]Cetacean Ecology and Acoustics Laboratories (CEAL), University of Queensland, 37 Fraser Street, Dunwich, Queensland 4183, Australia
[3]Department of Biological Sciences, University of New Brunswick, 100 Tucker Park Road, Saint John, New Brunswick, E2L 4L5, Canada
[4]KU Leuven – University of Leuven, Department of Neurosciences, ExpORL, Herestraat 49 bus 721, B-3000 Leuven, Belgium

Abstract

In one of a series of studies of noise-induced hearing loss to determine the frequency-dependent susceptibility of California sea lions (*Zalophus californianus*) to temporary hearing threshold shift (TTS), two subjects were exposed for 60 min to two different fatiguing sounds. These were continuous one-sixth-octave noise bands (NBs), centered at 2 kHz, at sound pressure levels (SPLs) of 138 to 167 dB re 1 µPa (resulting in sound exposure levels [SELs] of 174 to 203 dB re 1 μPa^2s), and at 4 kHz, at SPLs of 133 to 169 dB re 1 µPa (resulting in SELs of 169 to 205 dB re 1 μPa^2s). Using a psychoacoustic technique, TTSs were quantified at 2, 2.8, 4.2, 5.6, and 8 kHz (at the center frequency of each NB, at half an octave higher, and at one octave higher). After exposure to both NBs, higher SELs resulted in greater TTS at all hearing frequencies that were tested. TTSs and hearing recovery patterns were similar in both sea lions. The effect of fatiguing sound duty cycle on TTS was investigated with the NB at 4 kHz and with 1.6-s signal duration, at a mean SPL of 169 dB re 1 µPa. Duty cycle reduction from 100 to 90% resulted in the largest decrease in TTS, and no TTS occurred at duty cycles ≤ 60%. The equal-energy hypothesis was investigated with the NB at 4 kHz: five combinations of SPL and exposure duration that resulted in the same SEL (197 dB re 1 μPa^2s) produced similar initial TTSs in both sea lions. Susceptibility of California sea lions to TTS is higher than previously believed; for sounds around 2 and 4 kHz, it is similar to the susceptibility of harbor seals (*Phoca vitulina*). These data will contribute towards the development of an evidence-based underwater sound weighting function for the protection of Otariidae.

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

Introduction

Human activities in the Pacific Ocean and elsewhere are expected to increase in the coming decades. Many of these activities produce underwater sounds, which vary in duration and level. In some activities, producing sound is the goal (e.g., testing or using naval sonar and seismic surveys). In others, sound is a byproduct (e.g., shipping, offshore pile driving, blasting, and dredging).

The California sea lion (*Zalophus californianus*) is a pinniped (family Otariidae, eared seals) with its geographic range in the coastal waters of the northeast Pacific Ocean. It has acute underwater hearing between ~0.1 and ~50 kHz (Schusterman et al., 1972; Mulsow et al., 2012; Reichmuth & Southall, 2012; Reichmuth et al., 2013). Depending on the received level and the exposure duration, noise in the environment may reduce a sea lion's hearing either temporarily (temporary threshold shift [TTS]) or permanently (permanent threshold shift [PTS]; Melnick, 1991; Yost, 2007). Such noise-induced hearing loss, whether temporary or permanent, may affect the sea lion's fitness by interfering with its ability to detect biologically relevant sounds.

At present, data to assess the impact of underwater sound on California sea lion hearing are limited to the results of three studies. Kastak et al. (1999) exposed a sea lion to octave-band noise centered at 1 kHz (55 to 65 dB sensation level; actual sound pressure levels [SPLs] were not reported) and measured a mean initial TTS of ~4 dB. Finneran et al. (2003) exposed two sea lions to underwater impulses from an arc-gap transducer, which did not elicit TTS. Kastak et al. (2005) elicited ~6 dB TTS in sea lions exposed to an octave-band noise centered at 2.5 kHz. This last study suggests that the TTS onset sound exposure level (SEL; i.e., defined as 6 dB TTS by Southall et al., 2019) in California sea lions is ~20 dB less than the TTS onset SEL in harbor seals (*Phoca vitulina*) for sounds of similar frequency (Finneran, 2015; Houser et al., 2017). The large difference in TTS, as reported by Kastak et al. (2005), suggested a difference in susceptibility to hearing loss between the Otariidae and the Phocidae. However, this large difference is unexpected, as the two pinniped species that were tested have similar hearing thresholds over a large part of their underwater audiograms (Kastelein et al., 2009; Reichmuth et al., 2013) and are expected to have similar noise exposures as they occupy similar acoustic environments in their overlapping geographic ranges in the northeast Pacific Ocean.

Government regulators setting sound exposure criteria to protect the hearing of marine mammals have based weighting functions for Otariidae on the limited data from Kastak et al.'s (2005) study (Southall et al., 2007, 2019; Finneran, 2015, 2016; Houser et al., 2017; National Marine Fisheries Service [NMFS], 2018). To provide more data for the weighting functions and to allow comparison of susceptibility to TTS in the Otariidae and the Phocidae based on more data points, we began a research project on the susceptibility to TTS of the California sea lion over its entire hearing range. The resulting equal-TTS curves will form the basis of an evidence-based underwater sound weighting function for Otariidae (Houser et al., 2017). The research project is divided into four studies, each reporting on TTS caused by two fatiguing sound frequencies (0.5 and 1 kHz, 2 and 4 kHz [present study], 8 and 16 kHz, 32 and 40 kHz).

The following are the goals of the present study: (1) to quantify TTS and determine the TTS onset SEL after exposure of California sea lions to fatiguing sounds with center frequencies of 2 and 4 kHz at several SELs; (2) to determine which of three hearing frequencies (i.e., the center frequency of the fatiguing sound, and half an octave and one octave above that frequency) is most affected by exposure to fatiguing sound at each level; (3) to describe the pattern of hearing recovery after the fatiguing sounds stop; (4) to assess differences in susceptibility to TTS between two California sea lions; (5) to assess the effect of fatiguing sound duty cycle (with sound exposures of constant SPL and constant exposure duration) on TTS with the 4 kHz fatiguing sound; and (6) to test whether different combinations of SPL and exposure duration that result in the same SEL elicit the same initial TTS (i.e., to test the equal-energy hypothesis) with the 4 kHz fatiguing sound.

Methods

Subjects and Study Area

The subjects were an adult female California sea lion, identified as F01, and her juvenile male offspring, identified as M02. During the study, F01 was 8 to 9 y old, her total body length was 160 cm, and her body weight varied between 70 and 86 kg, depending on the season. M02 was 2 to 3 y old, his total body length increased from 126 to 140 cm, and his body weight increased from 38 to 61.5 kg during the study period. Both sea lions were healthy throughout the study.

The sea lions received about 80% of their daily fish ration during hearing test sessions. The remaining 20% was provided while they were performing husbandry tasks, some of which were associated with the gating procedures for the hearing tests. The two subjects had similar hearing thresholds, which, for the tested frequency range, were also similar to those of another California sea lion (Reichmuth & Southall, 2012; Reichmuth et al., 2013; see "Results"). Therefore, their hearing was assumed to be representative for their species. Variation in the subjects' performance was minimized by making weekly adjustments (usually in the order of 100 g) to their daily food ration, based on their weight, their recent performance in hearing tests and husbandry tasks, 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 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 haulout 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 transducer for the fatiguing sounds was placed (Figure 1). Sections of the pool were separated by net fences with gates. The floors of both pools were covered with a 20-cm-thick layer of sloping sand, and skimmers kept the water level constant so

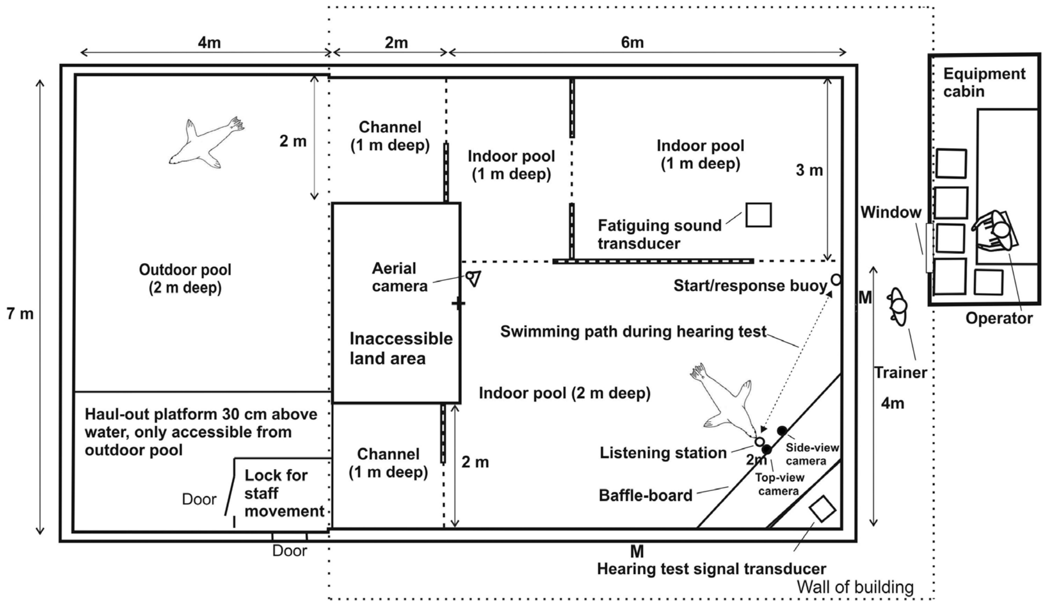

Figure 1. The pool complex in which the California sea lions (*Zalophus californianus*) were housed and the TTS study was conducted. The deep part of the indoor pool was where the sea lions' hearing was tested and where they were exposed to the fatiguing sounds. The fatiguing sound transducer was kept in the shallow part of the indoor pool behind a net fence to prevent the sea lions from approaching the sound source closely. During hearing tests, the sea lion not being tested was kept in the outdoor pool (as shown). The dashed lines in the net fences indicate net gates which could be opened or closed. The thin dotted line indicates the wall of the building containing the indoor pool and the channels. Two "M"s indicate the locations of the aerial microphones at the sides of the indoor pool.

that sound conditions were stable. Seawater was pumped directly from the nearby Eastern Scheldt, a lagoon of the North Sea, into the water circulation system. Recirculation through a sand filter ensured year-round water clarity. The average monthly water temperature varied during the study period between 1.5 and 24°C, and the salinity was around 3.4%. The water circulation system was made to be as quiet as possible and was switched off during the day (1 h before the first hearing test was conducted). During sound exposure sessions, the net gates were closed so that both sea lions were confined to the deep part of the indoor pool; they 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 Equipment—The ambient noise was measured and the fatiguing sound and hearing test signals were calibrated once every 3 mo during the study period by an acoustic consulting agency (TNO). The sound measurement equipment consisted of three hydrophones (Model 8106; Brüel & Kjaer [B&K], Nærum, Denmark) with a multichannel high-frequency analyzer (Model 3560D, B&K PULSE, B&K) and a laptop computer with B&K PULSE software (*Labshop*, Version 12.1). The system was calibrated with a pistonphone (Model 4223, B&K). The broadband SPL (dB re 1 µPa; American National Standards Institute [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.

The SPL in air was measured with two microphones (Model 4193, B&K) with pre-amplifiers (Model 2669, B&K), which were connected to the multi-channel, high-frequency analyzer mentioned above. The system was calibrated with a microphone calibrator (Model 4231, B&K).

Background Noise—Great care was taken to make the sea lions' listening environment as quiet as possible while their hearing thresholds were being measured. Only researchers involved in the hearing tests were allowed within 15 m of the pool complex during hearing test sessions, and they were required to stand still. The ambient noise in the indoor pool was very low and fairly constant in amplitude under test conditions (i.e., water circulation system off, no rain, and generally wind force Beaufort 4 or below; stronger wind from the southwest was sometimes acceptable, as a dike on one side of the pool sheltered the pool from these prevailing winds; Figure 2).

Fatiguing Sounds—Continuous (i.e., 100% duty cycle) one-sixth-octave noise bands (NBs) centered at 2 and 4 kHz, without harmonics, were used as fatiguing sounds (sounds intended to cause TTS) in most sessions. For the NB centered at 4 kHz, lower duty cycles were also used to assess the effect of duty cycle (see "Experimental Procedures"). The fatiguing sounds were selected because they were within the range of most sensitive hearing of California sea lions (Reichmuth et al., 2013). The digitally generated sounds (WAV file, sample rate: 768 kHz) were played back by a laptop computer (Model V5-552; Acer Aspire, Taipei, Taiwan.) with a program written in *LabVIEW* to an external data acquisition card (Model USB 6361; National Instruments, Austin, TX, USA), the output of which could be controlled in 1-dB steps with the *LabVIEW* program. The output of the card went through a custom-built buffer to a custom-built passive low-pass filter set to either 3 kHz (for NB center frequency 2 kHz) or 5 kHz (for NB center frequency 4 kHz), after which it went to a power amplifier (Model VPA2200MBN; HQ Power, Velleman, Gavere, Belgium) which drove the transducer (Model LL1424HP; Lubell, Whitehall, OH, USA) through an isolation transformer (Model AC1424HP, Lubell). The transducer was suspended in the shallow part of the indoor pool at 1 m depth, 5 cm above the pool floor. The linearity of the transmitter system producing the fatiguing sound was

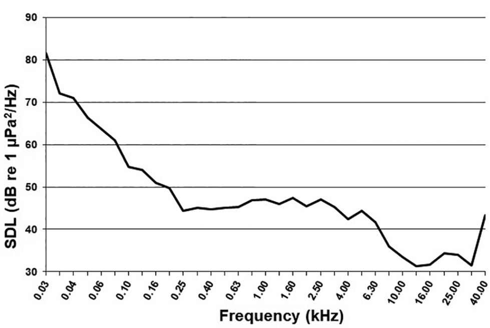

Figure 2. The general underwater ambient noise level in the indoor pool used for California sea lion hearing tests under test conditions. Measurements were recorded as one-third-octave bands and converted to spectrum density levels (SDLs).

checked during each calibration and was found to be consistent to 1 dB within a 42 dB range (overlapping the SPL range used in this study).

To determine the distribution of the fatiguing sounds in the deep part of the indoor pool (where the sea lions were during exposure sessions), the SPL was measured at 42 points: at 14 locations on a horizontal grid with cells of 1 × 1 m, at three depths per location (0.5, 1.0, and 1.5 m below the surface; Figure 3). To determine their acoustic dose, the sea lions were watched continuously via a camera system during fatiguing sound exposures. They swam throughout the entire indoor pool at all depths. Therefore, the average received SPL as 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 and location, and no gradient existed in the SPL in relation to the distance to the transducer, resulting in a very homogeneous sound field for both fatiguing sounds (Figure 3; standard deviations [SDs] were 2.9 dB at 2 kHz and 2.1 dB at 4 kHz; $n = 42$).

During sound exposure sessions, the one-sixth-octave NB centered at 2 kHz was projected for 60 min at various source levels, resulting in mean SPLs (assumed to be the mean received SPLs of the sea lions) ranging from 138 to 167 dB re 1 μPa (SEL range: 174 to 203 dB re 1 μPa^2s). The one-sixth-octave NB centered at 4 kHz was also projected for 60 min (but 10 to 80 min when testing the equal-energy hypothesis) at various source levels, resulting in mean SPLs ranging from 133 to 169 dB re 1 μPa (SEL range: 169 to 205 dB re 1 μPa^2s).

The sea lions mostly took single, short breaths while lifting only their noses out of the water. During occasional jumps, the sea lions' heads were entirely out of the water for fractions of a second. To ascertain how this affected the SEL to which they were exposed, the SPL in air was measured at two locations just outside the deep part of the indoor pool with microphones mounted on tripods 30 cm above the water surface (see the letters "M" in Figure 1). The aerial SPL was measured while the NBs at 2 and 4 kHz were being played back underwater at all SPLs used in the study. Per fatiguing sound SPL, the aerial SPL did vary at most 1 dB between the two measurement locations, so the mean of the two measurements can be assumed to be the SPL the sea lions were exposed to while their heads were completely out of the water (i.e., in-air SPL; Tables 1 & 2).

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 system were checked with an oscilloscope (Model 632FG; Voltcraft, Conrad Electronics, Berlin, Germany) and a voltmeter (Model GES927216GMD-8341;

a) 0.5 m depth (2 kHz)

	1	2	3	4	5
1	146	149	148	156	152
2	149	152	147	148	142
3	143	149	152	147	

d) 0.5 m depth (4 kHz)

	1	2	3	4	5
1	166	164	164	164	167
2	160	167	162	161	168
3	162	166	162	164	

b) 1.0 m depth T

	1	2	3	4	5
1	145	147	146	152	151
2	152	150	149	149	151
3	149	149	152	145	

e) 1.0 m depth T

	1	2	3	4	5
1	160	161	160	161	162
2	161	164	163	163	168
3	162	164	163	162	

c) 1.5 m depth

	1	2	3	4	5
1	143	148	145	149	147
2	152	149	151	150	151
3	151	151	153	149	

f) 1.5 m depth

	1	2	3	4	5
1	165	162	160	162	162
2	162	164	161	163	161
3	162	165	163	165	

Figure 3. Examples (not to scale) of the SPL distribution (values in dB re 1 µPa) in the deep part of the indoor pool (6 × 4 m, 2 m deep; see Figure 1) when the continuous one-sixth-octave noise bands (NBs) centered at 2 kHz (a to c) and 4 kHz (d to f), used as the fatiguing sounds, were being played. 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) SPL for 2 kHz (a to c) was 150 ± 2.9 dB re 1 µPa ($n = 42$); and for 4 kHz (d to f), it was 163 ± 2.1 dB re 1 µPa ($n = 42$). The letter "T" above the box in (b) and (e) indicates the approximate location of the transducer (at 1 m depth) in the adjacent shallow part of the indoor pool. The gray area indicates the location of the hearing test signal transducer and baffle-board; this part of the pool could not be accessed by the sea lions (see Figure 1).

GW Instek, New Taipei City, Taiwan) by producing a 2 or 4 kHz continuous tone from the laptop. The acoustic underwater signal was checked with a hydrophone (Model EC6073; Reson, Slangerup, Denmark), a pre-amplifier (Model 2365, B&K), and a spectrum analyzer (Model PCSU1000; Velleman, Gavere, Belgium). If the values obtained were the same as those obtained by the acoustic consulting agency during SPL calibrations, the SPLs were assumed to be correct, and a 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) and, thus, to more stable hearing thresholds. For TTS studies, precise hearing thresholds are very important, as sometimes only small threshold shifts occur and hearing usually recovers rapidly. The hearing test signals were generated digitally (*Adobe Audition*, Version 3.0). 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 amplitude of 50 ms. The WAV files used as hearing test signals were played on a laptop computer (Model CX623; MSI, Zhonghe District, Taipei, Taiwan) with a program written in *LabVIEW* to an external data acquisition card (Model USB6251, National Instruments). The output of the card was controlled in 1-dB steps with the *LabVIEW* program and went through a custom-built buffer, a custom-built passive low-pass filter, and a custom-built mixer, and then drove a balanced tonpilz piezoelectric acoustic transducer (Model LL916, Lubell) through an isolation transformer (Model AC202, Lubell).

The hearing thresholds were tested at the frequency of the fatiguing sound, half an octave higher, and one octave higher. Thus, for the NB at 2 kHz, the hearing test frequencies were 2, 2.8, and 4.2 kHz; and for the NB at 4 kHz, the hearing test frequencies were 4.2, 5.6, and 8 kHz. During the initial hearing tests, the SPL of the 4 kHz hearing test signal fluctuated more than usual (probably due to the water temperature, which slightly changed the transducer's output characteristics), and the SPL of a 4.2-kHz hearing test signal was more stable, so that hearing test signal frequency was used instead of the envisioned 4 kHz.

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 the sea lion 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 to 2 dB, depending on the test frequency. The mean SPL of the two hydrophones was used to calculate the stimulus level during hearing tests. Before each session, the SPL of the hearing test signal at the location of the listening station was measured with the same equipment as used to check the SPL of the fatiguing sound.

Experimental Procedures

Each hearing test trial began with one of the California sea lions at the start/response buoy (Figure 1). The level of the hearing test signal 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 sea lions were trained to swim from the start/response buoy to the listening station in response to a hand signal from the trainer and remain stationed there. They returned to the start/response buoy upon hearing the test signal in signal-present trials or the trainer's whistle in signal-absent trials. When they did not detect the hearing test signal, they were called back to the start/response buoy by the trainer lightly tapping three times on the side of the pool. 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 & Watson, 1973) produces a 50% correct detection threshold (Levitt, 1971). A switch from a test signal level that a sea lion responded to (a "hit"), to a level that it did not respond to (a "miss"), or vice versa, was called a reversal. Signals were produced at a random time 4 to 12 s after a sea lion stationed properly (i.e., in line with the beam of the transducer) at the listening station (Figure 1).

Each hearing test session consisted of ~25 trials and lasted for up to 12 min per sea lion. In each pre-exposure session, a minimum of 10 reversals was obtained. For each sea lion, the first test session after the fatiguing sound stopped was divided into three 4-min periods. In each of these periods, a minimum of three (mostly four) reversals was obtained. Any session for which the minimum number of reversals was not obtained was discarded. 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) a pre-exposure hearing test, (2) fatiguing sound exposure, and (3) one or more post-sound exposure (PSE) hearing tests, was conducted per day, starting at around 0900 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 otherwise have led to large changes in the sea lions' swimming patterns. The 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.

During most of the study, the two sea lions were tested in the same order to ensure a quick and efficient start after sound exposure stopped: first F01, then M02. To protect their hearing, the subjects were only exposed to fatiguing sounds once per day, so randomizing the order in which they were tested while maintaining the sample sizes would have doubled the study period. However, the order was reversed in four consecutive sessions to investigate individual differences in susceptibility to TTS. In these sessions, M02 was tested first after exposure to each of the fatiguing sounds at one high SEL: 203 dB re 1 μPa^2s dB for the NB at 2 kHz (with a hearing test signal of 2 kHz) and 205 dB re 1 μPa^2s for the NB at 4 kHz (with hearing test signals of 5.6 and 8 kHz).

To gain insight into the duration of the TTS, besides the magnitude of TTS immediately after the exposure, the subsequent changes in hearing (including recovery) were recorded. The hearing sensitivity of F01 was tested during up to six PSE periods: 1-4 (PSE_{1-4}), 4-8 (PSE_{4-8}), 8-12 (PSE_{8-12}), 60 (PSE_{60}), 120 (PSE_{120}), and, only for the NB at 4 kHz, 240 (PSE_{240}) min after the fatiguing sound exposure ended. The hearing sensitivity of M02 was tested 12-16 (PSE_{12-16}), 16-20 (PSE_{16-20}), 20-24 (PSE_{20-24}), 72 (PSE_{72}), 132 (PSE_{132}), and, only for the NB at 4 kHz, 252 (PSE_{252}) min after the fatiguing sound exposure ended. Testing was continued until hearing recovery was deemed to have taken place. Recovery was defined as a return to < 2 dB TTS, based on the fact that statistically significant initial TTS was generally above ~2 dB and reflected the precision with which the threshold could be measured.

Sample sizes (see "Results") were chosen to maximize the time available for testing SPLs in which TTS seemed to occur, to minimize the risk of hearing damage from repeated exposure to the loudest sounds, and to avoid repeated testing of SPLs for which it was clear without analysis that TTS did not occur.

The SEL of the fatiguing sound was carefully increased in steps of 6 dB; at each SEL, the hearing threshold was measured half an octave above the center frequency of the fatiguing sound until around 10 dB TTS_{1-4} occurred at that frequency. In the subsequent sessions, the threshold at the center frequency and at one octave above the center frequency of the fatiguing sound were then measured after exposure to that SEL. Depending on the results, thresholds were measured after exposure to higher and lower SELs with each of the three hearing test frequencies per fatiguing sound, thus always protecting the hearing of the sea lions. The lowest SEL tested per hearing frequency depended on the TTS generated: generally, once TTS_{1-4} was less than ~2 dB, lower SELs were not tested.

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 2, 2.8, 4.2, 5.6, or 8 kHz) but was followed by exposure to the normal (very low) ambient noise in the indoor pool for 60 min (Figure 2). The post-ambient exposure (PAE) hearing test session was divided into three periods per subject: 1-4 (PAE_{1-4}), 4-8 (PAE_{4-8}), and 8-12 (PAE_{8-12}) min after ambient noise exposure for F01, and 12-16 (PAE_{12-16}), 16-20 (PAE_{16-20}), and 20-24 (PAE_{20-24}) min after ambient noise exposure for M02. Pre-exposure hearing test sessions continued until 10 reversals were obtained, which occurred within ~8 min. The PAE test sessions were always 12 min. Control tests were randomly dispersed during the study period among the fatiguing sound exposure tests and also started at around 0900 h.

The effect of the duty cycle of the fatiguing sound was tested with the NB centered at 4 kHz at the highest SPL (169 dB re 1 μPa). Hearing was tested at 5.6 kHz—the hearing frequency that had the highest initial TTS after exposure to fatiguing sounds at 100% duty cycle. The fatiguing sound's signal duration was set to 1,600 ms, as this is the approximate signal duration presently used in the U.S. Navy's 53-C sonar system. Duty cycles envisioned to be tested were 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 5, and 2.5% (with inter-pulse intervals of 0, 0.2, 0.4, 0.7, 1.1, 1.6, 2.4, 3.7, 6.4, 14.4, 30.4, and 62.4 s, respectively). However, during the first series of tests with decreasing duty cycles, TTS was not found at 50% duty cycle, so 40, 30, 20, 10, and 5% duty cycles were not tested. The 2.5% duty cycle was tested because it is the duty cycle most commonly used in the U.S. Navy's 53-C sonar system. Total exposure duration for all seven duty cycles was 60 min (including inter-pulse intervals). The duty cycles were tested over 7 d in order, from the highest (100%) to the lowest (2.5%), after which this series was repeated once, resulting in $n = 2$ for each duty cycle. The variation in TTS in both sea lions was very small, so further repeats were deemed unnecessary.

To test the equal-energy hypothesis, which states that all combinations of SPL and exposure duration that result in the same SEL elicit similar initial TTS, the sea lions were exposed to several SPL and exposure duration combinations for the NB centered at 4 kHz. The combinations, all resulting in an SEL of 197 dB re 1 $\mu Pa^2 s$, were SPL of 169 dB re 1 μPa for 10 min, SPL of 166 dB re 1 μPa for 20 min, SPL of 163 dB re 1 μPa for 40 min, SPL of 161 dB re 1 μPa for 64 min, and SPL of 160 dB re 1 μPa for 80 min. The duty cycle was always 100%, and hearing was tested at 5.6 kHz, as the highest initial TTS occurred at this hearing frequency (see "Results"). Each SPL and exposure duration combination was tested four times.

Data for the NB centered at 2 kHz were collected between September 2019 and April 2020, and data for the NB centered at 4 kHz were collected between April and October 2020.

Data Analysis

The pre-stimulus response rates by the sea lions for both signal-present and signal-absent trials (in the latter, a whistle indicating the end of the test period was the stimulus) were calculated as the number of pre-stimulus responses as a percentage of the trials in each hearing test period.

The pre-exposure mean 50% hearing threshold ($PE_{50\%}$) for each hearing test session was determined by calculating the mean SPL of all reversal pairs in the pre-exposure hearing session. The TTS, quantified for each hearing test frequency 1 to 4 min after sound exposure stopped (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 initial 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). The level of significance was established by conducting a one-way ANOVA 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.

Recovery of hearing, individual differences in susceptibility to TTS, and the effect of duty cycle on TTS are described without formal statistical analysis. The equal-energy hypothesis was tested by conducting a one-way ANOVA on the initial TTS, separately for each sea lion, with the factor exposure duration and followed by Tukey multiple comparisons.

All analyses were conducted in *Minitab 18*, and data conformed to the assumptions of the tests used (equal variances, normal distribution of data, and residuals; Zar, 1999).

Results

Pre-Stimulus Response Rate

Before and after the 60-min sound exposure periods, the sea lions were always willing to participate in the hearing tests. The pre-stimulus response rates for both signal-present and signal-absent trials were similar in hearing tests in both pre- and post-exposure periods for both control and sound exposure sessions. Therefore, trials from different testing periods and from control and exposure sessions were pooled to calculate pre-stimulus response rate percentages. For F01, the pre-stimulus response rates for the pre-exposure sessions ranged between 5.4 and 12.7%. Her post-exposure pre-stimulus response rate (mostly PSE_{1-4} and some PSE_{12-16}) ranged between 2.7 and 14.5%.

For M02, the pre-stimulus response rates for the pre-exposure sessions ranged between 8.6 and 13.7%. His post-exposure pre-stimulus response rate (mostly PSE_{12-16} and some PSE_{1-4}) ranged between 7.0 and 14.4%.

Effect of SEL of the NB at 2 kHz on TTS Levels and Recovery Time

The one-way ANOVAs to investigate onset of TTS showed that both TTS_{1-4} (F01) and TTS_{12-16} (M02) were significantly affected by the 2 kHz fatiguing sound's SEL at all three hearing frequencies tested. Comparisons with the control revealed that the statistically significant onset of TTS varied depending on the sea lion and the hearing test frequency (Table 1).

No change in susceptibility to TTS was observed during the study. 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 (Table 1).

TTS and Recovery in California Sea Lions After Exposure to the NB at 2 kHz

With a hearing test signal of 2 kHz, statistically significant TTS_{1-4} occurred in F01 after SELs of ≥ 180 dB (re 1 μPa²s; Table 1; Figure 4a). Hearing recovered within 12 min after SELs of 180 and 186 dB and within 60 min after SELs of ≥ 192 dB (Figure 5a). With a hearing test signal of 2.8 kHz, statistically significant TTS_{1-4} occurred after SELs of ≥180 dB (Table 1; Figure 4a). Recovery of hearing occurred within 12 min after an SEL of 180 dB and within 60 min after SELs of ≥ 186 dB (Figure 5b). With a hearing test signal of 4.2 kHz, significant TTS_{1-4} occurred after SELs of ≥ 186 dB (Table 1; Figure 4a). Recovery of hearing occurred within 8 min after SELs of 186 and 192 dB, within 12 min after an SEL of 198 dB, and within 60 min after an SEL of 203 dB (Figure 5c).

With a hearing test signal of 2 kHz, statistically significant TTS_{12-16} occurred in M02 after SELs of ≥ 186 dB (re 1 μPa²s; Table 1; Figure 4b). Hearing recovered within 20 min after SELs of ≤ 198 dB and within 72 min after an SEL of 203 dB (Figure 6a). With a hearing test signal of 2.8 kHz, statistically significant TTS_{12-16} occurred after an SEL of ≥ 186 dB (Table 1; Figure 4b). Recovery of hearing occurred within 20 min after SEL of 186 dB, and within 24 min after all other SELs (Figure 6b). With a hearing test signal of 4.2 kHz, significant TTS_{12-16} only occurred after an SEL of 203 dB (Table 1; Figure 4b), and recovery occurred within 20 min (Figure 6c).

Individual Differences in TTS After Exposure to a 2 kHz NB at a High SEL

During four sessions, the order in which the California sea lions were tested at hearing frequency 2 kHz after exposure to the NB at 2 kHz (SEL 203 dB re 1 μPa²s) was reversed. The mean TTS_{1-4} in M02 (10.7 dB, SD = 1.8 dB, n = 4) was 0.2 dB higher than the mean TTS_{1-4} in F01 after exposure to the same SEL (10.5 dB, SD = 0.4 dB, n = 4). The recovery patterns were similar (Figure 7a). The mean TTS_{12-16} in F01 (6.2 dB, SD = 1 dB, n = 4) was 0.6 dB higher than the mean TTS_{12-16} in M02 after exposure to the same SEL (5.6 dB, SD = 1.3 dB, n = 4; Table 1; Figure 4b). The recovery patterns were similar (Figure 7b).

Table 1. The mean underwater and aerial SPLs during ambient (control) and exposure conditions, and the mean, standard deviation (SD), and range of initial TTS (TTS_{1-4} and TTS_{12-16} in F01 and M02, respectively) after exposure to a continuous one-sixth-octave noise band centered at 2 kHz for 60 min at several SELs, quantified at hearing frequencies 2, 2.8, and 4.2 kHz. No TTS occurred during control sessions. *TTS significantly different from control value; *n* = sample size. SELs were calculated from underwater SPLs because the sea lions kept their heads underwater most of the time.

Hearing test freq. (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*
2.0	Ambient	Control	47	0.1	0.8	-0.7-1.0	5	0.3	0.6	-0.3-1.3	5
	138	174	56	1.2	0.5	0.8-1.9	4	0.5	0.4	0.0-1.1	4
	144	180	61	3.7*	0.8	2.9-4.8	4	0.8	1.3	-0.3-2.6	4
	150	186	67	5.0*	1.0	3.6-5.8	4	2.8*	0.8	2.2-3.9	4
	156	192	74	7.9*	1.3	7.0-9.8	4	3.9*	0.7	3.3-4.8	4
	162	198	80	9.4*	1.1	8.3-10.9	4	3.6*	0.4	3.2-4.1	4
	167	203	85	10.5*	0.4	10.1-11.1	4	5.6*	1.3	4.1-7.7	5
2.8	Ambient	Control	47	0.2	0.7	-0.8-1.4	6	1.1	0.9	0.0-2.3	5
	138	174	56	0.0	0.9	-0.7-1.2	4	-0.2	1.5	-2.0-1.7	4
	144	180	61	3.6*	0.5	3.1-4.3	4	0.0	0.5	-0.5-0.5	4
	150	186	67	5.9*	1.0	4.6-6.8	4	3.0*	0.8	2.5-4.2	4
	156	192	74	9.7*	0.8	8.8-10.7	4	6.7*	1.6	4.7-8.5	4
	162	198	80	8.3*	1.2	7.0-9.6	4	5.8*	0.5	5.5-6.5	4
	167	203	85	10.2*	0.2	9.9-10.3	4	4.5*	0.4	4.1-5.2	5
4.2	Ambient	Control	47	1.0	0.7	0.2-1.7	5	0.3	1.2	-0.8-2.1	5
	144	180	61	0.9	0.9	0.2-2.5	5	1.1	1.3	-0.3-2.9	5
	150	186	67	3.2*	0.4	2.8-3.8	4	0.1	1.2	-1.3-1.5	4
	156	192	74	4.4*	1.0	3.4-5.8	4	0.4	0.4	-0.1-0.8	4
	162	198	80	4.9*	1.1	3.7-6.0	4	1.3	1.4	-0.6-2.6	4
	167	203	85	8.2*	0.8	7.5-9.1	4	3.8*	1.0	2.5-4.8	4

Effect of SEL of the NB at 4 kHz on TTS Levels and Recovery Time

The one-way ANOVAs to investigate onset of TTS showed that both TTS_{1-4} (F01) and TTS_{12-16} (M02) were significantly affected by the 4 kHz fatiguing sound's SEL at all hearing frequencies. 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. 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 (Table 2).

TTS and Recovery in California Sea Lions After Exposure to the NB at 4 kHz

With a hearing test signal of 4.2 kHz, statistically significant TTS_{1-4} occurred in F01 after exposure to SELs of ≥ 187 dB (re 1 µPa²s; Table 2; Figure 8a). Hearing recovered within 12 min after SELs of 187 and 193 dB, and within 60 min after SELs of 199 and 205 dB (Figure 9a). With a hearing test signal of 5.6 kHz, statistically significant TTS_{1-4} occurred after SELs of ≥ 175 dB (Table 2; Figure 8a). Recovery of hearing occurred within 8 min after an SEL of 175 dB, within 12 min after an SEL of 181 dB, within 60 min after SELs of 187, 193, and 199 dB, and within 120 min for an SEL of 205 dB (Figure 9b). With a hearing test signal of 8 kHz, significant TTS_{1-4} occurred after SELs of ≥ 187 dB (Table 2; Figure 8a). Recovery of hearing occurred within 12 min after an SEL of 187 dB, within 60 min after SELs of 193 and 199 dB, and within 240 min after an SEL of 205 dB (Figure 9c).

With a hearing test signal of 4.2 kHz, statistically significant TTS_{12-16} occurred in M02 after an SEL of ≥ 199 dB (re 1 µPa²s; Table 2; Figure 8b). Hearing recovered within 72 min (Figure 10a). With a hearing test signal of 5.6 kHz, statistically significant TTS_{12-16} occurred after SELs of ≥ 193 dB (Table 2; Figure 8b). Recovery of hearing occurred within

Table 2. The underwater and aerial SPLs during ambient (control) and exposure conditions, and the mean, standard deviation (SD), and range of initial TTS (TTS_{1-4} and TTS_{12-16} in F01 and M02, respectively) after exposure to a continuous one-sixth-octave noise band centered at 4 kHz for 60 min at several SELs, quantified at hearing frequencies 4.2, 5.6, and 8 kHz. No TTS occurred during control sessions. *TTS significantly different from control value; *n* = sample size. SELs were calculated from underwater SPLs because the sea lions kept their heads underwater most of the time.

Hearing test freq. (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*
4.2	Ambient	Control	45	0.1	1.0	-0.9-1.6	5	1.3	0.2	1.0-1.6	5
	145	181	62	0.6	1.0	-0.3-2.0	4	1.0	0.5	0.4-1.5	4
	151	187	68	5.3*	1.7	4.3-7.8	4	2.9	1.3	1.5-4.3	4
	157	193	74	6.6*	1.2	5.3-8.2	4	3.1	0.9	2.3-4.3	4
	163	199	80	9.1*	1.9	7.6-11.8	4	7.2*	1.2	6.3-9.1	4
	169	205	86	11.9*	1.3	10.5-13.4	4	9.4*	1.2	7.7-10.2	4
5.6	Ambient	Control	45	0.2	1.2	-1.5-1.7	8	-0.4	1.3	-2.5-1.4	8
	133	169	51	1.2	0.5	0.8-1.9	4	0.2	0.9	-1.2-1.0	4
	139	175	56	2.7*	1.2	1.4-4.3	4	0.7	0.6	-0.1-1.4	4
	145	181	62	8.3*	1.7	6.3-10.2	4	0.8	1.2	-0.7-2.1	4
	151	187	68	9.3*	0.7	8.6-10.2	4	1.7	1.0	0.4-2.4	4
	157	193	74	14.6*	1.3	13.0-15.9	4	4.9*	1.0	3.5-5.9	4
	163	199	80	18.1*	1.6	16.7-20.5	4	5.8*	1.4	4.5-7.9	4
	169	205	86	22.4*	1.9	19.6-24.1	5	9.8*	2.8	6.5-12.9	6
8.0	Ambient	Control	Ambient	-0.2	0.5	-0.5-0.5	4	0.0	0.4	-0.5-0.5	4
	139	175	56	1.0	0.7	0.3-1.8	4	-0.2	1.2	-1.9-0.7	4
	145	181	62	1.1	0.5	0.6-1.9	4	2.9*	1.0	1.7-4.2	4
	151	187	68	8.8*	0.8	8.0-9.9	4	4.4*	0.6	3.6-5.0	4
	157	193	74	8.4*	0.9	7.4-9.4	4	7.7*	2.2	4.8-9.8	4
	163	199	80	11.9*	1.1	10.4-12.8	4	8.3*	1.0	7.0-9.4	4
	169	205	86	18.9*	0.6	18.2-19.6	4	13.3*	1.1	11.9-14.7	4

20 min after SELs of 193 dB, within 24 min after an SEL of 199 dB, and within 72 min after an SEL of 205 dB (Figure 10b). With a hearing test signal of 8 kHz, significant TTS_{12-16} occurred after SELs of ≥ 181 dB (Table 2; Figure 8b). Recovery of hearing occurred within 20 min after SELs of 181 and 187 dB, within 24 min after an SEL of 193 dB, within 72 min after an SEL of 199 dB, and within 132 min after an SEL of 205 dB (Figure 10c).

Individual Differences in Susceptibility to TTS After Exposure to a NB at 4 kHz

During eight sessions (four per hearing test frequency), the order in which the California sea lions were tested at hearing frequencies 5.6 and 8 kHz after exposure to the NB at 4 kHz (SEL 205 dB re 1 µPa²s) was reversed.

The mean TTS_{1-4} of M02, measured at 5.6 kHz (21.3 dB, SD = 2.1 dB, *n* = 4), was 1.1 dB lower than the mean TTS_{1-4} of F01 after exposure to the same SEL (22.4 dB, SD = 1.9 dB, *n* = 5; Figure 11a). The recovery patterns were similar. The mean TTS_{12-16} of F01 measured at 5.6 kHz (12.4 dB, SD = 2.2 dB, *n* = 4) was 2.6 dB higher than the mean TTS_{12-16} of M02 after exposure to the same SEL (9.8 dB, SD = 2.8 dB, *n* = 6; Table 2; Figure 11b). The recovery patterns were similar.

The mean TTS_{1-4} of M02 measured at 8 kHz (19.8 dB, SD = 1.0 dB, *n* = 2) was 0.9 dB higher than the mean TTS_{1-4} of F01 after exposure to the same SEL (18.9 dB, SD = 0.7 dB, *n* = 4; Figure 12a). The recovery patterns were similar. The mean TTS_{12-16} of F01 measured at 8 kHz (12.6 dB, SD = 1.3 dB, *n* = 4) was 0.6 dB lower than the mean TTS_{12-16} of M02 after exposure to the same SEL (13.3 dB, SD = 1.1 dB, *n* = 2; Figure 12b). The recovery patterns were similar.

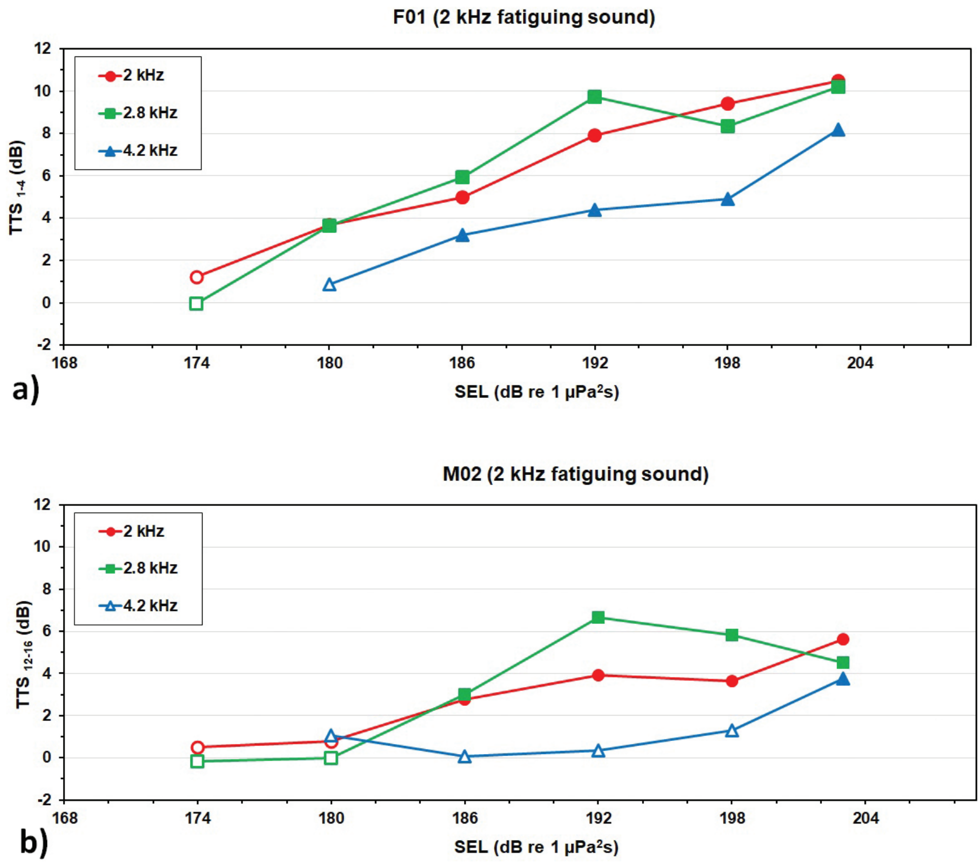

Figure 4. Mean TTS_{1-4} in F01 (a) and mean TTS_{12-16} in M02 (b) after exposure for 60 min to a continuous one-sixth-octave NB centered at 2 kHz at several SELs, quantified at hearing frequencies 2, 2.8, and 4.2 kHz (i.e., at the center frequency of the fatiguing sound, half an octave above it, and one octave above it). Solid symbols indicate significant TTS relative to the control sessions. Sample size varies per data point (see Table 1). For SPLs (dB re 1 µPa), subtract 36 dB from the SEL values. For control values, see Table 1 and Figures 5 & 6.

Effect of Fatiguing Sound Duty Cycle on TTS
The duty cycle of the fatiguing sound had a strong effect on TTS_{1-4} in F01 (Figure 13a) and on TTS_{12-16} in M02 (Figure 13b). The biggest reduction in TTS occurred when the duty cycle was reduced from 100 to 90%: the mean initial TTS decreased by 12.8 dB in F01 and by 6.5 dB in M02. At duty cycles ≤ 60%, no TTS occurred in the sea lions (Figure 13).

Testing the Equal-Energy Hypothesis
After exposure to five combinations of SPL and exposure duration that resulted in fatiguing sounds with the same underwater SEL (i.e., 197 dB re 1 µPa²s), hearing was tested at 5.6 kHz. Each combination was tested four times, and the initial TTSs were very similar (Figure 14). One-way ANOVAs were significant ($p < 0.001$) for both sea lions. Tukey multiple comparisons showed that similar TTSs occurred after all exposure combinations since they all differed significantly from the control and not from one another. In both subjects, recovery patterns were similar after exposure to all combinations (Figure 15).

Discussion

Baseline Hearing Thresholds, Performance, and Aerial Sound Exposure
During pre-exposure periods, the hearing thresholds of the two California sea lions for the hearing test signals (2, 2.8, 4.2, 5.6, and 8 kHz) were within 5 dB of the audiogram of a 3-y-old female California sea lion (Reichmuth et al., 2013; Figure 16). Thus, for the frequency range that was tested, the subjects of the present study probably

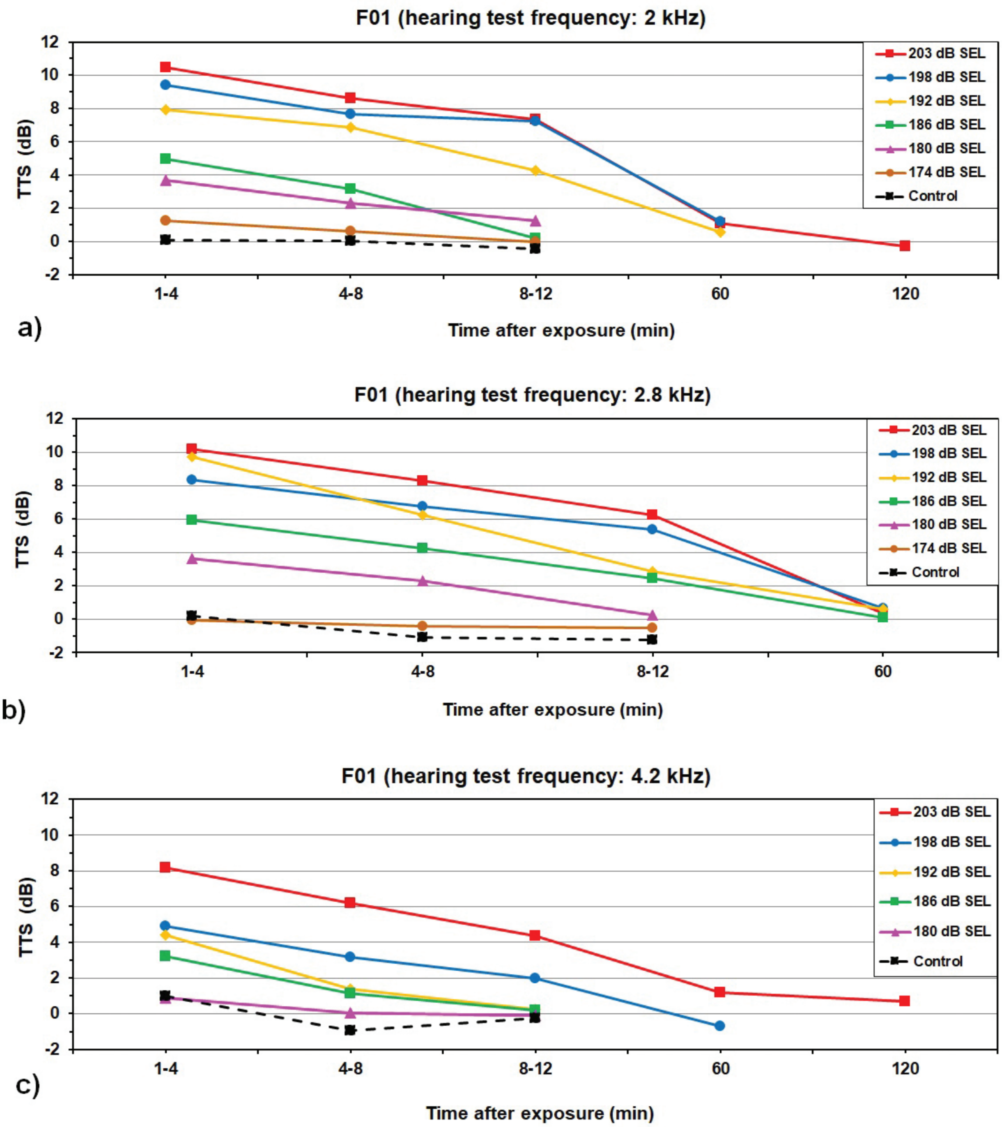

Figure 5. Changes in the hearing thresholds of F01, including recovery, tested at 2 kHz (a), 2.8 kHz (b), and 4.2 kHz (c) after exposure to a continuous one-sixth-octave NB centered at 2 kHz for 60 min at several SELs. Hearing was considered recovered once TTS was < 2 dB. Mean TTSs are shown. For sample sizes and SDs (only for $TTS_{1\text{-}4}$), see Table 1. The X-axis scale in (b) differs from those in (a) and (c). For average received SPLs (dB re 1 µPa), subtract 36 dB from the SEL values. The "TTS" values (no shift occurred) during control sessions are also shown.

had normal hearing representative of California sea lions.

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 subjects. This suggests that the sea lions used the same decision-making criteria in all conditions and that their decision-making process in the post-exposure hearing tests was not influenced by the sound exposure.

The susceptibility of terrestrial mammals to TTS may change over time (Kujawa & Liberman, 1997; Mannström et al., 2015), but such changes were not observed in the present study. Susceptibility to TTS may have been stable throughout the study period due to the relatively

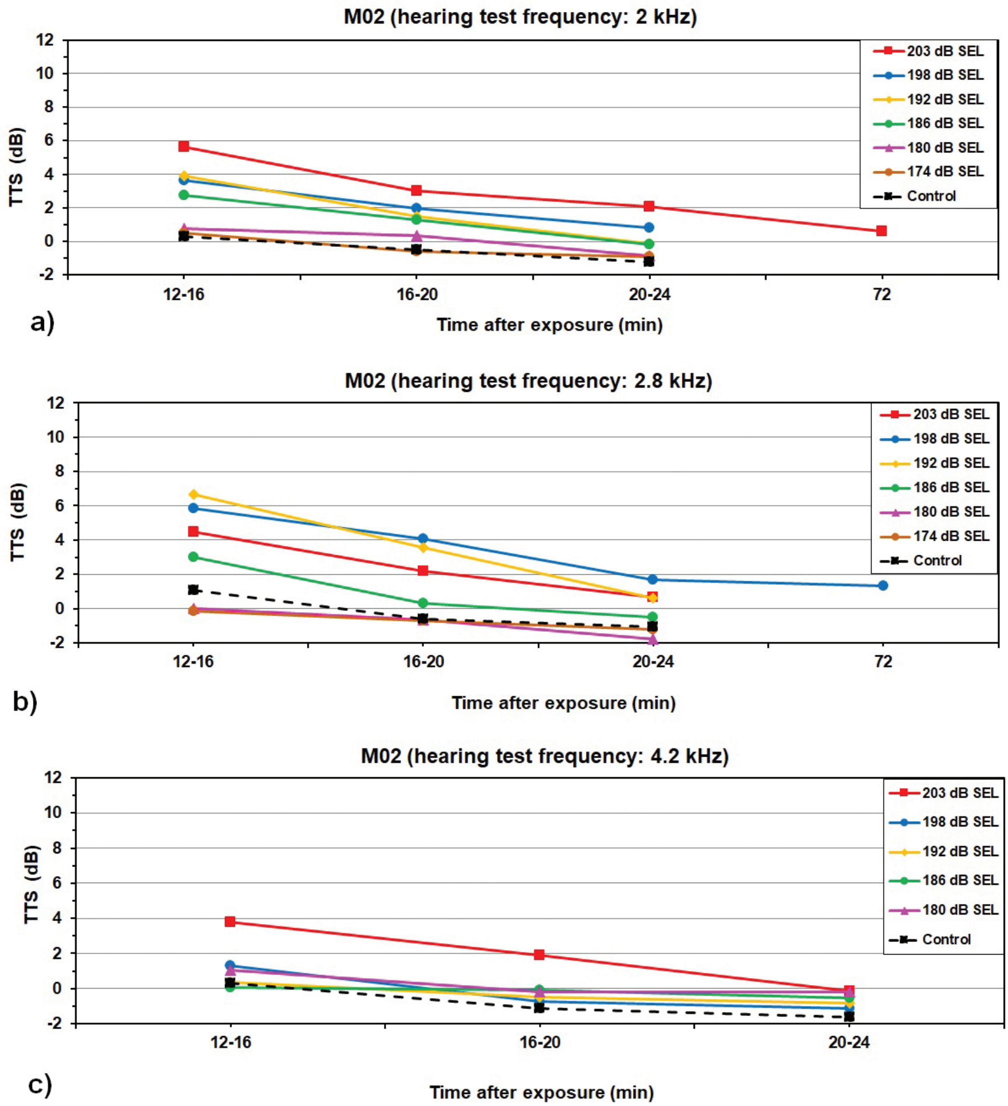

Figure 6. Changes in the hearing thresholds of M02, including recovery, tested at 2 kHz (a), 2.8 kHz (b), and 4.2 kHz (c) after exposure to a continuous one-sixth-octave NB centered at 2 kHz for 60 min at several SELs. Hearing was considered recovered once TTS was < 2 dB. Mean TTSs are shown. For sample sizes and SDs (only for TTS_{12-24}), see Table 1. The X-axis scale in (c) differs from those in (a) and (b). For average received SPLs (dB re 1 µPa), subtract 36 dB from the SEL values. The "TTS" values (no shift occurred) during control sessions are also shown.

short exposure periods and the relatively low TTSs elicited.

The projection of aerial fatiguing sound during exposure sessions was deemed unnecessary, as the sea lions swam mostly underwater, taking mainly single, short breaths while lifting only their noses out of the water, and occasionally breathing during a short jump which lasted a fraction of a second. It was assumed that acoustic energy reaches the ears as if the entire head were below the water surface, as long as the lower jaw is below the surface (as occurs in seals; Kastelein et al., 2018). Even when their lower jaws were above the water surface, the subjects were exposed to the fatiguing sound at high SPLs, as demonstrated by the SPLs measured in air during exposure periods (Tables 1 & 2). In many cases, the aerial SPLs were so high that the operator in the equipment cabin had to wear ear protectors.

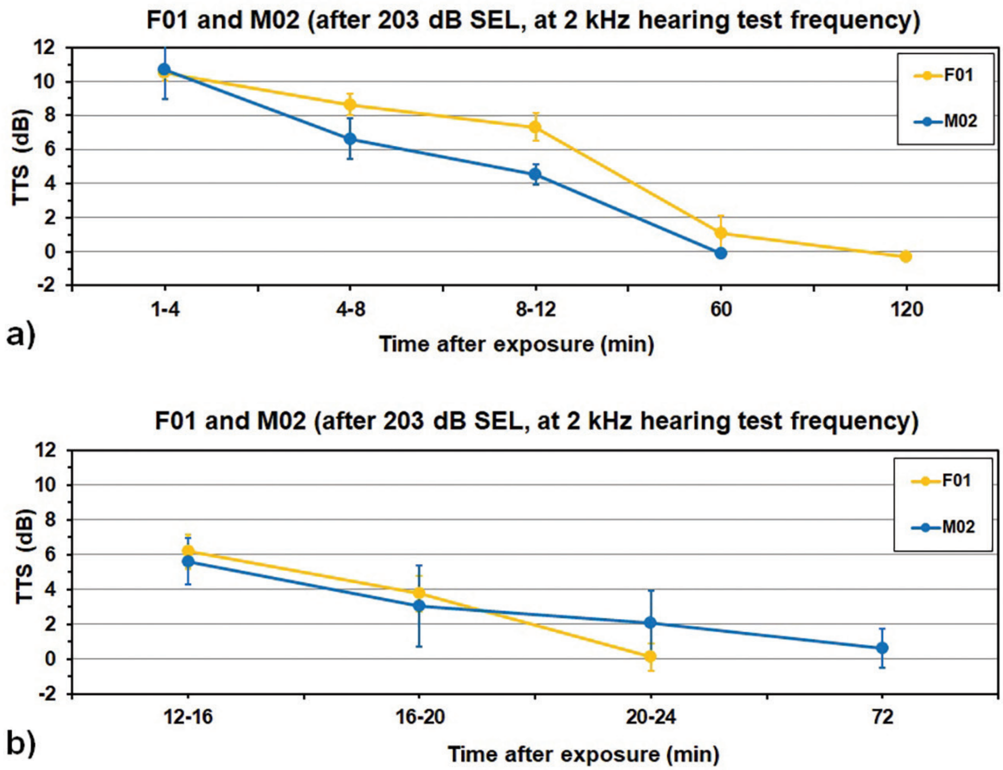

Figure 7. Mean TTS (± SD; *n* = 4) at 2 kHz in F01 and M02 measured 1 to 12, 60, and 120 min (a) and 12 to 24 and 72 min (b) after exposure for 60 min to the NB at 2 kHz at an SEL of 203 dB re 1 μPa^2s.

Magnitude of TTS and Onset SEL

Southall et al. (2019) proposed the lowest SEL required to elicit 6 dB TTS as a marker of TTS onset. By this definition, the onset of $TTS_{1\text{-}4}$ in F01 after exposure to the NB at 2 kHz occurred at an SEL of 186 dB re 1 μPa^2s (at 2.8 kHz); after exposure to the NB at 4 kHz, onset occurred at 179 dB re 1 μPa^2s (at 5.6 kHz). After exposure to the NB at 2 kHz, the onset of $TTS_{12\text{-}16}$ in M02 (after some recovery of hearing) occurred at 191 dB re 1 μPa^2s (at 2.8 kHz); after exposure to the NB at 4 kHz, onset occurred at 190 dB re 1 μPa^2s (at 8 kHz). These results 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 (*Phocoena phocoena*; Kastelein et al., 2021), Yangtze finless porpoises (*Neophocaena phocaenoides asiaeorientalis*; Popov et al., 2011), and harbor seals (Kastelein et al., 2020).

The SEL causing TTS onset (1 to 4 min; 6 dB) in the California sea lions in the present study by sound at frequencies of 2 and 4 kHz was ~14 to 20 dB lower than the SEL reported by Kastak et al. (2005; Figure 16) by 2.5 kHz, but similar to the SEL causing TTS onset in harbor seals by 4 kHz (Kastelein et al., 2012b, 2020). When Kastak et al.'s (2005) study was conducted, TTS measurements in marine mammals were still at an early stage. In the present study, some 15 y later, a well-established TTS measurement procedure was used (TTS measurements during 1 to 4 min after the fatiguing sound stopped at three hearing frequencies, followed by repeated measurements until hearing had recovered). The lower initial TTSs and higher TTS onset SELs reported by Kastak et al. could be due to a wide range (and combination) of factors, such as (1) the subject's lower susceptibility to TTS, (2) the lower duty cycle of the fatiguing sound, (3) the actual received SEL being lower than the stated SEL due to swimming behavior and distance of the subject to the fatiguing sound transducer, (4) longer delays between the end of sound exposure and start of post-exposure hearing tests, (5) more time used to quantify post-exposure hearing thresholds, (6) higher levels

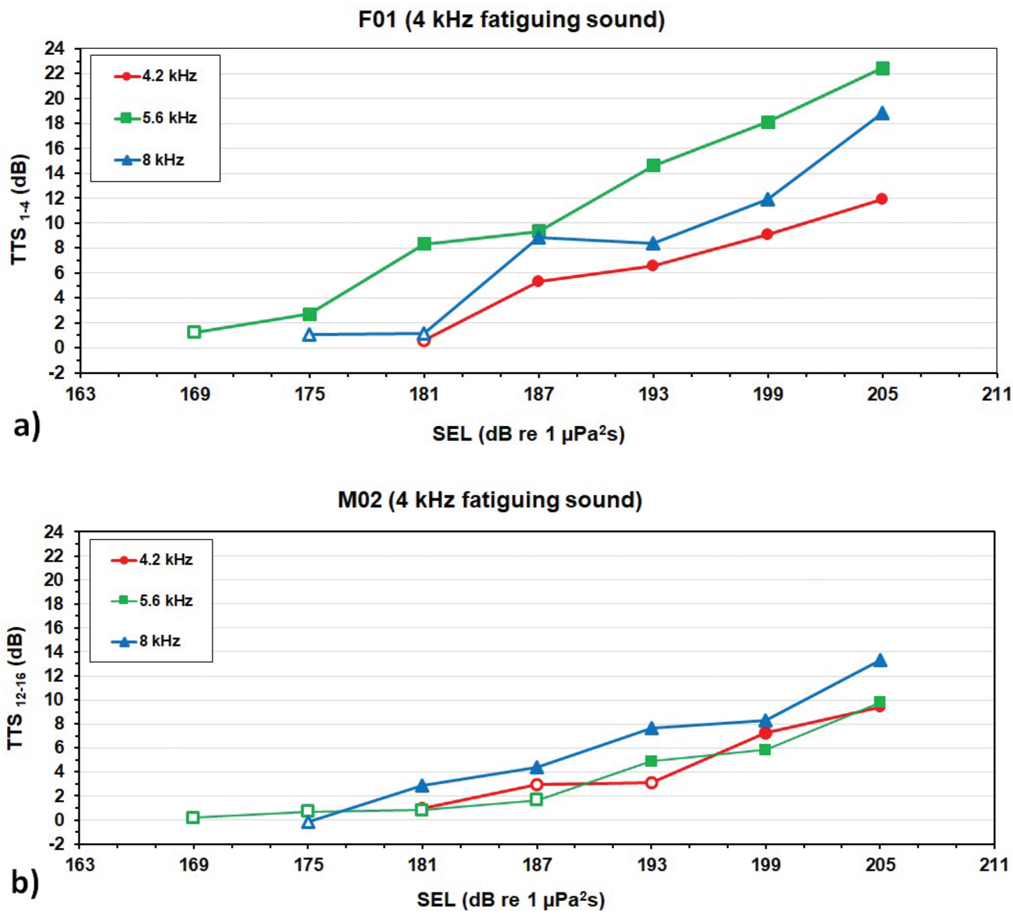

Figure 8. TTS_{1-4} in F01 (a) and TTS_{12-16} in M02 (b) after exposure for 60 min to a continuous one-sixth-octave NB centered at 4 kHz at several SELs, quantified at hearing frequencies 4.2, 5.6, and 8 kHz (i.e., at the center frequency of the fatiguing sound, half an octave above it, and one octave above it). Sample size (*n*) varies per data point shown. For SPLs (dB re 1 μPa), subtract 36 dB from the SEL values. For control values, see Table 2 and Figures 9 & 10. Solid symbols indicate significant TTS relative to the control sessions.

of background noise, and (7) the wider bandwidth of the fatiguing sound.

Individual variation in TTS susceptibility (factor 1) could explain the difference, but such big differences were not observed in studies with other marine mammals using comparable methodology (Kastelein et al., 2020, 2021), and little or no individual variation was observed in the present study. However, sample sizes in all studies were small, and the subjects in the present study are genetically related. For factor 2, Kastak et al.'s (2005) subject was exposed to sound 75% of the time (~75% duty cycle). Non-exposure time was included when calculating the SEL, but recovery of hearing during breaks in the exposure was not considered. As is clear from the results of the present study, the duty cycle of the fatiguing sound affects the elicited TTS significantly, which was also seen in a TTS study with harbor porpoises (Kastelein et al., 2014b). Hearing recovery in non-exposure times (pauses) in the study by Kastak et al. (2005) may therefore have led to lower initial TTSs and higher TTS onset SELs than observed in the present study (Figure 16). In relation to factor 3, Kastak et al.'s (2005) subject's location was at the transition of the near field (where complex interactions between the sound waves determine the SEL) and far field (where complex interactions are absent) of the transducer so that small differences in its head position may have resulted in large differences in sound exposure. In the

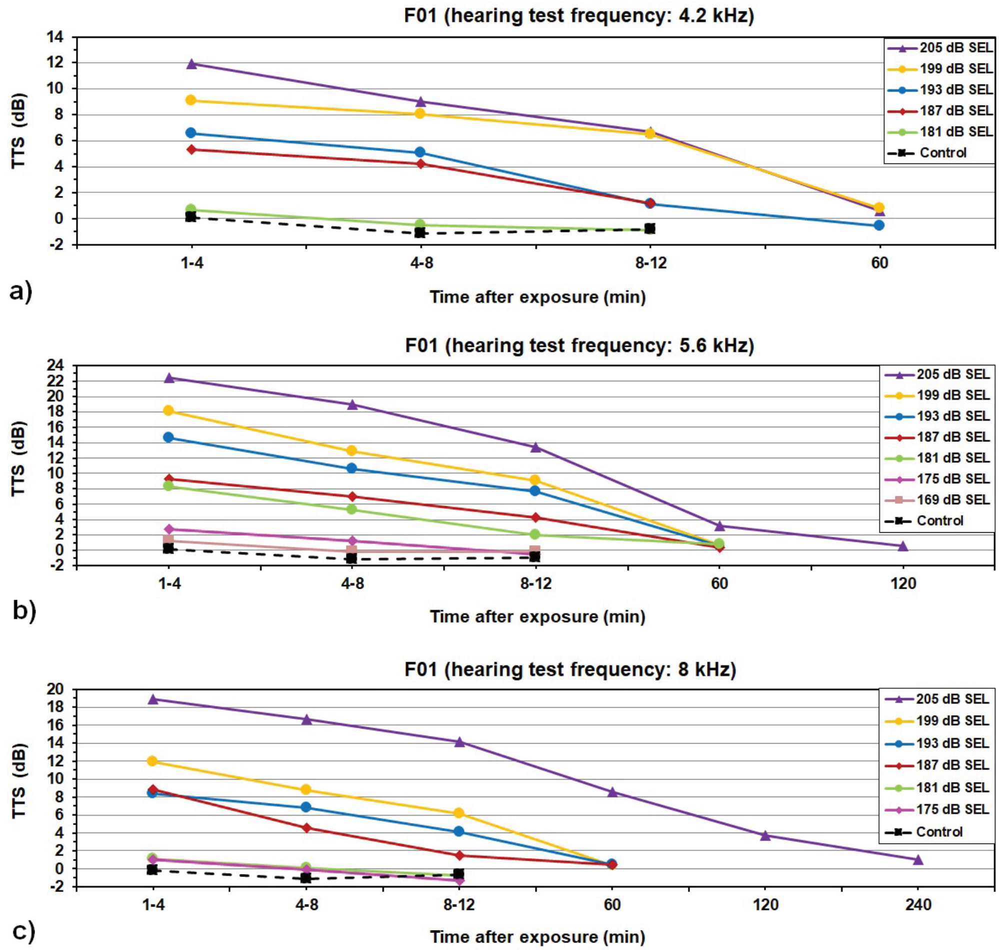

Figure 9. Changes in the hearing thresholds of F01, including recovery, tested at 4.2 kHz (a), 5.6 kHz (b), and 8 kHz (c) after exposure to a continuous one-sixth-octave NB centered at 4 kHz for 60 min at several SELs. Hearing was considered recovered once TTS was < 2 dB. Mean TTSs are shown. For sample sizes and SDs (only for TTS_{1-4}), see Table 2. Note that the X- and Y-axis scales differ in (a), (b), and (c). For average received SPLs (dB re 1 µPa), subtract 36 dB from the SEL values. The "TTS" values (no shift occurred) during control sessions are also shown.

present study, the subjects were in the far field of the transducer, swimming fast in the entire pool, where the SPL was fairly homogeneous during exposure periods (Figure 2). The duration of the delay between the end of fatiguing sound exposures and the start of post-exposure hearing tests (factor 4) was not reported by Kastak et al. (2005). If the delay was longer than in the present study, in which hearing tests started within 1 min after the sound stopped, the mean post-exposure hearing thresholds reported by Kastak et al. included more recovery of hearing than those in the present study. This would have resulted in lower initial TTSs and, thus, higher TTS onset SELs than observed in the present study in F01 (Figure 16). This phenomenon (i.e., lower initial TTS due to later start of TTS measurements after the fatiguing sound stopped) is actually seen in the TTS_{12-16} of M02 (Figure 16). The time required to quantify post-exposure hearing measurements (factor 5) by Kastak et al. (2005) was "generally 15 min" (p. 3157) so it was longer than the 4 min required in the present study. This means that the post-exposure hearing thresholds reported by Kastak et al. included more recovery of hearing than those in the present study. In the present study, recovery in TTS of up to 6 dB occurred between 1 to 4 and 8 to 12 min post-exposure, indicating that differences in measurement duration after exposure could affect

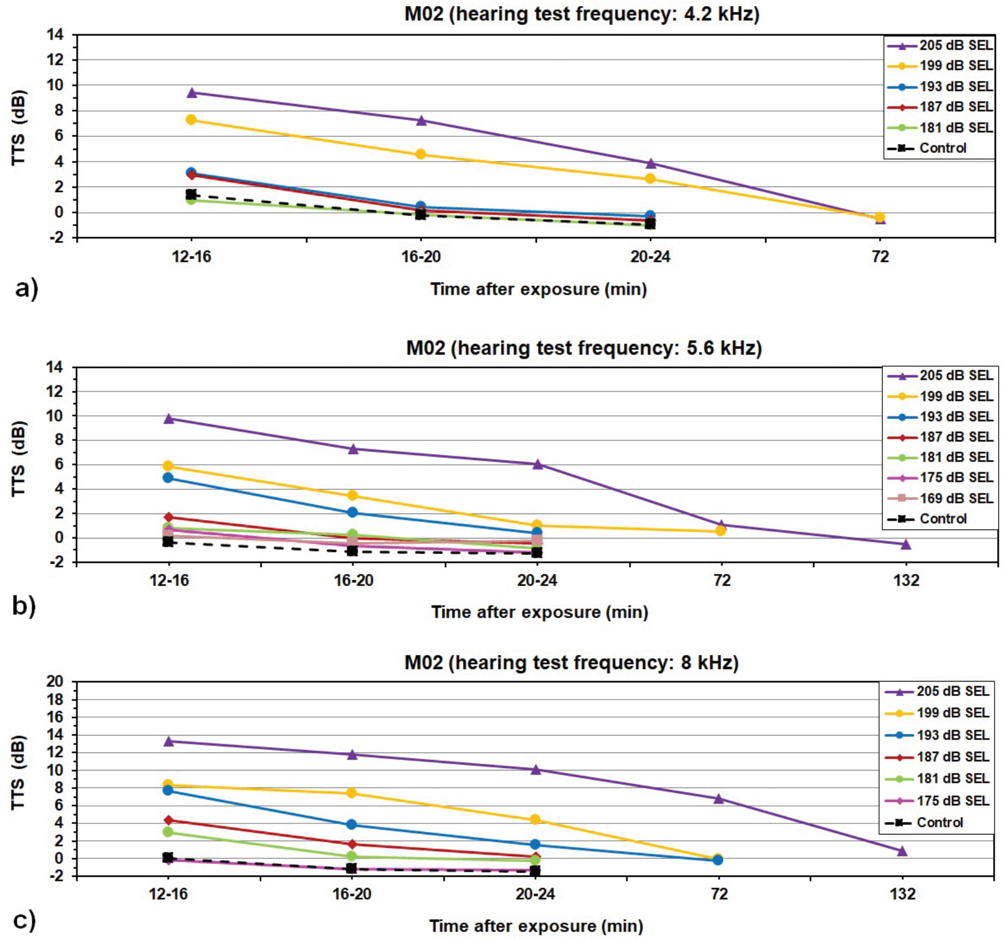

Figure 10. Changes in the hearing thresholds of M02, including recovery, tested at 4.2 kHz (a), 5.6 kHz (b), and 8 kHz (c) after exposure to a continuous one-sixth-octave NB centered at 4 kHz for 60 min at several SELs. Hearing was considered recovered once TTS was < 2 dB. Mean TTSs are shown. For sample sizes and SDs (only for TTS_{12-24}), see Table 2. Note that the X- and Y-axis scales differ in (a), (b), and (c). For average received SPLs (dB re 1 µPa), subtract 36 dB from the SEL values. The "TTS" values (no shift occurred) during control sessions are also shown.

the observed initial TTS. In relation to factor 6, the present study was conducted under very low background noise level conditions, so masking did not occur. Kastak et al. (2005) did not report the background noise level, but higher and more fluctuating levels than in the present study could have influenced the TTS measurements; masking during the pre-exposure hearing test could have reduced the initial TTS and, thus, would have resulted in higher TTS onset SELs. In relation to factor 7, the bandwidth of the fatiguing sound used by Kastak et al. (2005) was one octave (spanning about three critical bandwidths; Southall et al., 2003), whereas the bandwidth in the present study was one-sixth-octave (less than one critical bandwidth). However, TTS generated with octave-band noise centered at 4 kHz was similar to TTS generated with one-sixth-octave NBs centered at 2 and 6 kHz in harbor seals (Kastelein et al., 2012b, 2019, 2020).

Hearing Frequency Most Affected by Each Fatiguing Sound

TTS was elicited after exposure to both fatiguing sounds, at all three hearing frequencies, in both sea lions. In the SEL range tested with the NB at 2 kHz, the highest TTS in F01 occurred at the hearing frequency of the center frequency of the fatiguing sound (2 kHz) and in M02 at 2.8 kHz. In the SEL range tested with the NB at 4 kHz, the

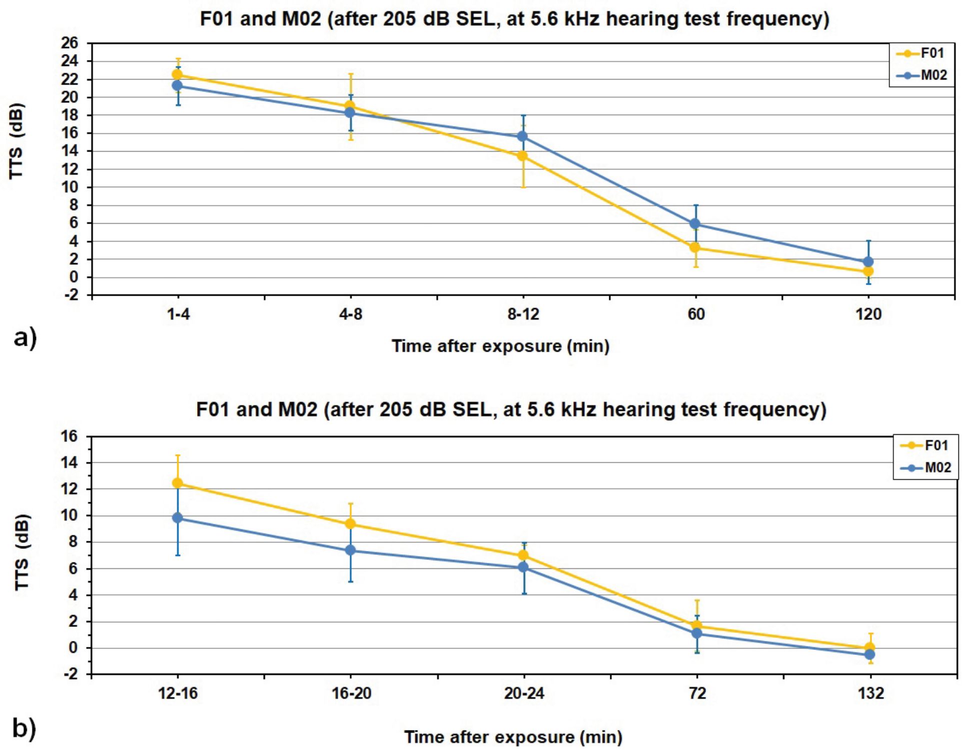

Figure 11. Mean TTS (± SD; *n* = 4) at 5.6 kHz in F01 and M02 measured 1 to 12, 60, and 120 min (a) and 12 to 24, 72, and 132 min (b) after exposure to the NB at 4 kHz at an SEL of 205 dB re 1 µPa^{2}s.

highest TTS occurred at the hearing frequency half an octave above the center frequency of the fatiguing sound (5.6 kHz) in F01, and one octave above the center frequency of the fatiguing sound (8 kHz) in M02.

TTS research in terrestrial mammals suggests that, in most cases, the maximum TTS is induced half an octave above the fatiguing sound's frequency (Cody & Johnstone, 1981; McFadden & 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 & Johnstone (1981) found TTS/SPL curves for guinea pigs (*Cavia porcellus*) to be much steeper for frequencies more than half an octave above the fatiguing sound's frequency than for frequencies less than half an octave above it. So far in marine mammals, the relationship between received SPL and affected hearing frequency has only been studied in one odontocete species, the harbor porpoise (Kastelein et al., 2014a), and in one phocid, the harbor seal (Kastelein et al., 2019). These studies show that the hearing frequency most affected depends on the SPL of the fatiguing sound and that TTS at the center frequency of the fatiguing sound stabilizes or decreases with increasing SEL. The TTS at half an octave above the center frequency of the fatiguing sound increases with increasing SELs, and the TTS at one octave above the center frequency follows the same pattern but generally starts to increase at higher SELs (Kastelein et al., 2014a, 2019). In the present study with California sea lions, the TTSs increased with increasing SEL at all three hearing frequencies after exposure to SELs of up to 203 dB re 1 µPa^{2}s (NB at 2 kHz; Figure 4; the only exception was TTS_{12-16} at hearing frequency 2.8 kHz) and up to 205 dB re 1 µPa^{2}s (NB at 4 kHz; Figure 8). This indicates that, for the fatiguing sounds and SELs tested in the present study, a large hearing frequency bandwidth was affected.

Pattern of Hearing Recovery

The pattern of hearing recovery in the present study, for all hearing frequencies and all fatiguing sounds, was for recovery from initial TTSs of 0 to 5 dB to take place within 10 min, TTSs of 5 to 10 dB within 60 min, TTSs of 10 to 15 dB within 120 min, and TTSs of 15 to 25 dB within

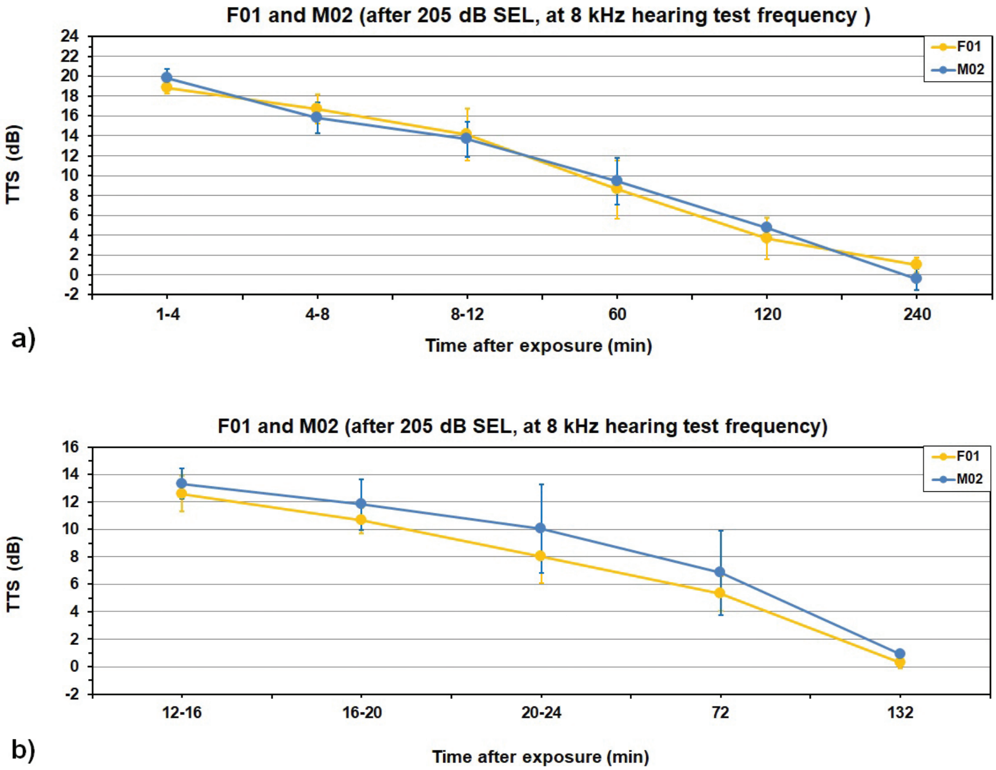

Figure 12. Mean TTS (± SD; *n* = 4) at 8 kHz in F01 and M02 measured 1 to 12, 60, 120, and 240 min (a) and 12 to 24, 72, and 132 min (b) after exposure to the NB at 4 kHz at an SEL of 205 dB re 1 μPa^2s.

240 min. Little more is known about hearing recovery rates in California sea lions. Kastak et al. (2007) exposed a California sea lion to a continuous octave-band noise centered at 2.5 kHz, but they started testing their subject 10 to 15 min after sound exposure stopped and did not test it again the same day unless initial TTS was higher than 20 dB. The TTSs of < 20 dB recovered within 24 h, and six instances of TTS > 20 dB required a longer recovery time. These TTSs followed a linear recovery rate of 8.8 dB per log(min), suggesting that a TTS of 25 dB would require almost 700 min to recover. The present study suggests that recovery from this TTS would take < 240 min. However, in the present study, initial TTSs were tested almost immediately after sound exposure stopped, and Kastak et al.'s (2007) initial TTSs would have been higher if they had been measured sooner after the sound stopped. This may explain the longer recovery time observed for TTSs > 20 dB by Kastak et al.

The pattern of hearing recovery after very high initial TTSs may be different from that after initial TTSs of ≤ 11 dB, as was observed in a harbor seal exposed for 60 min to an octave-band noise centered at 4 kHz at a higher SPL than intended (Kastelein et al., 2012b, 2013). This seal's TTS_{12-16} was 44 dB, and hearing recovered over the course of 4 days. No function was fitted to this recovery pattern, but it differed from observed recovery patterns in the same and another harbor seal for lower initial TTSs (Kastelein et al., 2013).

Individual Differences in Susceptibility to TTS

Testing the hearing of both California sea lions in the present study at the same time after the fatiguing sound stopped showed that TTSs and recovery patterns were similar (Figures 7, 11 & 12). Susceptibility to TTS in both subjects was similar for the NBs at 2 and 4 kHz; however, the sample size is too small to draw general conclusions about variability in susceptibility to TTS within the species, and the sea lions are genetically related (mother and son). 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;

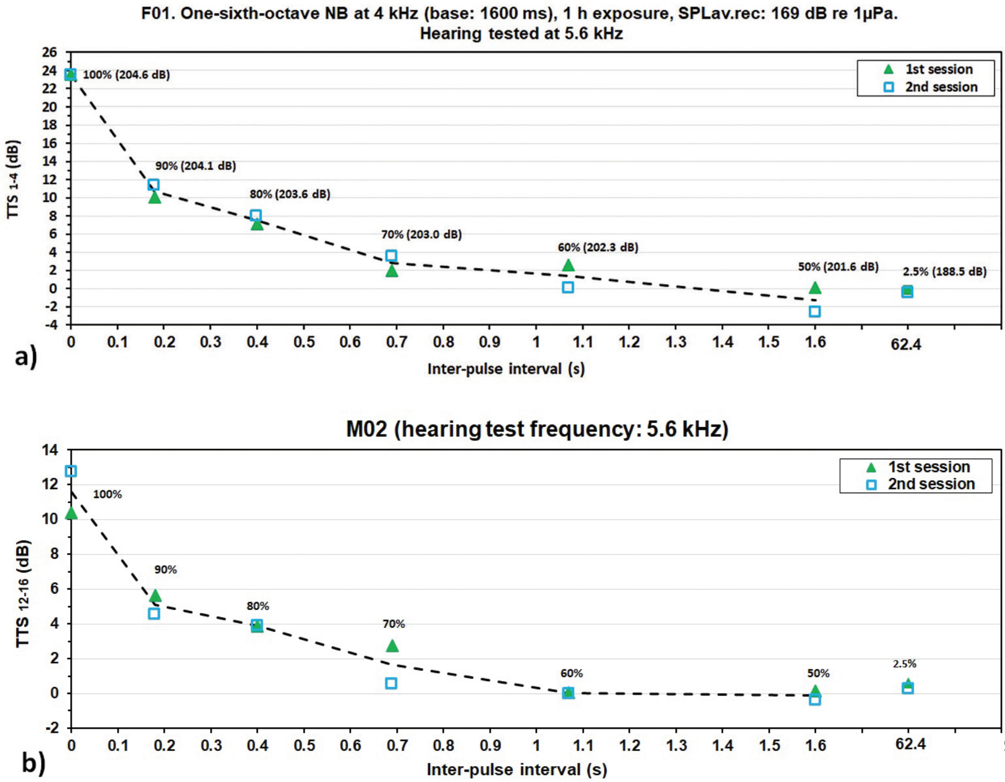

Figure 13. The TTS_{1-4} of F01 (a) and TTS_{12-16} of M02 (b), tested at 5.6 kHz hearing frequency after exposure for 60 min to one-sixth-octave NB centered at 4 kHz at an SPL of 169 dB re 1 µPa, presented with seven different duty cycles and, thus, seven inter-pulse intervals. The signal duration was 1,600 ms. Two sessions were conducted for each duty cycle. The dashed lines are based on the mean of the two measurements. In (a), the cumulative SEL (dB re 1 µPa^{2}s) is shown between parentheses after each duty cycle. No TTS was elicited with duty cycles < 60%. Of duty cycles < 50%, only 2.5% was tested because this duty cycle is generally used for U.S. Navy 53-C sonar.

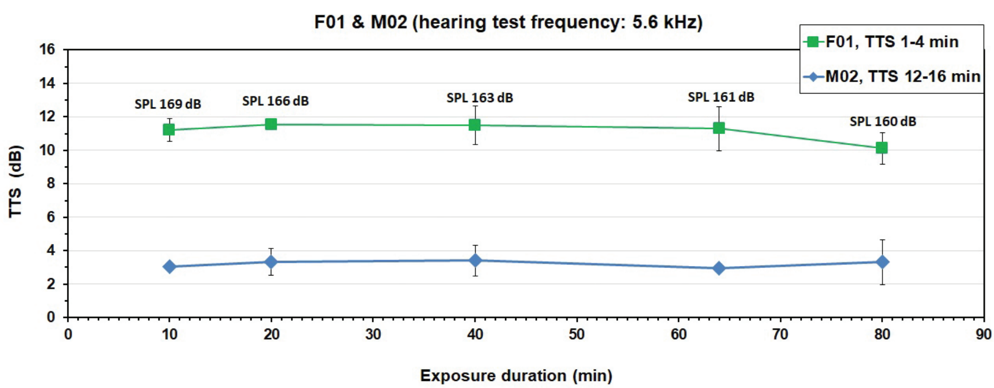

Figure 14. The mean (± SD; $n = 4$) TTS_{1-4} of F01 and mean (± SD; $n = 4$) TTS_{12-16} of M02 were tested at 5.6 kHz hearing frequency after exposure to one-sixth-octave NB centered at 4 kHz for 10 to 80 min, at SPLs of 160 to 169 dB re 1 µPa, resulting in identical SELs (197 dB re 1 µPa^{2}s). In some cases, the SD is so low that it is not visible due to overlap with the symbol.

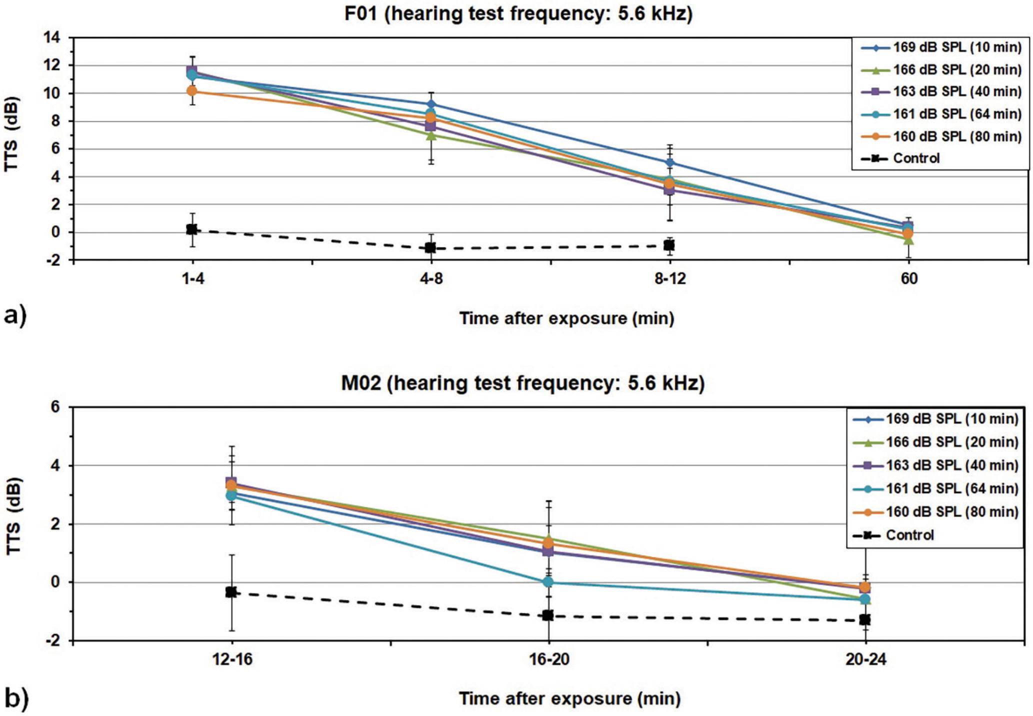

Figure 15. Mean TTS (± SD; *n* = 4) at 5.6 kHz of F01 1 to 12 and 60 min (a) and M02 12 to 24 min (b) after exposure to the NB at 4 kHz at an SEL of 197 dB re 1 µPa²s, composed of five different combinations of SPL and exposure duration.

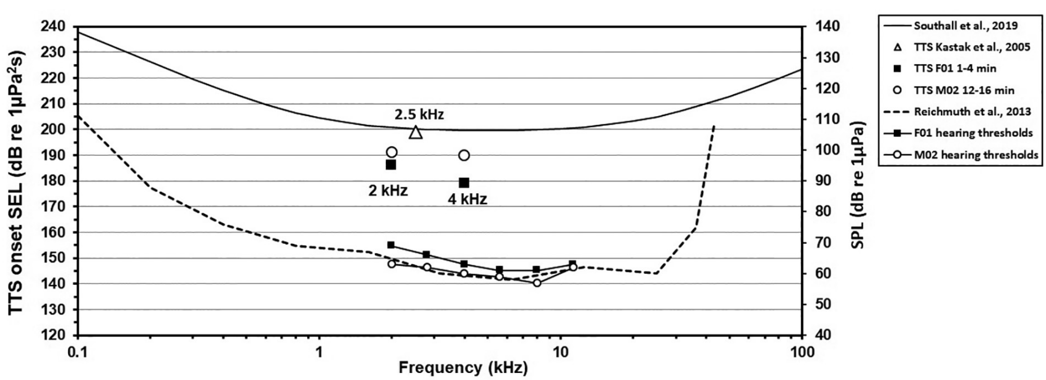

Figure 16. The published TTS onset curve for California sea lions (solid line; Southall et al., 2019), which was based on a study by Kastak et al. (2005; △) in which a California sea lion was exposed to a continuous one-octave NB centered at 2.5 kHz. The lowest SEL required to cause 6 dB TTS is defined as a marker of TTS onset (Southall et al., 2019). The SELs of one-sixth-octave NBs centered at 2 and 4 kHz, which caused 6 dB TTS_{1-4} in F01 (■) and 6 dB TTS_{12-16} in M02 (○), are derived from the present study. 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 in the present study between 2 and 11.3 kHz are also shown (Y-axis on right).

Spankovich et al., 2014). Therefore, further replication with more California sea lions is needed to assess the generality of the results obtained in the present study.

Effect of Fatiguing Sound Duty Cycle on TTS

The present study showed a dramatic impact of fatiguing sound duty cycle on initial TTS in both subjects. F01 went from 24 dB TTS_{1-4} after exposure for 60 min to the NB at 4 kHz with a 100% duty cycle to 11 dB TTS_{1-4} after exposure to the same sound at a 90% duty cycle (1,600-ms signals; 180-ms inter-pulse intervals). After exposure to this sound at a duty cycle of 100%, M02 had 12 dB TTS_{12-16}; when the duty cycle was 90%, he had 5 dB TTS_{12-16}. In both subjects, the TTS was reduced by ~50% by introducing an interval of only 180 ms between signals (reducing the duty cycle from 100 to 90%). Although the SEL_{cum} only dropped 0.5 dB from 204.6 dB re 1µPa^{2}s at 100% duty cycle to 204.1 dB re1µPa^{2}s at 90% duty cycle, the initial TTS in both sea lions was almost halved. This shows that the hearing of California sea lions can recover, at least partly, during brief intervals between fatiguing sound signals. The cumulative SEL decrease of 0.5 dB in F01 resulted in a TTS_{1-4} decrease of around 12 dB.

Kastelein et al. (2014b) did a similar study on the effect of duty cycle on the initial TTS in a harbor porpoise. They used repeated 1 s, 1 to 2 kHz sweeps as fatiguing sounds, an average received SPL of 168 dB re 1 µPa, and an exposure duration of 60 min. At 100% duty cycle, a TTS_{1-4} of 27 dB was elicited, but in order to reduce TTS by half, the duty cycle had to be reduced to around 35%. Kastelein et al. (2015) tested the effect of duty cycle in fatiguing sounds consisting of repeated 1 s, 6 to 7 kHz downsweeps at an average received SPL of 166 dB re 1 µPa on TTS_{1-4} in the same harbor porpoise as tested by Kastelein et al. (2014b). Two duty cycles were tested: 10% (9-s inter-pulse interval) and 100% (continuous). The number of sweeps per exposure, and thus the cumulative SEL, was varied. The higher the number of sweeps in a session, the bigger the difference in TTS_{1-4} between the 100 and 10% duty cycles, as relatively more intervals became available for the hearing to recover. This suggests that, along with duty cycle, the pattern of sound production is likely to affect hearing recovery. For instance, a 50% duty cycle sound composed of 1-s signals and 1-s inter-pulse intervals is likely to cause a different initial TTS than a 50% duty cycle sound composed of 10-s signals and 10-s inter-pulse intervals. In the present study, the SPL remained constant in the duty cycle tests, but the cumulative SEL was related to the duty cycle (Figure 13).

Understanding the effects of non-continuous fatiguing sounds and the combined effects of fatiguing sound duration, signal duration, inter-pulse interval duration, and duty cycle on TTS has great potential for developing methods to reduce hearing damage in wild marine mammals and should be explored in future studies.

Testing the Equal-Energy Hypothesis

The equal-energy hypothesis states that exposure to continuous (100% duty cycle) fatiguing sounds with the same energy (expressed in SEL) results in the same TTS (Southall et al., 2007). The present study showed that, for NBs at 4 kHz in the duration and SPL ranges tested, the equal-energy hypothesis is supported for California sea lion hearing.

Previous TTS studies with marine mammals have both supported and refuted the equal-energy hypothesis. Kastak et al. (2007), after exposing a California sea lion in air to a continuous octave-band noise centered at 2.5 kHz, found in support of the hypothesis that long-duration exposures induce the same TTS at lower SPLs as do short-duration exposures at higher SPLs. However, Kastelein et al. (2012b) refuted the hypothesis for TTS resulting from low-SPL, long-duration (1 h) sound exposures in harbor seals: different magnitudes of TTS resulted from exposure to sounds with identical SELs but consisting of different combinations of SPL and exposure duration. The hypothesis was also refuted for bottlenose dolphins (Mooney et al., 2009; Finneran & Schlundt, 2010), harbor porpoises (Lucke et al., 2009; Kastelein et al., 2012a), and belugas (*Delphinapterus leucas*; Popov et al., 2014). This refuting research suggests that increased SEL due to increased exposure duration has a different effect on the induced TTS than increased SEL of the same magnitude due to increased SPL, regardless of the method used: physiological (Lucke et al., 2009; Mooney et al., 2009; Popov et al., 2014) or psychoacoustic (Finneran & Schlundt, 2010; Kastelein et al., 2012a; present study). However, a study in which harbor porpoises were exposed to continuous 1 to 2 kHz sweeps showed that the same cumulative SELs (composed of various combinations of SPL and exposure duration) did elicit the same magnitude of TTS_{1-4}, as predicted by the equal-energy hypothesis, as long as the duty cycle remained constant (Kastelein et al., 2014b).

Within a certain range, the SEL of a fatiguing sound can be a predictor of the initial TTS it induces, but data obtained only from high-SPL, short-duration exposures might result in underestimation of the TTS induced as a function of the exposure duration, particularly if they are extrapolated to low-SPL, long-duration exposures. Testing the equal-energy hypothesis more fully is important for environmental impact assessments (EIAs), as it may allow the prediction of initial TTS in marine mammals that have been exposed to combinations of fatiguing sound SPL and exposure duration that have not been tested in controlled experiments. Further research will be needed to explore and quantify relationships between the combinations of SPL and exposure duration.

Towards Improved Protection of Otariidae from Underwater Anthropogenic Noise

In California sea lions, repeated temporarily reduced hearing or permanently reduced hearing may reduce the efficiency of biologically important activities, such as navigation, prey detection, communication with conspecifics, and predator avoidance, thus potentially reducing their fitness, reproductive output, and longevity. However, the specific biological effects of TTS for California sea lions are still poorly understood. Furthermore, biologically significant TTS may differ from statistically significant TTS as defined in the present study relative to the control sessions. The biological effect of hearing damage depends on the magnitude of the TTS, the duration of the exposure (as TTS begins to occur and increases during sound exposure), the frequency of occurrence of sound exposures, the time taken for hearing to recover, and the hearing frequency or bandwidth that is affected.

Protection from, and mitigation of, the effects of underwater anthropogenic noise can be achieved through legislation, but data are required to generate weighting functions and set allowable exposure thresholds. Our assessment of the effect of fatiguing sound duty cycle on TTS shows that significant reductions in hearing damage could be achieved by introducing short inter-pulse intervals. This would allow hearing to recover, at least partly, during sound exposure periods, and thus reduce TTS and its biological impacts.

After exposure to the NB at 4 kHz, F01 experienced 6 dB of TTS_{1-4} at an SEL of 179 dB re 1 µPa^{2}s. A conservative approach to protect the hearing of California sea lions, based on the TTS onset data of the present study while awaiting completion of the research project, would be to reduce the TTS onset thresholds for Otariidae (marine mammal group OCW; Southall et al., 2019) from a minimum of 199 dB re 1 µPa^{2}s at 2.5 kHz to 179 dB re 1 µPa^{2}s at 4 kHz.

Our assessment of individual variation suggests that TTS and recovery may be broadly consistent in California sea lions, although only two related individuals were assessed. However, the Otariidae family consists of 15 extant species, and it is unknown how similar the other species are to the California sea lion in their susceptibility to TTS. In future studies in this research project, more fatiguing sound frequencies will be tested in California sea lions to produce equal TTS curves. These curves will be used to generate weighting functions that can be used to set safety criteria for broadband sounds in the marine environment for California sea lions and perhaps for other species of the Otariidae family (as suggested by Houser et al., 2017).

Acknowledgments

We thank assistants Suzanne Cornelisse, Femke Kuiphof, and Stacey van der Linden; students Irna Huisjes, Anouk van der Horst, Roos de Lepper, Pepijn Degger, Luna Korsuize, and Eline Theuws; and volunteer Manouk Vermeulen for their help in collecting the data. We thank Arie Smink for the design, construction, and maintenance of the electronic equipment. We thank Bert Meijering (Topsy Baits) for providing space for the SEAMARCO Research Institute. Erwin Jansen (TNO, the Hague, the Netherlands) conducted the acoustic calibration measurements. We also thank Nancy Jennings (Dotmoth.co.uk) and the anonymous reviewers for their valuable constructive comments on this manuscript. Funding for this study was obtained from the U.S. Navy's Living Marine Resources program (Contract No. N-39430-20-C-2215). We thank Mandy Shoemaker and Anu Kumar for their guidance on behalf of the LMR program. The sea lions were made available for the research by Blijdorp Zoo, Rotterdam, the Netherlands. The training and testing of the California sea lions was conducted under authorization of the Netherlands Ministry of Economic Affairs, Department of Nature Management.

Literature Cited

American National Standards Institute (ANSI). (2013). *ANSI S1.1-2013 Acoustical terminology*. ANSI.

Cody, A. R., & Johnstone, B. M. (1981). Acoustic trauma: Single neuron basis of the "half-octave shift." *The Journal of the Acoustical Society of America*, *70*(3), 707-711. https://doi.org/10.1121/1.386906

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.

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. (2016). *Auditory weighting functions and TTS/PTS exposure functions for marine mammals exposed to underwater noise*. Space and Naval Warfare Systems Center Pacific, San Diego.

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. (2010). Frequency-dependent and longitudinal changes in noise-induced hearing loss in a bottlenose dolphin (*Tursiops truncatus*) (L). *The Journal of the Acoustical Society of America*, *128*(2), 567-570. https://doi.org/10.1121/1.3458814

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., 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., 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 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

Kastak, D., Reichmuth, C., Holt, M. M., Mulsow, J., Southall, B. L., & Schusterman, R. J. (2007). Onset, growth, and recovery of in-air temporary threshold shift in a California sea lion (*Zalophus californianus*). *The Journal of the Acoustical Society of America*, *122*(5), 2916-2924. https://doi.org/10.1121/1.2783111

Kastelein, R. A., Gransier, R., & Hoek, L. (2013). Comparative temporary threshold shifts in a harbor porpoise and harbor seal, and severe shift in a seal. *The Journal of the Acoustical Society of America*, *134*(1), 13-16. https://doi.org/10.1121/1.4808078

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., Schop, J., & Hoek, L. (2015). Effect of exposure to intermittent and continuous 6–7 kHz sonar sweeps on harbor porpoise (*Phocoena phocoena*) hearing. *The Journal of the Acoustical Society of America*, *137*(4), 1623-1633. https://doi.org/10.1121/1.4916590

Kastelein, R. A., Schop, J., Gransier, R., & Hoek, L. (2014a). Frequency of greatest temporary hearing threshold shift in harbor porpoises (*Phocoena phocoena*) depends on the noise level. *The Journal of the Acoustical Society of America*, *136*(3), 1410-1418. https://doi.org/10.1121/1.4892794

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., Hoek, L., Gransier, R., Rambags, M., & Claeys, N. (2014b). Effect of level, duration, and inter-pulse interval of 1–2 kHz sonar signal exposures on harbor porpoise hearing. *The Journal of the Acoustical Society of America*, *136*(1), 412-422. https://doi.org/10.1121/1.4883596

Kastelein, R. A., Wensveen, P. J., Hoek, L., Verboom, W. C., & Terhune, J. M. (2009). Underwater detection of tonal signals between 0.125 and 100 kHz by harbor seals (*Phoca vitulina*). *The Journal of the Acoustical Society of America*, *125*(2), 1222-1229. https://doi.org/10.1121/1.3050283

Kastelein, R. A., Helder-Hoek, L., Cornelisse, S. A., Defillet, L. N., Huijser, L. A. E., & Gransier, R. (2021). 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

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 deefferentation 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/10.1121/1.19123755

Lucke, K., Siebert, U., Lepper, P. A., & Blanchet, M-A. (2009). Temporary shift in masked hearing thresholds in a harbor porpoise (*Phocoena phocoena*) after exposure to seismic airgun stimuli. *The Journal of the Acoustical Society of America*, *125*(6), 4060-4070. https://doi.org/10.1121/1.3117443

Madsen, P. T. (2005). Marine mammals and noise: Problems with root mean square sound pressure levels for transients. *The Journal of the Acoustical Society of America*, *117*(6), 3952-3957. https://doi.org/10.1121/1.1921508

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

McFadden, D. (1986). The curious half octave shift: Evidence for a basalward migration of the traveling-wave envelope with increasing intensity. In R. J. Salvi, D. Henderson, R. P. Hamernik, & V. Colletti (Eds.), *Basic and applied aspects of noise-induced hearing loss* (pp. 295-312). Plenum Press. https://doi.org/10.1007/978-1-4684-5176-4_21

McFadden, D., & Plattsmier, H. S. (1983). Frequency patterns of TTS for different exposure intensities. *The Journal of the Acoustical Society of America*, *74*(4), 1178-1184. https://doi.org/10.1121/1.390041

Melnick, W. (1991). Human temporary threshold shifts (TTS) and damage risk. *The Journal of the Acoustical Society of America*, *90*(1), 147-154. https://doi.org/10.1121/1.401308

Mooney, T. A., Nachtigall, P. E., & Vlachos, S. (2009). Sonar-induced temporary hearing loss in dolphins. *Biology Letters*, *5*(4), 565-567. https://doi.org/10.1098/rsbl.2009.0099

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

National Marine Fisheries Service (NMFS). (2018). *2018 revisions to: Technical guidance for assessing the effects of anthropogenic sound on marine mammal hearing (Version 2.0): Underwater thresholds for onset of permanent and temporary threshold shifts* (NOAA Technical Memorandum NMFS-OPR-59). U.S. Department of Commerce, National Oceanic and Atmospheric Administration. 167 pp.

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

Robinson, D. E., & Watson, C. S. (1973). Psychophysical methods in modern psychoacoustics. In J. V. Tobias (Ed.), *Foundations of modern auditory theory* (Vol. 2, pp. 99-131). Academic Press.

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. (2003). Auditory masking in three pinnipeds: Aerial critical ratios and direct critical bandwidth measurements. *The Journal of the Acoustical Society of America*, *114*(3), 1660-1666. https://doi.org/10.1121/1.1587733

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

Southall, B. L., Bowles, A. E., Ellison, W. T., Finneran, J. J., Gentry, R. L., Greene, C. R., Jr., Kastak, D., Ketten, D. R., Miller, J. H., Nachtigall, P. E., Richardson, W. J., Thomas, J. A., & Tyack, P. L. (2007). Marine mammal noise exposure criteria: Initial scientific recommendations. *Aquatic Mammals*, *33*(4), 411-521. https://doi.org/10.1578/AM.33.4.2007.411

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

Yost, W. A. (2007). *Fundamentals of hearing: An introduction*. Academic Press. 326 pp.

Zar, J. H. (1999). *Biostatistical analysis*. Prentice-Hall. 718 pp.