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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

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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), two subjects were exposed for 60 min to two fatiguing sounds: continuous one-sixth-octave noise bands (NBs) centered at 8 kHz (at sound exposure levels [SELs] of 166 to 190 dB re 1 µPa^{2}s) and at 16 kHz (at SELs of 183 to 207 dB re 1 µPa^{2}s). Using a psychoacoustic technique, TTSs were quantified at 8, 11.3, 16, 22.4, and 32 kHz (at the center frequency of each NB, half an octave higher, and one octave higher). For both NBs, higher SELs resulted in greater TTSs. In the SEL ranges that were tested, the largest TTSs occurred when the hearing test frequency was half an octave higher than the frequency of the fatiguing sound. 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. The effect of fatiguing sound duty cycle on TTS was investigated with the 8 kHz NB, using 1,600 ms signals at a mean sound pressure level (SPL) of 154 dB re 1 µPa. Duty cycle reduction from 100 to 90% resulted in a large decrease in TTS; no TTS was observed at duty cycles ≤ 30%. The equal-energy hypothesis was tested with the 8 kHz NB and found to hold true: five combinations of SPL and exposure duration all resulting in a 182 dB SEL produced similar initial TTSs in both sea lions. These findings will contribute to the protection of otariid hearing from anthropogenic noise by facilitating the development of evidence-based underwater sound weighting functions. Our results also show that the introduction of short inter-pulse intervals to underwater sounds aids in the protection of otariid hearing by allowing recovery to take place.

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

Introduction

Underwater anthropogenic noise, whether produced intentionally (e.g., sonar and seismic blasts) or unintentionally (e.g., noise from shipping, dredging, and pile driving), has the potential to reduce the hearing abilities of marine mammals (Southall et al., 2019). California sea lions (*Zalophus californianus*) inhabit coastal areas of the northeast Pacific Ocean (Melin et al., 2018) where underwater anthropogenic noise is abundant. The California sea lion's underwater hearing is sensitive between 0.1 and 50 kHz (Schusterman et al., 1972; Mulsow et al., 2012; Reichmuth & Southall, 2012; Reichmuth et al., 2013). When exposed to high-amplitude sounds over certain time periods, a California sea lion's hearing can be reduced either temporarily (TTS; temporary threshold shift) or permanently (PTS; permanent threshold shift; Melnick, 1991; Yost, 2007). Noise-induced hearing loss can reduce a sea lion's fitness by interfering with its ability to detect biologically important sounds. Reduced fitness may eventually have population-level consequences.

No PTS studies have been conducted for ethical reasons, but there are presently four published TTS studies on California sea lions (Kastak et al., 1999, 2005; Finneran et al, 2003; Kastelein et al., 2021b). Kastak et al. (1999) exposed a California sea lion to a one-octave noise band (NB) 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 California sea lions to underwater impulses from an arc-gap transducer, but no TTS was elicited. A TTS of ~6 dB was found in California sea lions exposed to a one-octave NB centered at 2.5 kHz (Kastak et al., 2005). Results suggest that the TTS-onset sound exposure level (SEL) in California sea lions is ~20 dB higher (SELs) than in harbor seals (*Phoca vitulina;* Finneran, 2015; Houser et al., 2017). This lower susceptibility to TTS in California sea lions than in harbor seals was unexpected because the two pinniped species have similar hearing thresholds over most of their underwater audiograms (Kastelein et al., 2009a, 2009b; Reichmuth et al., 2013). However, the fourth study of TTS in California sea lions (Kastelein et al., 2021b) found that their susceptibility to noise-induced hearing loss around 2 and 4 kHz was similar to that of harbor seals (Kastelein et al., 2020). These contrasting findings demonstrate the need for consistent testing of susceptibility to TTS in different species, over the entire hearing range of each species. Systematic testing would allow evidence-based underwater sound weighting functions to be established for groups of closely related pinnipeds (e.g., for phocids and for otariids; Houser et al., 2017; National Marine Fisheries Service [NMFS], 2018).

The present study is part of a research project consisting of four studies on TTS and recovery in otariids, each reporting on susceptibility to TTS in California sea lions caused by two fatiguing sound frequencies: 0.5 and 1 kHz, 2 and 4 kHz (Kastelein et al., 2021b), 8 and 16 kHz (present study), and 32 and 40 kHz. When the full frequency range has been studied, the TTS data will form the basis of an evidence-based underwater sound weighting function for Otariidae (Houser et al., 2017; Southall et al., 2019). The goals of the present study are as follows: (1) to quantify the magnitude of TTS in two California sea lions and determine the TTS-onset SEL after exposure to fatiguing sounds with center frequencies of 8 and 16 kHz at several SELs; (2) to determine how different hearing frequencies (corresponding to the center frequency of each fatiguing sound [8 and 16 kHz], half an octave higher, and one octave higher) are affected by exposure to fatiguing sounds at each SEL; (3) to describe the pattern of hearing recovery after the fatiguing sounds centered at 8 and 16 kHz stop; (4) to assess differences in susceptibility to TTS between the two sea lions after exposure to fatiguing sounds centered at 8 and 16 kHz; (5) to assess the effect of fatiguing sound duty cycle on TTS using the fatiguing sound centered at 8 kHz; 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) using the fatiguing sound centered at 8 kHz.

Methods

Subjects and Study Area

The subjects were an adult female California sea lion, identified as sea lion F01, and her juvenile male offspring, identified as sea lion M02. During the study, F01 aged from 8 to 10 y and M02 from 2 to 4 y. F01's total body length was 160 cm, and her body weight varied between 70 and 86 kg, depending on the season. M02's total body length increased from 126 to 155 cm, and his body weight increased from 38 to 68 kg during the study period. Both sea lions were healthy throughout the study.

The California sea lions received about 75% of their daily fish ration during hearing test sessions. The remaining 25% was provided while they were performing husbandry tasks, some of which were associated with moving the subjects individually between pools so only one sea lion was present at a time in the indoor pool used for the hearing tests. The two subjects had similar hearing thresholds (see "Discussion"), which were also similar to those of five other California sea lions (Schusterman et al., 1972; Kastak & Schusterman, 1998; Southall et al., 2005; Mulsow et al., 2012; Reichmuth & Southall, 2012; Reichmuth et al., 2013). 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 body 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 California 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 it, 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 fatiguing sound exposure 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 (see Kastelein et al., 2021b, for a top view of the pool complex). 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, resulting in stable sound conditions. 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 between -0.5° and 24°C during the study period, and the salinity was ~3.4%. The water circulation system was switched off during the day at least 1 h before the first hearing test was conducted, allowing the pool water to settle and further reduce background noise. During fatiguing 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.

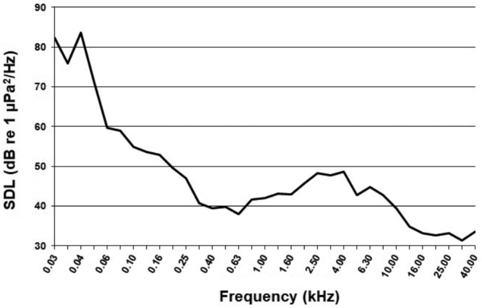


Figure 1. The general underwater ambient noise level under test conditions in the indoor pool used for California sea lion (*Zalophus californianus*) hearing tests. Measurements were recorded as one-third-octave bands and converted to spectrum density levels (SDLs).

Acoustics

Sound Pressure Level Measurement Equipment—The ambient noise was measured, and the fatiguing sound and hearing test signals were calibrated, once every 3 months during the study period by an acoustic consulting agency (TNO, the Hague, the Netherlands). The sound measurement equipment consisted of three hydrophones (Model 8106; Brüel & Kjaer [B&K], Nærum, Denmark), a multichannel high-frequency analyzer (B&K PULSE, Model 3560 D, B&K), and a laptop computer with B&K PULSE software (*Labshop*, Version 12.1). Three hydrophones were used to measure the fatiguing sound and two to measure the hearing test signals. 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 per location.

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 multichannel high-frequency analyzer mentioned above. The system was calibrated with a microphone calibrator (Model 4231, B&K).

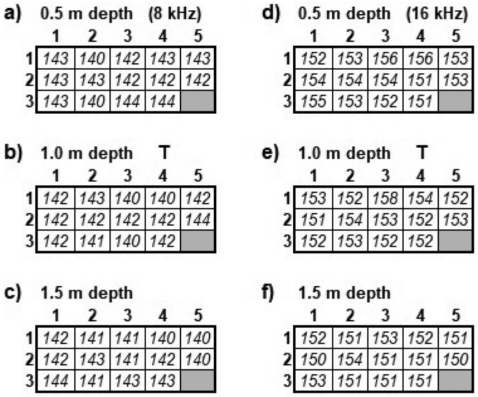
Background Noise—Great care was taken to make the California sea lions' listening environment as quiet as possible while their hearing thresholds were being measured. Only researchers involved in the hearing tests were allowed within 15 m of the pool complex during hearing test sessions and fatiguing sound exposures, and those involved were required to stand still. The ambient noise in the indoor pool was very low and fairly constant in amplitude under test conditions (Figure 1): water circulation system off, no rain, and wind force generally Beaufort 4 or below. Stronger wind from the southwest was sometimes acceptable, as a dike on one side of the pool sheltered it from these prevailing winds.

Fatiguing Sounds—Continuous (i.e., 100% duty cycle) one-sixth-octave NBs centered at 8 or 16 kHz, without harmonics, were used as fatiguing sounds (i.e., sounds intended to cause TTS) in most sessions. For the NB centered at 8 kHz, lower duty cycles were used to assess the effect of duty cycle on TTS (see "Experimental Procedures"). The fatiguing sounds were selected because they are one-octave spaced frequencies within the range of functional hearing of California sea lions (Reichmuth et al., 2013) and have not been tested before. The digitally generated sounds (WAV files; sample rate: 768 kHz) were played back by a laptop computer (Model V5-552; Acer Aspire, Taipei, Taiwan) with a program written in *LabVIEW* (National Instruments, Austin, TX, USA) to an external data acquisition card (Model USB 6361, National Instruments), the output of which could be controlled in 1 dB steps with the *LabVIEW* program. The output of the card went through a ground loop isolator, a custom-built buffer and a fixed low-pass filter, a variable passive low-pass filter, a digital attenuator, and (for the 8 kHz fatiguing sound only) an active low-pass filter (Model 3361; Krohn-Hite, Brockton, MA, USA), after which it went to a power amplifier (Model VPA2200MBN; HQ Power, Velleman, Gavere, Belgium) that drove the transducer (Model LL1424HP; Lubell, Columbus, 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, 10 cm above the pool floor. The linearity of the transmitter system producing the fatiguing sound was checked during each calibration and was found to be consistent to 1 dB within a 42 dB range (overlapping the SPL range used in this study).

To determine the distribution of the fatiguing sounds in the deep part of the indoor pool (where the California 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 2). 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 fairly homogeneous sound field for both fatiguing sounds (Figure 2).

During sound exposure sessions, the one-sixth-octave NB centered at 8 kHz was projected for 60 min (or between 10 and 80 min when testing the equal-energy hypothesis) at various source levels, resulting in mean SPLs ranging from 130 to 154 dB re 1 µPa (SEL range: 166 to 190 dB re 1 µPa^{2}s). The one-sixth-octave NB centered at 16 kHz was also projected for 60 min at various source levels, resulting in mean SPLs ranging from 147 to 171 dB re 1 µPa (SEL range: 183 to 207 dB re 1 µPa^{2}s).

The California 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 < 1 s. 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. The aerial SPL was measured while the NBs at 8 and 16 kHz were being played back underwater at all SPLs used in the study. Per fatiguing SPL, the aerial SPL varied by at most 2 dB between the two measurement locations, so the mean of the two measurements can be assumed to be the aerial SPL the sea lions were exposed to while their heads were completely out of the water (Tables 2 & 3).



a) 0.5 m depth (8 kHz)

	1	2	3	4	5
1	143	140	142	143	143
2	143	143	142	142	142
3	143	140	144	144	▨

b) 1.0 m depth T

	1	2	3	4	5
1	142	143	140	140	142
2	142	142	142	142	144
3	142	141	140	142	▨

c) 1.5 m depth

	1	2	3	4	5
1	142	141	141	140	140
2	142	143	141	142	140
3	144	141	143	143	▨

d) 0.5 m depth (16 kHz)

	1	2	3	4	5
1	152	153	156	156	153
2	154	154	154	151	153
3	155	153	152	151	▨

e) 1.0 m depth T

	1	2	3	4	5
1	153	152	158	154	152
2	151	154	153	152	153
3	152	153	152	152	▨

f) 1.5 m depth

	1	2	3	4	5
1	152	151	153	152	151
2	150	154	151	151	150
3	153	151	151	151	▨

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; figure not to scale) when the fatiguing sounds were being played. These were continuous one-sixth-octave noise bands (NBs) centered at 8 kHz (a-c) and 16 kHz (d-f). Measurements were taken at 14 locations on a horizontal grid with cells of 1 × 1 m (the outer hydrophone locations were 1.0 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 California sea lions experienced during sound exposures. In these examples, the mean (± standard deviation [SD]) SPL for 8 kHz (a-c) was 142 ± 1.2 dB re 1 µPa ($n = 42$); and for 16 kHz (d-f), it was 153 ± 1.7 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 baffleboard; this part of the pool could not be accessed by the sea lions (see Kastelein et al. [2021b] for a scale drawing of the pool).

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 GES927216 GDM-8341; GW Instek, New Taipei City, Taiwan) by producing an 8 or 16 kHz continuous tone from the laptop. The acoustic underwater signal was checked with a hydrophone (Model EC6073; Reson, Slangerup, Denmark) 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 California 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 constant-frequency pure tones because sweeps lead to more stable received SPLs at the listening station (Finneran & Schlundt, 2007) and, thus, to more stable thresholds. For TTS studies, precise hearing thresholds are very important for detecting small threshold shifts.

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 8 kHz, the hearing test frequencies were 8, 11.3, and 16 kHz, and for the NB at 16 kHz, the hearing test frequencies were 16, 22.4, and 32 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 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 ground loop isolator via a custom-built buffer to a custom-built passive low-pass filter, to a variable passive low-pass filter, and to a second custom-built buffer. Then, for the 8, 11.3, and 16 kHz hearing test signals (before and after exposure to the 8 kHz fatiguing sound), the output drove a balanced tonpilz piezoelectric acoustic transducer (Model LL916, Lubell) through an isolation transformer (Model AC202, Lubell). For the 16, 22.4, and 32 kHz hearing test signals (before and after exposure to the 16 kHz fatiguing sound), the output drove a cylindrical hydrophone (Model 337; EDO Western, Salt Lake City, UT, USA).

The free-field received SPL of each hearing test signal was measured at the position of the California 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 a session, the voltage output to the transducer was measured with a voltmeter (Model 3478A; Hewlett Packard, Spring, TX, USA), and the SPL at the listening station was checked with the equipment used to measure the SPL of the fatiguing sound, with the addition of a pre-amplifier (Model 2365, B&K).

Experimental Procedures

Pre-Exposure and Post-Exposure Hearing Tests—Each hearing test trial began with one of the California sea lions at the start/response buoy (see Kastelein et al., 2021b). 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) using 2 dB steps. 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 at the listening station (i.e., when it was in line with the beam of the transducer).

Each hearing test session consisted of ~25 trials and lasted for up to 12 min per California sea lion. In each pre-exposure session, a minimum of ten 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, 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). When a sea lion returned to the start/response buoy before either a test signal or a whistle (see "Experimental Procedures") was produced (i.e., a pre-stimulus response or false alarm), the sea lion was ignored for 10 s, after which testing was resumed. If a pre-stimulus response was clearly due to an external sound, the trial was repeated.

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, (2) a fatiguing sound exposure, and (3) one or more post-sound exposure (PSE) hearing tests. The SPL of the fatiguing sound was increased slowly during the first minute of the exposure period to avoid startle responses, which may otherwise have led to large changes in the sea lions' swimming patterns. 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 pool, so testing of the second sea lion could begin without delay.

During most of the study, the two California 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 sessions for each fatiguing sound frequency to investigate individual differences in susceptibility to, and recovery from, TTS. In these sessions, M02 was tested first after exposure to each of the two fatiguing sounds at one high SEL: 184 dB re 1 µPa^{2}s dB for the NB at 8 kHz (measured with an 11.3 kHz hearing test signal), and 207 dB re 1 µPa^{2}s for the NB at 16 kHz (measured with a 22.4 kHz hearing test signal).

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 measured. The hearing sensitivity of F01 was tested during up to six PSE periods: 1-4 min (PSE$_{1-4}$), 4-8 min (PSE$_{4-8}$), 8-12 min (PSE$_{8-12}$), 60 min (PSE$_{60}$), 120 min (PSE$_{120}$), and, only for the NB at 16 kHz, 240 min (PSE$_{240}$) after the fatiguing sound exposure ended. The hearing sensitivity of M02 was tested 12-16 min (PSE$_{12-16}$), 16-20 min (PSE$_{16-20}$), 20-24 min (PSE$_{20-24}$), and, only for the NB at 8 kHz, 72 min (PSE$_{72}$) after the fatiguing sound exposure ended. Testing was continued after the first PSE hearing test until hearing recovery had taken place. Recovery was defined here as a return to < 2 dB TTS, based on the fact that statistically significant initial TTS was above ~2 dB (see "Results") and reflecting the precision with which the threshold could be measured. Initial TTS was the TTS calculated from measurements taken 1 to 4 min and 12 to 16 min after sound exposure stopped (TTS$_{1-4}$ in F01 and TTS$_{12-16}$ in M02). TTSs calculated from measurements taken during subsequent PSE hearing test periods were referred to as TTS$_{4-8}$, TTS$_{8-12}$, TTS$_{16-20}$, TTS$_{20-24}$, etc.

Sample Sizes and Test Levels—Sample sizes ranged from $n = 2$ to $n = 6$ (see "Results") for each combination of test parameters. Sample sizes were chosen to (1) maximize the time available for testing SPLs in which TTS was expected to occur, (2) minimize the risk of hearing damage from repeated exposure to the loudest sounds, and (3) avoid repeated testing of SPLs for which it was clear without analysis that TTS was unlikely to occur. When determining suitable test levels per fatiguing sound, the SEL of the fatiguing sound was carefully increased in 6 dB steps. 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. The thresholds at the center frequency and one octave above the center frequency of the fatiguing sound were then measured after exposure to that SEL. Depending on the results, thresholds for each of the three hearing test frequencies were measured after exposure to higher and lower SELs, always keeping the protection of the hearing of the sea lions in mind. 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—Control tests were conducted in the same way as sound exposure tests but without fatiguing sound exposure. Each control test started at around 0900 h with a pre-exposure hearing test session (test signals centered at 8, 11.3, 16, 22.4, or 32 kHz) and was followed by a 60-min exposure to the normal, very low, ambient noise in the indoor pool (see Figure 1). The post-ambient exposure (PAE) hearing test session was divided into three periods per subject: 1-4 min (PAE$_{1-4}$), 4-8 min (PAE$_{4-8}$), and 8-12 min (PAE$_{8-12}$) after ambient noise exposure for F01; and 12-16 min (PAE$_{12-16}$), 16-20 min (PAE$_{16-20}$), and 20-24 min (PAE$_{20-24}$) after ambient noise exposure for M02. Pre-exposure hearing test sessions for control tests continued until ten reversals were obtained, which generally occurred within 8 to 12 min; the PAE test sessions were always 12 min. Control tests were randomly dispersed among the fatiguing sound exposure tests during the study period.

Effect of Duty Cycle and Testing the Equal-Energy Hypothesis—The effect of the duty cycle of the fatiguing sound was tested with the NB centered at 8 kHz at the highest SPL (154 dB re 1 µPa). Hearing was tested at 11.3 kHz, the hearing frequency that had the highest initial TTS after exposure to fatiguing sounds at 100% duty cycle (see "Results"). 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 53C 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_{1-4} was not found in F01 at 30% duty cycle, so this duty cycle was not tested for M02, and duty cycles of 20, 10, and 5% were not tested for either sea lion. However, the 2.5% duty cycle was tested for both sea lions because it is the duty cycle most commonly used in the U.S. Navy's 53C sonar system. The total exposure duration for all nine duty cycles that were tested was 60 min (including inter-pulse intervals). The duty cycles were tested over 9 days in order from the highest (100%) to the lowest (2.5%), after which this series was repeated three times, resulting in $n = 4$ for each duty cycle.

To test the equal-energy hypothesis, which states that all combinations of SPL and exposure duration that result in the same SEL elicit similar initial TTSs, the California sea lions were exposed to several SPL and exposure duration combinations for the NB centered at 8 kHz. The combinations, all resulting in an SEL of 182 dB re 1 μPa^2s, were as follows: SPL of 154 dB re 1 μPa for 10 min, SPL of 151 dB re 1 μPa for 20 min, SPL of 148 dB re 1 μPa for 40 min, SPL of 146 dB re 1 μPa for 60 min, and SPL of 145 dB re 1 μPa for 80 min. The duty cycle was always 100%, and hearing was tested at 11.3 kHz, as the highest initial TTS after the 100% duty cycle NBs occurred at this hearing frequency (see "Results"). Each combination was tested four times in random order.

Data Analysis

Data for the NB centered at 8 kHz were collected between March 2019 and January 2021, and data for the NB centered at 16 kHz were collected between January and May 2021.

The pre-stimulus response rate by the California sea lions was calculated as a percentage of the trials in each hearing test period. Both signal-present and signal-absent trials (in the latter, a whistle indicating the end of the test period was the stimulus) were included in the calculations. Trials of all three hearing test frequencies per fatiguing sound (NBs centered at 8 and 16 kHz) were pooled, as were post-exposure trials for each fatiguing SEL.

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 in the control tests (PAE_{1-4} or PAE_{12-16}), both relative to the pre-exposure thresholds. 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 ($p < 0.05$), the levels were compared to the control by means of Dunnett multiple comparisons. In one case, where there were only two levels (16 kHz NB for hearing test frequency 16 kHz; see "Results"), a *t* test was used instead of an ANOVA.

Recovery of hearing, individual differences in 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 California 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

The California sea lions always participated in the hearing tests before and after the 60-min sound exposure sessions. The pre-stimulus responses (or false alarms) by F01 and M02 during the pre-exposure and post-exposure hearing test periods with NBs at 8 and 16 kHz and during control tests are shown in Table 1.

Effect of SEL of the NB at 8 kHz on TTS Levels and Recovery Times

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 8 kHz fatiguing sound's SEL at all three hearing frequencies. Comparisons with the control revealed that the statistically significant onset of TTS varied depending on the sea lion (i.e., the timing of the post-exposure test) and the hearing test frequency (Table 2).

No change in susceptibility to TTS was observed during the study (i.e., there was no gradual increase or decrease in TTS over the course of the study). As expected, 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 After Exposure to an 8 kHz NB

With a hearing test signal of 8 kHz, statistically significant TTS_{1-4} occurred in F01 after SELs of ≥ 172 dB re 1 μPa^2s (Table 2; Figure 3a). Hearing

Table 1. The pre-stimulus response rates of California sea lions F01 and M02, calculated as percentages of the total number of trials (sample size). Trials of all three hearing test frequencies per fatiguing sound (noise bands [NBs] centered at 8 and 16 kHz) are pooled, as are post-exposure trials for each fatiguing sound exposure level. PSE = post-sound exposure; PAE = post-ambient exposure. The subscript numbers after the PSEs and PAEs indicate the time periods in minutes in which the measurements were conducted after exposure to the fatiguing sound or ambient sound.

Pre-stimulus response rate (NB at 8 kHz)							
F01							
Fatiguing sound	Pre-exposure	PSE$_{1-4}$	PSE$_{4-8}$	PSE$_{8-12}$	PSE$_{60}$	PSE$_{120}$	
	8%	8%	11%	8%	9%	0%	
Sample size	1,255	524	543	503	344	15	
Control	Pre-exposure	PAE$_{1-4}$	PAE$_{4-8}$	PAE$_{8-12}$			
	11%	11%	10%	8%			
Sample size	346	133	140	134			
M02							
Fatiguing sound	Pre-exposure	PSE$_{12-16}$	PSE$_{16-20}$	PSE$_{20-24}$	PSE$_{72}$		
	12%	8%	11%	15%	12%		
Sample size	1,220	455	452	460	190		
Control	Pre-exposure	PAE$_{12-16}$	PAE$_{16-20}$	PAE$_{20-24}$			
	12%	10%	16%	15%			
Sample size	307	102	107	115			
Pre-stimulus response rate (NB at 16 kHz)							
F01							
Fatiguing sound	Pre-exposure	PSE$_{1-4}$	PSE$_{4-8}$	PSE$_{8-12}$	PSE$_{60}$	PSE$_{120}$	PSE$_{240}$
	4%	6%	6%	7%	5%	7%	13%
Sample size	893	439	432	405	306	102	23
Control	Pre-exposure	PAE$_{1-4}$	PAE$_{4-8}$	PAE$_{8-12}$			
	7%	5%	7%	6%			
Sample size	274	139	135	127			
M02							
Fatiguing sound	Pre-exposure	PSE$_{12-16}$	PSE$_{16-20}$	PSE$_{20-24}$	PSE$_{72}$		
	9%	9%	11%	11%	14%		
Sample size	834	372	391	399	97		
Control	Pre-exposure	PAE$_{12-16}$	PAE$_{16-20}$	PAE$_{20-24}$			
	8%	7%	12%	14%			
Sample size	260	122	127	135			

recovered within 12 min after SELs of 172 and 190 dB, within 8 min after an SEL of 178 dB, and within 60 min after an SEL of 184 dB (Figure 4a). With a hearing test signal of 11.3 kHz, statistically significant TTS$_{1-4}$ occurred after SELs of ≥ 178 dB (Table 2; Figure 3a). Recovery of hearing occurred within 12 min after an SEL of 178 dB and within 60 min after SELs of 184 and 190 dB (Figure 4b). With a hearing test signal of 16 kHz, significant TTS$_{1-4}$ occurred after SELs of ≥ 184 dB (Table 2; Figure 3a). Recovery of hearing occurred within 12 min after an SEL of 184 dB and within 60 min after an SEL of 190 dB (Figure 4c).

With a hearing test signal of 8 kHz, statistically significant TTS$_{12-16}$ occurred in M02 after SELs of ≥ 184 dB (re 1 μPa^{2}s; Table 2; Figure 3b). Hearing recovered within 20 min after SELs of 184 and 190 dB (Figure 5a). With a hearing test signal of 11.3 kHz, statistically significant TTS$_{12–16}$ occurred after SELs of ≥ 184 dB (Table 2; Figure 3b). Recovery of hearing occurred within 72 min after SELs of 184 and 190 dB (Figure 5b). With a hearing test signal of 16 kHz, significant TTS$_{12-16}$ only occurred after an SEL of 190 dB (Table 2; Figure 3b), and recovery occurred within 24 min (Figure 5c).

Table 2. The mean, standard deviation (SD), and range of initial TTS (TTS$_{1-4}$ in F01 and TTS$_{12-16}$ in M02) after 60-min exposures to ambient noise (control) and a continuous one-sixth-octave NB centered at 8 kHz at several SELs, quantified at hearing frequencies 8, 11.3, and 16 kHz. Underwater SELs (calculated from underwater SPLs) and aerial SPLs are shown for each underwater SPL. TTS levels were calculated as the differences between mean pre-exposure and mean post-exposure hearing thresholds. No TTS occurred during control sessions. *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*
8	Ambient	Control	41	0.7	0.6	0.0-1.6	5	-0.3	1.7	-2.7-1.5	4
	130	166	47	1.5	0.6	0.8-2.3	4	0.9	2.1	-0.6-3.8	4
	136	172	53	5.7*	2.0	3.0-7.2	4	0.5	1.3	-1.0-2.3	4
	142	178	59	4.3*	1.1	3.1-5.8	5	2.2	0.6	1.6-3.0	4
	148	184	65	8.0*	1.0	6.8-8.9	4	3.0*	0.9	2.0-4.2	4
	154	190	71	6.4*	3.4	4.0-8.8	2	4.8*	0.7	4.3-5.4	2
11.3	Ambient	Control	41	0.7	2.1	-2.4-2.4	4	0.6	2.0	-1.8-2.9	4
	130	166	47	1.2	1.7	-0.8-3.3	4	1.7	2.4	-1.0-4.0	4
	136	172	53	2.9	1.4	0.9-4.3	4	1.3	2.2	-1.9-3.2	4
	142	178	59	7.1*	1.6	4.8-8.2	4	0.7	1.7	-1.6-2.5	4
	148	184	65	13.1*	0.4	12.4-13.4	5	5.6*	1.0	4.3-6.6	4
	154	190	71	18.0*	1.8	16.0-20.2	4	9.5*	2.6	7.0-13.0	4
16	Ambient	Control	41	1.2	1.1	-0.3-2.5	5	1.7	1.2	0.0-2.8	4
	142	178	59	1.7	1.1	0.7-3.0	4	0.6	1.3	-1.6-1.6	5
	148	184	65	5.2*	1.5	3.3-6.5	4	0.3	1.0	-0.5-1.7	4
	154	190	71	9.5*	2.0	7.2-11.3	4	4.8*	0.7	3.8-5.5	4

Individual Differences in TTS After Exposure to an 8 kHz NB

During four sessions, the order in which the California sea lions were tested at hearing frequency 11.3 kHz after exposure to the NB at 8 kHz (SEL 184 dB re 1 µPa²s) was reversed. The mean TTS$_{1-4}$ in M02 (12.1 dB, SD = 1.2 dB, *n* = 4) was 1.0 dB lower than the mean TTS$_{1-4}$ in F01 (13.1 dB, SD = 0.4 dB, *n* = 4) after exposure to the same SEL. The recovery patterns were similar (Figure 6a). The mean TTS$_{12-16}$ in F01 (8.7 dB, SD = 1.5 dB, *n* = 4) was 3.1 dB higher than the mean TTS$_{12-16}$ in M02 (5.6 dB, SD = 1.0 dB, *n* = 4) after exposure to the same SEL. Again, the recovery patterns were similar (Figure 6b).

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 and on TTS$_{12-16}$ in M02 (Figure 7). When the duty cycle was reduced from 100 to 90% (a cumulative SEL decrease of 0.5 dB), the mean initial TTS decreased by 3.3 dB in F01 and by 3.2 dB in M02. At a duty cycle of 50%, the cumulative SEL decreased by 3 dB and the TTS levels dropped by 12.3 dB for F01 and 7.5 dB for M02 relative to the 100% duty cycle. At duty cycles ≤ 60%, no TTS$_{12-16}$ occurred in M02. At a duty cycle of 30%, no TTS$_{1-4}$ occurred in F01. At a duty cycle of 2.5%, there was no TTS in either sea lion (Figure 7).

Testing the Equal-Energy Hypothesis

The equal-energy hypothesis held true for both California sea lions. After exposure to five combinations of SPL and exposure duration that resulted in 8 kHz NB fatiguing sounds with the same underwater SEL (i.e., 182 dB re 1 µPa²s), hearing was tested at 11.3 kHz—four times for each combination. One-way ANOVAs were significant ($p < 0.001$) for both sea lions. Tukey multiple comparisons showed that for each sea lion, similar TTSs occurred after all exposure combinations since they all differed significantly from the control and not from one another (Figure 8). In both subjects, recovery patterns were similar after exposure to all combinations of SPL and exposure duration (Figure 9).

Effect of SEL of the NB at 16 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 16 kHz fatiguing sound's SEL at hearing test frequencies of 22.4 and 32 kHz. Comparisons with the control revealed that the statistically significant onset of TTS varied depending on the sea lion (i.e., on the timing of the post-exposure test) and the hearing test frequency. The *t* tests showed that there was no effect at the hearing test frequency of 16 kHz; initial TTS was similar in control and test sessions (Table 3).

No change in susceptibility to TTS was observed during the course of the study. As expected, 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 similar (Table 3).

TTS and Recovery After Exposure to a 16 kHz NB

With a hearing test signal of 16 kHz, statistically significant $\text{TTS}_{1\text{-}4}$ did not occur in F01 even after an SEL of 207 dB (re 1 $\mu\text{Pa}^2\text{s}$; Table 3; Figures 10a & 11a). With a hearing test signal of 22.4 kHz, statistically significant $\text{TTS}_{1\text{-}4}$ occurred after SELs of ≥ 189 dB (Table 3; Figure 10a). Recovery of hearing occurred within 8 min after an SEL of 189 dB, within 60 min after SELs of 195 and 201 dB, and within 240 min after an SEL of 207 dB (Figure 11b). With a hearing test signal of 32 kHz, significant $\text{TTS}_{1\text{-}4}$ occurred after SELs of ≥ 201 dB (Table 3; Figure 10a). Recovery of hearing occurred within 12 min after an SEL of 201 dB and within 60 min after an SEL of 207 dB (Figure 11c).

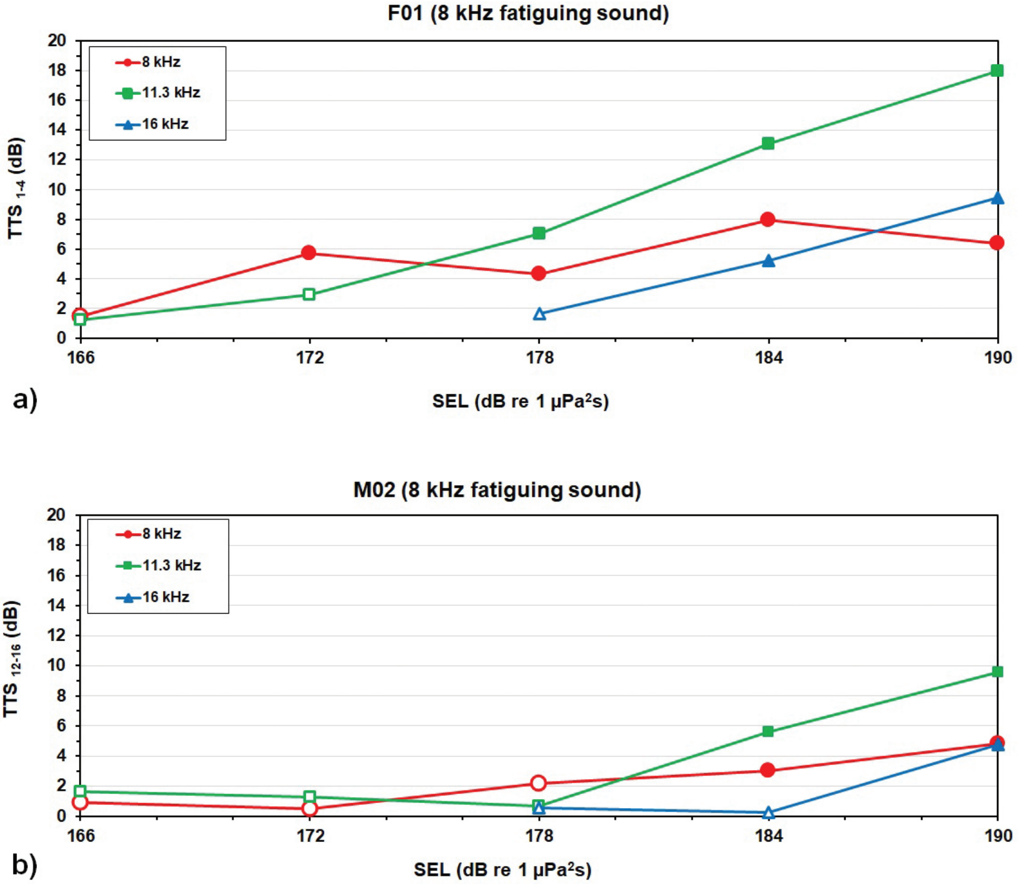


Figure 3. Mean $\text{TTS}_{1\text{-}4}$ in F01 (a) and mean $\text{TTS}_{12\text{-}16}$ in M02 (b) after 60-min exposure to a continuous one-sixth-octave NB centered at 8 kHz at several SELs, quantified at hearing frequencies 8, 11.3, and 16 kHz (i.e., at the center frequency of the fatiguing sound, 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 sessions. Sample size varies per data point (see Table 2). For SPLs (dB re 1 µPa), subtract 36 dB from the SEL values. For SDs and control values, see Table 2 and Figures 4 & 5.

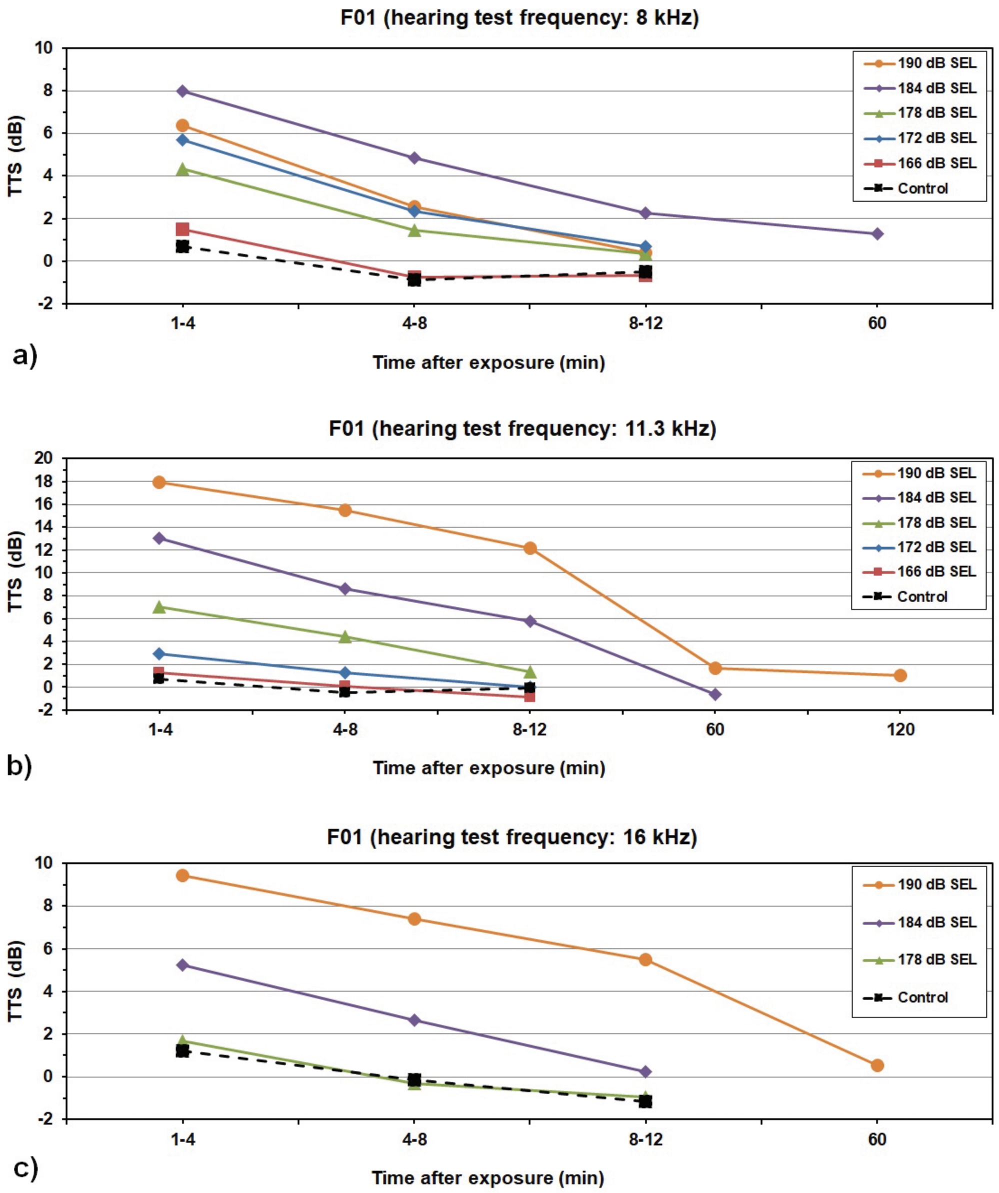
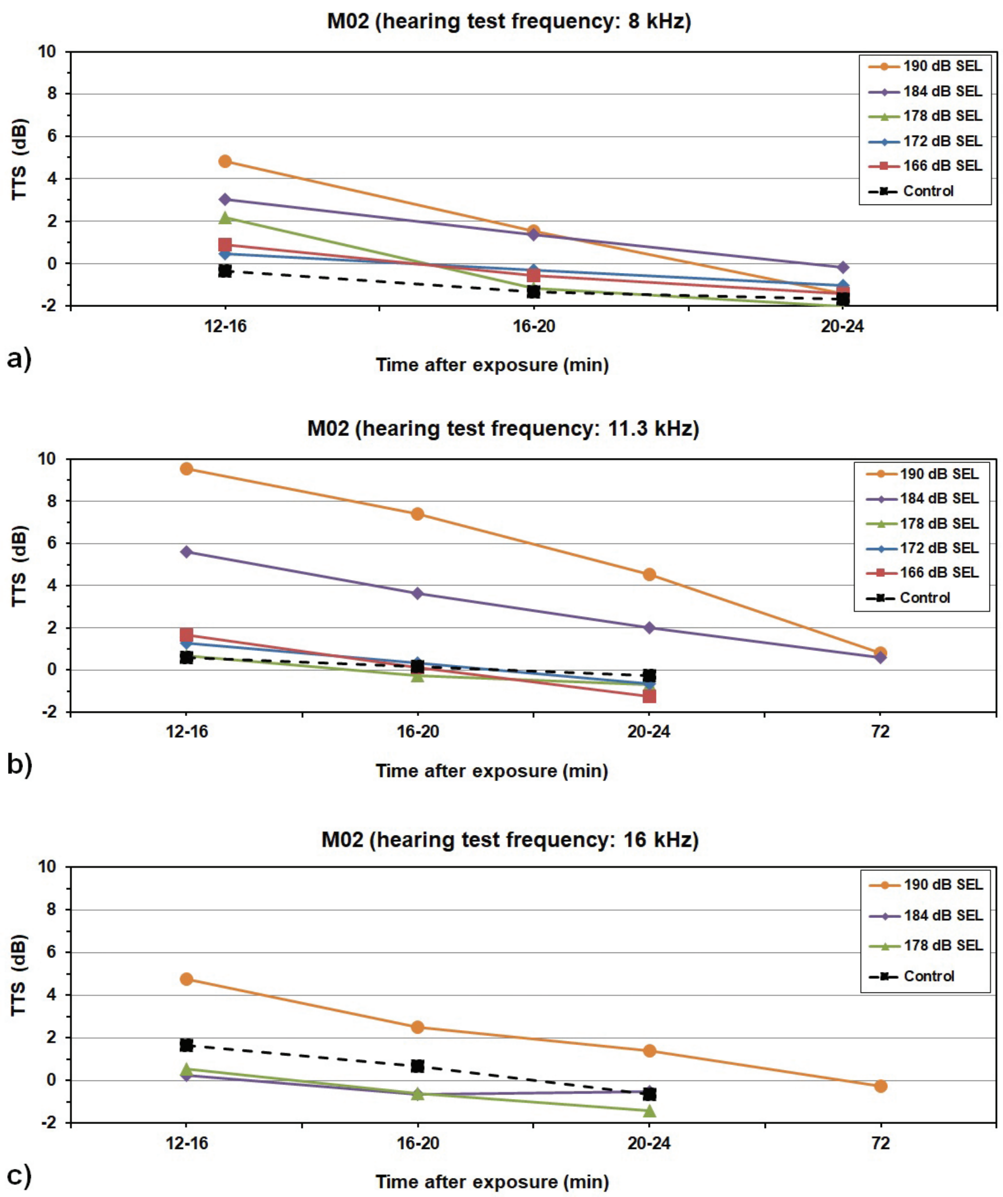


Figure 4. Changes in the mean TTS of F01, including recovery, tested at 8 kHz (a), 11.3 kHz (b), and 16 kHz (c) after 60-min exposure to a continuous one-sixth-octave NB centered at 8 kHz at several SELs. Hearing was considered recovered once TTS was < 2 dB. For sample sizes and SDs (only for $TTS_{1\text{-}4}$), see Table 2. Note that the x- and y-axis scales in (b) differ from those in (a) and (c). For average received SPLs (dB re 1 µPa), subtract 36 dB from the sound exposure level (SEL) values. The mean "TTS" values during control sessions (no shifts occurred) are also shown.


M02 (hearing test frequency: 8 kHz)

M02 (hearing test frequency: 11.3 kHz)

M02 (hearing test frequency: 16 kHz)

Figure 5. Changes in mean TTS of M02, including recovery, tested at 8 kHz (a), 11.3 kHz (b), and 16 kHz (c) after 60-min exposure to a continuous one-sixth-octave NB centered at 8 kHz at several SELs. Hearing was considered recovered once TTS was < 2 dB. For sample sizes and SDs (only for $TTS_{12\text{-}16}$), see Table 2. Note that the x-axis scale in (a) differs from those in (b) and (c). For average received SPLs (dB re 1 µPa), subtract 36 dB from the SEL values. The mean "TTS" values during control sessions (no shifts occurred) are also shown.

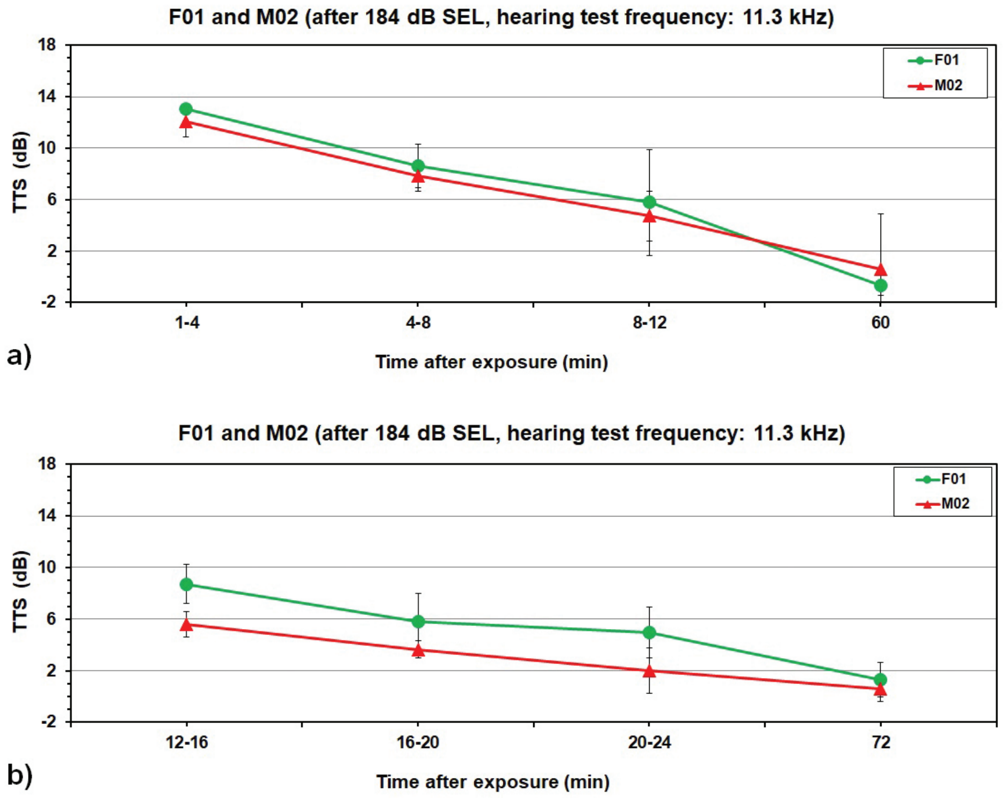


Figure 6. Testing individual differences in susceptibility to TTS. Mean TTS (± SD; *n* = 4) at 11.3 kHz in F01 and M02, measured 1 to 12 and 60 min (a) and 12 to 24 and 72 min (b) after 60-min exposure to the continuous NB at 8 kHz at an SEL of 184 dB re 1 μPa^{2}s.

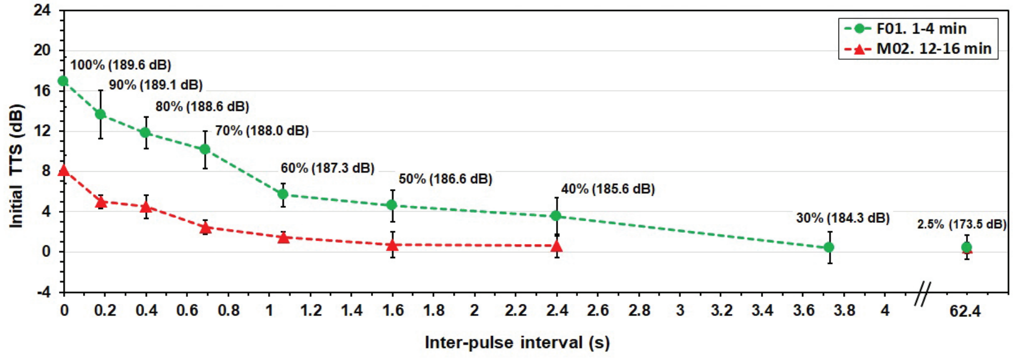


Figure 7. Testing the effect of duty cycle on TTS. The mean (± SD; *n* = 4) TTS_{1-4} of F01 and TTS_{12-16} of M02, tested at 11.3 kHz hearing frequency, after 60-min exposure to a one-sixth-octave NB centered at 8 kHz at SPL 154 dB re 1 μPa, presented with up to nine different duty cycles (i.e., with nine different inter-pulse intervals). The signal duration was 1,600 ms in all cases. The cumulative SEL (in dB re 1 μPa^{2}s) is shown between parentheses after each duty cycle. Duty cycle 2.5% was tested in both California sea lions (results overlap) as this is the most commonly used duty cycle in the U.S. Navy's 53C sonar system.

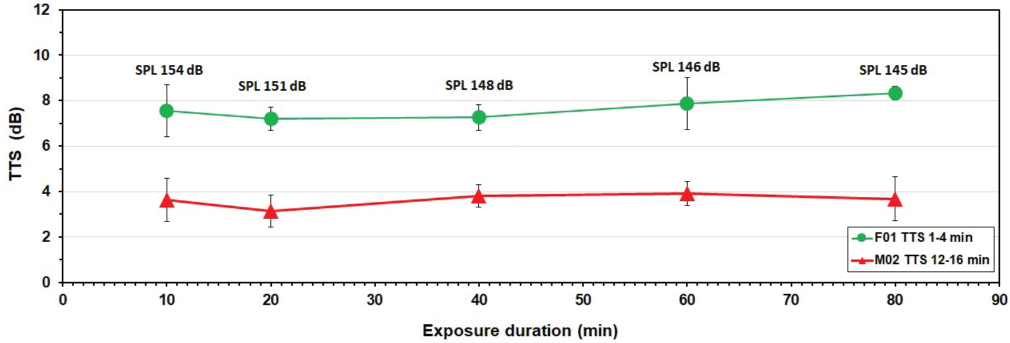


Figure 8. Testing the equal-energy hypothesis. The mean (± SD; *n* = 4) TTS_{1-4} of F01 and TTS_{12-16} of M02 at 11.3 kHz after exposure to a one-sixth-octave NB centered at 8 kHz for 10 to 80 min, at SPLs of 145 to 154 dB re 1 µPa, all combinations resulting in an identical SEL (182 dB re 1 μPa^2s).

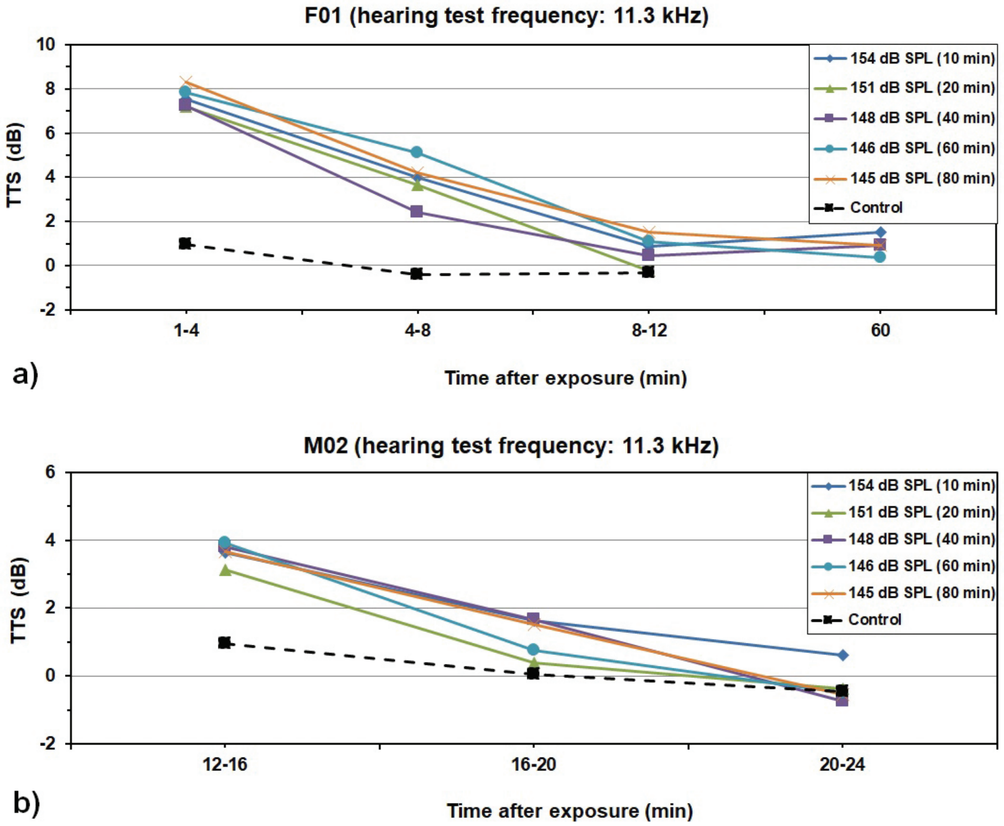


Figure 9. Testing the equal-energy hypothesis. The mean TTS (*n* = 4) at 11.3 kHz of (a) F01, measured 1 to 12 and 60 min after exposure to the NB at 8 kHz; and (b) M02, measured 12 to 24 min after exposure to the NB at 8 kHz. The SEL of 182 dB re 1 μPa^2s was composed of five different combinations of SPL (dB re 1 µPa) and exposure durations. The mean "TTS" values during control sessions (no shifts occurred) are also shown.

Table 3. 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 NB centered at 16 kHz at several SELs, quantified at hearing frequencies 16, 22.4, and 32 kHz. Underwater SELs (calculated from underwater SPLs) and aerial SPLs are shown for each underwater SPL. TTS levels were calculated as the differences between pre-exposure and post-exposure hearing thresholds. No TTS occurred during control sessions. *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 μPa^{2}s)	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*
16	Ambient	Control	36	0.2	0.2	0.0-0.5	4	0.6	1.2	-1.2-1.7	4
	171	207	74	0.5	0.7	-0.2-1.5	4	0.4	1.0	-0.7-1.7	4
22.4	Ambient	Control	36	-0.4	0.9	-1.4-1.1	5	-0.6	2.2	-3.3-2.3	5
	147	183	50	0.8	0.5	0.2-1.3	4	0.7	0.3	0.5-0.9	2
	153	189	55	3.1*	0.6	2.6-3.9	4	0.8	1.2	0.0-1.6	2
	159	195	61	7.6*	1.1	7.0-9.3	4	0.2	1.2	-1.2-1.8	4
	165	201	67	12.9*	1.2	11.3-13.8	5	4.8*	0.8	3.9-5.8	5
	171	207	74	16.3*	3.8	11.2-19.7	4	6.0*	0.6	5.2-6.7	6
32	Ambient	Control	36	0.9	0.7	-0.1-1.7	4	0.2	0.9	-1.0-1.1	4
	159	195	61	2.2	0.4	1.5-2.5	4	2.0*	1.0	0.6-2.8	4
	165	201	67	6.9*	1.7	5.1-8.5	4	3.9*	0.9	2.9-5.1	4
	171	207	74	12.0*	2.3	10.4-15.3	4	5.5*	0.7	4.6-6.1	4

With a hearing test signal of 16 kHz, statistically significant TTS$_{12\text{-}16}$ did not occur in M02 either, even after an SEL of 207 dB (re 1 μPa^{2}s; Table 3; Figures 10b & 12a). With a hearing test signal of 22.4 kHz, statistically significant TTS$_{12\text{-}16}$ occurred after SELs ≥ 201 dB (Table 3; Figure 10b). Recovery of hearing occurred within 24 min after both SELs (Figure 12b). With a hearing test signal of 32 kHz, significant TTS$_{12\text{-}16}$ occurred after an SEL of ≥ 195 dB (Table 2; Figure 10b). Recovery of hearing occurred within 20 min after SELs of 195 and 201 dB and within 24 min after an SEL of 207 dB (Figure 12c).

Individual Differences in TTS After Exposure to a 16 kHz NB

During four sessions, the order in which the California sea lions were tested at hearing frequency 22.4 kHz after exposure to the NB at 16 kHz (SEL 207 dB re 1 μPa^{2}s) was reversed. The mean TTS$_{1\text{-}4}$ of M02, measured at 22.4 kHz (14.5 dB, SD = 1.4 dB, *n* = 4), was 1.8 dB lower than the mean TTS$_{1\text{-}4}$ of F01 (16.3 dB, SD = 3.8 dB, *n* = 4) after exposure to the same SEL. The recovery patterns were similar, but the hearing of F01 took longer to recover (Figure 13a). The mean TTS$_{12\text{-}16}$ of F01 measured at 22.4 kHz (7.2 dB, SD = 1.2 dB, *n* = 4) was 1.2 dB higher than the mean TTS$_{12\text{-}16}$ of M02 (6.0 dB, SD = 0.6 dB, *n* = 6) after exposure to the same SEL. The recovery patterns were similar (Figure 13b).

Discussion and Conclusions

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 2 and 16 kHz differed by only a few dB (Kastelein et al., 2021b; present study) and were similar to the thresholds reported by Reichmuth et al. (2013) for those frequencies. Above 16 kHz, the thresholds resembled those of two California sea lions used in other research (Kastak & Schusterman, 1998; Southall et al., 2005; Mulsow et al., 2012). This suggests that the hearing of the sea lions in the present study was representative of their species.

The performance of both California sea lions was consistent throughout the course of the study period. Most TTS measurements had SDs of ≤ 2 dB (Tables 2 & 3). Mean pre-stimulus response rates by both sea lions were similar in all pre-exposure hearing tests, control tests, and hearing tests after exposure to the NBs at 8 and 16 kHz (Table 1), suggesting that performance was similar in all test

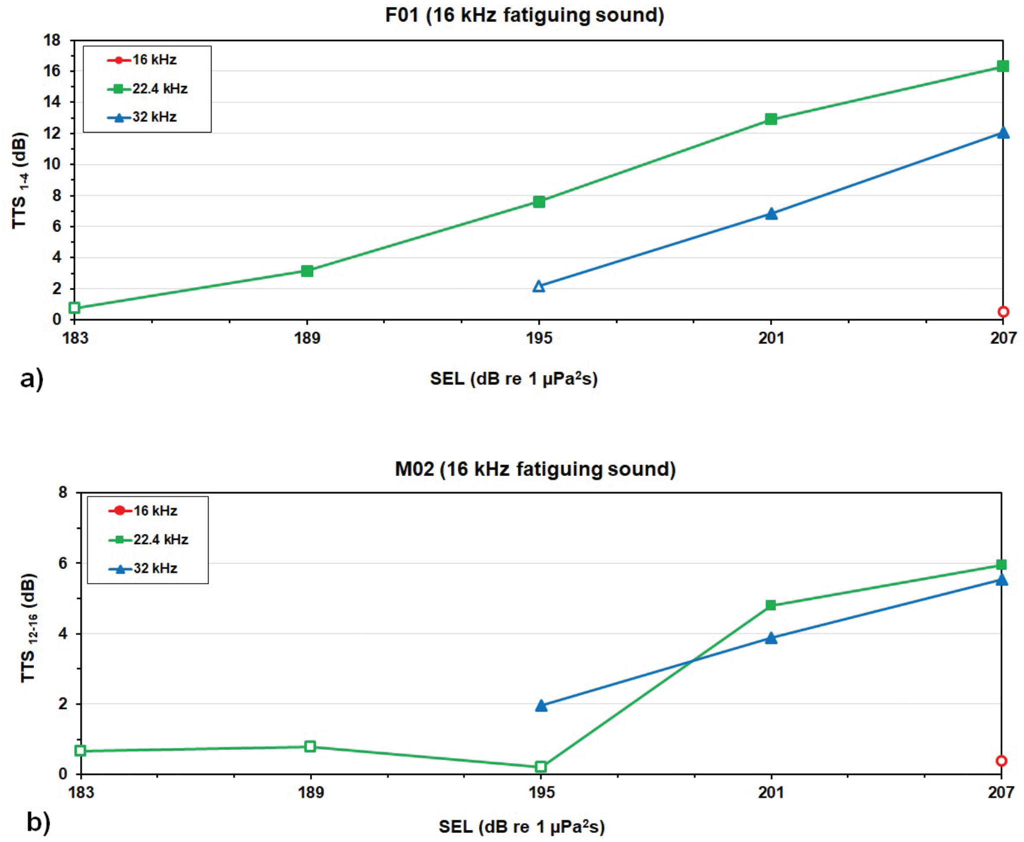


Figure 10. Mean TTS_{1-4} in F01 (a) and mean TTS_{12-16} in M02 (b) after 60-min exposure to a continuous one-sixth-octave NB centered at 16 kHz at several SELs (dB re 1 μPa²s), quantified at hearing frequencies 16, 22.4, and 32 kHz (i.e., at the center frequency of the fatiguing sound, half an octave above it, and one octave above it). Hearing frequency 16 kHz was tested only after exposure to 207 dB SEL (and no TTS occurred). Open symbols indicate thresholds similar to those in control tests (no TTS); solid symbols indicate statistically significant TTS relative to the control sessions. Sample size varies per data point (see Table 3). For SPLs (dB re 1 μPa), subtract 36 dB from the SEL values. For SDs and control values, see Table 3 and Figures 11 & 12.

periods. The TTS levels of F01 were slightly more variable than those of M02 over time. Overall, both sea lions exhibited consistent response patterns to the particular sound exposures in terms of initial TTS and recovery patterns. 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 short exposure periods and the relatively low TTSs elicited in the present study compared to those in the Kujawa & Liberman (1997) and Mannström et al. (2015) studies, as discussed by Houser (2021).

California sea lions are able to lift their heads out of the water. 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 lower jaws were above the water surface 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 2 & 3). The building around the pool had hard inside surfaces and caused the SPL in air to be fairly homogeneous due to reflections. In many cases, aerial SPLs during the fatiguing sound exposures were so high that the operator in the equipment cabin had to wear ear protectors (despite the dividing wall). Therefore, it was not considered

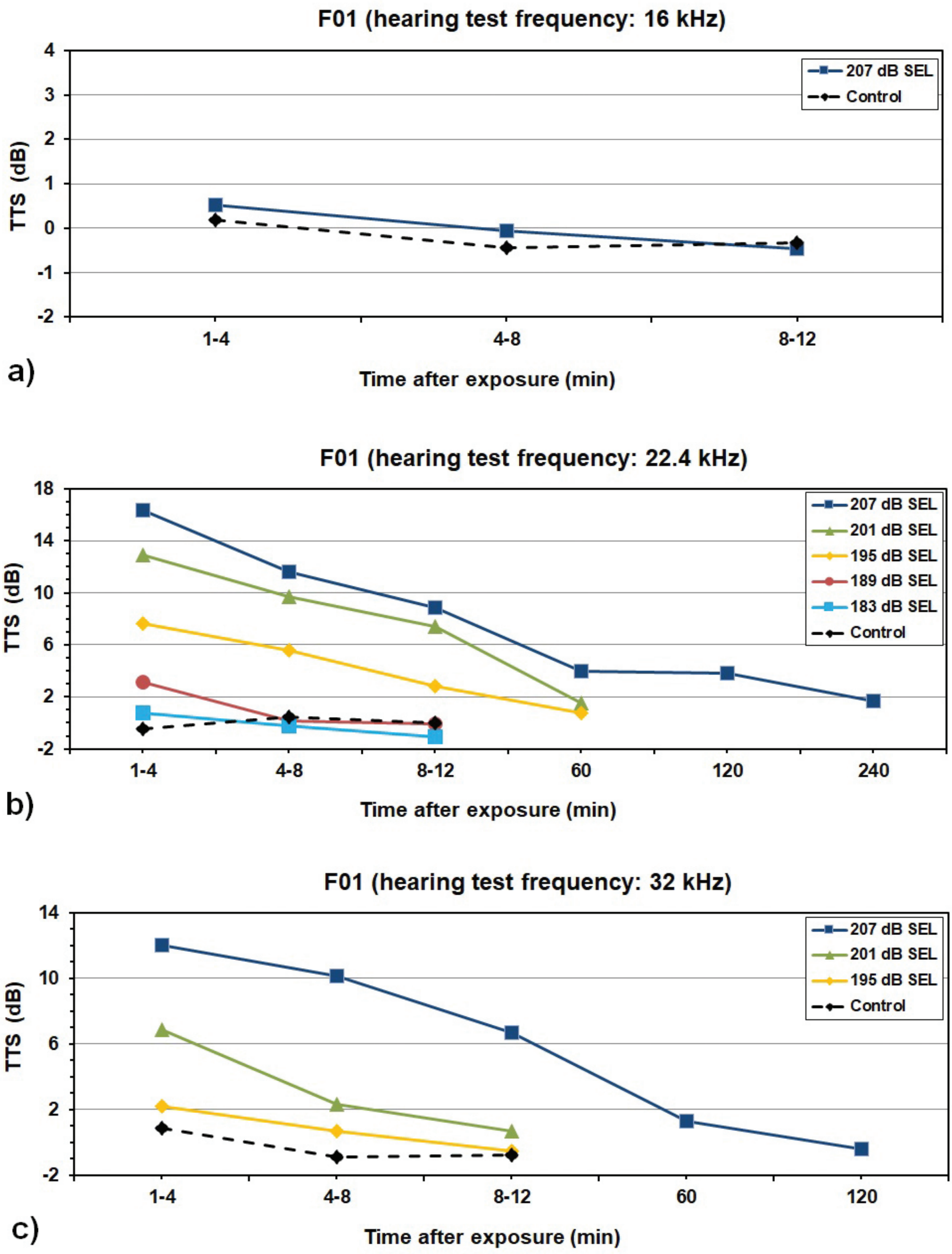


Figure 11. Changes in the mean TTS of F01, including recovery, tested at 16 kHz (a), 22.4 kHz (b), and 32 kHz (c) after 60-min exposure to a continuous one-sixth-octave NB centered at 16 kHz at several SELs (dB re 1 $\mu Pa^2 s$). Hearing was considered recovered once TTS was < 2 dB. For sample sizes and SDs (only for TTS_{1-4}), see Table 3. 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 mean "TTS" values during control sessions (no shifts occurred) are also shown.

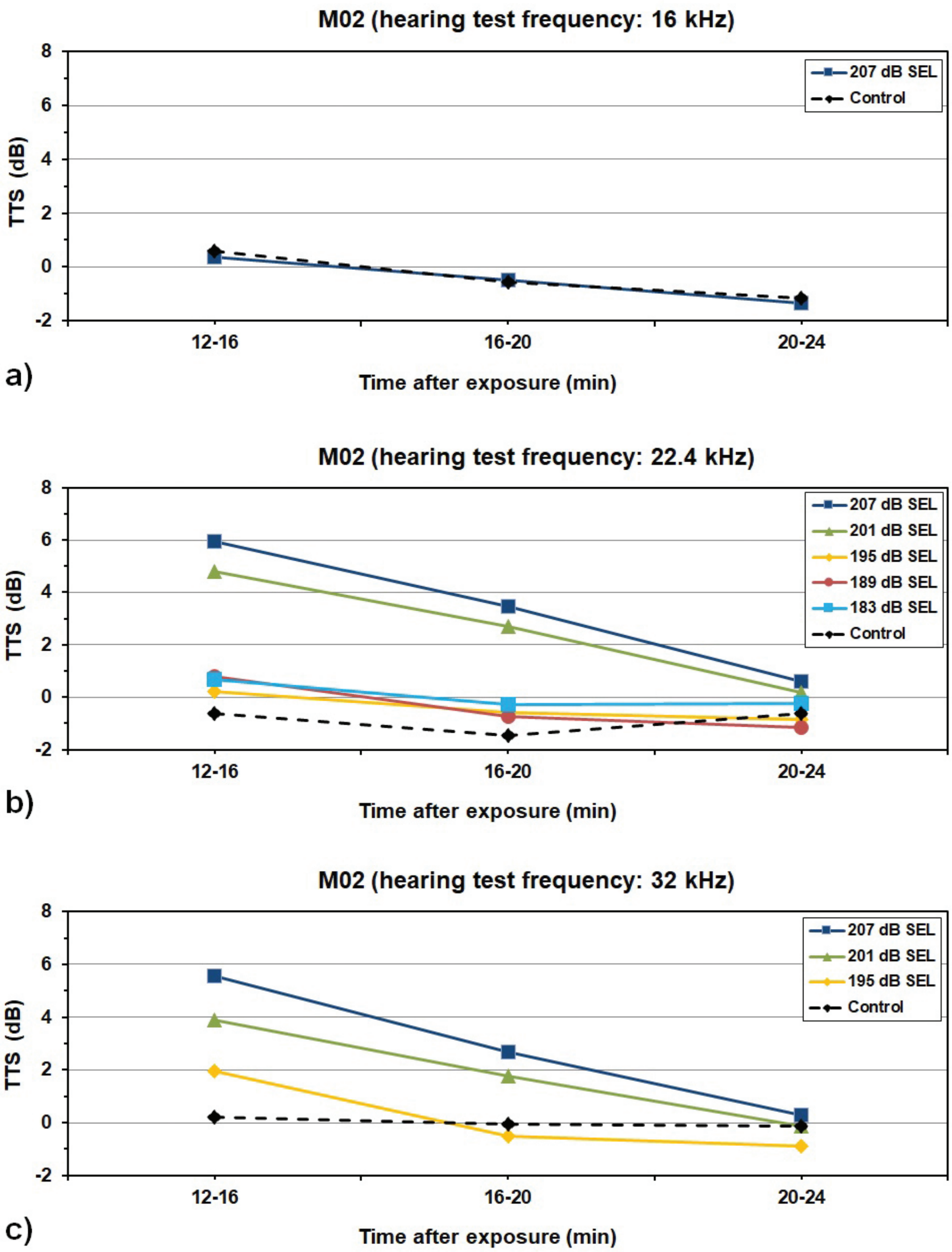


Figure 12. Changes in the mean TTS of M02, including recovery, tested at 16 kHz (a), 22.4 kHz (b), and 32 kHz (c) after 60-min exposure to a continuous one-sixth-octave NB centered at 16 kHz at several SELs (dB re 1 μPa^2s). Hearing was considered recovered once TTS was < 2 dB. For sample sizes and SDs (only for $TTS_{12\text{-}16}$), see Table 3. For average received SPLs (dB re 1 μPa), subtract 36 dB from the SEL values. The mean "TTS" values during control sessions (no shifts occurred) are also shown.

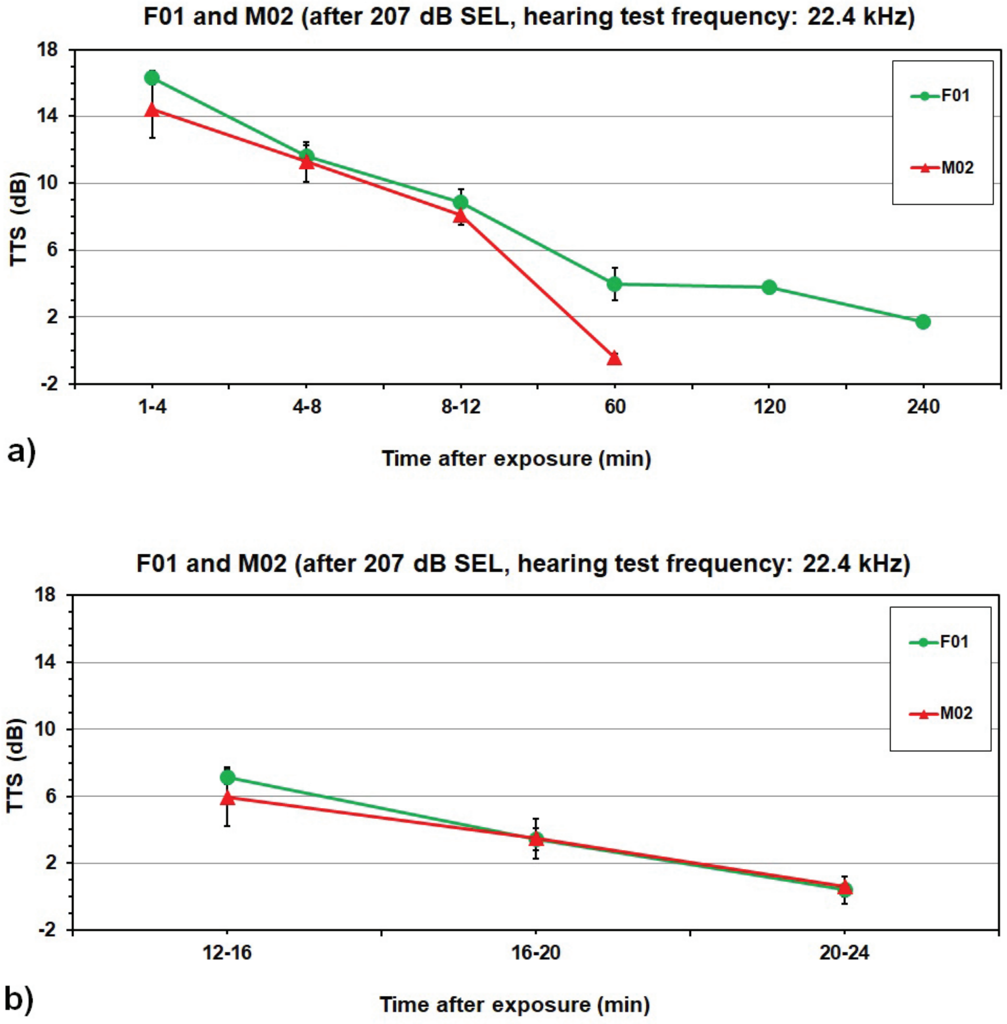


Figure 13. Testing individual differences in susceptibility to TTS. Mean TTS (± SD; $n = 4$) at 22.4 kHz in F01 and M02, measured 1 to 12, 60, 120, and 240 min (a) and 12 to 24 min (b) after 60-min exposure to the continuous NB at 16 kHz at an SEL of 207 dB re 1 $\mu Pa^2 s$.

necessary to project additional aerial fatiguing sound with aerial loudspeakers during exposure sessions. Even when the sea lions lifted their heads out of the water for short periods, the duty cycle of the fatiguing noise remained close to 100%, and the sea lions did not experience quiet intervals. Therefore, our underwater SELs (and, thus, TTS measurements) are assumed to be accurate.

Magnitude of TTS and Onset SEL

In both F01 and M02, the largest initial TTSs occurred when the hearing test frequency was half an octave higher than the center frequency of the fatiguing sound. 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 frequencies, the onset of $TTS_{1\text{-}4}$ in F01 after exposure to the NB at 8 kHz occurred at an SEL of 177 dB re 1 $\mu Pa^2 s$ (at 11.3 kHz); after exposure to the NB at 16 kHz, onset occurred at 193 dB re 1 $\mu Pa^2 s$ (at 22.4 kHz). After exposure to the NB at 8 kHz (followed by some recovery of hearing), the onset of $TTS_{12\text{-}16}$ in M02 occurred at 185 dB re 1 $\mu Pa^2 s$ (at 11.3 kHz); after exposure to the NB at 16 kHz, onset occurred at 207 dB re 1 $\mu Pa^2 s$

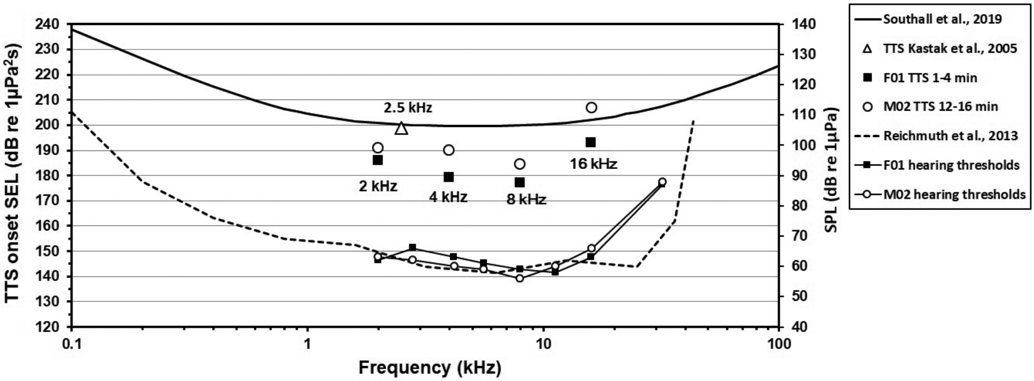


Figure 14. The SELs of one-sixth-octave NBs centered at 2 and 4 kHz (Kastelein et al., 2021b) and at 8 and 16 kHz (present study) which caused 6 dB TTS$_{1\text{-}4}$ in F01 (■) and 6 dB TTS$_{12\text{-}16}$ in M02 (○). In this figure, the lowest SEL required to cause 6 dB TTS is defined as a marker 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 2 and 32 kHz are also shown (right-hand y-axis, showing SPLs).

(at 22.4 kHz; Figure 14). These results, combined with data on TTS after exposure to fatiguing sounds centered at 2 and 4 kHz (Kastelein et al., 2021b), 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., 2021a), Yangtze finless porpoises (*Neophocaena phocaenoides asiaeorientalis*; Popov et al., 2011), and harbor seals (Kastelein et al., 2020).

The hearing thresholds for 8 and 16 kHz tonal signals of harbor seals and California sea lions are similar (Kastelein et al., 2009b; Reichmuth et al., 2013; present study; Figure 14). Susceptibility of California sea lions to TTS after exposure to sounds around 8 kHz is similar to that of harbor seals, but for sounds around 16 kHz, California sea lion hearing is less susceptible than harbor seal hearing (TTS onset requires a 13 dB higher SEL; Kastelein et al., 2020). Species-specific differences in susceptibility to TTS are important considerations in the protection of pinniped hearing from anthropogenic noise.

Individual Differences in Susceptibility to TTS

Testing the hearing of both California sea lions at the same times after the fatiguing sound stopped showed that TTSs and recovery patterns were similar (Figures 6 & 13). Susceptibility to TTS in both subjects was also similar for NBs at 2 and 4 kHz (Kastelein et al., 2021b). However, the sample size 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 is needed to assess the generality of the results obtained in the present study.

Effect of Fatiguing Sound Duty Cycle on TTS

Fatiguing sound duty cycle had a large impact on initial TTS in both subjects: a 10% reduction in duty cycle resulted in an 18% reduction in TTS$_{1\text{-}4}$ in F01 and a reduction of 35% in TTS$_{12\text{-}16}$ in M02 (Figure 7). In both subjects, the TTS was reduced by 3 dB by introducing an interval of only 180 ms between signals (i.e., by reducing the duty cycle from 100 to 90% and, thus, decreasing the cumulative SEL by only 0.5 dB). These findings show that the hearing of California sea lions can recover, at least partly, during brief intervals between fatiguing sound exposures. Thus, at the low duty cycle most commonly used in the U.S. Navy's 53C sonar system (2.5%), the SPL received by a California sea lion would need to be extremely high to elicit TTS. 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 the equal-energy hypothesis is supported for California sea lion hearing for NBs at 8 kHz in the duration and SPL ranges tested. Another study in this research project, with 4 kHz fatiguing sound, also supported the equal-energy hypothesis (Kastelein et al., 2021b).

Other TTS studies with marine mammals have both supported the equal-energy hypothesis (for California sea lions, Kastak et al., 2007; for harbor porpoises, Kastelein et al., 2014) and refuted the hypothesis (for harbor seals, Kastelein et al., 2012b; for bottlenose dolphins, Mooney et al., 2009; Finneran & Schlundt, 2010; for harbor porpoises, Lucke et al., 2009; Kastelein et al., 2012a; and for beluga whales [*Delphinapterus leucas*], Popov et al., 2014).

In beluga whales, a species for which the equal-energy hypothesis does not apply, when fatiguing SEL is equal, greater TTS occurs at higher SPLs with shorter exposures than at lower SPLs with longer exposures (Popov et al., 2014). Within a certain range, the SEL of a fatiguing sound can predict the initial TTS it induces. Data obtained only from high-SPL, short-duration exposures might result in overestimation of the TTS induced as a function of the exposure duration, particularly if they are extrapolated to low-SPL, long-duration exposures. Further ethical testing of the equal-energy hypothesis is needed for environmental impact assessments and to enable the prediction of TTS in marine mammals that are exposed to fatiguing sounds.

Towards Improved Protection of Otariidae from Underwater Anthropogenic Noise

Hearing damage, whether permanent or temporary (but repeated), may compromise an individual California sea lion's fitness and/or survival. The current study suggests that significant reductions in hearing damage from anthropogenic noise could be achieved by reducing the duty cycle by introducing short intervals of silence, allowing hearing to recover, at least in part, during periods of sound exposure. It is possible that even periods of low SPL would be sufficient. In addition, California sea lions are more susceptible to TTS than previously predicted: all SELs at which the (6 dB) onset of TTS_{1-4} has been observed in F01 so far (Kastelein et al., 2021b; present study) are below the (6 dB) TTS-onset SELs modeled and predicted by Southall et al. (2019) for Otariidae, as are most of the TTS_{12-16} onset SELs of M02. Methodological differences between the Kastak et al. (2005) and Kastelein et al. studies that may explain the differences in TTS-onset SELs are discussed in detail by Kastelein et al. (2021b). Based on the results presented in the present study, we recommend that the TTS-onset SEL thresholds for Otariidae (marine mammal group OCW; Southall et al., 2019) should be reduced, from a minimum of 200 dB re 1 μPa^2s (at 2.5 kHz) to 177 dB re 1 μPa^2s (at 8 kHz; see Figure 14).

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