Long-term evidence of noise-induced permanent threshold shift in a harbor seal (*Phoca vitulina*)[a]

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In psychophysical studies of noise-induced hearing loss with marine mammals, exposure conditions are often titrated from levels of no effect to those that induce significant but recoverable loss of auditory sensitivity [temporary threshold shift (TTS)]. To examine TTS from mid-frequency noise, a harbor seal was exposed to a 4.1-kHz underwater tone that was incrementally increased in sound pressure level (SPL) and duration. The seal's hearing was evaluated at the exposure frequency and one-half octave higher (5.8 kHz) to identify the noise parameters associated with TTS onset. No reliable TTS was measured with increasing sound exposure level until the second exposure to a 60-s fatiguing tone of 181 dB re 1 μPa SPL (sound exposure level 199 dB re 1 $\mu Pa^2 s$), after which an unexpectedly large threshold shift (>47 dB) was observed. While hearing at 4.1 kHz recovered within 48 h, there was a permanent threshold shift of at least 8 dB at 5.8 kHz. This hearing loss was evident for more than ten years. Furthermore, a residual threshold shift of 11 dB was detected one octave above the tonal exposure, at 8.2 kHz. This hearing loss persisted for more than two years prior to full recovery. https://doi.org/10.1121/1.5129379

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I. INTRODUCTION

Auditory effects of intense or chronic noise exposure in marine mammals are studied for conservation and management reasons. Such research seeks to identify the acoustic parameters of exposures that are expected to cause either temporary or permanent elevations in hearing threshold for different species (National Marine Fisheries Service, 2018; Southall *et al.*, 2019). Similar studies have been conducted with terrestrial mammals for many decades, primarily to model the effects of industrial and military noise on human hearing (see Salvi and Boettcher, 2008). In laboratory animals, it is common to expose many individuals to high-amplitude, long-duration noise to induce substantial (>30 dB) temporary threshold shifts (TTS) or to cause non-recoverable, permanent threshold shifts (PTS). This enables direct examination of exposure conditions and recovery periods associated with hearing loss. Ethical considerations and limitations of sample size preclude similar approaches with marine mammals. Instead, marine mammal studies use less intense fatiguing stimuli with psychoacoustic or electrophysiological methods of hearing assessment (for review, see Finneran, 2015). The aim is to induce relatively small, reliable, and recoverable hearing losses that can be used to describe TTS onset conditions and growth curves, and to predict—based on data from terrestrial mammals—the scaled-up conditions that would result in PTS. As a result, despite substantial concern for lasting hearing damage caused by human-generated noise in the oceans, there are no confirmed cases of noise-induced PTS in marine mammals.[1]

Threshold shifts of 30 to 60 dB have sometimes occurred during auditory studies with seals, sea lions, dolphins, and porpoises (Finneran *et al.*, 2007; Finneran and Schlundt, 2013; Kastak *et al.*, 2007; Kastelein *et al.*, 2013; Kastelein *et al.*, 2014a; Popov *et al.*, 2011b; Popov *et al.*, 2011a; Popov *et al.*, 2013; Popov *et al.*, 2014). The fatiguing sounds in these cases were tones or band-limited noise. Although the induced threshold shifts were larger than expected based on intended exposure parameters, complete recovery of hearing threshold occurred within several days. Progression of recovery was monitored where possible, showing trends that either appeared linear when referenced to logarithmic time scales, or that contained at least two regions with different slopes (see Finneran, 2015). In terrestrial mammals exhibiting even larger shifts in hearing, auditory recovery can occur over three temporal phases (Salvi and Boettcher, 2008). Given the limited available data, it remains unknown whether the time course and progression of hearing recovery can be predicted solely from the magnitude of initial threshold shifts.

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In 2007, during an effort to better understand the acoustic conditions associated with mild to moderate TTS, the underwater hearing sensitivity of a trained harbor seal (*Phoca vitulina*) was evaluated before and immediately following exposure to a 4.1-kHz tonal fatiguing stimulus. Rather than detecting the onset of TTS as a relatively small but repeatable reduction in hearing sensitivity as the exposure level was systematically raised over a three-month period, an unexpected and abrupt threshold shift >47 dB was observed one-half octave above the exposure frequency. This report provides the details of that event and the subsequent long-term tracking of auditory recovery, which revealed a permanent, focal (narrowband) hearing loss for this seal. As this is the first confirmed report of PTS following a known acoustic exposure event in a marine mammal, this information should be useful for estimating and mitigating the effects of human-generated noise on marine life.

II. MATERIALS AND METHODS

A. Study design

Studies of TTS in marine mammals often utilize noise titration procedures prior to undertaking systematic testing using balanced experimental designs (for review see Finneran, 2015). This initial ramp-up phase enables the specific acoustic conditions associated with TTS onset in individual animals to be conservatively determined by slowly increasing the fatiguing noise exposure from a level of no effect on hearing threshold to a level of repeatable threshold shift (TS). In this study, ramp-up testing was conducted prior to a planned experiment by measuring hearing thresholds in a single harbor seal before and after underwater exposure to a 4.1-kHz pure tone. Over the course of 93 days and 25 exposures, the duration of the fatiguing tone was increased from 12 to 60 s, and sound pressure level (SPL) was increased from 117 to 182 dB re 1 μPa. This generated a progressive range of unweighted, cumulative sound exposure levels (SEL) that extended from 128 to 199 dB re 1 $\mu Pa^2 s$. The first 24 exposures yielded no residual change in auditory sensitivity for the harbor seal, either at the center frequency of the fatiguing sound (4.1 kHz) or one-half octave higher (5.8 kHz). After an unexpectedly large TS was observed at 5.8 kHz following the final exposure, recovery was monitored closely during the first 72 h, almost daily during the following weeks and months, and intermittently in water and in air during subsequent years in order to thoroughly document recovery of hearing in this seal as well as long-term (permanent) hearing loss.

B. Subject and testing environment

The subject was a male Pacific harbor seal (*P. v. vitulina*) identified as *Sprouts* (NOA0001707). He was 19 years old at the start of testing (June 2007) and 30 years old at the conclusion of testing (September 2018). The seal was housed in flow-through saltwater pools with adjacent deck space at Long Marine Laboratory at the University of California Santa Cruz in Santa Cruz, CA. He participated in various psychoacoustic experiments prior to and during data collection for this study, including threshold audiometry (Cunningham and Reichmuth, 2016; Kastak and Schusterman, 1998; Reichmuth *et al.*, 2013; Southall *et al.*, 2005), auditory masking (Cunningham and Reichmuth, 2016; Southall *et al.*, 2000, 2003), and TTS assessment following exposure to broadband or octave-band noise (Kastak *et al.*, 1999; Kastak *et al.*, 2005a; Kastak and Schusterman, 1996; Kastak *et al.*, 2005b). His underwater hearing thresholds were comparable to those previously reported for other harbor seals (Kastelein *et al.*, 2009; Møhl, 1968; Terhune, 1988), and he showed no indication of age-related hearing loss during the study period.

Underwater testing occurred in a partially in-ground, seawater-filled, circular concrete pool (7.6 m diameter, 1.8 m depth). In-air testing occurred in a custom-built hemi-anechoic acoustic chamber (3.3 m long, 2.3 m wide, 2.2 m high). These testing environments have been used in other auditory studies and are further described by Sills *et al.* (2014).

To ensure that conditions were sufficiently quiet to measure absolute thresholds, ambient noise in the underwater testing environment was measured using a TC4032 low-noise hydrophone (0.01–80 kHz, +/− 2.5 dB; Reson A/S, Slangerup, Denmark) paired with a spectrum analyzer or a self-powered 2250 sound level meter (Brüel & Kjær A/S, Nærum, Denmark). Ambient noise in the acoustic chamber was similarly measured with a Brüel & Kjær 4189 free-field microphone (0.006 to 20 kHz) paired with the self-powered 2250 sound level meter.

C. Fatiguing stimulus

The fatiguing sound presented during the ramp-up phase was a 4.1-kHz pure tone with duration of 12, 16, 30, or 60 s including 3-s rise/fall times, and received level between 117 and 182 dB re 1 μPa (for details and corresponding SEL values, see Table I). The tone was played under water through a LL1424HP transducer (Lubell Labs, Columbus, OH) positioned near the bottom of the pool. A PVC chin rest mounted to the square frame of the transducer served as a station for the seal, who was trained to swim to the chin rest and remain in place for the duration of the exposure. The tone was generated using hearing test program (HTP) custom software (Finneran, 2003) in LABVIEW [National Instruments (NI) Corp., Austin, TX] and sent through an NI PXI-6229 data acquisition (DAQ) card with an attached BNC-2120 breakout box, a 3550 bandpass filter (Krohn-Hite, Brockton, MA), a P7000 amplifier (Hafler Professional, Tempe, AZ), and a Lubell Labs AC1424HP bridging transformer box prior to projection through the LL1424HP transducer.

The fatiguing tone was calibrated at the exposure station prior to each exposure event to ensure spectral content and level. It was received by the TC4032 hydrophone and passed through a Krohn-Hite 3364 anti-aliasing filter to the same NI system. Variability in the received sound field was less than +/− 3 dB within the area occupied by the seal's head when positioned at the station.

D. Audiometric signals

The signals used to evaluate TTS were 500-ms frequency-modulated (FM) upsweeps with 10% bandwidth (+/− 5% of

TABLE I. The sequence of progressively increasing tonal noise exposures at 4.1 kHz that culminated in an unexpectedly large threshold shift (TS) at 5.8 kHz after the 25th exposure. Exposure conditions (duration, SPL, and unweighted cumulative SEL) associated with each measured TS are specified, as is the time taken to complete threshold measurement (i.e., to reach the fifth descending miss) following cessation of the fatiguing tone. Note that final TS values are reported here, which were calculated as the difference between the pre-exposure and post-exposure hearing thresholds based on the first five hit-to-miss transitions of each. However, based on initial high misses, exposure numbers 21, 22, and 24 had indications of initial TS in excess of 26 dB and exposure number 25 had indications of initial TS greater than 57 dB prior to final threshold measurement. To illustrate the temporal spacing of auditory exposures, study day (referenced to exposure number 1) is also provided.

Exposure number	Study day	Exposure duration s	Exposure SPL dB re 1 μPa	Exposure SEL dB re 1 $\mu Pa^2 s$	Hearing frequency kHz	Threshold shift dB	Time to threshold min:s
1	0	12	117	128	4.1	0	8:19
2	2	16	121	133	4.1	−2	5:49
3	3	16	121	133	4.1	0	7:42
4	8	12	136	147	4.1	−1	7:29
5	9	16	136	148	4.1	7	8:35
6	10	16	133	145	4.1	−1	7:14
7	15	30	137	152	4.1	5	5:22
8	17	30	138	153	4.1	1	5:09
9	21	30	138	153	4.1	−2	5:38
10	22	30	152	167	4.1	−1	5:02
11	28	30	158	173	4.1	2	6:23
12	42	30	158	173	5.8	2	6:20
13	44	30	158	173	5.8	−1	4:16
14	45	30	164	179	5.8	10	3:38
15	50	30	164	179	5.8	4	7:44
16	52	30	169	184	5.8	−2	6:22
17	58	30	169	184	5.8	−1	5:30
18	65	30	171	186	5.8	0	5:07
19	72	30	174	189	5.8	2	5:30
20	73	30	178	193	5.8	0	6:02
21	74	30	182	197	5.8	8	9:23
22	77	30	182	197	5.8	2	12:42
23	84	30	181	196	5.8	−6	5:45
24	91	60	182	199	5.8	7	9:04
25	93	60	181	199	5.8	47	7:48

center frequency) and 20-ms rise/fall times. Audiometric signals were centered at 4.1 kHz (the exposure frequency), 5.8 kHz (one-half octave above the exposure frequency), or 8.2 kHz (one octave above the exposure frequency) and were generated using HTP software. Outgoing signals were sent from HTP software through an NI data acquisition system (PXI-6229 DAQ card with attached BNC-2120 breakout box, PXI-6070E DAQ card mounted in a PXI-1010 chassis with a SCB-68A connector block, or a USB-6259 BNC M-series DAQ module). The signals passed through a PA5 digital attenuator (Tucker Davis Technologies, Inc., Alachua, FL) and a Krohn-Hite 3550 or 3364 filter, and were transmitted into the pool from a 1042 projecting hydrophone (International Transducer Corporation, Santa Barbara, CA), the LL1424HP transducer with bridging transformer box, or a J-11 transducer (Naval Undersea Warfare Center, Newport, RI). In some cases, a Hafler P1000 amplifier was used in line before the projector.

Audiometric signals were projected into the pool at least 5 m from a water-filled PVC apparatus that served as a listening station. This apparatus included a chin rest where the seal could reliably position his head at a depth of 0.8 to 1 m, a response target he could press upon detection of a signal, a trial light to denote the duration of each test trial, and an underwater camera that enabled a remote experimenter to view each trial in real time. The signals were calibrated daily with the TC4032 hydrophone and returned through the 3364 filter and NI DAQ system to HTP software. The sampling rate on the incoming signal was at least 200 kHz. Variability in the received sound field was less than +/− 3 dB within the area occupied by the seal's head when positioned at the listening station.

In the year after the final exposure, hearing sensitivity was assessed in air in the acoustic chamber. Airborne signals were 500-ms, 5.8-kHz pure tones that were produced in the same manner as during underwater testing, but transmitted through a 2404 H speaker (JBL Incorporated, Northridge, CA) that was at least 0.8 m from the listening station. Similar to the underwater listening station, this setup included a chin rest, response target, trial light, and video camera. Daily calibration signals were received with a C550H microphone (0.02–20 kHz, +/− 2 dB; Josephson Engineering, Santa Cruz, CA) and returned to HTP software through the same hardware chain described above, with variability in the received sound field less than +/− 3 dB.

E. Threshold testing

The procedure for TTS testing involved (1) measurement of a pre-exposure hearing threshold, (2) voluntary exposure to the 4.1-kHz fatiguing tone, (3) measurement of a post-exposure hearing threshold within minutes of the exposure event, and (4) confirmation of normal hearing 24 h following exposure.

The psychophysical method used to estimate hearing thresholds was an adaptive up-down staircase procedure (Cornsweet, 1962). The seal was cued by a trainer to swim to the listening station and wait for a test tone during a 4-s interval denoted by the trial light. If the seal detected the tone during the trial interval, he indicated so by touching the response target with his nose. If no tone was detected, he remained in position at the station. Feedback for each correct response (reporting presence or absence of the signal as appropriate) was given in the form of an acoustic conditioned reinforcer, and the seal proceeded to the next trial. No feedback was provided following errors (reporting signal detections on signal-absent trials or reporting signal absence on signal-present trials). Following completion of a predetermined series of 1 to 5 correct trials, the seal was recalled to the surface by the trainer to receive primary reinforcement (one piece of fish for each correct response). Testing in air was conducted in the same manner, except that single trials were presented and followed individually by fish reinforcement for correct responses.

The proportion of signal-present trials in a session was between 50% and 75%. Signal-present and signal-absent trials occurred in a pseudorandom order. Each session began with several supra-threshold warm-up trials and ended with several supra-threshold cool-down trials intended to maintain subject motivation and behavioral control. The main portion of each session included trials that were used to estimate threshold. During this phase, the signal SPL was lowered by 2 dB following each correct detection, and raised by 2 dB following each failure to detect (miss). Testing proceeded in this up-down fashion until five hit-to-miss transitions were completed. Threshold was determined from performance on signal-present trials following the method of Dixon and Mood (1948). False alarm rate for each session was defined as the percentage of signal-absent trials on which the seal reported detection of a signal (responses prior to signal presentation on signal-present trials were also scored as false alarms).

Additional details of the testing configurations and methods used to measure hearing in air and under water can be found in Reichmuth *et al.* (2013).

F. Threshold shift and recovery assessment

Threshold shift was calculated for each exposure session as the difference between the pre-exposure hearing threshold and the post-exposure hearing threshold. The time taken to measure the post-exposure hearing threshold was estimated as the duration between the offset of the fatiguing tone and the fifth hit-to-miss transition of the session.

Following the large TS observed after the last exposure to the fatiguing tone, thresholds were repeatedly measured to track hearing recovery. Audiometric testing was conducted primarily at 5.8 kHz, but also at 4.1 and 8.2 kHz. Within the first 6 h (<400 min) following this exposure, hearing sensitivity was opportunistically measured four times at 5.8 kHz and once at 4.1 kHz. In the five days following the exposure (1000 to 7500 min) hearing was measured eight times at 5.8 kHz and four times at 4.1 kHz. Beyond that, thresholds were measured regularly over the next year and intermittently over the next 10 years, including an additional 136 sessions at 5.8 kHz, 35 sessions at 8.2 kHz, and 19 sessions at 4.1 kHz. To determine a TS for each of these "recovery" sessions, the threshold estimate for the session was referenced to the mean hearing threshold obtained at the same frequency prior to the exposure event.

In addition to tracking long-term recovery using the adaptive staircase method, audiometric testing was conducted periodically using the method of constant stimuli (Stebbins, 1970). This provided a robust measure of threshold at each frequency based on psychometric functions generated from pooled performance over multiple sessions, and data that could be directly compared to previous measurements obtained for this seal using the same method (Reichmuth *et al.*, 2013; Southall *et al.*, 2005).[2] Thresholds were measured over 2 to 3 sessions to ensure stable performance (95% confidence intervals of <3 dB for the pooled data). Hearing thresholds were measured in this manner under water at 4.1, 5.8, and 8.2 kHz at one or more intervals of 1, 2, 5, 8, and 10 years following the final exposure event.

To evaluate the effect of medium on the observed hearing loss, thresholds were measured at 5.8 kHz in air with the method of constant stimuli. The in-air and underwater thresholds measured at 5.8 kHz in the year following the last exposure event were then compared to thresholds obtained previously for this individual (estimated from Reichmuth *et al.*, 2013; Southall *et al.*, 2005).

III. RESULTS

A. Threshold shifts

Figure 1 shows the gradual increase in exposure level of the 4.1-kHz fatiguing tone and the corresponding threshold shifts measured for the harbor seal. The conditions specified for each exposure event (tone duration, SPL, and SEL), the hearing test frequency, and the time to threshold measurement are provided in Table I. The seal's behavior was consistent throughout successive exposures: he remained motionless at the exposure station, and never left the station prior to being cued to do so. His behavior during corresponding audiometric testing was also generally stable: mean false alarm rate was 0.14 (SD 0.15) for both pre- and post-exposure sessions. Threshold measurements began immediately following each exposure and were typically completed 6:42 min:s (SD 1:56) after cessation of the fatiguing tone.

For the first eleven exposures, the seal's hearing was tested at 4.1 kHz, the frequency of the fatiguing tone. In these sessions, stimulus duration was 12, 16, or 30 s while SPL increased from 117 to 158 dB re 1 μPa. The mean threshold shift associated with these exposures was 1 dB (SD 2.9), and there was no trend in increasing TS at 4.1 kHz with increasing SEL ($R^2 = 0.01$, $p = 0.76$). After exposure 11, the audiometric test frequency was increased to 5.8 kHz to monitor for the emergence of an anticipated threshold shift

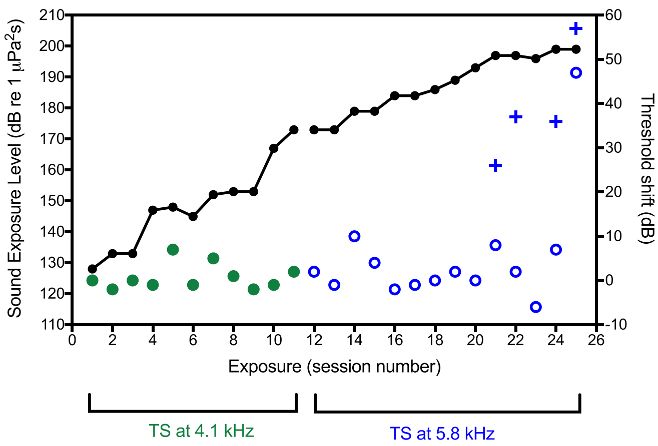

FIG. 1. (Color online) Ramp-up of the 25 tonal exposures at 4.1 kHz presented to the seal over the course of 93 days. Threshold shifts (TS, right *y* axis) measured at center frequency (4.1 kHz, closed circles) and one-half octave higher (5.8 kHz, open circles) are shown relative to the cumulative sound exposure level (SEL, black line corresponding to left *y* axis) of the fatiguing tone for each exposure session. See Table I for details. TS was determined for each session using an adaptive staircase method, with the five hit-to-miss transitions used to calculate threshold typically falling in the 3–7 min interval following exposure. Note that final thresholds measured for sessions 21, 22, 24, and 25 likely underestimate the magnitude of TS, as a result of the seal's failures to detect audiometric signals early in the post test (and the corresponding increased time required to measure threshold—see text). The crosses shown for these four sessions provide TS estimates based on the initial high misses (<1 min following exposure). After the last exposure (session 25), the seal demonstrated an initial TS of at least 57 dB (estimated within 1 min) and a final TS of 47 dB, measured within 8 min; after this, exposures were discontinued and hearing recovery was tracked over time.

one-half octave above the exposure frequency. During exposures 12 to 23, stimulus duration was 30 s while SPL was gradually increased to a maximum of 182 dB re 1 μPa. The mean TS associated with these exposures was 1 dB (SD 4.3). Again, there was no trend in increasing TS at 5.8 kHz with increasing SEL ($R^2 = 0.06$, $p = 0.46$). Between exposures 1 and 23, three shifts were noted in excess of 6 dB. In each case, repeated testing failed to confirm a TS of similar magnitude.

On exposures 24 and 25, the SPL of the fatiguing sound was 181–182 dB re 1 μPa and duration was 60 s. This doubling in duration from prior exposures corresponded to a 3-dB increase in SEL, to 199 dB re 1 μPa^2s. On exposure 24, a TS of 7 dB was measured. When the same condition was repeated two days later for exposure 25, a marked change in the seal's responsivity was observed. The seal failed to detect the highest signal level presented (117 dB re 1 μPa). Approximately 4 min following cessation of the fatiguing tone, the seal began to respond to warm-up signals and a TS of 47 dB was estimated based on the average of the hit-to-miss transitions.

In retrospective examination, the auditory performance of the seal on presumably supra-threshold warm-up trials in the final five exposure sessions (21–25) included some anomalous responses. While the seal typically made no errors on warm-up trials, on sessions 21, 22, 24, and 25 he initially failed to respond to the highest signal levels presented at the start of the session. At this point in the ramp-up progression, the SEL of the fatiguing tone was between 196 and 199 dB re 1 μPa^2s. Following these initial high misses, the seal began to detect the audiometric signal and then proceeded to complete the hearing test in a typical manner. In hindsight, these early errors may have indicated large but rapidly recovering shifts in sensitivity at 5.8 kHz. Our psychophysical method did not allow hearing to be fully assessed within the first few minutes (<3 min) following exposure. However, estimates of transient TS based on initial, high misses in the warm-up phases of these sessions likely provide a conservative approximation of temporary hearing loss within one to two minutes of the exposure event. *Post hoc* review of session data shows that such rapidly recovering shifts of at least 26, 37, 36, and 57 dB may have occurred after exposures 21, 22, 24, and 25, respectively, prior to the 47 dB shift that was ultimately measured following exposure 25.

B. Recovery of hearing and residual hearing loss

Prior to the final tonal exposure, the seal's underwater hearing sensitivity at 5.8 kHz was 62 dB re 1 μPa (SD 2.2, n = 24 sessions); his mean hearing threshold at 4.1 kHz was 59 dB re 1 μPa (SD 2.4, n = 23 sessions). These thresholds, determined by adaptive staircase testing, were the reference values used to track recovery following the large TS event. Figure 2 depicts the progression of auditory recovery at 4.1 and 5.8 kHz. Recovery is expressed in terms of time post exposure [log(min)], and extends over a period of 4006 days (nearly 11 years).

There are limited available data (24 sessions) to track hearing recovery at 4.1 kHz, the exposure frequency. However, these data show rapid and complete recovery of hearing within two days (<3000 min) of the final exposure event. Following this recovery period, there is no evidence of a residual threshold shift at 4.1 kHz (Fig. 2). The average TS at this frequency, measured from one to more than ten years following the exposure event, was −2 dB (SD 3.7).

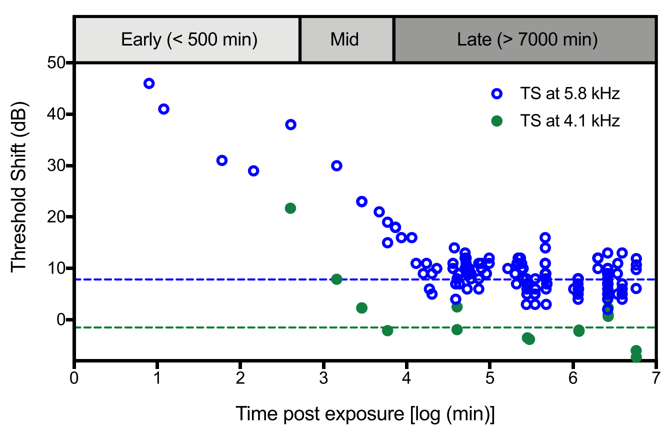

FIG. 2. (Color online) Recovery of hearing in the seal at 4.1 and 5.8 kHz following the final tonal exposure at 4.1 kHz. The plot shows residual threshold shifts (TS) determined from adaptive staircase testing expressed as a function of log(time). The amount of time represented by this plot is >10 years, which corresponds to 4006 days, 5 768 622 min, or 6.761 log(min). Dashed lines indicate mean TS calculated from data obtained more than 1 year [5.721 log(min)] following the exposure. The lower dashed line shows a TS of −2 dB at 4.1 kHz. The upper dashed line shows a TS of 8 dB at 5.8 kHz. Three identified phases of recovery are denoted above the data: early (<500 min), mid or intermediate (500 to ∼7000 min), and late (7000+ min); see text for details.

There are substantial data (148 sessions) available to examine recovery of hearing at 5.8 kHz. Thresholds measured at 5.8 kHz show a three-phase pattern of recovery that is generally consistent with that described by Salvi and Boettcher (2008). This consists of an early recovery phase (<500 min); a more rapid, intermediate-recovery phase (500 to ∼7000 min); and a gradual late-recovery phase followed by a plateau. At 5.8 kHz, hearing did not fully recover to baseline (pre-exposure) levels. The average TS, measured from one to more than ten years following the exposure event, was 8 dB (SD 2.8).

It is notable that the auditory data for 4.1 and 5.8 kHz (Fig. 2) demonstrate similar rates of recovery during the intermediate interval following the final exposure. Hearing recovered at a rate of −16 dB per log(min) at 4.1 kHz, and at rate of −17 dB per log(min) at 5.8 kHz during this interval.

Audiometric testing conducted at 8.2 kHz showed a persistent elevation in threshold (relative to what was expected based on interpolation from nearby frequencies) through the end of 2009 (average TS 11 dB, SD 3.6, n = 27 sessions). However, this shift was not evident during testing conducted in 2015 and 2018 (average TS of 2 dB, SD 2.3, n = 8 sessions), suggesting long-term (2+ years) recovery of hearing threshold at this frequency.

Threshold data obtained in water for this seal using the method of constant stimuli are provided in Fig. 3 for test frequencies of 4.1, 5.8, and 8.2 kHz, with threshold values obtained prior to the exposure event compared to those obtained in successive years. At 4.1 kHz (Fig. 3, upper panel), there was no residual change in sensitivity 1, 2, 5, or 10+ years following the exposure event (average TS −2 dB, SD 3.8). At 5.8 kHz (Fig. 3, middle panel), there was a significant, sustained loss of hearing (average TS 11 dB, SD 2.0) that was evident 1, 2, 5, 8, and 10+ years following the exposure event. This relative loss of underwater hearing sensitivity at 5.8 kHz was also expressed in the seal's ability to hear sounds in air, with a TS of 12 dB measured in the year following the exposure event (Fig. 4). A mixed picture emerges at 8.2 kHz (Fig. 3, lower panel). One year after the exposure event, a TS of 11 dB was measured. Similarly, two years after the exposure event, a TS of 10 dB remained. However, when tested 8 and 10 years later, hearing at 8.2 kHz had apparently returned to normal, with a residual TS of only 1 dB referenced to the estimated pre-exposure threshold.

For a broader perspective, the residual hearing loss can be evaluated in the context of the seal's entire underwater audiogram measured with the method of constant stimuli (Fig. 5). This composite audiogram includes hearing thresholds obtained prior to the tonal exposures at frequencies of 6.4 kHz and below, and thresholds obtained in the year following the exposures at frequencies of 4.1 kHz and above. The atypical notch in the audiogram shows elevated hearing thresholds at 5.8, 6.4, and 8.2 kHz, but not 4.1 kHz (the exposure frequency) or 12.8 kHz (more than an octave above the exposure). Aside from this focal area of hearing loss, the remainder of the audiogram appears normal (see Reichmuth *et al.*, 2013). Note again that the 8.2 kHz shift evident in this

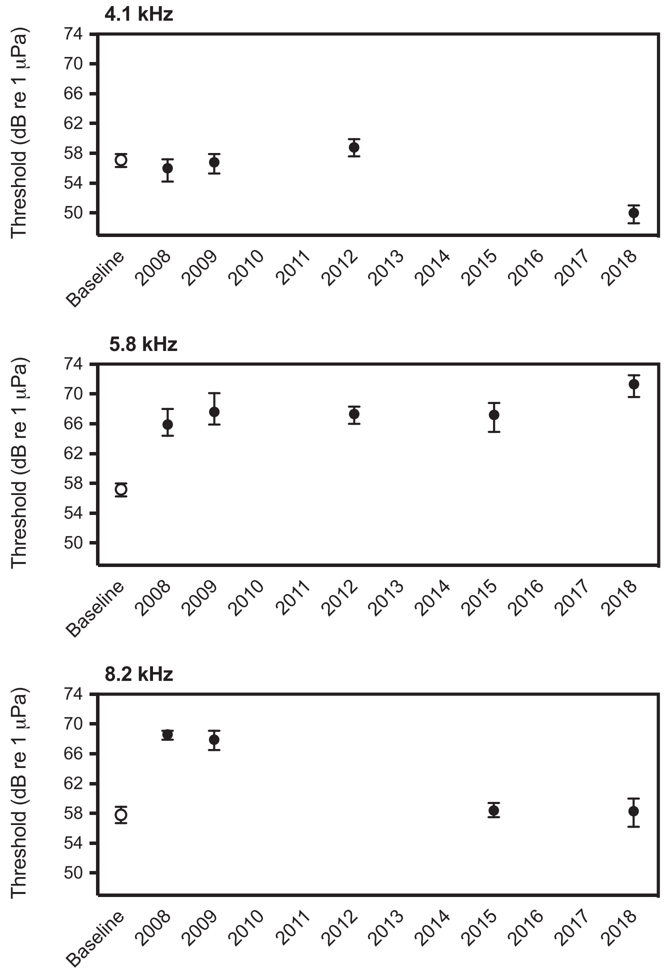

FIG. 3. Hearing thresholds obtained for the seal using the method of constant stimuli at 4.1 kHz (center frequency of exposure; upper panel), 5.8 kHz (one-half octave above exposure; middle panel), and 8.2 kHz (one octave above exposure; lower panel) in the years following the exposure event. For reference, baseline (pre-exposure) thresholds (open circles) were estimated from previously reported data at nearby frequencies (Southall *et al.*, 2005); see also Fig. 5. Error bars show 95% confidence intervals. The data demonstrate full hearing recovery at 4.1 kHz, incomplete recovery at 5.8 kHz with a stable threshold shift lasting at least 10 years, and what appears to be a persistent (>2 years) shift at 8.2 kHz that recovered sometime between 2- and 7-years post-exposure.

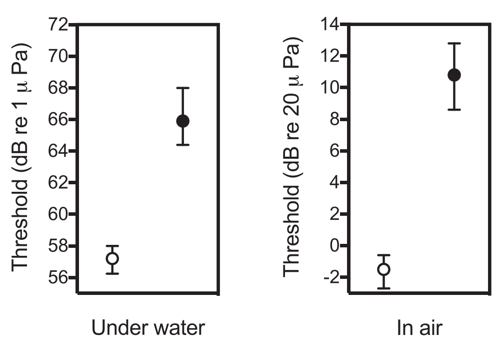

FIG. 4. Hearing thresholds obtained for the seal at 5.8 kHz both under water (left) and in air (right) using the method of constant stimuli. Baseline (pre-shift, open circle) thresholds were interpolated from previously reported data at surrounding frequencies (Southall *et al.*, 2005; Reichmuth *et al.*, 2013). Post-shift (closed circle) data were collected in the year after the final 4.1-kHz tonal exposure. Error bars show 95% confidence intervals. The comparison indicates similar, persistent threshold shifts (9 and 12 dB) under water and in air.

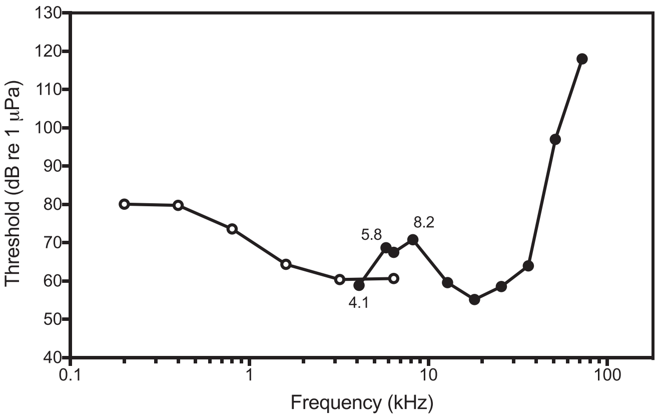

FIG. 5. Underwater composite audiogram for the seal demonstrating the change in hearing one year following the final tonal exposure. Hearing thresholds obtained using the method of constant stimuli are reported for frequencies between 0.2 and 72.4 kHz. Open circles show thresholds obtained prior to the 4.1-kHz tonal exposures (adjusted from Southall *et al.*, 2005). Closed circles show thresholds collected in 2008 during post-exposure audiometric testing (this study; adjusted from Reichmuth *et al.*, 2013). The prominent notch in the audiogram shows elevated thresholds between 5.8 and 8.2 kHz, one-half octave to one octave above the fatiguing tone.

audiogram in the year after the exposure event did eventually recover completely.

IV. DISCUSSION

One year following the second exposure to the 60-s, 4.1-kHz fatiguing tone of 181 dB re 1 μPa SPL (SEL of 199 dB re 1 $\mu Pa^2 s$), the seal's residual hearing loss of $\sim$ 10 dB spanned a frequency region from 5.8 to 8.2 kHz. While the loss of hearing one-half octave above the exposure frequency persisted for at least ten years, his hearing sensitivity one octave above the exposure exhibited very long-term recovery, eventually returning to pre-exposure values after more than two years. Thus, the hearing loss ultimately comprised an apparently permanent notch in the seal's audiogram one-half octave above the fatiguing tone.

The relatively small, permanent noise-induced hearing loss[3] in this harbor seal is well documented, both in terms of exposure parameters and long-term auditory effects. While the outcome was unanticipated, this study provides valuable data that contradict common assumptions about the relationship between TTS and PTS in marine mammals.

The most perplexing aspect of this case is the abrupt onset of high-magnitude (>47 dB) threshold shift that occurred during exposure ramp up without prior indication of smaller, recoverable threshold shifts. This indicates that a titration of SELs from a level of no effect toward a level of effect may not always result in predictable TTS. This finding is at odds with available data for other marine mammals using similar types of narrowband noise, which generally show gradual growth of TTS with increasing SEL, followed by more rapid growth in TTS above some critical exposure level (see Finneran, 2015; Kastelein *et al.*, 2014b; Kastelein *et al.*, 2019a; Kastelein *et al.*, 2019b). Also unexpected was the finding that—unlike some other studies that showed relatively high-magnitude threshold shifts in marine mammals (e.g., Finneran *et al.*, 2007; Kastak *et al.*, 2007; Kastelein *et al.*, 2013)—hearing losses observed in this seal either persisted for years or did not fully recover.

Subtle issues of session timing may help to explain the observed results. Here, threshold shift was measured in the interval between 3 and 7 min following the cessation of the fatiguing tone. This is comparable to the TTS_5 metric (i.e., TTS at 5 min post exposure) reported previously for seals and sea lions (Finneran *et al.*, 2003; Reichmuth *et al.*, 2016). However, this measurement window may have precluded detection of several large (>26 dB), rapidly recovering (<3 min) shifts in auditory sensitivity following exposure. A close review of within-session data suggests that the seal experienced three such shifts (exposures 21, 22, and 24) in the weeks prior to the final exposure. This pattern of large, repeated, and rapidly recovering shifts differs from expected results with terrestrial and marine mammals (see Finneran, 2015; Salvi and Boettcher, 2008). The significance of these events prior to the final exposure is unknown.

The physiological mechanisms underlying this seal's hearing loss are unclear. The data might suggest that hearing loss from the final exposure resulted from accumulation of noise-induced effects over the preceding exposures. However, metabolic stress, damage, or death of hair cells or primary auditory neurons would have been expected to yield smaller changes in threshold prior to the abrupt hearing loss. Perhaps accumulating auditory damage was reflected in the seal's immediate performance following exposures 21, 22, and 24, when he did not detect tones that were typically far above threshold. Rapid recovery of sensitivity (<3 min) following such high-magnitude hearing losses is unexpected, although anatomical evidence from terrestrial mammals suggests that auditory damage can be present even when behavioral hearing thresholds return to normal following noise exposure (Kujawa and Liberman, 2009).

Other metrics of auditory function besides behavioral threshold audiometry may have been more sensitive to the presence of underlying and accumulating damage in the seal's auditory system. These include measures of response time (a proxy for subjective loudness, Moody, 1970; Pfingst *et al.*, 1975) and auditory evoked potentials (AEPs). Reaction time measures were not collected during this study, but may have revealed subtle changes in the perceptual salience of supra-threshold auditory signals in sessions preceding the final exposure. AEP measurements are not presently feasible for amphibious mammals under water; thus, no electrophysiological data are available from this study. However, AEPs can sometimes reveal noise-induced changes in physiological thresholds in the absence of behavioral TTS (Finneran *et al.*, 2007; Finneran *et al.*, 2015; Kujawa and Liberman, 2009). As measurements of AEPs paired with behavioral hearing tests are possible in fully aquatic odontocetes, such dual assessments may help to identify physiological precursors to threshold shifts in marine mammals (see Finneran, 2015).

The three-phase pattern of auditory recovery at 5.8 kHz, which followed a complex multi-exponential function (see Finneran, 2015; Salvi and Boettcher, 2008) with resulting PTS, seems generally consistent with that reported for chinchillas (Saunders *et al.*, 1977). The highest rate of recovery was observed in the intermediate phase (500–7000 min post exposure), and was similar in magnitude to recovery rates reported for a dolphin and belugas after TTS exceeding 25 dB (Finneran *et al.*, 2007; Popov *et al.*, 2014). While the data available to describe hearing loss and fine-scale recovery for the seal one-half octave above the exposure frequency were extensive, the more limited data at 4.1 and 8.2 kHz prohibit a similarly thorough evaluation of recovery. Finneran (2015) modeled available TTS data for marine mammals using a simple relationship, with recovery occurring linearly with the logarithm of time. Such a function may describe the harbor seal's auditory recovery at 4.1 and 8.2 kHz based on the available data. Shifts at both of these frequencies recovered completely after several days to weeks, and thus can be classified as persistent hearing losses (Morfey, 2001). It is worth noting that the extensive time over which recovery was tracked in this study enabled detection of a return to baseline at 8.2 kHz after more than two years. Without such long-term testing, it is likely that this hearing loss one octave above the exposure frequency would have been considered a PTS.

These findings for a single harbor seal can be considered in the context of regulatory guidance for predicting the auditory effects of noise on marine mammals. At the start of this study in 2007, noise exposure criteria for seals and sea lions indicated PTS onset in water at a weighted level (i.e., taking into account the frequency response of the auditory system) of 203 dB re 1 μPa^2s (Southall *et al.*, 2007). This SEL criterion was recently adjusted to 201 dB re 1 μPa^2s for phocids (true seals) exposed to continuous sound (Finneran, 2016; National Marine Fisheries Service, 2016, 2018; Southall *et al.*, 2019).[4] The empirical data from the present study provide an opportunity to further consider these criteria, which assume that TTS will occur at exposure levels approximately 20 dB lower than those resulting in PTS. Although TTS onset for seals is predicted to occur at a weighted SEL of 181 dB re 1 μPa^2s, no TTS was observed for tonal exposures reaching 197 dB re 1 μPa^2s for this harbor seal. However, there was clear evidence of substantial TTS and residual PTS at 199 dB re 1 μPa^2s. Despite the lack of measurable TTS at predicted levels, current PTS onset criteria—which were derived independently of this study—are consistent with our findings.

Notably, the large TTS (and ultimately PTS) in this seal occurred at a level (199 dB re 1 μPa^2s) that was the same as that reported to induce 44 dB of TTS in another harbor seal exposed to mid-frequency (4 kHz), long-duration (60 min) octave-band noise (Kastelein *et al.*, 2013). Another recent report from Kastelein *et al.* (2019a) showed TTS onset following exposure to fatiguing tones (6.5 kHz) of the same duration at a sound exposure level that was about 10 dB lower, but still at higher levels than predicted. Given these findings, and the results of the present report, it is apparent that patterns of noise-induced hearing loss are not fully understood for seals, particularly for tonal exposures. The predictive relationship between TTS and PTS is more complicated than the extrapolations commonly used, and therefore should be applied conservatively.

V. CONCLUSIONS

The unexpected event described here is the only reported case of PTS in a marine mammal resulting from a known acoustic exposure. While there are relatively fewer studies of hearing loss in animals caused by exposure to tonal compared to broadband noise, the hearing loss experienced by this seal did not follow an expected pattern of TTS onset and growth. Instead, the transient TS of at least 47 dB (and likely >57 dB) and subsequent permanent loss of at least 8 dB one-half octave above the exposure frequency occurred without expected precursors in the exposure ramp-up sequence. This hearing loss also occurred without behavioral indicators during the seal's performance of a learned behavior (i.e., diving to a submerged apparatus and remaining stationary during exposures), demonstrating that permanent hearing damage can occur without measurable behavioral changes. The hearing loss induced under water manifested similarly in air, confirming that hearing losses in either medium have effects that are expressed amphibiously. These observations with a single, highly trained individual show that exposure to mid-frequency tones with high amplitudes—similar to intense, high duty-cycle military sonars (e.g., van Vossen *et al.*, 2011)—are dangerous to the auditory systems of seals.

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[1]But see Pacini *et al.* (2016) for a report of stranded odontocetes that may have been exposed to dynamite fishing. After stranding, auditory evoked potential measurements revealed elevated thresholds and a limited range of hearing for several individuals.

[2]The underwater hearing thresholds reported here have been adjusted to account for the frequency-specific sensitivity of the TC4032 hydrophone.

[3]Note that the residual hearing loss at 5.8 kHz for the harbor seal falls below the 20–25 dB hearing loss criterion for impairment in humans established by the World Health Organization (1991).

[4]Note that the updated weighting functions for seals that are associated with this SEL criterion emphasize frequencies below and above the range of most sensitive hearing. However, at mid-frequencies (1.6 to 22.4 kHz) like those used in this study, the absolute and weighted values are similar (within 3 dB).

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