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EVALUATING TEMPORARY THRESHOLD SHIFT ONSET LEVELS FOR IMPULSIVE NOISE IN SEALS

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JASA ARTICLE

Evaluating temporary threshold shift onset levels for impulsive noise in seals[a)]

Jillian M. Sills,[1,b)] Brandi Ruscher,[2] Ross Nichols,[2] Brandon L. Southall,[3] and Colleen Reichmuth[1]
[1]*Institute of Marine Sciences, Long Marine Laboratory, University of California Santa Cruz, Santa Cruz, California 95060, USA*
[2]*Department of Ocean Sciences, University of California Santa Cruz, Santa Cruz, California 95064, USA*
[3]*Southall Environmental Associates, Inc., 9099 Soquel Drive, Suite 8, Aptos, California 95003, USA*

ABSTRACT:
The auditory effects of single- and multiple-shot impulsive noise exposures were evaluated in a bearded seal (*Erignathus barbatus*). This study replicated and expanded upon recent work with related species [Reichmuth, Ghoul, Sills, Rouse, and Southall (2016). J. Acoust. Soc. Am. **140**, 2646–2658]. Behavioral methods were used to measure hearing sensitivity before and immediately following exposure to underwater noise from a seismic air gun. Hearing was evaluated at 100 Hz—close to the maximum energy in the received pulse, and 400 Hz—the frequency with the highest sensation level. When no evidence of a temporary threshold shift (TTS) was found following single shots at 185 dB re 1 μPa^2 s unweighted sound exposure level (SEL) and 207 dB re 1 μPa peak-to-peak sound pressure, the number of exposures was gradually increased from one to ten. Transient shifts in hearing thresholds at 400 Hz were apparent following exposure to four to ten consecutive pulses (cumulative SEL 191–195 dB re 1 μPa^2 s; 167–171 dB re 1 μPa^2 s with frequency weighting for phocid carnivores in water). Along with these auditory data, the effects of seismic exposures on response time, response bias, and behavior were investigated. This study has implications for predicting TTS onset following impulsive noise exposure in seals.
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I. INTRODUCTION

Over recent decades, the expansion of human activities in marine environments has resulted in an influx of noise throughout many of the world's oceans (McDonald *et al.*, 2006; Hildebrand, 2009). Oil and gas development, commercial shipping, and military operations often contribute significantly to underwater soundscapes. One of the many concerns arising from increased levels of underwater noise is the potential for noise-induced hearing loss in marine mammals—animals which rely on underwater sound for important life functions such as orientation, predator and prey detection, and communication.

Various studies have evaluated auditory sensitivity in marine mammals following exposure to noise (see, e.g., Finneran, 2015; Southall *et al.*, 2019, for recent reviews). Temporary changes in hearing (temporary threshold shifts, TTSs) are typically used as the primary measure of auditory effect in these controlled experiments. While such studies have largely focused on continuous (long-duration) fatiguing noise, there is growing interest in the effects of impulsive noise on marine mammal hearing. Impulsive noise is widespread in the marine environment and generated from a range of sources, including air guns used for seismic exploration and oil and gas production; short, intense pulses associated with underwater explosions (e.g., military operations and seal bombs used in fisheries); and impact pile driving for wind development projects, oil platforms and offshore energy installations, and construction in bays and harbors (Hildebrand, 2009). Impulsive sources in noise exposure studies have included playbacks of impact pile driving sounds (Kastelein *et al.*, 2015; Kastelein *et al.*, 2018) and actual or simulated seismic air guns (Lucke *et al.*, 2009; Finneran *et al.*, 2015; Reichmuth *et al.*, 2016; Kastelein *et al.*, 2017).

TTS research with impulsive noise has focused on odontocete cetaceans with specialized high-frequency hearing. However, pinnipeds (seals, sea lions, and walruses) and mysticete whales are likely more vulnerable to such exposures, as the energy content of anthropogenic impulsive noise primarily falls below 1 kHz (Richardson *et al.*, 1995). In particular, phocid (true) seals have the most sensitive low-frequency hearing of any marine mammal group tested to date (see Reichmuth *et al.*, 2013; Erbe *et al.*, 2016). Three studies have investigated the effects of impulsive underwater noise on pinniped hearing (Finneran *et al.*, 2003; Reichmuth *et al.*, 2016; Kastelein *et al.*, 2018). Of these, none demonstrated TTS onset—defined as threshold shift ≥6 dB (see Southall *et al.*, 2007; Finneran, 2016; National Marine Fisheries Service, 2018; Southall *et al.*, 2019)—following exposure to single or multiple pulses. Therefore, there is insufficient information available to determine TTS

[a)] Portions of this work were presented in "Auditory studies with bearded seals: sound sensitivity and the effects of noise," 5th International Conference of the Effects of Noise on Aquatic Life, Den Haag, Netherlands, July 2019 and in "Auditory detection, masking, and temporary threshold shift in bearded seals (*Erignathus barbatus*)," 6th International Meeting on the Effects of Sound in the Ocean on Marine Mammals, Den Haag, Netherlands, September 2018.

[b)] Electronic mail: jmsills@ucsc.edu, ORCID: 0000-0002-3110-4844.

onset conditions for any pinniped exposed to impulsive noise, which complicates efforts to manage noise effects on free-ranging individuals.

In the absence of direct data, current regulatory guidance (Finneran, 2016; National Marine Fisheries Service, 2018) is derived from the initial approach developed by Southall *et al*. (2007), supplemented by newer data available for pinnipeds through 2016 (see Southall *et al*., 2019). For the phocid carnivores in water (PCW) hearing group, TTS onset following impulsive noise exposures in water is predicted to occur at a peak sound pressure level of 212 dB re 1 μPa (corresponding to a nominal peak-to-peak sound pressure of 218 dB re 1 μPa) and a cumulative weighted sound exposure level[1] (SEL) of 170 dB re 1 μPa^2 s. However, these TTS onset values have not been empirically confirmed.

To inform regulatory guidance for seals and provide insight into appropriate noise-exposure guidelines for other marine mammals with presumed sensitive low-frequency hearing, we conducted a series of auditory experiments investigating TTS in seals. Due to the pervasiveness of seismic noise worldwide (e.g., Gedamke and McCauley, 2010; Nieukirk *et al*., 2012), this work focused on exposure to impulsive noise from seismic air guns. We initially evaluated underwater hearing in spotted (*Phoca largha*) and ringed (*Pusa hispida*) seals following exposure to single pulses from a small sleeve air gun (Reichmuth *et al*., 2016). In that study, behavioral audiometric testing was completed with trained seals at 100 Hz—approximately 1/2-octave above the peak energy of the broadband noise—in exposure conditions with received, unweighted SEL extending from 165 to 181 dB re 1 μPa^2 s (corresponding peak-to-peak sound pressures from 190 to 207 dB re 1 μPa). While the upper end of this SEL range reached the predicted *M*-weighted TTS-onset level from early noise exposure criteria (Southall *et al*., 2007), no TTS was observed in these seals.

Here, we expand upon this prior work with a set of three experiments to investigate TTS onset and the frequency-dependent effects of impulsive noise exposure. This work was completed primarily with one bearded seal trained for behavioral measurements of underwater hearing. Given underlying uncertainty about the auditory effects of impulsive noise, progressive testing toward predicted TTS-onset conditions proceeded conservatively. Experiment 1 began by replicating the single-shot exposure parameters used by Reichmuth *et al*. (2016) and extending these to higher target levels (received SEL up to 186 dB re 1 μPa^2 s; here, and for the remainder of this manuscript, SEL values are unweighted except when stated otherwise). When TTS onset was not identified at the primary test frequency of 100 Hz, experiment 2 evaluated hearing at a second frequency before and after exposure to the same received levels. This additional testing was completed at 400 Hz—the frequency of maximum sensation level, or greatest difference between the air gun exposure spectrum and the frequency-specific hearing threshold of the subject. This approach was based on other auditory studies with marine mammals, which suggest that maximum threshold shift may be observed at the frequency with the greatest offset between auditory sensitivity and fatiguing noise level (e.g., Kastak *et al*., 1999; Kastak *et al*., 2005; Kastak *et al*., 2007; Lucke *et al*., 2009; Kastelein *et al*., 2017).

In experiment 3, single-shot received SEL was maintained at a relatively constant exposure level (~185 dB re 1 μPa^2 s) while the number of consecutive exposures was increased from one to ten. Using multiple-pulse exposures had several advantages over continuing to increase SEL by raising the amplitude of single shots. First, these exposure sequences—with repetition rates comparable to real-world air gun arrays—simulated more realistic exposure scenarios in which free-ranging seals encounter multiple pulses while diving. Second, this method enabled the generation of impulsive sounds with characteristics more representative of distant seismic sources as opposed to the more complex acoustic conditions of larger single pulses in an enclosed tank. Finally, holding single-shot amplitude constant while increasing the cumulative exposure level avoided concerns for direct physiological harm (non-auditory effects) as a result of intense exposures at close range. While peak sound pressure level did not reach predicted TTS onset conditions for any single impulse, the received cumulative sound exposure level (cSEL) for the ten-shot exposure series reached the updated PCW-weighted level for predicted TTS onset in seals (Southall *et al*., 2019).

These auditory experiments yield improved predictions regarding TTS onset in seals following exposure to broadband noise from seismic air guns, and inform regulatory guidelines regarding impulsive noise in the marine environment.

II. MATERIALS AND METHODS

A. General methods

The primary goal was to identify the onset of repeatable, recoverable TTS (defined as threshold shifts ≥6 dB) in seals following exposure to impulsive underwater noise. The audiometric procedure involved four standard steps: (1) measurement of a pre-exposure hearing threshold at the target frequency; (2) voluntary exposure to calibrated air gun impulse(s), with number of pulses and received level determined by experiment and condition number; (3) measurement of a post-exposure hearing threshold at the target frequency within minutes of the exposure event; and in the event of a threshold shift, (4) measurement of a recovery hearing threshold at the target frequency 24 hours following exposure. The study design included both actual (air gun) exposures and control (no noise) exposures during each experimental condition.

1. Test subject

The subject was a subadult male bearded seal identified as *Noatak* (NOA0010270), who was 3–4 years old during testing. This seal's underwater hearing was evaluated previously with psychoacoustic methods (Sills *et al*., 2020); the resulting audiogram demonstrated sensitive auditory capabilities comparable to those of the related harbor (*Phoca vitulina*; Reichmuth *et al.*, 2013; Erbe *et al.*, 2016), spotted (Sills *et al.*, 2014), and ringed seals (Sills *et al.*, 2015). This bearded seal was maintained at a healthy body weight throughout training and data collection and received one-third to one-half of his prescribed diet (freshly thawed capelin and herring fish) during daily audiometric sessions. Research was conducted up to five days per week with a maximum of one exposure series per day; actual exposures could occur on consecutive days as long as hearing had returned to normal following exposure. Testing was conducted voluntarily with the subject's behavior under conditioned control, established by positive reinforcement training. The seal's participation in this study was approved by the Institutional Animal Care and Use Committee at the University of California Santa Cruz under authorization from the U.S. National Marine Fisheries Service (permit 18902) and the Ice Seal Committee.

2. Environment and apparatuses

Audiometric testing was conducted in a circular, partially in-ground pool (1.8 m deep, 7.6 m diameter) filled with ambient seawater (11 °C–17 °C).

Hearing thresholds were measured at the *listening station*, which was built from water-filled, acoustically transparent polyvinyl chloride (PVC). The listening station included a chin rest that positioned the seal's ears within a calibrated sound field at 1 m depth, 0.75 m from the edge of the pool. A response target, which the seal could press to indicate detection of a signal, was located 20 cm to the left of the chin rest. At the front of the chin rest was a switch that the seal depressed to initiate each trial, which enabled the automatic measurement of response time as the interval between signal onset and release of the switch. The listening station also included a light to indicate the duration of each individual trial and an underwater camera to provide a remote experimenter with a real-time view of the seal.

Exposure (and mock-exposure) events were conducted at the *exposure station*. This water-filled PVC station was suspended near the center of the test pool from an acoustically isolated steel pipe spanning the pool's diameter. The exposure station included a chin rest to position the seal's ears at 1 m depth, a TC4013 (Teledyne Reson A/S, Slangerup, Denmark) hydrophone (±3 dB response from 0.001 to 170 kHz, nominal sensitivity −211 dB re 1 V/μPa) coupled to the chin rest to quantify received exposure levels, a horizontal PVC bar that assisted the seal in maintaining his position, and an underwater camera to enable remote monitoring by the experimenter during all exposures. When positioned at the exposure station, the seal was 1 m from and on axis with the air gun.

3. Ambient noise measurements

Ambient noise measurements were made twice daily, as in Reichmuth *et al.* (2016), prior to each pre-exposure session and each noise exposure/post-exposure test sequence. One-minute, unweighted noise measurements were obtained for frequencies from 0.01 to 20 kHz with a Reson TC4032 low-noise hydrophone (±2.5 dB response from 0.01 to 80 kHz; nominal sensitivity −170 dB re 1 V/μPa with a frequency-specific sensitivity adjustment based on recent calibration). The hydrophone was mounted in the underwater testing enclosure and paired with a Reson EC6073 input module, Reson EC6069 battery module, and battery-powered 2270 sound analyzer (Brüel and Kjær A/S, Nærum, Denmark). Pre- and post-exposure 1/3-octave band levels containing the signal frequency were compared daily to ensure similar ambient noise backgrounds during pre- and post-exposure hearing tests.

These ambient noise measurements were pooled across the entire study ($n = 150$) to characterize background noise. Spectral density levels [dB re (1 μPa)^2/Hz] were determined from 1/3-octave band levels. Median spectral density values were used to represent typical ambient conditions across all sessions and evaluate whether audiometric thresholds could have been constrained by background noise. To describe the variance in background noise, percentile statistics (L10, L50, and L90) for 1/3-octave bands were calculated from equivalent continuous noise levels (Leq). Median (L50) noise levels were also compared for pre- versus post-exposure sessions to evaluate whether differences could have influenced estimates of threshold shift.

B. Experiment 1: The effect of single-shot exposures on hearing near the frequency of maximum exposure level

Experiment 1 evaluated hearing sensitivity at 100 Hz following escalating levels of single-shot exposures. This frequency was chosen to occur near the region of maximum energy for the broadband exposures (30–80 Hz). The single impulses received at close range to the source were intended to represent the acoustic conditions an animal would experience at farther distances from an operational array within a non-reverberant environment—to the greatest extent possible within the bounds of the experimental enclosure. As this was a continuation of work completed recently with spotted and ringed seals, many of the relevant methodological details can be found in Reichmuth *et al.* (2016).

At the beginning of experiment 1, *baseline audiometric testing* was conducted to determine a reference threshold at 100 Hz by confirming previous threshold measurements for the same subject, to describe typical variation in thresholds at 100 Hz, and to establish additional expertise in the subject. *Air gun exposure testing* then occurred over five successive noise exposure conditions (C1–C5). Conditions C1–C4 replicated previous testing with spotted and ringed seals (Reichmuth *et al.*, 2016) with a target SEL range of 165–181 dB re 1 μPa^2 s. Condition C5 extended SEL to 186 dB re 1 μPa^2 s. Corresponding received peak-to-peak sound pressure and peak sound pressure level ranges (dB re 1 μPa) for each of the testing conditions are provided in Table I, along with additional details of the experimental design.

TABLE I. Study parameters for single-shot exposure testing during experiments 1 and 2, showing the operating volume (in.^{3}) and pressure (psi) of the air gun, the horizontal distance from the air gun to the exposure station where the seal was located, the single-shot unweighted target SEL range (dB re 1 μPa^2 s), the corresponding received peak-to-peak sound pressure range (dB re 1 μPa), and the number of replicate audiometric testing sequences (n) conducted with the bearded seal subject under each condition at 100 and 400 Hz. Control conditions (full test sequences with mock noise exposures) were conducted in the same configuration as corresponding exposure sessions and interspersed with these exposures at a ratio of 1:4.

Exposure condition	Impulsive sound source	Air gun volume (in.^{3})	Air gun pressure (psi)	Distance (m)	Target exposure (dB SEL)	Corresponding exposure (dB peak-to-peak)	Replicate series at 100 Hz (n)	Replicate series at 400 Hz (n)
C1	Sleeve gun	10	30	1	165–168	190–193	4	—
C2	Sleeve gun	10	50	1	169–172	194–197	8	—
C3	Sleeve gun	10	70	1	173–176	199–202	8	—
C4	Sleeve gun	10	110	1	178–181	204–207	8	8
C5	BOLT	5	500	1.5	183–186	206–209	4	4
Control							8	3
Total							40	15

1. Audiometric signal generation and calibration

The signals used to evaluate hearing were 500 ms frequency-modulated upsweeps centered on 100 Hz, with narrow (10%) bandwidth and 5% linear rise and fall times. Signals were generated with the Hearing Test Program (HTP; Finneran, 2003) in LabVIEW [National Instruments (NI) Corp., Austin, TX] and sent through an NI 6259 data acquisition module, a 3364 bandpass filter (Krohn-Hite, Brockton, MA), a PA5 digital attenuator (Tucker-Davis Technologies, Alachua, FL), and a P1000 power amplifier (Hafler Professional, Tempe, AZ) prior to reaching a submerged J-11 low-frequency transducer (Naval Undersea Warfare Center, Newport, RI). Audiometric signals were calibrated prior to each session using the Reson TC4032 hydrophone at the position corresponding to the center of the subject's head while on the listening station. Measured signals were compared with expected sound pressure levels (SPLs) and evaluated in time and frequency domains to ensure signal quality. Spatial mapping of the received sound field was conducted at the start of the study to confirm acceptable variability (±3 dB) in the test stimulus within a 14 × 14 × 14 cm grid centered on the listening station.

2. Impulse noise generation and calibration

Two noise sources were used to generate impulsive stimuli. A custom 10 in.^{3} sleeve air gun (synthetic polymer, polyoxymethylene) was used for conditions C1–C4, and a BOLT 2800 LLX air gun (Teledyne Bolt, Houston, TX) with a 5 in.^{3} custom chamber was used to generate higher received levels for condition C5. In both cases, the air gun was suspended (with air supply and electrical lines secured) from a stainless-steel cable connected to a davit arm above the test pool. A portable air supply system was used to deliver an operational line pressure of 30–120 psi to the sleeve gun and 500 psi to the BOLT gun. The air gun was always pressurized before being submerged to a depth of 1 m. The horizontal distance relative to the exposure station was 1 m for the sleeve gun and 1.5 m for the BOLT air gun. The exact, fixed position of each source was determined through spatial characterization of received noise prior to the experiment; noise stimuli generated by either source were evaluated in terms of received level and acoustic characteristics to ensure the integrity and repeatability of received pulses at the exposure station in the pool. Reichmuth *et al.* (2016) provide additional details regarding sound source selection and consideration of these impulse stimuli relative to those generated by operational air gun arrays.

Single pulses from either air gun were triggered from a custom LabVIEW virtual instrument. The impulsive sounds were received by the Reson TC4013 hydrophone mounted at the exposure station, passed through a Reson VP2000 voltage preamplifier (with EC6069 battery module) and the NI 6259 data acquisition module, and measured in the LabVIEW software. Each noise exposure was quantified in terms of SEL, PCW-weighted SEL, peak-to-peak sound pressure, and peak sound pressure level.[2] Prior to each exposure session and without the subject present in the test pool, calibrated levels were determined and the operating pressure was adjusted to generate received levels that would fall within the target range established for the testing condition (see Table I). Every subject exposure during testing was also directly measured.

3. Hearing threshold measurements

Hearing was evaluated using a multiple-response go/no-go procedure to enable rapid assessment of hearing threshold. The subject was cued by a trainer to dive to the listening station and complete a series of signal detection trials before returning to the surface. During each trial, the trial light was illuminated to indicate the 4-s window within which a signal could occur. Correct responses—reporting a signal detection on a signal-present trial or remaining still on the station during a signal-absent trial—were marked by a conditioned acoustic reinforcer, after which the subject proceeded to the next trial. Incorrect responses—failure to report the signal on a signal-present trial (*miss*) or reporting a detection on a signal-absent trial (*false alarm*)—were not marked and the subject continued to the next trial. Each dive sequence consisted of 2–5 correct trials. An acoustic buzzer cued the subject to return to the surface following a correct response on the last trial of the dive, where a fish reward was delivered by the trainer in proportion to the number of correct responses during the preceding block of trials. The percentage of signal-present trials within a session was predetermined and varied between 50% and 65%.

An adaptive staircase method (Cornsweet, 1962) was used to estimate hearing threshold. Testing began with salient signals 15–20 dB above the expected threshold. During the warm-up period of each session, signal level was decreased by 3 dB after each correct detection until the first miss. Subsequently, signal SPL was increased by 3 dB following each miss and decreased by 3 dB following each correct detection until a total of five hit-to-miss transitions were completed. If initial misses were elevated—indicating a potential shift—testing was continued until performance stabilized. However, hearing threshold was always calculated from the trials between the first descending miss and the fifth descending miss (inclusive) of the session. Each session concluded with several trials at suprathreshold levels, which served to maintain subject motivation and behavioral control on the task.

Hearing threshold for a given session was determined from signal-present trials following the method of Dixon and Mood (1948). Threshold was defined as the SPL resulting in a 50% correct detection rate. Signal-absent trials were used to quantify response bias; false alarm rate was defined as the proportion of signal-absent trials on which the seal reported the detection of a signal (pre-stimulus responses on signal-present trials were also classified as false alarms).

4. Noise exposure training and testing

The subject was gradually conditioned to tolerate low-level impulsive sounds prior to participation in air gun exposure sessions. Details of the training process can be found in Reichmuth *et al.* (2016).

Once testing began, each exposure condition (including both actual and mock exposures) was completed prior to advancing to the next, higher level. The subject began with a pre-exposure threshold session at 100 Hz. He could advance to exposure testing if the pre-exposure threshold was within 3 dB of the reference threshold, the session false alarm rate was <30%, and the ambient noise level in the 1/3-octave band including 100 Hz was within 6 dB of the pre-exposure measurement. Provided these criteria were met, the subject moved on to either (1) exposure to impulsive noise at the exposure station (*exposure session*) or (2) an equivalent period on the exposure station with no air gun pulses (*control session*). The exposure was initiated 1–4 s after the seal positioned at the exposure station and no warning stimulus preceded the impulsive sound. Following the exposure event, the subject was cued to swim to the listening station and a post-exposure threshold session began immediately. The first failed detection (miss) typically occurred within 3 min of the air gun exposure, while the fifth descending miss was within 8–9 min of exposure. Threshold shift (TS) was calculated as the difference between the pre-exposure and the post-exposure thresholds. The ratio of control to exposure sessions was 1:4 throughout testing.

5. Behavioral response scoring

The subject's behavioral responses were recorded to video during all exposure and control events. These video recordings were later processed into individual clips that included the subject's behavior on the exposure station just prior to, during, and after the exposure/control event. Audio was stripped from each clip and a red circle was added as a visual marker to delineate the response window, which lasted from the start of the exposure/control event until the subject was prompted to the surface for reinforcement (fish). Additionally, a yellow "warning" circle appeared 0.5 s prior to the start of the response window. The video clip contained no visual indication of whether an event was an exposure or a control.

Videos were reviewed and scored by three experimentally blind observers at the end of the study. The observers rated the subject's reaction during the response window on a scale from 0 to 5. A score of "0" indicated no detectable change in stationing behavior, "1" indicated a just-detectable change (slight movement or flinch without breaking contact with the station), "2" indicated a momentary change (brief movement of the subject's head from the station), "3" indicated that the subject moved less than one-half of a body-length from the station and returned within the response window, "4" indicated that the subject moved greater than one-half of a body-length from the station and returned within the response window, and "5" indicated that the subject's stationing behavior was disrupted and did not recover within the response window.

Scores from the three observers were averaged for each exposure/control event. Exposure series scores were then grouped according to condition (C1–C5), while control session scores were pooled across all testing conditions.

C. Experiment 2: The effect of single-shot exposures on hearing at the frequency of maximum sensation level

To ensure that hearing loss was not occurring at frequencies above 100 Hz, experiment 2 evaluated TTS at 400 Hz—the frequency of greatest sensation level for the test subject (see Fig. 1). Experiment 2 replicated the two highest-amplitude exposure conditions (C4 and C5) from experiment 1 with 400 Hz as the hearing test frequency (see Table I). Experimental methods were identical to those applied in experiment 1 except where noted below.

As the subject's baseline threshold at 100 Hz was so similar to his previously measured audiogram threshold, his pretest thresholds at 400 Hz were referenced to his 400 Hz audiogram threshold (67 dB re 1 μPa) when determining whether to proceed to exposure testing.

Response time data from correct detections on signal-present trials were compared for pretest sessions relative to posttest sessions with paired t-tests. For this statistical

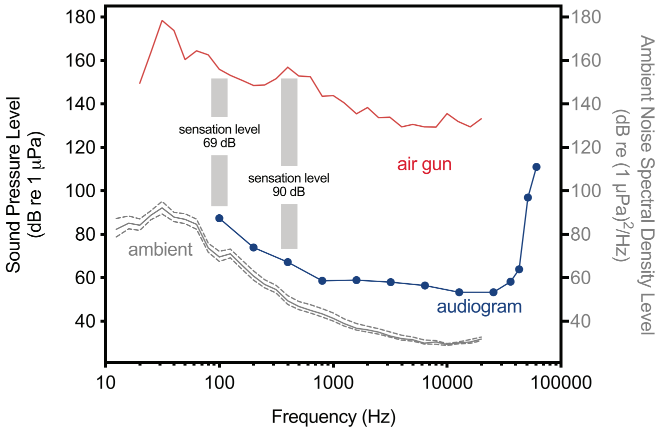

FIG. 1. (Color online) Average 1/3-octave band levels measured at the exposure station during condition C5 air gun testing are shown relative to the absolute underwater audiogram of the bearded seal subject (Sills *et al.*, 2020; left *y* axis). The air gun spectrum is an exemplar of the impulsive stimulus used (see Fig. 2, upper and lower panels); this stimulus was highly repeatable throughout testing (see Table III). The offset between the received air gun stimulus and the auditory threshold (i.e., the sensation level) is shown for both 100 and 400 Hz. Ambient noise spectral density levels were calculated from the median of measured 1/3-octave band levels ($n = 150$; see text and Fig. 2 lower panel), and are reported on the right *y* axis as the 50th percentile level of the noise distribution (L50, solid line). The 10th (L10, dashed line, above) and 90th (L90, dashed line, below) percentile levels are also given to characterize variability in background noise during the experiment.

comparison, data from experiments 1 (at 100 Hz) and 2 (at 400 Hz) were pooled to increase sample size and evaluated in terms of sensation level (i.e., SPL relative to threshold). While combining data for two frequencies may obscure absolute reaction times, this approach should have been sufficient to detect a change in latency from pre- to posttest sessions.

D. Experiment 3: The effects of multiple-shot exposures on hearing

Experiment 3 involved auditory testing with the bearded seal before and after exposure to multiple consecutive pulses from the BOLT air gun operated at the highest exposure condition (C5). Hearing was evaluated following two-, four-, and ten-shot exposure sequences. The inter-shot-interval was 10 s, which is representative of operational air gun arrays (International Association of Oil and Gas Producers, 2011; Gisiner, 2016).

Threshold testing was initially conducted at both 100 and 400 Hz. After a potential auditory effect was observed at 400 Hz during two- and four-shot testing, sessions were continued only at this frequency. As in experiment 2, the audiogram threshold served as the reference threshold for pretest sessions. However, testing proceeded more conservatively with a higher ratio of control sessions (∼1:2). In order to evaluate fine-scale patterns of auditory recovery, TTS was calculated both in terms of full session thresholds (based on five hit-to-miss transitions) and based on just the first miss of the pre- and posttest sessions.

As another precautionary measure, supplemental data were collected at a nearby frequency during four- and ten-shot testing. After 400 Hz, 800 Hz had the highest sensation level from the air gun exposure. Preliminary testing was conducted at 800 Hz in a second post-exposure session, immediately following the first post-exposure session. While these threshold data at 800 Hz were typically collected 11–15 min following the noise exposure, screening at this nearby frequency was conducted to ensure that any substantial shifts (which would not likely recover within this time frame) would be detected. Similarly, during four- and ten-shot testing, additional sessions following the primary post-exposure session were sometimes run at the two main test frequencies (100 and 400 Hz) to screen for large shifts.

III. RESULTS

A. Ambient noise during air gun exposure testing

Ambient noise measurements from 75 days of testing yielded 150 1-min samples. Median 1/3-octave band 50th percentile levels are shown in the lower panel of Fig. 2; corresponding noise spectral density levels are reported in Fig. 1 as the 10th, 50th, and 90th percentile levels of the noise distribution. Median L50 ambient noise spectral density levels within the 100 Hz 1/3-octave band for pre-exposure sessions were similar to those measured for post-exposure sessions on the same day (two-tailed paired *t*-test; $t_{1.2,74}$, $p > 0.05$, $n = 75$). Median L50 ambient noise spectral density levels within the 400 Hz 1/3-octave band for pre-exposure sessions were also similar to those measured for post-exposure sessions (two-tailed paired *t*-test; $t_{0.7,74}$, $p > 0.05$, $n = 75$).

Comparison of hearing thresholds to ambient noise spectral density levels demonstrated average threshold-to-noise offsets of 17 dB at 100 Hz (14–23 dB) and 17 dB at 400 Hz (10–22 dB). These offsets are similar to the previously measured critical ratios for the bearded seal at the same frequencies (Sills *et al.*, 2020). However, while noise in the 100 and 400 Hz 1/3-octave bands did fluctuate somewhat from day to day, threshold measurements were relatively stable (standard deviation 1.8 dB at 100 Hz and 2.0 dB at 400 Hz), suggesting that ambient noise did not substantively influence measured hearing thresholds. Furthermore, the lack of systematic differences in noise from pre- to post-exposure sessions indicated that measured TSs could not be explained by increasing noise.

B. Experiment 1: The effect of single-shot exposures on hearing near the frequency of maximum exposure level

The mean baseline threshold ($n = 12$) measured for the bearded seal subject at 100 Hz prior to the start of exposure testing was 86 dB re 1 μPa. This threshold, obtained using the multiple-response method, was within 1 dB of the threshold measured previously for the same subject using single-response audiometry (Sills *et al.*, 2020). Response bias was stable during testing with a mean session false alarm rate of 16%.

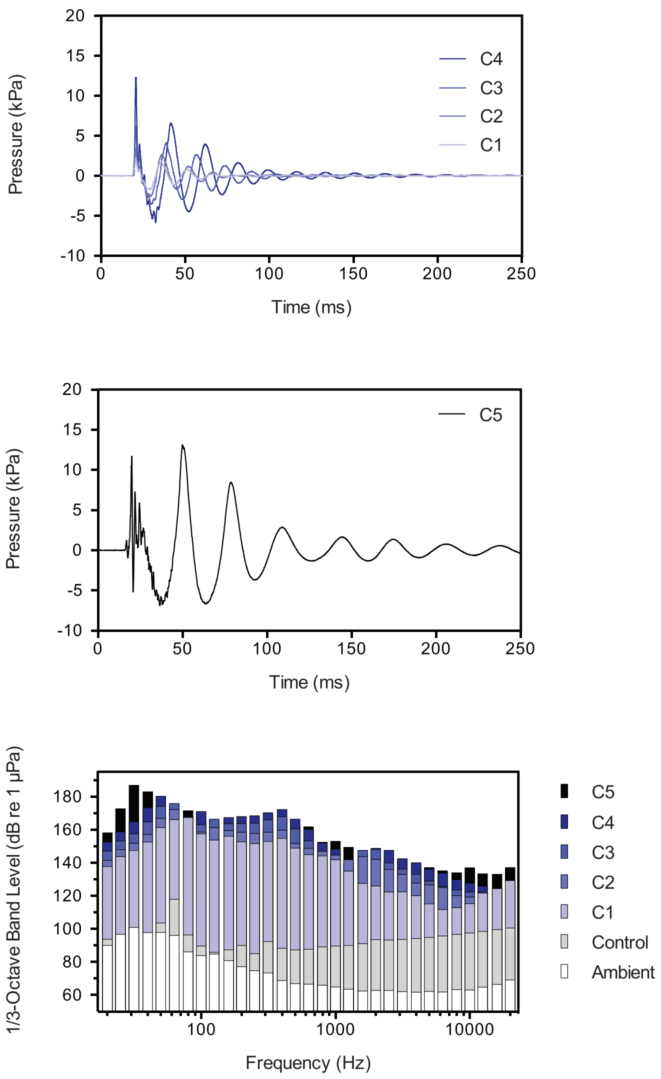

FIG. 2. (Color online) Air gun pulses received at the exposure station during testing. The upper panel shows examples of received waveforms from the four exposure conditions with the 10 in.^{3} sleeve gun (C1–C4) superimposed to align the primary pulse onsets. The center panel shows an exemplar received waveform for the highest exposure condition (C5) using the BOLT 2800 LLX air gun. The lower panel shows the frequency spectrum (0.01–20 kHz) of received 1/3-octave band levels from impulsive noise exemplars for each condition (C1–C5). These are the maximum, fast (125 ms time constant), unweighted noise levels for a 1-s period beginning at the onset of the pulse. Control bars show comparable levels measured with the same hydrophone during mock exposure conditions (note elevation over ambient levels due to the electrical noise of the measurement system). Ambient bars show the true background noise levels measured with a high-sensitivity hydrophone; these are median 1/3-octave band 50th percentile levels measured just prior to each pre-exposure and post-exposure session during the study period (1-min, unweighted noise samples, $n = 150$).

1. Received air gun exposures

Received exposure levels are reported as SEL, peak-to-peak sound pressure, and peak sound pressure level in Table II.[3] Representative waveforms for received noise stimuli are provided in Fig. 2. Data from conditions C1–C4 are combined in the upper panel of Fig. 2, while a representative condition C5 waveform is plotted in the middle panel. Noise exposures were reliable both within and across testing days within an experimental condition. Received stimuli from the sleeve air gun in conditions C1–C4 demonstrated the expected sharp-onset high-pressure peak followed by a negative pressure peak and subsequent bubble oscillations; this pattern is similar to that described in more detail in Reichmuth *et al.* (2016). Although received stimuli from the BOLT air gun used in condition C5 showed the same general patterns, there were some differences. The second positive peak (relative to hydrostatic pressure) was the highest, and there was comparatively greater energy in oscillations later in the time series. Another difference between the two seismic sources was the frequency distribution of received stimuli, which can be viewed in terms of maximum received 1/3-octave band levels in the lower panel of Fig. 2. Overall, there was more energy in lower frequency bands for the BOLT air gun used during condition C5 testing. For all exposures (C1–C5), measured peak-to-peak values were 3–5 dB higher than peak values.

2. Auditory responses

The bearded seal completed 32 exposures and 8 control sequences at 100 Hz, with median TSs from conditions C1–C5 provided in Table II. Individual and median TSs in each exposure and control condition are also depicted in the upper left panel of Fig. 3. All TSs were below the specified 6-dB criterion defining TTS onset, including at the highest exposure level of 185 dB re 1 μPa^2 s in condition C5. Median TS values of −1.5, +0.3, −0.4, +0.6, and +0.8 dB were observed for exposure sequences in conditions C1, C2, C3, C4, and C5, respectively, compared to a median TS of +0.8 dB in control sequences.

Also shown in Table II is a statistical measure of differences in false alarm rates for pre- and post-exposure threshold sessions. There were no significant differences in false alarm probability that could have affected TS measurements. There were no systematic trends in post-exposure audiometric data (as evaluated by linear regression) that would indicate possible recovery of hearing during these sessions.

3. Behavioral responses

Mean (rounded) behavioral scores for the bearded seal are shown for each testing condition in the upper right panel of Fig. 3. Mild but detectable behavioral responses were observed for the majority of exposure events, with no responses observed for the controls. Consistent avoidance responses were not observed. Mean responses did not exceed a behavioral score of *2* (with possible maximum of *5*); no individual response was scored higher than a *3*.

C. Experiment 2: The effect of single-shot exposures on hearing at the frequency of maximum sensation level

1. Received air gun exposures

The received noise stimuli in experiment 2 were similar to those in conditions C4 and C5 of experiment 1, shown in the upper two panels of Fig. 2. Received exposure levels for

TABLE II. Summary of received noise exposures for each single-shot condition (experiments 1 and 2), shown with corresponding TSs between pre- and post-exposure sessions. Received unweighted SEL (dB re 1 μPa^2 s), peak-to-peak sound pressure (pk-pk, dB re 1 μPa), and peak sound pressure level (pk, dB re 1 μPa) are shown as median values for each condition. TS is shown in dB as the median difference in absolute thresholds for each experimental (pre- to post-exposure) sequence, and ΔFA indicates the statistical difference in response bias from pre- to post-exposure sessions [two-tailed Fisher's exact test (0.05 α level); nonsignificant difference, ns; SD, standard deviation]. Control conditions conducted during 100 and 400 Hz testing are pooled.

Exposure condition	Test frequency (Hz)	Replicate exposure series *n*	Received SEL (SD)	Received pk-pk (SD)	Received pk (SD)	TS (SD)	ΔFA
C1	100	4	166 (0.5)	192 (0.5)	187 (0.9)	−1.5 (3.0)	ns
C2	100	8	171 (0.9)	196 (0.5)	191 (0.6)	+0.3 (1.3)	ns
C3	100	8	175 (0.8)	200 (1.5)	196 (2.0)	−0.4 (1.7)	ns
C4	100	8	179 (0.6)	206 (0.6)	202 (0.7)	+0.6 (2.7)	ns
C4	400	8	179 (0.5)	206 (0.5)	203 (0.6)	+0.4 (1.6)	ns
C5	100	4	184 (0.3)	206 (0.4)	203 (0.1)	+0.8 (1.6)	ns
C5	400	4	185 (0.4)	207 (0.4)	203 (0.5)	+0.5 (1.1)	ns
Control		11	—	—	—	+0.8 (1.6)	ns

experiment 2 are reported as SEL, peak-to-peak sound pressure, and peak sound pressure level in Table II.

2. Auditory responses

The bearded seal completed 12 exposures and 3 control sequences at 400 Hz, with median TSs from conditions C4 and C5 provided in Table II. Individual and median TSs for each exposure and control condition are also depicted in the lower left panel of Fig. 3. All TSs were below the 6-dB criterion defining TTS onset, including at the highest exposure level of 185 dB re 1 μPa^2 s in C5. Median TS values of +0.4 and +0.5 dB were observed at 400 Hz for exposure sequences in conditions C4 and C5, respectively, compared to a median TS of +0.8 dB in control sequences.[4]

A statistical measure of differences in false alarm rates for pre- and post-exposure threshold sessions is shown in Table II. As in experiment 1, there were no significant

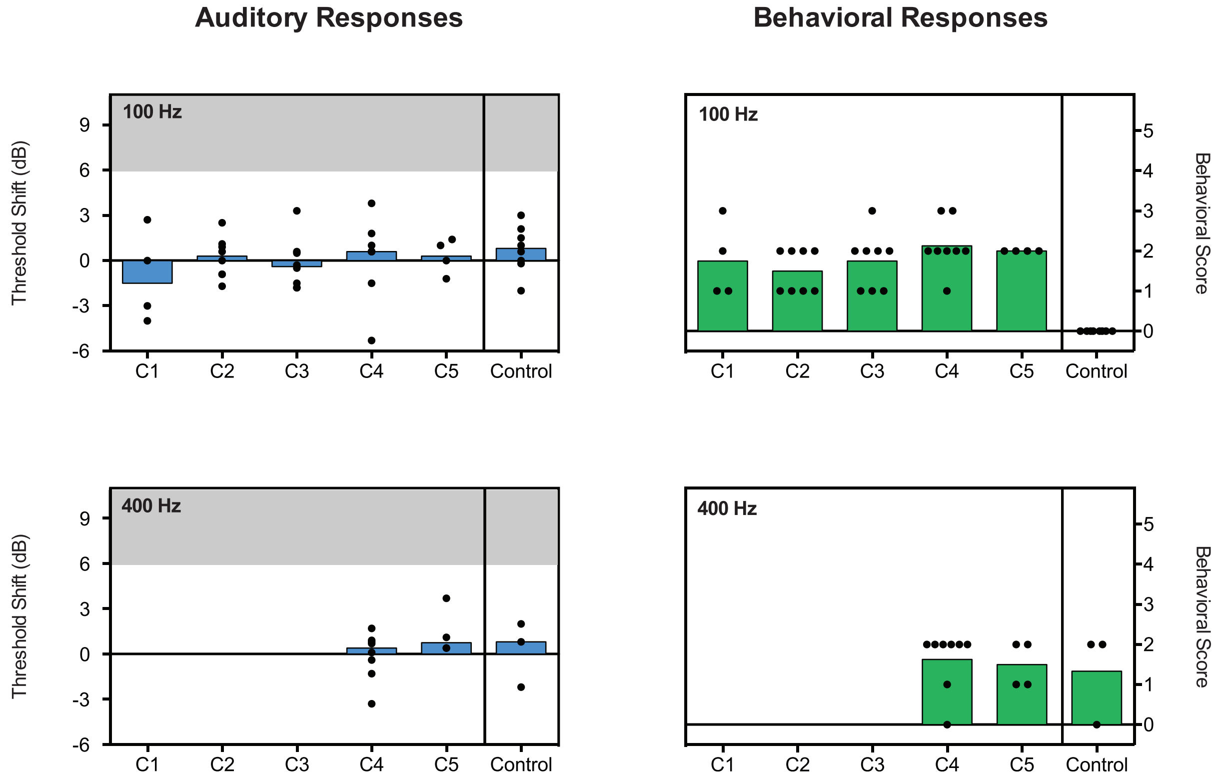

FIG. 3. (Color online) Auditory and behavioral responses of the seal are shown for each of the single-shot exposure conditions (C1–C5) and the control (no-exposure) condition. The upper panels depict testing at 100 Hz, while the lower panels provide results for testing at 400 Hz. Auditory responses (left panels) are shown as individual (points) and median (colored bars) TSs (dB). Median TSs did not exceed 1.0 dB, and no individual TS exceeded the 6-dB TTS onset criterion denoted by the shaded portion of the plot. Behavioral responses (right panels) are shown as individual (points) and mean (colored bars) behavioral scores obtained for the same exposure and control conditions. Score definitions for the 0–5 scale are provided in the text. During air gun exposure testing, the seal showed mean behavioral scores ≤2 in all exposure conditions, indicating relatively mild behavioral responses following training with lower amplitude impulsive sounds.

differences in false alarm probability that could have affected TS measurements, and there were no systematic trends in post-exposure audiometric data (as evaluated by linear regression) that would indicate possible recovery of hearing during these sessions.

Auditory reaction time data collected during threshold testing at 100 and 400 Hz (pooled for experiments 1 and 2) confirmed the lack of any effect in the highest testing condition (C5) as compared to control sessions. Auditory reaction times for sensation levels from 1 to 23 dB showed no systematic increase following noise exposure, suggesting that stimulus salience was similar before and after noise exposure. There was no significant difference in auditory reaction time in 18/19 paired pre- to post-exposure comparisons (*T*-test, $p > 0.05$); in one case, there was a detectable decrease in response time.

3. Behavioral responses

Behavioral scores for the bearded seal are shown in the bottom right panel of Fig. 3. Mild but detectable behavioral responses were observed for the majority of exposure events, with some (but fewer) responses for controls. None of the mean responses exceeded a behavioral score of *2* (with possible maximum of *5*); no individual response was scored higher than a *2*. As in experiment 1, consistent avoidance responses were not observed.

D. Experiment 3: The effects of multiple-shot exposures on hearing

1. Received air gun exposures

The individual pulses in experiment 3 were similar to those received in condition C5 of experiments 1 and 2, with single-shot SEL of approximately 185 dB re 1 μPa^2 s, peak-to-peak sound pressure of approximately 207 dB re 1 μPa, and a peak sound pressure level of approximately 203 dB re 1 μPa (see Table II, C5). Within a multiple-shot exposure series, the pulses were repeatable and well described by the middle plot in Fig. 2. Received levels for experiment 3 are reported for each exposure series in Table III, both as an unweighted cSEL value and with PCW-weighting applied. The separation between emitted shots in these sequences was 10 s except in the cases noted below.

2. Auditory responses

The bearded seal completed ten multiple-shot exposure sequences at 400 Hz (the primary test frequency), four exposure sequences at 100 Hz, and six control sequences. Of the exposures, four were two-shot exposure sequences, six were four-shot sequences, and four were ten-shot sequences. Table III provides the testing order, received noise exposure levels, and median TSs observed in each testing sequence in experiment 3. TS is reported both in terms of the full session (first five descending misses following noise exposure) and

TABLE III. Summary of multiple-shot noise exposure sequences during experiment 3. Received cumulative sound exposure level (cSEL, dB re 1 μPa^2 s) is provided for each exposure series both as an unweighted value and with PCW weighting applied (Southall *et al.*, 2019). For reference, the received unweighted SEL, peak-to-peak sound pressure, and peak sound pressure level values for the single pulses in each multiple-shot exposure were ~185 dB re 1 μPa^2 s, ~207 dB re 1 μPa, and ~203 dB re 1 μPa, respectively (see Table II, C5). TSs are reported in dB as the difference in absolute thresholds between pre- and post-exposure sessions, both for the full session (first five descending misses) and for the first miss only, which occurred 68–309 s (median 184 s) after the offset of the fatiguing noise (see Fig. 4). Behavioral responses are provided for each exposure series as the mean score across pulses; score definitions for the 0–5 scale are provided in the text. ΔFA indicates statistical difference in response bias from pre- to post-exposure sessions [two-tailed Fisher's exact test (0.05 α level); nonsignificant difference, ns; significant difference ($p < 0.05$), higher or lower].

Number of shots	Exposure series number	Test frequency (Hz)	Received cSEL (SD)	Received PCW-weighted cSEL (SD)	TS, session (SD)	TS, first miss (SD)	Behavioral score (SD)	ΔFA
2	1	100	188	163	−1.1	0.0	1.8 (0.2)	—
	2	100	187	163	−0.7	−6.0	2.0 (0.0)	—
	3	400	188	164	+5.4	+3.0	1.0 (1.4)	—
	4	400	188	164	+2.3	+3.0	1.8 (0.2)	—
	Summary ($n = 2$)	100	188 (0.3)	163 (0.1)	−0.9 (0.3)	−3.0 (4.2)	1.9 (0.1)	ns
	Summary ($n = 2$)	400	188 (0.2)	164 (0.1)	+3.9 (2.2)	+3.0 (0.0)	1.4 (0.6)	(higher)
4	1	400	191	167	+9.4	+15.0	1.8 (0.3)	—
	2	400	191	166	+5.6	+3.0	1.1 (0.7)	—
	3	100	191	166	+2.4	0.0	2.2 (0.4)	—
	4	400	191	167	+6.4	+3.0	1.6 (0.7)	—
	5	100	191	166	−0.9	−6.0	1.7 (0.3)	—
	6	400	191	167	−1.8	0.0	1.7 (0.6)	—
	Summary ($n = 2$)	100	191 (0.1)	166 (0.2)	+0.8 (2.3)	−3.0 (4.2)	1.9 (0.4)	ns
	Summary ($n = 4$)	400	191 (0.3)	167 (0.4)	+6.0 (4.8)	+5.3 (6.7)	1.5 (0.3)	ns
10	1	400	195	170	+4.1	+15.0	1.8 (1.2)	—
	2	400	194	171	+3.9	+12.0	2.0 (0.7)	—
	3	400	194	171	+0.5	+3.0	1.5 (0.4)	—
	4	400	194	171	−0.5	0.0	1.5 (0.7)	—
	Summary ($n = 4$)	400	194 (0.3)	171 (0.4)	+2.2 (2.4)	+7.5 (7.1)	1.7 (0.3)	ns
	Control (pooled, $n = 6$)	—	—	—	−0.2 (1.8)	−1.0 (3.1)	1.6 (0.4)	ns

in terms of the first miss only (post-exposure SPL referenced to pre-exposure SPL). Also provided in Table III is a statistical measure of differences in false alarm rates for pre- and post-exposure threshold sessions. As in the earlier experiments, there were no significant differences in false alarm probability that could have affected TS measurements.

At 400 Hz, TSs following two-shot exposures were below the 6-dB criterion defining TTS onset. However, testing did reveal shifts of +2.3 to +5.4 dB, which were higher than in earlier experiments. Subsequent four-shot exposure testing revealed a median shift of +6.0 dB at 400 Hz, with more variability in shifts measured in terms of the first miss (range 0.0 to +15.0 dB). Finally, ten-shot exposure testing resulted in a median shift of +2.2 dB, and a median shift of +7.5 dB when considering just the first miss.

Hearing at 400 Hz was also evaluated three times in secondary post-exposure sessions following primary four-shot testing at 100 Hz: twice after air gun exposures and once after a control sequence. In the two sessions following exposures, shifts of +7.8 and +3.7 dB were measured at 400 Hz relative to the audiogram threshold; initial TS was estimated at +13.0 and +7.0 dB for these sessions, respectively, based on just the first miss in each case. In the secondary post-exposure session following the control sequence, the measured TS was 0.0 dB. While these supplementary data are not summarized in Table III, they are included in Fig. 4, which depicts patterns in auditory performance at 400 Hz during four- and ten-shot exposure testing. Individual misses are plotted in Fig. 4 with respect to timing and signal SPL so that they can be evaluated in relation to the 6-dB TTS onset criterion. Threshold for these secondary post-exposure sessions was typically measured between 11 and 16 min following noise exposure.

At 100 Hz, all individual and median TSs during two- and four-shot exposure testing were below the 6-dB criterion defining TTS onset. Based on these results and because there were indications of a change in hearing sensitivity at 400 Hz at these levels, no further testing was conducted with 100 Hz as the primary test frequency. Hearing at 100 Hz was evaluated four times in secondary post-exposure sessions following primary testing at 400 Hz: three times after air gun exposure sequences and once following a control sequence. There was no indication of TS in any of these sessions.

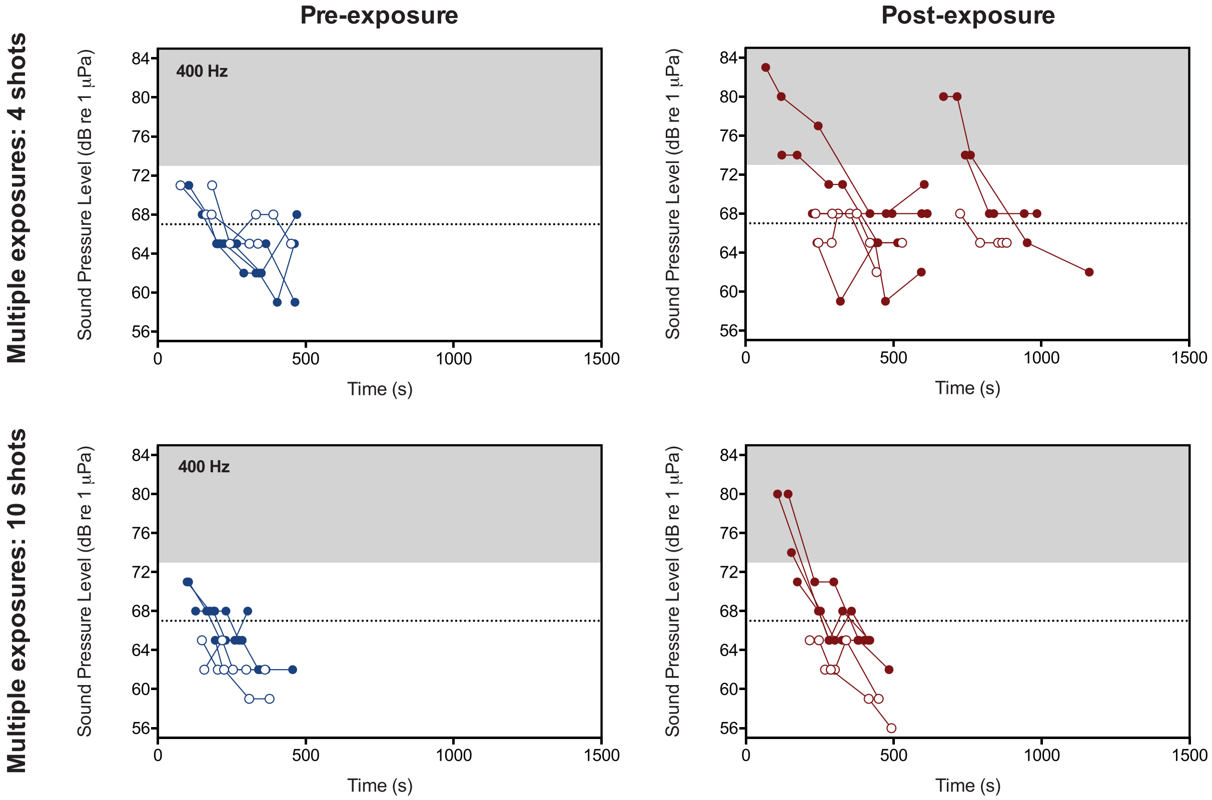

FIG. 4. (Color online) Patterns of performance during auditory testing at 400 Hz before and after exposure to multiple air gun pulses at condition C5 levels (experiment 3). Only failures to detect the audiometric signal (misses) are shown, with sequential misses from each individual session connected by a line. Misses are plotted in terms of timing and SPL of the audiometric signal. Sessions conducted before and after exposure to the air gun stimulus are shown with closed circles, while sessions conducted before and after control (mock) exposures are shown with open circles. Pre- and post-exposure sessions are shown in the left and right panels, respectively, for each four-shot (upper panels) and ten-shot (lower panels) testing series. Note that pre-exposure timing is referenced to the first trial of the session while post-exposure timing is referenced to the offset of the fatiguing (air gun) stimulus (shown at 0 s). The shaded portion of each plot denotes the 6-dB TTS onset criterion relative to the audiogram threshold at 400 Hz (67 dB re 1 μPa, dotted line). While the open circles (control data) show the typical patterns of performance during pre- and post-exposure sessions, elevated misses following air gun exposures (the closed circles in the shaded regions of the plots) demonstrate rapidly recovering shifts in auditory sensitivity at the test frequency.

Supplementary testing at 800 Hz was conducted four times during secondary post-exposure sessions: once following a four-shot exposure sequence, twice following ten-shot exposure sequences, and once following a ten-shot control sequence. In all cases, the post-exposure threshold showed no elevation relative to the audiogram threshold measured previously at the same frequency.

In a few cases, the timing of the interval between pulses varied somewhat from the nominal 10-s duration. If the subject's head was not positioned suitably at the exposure station, the experimenter would manually override the exposure. Once the subject re-stationed sufficiently, the experimenter would allow the sequence to continue. In three instances, this resulted in an interval of 12–20 s between two shots in an exposure sequence. For one session during four-shot testing, there was a delay of approximately 4 min between the first and second pulses in the series; the +6.4 dB TS measured in this case may, therefore, be a conservative estimate of TS.

No systematic differences in response times to 100 and 400 Hz audiometric stimuli were observed in experiment 3. Small sample sizes precluded reaction time comparisons within a single testing series, which may have revealed changes in response times following noise exposure events that produced TTS.

3. Behavioral responses

Behavioral scores for each exposure sequence and the control sequences are provided in Table III. Mild but detectable behavioral responses were observed for the majority of exposure events and controls during multiple-shot testing. As in prior experiments, consistent avoidance responses were not observed.

IV. DISCUSSION

There was no evidence of TS at 100 or 400 Hz in a bearded seal following exposure to single-shot air gun pulses with received SEL up to 185 dB re 1 μPa^2 s, peak-to-peak sound pressure up to 207 dB re 1 μPa, and peak sound pressure level up to 203 dB re 1 μPa. Similarly, multiple-shot exposures at this level, with cumulative SEL up to 191 dB re 1 μPa^2 s (PCW-weighted cSEL 167 dB re 1 μPa^2 s), caused no apparent change in auditory sensitivity at 100 Hz. However, the bearded seal's performance at 400 Hz—while somewhat variable—showed evidence of transient shifts in auditory sensitivity following exposure to four or more pulses with received cSEL of 191–195 dB re 1 μPa^2 s (PCW-weighted cSEL 167–171 dB re 1 μPa^2 s). The largest shift measured following exposure was +9.4 dB, whereas the largest shift based on the first miss following noise exposure was +15.0 dB. Hearing recovered quickly, and always returned to baseline levels during post-exposure testing ($\leq$16 min).

The rapid recovery of and variation in TSs measured at 400 Hz following four- and ten-shot exposure sequences make it difficult to precisely describe the onset of TTS. However, following seven of ten exposures (five with 400 Hz as the primary test frequency and two with 400 Hz as the secondary test frequency), initial misses were 6 dB or more above this subject's audiogram threshold. While recovery of hearing during these post-exposure sessions resulted in lower shifts when measured over the threshold session, there was clearly an auditory effect at this level of noise exposure.

These findings underscore the importance of timing in any study of TTS. Here, the timing of threshold measurement was generally similar to the TTS$_5$ metric used previously for pinnipeds (Finneran *et al.*, 2003; Reichmuth *et al.*, 2016; Reichmuth *et al.*, 2019). However, it is likely that some recovery of hearing has already occurred after five minutes (see, e.g., Reichmuth *et al.*, 2019). Despite generating a more variable result, direct comparison between the first miss of the pre-exposure session and the first miss of the post-exposure session may provide a more accurate assessment of the maximum, initial TS after noise exposure. For example, in this study the largest TTS measured in this way was +15.0 dB—at the frequency of maximum sensation level for the bearded seal subject—while the shift measured based on the full session threshold was +4.1 dB for the same exposure series. However, this approach requires many exposures to provide robust measures.

In addition to the auditory data directly evaluating TTS, reaction times were used as a secondary metric to confirm the absence of an effect on hearing when auditory TSs were not observed. No changes in reaction time were observed during experiments 1 and 2. Due to small sample sizes, we were not able to directly compare reaction times in experiment 3 for pre- and post-exposure threshold sessions in cases when a shift did occur. In these cases, a difference in response latency at a particular signal level would have been expected. In future studies, it would be useful to further explore the relationship between TSs and changes in response time. However, such an effort would also require a greater number of high exposure-level sessions than were conducted here (with a corresponding increase in response time measurements at each signal level), or else much higher TSs than induced in this study.

Similarly, the behavioral data complement the primary measurements of auditory sensitivity. The observed responses of the bearded seal were not suggestive of self-mitigation, which (if present) could have confounded measurements of TTS (see, e.g., Finneran *et al.*, 2015; Nachtigall *et al.*, 2016; Kastelein *et al.*, 2020). The seal was gradually trained to tolerate successively louder pulses before the start of exposure testing and, thus, had a unique exposure history relative to wild or naive individuals. The relatively mild behavioral responses of the bearded seal to these air gun stimuli should not be taken as an indication of how wild seals might react to an operational array. However, this seal's willingness to participate in exposure sequences that temporarily harmed hearing indicates that free-ranging seals may experience TTS (or permanent threshold shift, see Reichmuth *et al.*, 2019) in the absence of overt behavioral indicators.

A significant challenge for TTS studies is determining how to measure the fatiguing exposure in terms of the most biologically relevant metric. For impulsive noise, peak sound pressure level and SEL have been proposed as dual metrics to describe noise stimuli and predict TTS onset (Southall *et al.*, 2007; Finneran, 2016; National Marine Fisheries Service, 2018; Southall *et al.*, 2019), with the intent of addressing both intense instantaneous events and sustained or repeated exposures. The aim in the present study was to identify TTS onset conditions following impulsive noise exposure, with target exposure levels set primarily based on SEL. The experimental design was developed to reach predicted TTS onset levels for one exposure metric (SEL) while not overshooting the other (peak sound pressure level). If target levels had instead been set based on peak sound pressure level, the corresponding cSELs for multiple-shot exposures would have been considerably higher—well above predicted TTS onset levels and likely exceeding the behavioral tolerance of the subject. This SEL-based approach was sufficient to induce TTS, despite noise exposures with peak sound pressure levels reaching only 203 dB re 1 μPa, well below the predicted TTS-onset level of 212 dB re 1 μPa. While peak sound pressure level is certainly relevant for single high-amplitude exposures, SEL may be the more effective metric for most exposure scenarios with multiple, repeated impulses (Southall *et al.*, 2007; Southall *et al.*, 2019). Additional research is needed to confirm how best to characterize impulsive noise exposures with respect to hearing in marine mammals.

Another issue for TTS studies using impulsive, broadband noise is knowing where to look for auditory effects that may be distributed across frequencies. Auditory effects are suspected to occur at lower frequencies, in the region of greatest noise exposure; however, it is also in this frequency range that more time-consuming behavioral, rather than neurophysiological, methods are required. Behavioral studies conducted with spotted, ringed, and bearded seals exposed to impulsive noise (Reichmuth *et al.*, 2016; this study, experiment 1) considered auditory effects at frequencies just higher than the maximum energy in the broadband exposure (100 Hz). Evaluating hearing 1/2-octave above the exposure frequency is common in marine mammal studies (see Finneran, 2015). For example, recent work using tonal exposures has shown that—while the frequency of maximum TTS may vary with exposure level—auditory effects typically manifest at the center frequency of the exposure or 1/2-octave higher (Kastelein *et al.*, 2014; Kastelein *et al.*, 2019). Conversely, experiment 3 of the present study revealed the primary auditory effect following broadband, impulsive exposures at the frequency of greatest sensation level. Of two primary frequencies tested, the larger effect was observed at the frequency with the greater exposure level relative to the subject's auditory sensitivity. While we cannot rule out the possibility that more substantial TTS occurred at a higher frequency, preliminary screening at 800 Hz suggests this was not the case. Thus, when evaluating the effects of impulsive noise on hearing, it appears that expected patterns with respect to the frequency spread of TTS may not hold, and that considering the sensation level of the exposure may better predict the frequency (or frequencies) of maximum shift. The question of how broadband noise exposures manifest with respect to frequency-specific hearing effects is an important one, which should be evaluated through additional research. An improved understanding of the frequency of maximum TTS following impulsive exposures would both inform future empirical studies of noise-induced hearing loss and enable more accurate predictions of the auditory and ecological effects of impulsive noise on free-ranging seals.

This study highlights some of the difficulties involved in acquiring information about auditory responses in marine mammals, where sample size and exposure conditions are both constrained by time-consuming methods and significant expense. Experiment 1 of this study with one bearded seal extends the results of earlier work with spotted and ringed seals (Reichmuth *et al.*, 2016), demonstrating comparable responses to single-shot exposures. This expands available data from two to three species and from four to five individuals, which substantially increases the generality of these results. In addition to these data close to the frequency of maximum exposure, experiment 2 captures auditory effects at the frequency of maximum sensation level for one bearded seal—with opportunistic testing for one ringed seal[2]—lending additional confidence to the finding of no effect following single-shot exposures with SEL up to 185 dB re 1 μPa^2 s. Experiment 3 builds upon this research and hones in on the multiple-shot exposure conditions that produce auditory damage in seals. Although the results presented in experiment 3 are for a moderate number of multiple-shot exposures with a single individual, and are not conclusive with respect to the growth of TTS, the onset data provided are important and strengthened by the foundational data at lower received levels and with multiple species.

Considered together with the findings from Reichmuth *et al.* (2016), these results for five individuals significantly advance understanding of how impulsive noise affects the hearing of seals. With respect to current regulatory guidelines, this body of work suggests that the PCW-weighted TTS-onset level of 170 dB re 1 μPa^2 s SEL (predicted by Finneran, 2016; National Marine Fisheries Service, 2018; Southall *et al.*, 2019) is likely appropriate for seals in water. However, future work documenting larger TSs and patterns of auditory recovery following exposure to impulsive noise will be required to precisely define the exposure conditions resulting in TTS onset.

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[1] The frequency content of the exposure is weighted relative to auditory parameters for the functional hearing group. Frequencies within the range of best hearing are minimally weighted, whereas frequencies above and below this range are weighted according to the exposure function. See Houser *et al.* (2017) for details about the use of auditory weighting functions to predict the effects of noise on marine mammal hearing.

[2] The desired noise levels for these experiments were determined with a focus on SEL as the primary metric, with peak-to-peak sound pressure (which captured the asymmetrical nature of the received waveform) considered secondarily. For comparison to predicted TTS onset thresholds, PCW-weighted SEL and peak sound pressure level are also provided in Sec. III (Results).

[3] To supplement the primary acoustic pressure measurements, maximum broadband (0.01–1 kHz) particle velocity measurements (dB re 1 nm/s) were obtained along the main axis of transmission for typical received air gun exposures in conditions C4 and C5. A calibrated, negatively buoyant M20 velocity sensor (GeoSpectrum Technologies, Inc., Dartmouth, Nova Scotia, Canada) was suspended in a stable orientation from a polyurethane mounting rope affixed to a steel pipe spanning (but decoupled from) the pool. Three measurements per condition were obtained at the position of the exposure station with the sensor oriented to maximize directional sensitivity. As with the measured pressure values, maximum particle velocities were consistent between shots. The median of maximum particle velocity measurements for representative exposures was 162 dB re 1 nm/s in condition C4 and 163 dB re 1 nm/s in condition C5.

[4] A second subject, a 7-year-old female ringed seal identified as *Nayak* (NOA0006783), participated in a portion of experiment 2 in addition to the bearded seal. *Nayak* had previously completed single-shot air gun TTS testing at 100 Hz, up to condition C4 received levels (Reichmuth *et al.*, 2016). Here, she repeated condition C4 testing at 400 Hz. *Nayak* completed four exposures and one control sequence at 400 Hz. This ringed seal had a median TS value of +1.2 dB at 400 Hz for exposure sequences in condition C4, compared to a TS of −0.3 dB in the control sequence. There were no systematic trends in her post-exposure audiometric data that would indicate possible recovery of hearing during these sessions. These supplemental data confirmed the absence of an auditory effect at 400 Hz following single-shot noise exposures with levels up to 180 dB re 1 μPa^2 s.

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