Low-frequency temporary threshold shift not observed in spotted or ringed seals exposed to single air gun impulses[a)]

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Underwater hearing thresholds were measured at 100 Hz in trained spotted (*Phoca largha*) and ringed seals (*Pusa hispida*) before and immediately following voluntary exposure to impulsive noise from a seismic air gun. Auditory responses were determined from psychoacoustic data and behavioral responses were scored from video recordings. Four successive exposure conditions of increasing level were tested, with received unweighted sound exposure levels from 165 to 181 dB re 1 μPa^2 s and peak-to-peak sound pressures from 190 to 207 dB re 1 μPa. There was no evidence that these single seismic exposures altered hearing—including in the highest exposure condition, which matched previous predictions of temporary threshold shift (TTS) onset. Following training at low exposure levels, relatively mild behavioral responses were observed for higher exposure levels. This demonstrates that individuals can learn to tolerate loud, impulsive sounds, but does not necessarily imply that similar sounds would not elicit stronger behavioral responses in wild seals. The absence of observed TTS confirms that regulatory guidelines (based on M-weighting) for single impulse noise exposures are conservative for seals. However, additional studies using multiple impulses and/or higher exposure levels are needed to quantify exposure conditions that do produce measurable changes in hearing sensitivity.
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I. INTRODUCTION

Widespread expansion of industrial activities into Arctic regions has introduced anthropogenic noise into many previously undisturbed acoustic habitats. Underwater soundscapes that once reflected oceanographic dynamics and the acoustic behavior of marine life are increasingly influenced by human-generated sounds from shipping, sonars, and seismic activities during prolonged periods of reduced sea ice (Moore *et al.*, 2012). In particular, survey activities associated with bathymetric mapping and oil and gas exploration generate high-amplitude, impulsive sounds that propagate over large areas (Gedamke and McCauley, 2010; Nieukirk *et al.*, 2012). Understanding the behavioral and auditory effects of seismic operations on marine life is important to all those involved in the assessment and mitigation of associated environmental impacts (e.g., Nowacek *et al.*, 2015). However, few studies have addressed the extent to which these impulsive sounds influence hearing in Arctic marine mammals.

While mysticete whales are suspected to have high sensitivity to low-frequency sounds, phocid (true) seals exhibit the most sensitive low-frequency hearing abilities among the marine mammal species for which audiometric data are available (Reichmuth *et al.*, 2013; Erbe *et al.*, 2016). Thus, seals may be especially vulnerable to noise produced by air guns during seismic surveys, which is predominately concentrated below 1 kHz. Recently published hearing profiles for spotted seals (*Phoca largha*) and ringed seals (*Pusa hispida*) (Sills *et al.*, 2014, 2015) show sensitive underwater hearing (~50 to 70 dB re 1 μPa) across a broad range of frequencies (~300 Hz to >50 kHz), with a gradual low-frequency roll-off in hearing extending to a threshold of ~90 dB re 1 μPa at 100 Hz. The audiograms of these Arctic seals are notably consistent with those measured for the temperate-living harbor seal (*Phoca vitulina*), suggesting that exposure to noise below 1 kHz—and extending to <100 Hz—may be problematic for many phocid seals. Finally, the acute hearing abilities of these true seals establishes them as conservative models for all other marine carnivores and perhaps mysticete whales.

The auditory effect that has been used most commonly to predict when noise becomes "harmful" to marine mammals is hearing loss [e.g., Southall *et al.*, 2007; Finneran and Jenkins, 2012; National Marine Fisheries Service (NMFS), 2016]. There have been several efforts to predict noise exposure levels that induce recoverable, or temporary, threshold

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shifts (TTS) in pinnipeds (true seals, fur seals, sea lions, and walruses). These studies, reviewed recently by Finneran (2015a), include data for harbor seals, northern elephant seals (*Mirounga angustirostris*), and California sea lions (*Zalophus californianus*). These have primarily focused on continuous broadband or octave-band fatiguing noise, with controlled exposures lasting from minutes to hours (Kastak and Schusterman, 1996; Kastak *et al.*, 1999; Kastak *et al.*, 2005; Kastak *et al.*, 2007; Kastelein *et al.*, 2012; Kastelein *et al.*, 2013). Only one study has evaluated hearing in pinnipeds following impulsive noise exposure. Finneran *et al.* (2003) tested two California sea lions with an arc-gap transducer (pulsed power device) and found no measurable TTS after single underwater impulses with sound exposure levels (SEL) of up to 163 dB re 1 μPa^2 s and peak-to-peak sound pressures up to 205 dB re 1 μPa.

In the absence of other available data concerning temporary hearing loss following exposure to impulse noise, Southall *et al.* (2007) derived acoustic exposure criteria for all pinnipeds using an intentionally conservative approach based on data extrapolations from other taxa. The impulse exposure levels reported by Finneran *et al.* (2003) to have no residual effect on hearing in sea lions were considered in the context of TTS onset data available for other marine mammals. Through extrapolation of data available for mid-frequency cetaceans (beluga whales, *Delphinapterus leucas*), Southall *et al.* (2007) predicted that TTS onset in pinnipeds would occur at a single-impulse (single-shot) M-weighted[1] SEL of 171 dB re 1 μPa^2 s. With respect to unweighted peak sound pressure level, TTS onset was predicted at 212 dB re 1 μPa (corresponding to a nominal peak-to-peak sound pressure of 218 dB re 1 μPa). The acoustic criteria for injury proposed by Southall *et al.* (2007) were then derived from these TTS onset values through extrapolation from terrestrial animal data. To date, the underlying assumptions in this process remain untested. Direct measurements of how impulsive noise affects hearing in pinnipeds (and specifically, phocid seals) are needed, particularly for protected species inhabiting regions where such noise sources are commonly used.

The objective of this study was to identify received noise levels for single impulsive exposures that result in TTS onset for Arctic seals trained to cooperate in controlled behavioral measurements of underwater hearing. Because no such data are presently available, a precautionary approach—using a series of four sequentially increased exposure conditions—was taken. Four individual seals, two ringed seals and two spotted seals, were tested to evaluate potential species and individual differences. The range of single-shot exposure levels presented to the seals included relatively low exposure levels that were not predicted by Southall *et al.* (2007) to result in TTS, and extended to levels meeting the predicted M-weighted TTS onset value of 171 dB re 1 μPa^2 s.

II. MATERIALS AND METHODS

A. Experimental design

Our goal was to identify impulsive noise exposure levels from single seismic air gun transmissions that would induce TTS (defined as a repeatable, recoverable threshold shift $\geq$6 dB) in Arctic seals. The experimental design and testing procedures were conservatively developed to enable safe and voluntary participation of trained ringed and spotted seals, and to obtain audiometric measurements from these individuals with minimal variability to allow for detection of relatively small shifts in hearing. The underwater hearing thresholds obtained during the study were measured using narrowband signals centered at 100 Hz. This hearing test frequency was approximately 1/2-octave above the maximum energy in the received impulse, where TTS could reasonably be expected to manifest (see, e.g., Davis *et al.*, 1950).

The study had two phases. The first included a series of psychophysical threshold measurements with each subject to measure typical—or baseline—hearing thresholds at 100 Hz. *Baseline testing* was conducted to confirm the 100 Hz threshold measurements previously obtained from the same subjects, to establish additional expertise in the trained subjects, and to describe the variation in thresholds measured repeatedly at a single frequency.

The second phase included similar threshold measurements at 100 Hz, obtained just prior to and immediately following presentation of calibrated air gun noise. *Air gun exposure testing* was conducted to determine whether a threshold shift occurred as a result of noise exposure and, if so, to what extent. Air gun exposure testing occurred over four successive noise exposure conditions—each characterized by an incrementally increased target received noise exposure level that was determined *a priori*. The general procedure for air gun exposure testing involved four steps adapted from Ridgway *et al.* (1997), Kastak *et al.* (2007), and others.

(1) Measurement of a pre-exposure hearing threshold at 100 Hz.
(2) Voluntary exposure to calibrated air gun impulse noise, with target received level determined by condition number.
(3) Measurement of a post-exposure hearing threshold at 100 Hz within minutes of the exposure event.
(4) In the case of a threshold shift, measurement of a recovery hearing threshold at 100 Hz, 24 h following exposure.

Several aspects of the general study design ensured data quality and utility. The ambient (background) noise in the underwater testing environment was measured twice daily to confirm an adequately low noise floor for measurement of absolute (unmasked) hearing thresholds at 100 Hz, and to confirm similar background noise levels across pre- and post-exposure sessions. The impulsive sounds produced by the air gun were spatially characterized in the reverberant testing environment to ensure integrity and repeatability of received impulses. Control (or mock-exposure) sessions were included in the testing schedule to allow for comparisons of auditory measurements obtained in the absence of impulses to those obtained within and across exposure conditions. Finally, the behavior of subjects during both exposure and control sessions was recorded and scored to enable separate evaluation of potential auditory and behavioral effects resulting from noise exposure.

B. Test subjects

Four healthy ice seals living in human care participated in the study. Subjects included two three-year-old male spotted seals, identified as *TUNU* (NOA0006674) and *AMAK* (NOA0006675). Their aerial and underwater hearing had been previously measured using behavioral methods (Sills *et al.*, 2014). Two ringed seals were also tested: a sixteen-year-old adult male *NATCHEK* (NOA0005618) and a two-year-old female *NAYAK* (NOA0006783), who had both recently completed underwater and aerial hearing studies (Sills *et al.*, 2015). Thus, all four subjects were experienced in behavioral audiometry. The recently published underwater audiograms for these seals showed similar best hearing (minimum thresholds of 49–51 dB re 1 μPa between 12.8 and 25.6 kHz), similar low-frequency hearing (minimum thresholds of 88–91 dB re 1 μPa at 100 Hz), and comparable low-frequency roll offs in sensitivity (~10 dB/octave below 800 Hz). The underwater audiogram reported for *NATCHEK* showed elevated thresholds above 25.6 kHz relative to those measured for the other ringed seal subject, suggesting some degree of high-frequency hearing loss. Therefore, the four subjects were considered to have species-typical hearing at frequencies $\leq$25.6 kHz.

The seals were maintained at healthy body weights and fed a mixture of freshly thawed and cut herring and capelin fish. They received approximately half of their scheduled diets during daily testing. The participation of the seals in this research was approved by the Institutional Animal Care and Use Committee at the University of California Santa Cruz, with permission from the Ice Seal Committee and federal authorization from the U.S. National Marine Fisheries Service (marine mammal research permit 14535).

C. Testing environment

Experiments were conducted in the same circular, partially in-ground seawater pool (1.8 m deep, 7.6 m diameter) used in previous measurements of underwater hearing for these subjects (Sills *et al.*, 2014, 2015). Two experimental stations were constructed from PVC and mounted within the testing pool for the two behavioral tasks to be performed by the subjects (see supplementary Fig. 1).[2] Both stations were designed to flood with water when submerged, making them acoustically transparent.

Hearing thresholds were measured with each subject positioned at the *threshold station*, which was mounted near the pool wall. The threshold station was equipped with a chin rest, which was individually customized for each seal and designed to ensure that each subject's head remained in a fixed position at 1 m depth within the calibrated sound field. Located at the front of the chin rest was a magnetic switch that the subject depressed with its nose while in position on the station. A response target, which the subject touched to indicate signal detection, was located 20 cm to the subject's left. This configuration allowed for response time to be automatically measured as the time (in ms) between signal onset and when the subject released the switch to touch the response target. The threshold station was also equipped with an underwater camera to remotely monitor the subject's behavior and responses, an underwater trial light to delineate the listening interval on each trial, and a buzzer that served as a conditioned reinforcer to indicate each correct response. The experimenter operated the camera, light, and buzzer from a control room located near (but visually isolated from) the pool.

Single impulsive exposures from the air gun were presented to individual subjects voluntarily positioned at the *exposure station*, a submerged PVC apparatus located near the center of the test pool. The exposure station was suspended from a steel pipe that spanned the diameter of the pool and was mounted to provide acoustic isolation from the pool walls. This station was equipped with a chin rest (similar to the one described above) located at 1 m depth, a horizontal PVC bar that enabled positively buoyant subjects to maintain a fixed position during noise exposures, and an underwater camera that allowed the experimenter to remotely monitor the animals during each exposure event and to record their behavior at the exposure station.

D. Ambient noise measurement

Ambient noise was measured using a Reson TC4032 (A/S, Slangerup, Denmark) low-noise hydrophone ($\pm$2.5 dB re 1 μPa from 0.01–80 kHz; nominal sensitivity −170 dB re 1 V with frequency-specific sensitivity adjustment based on recent calibration) and battery-powered Brüel and Kjær 2270 sound analyzer (Brüel and Kjær A/S, Nærum, Denmark). The hydrophone was mounted at the location corresponding to the center of the animal's head on the threshold station, and connected to the sound analyzer via a Reson EC6073 junction box, which also provided power to the hydrophone from a 12 V gel cell battery.

Broadband (0.01–20 kHz) ambient noise data were collected over 1 min intervals, from which unweighted, equivalent noise levels (L_{eq}) were determined. Median ambient noise spectral density levels (dB re 1 μPa^2/Hz) were determined from 1/3-octave band levels (dB re 1 μPa) using frequency-specific bandwidths. Ambient noise percentile levels (10th, 50th, and 90th percentiles, or L10, L50, and L90, respectively) were calculated across multiple sessions to determine variance in ambient noise. Median L50 spectral density values were used to represent typical background noise conditions across multiple sessions.

During baseline testing, ambient noise was measured just prior to each session in the absence of the subject. These data were later pooled for each individual in order to evaluate whether ambient noise in the underwater testing environment could have constrained the baseline threshold estimate. During air gun exposure testing, ambient noise was measured once prior to pre-exposure testing, and again prior to noise exposure/post-exposure testing. Pre- and post-exposure 100 Hz 1/3-octave band noise levels were compared daily to ensure similar ambient noise backgrounds during pre- and post-exposure threshold sessions. Following the completion of testing, ambient noise measurements were pooled for all individuals, and median (L50) levels were compared for pre- and post-exposure threshold measurements to determine if there were significant differences between the two session types that could influence estimates of threshold shift.

E. Audiometric signal generation and calibration

Audiometric signals used to measure hearing at 100 Hz were generated using National Instruments (NI) LABVIEW software (National Instruments Corp., Austin, TX) with the Hearing Test Program (HTP) virtual instrument (Finneran, 2003) run on a custom-built personal computer. Signals were transmitted through an NI USB-6259 BNC M-series data acquisition module, a Krohn-Hite 3364 anti-aliasing filter (Krohn-Hite, Brockton, MA), a TDT PA5 digital attenuator (Tucker-Davis Technologies, Alachua, FL), and a Hafler P1000 power amplifier (Hafler Professional, Tempe, AZ) to the underwater transducer. A J-11 low-frequency transducer (Naval Undersea Warfare Center, Newport, RI) was suspended from a stainless steel cable connected to a davit arm above the test pool, and positioned 5.5 m behind the subject at the listening station (see supplementary Fig. 1).[2]

Audiometric signals were 500 ms, frequency-modulated upsweeps centered at 100 Hz, with 10% bandwidth and 25 ms linear rise/fall times. Prior to every session, the signals were calibrated in terms of sound pressure level (SPL, dB re 1 μPa) across a 50 dB dynamic range using an automated, step-wise calibration method in HTP. The Reson TC4032 hydrophone was used to calibrate the projected test signals received at the position corresponding to the center of the subject's head while on the threshold station. Calibration measurements were obtained just prior to each session in the absence of the subject.

F. Impulse noise generation and calibration

A custom 10 in.^{3} sleeve air gun made from a synthetic polymer (polyoxymethylene) was used to generate impulsive noise stimuli. Prior consideration of various impulse noise sources had indicated that the single 10 in.^{3} custom air gun was the best-suited option to achieve the desired noise exposure levels, with the focus on SEL as the most relevant practical metric for these single-impulse exposures (as described in Southall *et al.*, 2007). The air gun was suspended at 1 m depth from the davit arm above the test pool, with electrical and air supply lines affixed to a supporting stainless steel cable. A portable air supply system was used to deliver an operational line pressure of 30–100 psi to the air gun. The precise position (horizontal distance relative to exposure station) and operating pressure of the air gun were predetermined, and varied with testing condition (see Table I).

A single transmission from the air gun was triggered using a custom LABVIEW virtual instrument. Sound exposures were simultaneously received by two Reson TC4013 low-sensitivity hydrophones (0.01–100 kHz frequency response; −201 dB re 1 V nominal sensitivity). One hydrophone was located near the subject (on the chin rest of the exposure station) and served to quantify received exposure levels; the other was located near the sound source (0.5 m directly above the air gun shuttle at 0.5 m depth) and served as a consistent reference for air gun exposures. Each hydrophone was connected through a Reson VP2000 voltage preamplifier, and sound exposures were quantified using the custom LABVIEW software. Measurements were made in units of sound exposure level (dB SEL re 1 μPa^2 s) as well as peak-to-peak sound pressure (dB re 1 μPa). Calibrated levels of the air gun impulse were obtained prior to each exposure session, without subjects present in the test pool, and the operating pressure was adjusted to generate the desired received levels for each testing condition.

G. Air gun exposure conditions

Prior to the experiment, several operating pressure levels (psi) and air gun positions (relative to the exposure station) were evaluated in terms of the received level and the acoustic characteristics of the impulse noise, as well as the spatial and temporal variability of these parameters. Using these data and the predicted TTS onset level identified by Southall *et al.* (2007), four configurations of air gun operating pressures and placements were identified that would produce received levels spanning the unweighted SEL range of 165–181 dB re 1 μPa^2 s (Table I). The upper end of this range includes the M-weighted SEL predicted to result in TTS onset in pinnipeds.

The target exposure levels for conditions 1 through 4 were defined by SEL metrics and corresponding peak-to-peak sound pressures, with a 3 dB allowable range for each condition (Table I). The number of exposure sessions was four for the lowest exposure condition (C1), and eight for all other exposure conditions (C2–C4). Control sessions were also conducted; the experimental procedure was identical except that, instead of firing the air gun, a mock-exposure event was triggered and received levels were recorded from the hydrophones. One control session was conducted for every four exposure sessions, and placement of the control sessions within the session sequence was pseudorandom.

H. Hearing threshold measurements

Baseline hearing thresholds were measured for each subject and defined as the mean threshold for the 100 Hz signals obtained from a total of 12 sessions. Following completion of baseline threshold assessment, subjects proceeded to the exposure phase of testing. The procedure for obtaining absolute (unmasked) hearing thresholds during baseline testing and air gun exposure testing (*pre-exposure*, *post-exposure*, and *recovery* thresholds) was identical for all session types.

The method employed to measure hearing thresholds was a go/no-go signal detection procedure using a multiple-response paradigm. During multiple-response audiometry, the subject dove to the underwater threshold station and completed a sequence of several consecutive signal detection trials before returning to the surface and receiving a food reward. This method was chosen because of its utility in rapid assessment of hearing threshold, necessary for detecting a quickly recovering, post-exposure shift (see Kastak and Schusterman, 2002; Finneran *et al.*, 2005). Two trial types, each 4 s in duration, were presented: *signal trials*, during which the 100 Hz test signal was projected at varying onset times within the trial interval; and *catch trials* during which no signal was presented. Each 4-s trial interval was delineated by the illumination of the trial light. *Correct detections* (subject touched the response target during a signal trial) and

TABLE I. Experimental design for air gun exposure testing showing the operating pressure (psi) of the air gun, the distance (m) from the air gun to the exposure station where each subject was located, the unweighted target sound exposure level (SEL) range (dB re 1 μPa^2 s), the estimated corresponding received peak-to-peak sound pressure range (dB re 1 μPa), and the total number of experimental sessions (n) conducted under each condition. The ratio of control sessions (full test sequences without noise exposure) to exposure sessions was 1:4 throughout testing.

Condition	Pressure psi	Distance m	Target exposure dB SEL	Corresponding exposure dB pk-pk	n
C1	30	1.5	165–168	190–193	4
C2	30	1	169–172	194–197	8
C3	50	1	173–176	199–202	8
C4	100	1	178–181	204–207	8
Control	0	1	—	—	7
Total					35

correct rejections (subject remained still on the chin rest during a catch trial) were marked by a buzzer that served as a conditioned reinforcer. Incorrect responses, including a *miss* (subject failed to respond during a signal trial) and a *false alarm* (subject responded during a catch trial), were ignored and the subject was allowed to reposition at the station and move on to the next trial. Each dive sequence contained two to five correct trials. Following a correct response on the last trial of the sequence, the subject was cued by the trainer to return to the surface, where a reward was delivered that was proportionate to the number of correct responses during the multiple-trial dive sequence (i.e., the number of pieces of fish given was equal to the number of conditioned reinforcers earned). The proportion of signal trials to catch trials within a given session (50%–70%) was pre-determined and adjusted between sessions as necessary to maintain consistent subject response bias. The sequence of signal and catch trials was pre-determined using a quasi-random selection method within the HTP software based on the *a priori* trial ratio.

Thresholds were estimated using an adaptive staircase method (Cornsweet, 1962). Each threshold session began with a warm-up phase, during which test signals were initially set to a SPL of 116 dB re 1 μPa (an easily detected level $\sim$25 dB above the 100 Hz baseline thresholds for all subjects). The experimenter would subsequently decrease signal amplitude in 3 dB steps following each correct detection, until the first miss. Signals were then adaptively adjusted in an up/down manner based on subject performance—such that they increased by 3 dB after each miss and decreased by 3 dB after each correct detection—until a total of five *low misses* occurred (i.e., descending misses). The trials between (and including) the first low miss and the fifth low miss constituted the test phase data for the session. The signal trials within the test phase were used to calculate the hearing threshold, defined as the signal sound pressure level corresponding to the 50% correct detection rate (Dixon and Mood, 1948). Each session concluded with a cool-down phase including easily detectable signals.

False alarm rates were used to measure subject response bias, and were defined as the percentage of false detections out of the total number of catch trials, calculated for the entire session.[3] Sessions where the false alarm rate exceeded 30% were not included in baseline hearing threshold measurement or used as pre-exposure sessions during exposure testing.

I. Noise exposure training and testing

The subjects were conditioned to tolerate low-level impulsive sounds prior to participation in air gun exposure sessions. The seals were trained to swim to the exposure station and maintain their position on this station for a 10 s interval that eventually included an impulsive sound produced by a partially submerged percussive device. The received level of the training impulse was monitored with the same equipment used to measure and calibrate the air gun noise (described above). The amplitude of this stimulus was gradually increased through successive approximation over the course of several weeks. Once the seals were able to tolerate impulses at received peak-to-peak sound pressure values of up to 185 dB re 1 μPa, training sessions began with the 10 $in.^3$ air gun positioned 4 m from the seal and operated at the lowest possible firing pressure (30 psi). As before, the seals were rewarded for maintaining their position on the exposure station for the 10 s interval including the air gun impulse. No warning stimulus or predictable temporal pattern preceded the impulsive sound; this was the case both during training and exposure testing. Received levels were increased over successive days by moving the air gun progressively closer to the exposure station (from 4 to 2 m) following demonstration of behavioral tolerance. Data collection for C1 began once each seal subject was able to tolerate air gun exposures at peak-to-peak sound pressures of up to 189 dB re 1 μPa. Throughout training, pre-exposure and post-exposure thresholds were compared daily to confirm the absence of threshold shifts (TS) of $\geq$6 dB. Testing then proceeded conservatively, with the completion of each exposure condition prior to advancing to the next condition.

The procedure used during exposure testing was similar to that in previous TTS studies with pinnipeds using various types of fatiguing noise (Kastak and Schusterman, 1996; Kastak *et al.*, 1999; Kastak *et al.*, 2005; Kastak *et al.*, 2007). On a given testing day, each subject participated in (1) a pre-exposure threshold session, followed by (2) *either* exposure to the air gun impulse at the exposure station (noise exposure session) *or* a short period of rest on the exposure station with no air gun impulse (control session), which was immediately followed by (3) a post-exposure threshold session. The subject could only advance from pre-exposure testing to a noise exposure or control session if the pre-exposure threshold measurement was within 3 dB of the previously measured baseline threshold, the pre-exposure total false alarm rate was less than 30%, and the pre- and post-exposure 100 Hz 1/3-octave band ambient noise levels were within 6 dB of one another. Provided these criteria were met, the post-exposure threshold session began within 1–2 min following the exposure event and the exact time, referenced to the exposure (or control) trigger, was recorded for all low misses during the test phase. Following completion of this testing series each day, the pre-exposure and post-exposure thresholds for each subject were compared, and the resulting TS was calculated as the difference between the two threshold estimates.

J. Behavioral response scoring

The subjects' behavioral responses during exposure and control events were recorded to video so that the reactions of each animal could be evaluated across all conditions. The videos from the camera mounted on the exposure station were processed into standardized clips using video editing software. The recordings were shortened to include the subject's behavior on the exposure station prior to, during, and following each exposure/control event, and the audio was removed from each video clip. Visual markers were inserted onto the video to delineate the behavioral response time window to be scored by the observer. The *response window* (3–5 s) was marked by a red circle that appeared at the start of the exposure event and remained on the screen until the subject was recalled from the exposure station to receive reinforcement from the trainer. A yellow circle appeared 0.5 s before the red circle to alert the observer that the response window was about to begin. Video footage of control sessions was similarly edited based on the timing of the mock-exposure event. Following the experiment, session clips were arbitrarily coded and shuffled according to a random sequence.

An observer who was blind to experimental condition reviewed and scored the prepared video clips. The observer was instructed to carefully monitor each subject and to assign a score corresponding to the subject's behavior during the response window. The observer used a scoring scale that ranged from 0 to 5, where 0 indicated no detectable change in the subject's stationing behavior; 1 indicated a just-detectable change (slight movement or flinch without breaking contact with the station); 2 indicated a momentary change (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. The observer was permitted (but not required) to view each session a total of three times before assigning a score. If a session was given a score of 2–5, the observer recorded a brief description of the animal's behavior.

Behavioral response scores were grouped according to session type. For each subject, control session scores were pooled across testing conditions and exposure session scores were grouped according to exposure condition.

III. RESULTS

A. Baseline hearing thresholds

Baseline thresholds for the four subjects (Table II) revealed hearing sensitivity of 88 to 89 dB re 1 μPa at 100 Hz. These thresholds obtained using the multiple-response method were consistent with those measured previously for the same subjects using single-response audiometry—within 1 dB for the spotted seals (Sills *et al.*, 2014) and 3 dB for the ringed seals (Sills *et al.*, 2015). Thresholds were obtained in 7 to 8 min, as opposed to 11 to 18 min using single-response audiometry. Response bias was stable during testing, with mean session false alarm rates between 4% and 21% across subjects. Comparison of hearing thresholds to measured ambient noise spectral density levels demonstrated threshold-to-noise differences of 18 to 21 dB. These offsets exceeded previously measured critical ratios for each subject (Sills *et al.*, 2014, 2015) by 2–8 dB, enabling confirmation that ambient noise in the test enclosure was sufficiently low to reliably measure unmasked hearing thresholds and to reveal potential differences in sensitivity following noise exposure.

B. Ambient noise during air gun exposure testing

In addition to the ambient noise data collected during baseline testing, paired measurements were taken on all exposure testing days to characterize ambient noise for both pre- and post-exposure sessions. Median L50 ambient noise spectral density levels (dB re 1 μPa^2/Hz) within the 100 Hz 1/3-octave band for pre-exposure sessions were not

TABLE II. Summary of hearing data for each subject at 100 Hz. Previously published thresholds are provided for the two spotted seals (Sills *et al.*, 2014) and two ringed seals (Sills *et al.*, 2015), for comparison to mean baseline thresholds (n = 12) measured in this study. Corresponding standard deviations (SD), false alarm rates (%), and median ambient noise spectral density levels (dB re 1 μPa^2/Hz) for the 100 Hz 1/3-octave band are given. Resulting threshold-to-noise level offsets exceed previously published 100 Hz critical ratios for all subjects (Sills *et al.*, 2014, 2015).

	Subject	Published threshold dB SPL re 1 μPa	Baseline threshold dB SPL re 1 μPa (SD)	False alarm rate %	Ambient noise dB re 1 μPa^2/Hz	Threshold-to-noise offset dB	Published critical ratio dB
Spotted seals	*AMAK*	90[a]	89 (1.9)	9	71	18	16
	TUNU	89[a]	88 (1.6)	4	68	20	12
Ringed seals	*NATCHEK*	88	89 (1.6)	21	68	21	16
	NAYAK	91	88 (1.0)	12	68	20	14

[a]Note that published threshold values for the two spotted seals (Sills *et al.*, 2014) were corrected based on subsequent re-calibration of the hydrophone. Corrected values are shown here.

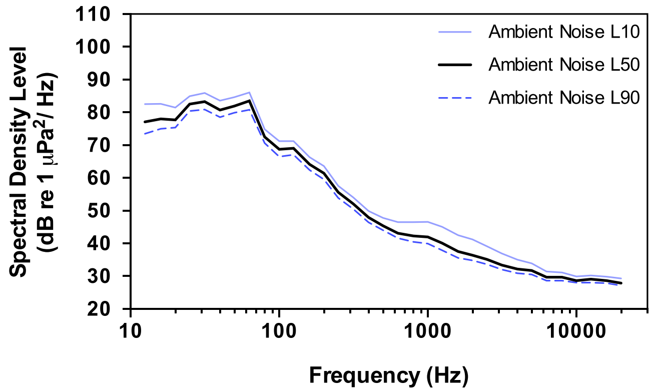

FIG. 1. (Color online) Ambient noise spectral density levels (dB re 1 μPa^2/Hz) measured in the testing pool from 0.01–20 kHz. Noise was measured daily prior to the pre-exposure session ($n = 96$) and again prior to the post-exposure session ($n = 96$). These measurements were pooled to characterize the background noise during the experiment. Spectral density levels were calculated from the median of 1/3-octave band levels, and reported as the 50th percentile level of the noise distribution (L50, bold line); the 10th (L10) and 90th (L90) percentile levels are provided for reference.

significantly different than those measured for post-exposure sessions on the same day (two-tailed paired t-test; $t_{1,95}$, $p > 0.05$, $n = 96$).

Given the observed similarities in pre- and post-exposure measures of ambient noise, the 192 measurements made before and after air gun exposure testing were pooled to quantify median ambient noise conditions and statistical variance metrics during the experiment. Median ambient noise spectral density level values for L10, L50, and L90, calculated from 1/3-octave band levels for frequencies from 0.01 to 20 kHz, are shown in Fig. 1. Typical ambient noise during air gun exposure testing (L50) is shown in bold, and variability from quieter conditions (L90: levels exceeded 90% of the time) to louder conditions (L10: levels exceeded 10% of the time) is also represented. The typical spectral density level (L50) at the hearing test frequency was 69 dB re 1 μPa^2/Hz during exposure testing.

C. Air gun exposure results

1. Received air gun exposures

The noise exposures received by the subjects were within the *a priori* target ranges specified for the four experimental conditions (Table I). Received exposure levels are reported as the actual (unweighted) values for both SEL and peak-to-peak sound pressure (Table III). Representative time-series waveforms are provided for each exposure condition [C1–C4; Fig. 2(A)], as well as for all air gun exposures in the highest condition [C4; Fig. 2(B)]. Across conditions, received waveforms show a sharp-onset (rapid rise time), high pressure peak that is characteristic of air gun sources. The initial "positive" (relative to hydrostatic pressure) peak is immediately (within 2 ms) followed by a sharp decrease in relative pressure, which results from the phase-inverted surface reflection. This reduction in pressure is followed by the remainder of the initial peak and the subsequent "negative" pressure in the waveform. Small multipath reflections are evident during this cycle as a result of reverberation and interference patterns within the constrained testing enclosure. Following the initial cycle is a relatively symmetrical and progressively dampened pattern (with period of

TABLE III. Summary of individual noise exposures for each subject in each condition are shown with corresponding threshold shift between pre- and post-exposure sessions. Received unweighted SEL (dB re 1 μPa^2 s) and peak-to-peak sound pressure (dB re 1 μPa) are shown as median values for each condition; peak sound pressure level (not shown) was on average 3 to 4 dB lower than peak-to-peak pressure for the same exposures. Threshold shift is shown as the median difference in thresholds, while ΔFA indicates statistical difference in response bias from pre- to post-exposure sessions [two-tailed Fisher's exact test (0.05 alpha level); non-significant difference = ns, significant difference ($p < 0.05$) = higher or lower].

	Subject	Condition (n)	Received exposure dB SEL (SD)	Received exposure dB pk-pk (SD)	Threshold shift dB (SD)	ΔFA
Spotted seals	*AMAK*	C1 (4)	166 (2.2)	190 (0.6)	+1.2 (1.4)	ns
		C2 (8)	169 (0.6)	195 (0.6)	−0.6 (1.9)	ns
		C3 (8)	173 (0.3)	200 (0.5)	+0.9 (1.7)	(lower)
		C4 (8)	178 (0.6)	205 (0.2)	+0.4 (1.9)	ns
		Control (7)	—	—	−0.4 (2.1)	ns
	TUNU	C1 (4)	167 (0.6)	191 (0.7)	−0.6 (2.1)	(higher)
		C2 (8)	170 (0.4)	196 (0.6)	−0.6 (2.4)	ns
		C3 (8)	175 (0.5)	201 (0.5)	−0.9 (1.5)	ns
		C4 (8)	180 (0.2)	206 (0.2)	+0.7 (2.9)	ns
		Control (7)	—	—	0.0 (2.7)	ns
Ringed seals	*NATCHEK*	C1 (4)	167 (0.8)	191 (1.4)	+0.2 (1.7)	ns
		C2 (8)	171 (0.8)	197 (0.7)	0.0 (3.9)	ns
		C3 (8)	174 (0.9)	200 (0.8)	+0.1 (2.5)	ns
		C4 (8)	180 (1.2)	206 (3.0)	+1.0 (1.9)	ns
		Control (7)	—	—	−1.7 (0.7)	ns
	NAYAK	C1 (4)	166 (0.3)	190 (0.8)	+0.6 (1.0)	ns
		C2 (8)	171 (0.5)	197 (0.1)	−0.3 (1.5)	ns
		C3 (8)	175 (0.7)	201 (0.6)	−0.9 (2.3)	ns
		C4 (8)	180 (0.1)	206 (0.3)	+0.7 (1.3)	ns
		Control (7)	—	—	−0.9 (2.1)	(higher)

15–20 ms) that is coincident with bubble oscillations from the air released by the firing of the air gun.

Received waveforms were consistent across the experimental sessions within each exposure condition [e.g., for condition 4, see Fig. 2(B)]. This was particularly evident in the initial positive and negative peak pressure pattern described above, which contained most of the impulsive energy, with somewhat more variability in the subsequent bubble oscillation patterns. For all exposures, the measured peak-to-peak sound pressures were 3 to 4 dB higher than peak sound pressure levels.

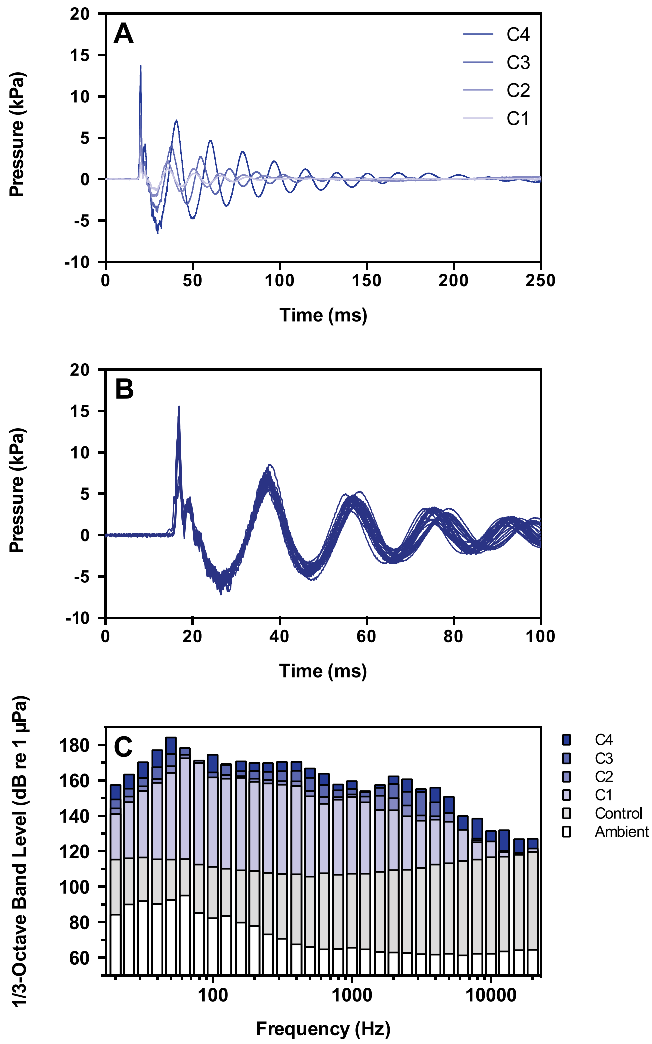

FIG. 2. (Color online) Air gun impulses received at the exposure station during testing. Panel (A) shows a representative waveform from each of the four exposure conditions (C1–C4) superimposed to match the primary pulse onset. Panel (B) shows all of the waveforms ($n = 32$, 8 each for 4 subjects) for the highest exposure condition (C4) to illustrate signal replicability. Note the shorter duration of panel B (100 ms) relative to A (250 ms) to increase resolution on the primary pulse and subsequent bubble oscillations. Panel (C) shows the frequency spectrum (0.01–20 kHz) of received 1/3-octave band levels from the same impulse noise exemplars shown in panel (A) (C1–C4). Ambient bars show the true background noise levels (corresponding to the spectral density levels in Fig. 1) measured with a high-sensitivity, low-noise hydrophone prior to each of the pre-exposure and post-exposure sessions. Control bars show levels measured during the control (no-exposure) conditions with the low-sensitivity hydrophone used to capture the impulsive events.

2. Auditory responses

The four subjects completed scheduled testing in C1–C4 for a total of 28 exposure sequences and 7 control sequences per individual. Video recordings of representative exposure events during testing under C4 with spotted seal *AMAK* and ringed seal *NATCHEK* are provided in Mm. 1. Table III summarizes the median threshold shifts observed in each condition, along with corresponding noise exposure levels. These auditory responses to air gun exposures are graphically depicted in Fig. 3, where the subjects' median threshold shifts are plotted for both experimental and control conditions. All of the median threshold shifts obtained under testing conditions 1–4 (as well as controls) were below the specified 6 dB criterion defining TTS. Also provided in Table III is a statistical measure of differences in false alarm rates between pre- and post-exposure sessions. There were no systematic differences in response bias that would explain the lack of measured TTS in any subject for any exposure condition.

Mm. 1. Air gun exposure video file. Air gun exposure events during condition 4 are shown for spotted seal *AMAK* and ringed seal *NATCHEK*. The video shows each seal swim to the exposure station to receive the air gun impulse. Following exposure, the seal stays at the station until a buzzer cues the seal to return to the trainer for a fish reward prior to starting post-exposure threshold testing. The file was recorded by a GoPro HERO2 video camera (GoPro, San Mateo, CA), and thus the audio was not captured by a calibrated receiver with flat frequency response. This file is type "mp4" (10.3 MB).

An important consideration in the TS measurements was time from the exposure event to threshold estimation, as recovery of hearing could theoretically occur within minutes of the exposure. In this respect, the multiple-response method served the need for rapid threshold measurement. During post-exposure testing, subjects descended to the first failed detection (miss) within 3–4 min of the air gun exposure, while the final miss of the test phase occurred 6–9 min after exposure. The specific interval denoting the test phase for threshold determination is provided for individual subjects below. There were no systematic trends in the post-exposure audiometric data (evaluated by linear regression) that would indicate possible recovery of hearing during these sessions.

Of the spotted seals, *AMAK* had median TS values of 1.2, −0.6, 0.9, and 0.4 dB for exposure sequences in conditions C1–C4, respectively, compared to a median TS of −0.4 dB in control sequences. For the majority of conditions there was no significant difference in the false alarm probability for pre- versus post-exposure threshold sessions; false alarm probability was significantly lower during C3 sequences. *AMAK*'s hearing threshold was determined in the interval from 3.6 to 7 min after exposure. *TUNU* had median TS values of −0.6, −0.6, −0.9, and 0.7 dB for exposure sequences in conditions C1–C4, respectively, compared to a median TS of 0.0 dB in control sequences. For the majority of conditions there was no significant difference in the false alarm probability for pre- versus post-exposure threshold sessions; false alarm probability was significantly higher during C1 sequences. *TUNU*'s hearing threshold was determined 3.2 to 6.3 min after exposure.

Of the ringed seals, neither *NATCHEK* nor *NAYAK* had median TS values greater than 6 dB in any of the four conditions; *NATCHEK* had a single session in C2 with a 10 dB TS. *NATCHEK* had median TS values of 0.2, 0.0, 0.1, and 1.0 dB for exposure sequences in conditions C1–C4, respectively, compared to a median TS of −1.7 dB in control sequences. For all conditions there was no significant difference in the false alarm probability for pre- versus post-exposure threshold sessions. *NATCHEK's* hearing threshold was determined 3.9 to 8.9 min after exposure. *NAYAK* had median TS values of 0.6, −0.3, −0.9, and 0.7 dB for exposure sequences in conditions C1–C4, respectively, compared to a median TS of −0.9 dB in control sequences. For the majority of conditions there was no significant difference in the false alarm probability for pre- versus post-exposure threshold sessions; false alarm probability was significantly higher during control sequences. *NAYAK's* hearing threshold was determined 3.8 to 7.6 min after exposure.

Although threshold shift was the primary measure of auditory response, one additional metric confirmed the finding of no effect in the highest exposure condition (C4) compared to control sessions. The auditory reaction times measured for correct signal detections in the signal-detection task were compared for pre- and post-exposure sessions, for each subject and SPL, with the assumption that a reduction in sensitivity would increase reaction time for the same SPL. Reaction times for signal SPLs from 89 to 116 dB re 1 μPa showed no systematic increase following noise exposure. There was no significant difference in auditory reaction time in 35/38 paired pre- to post-exposure comparisons (T-test, $p > 0.05$); in two cases, there was a detectable decrease in response time, and in one case there was an increase.

3. Behavioral responses

Blind observers scored behavioral responses during noise exposure and control sessions; mean behavioral scores for each subject and testing condition are shown in Fig. 3. No detectable behavioral responses were observed for any subject in the majority of mock-exposure events for control conditions. In contrast, all subjects exhibited relatively mild—but detectable—behavioral responses for the majority of exposure events. For three of the four research subjects (spotted seals *AMAK* and *TUNU* and ringed seal *NATCHEK*), none of the individual or mean responses exceeded a behavioral score of 2 (with possible maximum of 5). The ringed seal *NAYAK* was the most responsive to noise exposure, with mean response scores between 2 and 3 for all noise exposure conditions, and at least one response score of 4 in each condition (most occurring in C1). Despite the relatively low scores overall, there appears to be a slight trend toward higher response scores with increasing exposure level (from C1–C4) for these individuals.

IV. DISCUSSION

There was no evidence of low-frequency threshold shift in two spotted and two ringed seals following voluntary exposure to single-shot air gun impulses with received unweighted SEL up to 180 dB re 1 μPa^2 s and received peak-to-peak sound pressure up to 206 dB re 1 μPa. Measured hearing thresholds and auditory reaction times were not different before and immediately after these impulsive noise exposures. The subjects were highly trained for audiometric testing and were gradually conditioned to tolerate progressively more intense seismic exposures. Following training, they exhibited relatively mild behavioral responses to the air gun exposures during testing. This is the first study to evaluate the combined auditory and behavioral effects of impulse noise on phocid seals, and the data presented here add substantively to the limited available information concerning TTS in marine mammals.

The assessment of potential TTS following exposure to impulse noise depends on reliable measurements of unmasked hearing. Some variation in threshold measurements within and across test subjects is expected (see Yost and Killion, 1997), but must be minimized to the extent possible in order to discern potentially small changes in hearing attributable to noise exposure. We overcame this challenge in several ways. First, the subjects had extensive experience with cooperative psychoacoustic methods, and their complete underwater audiograms were available (Sills *et al.*, 2014, 2015); this enabled comparison of baseline thresholds measured with multiple response audiometry to published values obtained with standard audiometric methods. Second, baseline hearing thresholds measured prior to air gun exposure testing allowed us to identify typical variance in thresholds at 100 Hz and thus establish appropriate criteria for progression to noise exposures. Furthermore, false alarm rates were carefully monitored throughout testing to ensure that threshold shifts were not attributable to systematic changes in response bias. Finally, pre- and post-exposure measurements of low-frequency ambient noise ensured that thresholds were not constrained by background noise, and that threshold shifts could not be attributed to changes in ambient conditions. Explicit, empirically based criteria for allowable variance in pre-exposure hearing thresholds, subject response bias, and low-frequency ambient noise were successfully implemented to support audiometric testing.

Another significant challenge encountered during this study was that of generating consistent impulsive noise in the reverberant test enclosure, with acoustic features (e.g., rapid rise time) similar to actual air gun impulses but with amplitude scaled to achieve the specified target ranges (165–181 dB re 1 μPa^2 s SEL, with corresponding peak-to-peak sound pressure of 190–207 dB re 1 μPa). Simulated seismic exposures using playbacks of recorded air guns through underwater transducers lacked a sufficiently impulsive signal onset and were well below the specified received levels. Conversely, commercially available "off-the-shelf" seismic air guns were almost certain to exceed the target levels based on operating specifications and measurements in a related study (Finneran *et al.*, 2015). Consequently, a custom 10 in.^{3} sleeve air gun

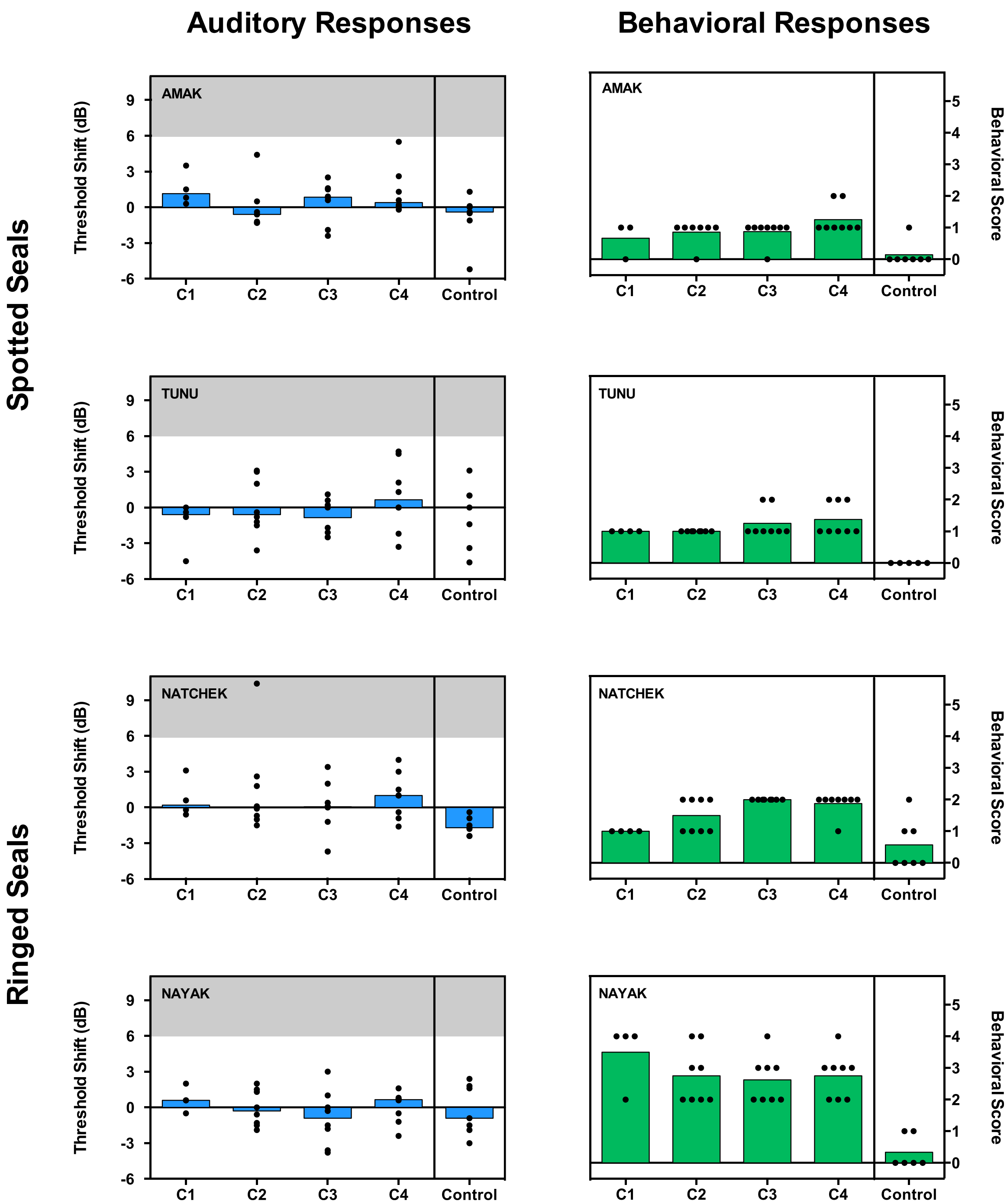

FIG. 3. (Color online) Auditory and behavioral responses during air gun exposure testing are shown for each subject for each of the four experimental conditions. Auditory responses (left panels) are shown as individual (points) and median (colored bars) threshold shifts (dB) obtained at 100 Hz for each of the exposure (C1–C4) and control (no-exposure) conditions. For both species and all subjects, median threshold shifts did not exceed 1.2 dB. Of the 140 total individual threshold shifts measured, only one exceeded the 6 dB TTS onset criterion, denoted by the shaded portion of the plots. Behavioral responses (right panels) are shown as individual (points) and mean (colored bars) behavioral scores obtained for each of the exposure and control conditions. Score definitions are provided in the text. During air gun exposure testing, three of the four subjects showed scores ≤ 2 in all exposure conditions, indicating only mild behavioral responses. One of the four subjects (*NAYAK*) exhibited behavioral responses >2 in all exposure conditions. This subject's behavioral scores were highest in C1, which had the lowest exposure levels, but was conducted first.

was selected as the sound source; similar versions are used to generate impulsive signals to calibrate operational air gun arrays. Operating conditions involving variation in source-receiver distance and chamber air pressure were identified to achieve the specified signal parameters at the exposure station. The resulting impulse exposures were highly repeatable within experimental conditions in terms of both waveform characteristics and received levels. However, while these exposures were in many ways similar to those generated by operational air gun arrays, it should be noted that both the reverberant nature of the enclosed testing environment and the close proximity of the sound source influenced the received noise waveform. There were certainly acoustic and contextual differences in this artificial testing environment relative to impulsive noise received by free-ranging animals from air gun arrays operating at greater ranges, even if overall received levels were similar.

In this laboratory setting, the ringed and spotted seals completed planned auditory testing with seismic impulses at sound exposure levels predicted by Southall *et al.* (2007) to result in TTS onset. However, the seals showed no evidence of TTS at the test frequency. None of the subjects had median threshold shifts exceeding 6 dB in any condition, and all subjects showed similar auditory responses in control and exposure sessions. One of the ringed seal subjects (*NATCHEK*) did demonstrate a threshold shift of 10 dB in a single testing session within exposure condition C2. Additional trials were run based on the elevated SPL of *NATCHEK's* initial misses, and recovery was observed within the post-exposure session. On the following day, *NATCHEK's* threshold was within normal limits for his baseline hearing threshold. No other threshold shifts occurred for this subject—or any of the other subjects—during testing, even in the two higher exposure conditions. It is unclear whether this single shift represents variable subject performance or an actual shift in hearing sensitivity following noise exposure.

Given the environmental considerations and experimental constraints applied during testing, the lack of measured TTS does not appear to be a function of auditory masking or subject performance. Additionally, the lack of a predictive stimulus or temporal cue for the air gun exposure makes it unlikely that a self-protective reflex (e.g., a head turn as observed in Finneran *et al.*, 2015) or gain control (Nachtigall *et al.*, 2016) would occur in this case and confound measurements of TTS. Timing of threshold measurement, however, may be a factor. In comparative studies of noise-induced threshold shift, TTS is measured when possible within two minutes of noise exposure (TTS_2: Kryter *et al.*, 1966). In this study, despite the multiple-response method used for audiometry, threshold shift was measured in the interval from three to nine minutes following noise exposure. This is generally similar to the TTS_5 measure reported for sea lions by Finneran *et al.* (2003). While the potential for some recovery of hearing thresholds during these few minutes cannot be ruled out, the lack of measured TTS, the absence of trends suggesting recovery following exposure, and patterns of recovery from measurable TTS in other animals (Salvi and Boettcher, 2008) all suggest that TTS onset occurs at some higher exposure level. Alternatively, TTS may occur above the 100 Hz test frequency, as broadband noise exposures can produce broadband TTS (Finneran, 2015a). The gradual increase in auditory sensitivity over the frequency region of the air gun spectrum may further influence the expected upward frequency spread of TTS. Despite some uncertainties, the results suggest that the estimated TTS onset levels for M-weighted single impulse exposures in pinnipeds (Southall *et al.*, 2007) are precautionary. The potential effects of multiple exposures remain unclear.

The M-weighting scheme advocated by Southall *et al.* (2007) filters low and high frequency portions of the sound exposure that fall outside the region of good hearing sensitivity. In the present study, application of this weighting reduced the effective maximum (broadband) sound exposure level within C4 from a median value of 179 dB re 1 μPa^2 s (unweighted) to 171 dB re 1 μPa^2 s (M-weighted). More recently proposed weighting functions for marine mammals that are designed to measure potentially harmful noise exposures (Finneran, 2015b; NMFS, 2016) further attenuate the effective maximum sound exposure level of these air gun impulses to 156 dB re 1 μPa^2 s; that is, 23 dB less than the actual (unweighted) sound exposure level, and 30 dB less than the level expected to cause permanent hearing damage (permanent threshold shift, or PTS) to seals (NMFS, 2016). Given the absence of evidence for the onset of TTS following impulse noise exposure in seals, it is as yet unknown whether this substantial weighting of impulsive noise exposures is appropriate.

Aside from the measured auditory responses in this study, the behavioral responses of these subjects to the exposure conditions could be viewed as consistent with the finding of no measurable TTS. Three of four seals showed responses that were considered mild, and even the seal with the highest response scores (ringed seal *NAYAK*) always returned quickly to the exposure station. Of the four subjects, *NAYAK* was the youngest and had spent the least amount of time in captivity. Her behavioral responses, which show a general declining trend in severity with increasing exposure level, can be explained by gradual habituation through counter-conditioning to the air gun stimulus. The absence of behavioral responses to mock exposures during control sessions confirms that observed responses to air gun exposures were due to the stimulus and not some other anticipatory factor. However, as subjects faced the air gun during noise-exposure events, it is impossible to determine whether behavioral responses were elicited only by the auditory (rather than the visual or somatosensory) aspect of the stimulus. It is important to note that, although the air gun exposures did not induce strong behavioral responses in the subjects following training, it is likely that wild seals without similar exposure histories would exhibit heightened reactions when exposed to similar levels of impulsive noise. Therefore, the behavioral results must be considered contextually.

This study has implications for ice-living seals and issues related to the industrial development of the Arctic. The lack of observed auditory responses at levels predicted to cause TTS indicates that initial predictions based on extrapolations (Southall *et al.*, 2007) were sufficiently precautionary. Furthermore, these findings suggest that the auditory systems of Arctic seals may be relatively resistant to impulse noise exposure at low frequencies. This is unexpected in light of their sensitive auditory thresholds and small critical ratios below 1 kHz (Sills *et al.*, 2014, 2015) relative to other marine mammals, including other pinnipeds (see Erbe *et al.*, 2016; Reichmuth *et al.*, 2013). Despite this enhanced ability to hear low-frequency sounds, the spotted and ringed seals in this study did not show greater auditory vulnerability to air gun sounds than bottlenose dolphins exposed to multiple shots of comparable low-frequency seismic noise, when tested at frequencies above 500 Hz (Finneran *et al.*, 2015).

The negative TTS results reported here should not be taken as an indication that exposures to seismic air guns do not adversely affect free-ranging ice seals. While our results suggest that auditory responses of spotted and ringed seals occur at higher levels than predicted by Southall *et al.* (2007) for single-shot exposures, repeated or higher amplitude exposures will almost certainly result in TTS at some (as yet unknown) exposure level. With increasing oil and gas exploration in high-latitude regions, ice-living seals and other polar species are increasingly exposed to intermittent impulsive noise. Typical seismic operations take place over periods of weeks to months with pulses occurring every 10 to 12 s (International Association of Oil and Gas Producers, 2011); if individual animals are not displaced from these areas, they will be exposed to many pulses at varying received levels over time. The effects of multiple exposures remain difficult to predict. Furthermore, in addition to the potential for hearing loss, it is important to consider the auditory effect of masking from air gun exposures, as seismic noise may interfere with the ability of seals to hear biologically relevant low-frequency sounds (Sills *et al.*, 2013) over much longer physical ranges (Guan *et al.*, 2015).

This study offers a conservative starting point for understanding how impulsive noise affects seals and other pinnipeds. Clearly, additional research is necessary. Most significantly, additional studies are needed using multiple air gun exposures, higher received levels, and additional test frequencies to identify the conditions and frequency regions in which TTS onset will occur. Potential physiological measurements (e.g., stress hormones) made simultaneously in these studies may provide additional insights into possible non-auditory noise effects. Behavioral studies with captive, but unconditioned individuals may also reveal aspects of behavioral disturbance in response to impulsive noise (see, e.g., Hastie *et al.*, 2014). Additionally, audiometric measurements and assessments of TTS in at least a few more seal species—particularly bearded seals (*Erignathus barbatus*), given their phylogenetic distinction from other phocid seals—may provide the basis of a functional hearing group that would justify the extrapolation of results to related, but untested, seal species. Finally, while challenging, the results of hearing studies paired with controlled noise exposures in laboratory conditions should be directly compared using a variety of exposure metrics (both acoustic and contextual) to measured noise exposures for free-ranging animals exposed to real air gun arrays.

V. CONCLUSIONS

(1) The thresholds obtained at 100 Hz reflect absolute auditory sensitivity for two spotted and two ringed seals, and were not influenced by background noise.
(2) The 100 Hz thresholds were consistent with prior measures for the same individuals, and were similar to one another.
(3) There was no residual change in auditory sensitivity measured across the four impulse noise exposure conditions, including at levels predicted by Southall *et al.* (2007) to cause temporary threshold shifts. These findings are not surprising given that these predictions were based entirely on extrapolations from other taxa, with conservative assumptions.
(4) The relatively low-magnitude behavioral responses observed during noise exposures indicate that individual animals can learn to tolerate loud, impulsive sounds, but do not imply that similar sounds would not elicit stronger behavioral responses in wild individuals.
(5) The findings are not surprising given that the regulatory criteria recommended by Southall *et al.* (2007) for impulsive noise in pinnipeds were based entirely on extrapolations from other taxa with conservative assumptions.
(6) Additional studies with trained individuals using multiple impulse noise exposures and/or higher exposure levels are needed to determine the actual noise conditions resulting in the onset of TTS.

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[1]Southall *et al.* (2007) developed auditory weighting functions (termed M-weightings) to account for frequency-specific differences in hearing for different groups of marine mammals; these functions, which de-emphasize noise energy contained in frequency regions with apparently reduced auditory sensitivity, were used to establish the first biologically relevant noise exposure limits for marine mammal taxa. A common approach in assessments of TTS is to provide unweighted (absolute) exposure levels as well as taxa-specific weighted values.

[2]See supplementary material at http://dx.doi.org/10.1121/1.4964470 for photograph of test pool showing equipment configuration during air gun exposure testing.

[3]While false alarm rates measured during the test phase of audiometry sessions provide the best estimate of response bias at threshold, we considered false alarm rates over the total session to minimize the effects of relatively few catch trials in the test phase when comparing response bias between individual sessions.

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