Temporary threshold shift (TTS) in odontocetes in response to multiple air gun impulses

Final Report

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I. Executive Summary

To investigate the auditory effects of multiple underwater impulses, auditory thresholds were measured in bottlenose dolphins before and after exposure to a series of impulses produced by a seismic air gun. The pre- and post-exposure hearing thresholds were compared to determine the amount of temporary hearing loss, called a temporary threshold shift (TTS), as a function of exposure conditions.

Three dolphins (BLU, TYH, and OLY) participated in the study. Initial exposures for each subject were designed to acclimate the dolphins to the sequence of underwater impulses and featured between 2 and 5 impulses. This initial test phase ranged from several months (BLU, TYH) to approximately 1 week (OLY), after which the primary data collection (Phase 1 testing) began. During this phase, subjects were exposed to a sequence of 10 impulses, delivered at a rate of one impulse every 10 seconds. Before and after each exposure sequence, thresholds were measured at several frequencies using psychophysical (behavioral) and/or electrophysiological [auditory evoked potential (AEP)] methods and compared to their pre-exposure values to determine the amount of TTS induced. After no substantial differences were seen between the post- and pre-exposure thresholds, the exposure level was increased (the dolphin was moved incrementally closer, and/or the air gun pressure/volume were increased) on the next exposure day and the process repeated.

During Phase 1, the dolphin BLU participated in a total of 46 exposure sessions and 45 control sessions; TYH participated in 57 exposure sessions and 125 control sessions; and OLY participated in 45 exposure and 122 control sessions. The maximum exposure for all three subjects was 10 impulses, at a distance of 3.9 m from the air gun operating at 2000 psi with a volume of 150 in^3 (the maximum output configuration for the air gun with the dolphin at the shortest practical distance). The mean cumulative sound exposure level (SEL) for the 150 in^3, 2000 psi, and 3.9 m exposure conditions was 195, 194, and 189 dB re 1 μPa^{2}s (for 10 impulses), for BLU, TYH, and OLY, respectively.

No substantial TTS was observed in any subject, at any test frequency, for any combinations of range, volume, or pressure during psychophysical testing. The electrophysiological measurements were inconclusive. No measurable TTS was seen in BLU or OLY; however, TYH's electrophysiological data did show a small TTS after exposure to 10 impulses from the air gun at the 150 in^3, 1500 psi, and 3.9 m range condition (193 dB re 1 μPa^{2}s cumulative SEL). Exposure at a higher level produced a smaller TTS, and later testing using tone-pip stimuli failed to produce any TTS. It is not known to what extent TYH's "anticipatory" behavior during the highest exposures (see below) affected the TTS results (i.e., whether he was able to mitigate the effects of the exposure).

The results of this study contrast with previous TTS testing with a seismic watergun, where (behavioral) TTS was observed in a beluga after an exposure with SEL of 186 dB re 1 $\mu Pa^2 s$. The differences between the auditory and behavioral effects of the watergun and air gun may have been a result of the relatively low frequency content of air gun impulses compared to the relatively high-frequency hearing ability of dolphins, or the lower peak-peak pressures produced by the air gun.

No behavioral reactions were observed in BLU after exposure to the air gun impulses. Behavioral reactions were observed in both TYH and OLY after exposure to the air gun impulses at the maximum output level. Both subjects appeared to anticipate the next exposure in a sequence and may have been attempting to mitigate the effects of the impulses.

After the Phase 1 testing, follow-on testing was conducted with OLY and TYH to explore the use of alternate evoked potential methods and to further examine the anticipatory behavioral reactions. During these tests, no substantial differences were seen in the AEP data when using tone-pip stimuli compared to the sinusoidal amplitude modulated tone stimuli used previously. In OLY, evoked potential data collected in response to an external sound stimulus presented during the air gun exposures showed a temporal correlation with the air gun impulses — the AEP amplitude followed a cyclical pattern of decreasing after each impulse. This pattern of decreasing hearing sensitivity is believed to have been a result of either the middle ear reflex, a short-term TTS, or active self-mitigation of the air gun noise exposure by the dolphin.

II. Background

It is becoming increasingly clear that intense anthropogenic (human-generated) underwater sound may adversely affect the hearing and behavior of many marine mammals, including dolphins and whales. Unfortunately, there are few data regarding the effects of intense sound on these mammals, making it difficult to predict safe exposure levels.

Exposure to intense sound may produce an elevated hearing threshold, also known as a noise-induced threshold shift. If the threshold returns to the pre-exposure level after a period of time, the threshold shift is known as a temporary threshold shift (TTS); if the threshold does not return to the pre-exposure level, the threshold shift is called a permanent threshold shift (PTS). PTS results in damage to the auditory system and sensory hair cell loss. Moderate, occasional TTS is fully reversible and does not result in permanent injury; TTS may thus be used to estimate safe limits of exposure to avoid PTS.

Studies of PTS and TTS in terrestrial mammals were instrumental in establishing noise exposure limits in humans; however, there are no PTS data and few TTS data for marine mammals. The majority of existing marine mammal TTS data concern the effects of single, continuous exposures to steady-state signals such as tones (e.g., Finneran et al., 2010a; Finneran et al., 2005; Finneran et al., 2007; Ridgway et al., 1997; Schlundt et al., 2000) or broadband noise (e.g., {Nachtigall, 2003 #2266;Nachtigall, 2004 #13379;Kastak, 1999 #1703;Kastak, 2005 #3559;Popov, 2011 #24096;Mooney, 2009 #21748; Popov, 2013 #28013; Kastelein, 2012 #28015; Kastelein, 2012 #28016}). Although pure tones may approximate many military and commercial sonars and broadband noise allows comparison with existing terrestrial data obtained under similar conditions, many anthropogenic sources of intense sound produce impulsive signals, i.e. transient signals with rapid rise times and high peak pressures. Impulsive sources include impact pile driving, underwater explosions, and seismic air guns. TTS data obtained with broadband noise or tones may suggest the effects of impulsive sounds; however, the relationship between hearing loss and the fundamental parameters of sound such as peak frequency, duration, rise time, peak pressure, and total energy are unknown, thus TTS measurements with impulsive stimuli are needed for direct predictions. Limited data exist regarding marine mammals exposed to impulsive sounds produced by an "explosion simulator" (Finneran *et al.*, 2000), a seismic watergun (Finneran et al., 2002), an arc-gap transducer (Finneran *et al.*, 2003), and a seismic air gun (Lucke et al., 2009). All of these studies featured exposures consisting of single impulses only; there are no TTS data for marine mammals exposed to multiple impulses.

III. Objectives of this study

The objectives of this study were to measure the amount of TTS induced in trained bottlenose dolphins (*Tursiops truncatus*) after exposure to multiple impulses produced by a seismic air gun. Two test phases were planned: (1) Determine the most susceptible frequency to TTS after exposure to 10 air gun impulses, and (2) determine the relationship between TTS, the received sound pressure level (SPL), and the number of impulses.

During Phase 1 testing no substantial effects were observed after exposure to 10 impulses at the highest exposure levels, therefore, Phase 2 testing was not performed. Instead, follow-on testing was conducted to examine different evoked potential measurement approaches and the behavioral reactions exhibited by the subjects.

IV. Subjects and test environment

Three Atlantic bottlenose dolphins participated in the experiments: BLU (female, approximately 45–46 y at the time of testing, ~200 kg), TYH (male, 30–32 y, 200 kg), and OLY (male, 27–29 y, 200 kg). All three animals had experience with cooperative psychophysical testing, and BLU and TYH previously participated in TTS experiments (Finneran *et al.*, 2010a; b; Finneran and Schlundt, 2010; Finneran *et al.*, 2007). During the course of the study, BLU became pregnant and, as a result, her participation ended somewhat sooner than originally planned.

The experiments were conducted in floating, netted enclosures located within San Diego Bay (Fig. 1). Water depth at the test site was approximately 7 m. The 4 m × 9 m test enclosure contained two “underwater listening stations” for behavioral hearing tests and one for AEP measurements (Fig. 2). The underwater listening stations were composed of wood and PVC pipe and contained an underwater sound projector, receiving hydrophone, and “biteplate” — a neoprene-covered plastic plate upon which the subjects were trained to position themselves (Fig. 3). The biteplate enabled the subject’s head (and ears) to be in a known position with respect to the sound sources and for the received sound levels to be accurately calibrated. Two stations, designated “S1” and “S2”, were used so the site of the impulsive sound exposures (the S1 station) differed from the location of the behavioral hearing tests (the S2 station). This was to ensure that if a dolphin refused to return to the exposure site, it would not prevent the post-exposure thresholds from being measured. The more shallow listening station, designated “S3”, was positioned just below the surface so that the blowhole remained above water and the dolphins could breathe freely during the longer auditory evoked potential measurements.

FIGURE 1. The test site in San Diego Bay, consisting of a floating netted enclosure.

FIGURE 2. Underwater listening stations used during the behavioral and AEP hearing measurements. From left to right: S2, S1, S3.

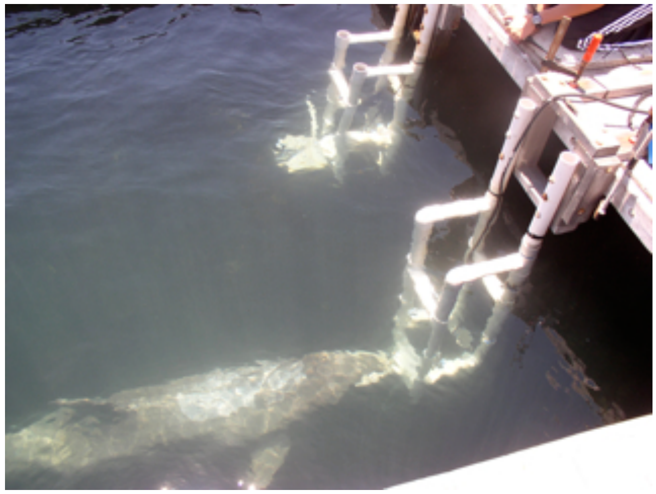

FIGURE 3. A dolphin positioned on the S1 listening station.

V. Methods

The test methodology was based on previous TTS studies at the US Navy Marine Mammal Program (Finneran *et al.*, 2010a; b; Finneran *et al.*, 2005; Finneran and Schlundt, 2010; Finneran *et al.*, 2007; Finneran *et al.*, 2000; Finneran *et al.*, 2002). Each subject participated in a single experimental session (control or exposure) each day. Each session consisted of a pre-exposure hearing test, exposure to either multiple air gun impulses (exposure session) or a "mock" exposure (control session), then a post-exposure hearing test. The difference between the post-exposure and pre-exposure hearing thresholds (in dB) indicated the amount of TTS produced by the exposure. *Note that TTS is defined as the difference between the post-exposure hearing threshold and the pre-exposure threshold, regardless of whether the difference is significant (TTS can be zero or negative).* Exposure conditions were repeated over a number of days to provide replicates and to test multiple frequencies after each exposure condition.

Both behavioral and auditory evoked potential (AEP) hearing test methods were employed. The notation "(behavioral) TTS" and "(AEP) TTS" are used in this document to distinguish between TTS values calculated from hearing thresholds measured behaviorally and those measured with auditory evoked potentials, respectively.

For behavioral hearing tests, subjects "whistled" in response to audible hearing test tones and remained quiet otherwise. Tone amplitudes were adjusted from one trial to the next using an adaptive staircase procedure where tone levels were reduced after the subject responded to a tone trial (a "hit") and increased when the subject failed to respond to a tone trial (a "miss"). Hearing thresholds were based on the average tone level of at least five hit-miss or miss-hit reversal points and could generally be estimated within 2–4 min per frequency tested. Up to three behavioral test frequencies were tested immediately before and immediately after the exposures (or mock exposures) in each session. When more than one frequency was tested, the frequencies were presented to the subject in reverse order in the pre- and post-exposure sessions so that the frequency tested immediately before the exposure was also tested immediately after the exposure.

AEP hearing tests utilized the single (TYH and OLY) and multiple (BLU) auditory steady state response technique (ASSR; Finneran *et al.*, 2007), where sinusoidal amplitude modulated (SAM) tones were presented using a descending method of limits and the evoked responses at the modulation rates tracked to estimate thresholds at individual tone frequencies. AEPs were typically measured before and after the behavioral tests which surrounded the exposures (or mock exposures). The multiple ASSR method allowed hearing to be simultaneously tested at four frequencies simultaneously. Two frequencies were normally tested when the single ASSR technique was employed. As with the behavioral frequencies, AEP test frequencies were presented in reverse order before and after the (mock) exposures. The ASSR approach does not work particularly well at low frequencies (below ~ 8 kHz) and is less precise compared to the behavioral approach.

Because the subjects' reactions to the exposures were unknown, they were trained to wear a self-contained hydrophone dosimeter (BLU) or cabled hydrophones attached to suction cups (TYH and OLY) during the impulsive sound exposures. This allowed estimates of the received sound levels regardless of the subject's location within the test enclosure.

VI. Accomplishments

1. Environmental Assessment

This project was performed via a Cooperative Research and Development Agreement (CRADA) between the US Navy and the International Association of Oil and Gas Producers (OGP). The CRADA specified that the seismic air gun could not be operated in San Diego Bay until the requirements of the National Environmental Policy Act (NEPA) were met; i.e., until the potential environmental effects of the project were analyzed. To satisfy the NEPA requirements, we conducted an environmental impact analysis and prepared an Environmental Assessment (EA). After reviewing the EA, a finding of 'No Significant Impact' was reached by the US Navy, Chief of Naval Operations (N456).

2. Monitoring and mitigation

The EA specified that the air gun could be fired a maximum of three days per week, with a maximum of 50 impulses per day. The EA also specified a monitoring plan and mitigation measures (i.e., do not fire the air gun if protected animals are within specified safety zones) to ensure that wild marine mammals and other protected species would not be not exposed to harmful sound levels.

Over the course of the study, the air gun was fired on 215 separate days, with a total of 3,113 impulses generated. Occasionally, multiple efforts (< 50 impulses total) were conducted within a single day. Mitigation measures were implemented on 73 of the 215 days, with a total of 79 mitigation actions. Twelve of the mitigation actions involved stopping the operation of the air gun during an ongoing shot sequence or canceling an effort prior to shots being fired. The other 67 events involved delaying the operation of the air gun until the safety zones were clear, after which operations continued. Thirty-three of the delayed mitigation actions involved other Navy activities (e.g., pausing operation of the air gun until Navy divers or swimmers left the water). The remaining 34 of the delayed mitigation actions involved pausing operation of the air gun until protected wildlife left the safety zone. Fourteen of the 34 delayed mitigation actions were the result of a specific harbor seal that repeatedly “hauled-out” on the floating enclosures near the test site (within the safety range for harbor seals). There were no significant changes in behavior noted in any protected wildlife during operations of the air gun.

3. Air gun output characterization

The air gun was a Sercel G-Gun 150, with an adjustable volume from 40 to 150 in^3. The air gun was deployed from a wench attached to a floating platform positioned in front of the S1 listening station. The air gun was always deployed to a depth of 2 m and suspended from the front and rear flanges so the long axis of the air gun was horizontal in the water column.

The air gun was pressured from a high-pressure air system, consisting of a compressor, storage tanks, and various valves and regulators. The high-pressure system we received with the air gun included a gasoline compressor and relatively small storage volume. Initial tests revealed that the system could not supply enough air at a sufficient rate to fire 10 or more impulses at a 10-s interval with an air gun volume of 150 in^3 and pressure of 2000 psi. We therefore designed and fabricated a new high-pressure air system (Fig. 4), which featured an electric compressor operating at ~3000 psi and included four large “K-style” gas bottles with a storage volume of approximately 1200 cubic feet. In the Fall of 2011, the 220-V electrical service to the test site was discontinued; from this time forward, the gasoline compressor was therefore used to pressurize the four gas storage bottles. The air pressure supplied to the air gun was regulated within the range of 1000–2000 psi, depending on the air gun volume, subject range, and desired received level. During a sequence of 10 impulses, the air gun pressure remained within 5% of the desired (initial) static operating pressure.

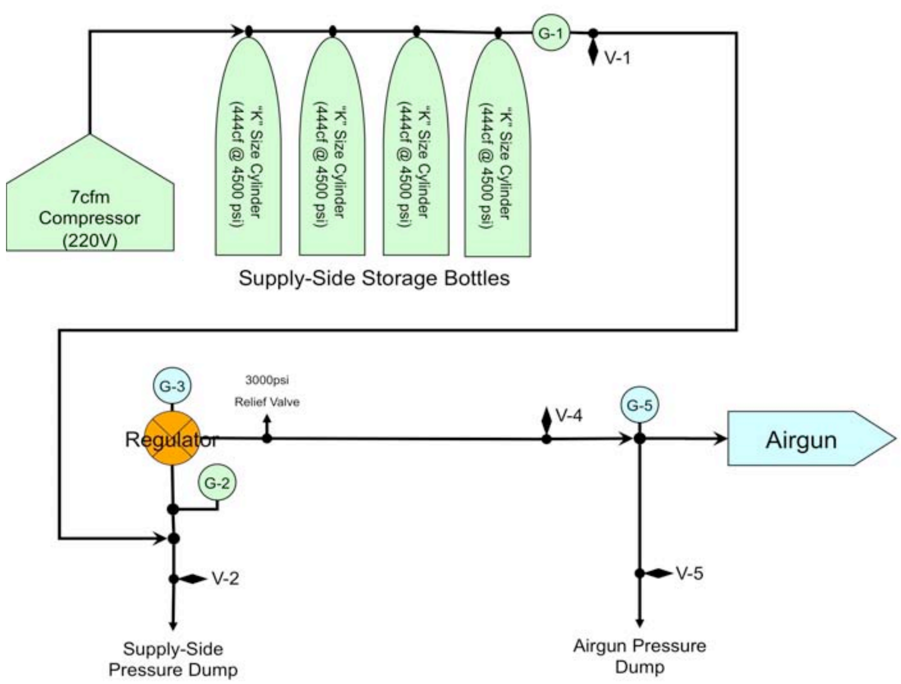

FIGURE 4. Schematic of the high-pressure air system.

The air gun output at the test site was characterized using hydrophones (Reson TC4013) positioned at the source (2-m distance, the closest practical distance given our arrangement) and at a variety of larger ranges. The hydrophone signals were filtered (1 Hz to 150 kHz, Reson VP1000 and TDT FT-5) and amplified if necessary (Reson VP1000) before being digitized at 300 kHz with 16-bit resolution (National Instruments USB-6251) and stored to disk using custom software. Figure 5 shows some example pressure-time waveforms recorded at various distances from different combinations of air gun volume and pressure. Figure 6 provides the sound exposure spectral density level as a function of frequency for each of the waveforms shown in

Fig. 5. Figure 7 shows the rms SPL, peak-peak SPL, and sound exposure level (SEL) for each of the waveforms in Fig. 5.

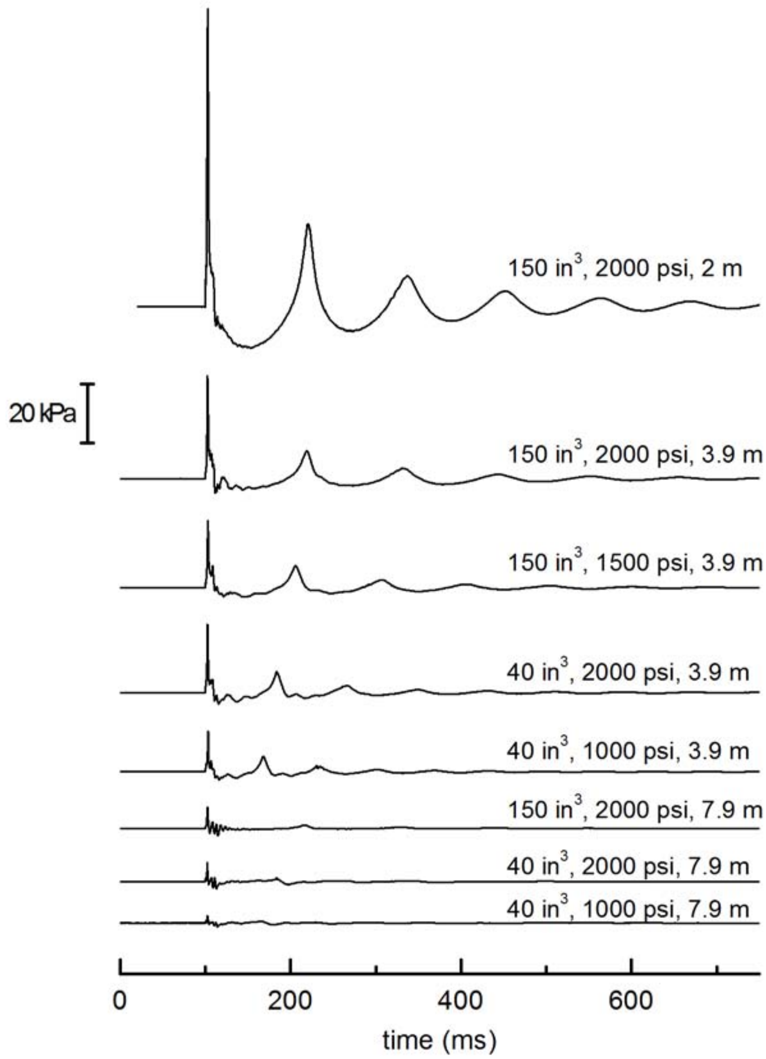

FIGURE 5. Example pressure-time waveforms for air gun impulses produced at various combinations of range, air gun volume, and air gun static pressure. The air gun depth was 2 m; water depth was approximately 7 m. The receiver depth was 1.3 m (the biteplate depth) for all recordings except for the top trace, where the receiver depth was 2 m.

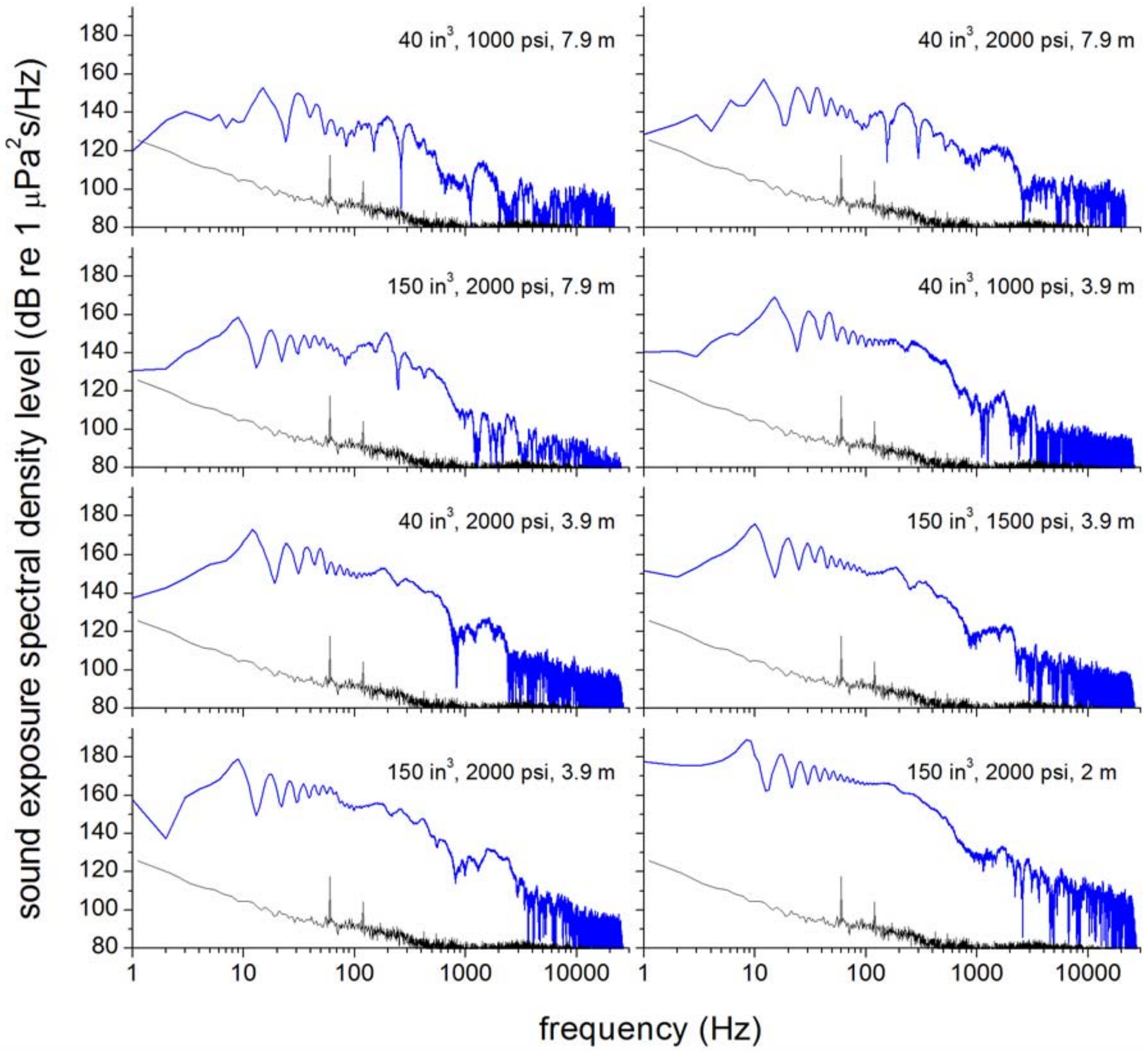

FIGURE 6. Sound exposure spectral density levels for air gun impulses produced at various combinations of range, air gun volume, and air gun static pressure (blue), along with average ambient noise energy spectral density measured over a comparable time interval (black). The line spectra within the noise correspond to the power line frequency and its harmonics. The air gun depth was 2 m; water depth was approximately 7 m. The receiver depth was 1.3 m for all recordings except the 150 in^3, 2000 psi, 2 m condition, where the receiver depth was 2 m.

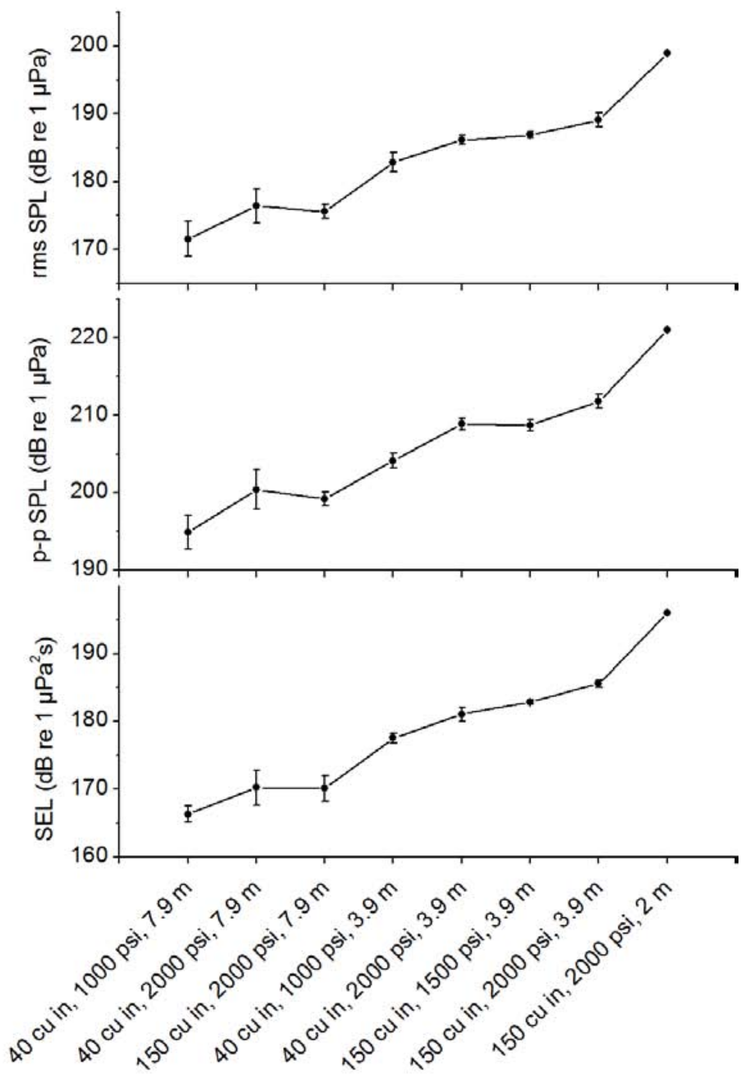

FIGURE 7. Mean values for rms SPL, peak-peak SPL, and SEL for air gun impulses produced at various combinations of range, air gun volume, and air gun static pressure. The air gun depth was 2 m; water depth was approximately 7 m. The receiver depth was approximately 1.3 m for all recordings except the 150 in^3, 2000 psi, 2 m condition, where the receiver depth was 2 m.

In addition to measuring the air gun output at relatively short ranges, we also performed some measurements at larger ranges. For relatively close ranges, the hydrophone was suspended from the floating enclosures to a depth of 2 m. At larger ranges, the hydrophone was suspended (2 m depth) from a boat whose position was recorded via GPS. Figure 8 shows example GPS tracks for several sequences of measurements; Fig. 9 summarizes the SEL measured along the various transects. We used nonlinear regression to fit a function of the form

$$RL = SL - m \log R, \tag{1}$$

where *RL* is the received level, *SL* is the source level, *R* is range (m), and *m* is the slope, to the plots of SEL versus range. The best fit values for the slope were between 24 and 25 for the short range measurements along transects 1–3 and between 19 and 20 for the longer range measurements along transects A–C. This means that at ranges from a few hundred meters to a few kilometers, the transmission loss along these transects in San Diego Bay was modeled reasonably well using the simple spherical spreading relationship (m = 20); however, closer to the air gun the spreading loss was larger.

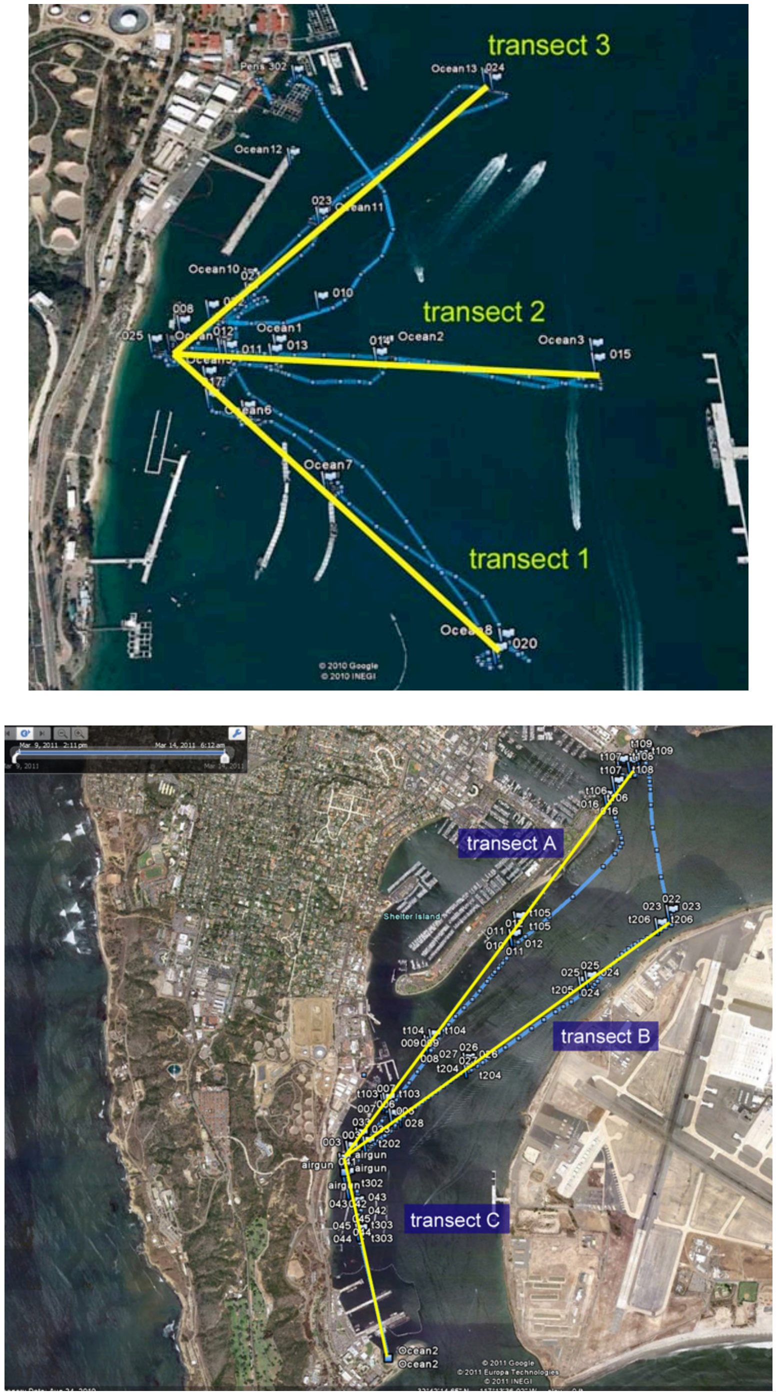

FIGURE 8. Transects originating at the test site used for long distance characterization of the air gun output. Transects 1, 2, 3 were relatively short-ranged (< 1 km); transects A, B, C covered longer ranges (> 1 km).

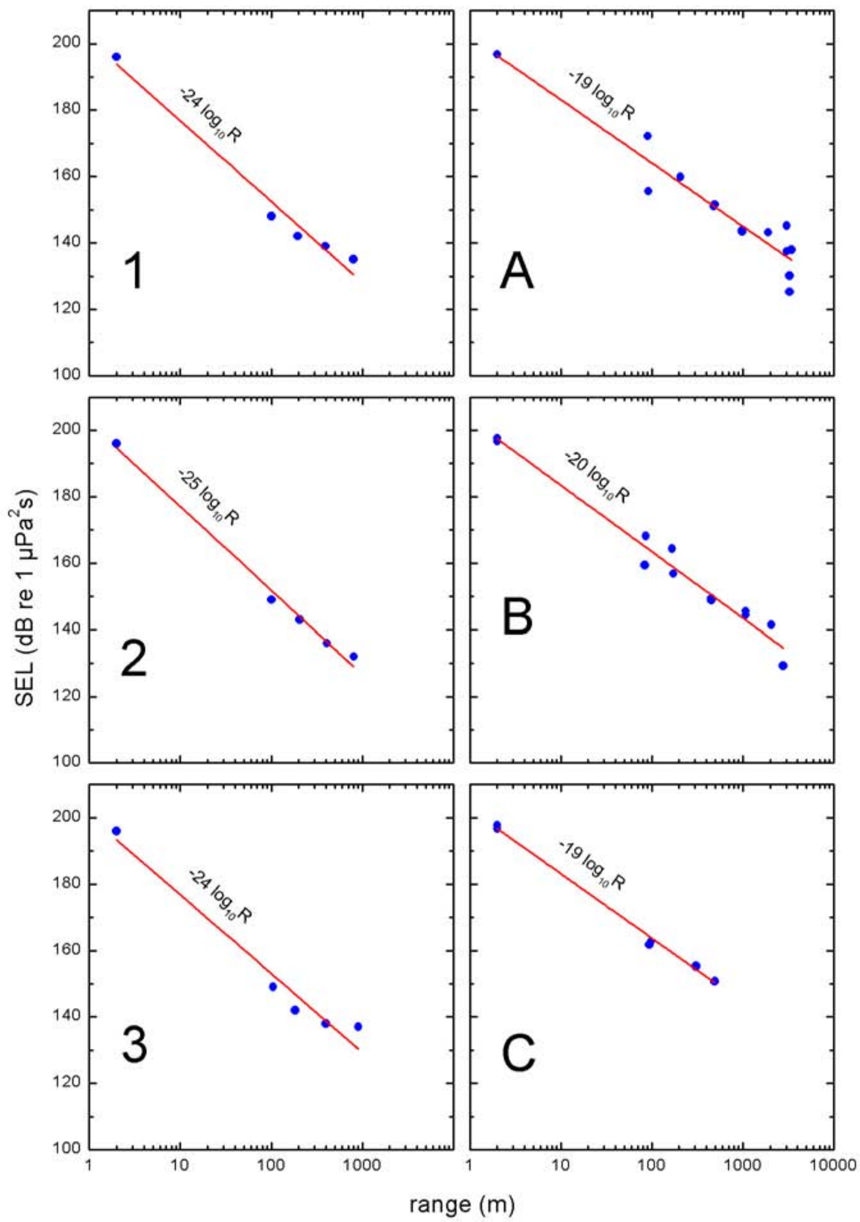

FIGURE 9. SEL generated by the air gun (volume = 150 in^3, static pressure = 2000 psi, depth = 2 m) along the transects shown in Fig. 8.

4. Baseline hearing thresholds

Although audiograms in a quiet pool existed for BLU and TYH, and OLY's hearing ability was known from previous AEP measurements, hearing thresholds had not been measured in San Diego Bay at the test site, where the ambient noise levels were relatively high and variable (Fig. 10). We therefore measured baseline hearing thresholds for each subject, at the test site, using behavioral techniques (the most accurate method). The frequency range for baseline hearing tests was 250 Hz to the upper cutoff frequency for each subject (50–120 kHz). Thresholds at each frequency were replicated over a period of several weeks. Figure 11 shows the resulting audiograms for each subject. TYH's hearing was normal for a dolphin, with an upper cutoff frequency of ~ 120–130 kHz. OLY had high-frequency hearing loss above ~ 70 kHz, and BLU has substantial high-frequency hearing loss above 40–50 kHz. The high-frequency hearing loss in OLY and BLU is not unusual for dolphins of their age (Houser and Finneran, 2006).

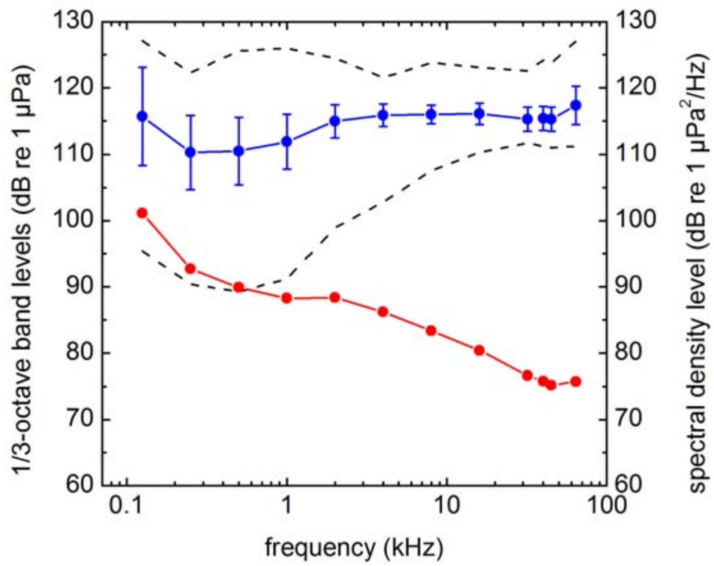

FIGURE 10. Ambient noise levels measured at the test site from 31 August 2011 to 7 December 2012. Blue symbols represent the mean SPL within 1/3-octave bands centered at the hearing test frequencies; error bars show the standard deviation and the dashed lines show the minimum and maximum values. Red symbols show the pressure spectral density levels. Data were collected during no-signal test trials during the dolphin hearing tests. The number of samples at each frequency ranges from 1506 to 1592. The main sources of ambient noise were small boats/ships and snapping shrimp.

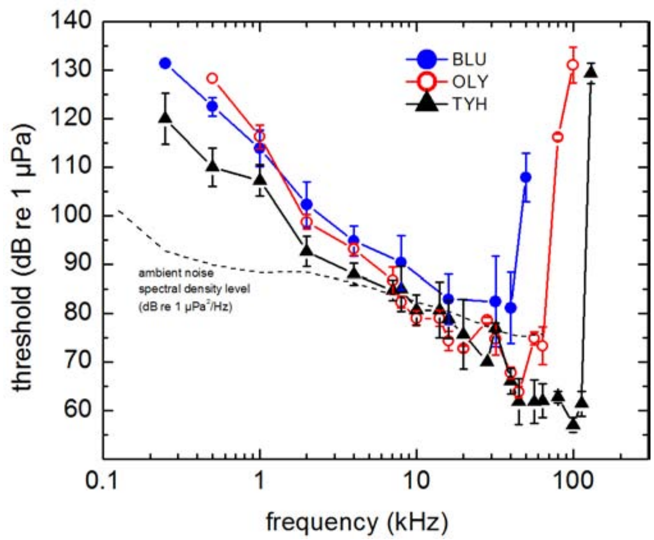

FIGURE 11. Baseline (behavioral) hearing thresholds for each subject measured at the test site. The symbols represent mean values and the error bars indicate the standard deviations.

5. Exposure sequence and sound exposure levels

Because the air gun sound source was novel to the dolphin subjects and we had no data to indicate exactly what exposures were likely to produce TTS, testing began with an exploratory stage, where exposures began at low received levels and were gradually increased over weeks and/or months to desensitize each subject to the impulsive sounds and to ensure that no hearing damage occurred. Because of the variable noise in San Diego Bay, we anticipated relatively high dispersion in the TTS data. Therefore, to identify the onset of TTS, exposure levels were slowly increased over weeks and months, and TTS (based on behavioral hearing thresholds) was measured at each hearing test frequency, with the goal of obtaining a TTS of approximately 10–15 dB (an amount clearly larger than the variability about the mean TTS found during control sessions). During this exploratory stage, few replications were performed at each frequency, but the increases in received level were small, so that large changes in auditory effects between levels were not expected (based on our prior experience with TTS in dolphins and belugas). If a clear TTS was obtained, the exposure level would be repeated over multiple days to replicate the TTS measurements at each hearing test frequency immediately after exposure. To identify the onset of TTS, an appropriate mathematical model would be fit to the TTS versus exposure level data and interpolation would be performed to identify the exposure level corresponding to some arbitrary amount of TTS (e.g., 6 dB). In this fashion, the variability in the data that makes identifying very small amounts of TTS difficult would not prevent the identification of the exposure conditions corresponding to the “onset” of TTS.

Eight different exposure “levels” — configurations of volume, pressure, and distance between the air gun and the subject — were used during the study. Mean p-p SPLs, rms SPLs, and cumulative SELs (for 10 impulses) for each of these configurations measured during the exposure sessions for each subject are provided in Tables I—III.

At least one calibration impulse was fired before a subject arrived at the test site to ensure the air gun was operational and that output levels remained consistent throughout the study. During initial tests with BLU, calibration impulses were recorded by one Reson 4013 hydrophone placed at the position of the midpoint between the subject’s ears when properly stationed underwater on the S2 biteplate. Calibrations during the period of subsequent testing with TYH and OLY were measured using two Reson 4013 hydrophones placed near the position of the subject’s ears when stationed on the S1 biteplate. Calibration SELs measured between the two hydrophones were normally within 1 dB of each other.

BLU was the first subject to participate in the study. Since the air gun effect on her hearing and behavior was unknown, BLU was free swimming in an enclosure approximately 50 m from the air gun, which was charged to 1000 psi with a volume of 40 in^3, for her initial exposure. Over a period of several weeks, BLU was moved closer to the air gun, the number of impulses increased, and the air gun volume and pressure were increased. After each exposure sequence, behavioral and AEP hearing thresholds were measured at several frequencies and compared to their pre-exposure values. After no substantial differences were observed between the post- and pre-exposure thresholds, the exposure level was increased (BLU was moved incrementally closer, and/or the air gun pressure/volume were increased) on the next exposure day and the process repeated. Exposure sessions with the dolphins TYH and OLY began after BLU was finished, therefore we were able to use the results obtained with BLU to develop a reduced exposure sequence for use with TYH and OLY (see Tables II and III). Again, exposures began at relatively low received levels and incrementally increased as no TTS was observed.

Exposure levels for BLU presented in Table I were measured by a single Reson 4013 hydrophone mounted to a self-contained acoustic dosimeter package and attached to a nylon harness which BLU wore during the control and exposure sessions (Fig. 12). Because we initially believed the animals might show aversive behavior and swim about the enclosure during the exposures, the dosimeter package allowed BLU to swim freely and record impulses regardless of her position in the test enclosure.

To measure the received levels during TYH and OLY’s exposure sessions, we replaced the dosimeter with cabled hydrophones attached via suction cups near the location of the ears (Fig. 13). This allowed a more accurate measurement of the exposure levels using higher sampling rates. The cabled hydrophones were used in both control and exposure sessions, and allowed impulses to be recorded regardless of the subject’s location in the test enclosure. TYH had previous experience wearing the cabled hydrophones and wore them throughout the study. OLY did not begin wearing the hydrophones until testing at Level 7; during Levels 1, 2 and 5, the hydrophones were placed near the calibration position on the S1 station and did not move during the exposure even if OLY may have. Custom software allowed for real-time measurement of individual impulses received at each ear (or at the S1 station). On occasion, one channel of the hydrophone inputs experienced a faulty/noisy connection and those data were dropped from analysis. Similarly, measurements that occurred when a hydrophone was out-of-water due to the animal’s movement around the test enclosure during the exposure were discarded as well (see Sect. 7 – Behavioral Results). If poor data quality prevented analyzing both hydrophone signals for a particular impulse, the SEL for that particular impulse was estimated using the mean SEL from the other impulses within the sequence. At the higher exposure levels, there was much more variation in the received levels for TYH and OLY when behavioral reactions to the exposures were observed (see Sect. 7 – Behavioral reactions).

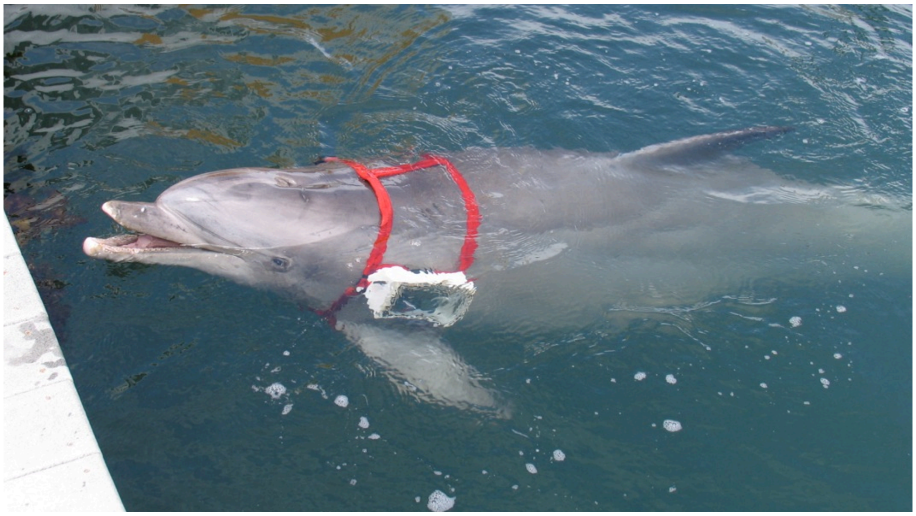

FIGURE 12. BLU wearing the air gun receive hydrophone mounted to a self-contained acoustic dosimeter package and attached to a nylon harness.

FIGURE 13. A dolphin at the S1 listening station wearing the cabled hydrophones attached near the location of the ears via suction cups (“ear cups”).

TABLE I. Number of exposure and control sessions and mean (sd) exposure levels for BLU. Exposure values are based on only those sessions with 10 air gun impulses.

Level	Range (m)	Vol. (in^3)	Pressure (psi)	Con.	Exp.	p-p SPL (dB re 1 μPa)	rms SPL (dB re 1 μPa)	cum. SEL (dB re 1 $μPa^2s$)
1	7.9	40	1000	10	1	193	170	175
2	7.9	40	2000	3	5	198 (0.3)	173 (2.2)	178 (0.2)
3	7.9	150	1500	1	4	198 (3.2)	175 (2.6)	180 (2.6)
4	7.9	150	2000	16	18	199 (0.8)	176 (1.0)	180 (1.8)
5	3.9	40	1000	2	4	204 (0.8)	183 (1.4)	188 (1.0)
6	3.9	40	2000	6	4	209 (0.0)	186 (0.0)	191 (0.0)
7	3.9	150	1500					
8	3.9	150	2000	7	10	212 (0.6)	189 (0.5)	195 (0.5)

TABLE II. Number of exposure and control sessions and mean (sd) exposure levels for TYH. Exposure values are based on only those sessions with 10 air gun impulses.

Level	Range (m)	Vol. (in^3)	Pressure (psi)	Con.	Exp.	p-p SPL (dB re 1 μPa)	rms SPL (dB re 1 μPa)	cum. SEL (dB re 1 $μPa^2s$)
1	7.9	40	1000	15	4	193 (0.5)	170 (2.4)	176 (0.8)
2	7.9	40	2000	11	5	200 (0.9)	176 (0.9)	180 (1.5)
3	7.9	150	1500					
4	7.9	150	2000					
5	3.9	40	1000	13	7	202 (0.8)	182 (0.8)	186 (0.5)
6	3.9	40	2000					
7	3.9	150	1500	35	22	208 (0.7)	188 (0.8)	193 (0.7)
8	3.9	150	2000	51	19	208 (1.4)	187 (1.6)	194 (0.8)

TABLE III. Number of exposure and control sessions and mean (sd) exposure levels for OLY. Exposure values are based on only those sessions with 10 air gun impulses.

Level	Range (m)	Vol. (in^3)	Pressure (psi)	Con.	Exp.	p-p SPL (dB re 1 μPa)	rms SPL (dB re 1 μPa)	cum. SEL (dB re 1 $μPa^2s$)
1	7.9	40	1000	16	4	197 (2.4)	174 (2.0)	176 (0.6)
2	7.9	40	2000	8	3	203 (2.9)	179 (2.1)	182 (1.0)
3	7.9	150	1500					
4	7.9	150	2000					
5	3.9	40	1000	12	6	206 (0.8)	185 (0.8)	189 (0.8)
6	3.9	40	2000					
7	3.9	150	1500	26	18	208 (6.8)	186 (7.1)	193 (4.5)
8	3.9	150	2000	60	14	199 (4.4)	177 (4.8)	189 (2.9)

6. TTS results

a. Control and exposure sessions

BLU participated in 10 desensitization exposure sessions, where the distance to the air gun was more than 8 m and/or the number of impulses was between 2 and 5. BLU then participated in a total of 46 exposure sessions and 45 control sessions (Table I). At the cessation of testing, BLU was 3.9 m from the air gun operating at 2000 psi with a volume of 150 in^3 (Level 8, the maximum output configuration for the air gun). The mean cumulative SEL for these 10-impulse exposures was 195 dB re 1 $\mu Pa^2 s$. Two additional sessions were also conducted at the same range (3.9 m) and air gun configuration (150 in^3, 2000 psi), but with the number of impulses increased to 20. [No measurable TTS was observed after these exposures, although with only two sessions, threshold measurements could not be repeated at any of the test frequencies.] Additional exposures could not be conducted because BLU's pregnancy prevented her from participating in future experiments.

TYH participated in 57 exposure sessions and 125 control sessions and OLY participated in 45 exposure sessions and 122 control sessions, respectively. For both dolphins, testing was conducted through Level 8, the maximum output for an exposure of 10 impulses, at a range of 3.9 m, with the air gun volume and static pressure at 150 in^3 and 2000 psi, respectively. The mean cumulative SEL for these exposures was 194 and 189 dB re 1 $\mu Pa^2 s$ for TYH and OLY, respectively. The lower cumulative SEL for OLY was a result of his movement pattern during the exposure sequence. The number of impulses fired was not increased beyond 10 due to alterations in these subjects' trained behaviors (see Sect. 7 – Behavioral results). In lieu of increasing the number of impulses fired, additional control and exposure sessions were conducted as part of follow-on tests described in Sect. 8.

b. Psychophysical (behavioral) testing

Pre-exposure behavioral thresholds were normally within ± 3 dB of baseline thresholds. Figure 14 depicts the mean time at which the first, second, and third pre- and post-exposure thresholds were estimated relative to the exposure (time zero) for each subject during control and exposure sessions. The first post-exposure threshold was typically estimated within 2–4 minutes. In the case of BLU and TYH, at least two frequencies were normally tested within 5 min after the final air gun impulse was fired. In most cases, post-exposure thresholds were measured more quickly than pre-exposure thresholds. The between-subject differences can be attributed to both the two different computer operators who ran the tests for BLU and for TYH and OLY, and to individual differences in the animals' movements between the underwater listening stations, response time, and response duration.

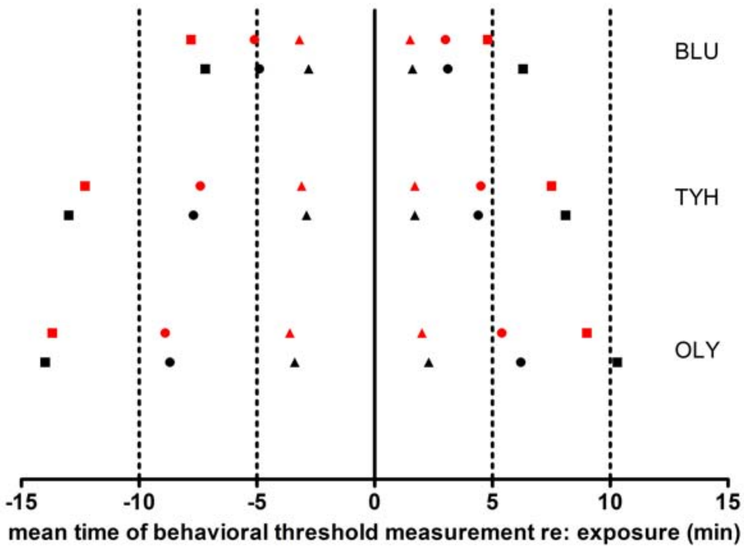

FIGURE 14. Mean times at which the first, second, and third pre- and post-exposure thresholds were estimated relative to the (mock) exposure (time zero) for each subject during control and exposure sessions. Red symbols-exposure sessions; black symbols-control sessions. Frequencies were tested in reverse order before and after exposures. Triangles-first frequency; circles-second frequency; squares-third frequency.

Figure 15 shows examples of the mean (behavioral) TTS measured at each hearing test frequency for BLU, TYH, and OLY. These data capture the main trends in the psychophysical data: no substantial amounts of (behavioral) TTS were observed in any of the dolphins after exposure to 10 air gun impulses, compared to that measured during control sessions for any combinations of range, air gun volume, or air gun pressure. Figure 16 summarizes the (behavioral) TTS results for all subjects and exposure levels. These data are plotted as the mean TTS as a function of exposure level for each subject and frequency. Mean values of (behavioral) TTS for all subjects were within ± 5 dB. There were no systematic differences between the control and exposure data; any meaningful TTS would have been identifiable as an exponential increase of TTS with increasing exposure level. The relatively small mean values for TTS, similarity between the control and exposure data, and the poor correlation between exposure level and mean TTS all indicate that the air gun exposures did not result in any measurable TTS when using psychophysical (behavioral) hearing test methods.

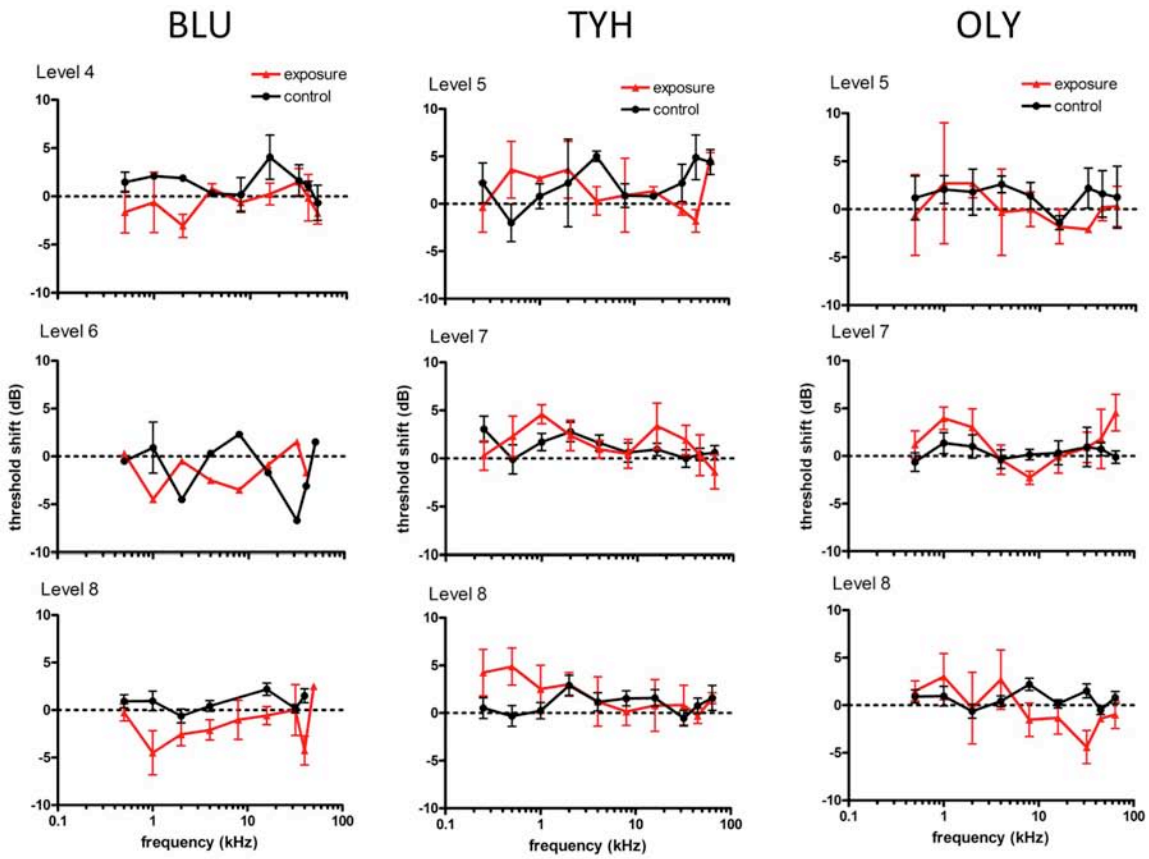

FIGURE 15. Examples of the amounts of (behavioral) TTS measured in the dolphins BLU, TYH, and OLY during control and exposure sessions. Hearing thresholds were obtained using a behavioral response paradigm.

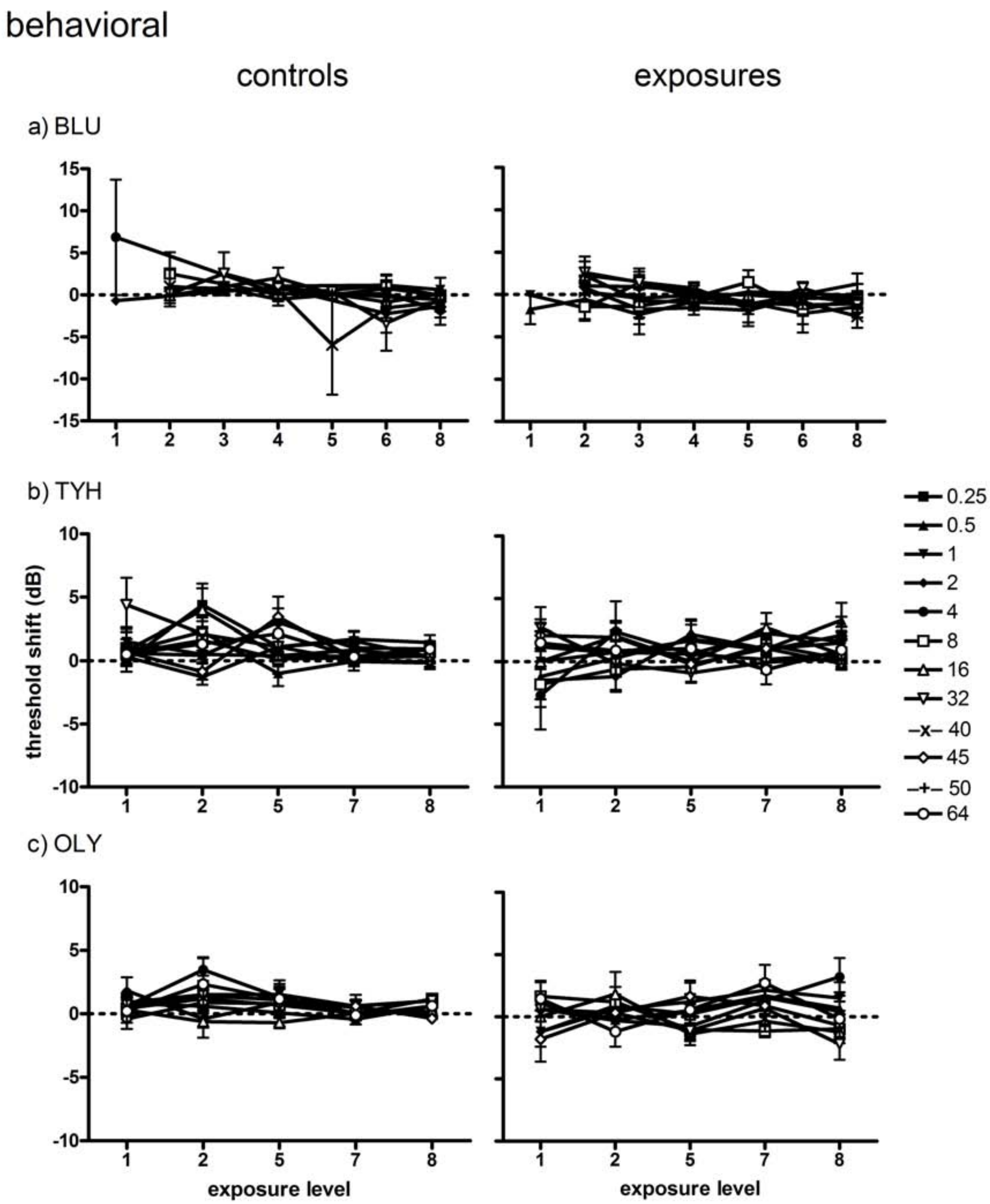

FIGURE 16. (behavioral) TTS measured in the dolphins BLU, TYH, and OLY at the test frequencies for control (left panels) and exposure (right panels) sessions at all exposure conditions.

c. AEP testing

Pre- and post-exposure AEP hearing tests were conducted before and after the behavioral hearing tests at Levels 4–8 for BLU, and at Levels 7 and 8 for TYH and OLY. Figure 17 shows examples of the mean (AEP) TTS thresholds measured at each hearing test frequency for BLU, TYH, and OLY. Figure 18 summarizes the (AEP) TTS results for all subjects and exposure levels. Figures 17 and 18 are analogous to Figs. 15 and 16 for the behavioral data. Figures 19, 20, and 21 show the relationship between the mean ASSR amplitude and the SAM tone SPL (called the input-output, or IO function) for BLU, TYH, and OLY, respectively, for control and Level 8 exposure sessions. The AEP data were more variable than the behavioral data and many of the AEP IO functions were shallow, especially for TYH, which made statistical detection of AEPs near threshold difficult. Overall, there were no clear differences between the control and exposure AEP thresholds for BLU or OLY. The IO functions for the pre- and post-exposure sessions were also very similar for BLU and OLY. For TYH, there were no differences between mean AEP thresholds except at 8 kHz, where TTSs of 9 and 6 dB were measured after the Level 7 and Level 8 exposures, respectively. Standard deviations about these mean values were high (10 and 7 dB, respectively) and there was little difference between the pre- and post-exposure IO functions. It is unusual that the mean TTS actually decreased from Level 7 to Level 8; however, the exposure SEL increased only 1 dB between these conditions and TYH’s anticipatory behavior may have affected the resulting TTS (see Sections 7 and 8).

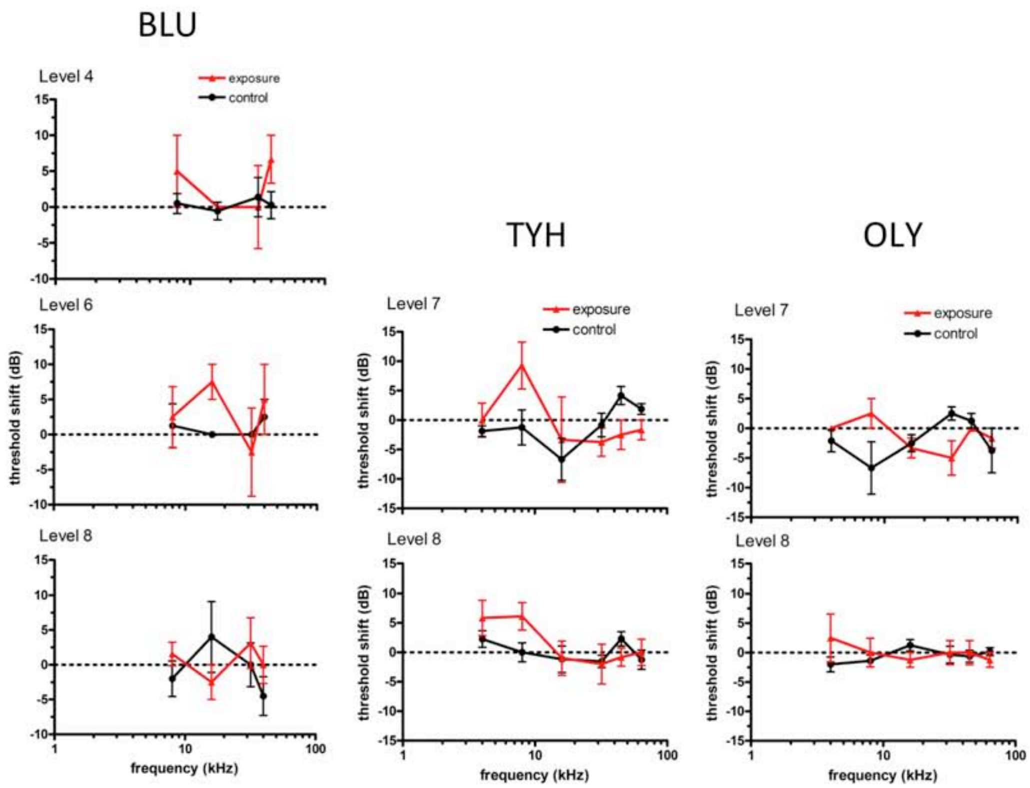

FIGURE 17. Examples of the amounts of (AEP) TTS measured in the dolphins BLU, TYH, and OLY during control and exposure sessions. Hearing thresholds were obtained using AEP (auditory steady state response) measurements and SAM tone stimuli.

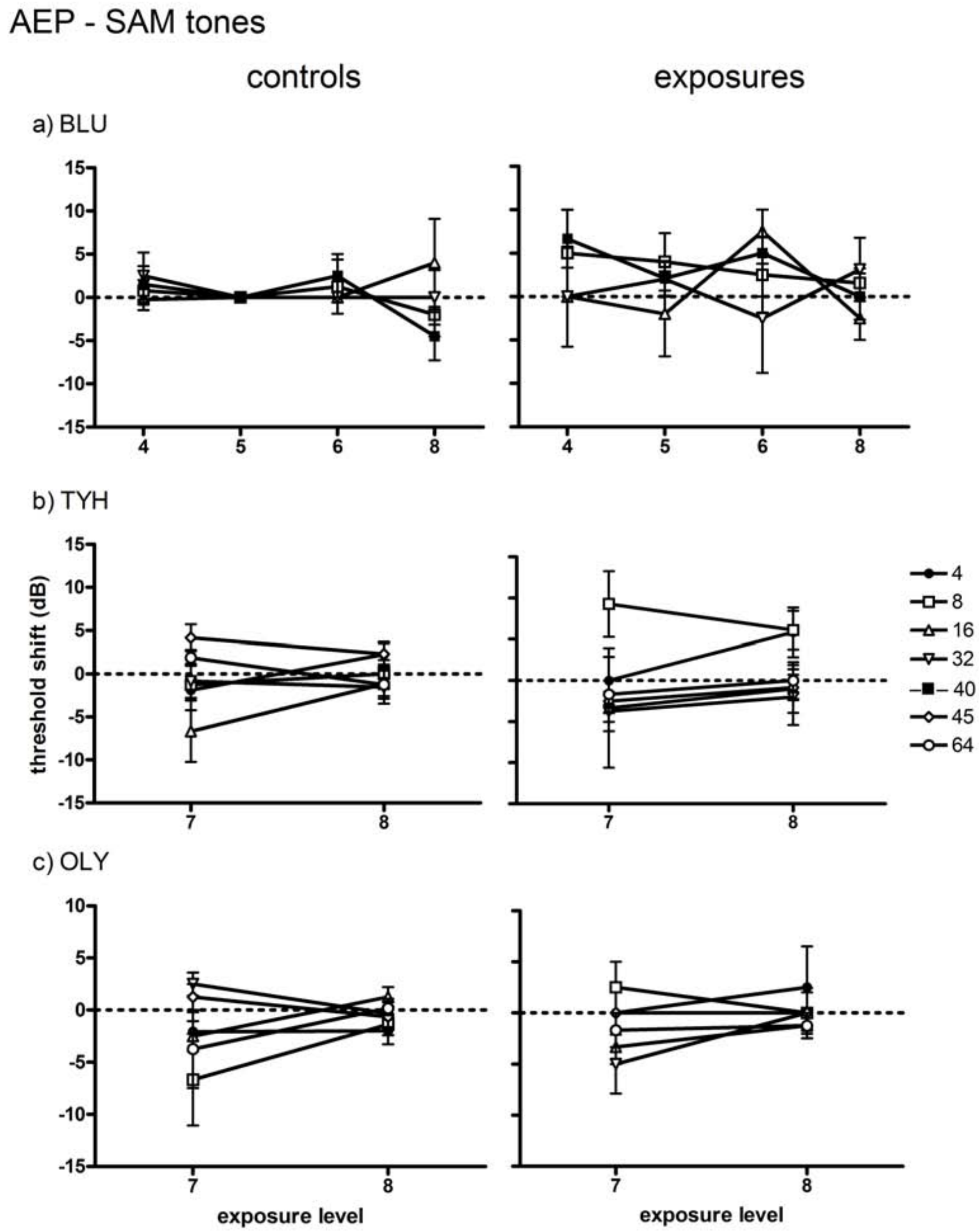

FIGURE 18. TTS measured in the dolphins BLU, TYH, and OLY at the AEP test frequencies for control sessions (left panels) and exposure sessions (right panels) at all exposure conditions. Hearing thresholds were obtained using AEP (auditory steady state response) measurements.

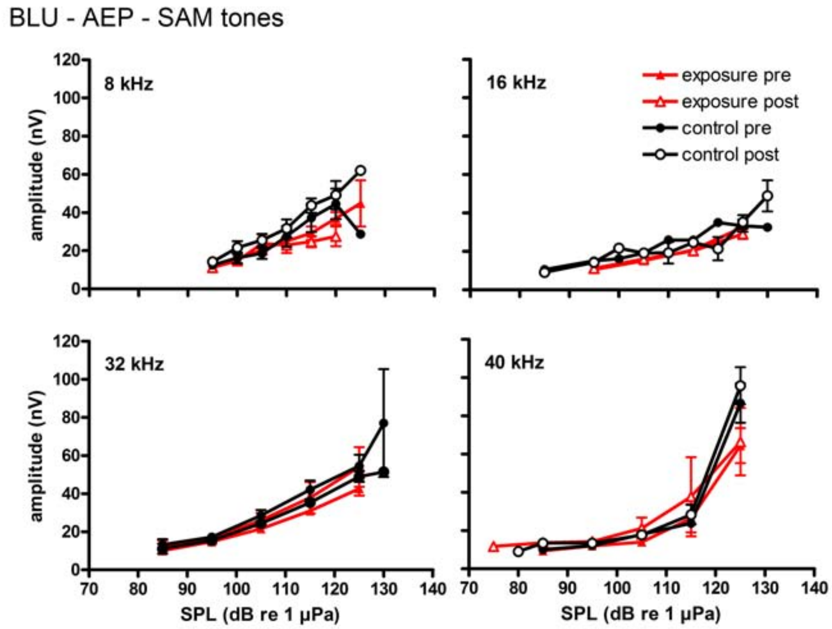

FIGURE 19. Pre- and post exposure AEP input-output (IO) functions for the frequencies tested during control (black) and exposure (red) sessions for BLU at Level 8 using SAM tone stimuli.

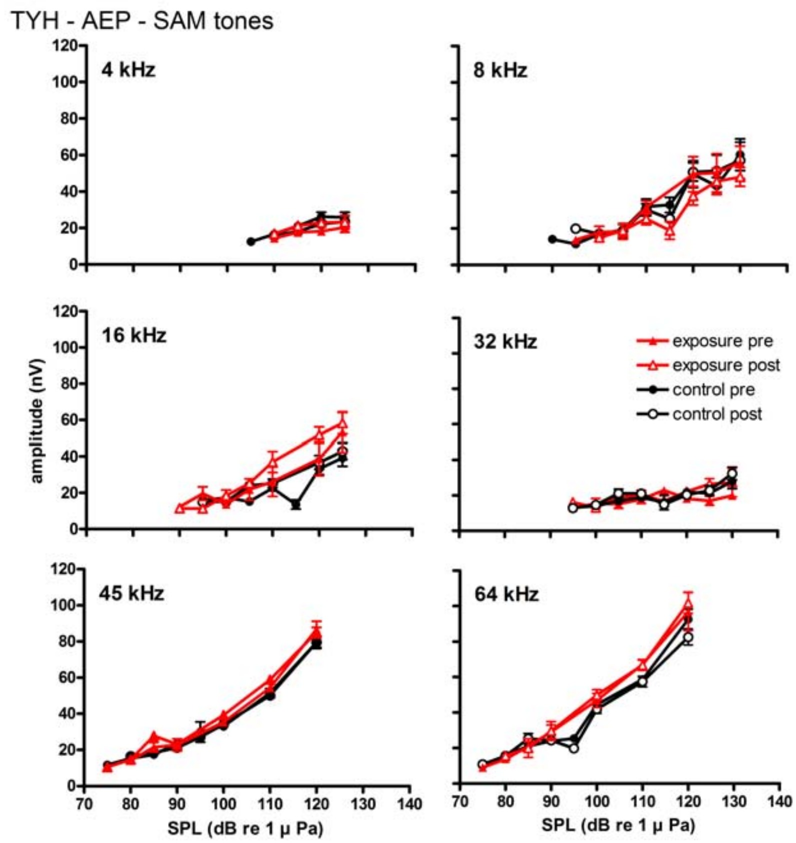

FIGURE 20. Pre- and post exposure AEP input-output (IO) functions for the frequencies tested during control (black) and exposure (red) sessions for TYH at Level 8 using SAM tone stimuli.

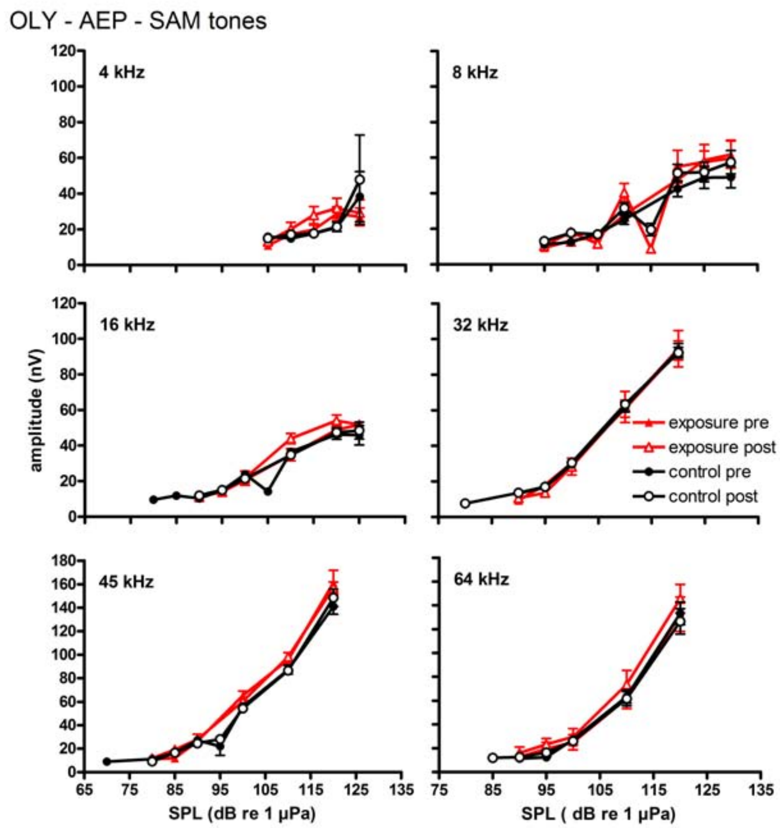

FIGURE 21. Pre- and post exposure AEP input-output (IO) functions for the frequencies tested during control (black) and exposure (red) sessions for OLY at Level 8 using SAM tone stimuli.

7. Behavioral reactions

We did not observe significant behavioral reactions in BLU after exposure to the air gun impulses. During the exposures, BLU's only reaction was to leave the S1 station upon hearing the first impulse of the exposure sequence and position herself on the S2 station (the hearing test site) and wait for the exposures to cease (similar to her normal response to an S1 signal). This reaction was observed during every exposure session beginning with the first exposure of Level 1 testing. During all control sessions, BLU remained on the S1 station for the 90-s mock exposure. The lack of a reaction from BLU is somewhat surprising, since she was exposed on multiple days to 10 or more impulses at the highest air gun output (150 in^3 and 2000 psi), at a distance of only 3.9 m. Because we initially believed the animals might show aversive behavior and swim about the enclosure during the exposures, with BLU we used the self-contained acoustic dosimeter package to measure the instantaneous sound pressure during each exposure. At relatively low exposure levels, TYH and OLY both remained on the S1 listening station for the 10-impulse exposure sequences. After TYH and OLY showed little reaction to the initial exposures, we replaced the dosimeter with cabled hydrophones attached to the subject via suction cups near the location of the ears. This allowed a more accurate measurement of the exposure levels using higher sampling rates.

TYH continued to show little reaction to the exposures from the 10 air gun impulses through Level 7. For almost all of the 40 exposure sessions at Levels 1, 2, 5, and 7, TYH remained on the S1 listening station for the 10 impulses, then responded to an underwater buzzer sounded by the trainer to come to the surface for fish reward before continuing to the S2 station. This was TYH's "usual" behavior in previous TTS experiments conducted in an indoor pool. During TYH's first Level 8 exposure, with a mean SEL approximately 3 dB higher than his Level 7 exposures, he left the S1 station after the first impulse and came to the surface. He was at the surface with his ears and suction-cup hydrophones underwater for the second impulse, was on his way back down to the S1 station when the third impulse fired, and was back on the S1 station for the remaining seven impulses. This behavior of leaving S1 and going to the surface after the first impulse occurred for all remaining exposure sessions. Usually he was at the surface for one or two impulses before heading back down to station on the S1 biteplate for the rest of the impulse sequence. On a couple of occasions, he came to the surface twice in the impulse sequence, but returned to the listening station each time.

On the ninth exposure session at Level 8, TYH began the impulse series as he had in the previous eight exposures. Then, after returning to the S1 station after coming up to the surface, TYH first stationed on S1, then backed off the S1 biteplate and turned his head to the (left) side while the impulse fired, and then stationed back on the biteplate. Just before the next impulse fired, he backed off again, turned to the side for the next impulse, and then re-stationed on S1. He did this on/off/on behavior — apparently anticipating the next impulse based on the fixed 10-s inter-impulse interval — for impulses number 7 through 10 of this series. TYH continued to do this timing sequence for all remaining exposures at Level 8, and for the follow-on tests at Levels 5, 7 and 8 (see Sect. 8 – Follow-on tests).

OLY's behavioral reactions to the exposures were more pronounced than BLU and TYH. In most cases for Levels 1, 2 and 5, OLY came up to the surface after the first impulse, was at the surface for the second impulse, and then headed back down to S1 and stationed there for the remaining seven or eight impulses. For OLY's first exposure at Level 7, he left the S1 station twice during the impulse sequence, but returned each time. For his next three exposure sessions, he reverted to only leaving the S1 station once after the initial impulse, and then remained on the biteplate. On his fifth exposure at Level 7, OLY left the S1 station after the first impulse and swam a circle around the test enclosure before re-stationing for impulses 4–10. For the next several Level 7 exposures, OLY surfaced either once or twice during the exposure sequence, and sometimes swam a circle before re-stationing on S1. OLY began wearing the suction cup hydrophones on his 11th exposure session at Level 7. Beginning with the 15th Level 7 exposure, OLY left the S1 station after the first impulse and floated at the surface generally facing away from the trainer and approximately 3.8 m farther away from the S1 station and the air gun for the remaining impulses before beginning his post-exposure hearing tests. Although he occasionally returned to the S1 station for a couple of impulses before resurfacing, floating near the surface was his typical reaction for the remainder of Phase 1 tests and for the follow-on tests. During OLY's 11th exposure at Level 8, he exhibited the anticipatory behavior for impulses 7–10 that TYH had shown: stationing on S1, backing off and turning sideways (left) for the impulse, then re-stationing on S1 until the next impulse fired (Fig. 22). Unlike TYH who just moved his head off to the side during the impulses, OLY turned his whole body to the side. OLY did this behavior on only a couple of occasions for the remainder of Phase 1 testing, but he did it on several occasions during the follow-on tests (see Sect. 8 – Follow-on tests).

Due to alterations in the trained behavior at the highest level(s) in these two subjects, no attempt was made to increase the number of impulses beyond 10. As can be seen by the SELs and standard deviations at Levels 7 and 8 for TYH and OLY, it was clear that their movement around the S1 station the test enclosure was having some dampening effect on their received levels. Increasing the number of impulses to increase the cumulative sound exposure in an attempt to elicit a measureable TTS would be ineffective if the subjects were able to mitigate exposure levels.

It is important to note that this study was designed to measure TTS, not behavioral reactions, and substantial effort was made to desensitize two of the three dolphins (BLU and TYH) to the air gun exposures. Thus, any behavioral observations must be treated with caution and they may not be representative of wild and/or naïve animals. However, the behavioral data do have some relative value, in that we have previously seen much more dramatic behavioral reactions in trained dolphins exposed to other impulsive and steady-state noise conditions (e.g., Finneran *et al.*, 2002; Schlundt *et al.*, 2000).

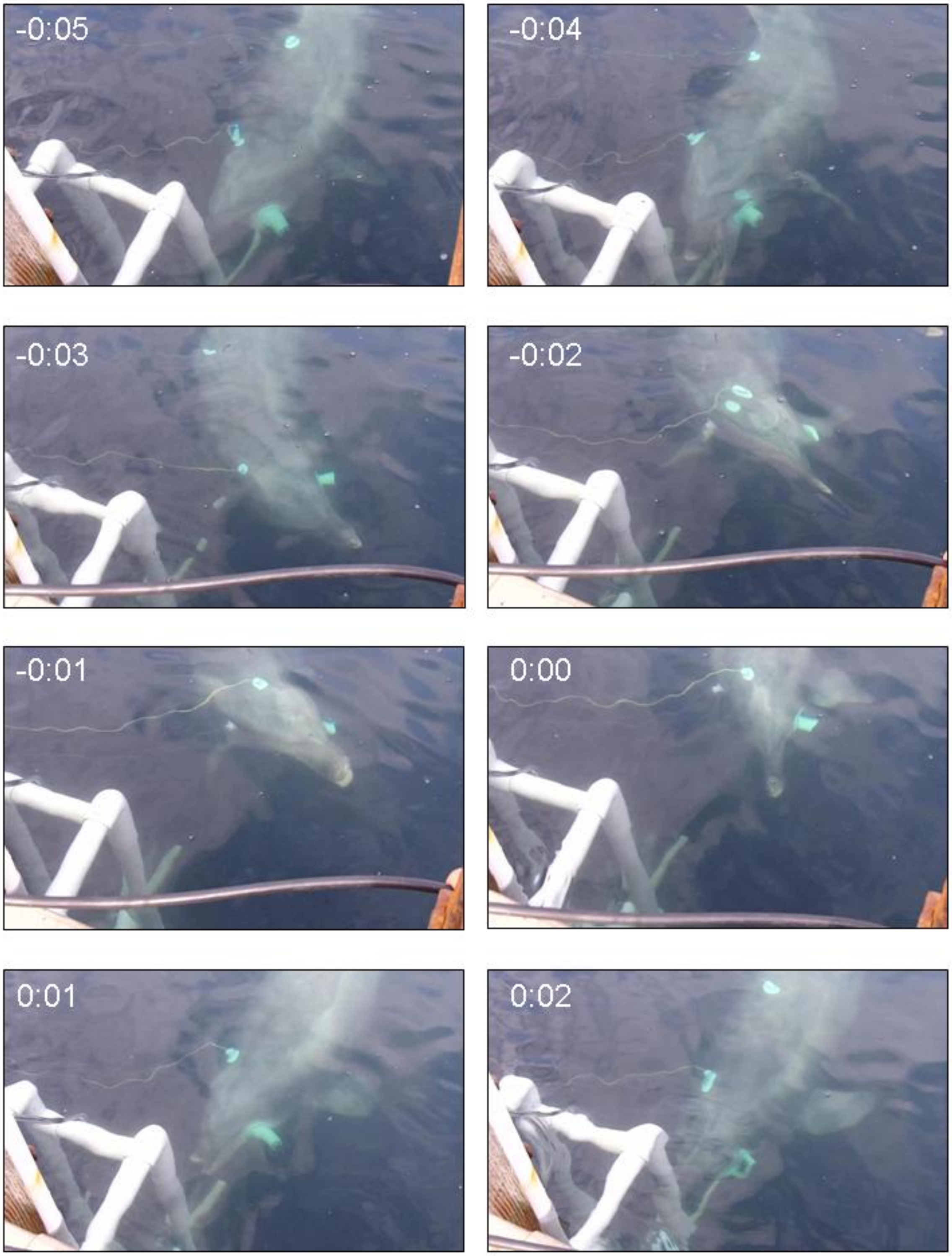

FIGURE 22. Example of anticipatory behavior in the dolphin OLY. The numbers in each panel indicate the time (in seconds) relative to an air gun impulse during a sequence with a 10-s inter-impulse interval.

The anticipatory behavior exhibited by both TYH and OLY is intriguing. Both subjects obviously learned the timing characteristics of the exposure sequences and eventually adopted the same posture during the exposures, even turning their heads the same direction. This occurred despite the two subjects never having been at the test enclosure at the same time during testing; i.e., they could not have observed the other animal's behavior during any exposures.

8. Follow-on tests

a. Repeat exposures at lower levels to assess alterations in behavior

At the completion of Phase 1 tests through Level 8, TYH and OLY were re-tested at Levels 7 and 5 with the intent of monitoring their behavior, specifically to see whether they might resume their previous behavior at those levels and remain on the S1 station during exposure. Both behavioral and AEP thresholds were measured pre- and post-exposure as in Phase 1 tests. Level 7 was repeated first. TYH participated in three exposure sessions and OLY participated in two exposure sessions. Their behavior during the impulsive sound exposures remained the same as during the Level 8 exposures in the weeks before. Both subjects came to the surface for several impulses, and if they returned to the S1 station, they left the S1 biteplate just before the impulse fired and returned during the inter-stimulus interval. The same behaviors were observed when TYH and OLY each participated in five additional exposures at Level 5. Although TYH stayed on the S1 biteplate for some consecutive impulses, he continued the behavior of surfacing at least once during the exposures and anticipated many of the remaining impulses. Received exposure levels during this repeat testing for TYH were very similar to those shown in Table II for Levels 5 and 7. The SELs for OLY at Level 7 were 4 dB lower than in the Phase 1 tests, and at Level 5 they were 6 dB lower. Because the data collected in these follow-on tests were not consistent with data in Phase 1 tests before the altered behavior began and the SELs reduced, they were not included in the TTS results presented above. Control sessions were interspersed between these follow-on exposures and because the subjects' behavior was consistent with prior controls, the behavioral and AEP threshold data from the control sessions were included in the Phase 1 results.

b. Repeat AEP measurements using tone pip stimuli

The IO functions in response to the SAM tones were particularly shallow, especially in the case of TYH, so additional AEP measurements were conducted at Level 8 using tone pip stimuli which typically generate a larger brainstem response and could reveal more obvious differences between pre- and post-exposure IO functions, if any existed. The data for 11 control and 10 exposure sessions for TYH, and eight control and six exposure sessions at Level 8 for OLY are provided in Fig. 23. IO functions for TYH and OLY obtained with tone-pip stimuli are shown in Figs. 24 and 25, respectively. No differences were seen in the (AEP) TTS values for either subject at any of the test frequencies. The amplitudes and slopes of the IO functions in response to the tone pips were improved for both subjects, but the results were still variable at the lowest frequencies tested. The small TTSs that had been observed in TYH's ASSR thresholds were not reproduced during tone-pip testing.

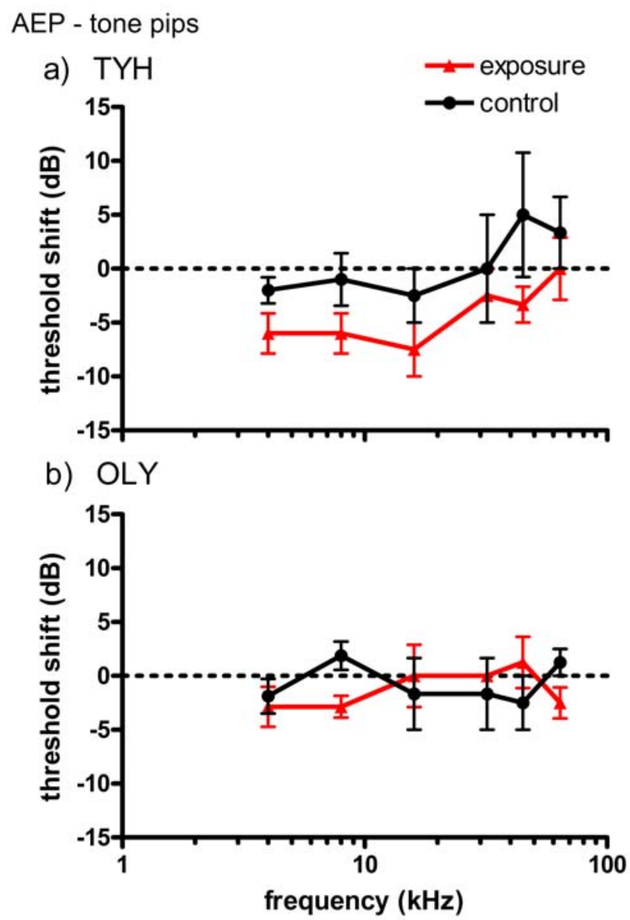

FIGURE 23. Examples of the amounts of (AEP) TTS measured in the dolphins TYH and OLY during control and exposure sessions at Level 8. Hearing thresholds were obtained using AEP (auditory steady state response) measurements and tone pip stimuli.

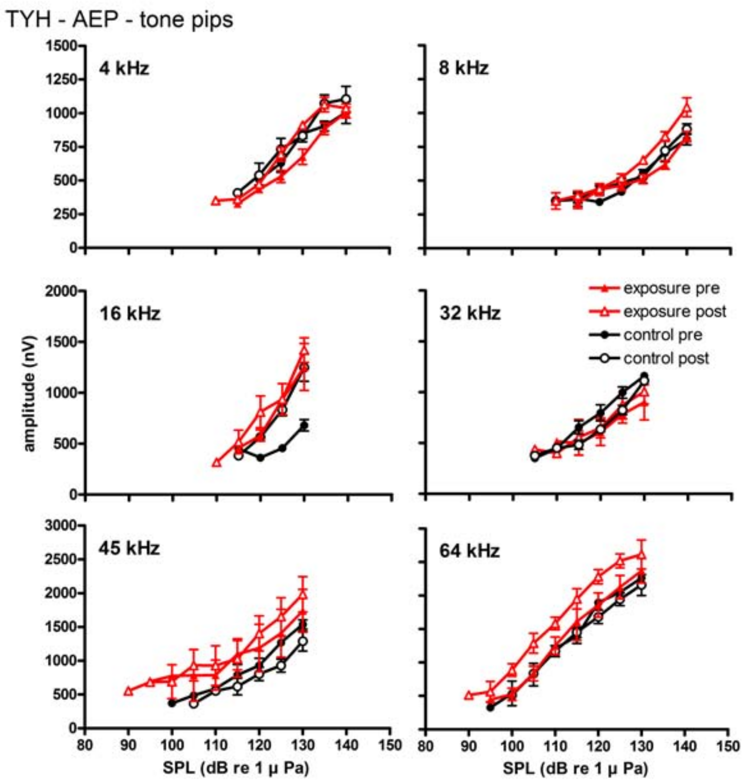

FIGURE 24. Pre- and post exposure AEP input-output (IO) functions for the frequencies tested during control (black) and exposure (red) sessions for TYH at Level 8 using tone pip stimuli.

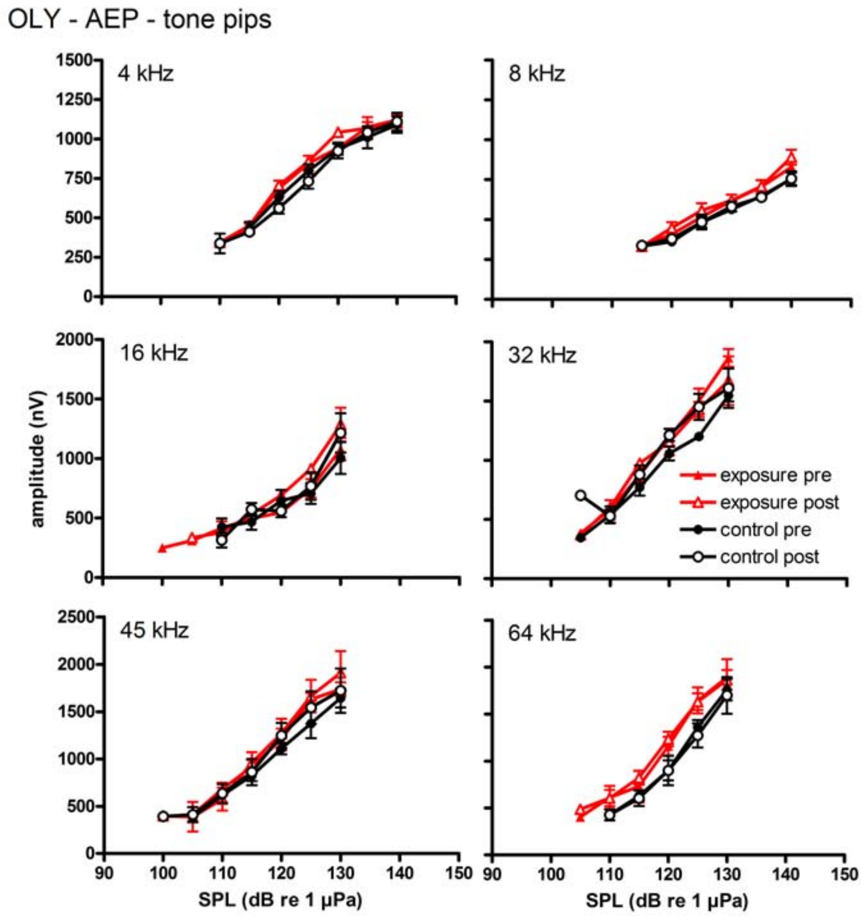

FIGURE 25. Pre- and post exposure AEP input-output (IO) functions for the frequencies tested during control (black) and exposure (red) sessions for OLY at Level 8 using tone pip stimuli.

No behavioral thresholds were collected during this follow-on test. TYH exhibited the "anticipatory behavior" to these exposures at the S1 station. OLY typically came off of the S1 biteplate and floated at the surface during these exposures, but returned to the trainer for his post-exposure hearing tests on the S3 station as soon as the impulse sequence ended. During some of these exposures OLY chuffed as the impulses were generated. Since both animals exhibited alterations in their behavior which may have affected the received SELs during this follow-on test, the SELs measured during these additional exposures are not included in the means presented for Level 8 in Tables II and III.

c. Steady-state ASSR measurements during exposures

It seems likely that the “anticipatory” behavior displayed by both TYH and OLY was related in some manner to mitigating the perceived level and/or auditory effects of the impulsive noise exposure. To investigate whether hearing sensitivity in TYH and OLY actually changed while they performed this behavior, exposures were conducted while hearing sensitivity was simultaneously monitored via the ASSR in response to a continuous sinusoidal amplitude modulated (SAM) tone. The SAM tone carrier frequencies (i.e., the hearing test frequency) were the ones that produced the largest ASSRs: 40 kHz for OLY and 60 kHz for TYH. The SAM tone modulation rates were 1 kHz. SAM tones were continuously delivered to each subject via a “jawphone” transducer — a piezoelectric transducer embedded in a suction cup and attached to the lower jaw. The jawphone was used to maintain a constant stimulus level even if the subjects moved. The instantaneous EEG was obtained from the potential difference between surface electrodes placed on the head and back and saved to disk over 120-s epochs before, during, and after exposure to the air gun impulses. The synchronization pulse used to trigger the air gun was also saved to disk. During offline analysis, the instantaneous EEG was first divided into 30-ms epochs. Time-averaging was then performed on each collection of 128 sequential epochs. Each average was then Fourier transformed to obtain the frequency spectrum. The ASSR amplitude was defined as the spectral amplitude at the SAM tone modulation rate (1 kHz). Successive averaging periods were overlapped by 50%. To avoid electrical or acoustical artifacts from the air gun triggering or impulse, epochs existing within ±200 ms of the air gun impulses were excluded from averaging. Measurements were made seven times with OLY and TYH. Each measurement consisted of a pre-exposure, exposure, and post-exposure session. The air gun firing was simulated, and the air gun trigger pulse recorded to disk but not used to fire the air gun, during the pre- and post-exposure sessions.

Figures 26 and 27 show the ASSR amplitudes as functions of time for the pre-exposure, exposure, and post-exposure sessions for OLY and TYH, respectively. Within each plot, ASSR measurements from different days are overlaid. If the subject remained stationary, one would expect the ASSR amplitude to remain relatively constant over time and there should be no correlation between the air gun exposures (or simulated air gun exposures) and the ASSR amplitudes. For OLY, the ASSR amplitudes measured during the pre-exposure and post-exposure sessions were relatively stable over time (as expected) and were not correlated with the timing of the simulated air gun exposures. Salient peaks in the post-exposure data (e.g., OLY’s post-exposure data at 30-s) correspond to the subject coming to the surface and putting one or more electrodes above water, which increased the measured AEP. For the exposure sessions, there was high variability during the first 5 exposures. This period of time corresponded to the time OLY came to the surface and moved to the opposite side of the pen. By the exposures 5–7, OLY was normally back on the S1 biteplate, performing his anticipatory head-turning behavior. The ASSRs measured during this time period were correlated with the air gun exposures: the ASSR amplitude decreased between exposures and increased during or just after the exposures. ASSRs for TYH were smaller in amplitude and more variable and no systematic patterns were seen in any of the data. The underlying cause of the pattern seen in OLY's data is not known; however, some potential hypotheses are:

(1) *Electrical artifacts from the air gun triggering pulse*. It is well known that AEP measurements can be affected by nearby electrical fields; however, the present measurements ignored the EEG data from 200 ms before to 200 ms after the air gun impulse. Also, oscillations in the ASSR did not appear in TYH's data. This makes it unlikely that the resulting ASSR oscillations were related to the trigger pulse signal.

(2) *Acoustic artifact from the air gun impulse* (i.e., the air gun impulse creates an AEP with energy at 1 kHz). Any transient short-latency AEP resulting from the air gun impulse would be < 10 ms in duration. The primary component of the air gun impulse itself was < 200 ms. Since the EEG data were ignored from 200 ms before to 200 ms after the air gun impulse, and the pattern was not seen in TYH's data, it seems unlikely that the pattern seen in the ASSR data was a result of an acoustic artifact from the air gun.

(3) *Movement artifact related to head movement*. OLY performed a stereotyped head turning motion just before every shot, so it is possible that his head movement caused a fluctuation in the ASSR amplitudes. ASSR amplitudes changed dramatically when the dolphins came to the surface and the electrodes were above the water; however, it is not obvious how changes in the dolphin's position, while remaining submerged, would cause the ASSR amplitude to change. Plus, TYH also performed the stereotyped head movement, but no oscillations in the ASSR were seen in TYH.

(4) *Middle-ear reflex*. The air gun impulse may have caused contraction of the stapedius muscle and a change in stiffness in the middle ear complex, resulting in a loss of sensitivity of the ear. The lower ASSR amplitudes in TYH may have prevented us from seeing the same effect during his exposures.

(5) *Short-term TTS*. The oscillations in the ASSR amplitude may reflect a very short-term TTS and recovery. The failure to see the same effect in TYH could have been a result of the different test frequencies (40 kHz for OLY and 60 kHz for TYH) or the lower ASSR amplitudes in TYH.

(6) *Self-mitigation of the air gun exposure*. Nachtigall *et al*. {, 2012 #28903} demonstrated that a false killer whale decreased its hearing sensitivity when signaled that a loud sound was about to arrive. The behavioral reactions observed in OLY and TYH clearly show that the animals learned to anticipate the air gun exposures. It is therefore possible that TYH and OLY learned to decrease hearing sensitivity to mitigate the effects of the air gun impulses, in a manner similar to the false killer whale tested by Nachtigall *et al*.

Additional experiments would be required to test hypotheses (3)–(6). For example, air gun impulses could be delivered on a random schedule, so the subjects could not anticipate the exposure time. If the decrement in ASSR amplitude persisted, this would suggest a direct effect from the exposure (stapedial reflex and/or TTS). Mock/simulated exposures could also be inserted into a sequence of impulses delivered at a fixed interval. If the decrement in ASSR amplitude was still observed, this would suggest a purely anticipatory response (i.e., self-mitigation). Testing at a variety of exposure levels would help separate self-mitigation from auditory effects, since the magnitude of TTS would scale with exposure. Testing at a variety of SAM tone frequencies would help separate self-mitigation and TTS from a stapedial reflex, since these phenomena would exhibit different frequency-dependencies. Finally, if self-mitigation appears to be occurring, specific mechanisms responsible could be evaluated by simulating air gun exposure sequences with dolphins out-of-water undergoing a computerized tomography (CT) scan to evaluate the positions of air spaces around the head as a function of time relative to exposures within an ordered sequence.

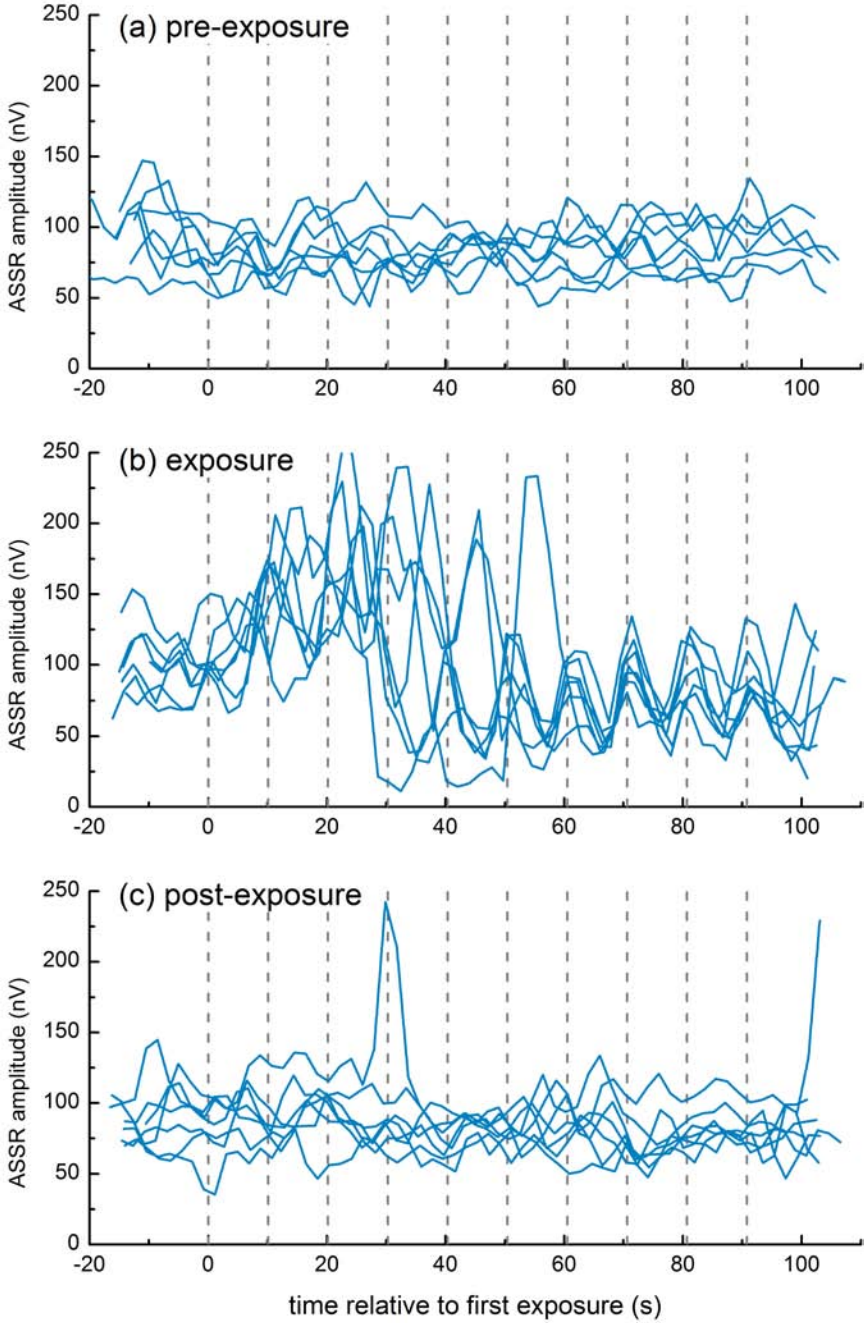

FIGURE 26. ASSR amplitudes as a function of time (a) before, (b) during, and (c) after exposure to air gun impulses for OLY. The vertical dashed lines indicate the times of the air gun exposures in (b) and the simulated exposures in (a), (c). Each trace represents an ASSR measurement from a different day.

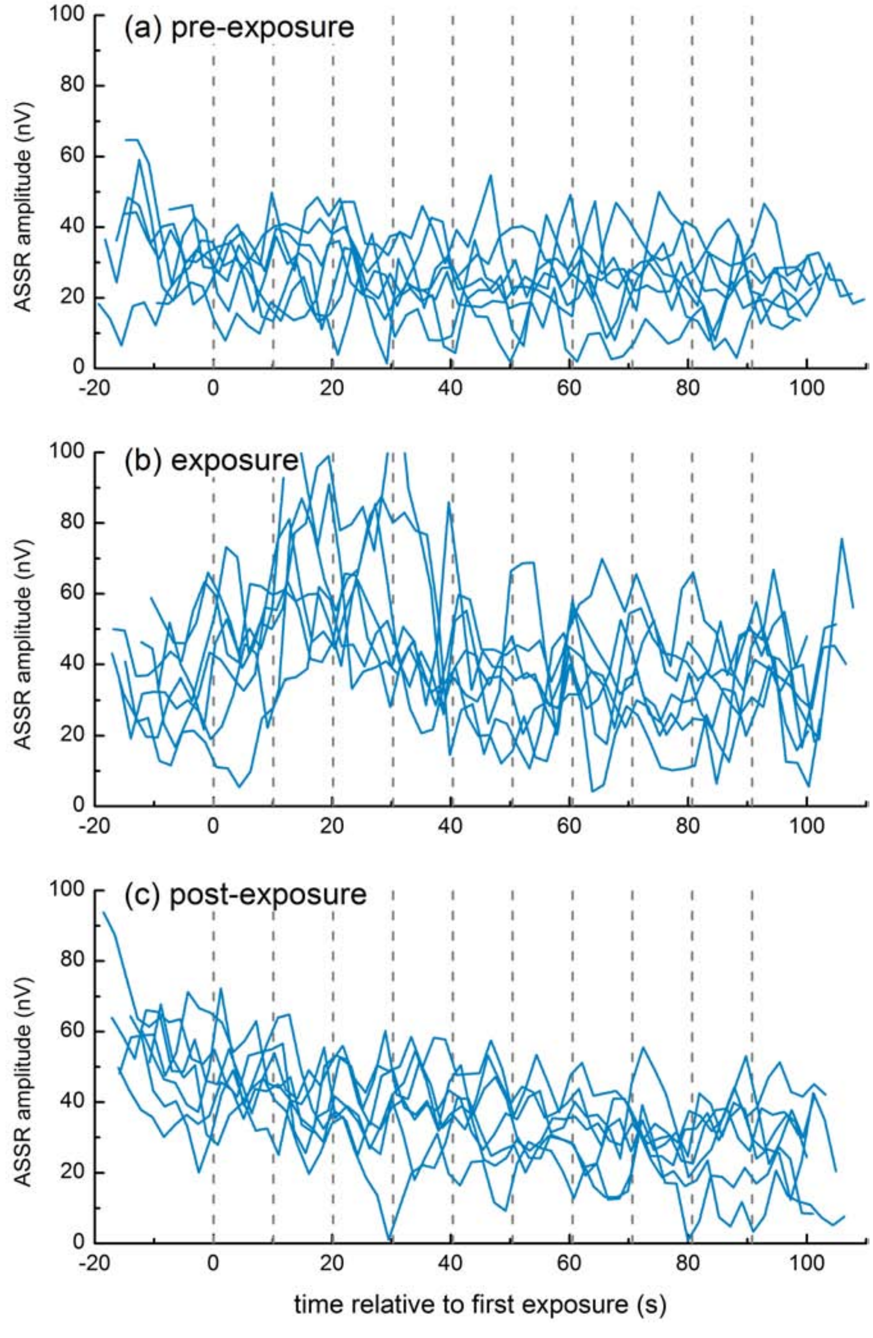

FIGURE 27. ASSR amplitudes as a function of time (a) before, (b) during, and (c) after exposure to air gun impulses for TYH. The vertical dashed lines indicate the times of the air gun exposures in (b) and the simulated exposures in (a), (c). Each trace represents an ASSR measurement from a different day.

VII. Significance

At the cessation of the study, the dolphins BLU, TYH and OLY showed no clear (behavioral) TTS after exposure to 10 impulses from a single, 150 in^3 air gun operating at 2000 psi and located at a range of 3.9 m (total cumulative SEL = ~189–195 dB re 1 μPa^2s). These data contrast with earlier studies where a beluga experienced TTS after exposure to a single impulse from a watergun (SEL = 186 dB re 1 μPa^2s; Finneran *et al.*, 2002) and a harbor porpoise exhibited TTS after exposure to a single air gun pulse (SEL = 164 dB re 1 μPa^2s; Lucke *et al.*, 2009). The occurrence of TTS in the porpoise exposed to lower level air gun exposures is consistent with the increased susceptibility of porpoises to noise (compared to dolphins) observed in TTS studies utilizing broadband noise (Kastelein *et al*., 2011; Popov *et al.*, 2011). The differences between the watergun effects on the beluga and the air gun effects on the dolphins BLU, TYH, and OLY are a little more surprising, since they were exposed to 10 impulses and the SEL from a single impulse was close to that of the watergun.

Potential explanations why (behavioral) TTS was not observed in BLU, TYH, or OLY at any of the exposure conditions include the following:

(1) Dolphins may be relatively insensitive to impulsive noise exposures. This idea is bolstered by the lack of TTS in the dolphin exposed a watergun impulse with SEL = 188 dB re 1 μPa^2s, but contradicted by anatomical data, auditory capability data, and tonal TTS data for dolphins and belugas which show very similar results between dolphins and belugas.

(2) There was little to no accumulation of effect across the multiple exposures. The single impulse SEL values for the airgun exposures were slightly lower than those from the watergun. It is possible that the inter-impulse interval was long enough to prevent accumulation of effects for the 10 impulses.

(3) The air gun exposures were inherently less hazardous than watergun exposures with the same SEL. Although predictions based on the single impulse SEL from the watergun data suggested that TTS should have occurred, the exposure scenarios may have actually been well below those capable of inducing TTS; i.e., unweighted SEL may not the best metric to predict auditory effects from impulsive sources like waterguns and air guns.

The two primary differences between the air gun and watergun exposures were the peak pressure and the frequency content. The p-p SPL of the watergun impulse that produced TTS was 228 dB re 1 μPa, 16–29 dB higher than the maximum p-p SPLs the dolphins were exposed to in the present study. If p-p SPL was used as the main predictor of TTS, we should not be surprised that the watergun produced TTS and the air gun exposure did not. The extent to which the p-p SPL of the air gun impulse was affected by the test environment is unknown; i.e., it is not known if the same relationship between p-p SPL for an air gun and watergun impulse with the same SEL would exist in other environments.

The relatively low frequency content in the air gun impulses may also have little effect on dolphins, whose best hearing sensitivity occurs at much higher frequencies. The frequency-dependent effects of noise are typically handled by applying a “weighting function” that adjust the frequency spectrum of the noise to emphasize noise frequencies where the listener is sensitive to noise and to de-emphasize frequencies where sensitivity to noise is low. For humans, the most commonly used weighting functions are the “A-weighting” and “C-weighting” functions [American National Standard Institute (ANSI), 2006]. These functions were derived from equal loudness contours for human listeners—curves representing the combinations of SPL and frequency that are perceived as equally loud to human listeners.

Historically, equal loudness contours did not exist for animals, therefore, auditory weighting functions for marine mammals were based on auditory sensitivity curves (e.g., Nedwell et al., 2007) or known or suspected audible bandwidths (e.g., Southall et al., 2007). Recently, subjective loudness level measurements have been performed with the bottlenose dolphin TYH, and the resulting data have been used to derive equal loudness contours and auditory weighting functions (Fig. 28; Finneran and Schlundt, 2011b) that agree closely with dolphin TTS onset data obtained from tonal exposures at 3 to 56 kHz (Finneran and Schlundt, 2011a). We can use this weighting function to estimate the “effective” exposure level for the air gun impulses compared to other noise sources.

Figures 29 and 30 show representative examples of the pressure-time waveforms and frequency spectra for the watergun exposure that caused TTS in a beluga (Finneran *et al.*, 2002) and the maximum single air gun impulse received by BLU in the present study. The mean SELs for these two exposure conditions were identical: 186 dB re 1 $\mu Pa^2 s$. However, the watergun exposure had a larger peak-peak pressure, faster rise-time, shorter duration, and more high-frequency content compared to the air gun exposure. When the dolphin weighting function is applied, the weighted SELs fall to 173 dB re 1 $\mu Pa^2 s$ for the watergun impulse and 144 dB re 1 $\mu Pa^2 s$ for the air gun. So, when taking into account the frequency-dependent sensitivity of the dolphin auditory system, the air gun impulses appear much less hazardous than the watergun impulses. Of course, the equal loudness data from which the dolphin weighting function was derived do not extend below 2.5 kHz, so the weighted SELs should be treated with some caution. However, the main point is that there is a frequency-dependence to the effects of noise. Noise with frequencies within an animal's best hearing range is more hazardous than noise with frequencies where an animal does not hear well. Because the energy within the air gun impulses is concentrated at relatively low frequencies, air gun signals may not be particularly hazardous to dolphin hearing. By contrast, the watergun impulses had more high frequency content; this may explain why the watergun produced TTS while the air gun did not.

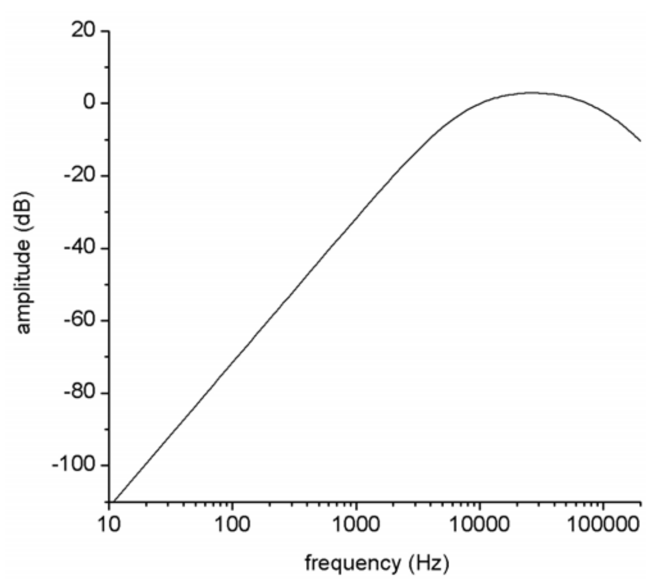

FIGURE 28. Auditory weighting function for bottlenose dolphins derived from the equal loudness contour passing through an SPL of 90 dB re 1 μPa at 10 kHz (Finneran and Schlundt, 2011b).

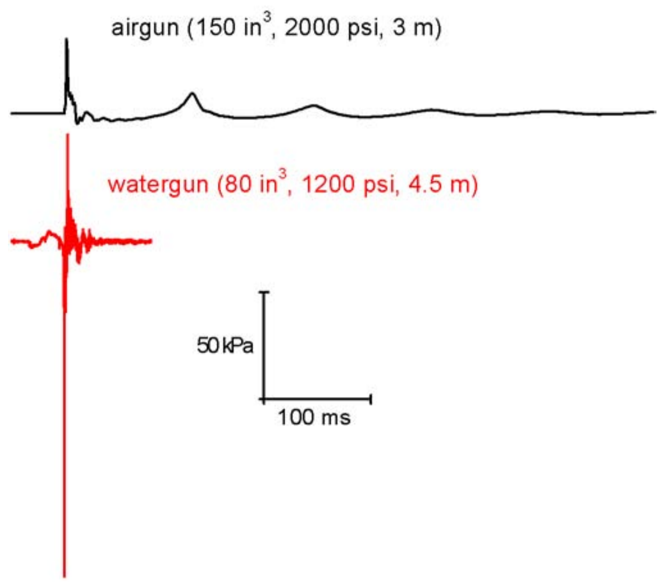

FIGURE 29. Representative pressure-time waveforms for the watergun impulsive noise exposure that caused TTS in a beluga (Finneran *et al.*, 2002) and the maximum air gun exposure for BLU from the present study.

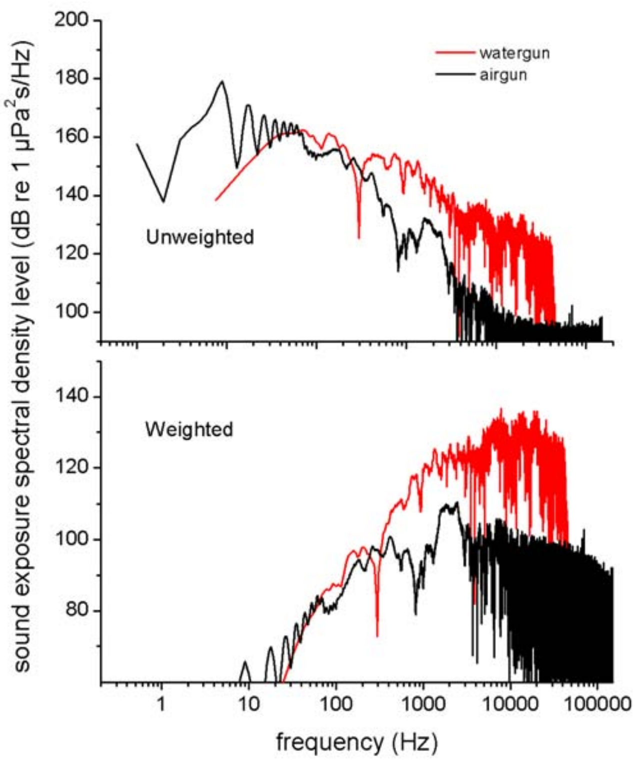

FIGURE 30. Representative spectra for the watergun impulsive noise exposure that caused TTS in a beluga (Finneran *et al.*, 2002) and the maximum air gun exposure for BLU from the present study (150 in^3, 2000 psi, 3.9 m). The sound exposure spectral density levels in the lower panel have been "weighted" by applying the dolphin weighting function based on the 90 dB re 1 μPa equal loudness contour (Finneran and Schlundt, 2011b).

Overall, the AEP measurements were inconclusive. No measurable TTS was seen in BLU or OLY. A small mean TTS was produced in TYH after the Level 7 exposures, but this could not be replicated at higher exposure levels or using tone-pip stimuli. The interpretation of these results is clouded by TYH's behavioral reactions — it is possible that the failure to reproduce the TTS seen at 8 kHz was due to TYH's self-mitigation of the noise exposure.

VIII. Conclusions

This project demonstrated that an air gun could be safely operated within San Diego Bay using visual observers and safety ranges to prevent exposure of protected species to potentially harmful levels. TTS measurements can be safely conducted with dolphins exposed to various combinations of air gun volume and pressure and at various distances, and, if the exposure levels are gradually increased, dolphins may show little reaction to the air gun impulses, even at ranges as close as 3.9 m and with the air gun operating at 150 in^3 and 2000 psi. At the highest exposure levels, two of the dolphins anticipated the exposures and adopted postures which suggested that they were attempting to mitigate the effects.

Exposures of up to 10 impulses from a 150 in^3 air gun operating at 2000 psi (cumulative SEL of 189–195 dB re 1 $\mu Pa^2 s$) did not produce clear, reliable TTS in any of the three dolphins; this may be a result of the relatively low frequency content of the air gun impulses compared to the dolphin's range of best hearing or the lower p-p SPL compared to earlier impulsive sources. The data from this study are consistent with the weighted SEL and p-p SPL criteria from Southall et al. (2007).

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