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An animal-borne active acoustic tag for minimally invasive behavioral response studies on marine mammals

Fregosi *et al.*

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An animal-borne active acoustic tag for minimally invasive behavioral response studies on marine mammals

Selene Fregosi^{1*} , Holger Klinck^{1,2}, Markus Horning³, Daniel P. Costa⁴, David Mann⁵, Kenneth Sexton⁶, Luis A. Hückstädt⁴, David K. Mellinger¹ and Brandon L. Southall^{7,8}

Abstract

Background: There is a variety of evidence that increased anthropogenic noise (e.g., shipping, explosions, sonar) has a measureable effect on marine mammal species. Observed impacts range in severity from brief interruptions of basic life functions to physiological changes, acute injury, and even death. New research tools are needed to better measure and understand the potential effects of anthropogenic noise on marine mammals. Current behavioral response studies typically utilize ship-based sound sources to study potential acute behavioral responses in tagged animals experimentally exposed to noise. Integrating the sound source within animal-mounted passive acoustic and motion-sensing tags provides a novel tool for conducting additional highly controlled response studies.

Results: We developed and conducted pilot field trials of a prototype tag on five juvenile northern elephant seals, *Mirounga angustirostris*, using experimental exposures to both natural and anthropogenic noise stimuli. Results indicate behavioral responses were elicited in tagged individuals. However, no pattern was found in the occurrence and types of response compared to stimulus type. Responses during the ascending dive phase consisted of a dive inversion, or sustained reversal from ascending to descending (8 of 9 exposures). Dive inversions following exposure were 4–11 times larger than non-exposure inversions. Exposures received during the descending dive phase resulted in increased descent rates in 9 of 10 exposures. All 8 exposures during dives in which maximum dive depth was limited by bathymetry were characterized by increased flow noise in the audio recordings following exposure, indicating increased swim speed.

Conclusions: Results of this study demonstrate the ability of an animal-mounted sound source to elicit behavioral responses in free-ranging individuals. Behavioral responses varied by seal, dive state at time of exposure, and bathymetry, but followed an overall trend of diving deeper and steeper and swimming faster. Responses did not consistently differ based on stimulus type, which may be attributable to the unique exposure context of the very close proximity of the sound source. Further technological development and focused field efforts are needed to advance and apply these tools and methods in subsequent behavioral response studies to address specific questions.

Keywords: Acoustic, Tag, Behavioral response, Northern elephant seal, Controlled exposure experiment

Background

Marine mammals rely on acoustic cues for many life functions including navigation, foraging, communication,

and predator avoidance [1]. Anthropogenic activities have contributed to increased ocean ambient noise levels in certain areas [2, 3]. The potential adverse effects of both acute and chronic human-generated noise on marine mammals are a major conservation concern [e.g., 4–14]. Naval sonar activities have been linked to cetacean stranding events, where necropsies have shown physical damage to vital organs [5, 6]. Noise has also been shown

*Correspondence: selene.fregosi@oregonstate.edu

¹ Cooperative Institute for Marine Resources Studies, Hatfield Marine Science Center, Oregon State University and NOAA Pacific Marine Environmental Laboratory, 2030 SE Marine Science Drive, Newport, OR 97365, USA

Full list of author information is available at the end of the article

to increase stress hormone levels [9], reduce foraging activity [8, 15], alter migration routes [16], mask communication sounds [10], and displace marine mammals from primary feeding and breeding grounds [11, 12, 15]. Understanding the differential responses of marine mammals to noise stimuli is necessary for managing human impacts in the ocean [17, 18].

Behavioral response studies (BRSs) using a controlled exposure experiment (CEE) paradigm have emerged as an effective tool to study discrete behavioral responses of individual, free-ranging marine mammals to particular sounds [19, 20]. These studies typically use animal-mounted archival motion-sensing (via pressure sensors and triaxial accelerometers and magnetometers) and acoustic recording tags as well as visual observations to measure individual focal animal response to a controlled exposure from a sound source deployed nearby. Measurements of swim speed, dive depth, dive duration, heading, vocal behavior, group spacing, ascent and descent rate, and other metrics are made before, during, and after the CEE in order to assess response [e.g., 21–25].

Combining the sound source with behavioral sensors and an acoustic recorder into a single animal-mounted tag could enable an alternative BRS methodological approach. Such an instrument could offer a more cost-effective tool that would allow for better control of exposure sound levels. While the exposure context is unique in being physically attached to the animal, this context is consistent for all exposures whereas other BRS approaches often have variability in the context of different exposures. Finally, it could enable investigation of the effects of varied sound levels, multiple or sustained exposures, and behavioral habituation.

The northern elephant seal, *Mirounga angustirostris* [26], presents an ideal study species to test the effectiveness of such a tag. Northern elephant seals are accessible as they haul out twice a year to breed and molt [27, 28]. Elephant seal diving behavior is well studied and found to be highly stereotypic, with almost continuous, repetitive deep diving [28–31]. They regularly reach depths similar to many of the cetacean species thought to be most affected by anthropogenic noise (i.e., beaked whales; [28, 32]) and dive as deep as or deeper than the deep sound channel (~1000 m). Previous studies have shown that carrying relatively large instruments does not inhibit the ability of juvenile seals to swim or forage, and instrument recovery rate is above 90 % [28, 33, 34]. Additionally, northern elephant seals have acute underwater hearing sensitivity with relatively low hearing thresholds occurring over a very broad frequency range [35, 36]. High-resolution behavioral responses to sound stimuli from our device can be collected for elephant seals using sensors

that measure changes in pressure, acceleration, strength of magnetic field, and turn rate [13, 37].

Here we describe a pilot study testing an animal-mounted active acoustic and motion-sensing tag on free-ranging juvenile northern elephant seals. The main goals of this study were (1) to test whether an animal-borne acoustic tag elicits any behavioral response and if so (2) to determine whether responses are related to stimulus content. Development and application of an active acoustic tag in a behavioral response paradigm could answer targeted questions regarding the nature and magnitude of responses of marine mammals to anthropogenic noise and their potential physiological consequences, leading to better informed evaluation of human-generated sound.

Methods

Tag development

The prototype tag contained three subsystems: an active acoustic playback system, a passive acoustic recording system, and a motion monitoring system (Fig. 1). An OpenTag single board controller (SBC, Loggerhead Instruments, Inc, Sarasota, FL, USA) controlled playbacks and contained a three-axis accelerometer, magnetometer, and gyroscope. It was programmed (Loggerhead Instruments, Arduino programming language) to execute a playback of a single stimulus at a predetermined time interval, using sound files stored on a SOMO playback module (4D Systems, Minchinbury, NSW, Australia), through an amplifier board (LM48580 Evaluation Board, Texas Instruments, Dallas, TX, USA) and two or three piezo electric ceramic cylinder transducers (Steiner & Martin, Inc, Doral, FL, USA; 26 mm external diameter × 22 internal diameter × 13 mm high, resonant frequency 43 ± 1.5 kHz). The playback system was powered by a 3.7 V, 3000 mAh lithium-ion polymer rechargeable battery (Tenergy, Fremont, CA, USA).

Six playback stimuli were used: sperm whale (*Physeter macrocephalus*) clicks, common dolphin (*Delphinus* sp.) whistles, killer whale (*Orcinus orca*) whistles and clicks, simulated mid-frequency active sonar, and white noise. White noise and the simulated mid-frequency sonar were created using Adobe Audition 3 (Adobe System Incorporated, San Jose, CA, USA). Sperm whale clicks and common dolphin whistles were obtained from the Discovery of Sound in the Sea sound library.¹ Both killer whale vocalizations were obtained from the Vancouver Aquarium.² An inverse of the transducer's transmitting sensitivity function was generated and used to normalize each playback's output over the entire frequency bandwidth.

¹ <http://www.dosits.org/audio/marinemammals/toothedwhales/>.

² <http://killerwhale.vanaqua.org/page.aspx?pid=1331>.

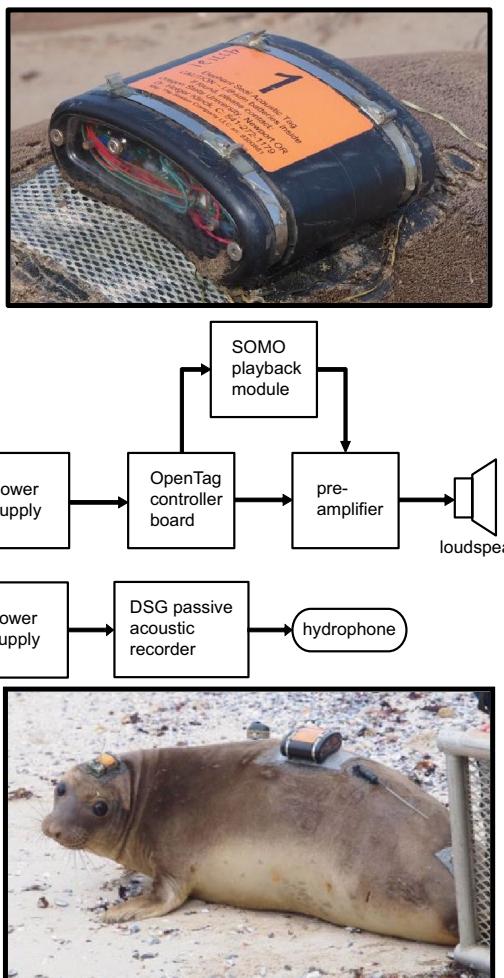


Fig. 1 Prototype tag was built, calibrated, and field-tested on northern elephant seals (center black and orange tag). Tag components included a DSG passive acoustic recorder and hydrophone, an active acoustic preamplifier, playback module, transducers, and OpenTag single board controller with a nine-axis motion sensor (three axes each for accelerometer, magnetometer, and gyroscope). A Wildlife Computers Mk10-AF TDR was mounted on the head. An additional TDR was attached to the shoulder but was not used in this study

Playbacks of stimuli were limited to 30 s to reduce the possibility of seals associating the stimulus with the device mounted on their back. Playback track source levels were measured using a calibrated hydrophone system (G.R.A.S. 42AC High Pressure Pistonphone, G.R.A.S. Sound & Vibration A/S, Holte, Denmark) and ranged from 121 to 132 dB re 1 μPa at 1 m, depending on exposure type (Table 1). Estimated received levels were calculated assuming spherical spreading, based on the distance of tag from the animals' ears.

A DSG acoustic recorder (Loggerhead Instruments, Inc, Sarasota, FL, USA) connected to an HTI-96-MIN

Table 1 Calibrated source levels for exposure stimuli

Stimulus	Source level
White noise	125.56 (0.94) @ 1 m (dB_{RMS} re 1 μPa)
Common dolphin whistles	130.66 (1.48) @ 1 m (dB_{RMS} re 1 μPa)
Sperm whale clicks ^a	131.77 (1.89) @ 1 m ($\text{dB}_{0\text{-peak}}$ re 1 μPa)
Killer whale whistles	121.06 (4.60) @ 1 m (dB_{RMS} re 1 μPa)
Killer whale clicks ^b	Estimated 126 @ 1 m ($\text{dB}_{0\text{-peak}}$ re 1 μPa)
Sonar	124.27 (0.58) @ 1 m (dB_{RMS} re 1 μPa)

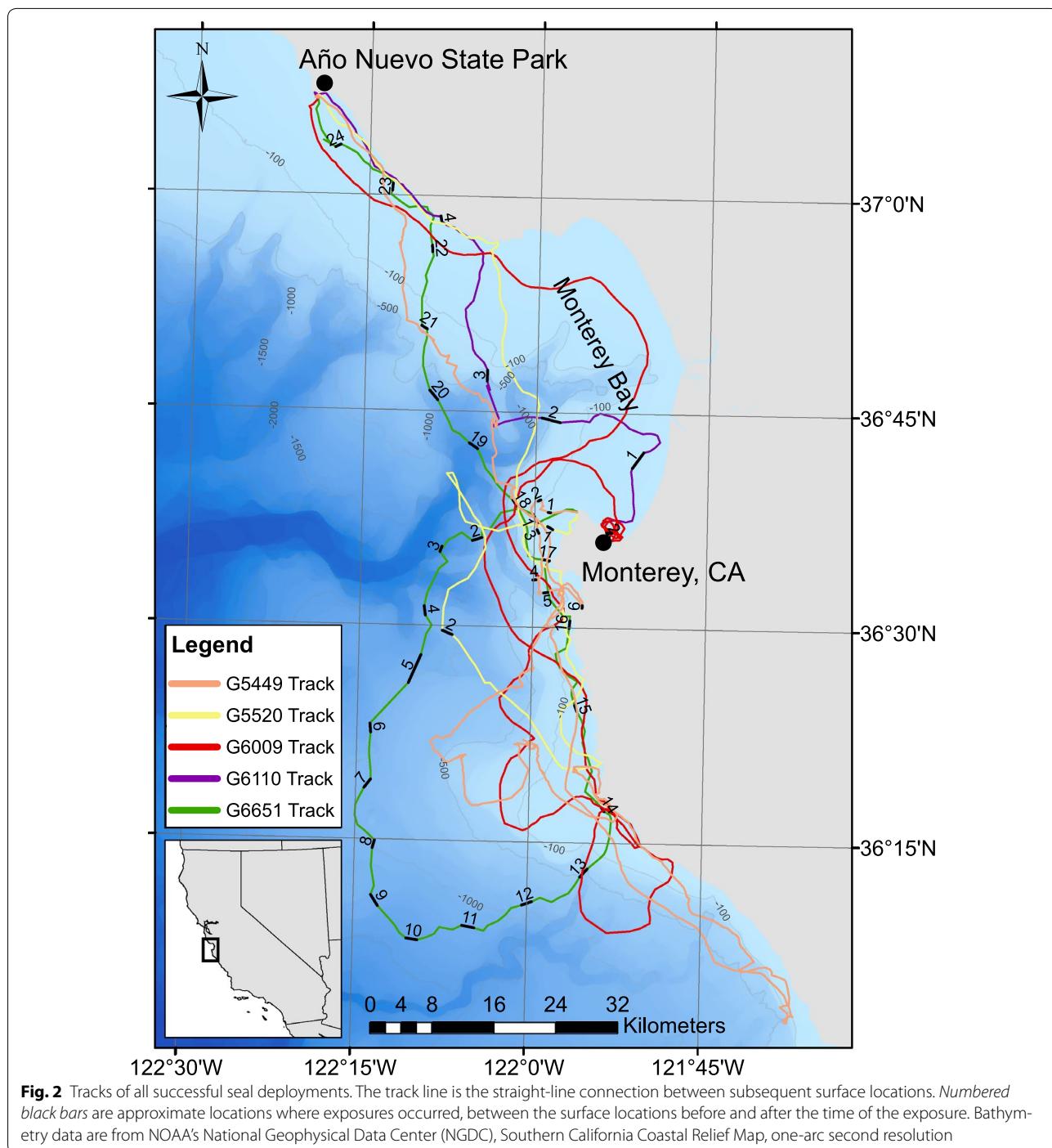
Mean and standard deviation of two calibrated recordings are given. All sources levels are given in dB_{RMS} re 1 μPa @ 1 m over the stimulus duration, except for the sperm whale click track^a, which was measured in $\text{dB}_{0\text{-peak}}$ re 1 μPa @ 1 m and the killer whale click track^b which was measured by relative comparison to killer whale whistles

hydrophone (High Tech, Inc, Long Beach, MS, USA; sensitivity -190.5 dB re 1 V μPa^{-1} ; frequency response 2 Hz–30 kHz) recorded for 30 min starting 15 min prior to each scheduled playback. This provided a recorded confirmation that a single playback occurred and allowed detection of other environmental noise events occurring at the time of the playback. Recordings were sampled at 32 kHz (16 bit resolution) with 20 dB of gain. The passive acoustic system was powered by a second 3.7 V, 3000 mAh lithium-ion polymer battery (Tenergy, Fremont, CA, USA).

All tag components were placed in an oil-filled Delrin® housing, rated to 1500 m depth. Clear, food-grade mineral oil was used as a non-compressible and non-conductive filler so no air remained in the housing and to limit acoustic impedance between the tag and seawater. The tag package weighed approximately 1.5 kg (<1.2 % total body mass of smallest seal) and was slightly negatively buoyant. The original tag design was modified following the first three deployments. Changes in pressure and temperature between the surface and at depth caused changes in mineral oil density. The slight flexibility of Delrin® caused a pumping action to develop that moved seawater past the o-ring seals into the oil-filled tag body. Larger o-rings were used, and flexible tubing with small air pockets was attached to the outside of the housing to allow minor changes in mineral oil density and limit seawater intrusion.

Field efforts

We conducted field experiments in March and April 2012 on juvenile northern elephant seals at Año Nuevo State Park, San Mateo County, CA, USA (Fig. 2; Table 2). Seals were instrumented with the active acoustic tag placed on the animal's back (30–60 cm from the ears) and an ARGOS-Fastloc GPS time-depth recorder (TDR) on the head (Mk10-AF, Wildlife Computers, Redmond, WA, USA). Tags were attached by gluing a flexible mesh



base to the animal's fur using quick-cure adhesive (Devcon 14270—5 Minute® Epoxy, ITW Polymers Adhesives North America, Danvers, MA, USA), weaving stainless steel locking ties through the mesh, and securing the tag with the locking ties (similar to [30, 38]). Animals were transported approximately 100 km south to Monterey,

CA, USA, and released. Seals were recaptured upon return to Año Nuevo, and tags (including the metal ties) were manually removed from the flexible mesh base. Seals molted off the base shortly after tag recovery. Depth was sampled every 1 s for seal G5449 and every 4 s for all other seals. Resolution of TDRs were 0.5 m and accuracy

Table 2 Summary dive statistics for all study individuals

Seal	Age	Sex	Body mass (kg)	Deployment duration (h)	Number of dives recorded	Number of dives deeper than 100 m (% of total)	Mean dive depth (m)		Mean dive duration (s)		Median dive duration (s)
							All dives	Dives deeper than 100 m	All dives	Dives deeper than 100 m	
G5449	1 ^a	M	134	207.22	972	509 (52.4 %)	136.618 (\pm 106.0357)	217.114 (\pm 84.7149)	646.6337 (\pm 267.9752)	829.34 (\pm 191.822)	637
G5520	1	F	167	63.11	402	20 (5.0 %)	366.157 (\pm 53.6568)	223.425 (\pm 116.878)	365.34.33 (\pm 251.5771)	958.0 (\pm 398.2795)	340
G6009	1	F	182	121.26	518	124 (23.9 %)	94.9913 (\pm 128.4333)	290.395 (\pm 129.899)	608.7259 (\pm 436.05)	1185.8 (\pm 324.3298)	556
G6110	2 ^a	F	262	30.24	148	28 (18.9 %)	75.0811 (\pm 108.5156)	258.375 (\pm 165.32)	585.4324 (\pm 379.2734)	1146.3 (\pm 279.5604)	526
G6651	1 ^a	F	206	73.01	321	115 (35.8 %)	145.829 (\pm 167.2949)	339.274 (\pm 136.802)	748.2991 (\pm 580.3701)	1419.1 (\pm 364.688)	1488

Age was either known from initial tagging as a pup or estimated^a in the field based on standard length and weight. Sex was assessed and body mass was measured before instrumentation. Deployment duration was dependent on the time it took the seal to return to the beach. Number of dives recorded includes all instances where the animal dove deeper than 4 m. Mean (and SD) dive duration, in min, are given for all dives and the subset of dives deeper than 100 m

± 1 m. The accelerometer, magnetometer, and gyroscope were all sampled at 50 Hz.

Playbacks occurred every 6 h for the first two seals and every 3 h for the remaining seals. Each playback consisted of one exposure to a single stimulus. The order of stimuli presentation varied between seals, but remained the same within an individual seal, unless the tag reset itself, at which point it started back at the beginning of the sequence.

Behavioral analysis

Passive recordings were screened for successful exposures (Table 3), and timing of exposures across multiple tag systems was synced. Depth data from the TDRs were examined for possible seal responses to exposures (Fig. 3). To account for zero offset drift, dive data were analyzed using the IKNOS toolbox (Y. Tremblay, unpublished) in MATLAB (MathWorks, Inc, Natick, MA, USA). Dives were defined as any instance where the individual dove deeper than 4 m. This threshold was chosen to be greater than the sum of the accuracy of the TDR (± 1 m) and the body length of an individual.

We hypothesized that responses would differ according to dive type and state, so different dives and exposures were analyzed separately. Dive shapes were used to identify different dive types (e.g., transit vs. foraging [39]). Dives were grouped into deep (>100 m) and shallow (≤ 100 m) dives (Table 3). Exposures were categorized by dive phase (descent, ascent, bottom phase, and surface; Table 3). Descents were defined as the segment from 0 m to the point at which the seal descended at a rate <0.1 m s $^{-1}$ for 20 s. The beginning of an ascent was defined as the point at which an individual ascended for more than 4 m over 20 s (an ascent rate of 0.2 m s $^{-1}$). Bottom phases were defined as the segment between the descending and ascending segments. Exposures that occurred at depths shallower than 20 m were labeled as “surface” and excluded from all analyses due to increased

ambient noise levels, and thus a decreased signal-to-noise ratio of the low-level stimulus, near the water’s surface.

To determine potential responses during ascent or bottom phases of deep (>100 m) dives, dive inversions (Fig. 4) were measured and compared. Dive inversions were defined as a change from ascending or horizontal swimming to descending, with a change in depth >3 m over 4 s. Change in depth and change in time from the inversion point to the next maximum depth were measured and normalized by maximum depth and total dive duration, respectively. In order to statistically compare dive inversions following exposures to dive inversions outside of exposure events, an Anderson–Darling k-sample (ADK; [40]) test was run on seal G6651, the only seal to receive multiple exposures during the ascending phase. The ADK test was selected because it is distribution free and does not rely on equal sample sizes; however, it requires that samples are taken from independent distributions, in this case, different seals. G5449 was chosen for comparison as it had the largest deployment duration and therefore the most non-exposure dive inversions. To first test whether different seals could be directly compared, an ADK test was used to compare non-exposure dive inversions for seals G6651 and G5449. The magnitude of the dive inversion following the single playback during the bottom phase of a deep dive was measured and reported.

Descent rates for descending phases of deep dives were measured and compared to identify possible responses during descents. Average descent rate over 60 s before and 60 s after exposure onset were calculated. A time window of 60 s was selected in order to differentiate sustained changes in descent rate from brief startle responses during the playback only. Because an increase in descent rate is considered a subtle response that likely happens regularly in non-exposure dives, statistical analysis of changes in descent rate was not performed.

Table 3 Deployment duration and number of exposures for the successful deployments of the prototype tag

Seal	Exposure interval (h)	Number of exposures received	Exposures by dive stage					
			Descent		Bottom phase		Ascent	
			Deep	Shallow	Deep	Shallow	Deep	Shallow
G5449	3	6	2	–	–	1	1	–
G5520	3	2	–	–	–	1	–	1
G6009	6	2	–	–	–	–	–	2
G6110	6	4	1	–	–	–	1	–
G6651	3	24	7	1	1	3	7	1
								4

The number of exposures was dependent on the success of the tag and the length of the deployment. Exposures during descent, bottom phase, and ascent are the subset of exposures used for analysis in each respective phase, split by shallow (<100 m) and deep (>100 m) exposures. Surface exposures were excluded from analysis

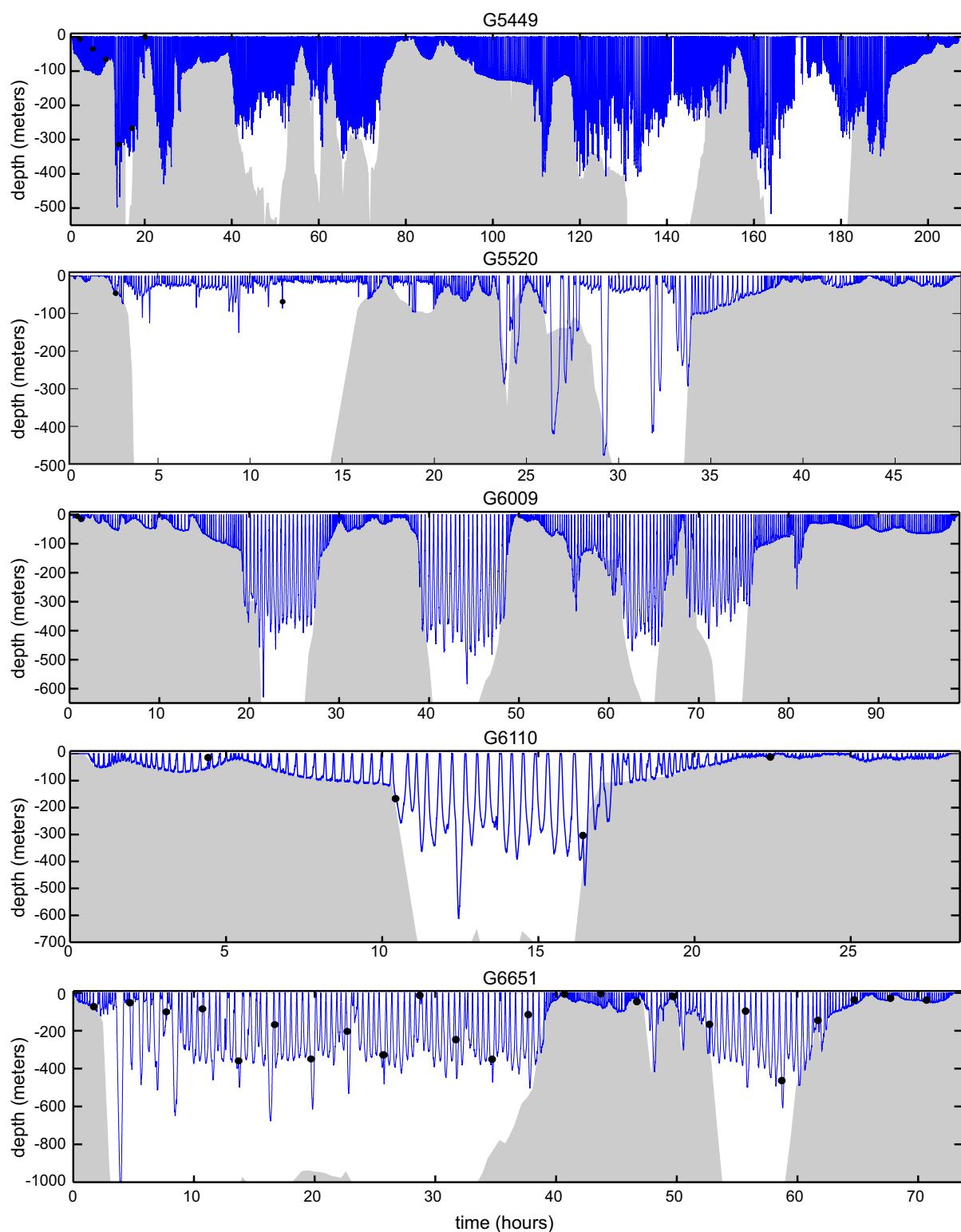
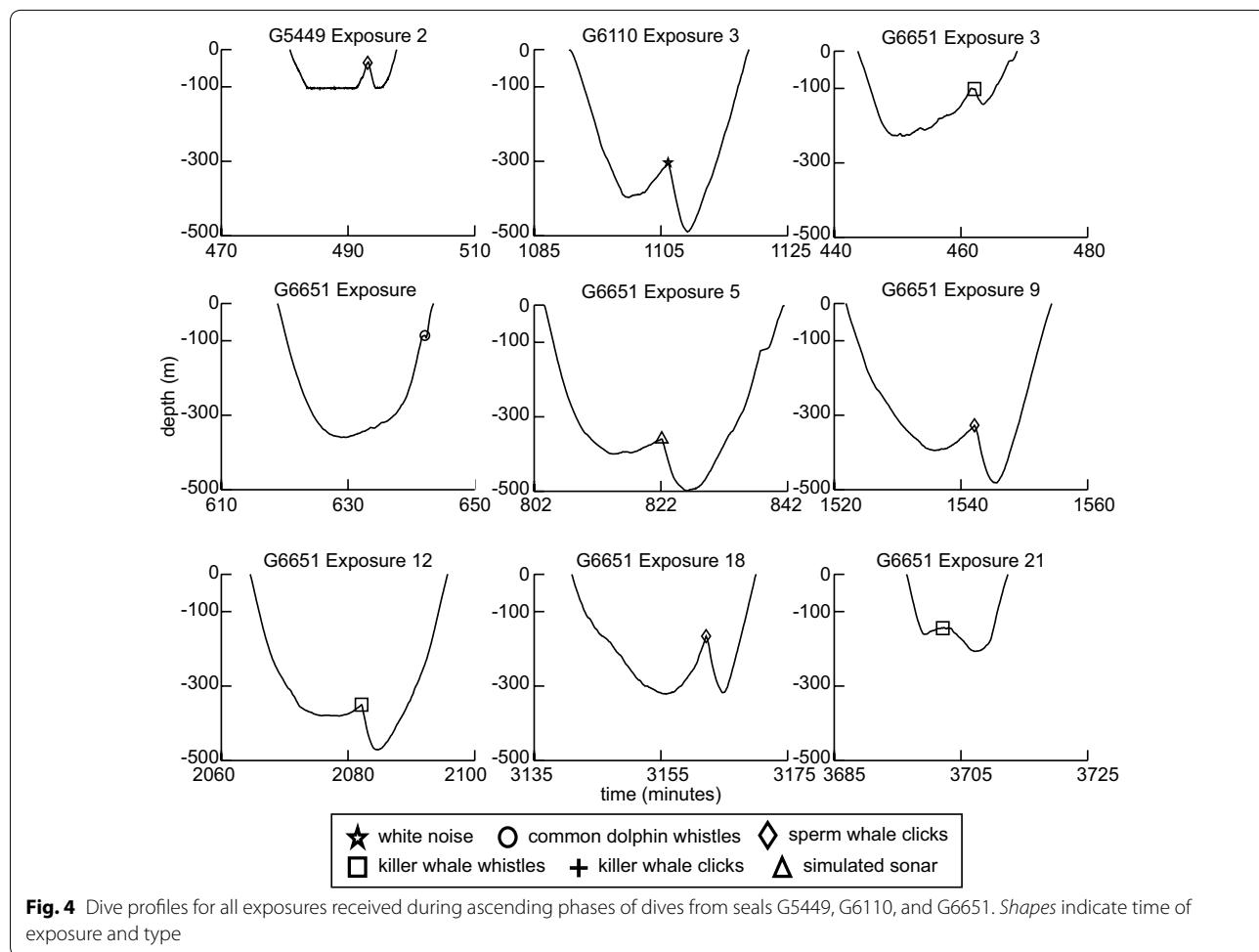


Fig. 3 Depth profiles and seafloor bathymetry for complete deployments for all seals. Seal dive profile is in blue, seafloor bathymetry is in gray, and exposures are indicated by black dots. Bathymetry data are from NOAA's NGDC, Southern California Coastal Relief Map, one-arc second resolution. Instances where a dive profile overlaps the seafloor are an artifact of data resolution mismatch; bathymetry data were extracted only for known GPS surface positions and linearly connected in the gray bathymetry plot, leading to areas with reduced detail



Shallow dives occurred along the shelf edge and were limited by the seafloor so diving deeper or reversing an ascent was not possible. To identify possible responses for these shallow dives, changes in flow noise on the passive acoustic recorder (a proxy for changes in swim speed) were measured and compared. Previous studies have found an 18- to 20-dB increase at very low frequencies (8–18 Hz) corresponds to a doubling of current flow, regardless of tag or hydrophone design [34, 41]. Average RMS in the 8–18 Hz frequency band was measured for 30–60 and 0–30 s before exposure and 0–30 and 30–60 s after the exposure ended (Adobe Audition CC, Adobe Systems Incorporated, San Jose, CA, USA). The before and after periods were divided to detect shorter-term changes in swim speed, but the entire 60-s period was taken into account when assessing a response.

The normalized change in depth for exposure and non-exposure dive inversions for seal G6651 was plotted against time to investigate possible habituation to multiple playbacks over the duration of the deployment. To investigate differential response of all seals to

different stimuli, each playback was categorized as either “response” or “no response,” using a minimum level of response based on percent change, which varied for each dive state (ascending, bottom, descending, or shallow/bottom-limited). Ascending or bottom-phase playbacks were categorized as “response” if there was a dive inversion magnitude >2 SDs from non-playback inversions. Descending playbacks were considered a response if descent rate increased by $>50\%$ following exposure, and for shallow dives, an increase in flow noise of $>10\%$ was considered a response. Percent of overall response was calculated.

Results

Tag development

Seven deployments of the prototype tag resulted in successful CEEs for five individuals (Table 2). Received levels at the individuals’ ears, estimated from the source level and assuming spherical spreading, ranged from 128 to 138 dB re 1 μ Pa, depending on seal and stimulus type. Mechanical (flooding) or electrical (tag control board

reset) tag failure rendered the remaining two unsuccessful. The playback control of the tag would occasionally experience a reset for undetermined reasons, resulting in a stimulus that was played out of sequence. In some cases, this reset caused the tag to stop working entirely.

Deployments and basic dive behavior

Five successful deployments ranged from 30 to 207 h in duration. Animals received 2–24 playbacks, each a single 30-s exposure, at an interval of 3 or 6 h, and non-exposure dives served as control dives (Table 3; Fig. 2). Four of five animals dove along the seafloor (depths up to ~100 m) until reaching the continental shelf edge, after which they exhibited deeper, pelagic transit dives with mean depths of 217–340 m over the deep water of Monterey Canyon. They returned to the shallow dive pattern when returning to shallow coastal waters near Año Nuevo State Park (Fig. 3). We found no evidence of foraging or drift dives, and the observed dive patterns were similar to those of other translocated juveniles [13, 42]. Dive depths <100 m were likely limited by the seafloor, while deeper dives were not bottom-limited, which may have an effect on responses measured (Fig. 3) [43]. One individual (G5520) rarely dove deeper than 100 m (5.0 % of all dives), even over deep water (Fig. 3). We found no significant difference in magnitude of non-exposure dive inversions for seals G5449 and G6651 (ADK, $p = 0.14$, $n = 283$ non-exposure inversions, seal G5449 and $n = 30$ non-exposure inversions, seal G6651). Both exposures to seal G6009 were received at <10 m depth, so they were excluded from further analyses. After this exclusion, there were 28 usable exposures on four seals, made up of 9 ascending, 10 descending, 1 bottom phase, and 8 shallow water exposures.

Controlled exposure experiment

We observed dive inversions following all playbacks that occurred during the ascending portion of a deep dive. Individuals descended an additional 116 m ($SD \pm 51.6$ m) on average (Fig. 4). 8 of 9 exposures resulted in changes in depth >2 SDs from the mean of all non-exposure inversions (Figs. 4, 5; Table 4). Seal G6651 was the only seal which received multiple exposures during ascent ($n = 7$ exposures), and the magnitudes of dive inversions following these exposures were statistically different than non-exposure inversions (Fig. 5; Table 4; ADK test, $p < 0.001$, $n = 283$ non-exposure inversions, seal G5449 and $n = 7$ exposure inversions, seal G6651). We observed this dive inversion response for white noise, sperm whale clicks, killer whale whistles, and simulated mid-frequency sonar exposure, but not following an exposure to common dolphins, received by seal G6651 (Fig. 4, G6651 Exposure 4). Seal G6651 was the only seal that received an exposure

during the bottom phase of a deep dive (351 m). She dove to 614 m immediately following exposure, a 75 % increase in maximum depth of dive (killer whale whistle exposure, Table 3).

Three seals received exposures during the descending phase of a deep dive, and two of three (G5549 and G6651) exhibited an increased descent rate (35.9–271.9 %; mean 125.2 %; ± 95.8 %) following exposure (Fig. 6; 9 of 10 total deep descending exposures). Seal G6110 had a lower descent rate following exposure to sonar (72.7 % decrease).

Flow noise (swim speed) increased by 10.0 (± 8.0) dB re 1 μ Pa from 0 to 30 s before exposure to 30–60 s after exposure (30.3 ± 14.7 % increase) in all 8 exposures (to three seals) that occurred in shallow water (<100 m). For two of the exposures, flow noise initially decreased in the 0–30 s immediately after exposure but by 60 s after, reached above pre-exposure levels.

Dive inversion responses seal G6651 were as pronounced as or more pronounced than those later in the deployment compared to earlier (Fig. 7). Seals responded to 23 of 28 total exposures (82 %), regardless of stimulus type, indicating no differential response.

Discussion

We identify behavioral responses in juvenile northern elephant seals elicited by an animal-mounted sound source. This is the first dedicated BRS conducted on any marine mammal using an animal-borne sound source (see [44] for incidental BRS with an active sonar tag). This approach has the potential to expand and complement current CEE methodologies. Such data will provide enhanced understanding of longer-term effects of anthropogenic noise.

Tag performance

Deployments that resulted in unsuccessful or reduced duration CEEs were due to issues with either the SBC or the housing. We were unable to resolve the cause of the SBC reset, but believe it was caused by the formation of a ground loop due to differential ground potentials of the independent hardware components comprising the single tag system. Although the housing was rated to 1500 m depth, either changes in temperature and pressure with depth or incorrectly sized o-rings likely caused failure of the slightly flexible Delrin® housing, allowing seawater to enter the housing and short circuit the electronics. Future tags would benefit greatly from being potted in solid resin; however, the non-permanent setup used in this trial allowed us to monitor and modify physical tag components and programming as needed during the field effort. Additionally, we found no evidence of instrumentation or drag affecting normal swimming behavior, as

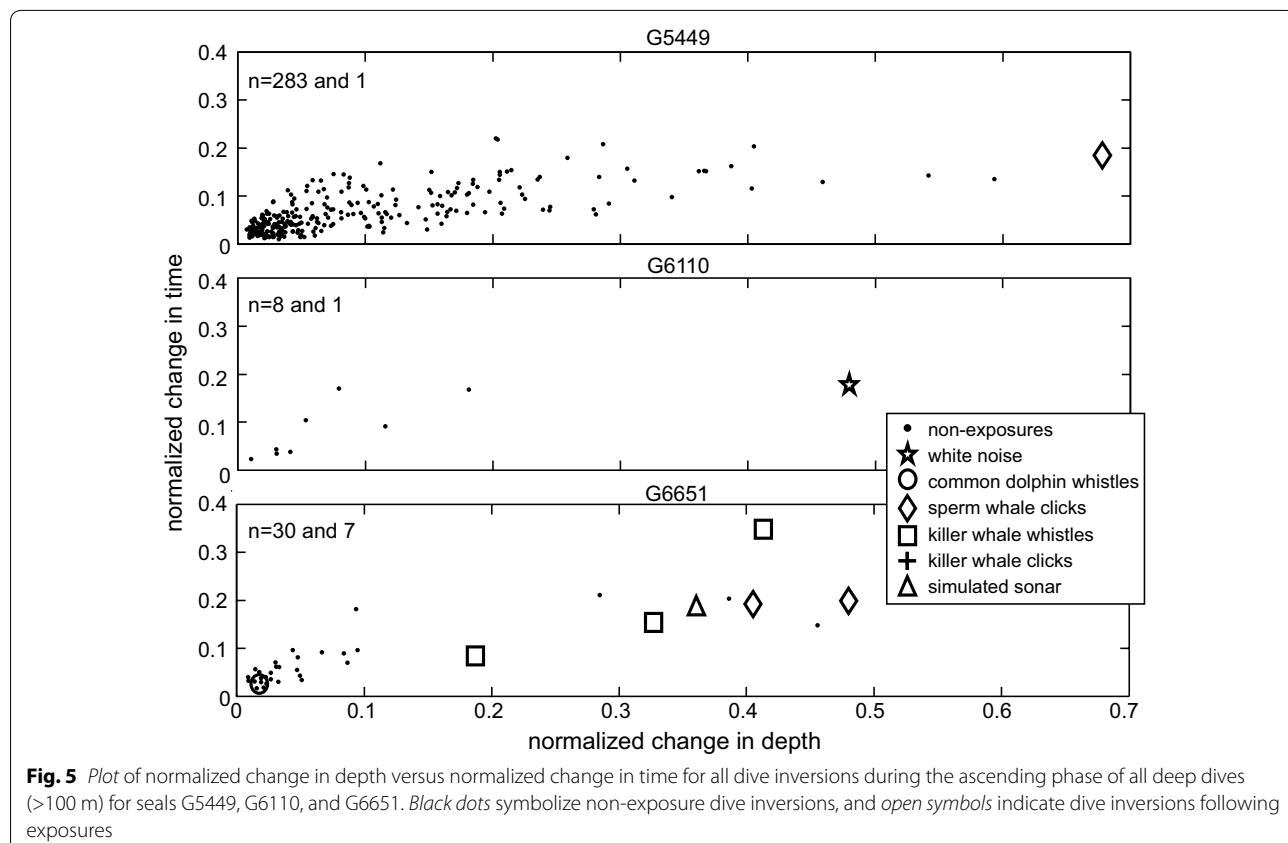


Table 4 Mean change in depth for non-exposure and exposure dive inversions

Seal	Mean change in depth for non-exposure inversions (m)	n	Mean change in depth for exposure inversions (m)	n	Anderson–Darling k-sample test
G5449	14.875 (± 13.997)	n = 283	70.5	n = 1	n/a
G6110	16.749 (± 16.293)	n = 8	186	n = 1	n/a
G6651	24.867 (± 39.981)	n = 30	96.36 (± 59.23)	n = 7	$p = <0.001$

Dive inversions are defined by a change from ascending to descending >3 m over 4 s, for all dives deeper than 100 m. Standard deviation is given in parentheses, and n is given for each. Results from an Anderson–Darling k-sample test comparing the change in depth of an inversion from ascending to descending, normalized by maximum depth of dive, for exposure inversions of seal G6651 to non-exposure inversions from seal G5449 ($n = 283$) are given

dive profiles were similar to those of other translocated seals carrying smaller devices [13, 42, 45].

Animal response to disturbance

Behavioral responses measured during CEEs varied with dive state at the time of exposure. Most responses involved seals diving deeper and/or longer after exposure. The clearest responses (Fig. 4; Table 4) involved dive inversions (reversal of an ascent) followed by the seal diving deeper than the initial maximum dive depth. These extended dive inversions served as strong indicators of response. Quantitatively, dive inversions resulted in greater depths and longer dive durations, when compared

to smaller-scale dive variations seen naturally in these and other translocated seals carrying a depth sensor [13, 46]. The exposure context here is unique in terms of the extreme proximity of the sound source to the animal, and the extrapolation of results to longer-range exposures should thus be considered cautiously. However, this context is more constant than in previous studies providing a stronger basis for the comparison of responses within an individual across exposure conditions.

Responses observed are similar to hypothesized elephant seal behavior to escape predators by diving deeper [37], and responses to anthropogenic sound sources are not unexpected [13, 44]. Responses elicited during CEEs

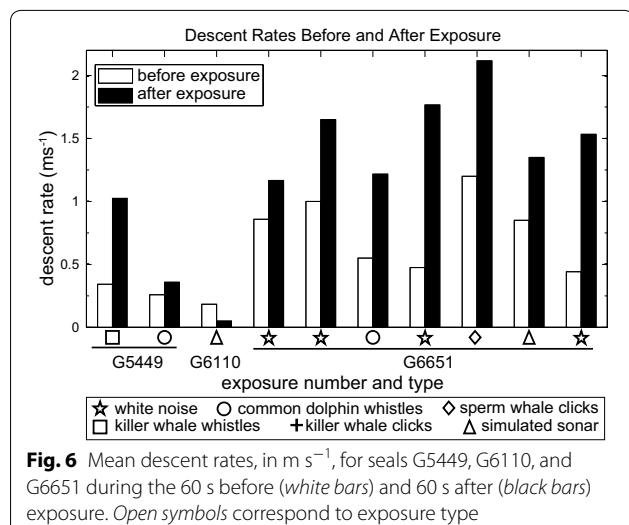
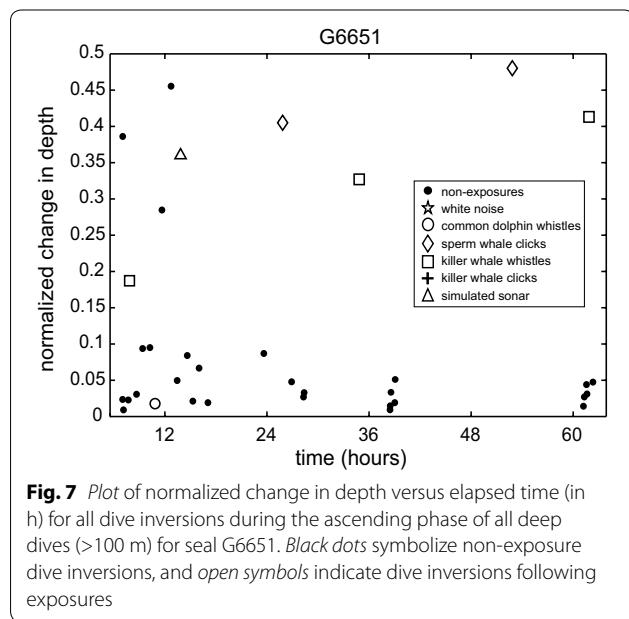


Fig. 6 Mean descent rates, in m s^{-1} , for seals G5449, G6110, and G6651 during the 60 s before (white bars) and 60 s after (black bars) exposure. Open symbols correspond to exposure type



is perceived to be coming from. Future testing using playback stimuli that are filtered to simulate different source locations could examine the effects of reverberation and localization in detail. Comparable parallel CEEs would need to be conducted on pinnipeds, using an external sound source that closely approximate actual signals, to fully evaluate the potential of this tag for BRS.

While individual differences could also contribute to different responses, no single seal received multiple exposures of all exposure types because of tag failures, which precludes further examination of this possibility. BRS exposing animals to multiple exposures of all stimuli are needed to investigate individual differences to stimuli. A differential response to dolphin whistles was recorded for seal G6651, the only seal exposed to two playbacks of dolphin whistles while ascending from a dive. No extreme dive inversions were recorded for dolphin whistles, while other stimuli resulted in extreme evasive behavior. This anecdotal example provides evidence for the possibility of differential response to different stimuli.

Repeated exposures to various stimuli may cause habituation over time [51]. Although there was no evidence of habituation over short-term deployments (<3 days), we cannot rule out the possibility of a sensitization response. The consistent response to most exposures independent of stimulus type and the apparent lack of reduction in response within individuals suggest either that seals are generally sensitive to a relatively wide range of audible exposures or possibly that seals became sensitized to subsequent exposure. Preliminary evidence from captive elephant seals suggests some sensitization, as opposed to habituation, upon repeated exposure to certain acoustic stimuli [52]. However, the sound source placement, context, and limited number of exposures of each stimulus type to each individual limited our ability to test for potential sensitization.

The tag and method may be useful in physiological studies of dive limits and studies of hearing ranges. The tag could be used as a mobile, programmable sound source to study frequency-dependent hearing in marine mammal species that cannot be studied using captive psychophysical methods. Little is known about hearing in many marine mammals because laboratory studies of hearing are not feasible due to constraints with keeping very large, migratory, deep-diving, and social species in captivity, but using controlled, incrementally increasing exposure levels of tonal sounds that trigger drastic changes in behavior could investigate differences in hearing sensitivities in previously inaccessible species.

Additionally, this tag could be used to study the physiological effects of unanticipated extensions of dives, another important research need for understanding the effects of noise on marine mammals [18]. We are

beginning to better understand how animals behaviorally respond to sound, but we still do not fully understand the underlying physiological changes that are linked to these behavioral changes (e.g., oxidative stress). Physiological parameters such as oxygen utilization, heart rate, and post-dive recovery times can be monitored in free-ranging animals [53, 54], and with this tag, dives can be experimentally extended, allowing quantitative investigation of physiological responses. To support the potential use of this tag to study physiological effects of extended dives possibly related to responses to anthropogenic noise, it is promising that the responses were similar to other deep-diving species of interest. For species that are specialized for oxygen efficiency during prolonged, deep dives, physiological responses to changes in planned dive duration could help physiologists better understand these adaptations [55].

Conclusions

This pilot study was the first of its kind to investigate the potential use of an animal-borne sound source to conduct BRS on marine mammals to further investigate the effects of anthropogenic noise. Five juvenile northern elephant seals were instrumented and translocated south of their colony at Año Nuevo State Park. They received playbacks of multiple stimulus types (both man-made and natural) from the tag while diving continuously on their return to the colony. Dive behavior before and after exposures were compared to assess whether animal-borne tags holding sound sources were capable of eliciting a response from a free-ranging animal and whether potential responses differed with stimulus content.

Projecting sound from a tag mounted to the back of a free-ranging juvenile northern elephant seal did elicit behavioral responses; however, the responses were not consistently different with different stimulus types. Responses varied by seal, by dive state, and by the bathymetry where the exposure occurred, but in general, seals dove deeper following playback. Animals responded to 82 % of exposures overall, with no clear evidence of a reduction in response to repeated exposures of various stimuli. The unique and biologically unrealistic context of a sudden sound exposure coming from directly behind the animal is likely the greatest limiting factor preventing use of this tag in examining differential responses to particular sounds and relating them to responses that may occur over more realistic source-animal ranges. Making conclusions about the effects of particular sounds on juvenile northern elephant seals was beyond the scope of this pilot study, but additional tests using this technology may help develop additional questions and hypotheses in the future.

Tag improvements and additional field testing could strengthen results found here, but the concept of an animal-borne sound source for triggering behavioral responses has been validated. The use of this novel animal-borne sound source in future studies could help increase the ability to measure and evaluate the type and probability of response for various sound exposures within a constant context, connecting vital rates to long-term health effects, evaluating potential habituation or sensitization from repeated exposures, and even evaluating basic aspects of hearing (e.g., frequency ranges) for marine mammals previously unstudied. Each of these would represent important steps in better understanding the effects of anthropogenic noise on marine mammals.

Abbreviations

ADK: Anderson–Darling k-sample; BRS: behavioral response study; CEE: controlled exposure experiment; ESA: Endangered Species Act; MMPA: Marine Mammal Protection Act; NGDC: National Geophysical Data Center; NMFS: National Marine Fisheries Service; NOAA: National Oceanic and Atmospheric Association; NRC: National Research Council; SBC: single board controller; TDR: time-depth recorder.

Authors' contributions

SF and HK were involved in securing funding, tag development, field work, analysis, and drafting the manuscript. MH provided field, analysis, and writing support. DPC provided field assistance and permitting. DM provided tag development and analysis support. KS participated in tag development and field efforts. LAH provided field and data analysis support. DKM helped secure funding and participated in tag development. BLS provided analysis, theory, and writing support. All authors read, edited, and approved the final manuscript.

Author details

¹ Cooperative Institute for Marine Resources Studies, Hatfield Marine Science Center, Oregon State University and NOAA Pacific Marine Environmental Laboratory, 2030 SE Marine Science Drive, Newport, OR 97365, USA. ² Bioacoustics Research Program, Cornell Laboratory of Ornithology, Cornell University, 159 Sapsucker Woods Road, Ithaca, NY 14850, USA. ³ Marine Mammal Institute, Hatfield Marine Science Center, Oregon State University, 2030 SE Marine Science Drive, Newport, OR 97365, USA. ⁴ Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, 100 Shaffer Road, Santa Cruz, CA 95060, USA. ⁵ Loggerhead Instruments, 6576 Palmer Park Circle, Sarasota, FL 34238, USA. ⁶ The Sexton Corporation, 2130 Davcor St SE, Salem, OR 97302, USA. ⁷ Southall Environmental Associates, 9099 Soquel Drive Suite 8, Aptos, CA 95003, USA. ⁸ Long Marine Laboratory, Institute of Marine Sciences, University of California, Santa Cruz, 100 Shaffer Road, Santa Cruz, CA 95060, USA.

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Competing interests

The authors maintain no competing financial interests for this study.

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