Agenda Item 3.5 | Implementation Review: Research

Investigation of the Effects of Anthropogenic Sounds on Harbour Porpoises (Action 11)

Information Document 3.5c | The use of Seal Scarers as a Protective Mitigation Measure can induce Hearing Impairment in Harbour Porpoises

Action Requested | Take note

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The use of seal scarers as a protective mitigation measure can induce hearing impairment in harbour porpoises[a)]

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Acoustic deterrent devices (ADDs) are used to deter seals from aquacultures but exposure of harbour porpoises (*Phocoena phocoena*) occurs as a side-effect. At construction sites, by contrast, ADDs are used to deter harbour porpoises from the zone in which pile driving noise can induce temporary threshold shifts (TTSs). ADDs emit such high pressure levels that there is concern that ADDs themselves may induce a TTS. A harbour porpoise in human care was exposed to an artificial ADD signal with a peak frequency of 14 kHz. A significant TTS was found, measured by auditory evoked potentials, with an onset of 142 dB re 1 μPa^{2}s at 20 kHz and 147 dB re 1 μPa^{2}s at 28 kHz. The authors therefore strongly recommend to gradually increase and down regulate source levels of ADDs to the desired deterrence range. However, further research is needed to develop a reliable relationship between received levels and deterrence. © *2019 Acoustical Society of America.*
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I. INTRODUCTION

Acoustic deterrent devices (ADDs, e.g., seal scarers) are applied in two different scenarios. These devices are mainly applied to deter seals from fish farms with the aim to prevent economic loss, due to seal depredation and damage to fishing gear. In European areas with extensive fish farms (e.g., along the west coast of Scotland), ADDs are a chronic source of anthropogenic noise pollution (Findlay *et al.*, 2018). Coastal areas where aquacultures are located are a typical habitat of harbour porpoises (*Phocoena phocoena*) (Brandt *et al.*, 2018; Findlay *et al.*, 2018; Gilles *et al.*, 2016; Hammond *et al.*, 2017; Peschko *et al.*, 2016; Viquerat *et al.*, 2014). Harbour porpoises have a very wide hearing range and are capable of hearing seal scarer signals (Kastelein *et al.*, 2002; Ruser *et al.*, 2016). While ADDs show highly varying success in seal deterrence (Götz and Janik, 2013) and could even attract animals, harbour porpoises exhibit strong avoidance reactions (Mikkelsen *et al.*, 2017). The much further deterrence of harbour porpoises occurs as an unwanted side effect in cases where ADDs are deployed to deter seals from fish farms (Brandt *et al.*, 2013).

The second scenario of ADD application is the use as a deterrent device prior to pile driving activities in offshore wind farms with the aim to deter harbour porpoises as a target species. Offshore wind farm construction is substantially increasing in Europe, providing a promising alternative to fossil fuels and nuclear power. In total, 92 offshore wind farms have been constructed to date in 11 European countries including sites with partial grid connection, amounting to 4149 connected wind turbines (Remy and Mbistrova, 2018). The majority of wind turbines are bottom mounted. High levels of impulsive noise arise when piles are driven into the seabed (Bailey *et al.*, 2010; Brandt *et al.*, 2018; Tougaard *et al.*, 2009). The increasing human encroachment which accompanies the construction of offshore wind farms has negative effects on key ecological species such as the harbour porpoise (Brandt *et al.*, 2018, 2012; Dähne *et al.*, 2013; Tougaard *et al.*, 2009), which inhabits coastal areas.

Due to its high sensitivity toward anthropogenic noise, the harbour porpoise can be regarded as an indicator species in noise impact evaluations (Southall *et al.*, 2007; Tougaard *et al.*, 2015). Harbour porpoises face the risk of a temporary hearing impairment if they stay in areas close to the pile driving site since impulsive noise has the potential to induce a temporary threshold shift (TTS) from single (Lucke *et al.*, 2009) or multiple exposures (Kastelein *et al.*, 2015a, 2016). Implementation of noise exposure criteria into national legislation differs between countries (Stöber and Thomsen, 2019). In particular, injury is defined differently between national policies (e.g., by the Habitats Directive in Europe or by the U.S. Marine Mammal Protection Act in the United States). In Germany, a temporary loss of hearing after the exposure to pile driving noise (TTS) is considered as an injury (BMU 2014), whereas most other states in Europe or in the United States only regard a permanent threshold shift (PTS) as an injury. Harbour porpoises are protected throughout Europe and are listed in Annexes II and IV of the European Union Habitats Directive (Council Directive 92/43/EEC on the Conservation of natural habitats and of wild fauna and flora).

Although several countries established criteria frameworks which define limits for hearing impairments, regulations pertaining to the use of deterrent devices differ between

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countries [reviewed for Germany, Denmark, and the United States in Stöber and Thomsen (2019)]. In German waters single strike sound exposure level (SEL_{SS}) of 160 dB re 1 $\mu Pa^2 s$ and a peak pressure level (L_p) of 190 dB re 1 μPa must not be exceeded at a distance of 750 m to the pile driving site (BMU, 2014). In order to prevent physical damage harbour porpoises must be deterred from the near-field where sound exposure thresholds can exceed the threshold (BMU, 2014). Following German legislation, permissions for offshore wind farm constructions generally include the condition to deter harbour porpoises prior to pile driving activities (BMU, 2014). ADDs are regularly applied as a tool to deter harbour porpoises prior to pile driving activities. ADDs emit signals between 10 and 40 kHz, corresponding to the range of best under water hearing in seals (Kastak and Schusterman, 1998; Kastelein *et al.*, 2018, 2009; Reichmuth *et al.*, 2013), at very high source levels up to 193 dB re 1 μPa (Lepper *et al.*, 2004). Seal scarer signals leads to deterrence effects in harbour porpoises, measured by surfacing distance to sound source (Brandt *et al.*, 2013; Mikkelsen *et al.*, 2017), aerial surveys (Brandt *et al.*, 2012) or by echolocation activity (Brandt *et al.*, 2012).

The aim of this study was to test whether a seal scarer has the potential to induce a TTS in harbour porpoise hearing. Additionally, we determined the threshold distance at which single exposures to seal scarer signals can induce a TTS, in order to estimate hazard zones for seal scarers. We provide critically needed information to develop protective measures which ensure both sufficient deterrence and the avoidance of hearing impairment inflicted by ADD signals.

II. METHODS

A. Study area and animal subject

All measurements were conducted at the Fjord & Bælt Centre in Kerteminde (Denmark). One harbour porpoise was kept in the 36 × 15 m semi-natural outdoor enclosure during this study. The enclosure was constructed of nets with a mesh size of 10 cm^2, allowing for a natural flow of seawater from the Kerteminde Fjord and the Great Belt. Water depth within the enclosure varied between 3 and 4 m, depending on position and tide. Measurements were conducted in a 6 × 4 × 1.9 m floating holding pool. A gate on one side was always open, allowing the animal to leave whenever it wanted. The study subject was a female harbour porpoise born in 1995, which has been kept in human care at Fjord & Bælt since 1997 after it was rescued from a pound net.

The harbour porpoise is kept under human care by the Fjord & Bælt in Kerteminde (Denmark) under Permit No. SVANA-610-00084 from the Danish Ministry of Food, Agriculture and Fisheries. All trials were conducted adhering to the respective ethical principles as well as to the relevant international and national guidelines for animal experiments and under constant supervision of experienced biologists and animal trainers. Experienced animal trainers monitored animal condition and signs of stress of the animal throughout all experiments. The harbour porpoise has been trained 2 times a day through standard operant conditioning and positive reinforcement techniques by a team of professional animal trainers. Trials were only conducted if visibility allowed for observing the harbour porpoise underwater at the bite plate throughout the whole experiment. During all exposure sessions the animal was visually observed by the trainer from above and additionally recorded by an underwater camera. In case of an observed stress response the experiments would have been stopped immediately.

B. Background noise recordings

Vessels passing close to the enclosure increased background noise levels since the semi-natural enclosure was solely separated from the harbour of Kerteminde by nets. Therefore, background noise was continuously monitored during measurements. Experiments were only conducted if no vessel was passing by or no other unwanted noise source was present. Background noise was recorded using a custom-made software application (LabVIEW, USA) with a hydrophone (TC4032, Reson Teledyne, Denmark), pre-amplified by 20 dB and bandpass filtered (100 Hz–180 kHz, B1501 Hydrophone amplifier, ETEC, Frederiksværk, Denmark). Recordings were digitized with a data acquisition card (NI USB 6251, National Instruments, Austin, TX) sampling at 400 k samples s^{-1} and at a 16 bit resolution.

C. Experimental procedure to measure hearing thresholds

Hearing thresholds were measured by monitoring the auditory evoked potential (AEP) response, a commonly used non-invasive electrophysiological technique (Finneran, 2018; Nachtigall *et al.*, 2017; Ruser *et al.*, 2016). In principle, a hearing stimulus (1) is presented to the test individual. If the acoustic stimulus is above threshold levels, the neurons within the acoustic pathway are stimulated and the neuronal discharges can be detected by electrodes placed on the head (2).

1. Hearing test stimulus

Hearing thresholds of the animal were determined at 20 and 28 kHz. Short tones centred at these frequencies were emitted, while AEPs of the brainstem were simultaneously recorded. A rugged notebook (Panasonic Toughbook CF30) computer was used to digitally generate stimuli, which were converted to analogue by a USB multifunction data acquisition card (NI USB 6251, National Instruments, Austin, TX). The stimuli were updated at a 1 MHz rate with a 16 bit resolution and bandpass filtered (100 Hz–250 kHz, 24 dB/octave, Krohn Hite, Brockton, MA) before emission by the transducer. The generated stimuli were emitted by a TC4033 transducer (Teledyne Reson, Denmark), placed at depth of 0.8 m and a distance of 1 m in front of the animal. Generated stimuli consist of 1024 tone pips for each tested sound intensity. Each pip consists of two sine rise, one sine steady, and two sines fall, with an epoch length of 17 ms (Fig. 1). Accordingly, the exposure per tested sound pressure level (SPL) had a duration of 17.4 s. The targeted maximum dive duration of 40 s allowed for testing two different SPL values per dive. All playbacks of hearing test stimuli and recordings of AEPs were conducted with a custom written software [Evoked Response Study Tool (EVREST) (Finneran, 2009;

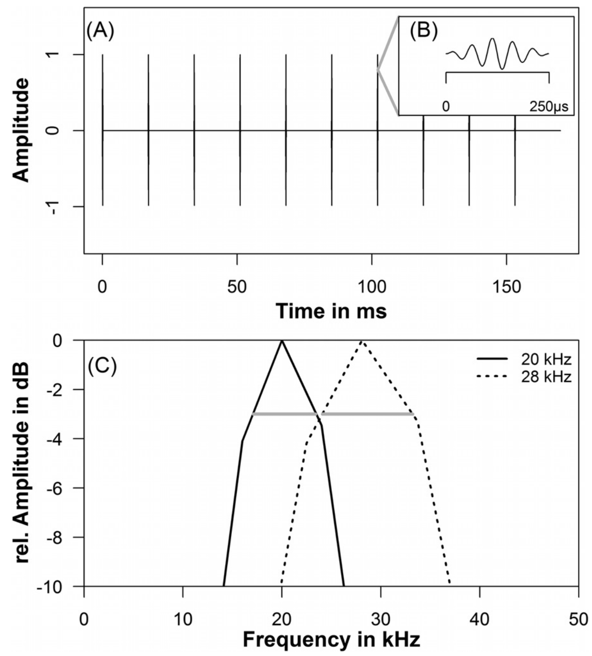

FIG. 1. Tone pips, used as hearing stimuli. (A) Section with ten tone pips out of a complete train consisting of 1024 repetitions. (B) Detail view of one pip (five cycles of 20 kHz carrier) within the pip train. (C) Frequency spectra of the 20 and 28 kHz tone pips with −3 dB bandwidth levels (gray horizontal lines).

Finneran *et al.*, 2008)]. Received SPL of test stimuli were calibrated by averaging 1024 stimuli prior to each trial at an SPL of 111 dB re 1 μPa with a TC4013 hydrophone (Teledyne Reson, Denmark) placed at the bite plate, close to the position of the porpoise during stimulus presentation. Recorded stimuli signals were pre-amplified by 60 dB (ETEC amplifier, Denmark), bandpass filtered from 1 to 180 kHz (ETEC B 1501, Denmark) and then digitized at 500 kHz with a 16 bit DAQ-card (NI USB 6251, National Instruments, Austin, TX).

2. Brain potential acquisition and hearing sensitivity assessment

AEPs were measured using 10 mm silver-plated electrodes imbedded in suction cups which were gently attached to the body surface along with standard conductive gel, which is regularly used in human electroencephalography recordings. Small electrical charges generated by the brain in response to the acoustic stimuli could be measured by placing the active (+) electrode behind the blowhole, the inverting electrode (−) along the dorsal midline of the porpoise between the blowhole and dorsal fin and the ground electrode (⏚) on top of that suction cup. Impedance between electrodes was tested with an impedance meter (Temec Instruments BV, NL) to ensure that impedance was <3 k Ohm during all measurements. Electrodes were connected with 10 m long shielded cables to a bio-potential amplifier (CP511, GRASS Technologies, West Warwick, RI) in order to amplify (+100 dB) and filter (0.3–3 kHz bandpass filter) the measured voltage between the inverting and non-inverting electrodes. The signal was digitized at 50 kHz and 16 bit resolution by the data acquisition card, connected to the computer. AEPs were recorded with the custom-made software (EVREST) that was also used to emit stimuli.

3. Experimental setup

First, the animal had to get accustomed to wearing suction cups on the skin. Suction cups contained silver-plated electrodes which were attached via cables to the computer. Second, the animal was trained to dive to a bite plate at a depth of 0.8 m and be stationary.

Two separate stations approximately 4 m apart at the right and left corner of the holding pool were used for the trials. Both bite plates were positioned at a fixed distance of 1 m to the projecting transducer. At the left bite plate the animal could be exposed to the fatiguing sound, and hearing tests were conducted solely at the right bite plate. Each trial to determine a hearing threshold included four dives to the bite plate, each lasting 40 s. During a dive, two different sound amplitudes could be tested, allowing for testing eight intensities in four dives. Sound amplitude started at 80 dB re 1 μPa [root-mean-square (rms)] for baseline measurements and was increased by the EVREST software internally in steps of 5 dB up to 120 dB re 1 μPa (rms).

D. Fatiguing stimulus and experimental procedure

We aimed at testing if seal scarer signals can induce a temporary hearing shift in harbour porpoises. Therefore, we exposed the animal to artificial seal scarer signals at a range of SELs and tested if post-exposure hearing thresholds differed from baseline hearing thresholds. One complete session consisted of three trials which were conducted on the same day. Three trials were needed to determine (1) the pre-exposure baseline hearing at experimental day, (2) the post-exposure hearing, and (3) the recovery. In trials 1 and 3 a hearing threshold was determined as described, whereas prior to trial 2, the animal was exposed to a fatiguing stimulus. During all exposure sessions the animal was visually observed by the trainer from above and additionally recorded by an underwater camera.

In all trials the harbour porpoise was first sent to the exposure station at the left bite plate. The animal stayed there upon receiving an acoustic signal from the trainer. For the baseline hearing or recovery measurements the animals were not exposed, but prior to exposure trials the animal received a fatiguing stimulus while staying at the bite plate. To allow for a complete recovery of the hearing system before the next trial the animal was exposed only once a day. Recovery measurements of the hearing thresholds took place 2 h after exposure to the fatiguing stimulus.

We exposed the animal to an artificial seal scarer signal comparable to a Lofitech (Lofitech AS, Leknes, Norway) seal scarer as fatiguing stimuli. This artificial seal scarer signal was built following Lofitech seal scarer signals, recorded at a distance of 130 m (Brandt *et al.*, 2013). The main frequency component of the 0.5 s signal was set to 14 kHz and four harmonics (28, 42, 56, and 70 kHz), and gradually decreasing sound levels were added. The artificial signal was generated in R (R Core Team, 2019), using the package "seewave" (Sueur *et al.*, 2008). The stimulus level of each frequency component was calibrated before the first exposure of this study, meeting the gradually decreasing components in Brandt *et al.* (2013). The same signal was used for all exposures within this study.

Initially the fatiguing sounds were presented at very low exposure levels and then subsequently increased by a maximum of 3 dB between days. The signals were transmitted by an ITC-1001 transducer (International Transducer Corporation, Santa Barbara, CA). A power amplifier (PA1001, ETEC, Denmark) was used to increase sound energy emission in steps of 3 dB. Signals were amplified (PA1001, ETEC, Denmark) and recorded at the bite plate with a TC4013 hydrophone (Reson Teledyne, Denmark), pre-amplified by 40 dB (ETEC-B, 1501 amplifier, Denmark) and bandpass filtered with a passband from 1 to 180 kHz. Fatiguing stimuli were played back by custom written software (LabVIEW, USA), which was also used for background recordings. The SEL was determined using this software. The frequency spectrum of the artificial seal scarer signal, used as a fatiguing stimulus on October 18, is presented in Fig. 2.

In further analysis, recordings of seal scarer exposures were additionally frequency weighted, adjusting the signal to perceived loudness based on the harbour porpoise hearing spectra. We used the proposed NOAA_{HF} (National Oceanic and Atmospheric Administration) frequency weighting, which has been recommended to evaluate effects of underwater noise on the hearing of marine mammals (National Marine Fisheries Service, 2018; Southall *et al.*, 2019). Frequency weighting of raw wav recordings has been conducted by the recently published MATLAB (MathWorks,

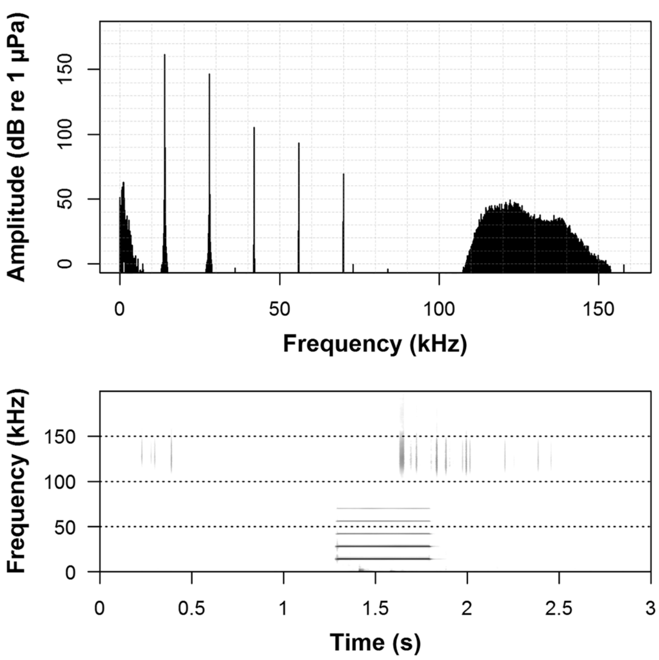

FIG. 2. Recorded frequency spectrum of one generated artificial seal scarer signal (top), which was used for animal exposure. The STFT of the recorded signal (bottom) shows the peak frequency of the seal scarer signal at 14 kHz with 4 octave overtones. Porpoise clicks around 130 kHz were recorded prior to and after exposure. Overlap = 87.5%, window length = 1048 samples.

Natick, MA) function "NOAAweighted" (Tougaard and Beedholm, 2018) within the recommended Marine Mammal Noise Exposure Criteria. Southall *et al.* (2019) refer to this same weighting function as "very high frequency cetaceans." The SEL was determined afterwards by the same software used for the playback of fatiguing sounds.

E. TTS definition

All hearing thresholds with no prior exposure were used to determine the baseline. Due to the small sample size of baseline measurements at 28 kHz, we included hearing thresholds which have been measured 2 h post-exposure in recovery trials. These two events have been tested by a Welch two sample *t*-test for unpaired samples for differences compared to the eight baseline measurements. The thresholds measured in recovery trials did not show significant differences from the baseline trials. Therefore, hearing thresholds at 28 kHz measured in the recovery trials were pooled with the baseline measurements. This pooling is assumed to be conservative in the detection of a TTS since only higher and no lower hearing thresholds are expected in the post-exposure trials. The baseline-hearing threshold was defined as the mean of all these trials. The post-exposure threshold measurement procedure was the same as for baseline hearing trials. Hearing thresholds were measured immediately after exposure and were gathered in an interval of 1 to 8 min after exposure. Using the mean hearing threshold and its standard deviation we obtained the threshold for a significant hearing shift ($p = 0.05$). A TTS was defined as a hearing threshold exceeding the mean threshold by 1.65× the standard deviation ($p = 0.05$, one-sided test). A typical TTS definition is a hearing threshold 6 dB above the baseline (Southall *et al.*, 2019). However, this threshold is based on the smallest shift which was clearly distinguishable due to the variability of 3–4 dB in baseline measurements in the study of Schlundt *et al.* (2000). Although we follow the TTS definition as a significant shift from baseline hearing [see, e.g., Finneran *et al.* (2005); Kastelein *et al.* (2014b, 2016)], the 6 dB criterion onset can be obtained from the provided TTS regression.

F. Data analysis

Data recorded by the electrodes were first checked for quality in order to exclude obvious disturbances which can be produced by environmental effects (waves, rain) or technical problems (crosstalk between cables). Recorded signals with peak voltages above 20 μV were rejected in order to exclude myogenic artefacts with large amplitudes originating from movements or respiration of the animal. Data were post-processed with a digital Butterworth bandpass filter of eighth order, to get a clear signal between 0.3 and 3 kHz. To objectively determine if stimuli at a certain SPL could be heard by the animal, we tested if the recorded brainstem signal differed from background noise within the recordings by a single point F-test (Don *et al.*, 1984; Elberling and Don, 1984). This method uses a variance analysis in determining the ratio of the magnitude of the ABR to the estimated averaged background noise. Afterwards, all responses were verified by two trained assessors searching for stereotypic patterns manually. The stereotypic wave V in an AEP has been proven to have a significant relationship to loudness (Serpanos *et al.*, 1997) and therefore served as an indicator for sound perception. A signal was determined as perceived ("hit") if wave V was determined in a time window of 3.8 to 5.2 ms after exposure. If no wave V was found the signal was determined as "miss." Hearing thresholds were defined as the mean SPL of the lowest hit and the highest miss (Fig. 3). All analyses were conducted and figures created with R Studio (R Core Team, 2019).

III. RESULTS

A. Baseline hearing thresholds

Baseline hearing at 20 kHz was measured on 25 days (see Table II in Appendix A), resulting in a mean baseline hearing threshold of 90.0 dB re 1 μPa $\pm$ 3.7 dB. An inspection of quantile-quantile plots verified that baseline hearing thresholds were normally distributed and a critical value ($p < 0.05$) of 96.1 dB re 1 μPa was obtained. Therefore, a hearing shift higher than 6.1 dB was regarded as a significant hearing elevation.

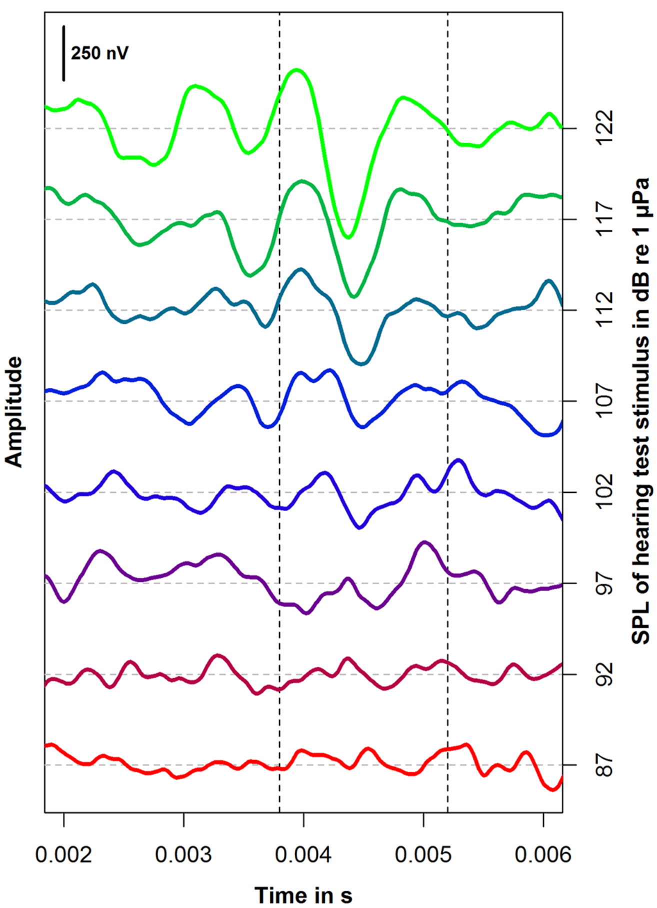

FIG. 3. (Color online) Example of measured AEP for 8 SPLs (color coded) between 87 and 122 dB re 1 μPa. Data were recorded after an exposure to a seal scarer signal with an SEL of 147.3 dB re 1 μPa^{2}s. The dashed black vertical lines indicate the time window where the wave V was searched. The post-analysis with the single point *F*-test and visual screening of two experienced assessors determined the lowest hit at a SPL of 97 dB re 1 μPa and the highest miss at 92 dB re 1 μPa. The hearing threshold was determined in the middle of these values and corrected for the results of the SPL calibration (-5.3 dB), resulting in a threshold of 89.2 dB re 1 μPa. The vertical black line in the top-left corner represents the voltage scale.

Baseline measurements for stimulation at 28 kHz were conducted on 6 days, resulting in 10 measurements (see Table II in Appendix A). Since the measurements of baseline and recovery sessions were not significantly different ($p = 0.9713$), pre- and post-exposure hearing thresholds were pooled to determine a baseline hearing threshold for 28 kHz. The mean baseline hearing threshold was 85.4 dB re 1 μPa $\pm$ 2.9 dB. An inspection of quantile-quantile plots verified that baseline hearing thresholds were normally distributed and a critical value ($p < 0.05$) of 90.4 dB re 1 μPa was obtained. Therefore, a hearing shift higher than 4.8 dB was regarded as a significant hearing elevation.

B. Post-exposure thresholds

The harbour porpoise showed an aversive reaction to the presentation of the fatiguing stimulus in almost all cases with an exposure exceeding 143 dB re 1 μPa^{2}s. It consisted of a short backward movement without leaving the bite plate. This reaction could represent the acoustic startle reflex which mirrors the audiogram 80–90 dB above the hearing threshold (Pilz *et al.*, 1987). Acoustic signals with a short rise time can elicit an oligo-synaptic reflex arc in the brainstem, provoking the contraction of refractor muscles (Koch and Schnitzler, 1997). This reaction did not occur during all trials. The harbour porpoise stayed at its position until it was called back by the trainer during all exposure sessions.

For hearing tests at 20 kHz, exposure trials were conducted on 9 days with SELs between 137.6 and 157.5 dB re 1 μPa^{2}s. In 5 trials a significant temporary hearing shift was measured at 20 kHz, as the post-exposure threshold was above 96.1 dB re 1 μPa, which was determined as the critical value for a TTS at 20 kHz. Calculating linear regression for these determined hearing shifts, we obtained a $\text{TTS}_{\text{onset}}$ at a SEL of 141.8 dB re 1 μPa^{2}s (Fig. 4).

Post-exposure hearing at 28 kHz was tested in 5 trials with SELs from 146.9 to 155.6 dB re 1 μPa^{2}s. In 4 trials a significant TTS, defined as a hearing threshold above 90.2 dB re 1 μPa, could be measured at 28 kHz. We inferred a $\text{TTS}_{\text{onset}}$ at a SEL of 146.9 dB re 1 μPa^{2}s, using linear regression (Fig. 4).

We obtained a SPL-$\text{TTS}_{\text{onset}}$ for each SEL-$\text{TTS}_{\text{onset}}$ by a linear regression of measured SPL and SEL (see Fig. 6 in Appendix C). NOAA_{HF} weighted $\text{TTS}_{\text{onset}}$ were derived from the calculated regression of frequency weighted exposure recordings and the determined TTS (see Fig. 7 in Appendix D). All results are summarized in Table I.

IV. DISCUSSION

A. Application of seal scarers

ADDs are widely used for instance to counteract the economic loss by seal depredation in aquacultures. Within the past 30 yr the worldwide farming of finfish species has substantially increased (FAO, 2018; Findlay *et al.*, 2018) and so has the economic loss by seal depredation. These sites represent an easily accessible food source with a high

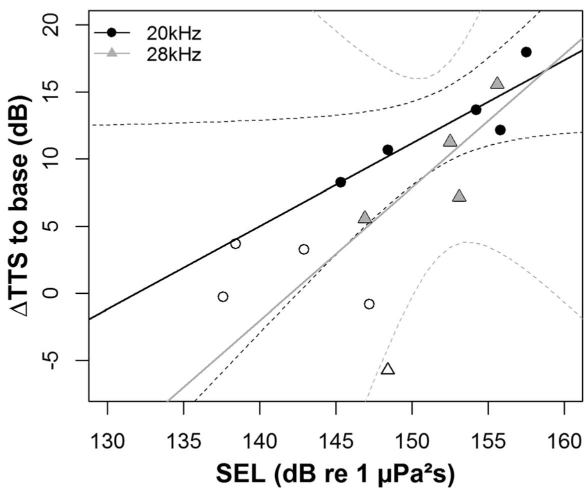

FIG. 4. Effects of seal scarer exposure (at 14 kHz, with four harmonics) on hearing thresholds at 20 kHz (black circles) and 28 kHz (gray triangles). Filled symbols indicate a significant shift from baseline hearing, which was determined at >6.1 dB for 20 kHz and >4.8 dB for 28 kHz. Unfilled symbols indicate hearing thresholds, which were not significantly shifted after exposure. A linear regression for each hearing frequency, using only exposures which led to a TTS, was calculated in order to estimate the TTS_{onset}. The 95% confidence intervals are shown by dashed lines. The TTS_{onset} at 20 kHz was determined at 141.8 and at 28 kHz at 146.9 dB re 1 $\mu Pa^2 s$.

profitability if no anti-predator control methods are applied. Although ADDs are extensively applied where fish farms exist in Europe, e.g., along the west coast of Scotland, no official statistics exist on the number, types, and duration of ADD usage (Findlay *et al.*, 2018). Furthermore, its deterrence efficiency is highly variable and may also decrease over time, due to habituation (reviewed in Götz and Janik, 2013).

In contrast to seals, harbour porpoises show strong avoidance behaviour to ADDs (Brandt *et al.*, 2013; Mikkelsen *et al.*, 2017; Olesiuk *et al.*, 2002). Seal scarer operators should be aware that the behavioural manipulation of seals as a target species might also affect non-target species which is widely overlooked when ADDs are applied around aquacultures. The rather unspecific signals of seal scarers are not specifically tuned to the auditory abilities of seals. Areas where aquacultures are located regularly overlap with harbour porpoise occurrence (Findlay *et al.*, 2018). Harbour porpoises are especially sensitive for underwater noise (Southall *et al.*, 2019). For the case of fish farms, harbour porpoises (*Phocoena phocoena*) are regarded as a non-

TABLE I. Summary of the determined TTS_{onset} after exposure to artificial seal scarer signals (14 kHz, with four harmonics) at 20 and 28 kHz hearing frequency for unweighted and auditory based $NOAA_{HF}$ (National Oceanic and Atmospheric Administration; National Marine Fisheries Service, 2016) weighted exposures.

Frequency (kHz)	Weighting	Intercept	slope	SEL-TTS_{onset} (dB re 1 $\mu Pa^2 s$)	SPL-TTS_{onset} (dB re 1 μPa)
20	—	−81.7	0.6	141.8	155.2
20	$NOAA_{HF}$	−78.9	0.6	138.4	152.9
28	—	−103.9	0.8	146.9	160.3
28	$NOAA_{HF}$	−136,6	1.0	143.6	157.4

target species because there is no evidence that these animals feed on farmed fish or damage fishing gear (Götz and Janik, 2013). The strong reactions of harbour porpoises to ADDs have been exploited in other applications such as a deterrent device prior to pile driving activities with the aim to prevent hearing impairment from the high noise levels of pile driving strikes. Indeed, a far reaching avoidance behaviour occurred up to distances of 7.5 km (Brandt *et al.*, 2012) or even 12 km (Dähne *et al.*, 2017). Although the exploitation of renewable energy represents an important component toward a more environmentally friendly power production, impacts on marine fauna caused by anthropogenic noise during and prior to the construction have to be considered.

B. Effect on harbour porpoise hearing

Caution is required when using deterrent devices with high source levels for both the application around fish farms and as deterrent devices to prohibit TTS from pile driving strikes. We could show that these signals itself have the potential to induce a significant temporary hearing shift in a harbour porpoise both at 20 and 28 kHz hearing frequency. Harbour porpoises critically rely on hearing to navigate (Villadsgaard *et al.*, 2007), find and catch prey items (DeRuiter *et al.*, 2009; Wisniewska *et al.*, 2016), and communicate (Clausen *et al.*, 2010; Sørensen *et al.*, 2018). Disturbance effects arise from numerous sources in addition to noise pollution (Andreasen *et al.*, 2017; ASCOBANS, 2002, 2012; Beineke *et al.*, 2005; Das *et al.*, 2006; Jepson *et al.*, 2016; Mahfouz *et al.*, 2014; Reeves *et al.*, 2013). Affected hearing can influence the survival rate of single individuals (Mann *et al.*, 2010; Morell *et al.*, 2017). Disturbance effects may even result in decreased individual fitness and could lead to long-term population consequences (King *et al.*, 2015).

Although the experiments were conducted in a semi-natural enclosure with a sound field that we could not control, the measured TTS was most likely induced by the exposure of the seal scarer signal only. While passing vessels can lead to behavioural reactions of harbour porpoises (Dyndo *et al.*, 2015; Wisniewska *et al.*, 2018), there is no evidence for the potential to induce a TTS by this continuous noise source. Experiments were only conducted in the absence of anthropogenic noise. Therefore, we can exclude the possibility that the induced TTS derived from another noise source than the seal scarer signal.

The pre-exposure baseline hearing threshold at 20 kHz is about 40 dB higher than measured by Kastelein *et al.* (2010). This difference is substantially caused by differences in the applied methods to measure hearing thresholds. Using psychophysical techniques instead of AEP measurements can lead to thresholds, which are 1–31 dB lower (Mulsow and Reichmuth, 2010). Hearing thresholds of wild harbour porpoises from the Inner Danish Waters which have been determined by AEP measurements are about 10 dB lower (Ruser *et al.*, 2016) than the baseline hearing of this study at 20 kHz. Our data were derived from one animal, at a senior age of 23 yr, in a semi-natural enclosure restricted by nets from the harbour solely, but is still within the 1.5

interquartile range of wild harbour porpoise hearing thresholds (Ruser *et al.*, 2016).

We tested the effect of the artificial seal scarer signal on harbour porpoise hearing at 20 kHz, although the main frequency component of the fatiguing stimulus was at 14 kHz, which is ∼0.5 octaves below. This was due to technical difficulties in measuring hearing thresholds below 20 kHz in the semi-natural environment and with this certain animal. The hearing threshold at 28 kHz is at the frequency of the first harmonic of the artificial seal scarer signal. Conclusively, this limits the comparability between the estimated onsets for 20 and 28 kHz because a TTS is expected to be greatest at 0.5 octaves above the fatiguing stimulus (Kastelein *et al.*, 2014b; McFadden and Plattsmier, 1983; Popov *et al.*, 2011).

In one case for each 20 and 28 kHz trials we did not measure a TTS after the exposure even if the SEL exceeded the TTS_{onset} (Fig. 4, unfilled circle, unfilled triangle). Although we took care in counteracting any potential conditioning behaviour, the unaffected hearing threshold after exposure could be a result of a self-protective mechanism which actively dampens hearing sensitivity, in expectation of an impending loud noise event. This self-mitigation has previously been shown for four Odontocete species (Nachtigall *et al.*, 2017). The harbour porpoise in this study could have reduced its hearing abilities in order to prevent a TTS during exposure. Since this effect seems to be of short duration (Finneran, 2018; Nachtigall *et al.*, 2016), we could not find a TTS when we measured the hearing in the period after the exposure, although expected for these exposure levels, because either a TTS was not induced or the self-mitigation was not active anymore.

The study design was adjusted to counteract any conditioning behaviour by the porpoise to reduce hearing sensitivity in expectation of sound exposure. This was done by keeping all experimental processes stable in exposure and non-exposure trials, besides sound exposure.

The measured hearing shifts are rather a result of a fatigued hearing after exposure than self-mitigation. This phenomenon only occurred after conditioning the animals to expect an unpleasant signal after a preceding warning stimulus (Nachtigall *et al.*, 2017). In this study, we tested hearing after exposure so there was no expectation of an upcoming unpleasant event.

C. Estimated hazard zones

Based on the determined SPL-TTS_{onset} of 155.2 dB re 1 μPa (*p-p*, Table I), we estimated a hazard zone where a TTS could be induced in harbour porpoise hearing after the reception of a single seal scarer signal. To estimate hazard zones, a theoretical sound propagation of seal scarer signals was modelled. Specified by the manufacturer, the source level of the Lofitech seal scarer is between 189 and 193 dB re 1 μPa (Ocean Science Consulting LTD, http://www.lofitech.co.uk/). To consider the most precautionary approach, we assumed the highest reported source level of 193 dB re 1 μPa. Based on this source level a theoretical sound propagation was modelled as a simple logarithmic regression. A similar approach with a simple logarithmic regression close to spherical spreading proved to be valid for seal scarer sound propagation in the German North Sea (Brandt *et al.*, 2012). The theoretical propagation was modelled for deep [spherical spreading with a transmission loss of $20 \times \log_{10}(r)$] and shallow water [cylindrical spreading with a transmission loss of $10 \times \log_{10}(r)$]. A practical spreading with $15 \times \log_{10}(r)$ in between deep and shallow spreading was additionally modelled, resulting in three tested factors for the slope of the transmission loss. The absorption coefficient α was estimated for typical North Sea parameters (assuming 15 °C water temperature, salinity of 35 ppt, a depth of 20 m, acidity of pH = 8) and a peak frequency of 14 kHz, at 1.5 dB km^{-1} (Ainslie and McColm, 1998). Accordingly, for the received level we obtained $RL = SL - slope \times \log_{10}(r) - \alpha \times r$.

Since the source level of 193 dB re 1 μPa corresponds to a rms value, the SPL-TTS_{onset} (*p-p*) was corrected. For the SPL-TTS_{onset} of 155.2 dB re 1 μPa (*p-p*,) we obtain a rms SPL-TTS_{onset} of 146.2 dB re 1 μPa (Fig. 5, horizontal dashed line), by subtracting 9 dB (Madsen, 2005). Single seal scarer signals with a source level of 193 dB re 1 μPa are assumed to induce a TTS in harbour porpoises up to distances between 211 m (spherical spreading in deep water) and 5.9 km (cylindrical spreading in shallow water), depending on theoretical sound propagation (Fig. 5).

D. Cumulative effects of multiple exposure

The determined SEL TTS_{onset} was determined for single exposures with 0.5 s long signals and is assumed to be higher for multiple exposure, like it has been shown for the exposure with single (Lucke *et al.*, 2009) and multiple impulsive low frequency noise (Kastelein *et al.*, 2015a, 2016). The

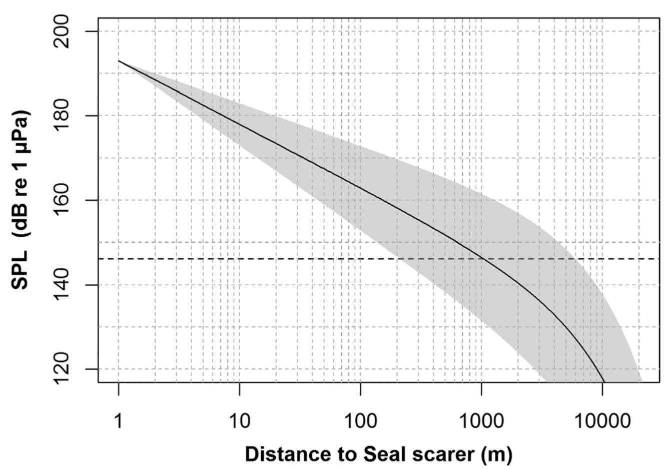

FIG. 5. Estimated hazard area where single seal scarers signals exceed the determined SPL TTS_{onset} of 155.2 dB (pp), corresponding to a SPL TTS_{onset} of 146.2 dB re 1 μPa (rms, dashed line). The sound propagation of a seal scarer signal with a source level of 193 dB re 1 μPa (rms) was estimated by a simplistic logarithmic regression. The gray shaded area represents the range between cylindrical [$10 \times \log_{10}$(distance)] and spherical [$20 \times \log_{10}$(distance)] spreading. The solid line represents an assumed practical [$15 \times \log_{10}$(distance)] sound propagation. The absorption coefficient was estimated at 1.526 dB km^{-1} for the main frequency component of 14 kHz, a water temperature of 15 °C, a salinity of 35 ppt, a depth of 20 m, and acidity of pH = 8 (Ainslie and McColm, 1998). A temporary hearing impairment for harbour porpoises can be induced at distances up to 211 m or 5.9 km.

equal energy hypothesis, meaning that the same amount of energy regardless of how many events will always induce a TTS, is assumed to be inapplicable (reviewed in Southall *et al.*, 2007). An equal hearing shift can only be expected for continuous fatiguing noises or for exposures with similar duty cycles (e.g., Finneran *et al.*, 2010a,b, Kastelein *et al.*, 2015b, 2014b; Mooney *et al.*, 2009; Popov *et al.*, 2014). In fact, the TTS_{onset} decreases with increasing SPL and duty cycle (Kastelein *et al.*, 2015b, 2014a).

The inter pulse intervals of seal scarers are usually randomized at intervals between 0.6 and 90 s to counteract potential habituation effects [e.g., Lofitech used by Brandt *et al.* (2013)]. Within these varying inter pulse intervals the hearing can recover. Since higher duty cycles induce a TTS at a lower SEL_{cum}, a TTS is more likely for multiple pulses with short inter pulse intervals. However, further experiments are needed to determine the TTS_{onset} for varying SELs and inter pulse intervals. This is critically needed to reliably predict a TTS potential for fleeing harbour porpoises from multiple exposures.

E. Management approach

We could demonstrate that a single exposure to seal scarer signals can lead to hearing impairment of non-target species at hundreds of meters. Negative effects could be even more dramatic if multiple ADDs are applied simultaneously on multiple cages within a single aquaculture or at further adjacent sites. We obtained a frequency weighted TTS_{onset} of 138 dB re 1 $\mu Pa^2 s$, which is surprisingly 15 dB lower than the updated recommended marine mammal noise exposure criteria for continuous noise (Southall *et al.*, 2019). Following these recommendations, a PTS_{onset} is estimated 20 dB above the TTS_{onset} level, which corresponds to 158 dB re 1 $\mu Pa^2 s$ according to our results. Regarding the high source level of commercially available seal scarers, even a PTS can be induced at very close distances. Given the evidence that seal scarers can induce an injury in harbour porpoises, no matter if defined as TTS (e.g., German regulation; BMU, 2014) or PTS [most other states in Europe and in the United States, e.g., Southall *et al.* (2019)], there is a clear requirement to manage the application of seal scarers.

For the application of seal scarers to deter seals around aquacultures, a shift to lower frequencies could decrease the risk for hearing impairment for the harbour porpoise as a non-target species. A shift to lower frequencies, as also proposed by Götz and Janik (2013), would be beneficial since the hearing abilities for lower frequencies are better for seals than for porpoises (Kastelein *et al.*, 2002; Ruser *et al.*, 2016). Deterring signals for seals should not contain much energy above 5 kHz if odontocetes use habitats around the fish farm (Götz and Janik, 2013). On the contrary, using lower frequencies could be worse for baleen whales. Therefore it has to be considered if baleen whales inhabit areas around the site where ADDs are applied. In case of further developments of ADDs, the target specificity must be validated by independent studies prior to deployment around aquacultures.

Although ADDs are a significant and chronic source of underwater noise pollution along the Scottish west coast around aquacultures with a steady increase in ADD usage and substantial geographic expansion (Findlay *et al.*, 2018), neither license is required to deploy these devices nor any statistics on usage exist (Coram *et al.*, 2014). An unwanted deterrence of harbour porpoises has been regarded as a comparatively benign side effect, while ADDs were deployed to counteract economic loss by seals. While behavioural responses and exclusion from key habitats of harbour porpoises were evaluated as insufficient arguments to regulate the use of ADDs, the evidence that a TTS or even PTS can be induced must lead to a regulation system.

Additionally, an adjustment of the source level of ADDs has to be considered to reduce the potential impact on harbour porpoise hearing. This adjustment should be taken into account for the use around aquacultures, but also as a deterrent device for harbour porpoises, prior to pile driving activities. From a conservation point of view, this deterrence should be adjusted to the expected TTS hazard zone from pile driving strikes. While received levels above the presented TTS_{onset} are assumed to induce increased hearing thresholds, the deterrence efficiency would be decreased accordingly, which is a problem from a commercial perspective (Götz and Janik, 2013). We therefore advise to down regulate source levels as a protective measure and use an amplitude ramp up, giving harbour porpoises sufficient time to leave hazardous areas.

V. CONCLUSION

Seal scarer signals have the potential to impair harbour porpoise hearing. The TTS_{onset} was determined at an SEL of 141.8 dB re 1 $\mu Pa^2 s$ at the hearing threshold of 20 kHz and at a 146.9 dB re 1 $\mu Pa^2 s$ for 28 kHz hearing frequency. The frequency weighted TTS_{onset} was 15 dB below the recommended Marine Mammal Noise Exposure Criteria (Southall *et al.*, 2019). Hazard zones, where a TTS can be induced by a single exposure, are dependent on sound propagation but are expected to be between 211 m and 5.9 km for reported source levels of up to 193 dB re 1 μPa. Based on our findings, effects of multiple exposure cannot be predicted due to the random inter pulse intervals. In order to use seal scarers to deter harbour porpoises instead of impairing their hearing, we suggest to down regulate source levels to the desired deterrence range and to slowly increase the source level, giving harbour porpoises time to flee.

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APPENDIX A: MEASURED HEARING THRESHOLDS FOR BASELINE HEARING MEASUREMENTS

Table II shows the results for the hearing tests which were conducted without any prior exposure. The measured hearing thresholds were used to determine the baseline hearing threshold.

TABLE II. Measured hearing thresholds, which have been used to calculate mean hearing thresholds.

Date	Frequency kHz	Threshold dB re 1 μPa
13.07.2017	20	87.6
13.07.2017	20	93.0
04.09.2017	20	85.8
04.09.2017	20	86.2
22.09.2017	20	86.5
27.06.2018	20	91.8
28.06.2018	20	82.1
28.06.2018	20	86.5
06.07.2018	20	95.7
06.07.2018	20	88.2
06.07.2018	20	92.0
17.07.2018	20	87.2
19.07.2018	20	89.6
20.07.2018	20	96.4
22.08.2018	20	93.6
23.08.2018	20	92.6
04.09.2018	20	92.6
13.09.2018	20	92.2
18.09.2018	20	87.9
19.09.2018	20	93.0
29.06.2017	28	84.0
10.10.2018	28	87.2
10.10.2018	28	84.7
10.10.2018	28	89.9
11.10.2018	28	89.0
17.10.2018	28	82.4
17.10.2018	28	83.7
18.10.2018	28	86.1
18.10.2018	28	87.3
25.10.2018	28	80.0

APPENDIX B: MEASURED HEARING THRESHOLDS AFTER EXPOSURE

Table III shows the measured hearing thresholds after the exposure to the artificial seal scarer signal.

TABLE III. Measured hearing thresholds after exposure, which resulted in a significant hearing shift, relative to the baseline. Asterisks indicate levels of significance (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

Date	Frequency kHz	Threshold dB re 1 μPa	Exposure dB re 1 μPa^{2}s	*z-value*	*p-value*
17.07.2018	20	93.73	138.4	1.01	0.1570
19.07.2018	20	89.75	137.6	−0.08	0.5300
20.07.2018	20	100.7	148.4	2.90	0.0019**
22.08.2018	20	89.2	147.2	−0.22	0.5889
31.08.2018	20	108	157.5	4.89	0.0000***
04.09.2018	20	103.7	154.2	3.72	0.0001***
13.09.2018	20	102.2	155.7	3.31	0.0005***
18.09.2018	20	93.3	143.1	0.89	0.1867
19.09.2018	20	98.3	145.3	2.25	0.0122*
11.10.2018	28	91	146.9	1.92	0.0273*
17.10.2018	28	92.6	153.1	2.47	0.0067**
18.10.2018	28	101	155.6	5.37	0.0000***
24.10.2018	28	96.7	152.5	3.89	0.0001***
25.10.2018	28	79.7	148.4	−1.98	0.9760

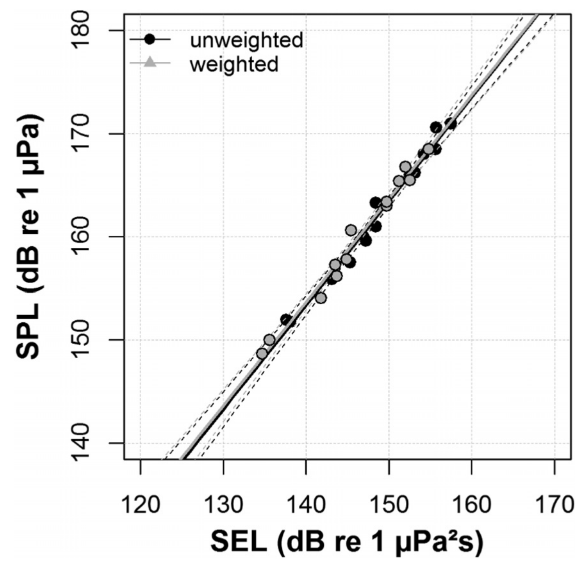

FIG. 6. Linear regression of SEL–SPL of all played back seal scarer signals during hearing tests at 20 and 28 kHz. Black points represent the unweighted SEL of all exposures. The circles represent the $NOAA_{HF}$ frequency weighted SEL of all exposures. The regression was used to estimate the corresponding SPL-TTS_{onset} from the SEL-TTS_{onset}.

APPENDIX C: LINEAR REGRESSION OF SEL–SPL

Figure 6 shows the modeled correlation between sound exposure and sound pressure levels of the recorded artificial seal scarer sounds. This linear regression allowed for the transfer of the determined SEL-TTS_{onset} to the SPL-TTS_{onset}.

APPENDIX D: AUDITORY FREQUENCY BASED WEIGHTING OF EXPOSURES AND RESULTING WEIGHTED TTS ONSET

Figure 7 shows the auditory based frequency weighted ($NOAA_{HF}$) sound exposure levels of the fatiguing stimuli and the measured hearing thresholds after exposure. Based on these results, a frequency weighted TTS_{onset} was determined with a linear regression.

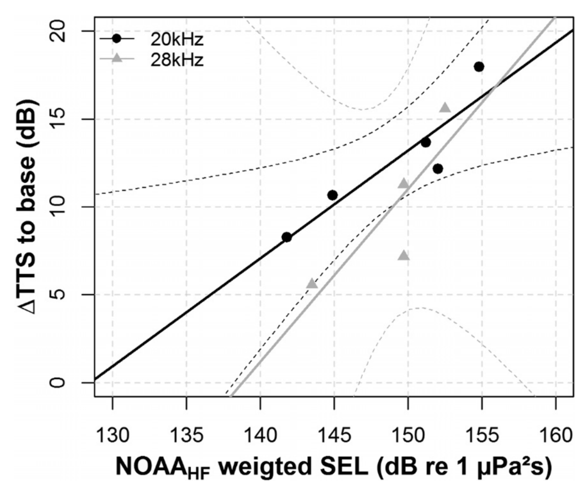

FIG. 7. Effects of seal scarer exposure on hearing thresholds at 20 (circles) and 28 kHz (triangles). The TTS_{onset} was determined with a linear regression for 20 kHz at 138.4 and for 28 kHz at 143.9 dB re 1 μPa^{2}s.

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