

Temporary shift in masked hearing thresholds in a harbor porpoise (*Phocoena phocoena*) after exposure to seismic airgun stimuli

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An auditory study was conducted to derive data on temporary threshold shift (TTS) induced by single impulses. This information should serve as basis for the definition of noise exposure criteria for harbor porpoises. The measurements of TTS were conducted on a harbor porpoise by measuring the auditory evoked potentials in response to amplitude-modulated sounds. After obtaining baseline hearing data the animal was exposed to single airgun stimuli at increasing received levels. Immediately after each exposure the animal's hearing threshold was tested for significant changes. The received levels of the airgun impulses were increased until TTS was reached. At 4 kHz the predefined TTS criterion was exceeded at a received sound pressure level of 199.7 dB_{pk-pk} re 1 μ Pa and a sound exposure level (SEL) of 164.3 dB re 1 μ Pa² s. The animal consistently showed aversive behavioral reactions at received sound pressure levels above 174 dB_{pk-pk} re 1 μ Pa or a SEL of 145 dB re 1 μ Pa² s. Elevated levels of baseline hearing sensitivity indicate potentially masked acoustic thresholds. Therefore, the resulting TTS levels should be considered masked temporary threshold shift (MTTS) levels. The MTTS levels are lower than for any other cetacean species tested so far.

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I. INTRODUCTION

Anthropogenic sound resulting from shipping, industrial and military activities and many other sources has led to a substantial increase in the underwater background noise in the oceans over the past decades (Hildebrand, 2004). The North and Baltic Seas are among the most intensively used and consequently noisiest marine areas (OSPAR Commission, 2000). Seismic surveys are one of the most prominent contributors to the overall noise budget in these areas, as in almost all oceans. Consequently, these surveys moved into the focus of interest of scientists as well as policy makers due to the intensity of the emitted sounds and spatiotemporal scale of these activities. Seismic surveys are conducted covering vast areas while searching for hydrocarbon deposits—in the central North Sea the most recent campaign was conducted at the Doggerbank area in spring/summer 2007. The total source level of airgun arrays used as sound source during these surveys depends on size, number, and timing of the individual airguns. With source levels ranging from 225 to 255 dB re 1 μ Pa_{peak} (Richardson *et al.*, 1995), seismic surveys are routinely conducted continuously over several weeks, with repetition rates of several signals per minute.

The acoustic emissions produced during these programs may reach intensities with a potential of causing a variety of effects in the marine fauna at considerable distances—from behavioral reactions (McCauley *et al.*, 2000; Tougaard *et al.*, 2003) and potential stress to physiological effects (Finneran *et al.*, 2002), injury (McCauley *et al.*, 2003), and possibly death (Ketten *et al.*, 1993).

Most odontocete species are known to produce, and be sensitive to, sound (see review in Richardson *et al.*, 1995; Wartzok and Ketten, 1999). They are represented in the central and southern North Sea, the Baltic Sea, and especially in German waters by the harbor porpoise (*Phocoena phocoena*) as the only resident cetacean species. Harbor porpoises have a very acute sense of hearing underwater (Andersen, 1970; Kastelein *et al.*, 2002) and have been shown to use echolocation to find their prey (Busnel *et al.*, 1965) as well as for spatial orientation and navigation underwater (Verfuß *et al.*, 2005). Their acoustic sense has evolved to be their likely dominant sense vital to their survival. Any impairment or damage to their auditory system may have deleterious consequences for the affected individuals.

Auditory studies on terrestrial animals have shown that the exposure to intense impulsive sounds could exceed the tolerance of their auditory system and lead to an increased

hearing threshold (Ahroon *et al.*, 1996; Kryter, 1994; Yost, 2000). Such a noise-induced threshold shift (TS) can either be temporary (TTS) or permanent (PTS), depending on the hearing system's capacity for recovery once the sound has ceased. A similar cause-effect relationship has been found in odontocetes as TTS has been demonstrated in bottlenose dolphins (*Tursiops truncatus*) and belugas (*Delphinapterus leucas*) (Schlundt *et al.*, 2006; Finneran *et al.*, 2002; Nachtigall *et al.*, 2003, 2004) after exposure to intense intermittent or continuous noise. The TTS data obtained so far indicated that the energy flux density [i.e., the acoustic energy over time or sound exposure level (SEL)] of a signal can be used in combination with a maximum peak pressure to determine noise exposure criteria for marine mammals. As SEL is calculated by integrating the squared pressure over a standard unit of time, the duration of a signal plays an important role with regard to TTS. It is still unclear whether the dose-response function follows an "equal-energy rule" in marine mammals, but in the absence of specific data it can be used as a first-order approximation, as pointed out by Southall *et al.* (2007).

Based on these TTS data, a peak pressure of 224 dB_{peak} re 1 μPa and a SEL of 195 dB re 1 μPa² s were initially proposed as noise exposure criteria for mid-frequency cetaceans (e.g., bottlenose dolphins and belugas) for exposures to pulsed sounds (Ketten and Finneran, 2004). With the noise exposure criteria proposed by Southall *et al.* (2007), the focus of marine mammal policy has shifted toward PTS and the onset of behavioral disruption. They proposed appropriate interim noise exposure criteria for all toothed whale species based on the dose-response functions found in the two cetacean species tested for their TTS limit so far (see above). The relevant PTS level for single impulses is set for all toothed whale species to a peak pressure of 230 dB_{peak} re 1 μPa and a SEL of 198 dB re 1 μPa² s. A criterion for SEL has also been set for the first time for multiple exposures to impulsive sounds, which are likely to lead to a reduced tolerance of the auditory system (Ahroon *et al.*, 1996). This threshold (198 dB re 1 μPa² s) is identical to the SEL criteria for single impulses. The subjects from former TTS studies are categorized as mid-frequency cetaceans with the main energy of their echolocation clicks and their range of best hearing sensitivity <100 kHz. Harbor porpoises, in contrast, are categorized as high-frequency cetaceans (Ketten, 2000; Southall *et al.*, 2007), with a best hearing sensitivity at frequencies above 100 kHz (Andersen, 1970; Kastelein *et al.*, 2002) and an energy maximum of their echolocation signals in the range 110–140 kHz (Verboom and Kastelein, 1995). There are no TTS data available for this species, or for any other high-frequency cetacean species. These differences in their acoustic and auditory characteristics may also be reflected in differences in the overall tolerance of their auditory systems to intense noise. Accordingly, a transfer of the first-order approximated auditory dose-response function to the harbor porpoise could be questionable. The same applies to an application of the noise exposure criteria proposed by Southall *et al.* (2007) to assess effects of pile driving impulses on harbor porpoises (as generated, e.g., during the construction of wind turbines).

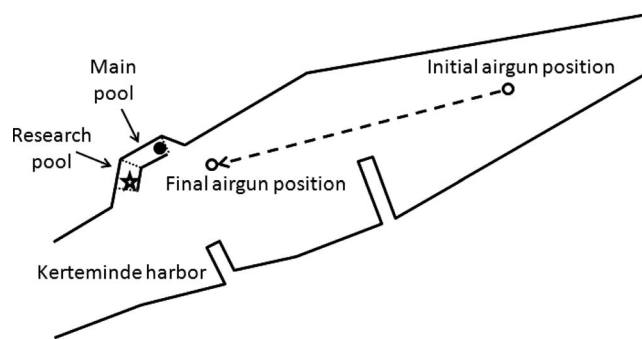


FIG. 1. Schematic overhead view of the experimental setup. Symbols indicate the approximate position of the harbor porpoise during the exposures to the airgun impulses in the main pool (filled circle), its position during the hearing tests (star), and the initial as well as the final location of the airgun (open circles) within the harbor of Kerteminde.

To base the assessment of acoustic effects of impulsive noise on species-specific data, a dedicated TTS study was conducted on one harbor porpoise. A key element for the planned study was access to a harbor porpoise trained to participate in experiments so that the experiments could be conducted under controlled conditions and definitive information on the dose-response function gathered. The aim of this acoustic study was to define the tolerance limit of the auditory system of the harbor porpoise to single impulsive sounds. Such data would enable regulatory agencies to define "zones of impact" (Richardson *et al.*, 1995) around the construction sites. At the same time, such data could be applied as a more robust baseline in the definition of noise exposure criteria for other high-frequency cetacean species [see outline by Southall *et al.* (2007)].

II. METHODS

A. Subject and facility

A male harbor porpoise held under human care in the Fjord & Bælt Centre (F&B) in Kerteminde, Denmark was chosen as subject for the studies. This animal, named *Eigil*, was estimated to be between 9 and 10 years old, with a length of 143 cm and an average weight of 40 kg in 2005 when the study began. A comprehensive medical record of all treatments exists for *Eigil* for almost his entire life. He was held in this facility with two female harbor porpoises at that time. The older female was pregnant twice during the study period from 2005 until 2007 and gave birth to a female calf right after the end of the studies in summer 2007. The design of the auditory experiments was altered due to the pregnancies and thus they are relevant for discussion of the results.

The animals were held together at the F&B in a semi-natural outdoor pool of 30 × 20 m² and an average depth of 4 m. Their enclosure stretches along the entrance from the Baltic Sea to a small fjord on one side of the busy fishing harbor of Kerteminde. It has a natural sea bottom and solid walls of concrete and steel on the two long sides. It is separated from the harbor on its narrow ends by nets, thereby providing a constant water exchange with the Baltic Sea (Fig. 1).

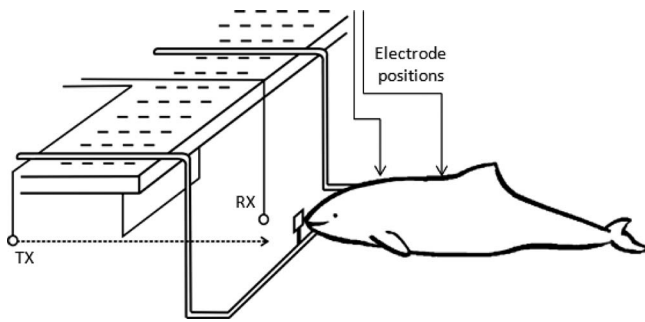


FIG. 2. Schematic plot of the research setup for the AEP measurements with the animal positioning itself at 1.5 m water depth in front of its underwater station and with its body in a straight line with the sound path of the incoming AEP stimuli. The direct sound path is indicated by a dashed line between sound source (TX) and monitoring hydrophone (RX) and the animal's position.

The enclosure is divided into two compartments (“main pool” and “research pool”), allowing separation of the animals for experiments. A floating pen ($4.5 \times 4.5 \times 1.5 \text{ m}^3$) in the research pool was wrapped with sound absorbing foam, providing an acoustic shelter for the two females during the planned exposures to intense sound in the later stage of the study.

All experiments were conducted with Eigil, who was separated temporarily from the two females to avoid behavioral or acoustic interference between the animals during the research. Eigil was trained to accept the electrodes that were attached to his head and back with suction cups and to dive on command to an underwater station at 1.5 m water depth. The training method used was based on operant conditioning and positive reinforcement (Pryor, 1984; Ramirez, 1999). No food deprivation was used during these experiments. He stationed himself actively at the setup with his rostrum touching a $4 \times 4 \text{ cm}^2$ polyvinylchloride (PVC) plate in front of the sound transducers (Fig. 2) for the hearing tests. He stayed there for 100 s on average until he was called back to the surface by the trainer to receive reinforcement. This experimental sequence was called a “send.” A complete research session was comprised of four sends on average. The number of research sessions per day depended on weather conditions and varied between one and four during the study period with an average of two sessions, ideally one in the morning and one in the afternoon.

B. Study design

The study was divided into two modules: The first consisted of measurements of the animal's absolute hearing thresholds over almost its entire functional frequency spectrum, thus providing a baseline for the second module, a tolerance test of the animal's hearing. This TTS test was designed to follow the same procedural structure as the experiments conducted by Finneran *et al.* (2002). The animal's hearing thresholds were measured in half octave steps over ~ 5.5 octaves with the lower-frequency limit set by the methodological parameters of the auditory evoked potential (AEP) stimulation. The threshold measurements were repeated several times at three selected frequencies (representing the low, mid-, and high frequencies of its functional

hearing range) to measure normal variation. This would subsequently allow definition of a frequency-specific TTS criterion.

C. Measurement of auditory sensitivity (AEP method)

The measurement of AEPs (AEP method) was chosen to measure the hearing thresholds in the harbor porpoise as it allows a comparatively rapid data acquisition and is non-invasive. For this reason the technique has been widely adopted in human patients and is also used for screening newborns (Hall, 2006). This technique is based on the presentation of acoustic stimuli, which will generate neuronal potentials in the acoustic system upon perception of these stimuli (Picton, 1987). Two surface electrodes are placed on the animal's skin using suction cups—one near the blowhole and the other near the dorsal fin—to record the neural responses evoked within the auditory system (Supin *et al.*, 2001). These potentials are generated within neuronal nuclei at different positions in the auditory system, thereby forming an electric field, which can be detected and recorded even on the skin surface. AEPs are useful for measuring the functioning of the auditory system and examining important aspects of auditory processing. To distinguish these comparatively small electric potentials from the overall neuronal activity—i.e., electric activity of the animal's musculature, other sensory inputs, etc.—the acoustic test stimuli are presented at a high repetition rate. By coherently averaging the evoked potentials (e.g., more than 500 AEPs), non-acoustic neuronal signals and incoherent acoustic signals not associated with the acoustic stimuli are reduced or eliminated.

A refined methodological approach is based on the use of rhythmic sound modulations. By sinusoidally modulating the amplitude of carrier tone or sound pulse sequence, it is possible to elicit a neuronal response, which includes a specific frequency component correlated with the modulation frequency used. This effect occurs because the auditory system is capable of following the envelope of a sinusoidal signal and producing corresponding neuronal potentials, called an envelope-following response (EFR). By applying a fast-Fourier transformation (FFT) analysis, the modulation frequency component can be identified and quantified. The resulting amplitude of the EFR represents the energy content of the neuronal response at the given modulation frequency. The strength of this EFR can simultaneously be taken as a relative measure for the perception of the carrier frequency of the amplitude-modulated (AM) signal. At each frequency, the stimuli were presented in decreasing intensity, starting at a clearly audible level, until a (neuronal) response was no longer detected. The resulting data were statistically tested for significance by using an F-test to identify EFRs from arbitrarily occurring noise at the given AM frequency (cf. Finneran *et al.*, 2007).

D. Sound generation and data acquisition

The animal's hearing was tested at frequencies between 4 and 160 kHz with sinusoidally amplitude-modulated (modulation rate: 1.2 kHz; duration: 25 ms) signals as AEP stimuli. The signals were of 25 ms duration with a modula-

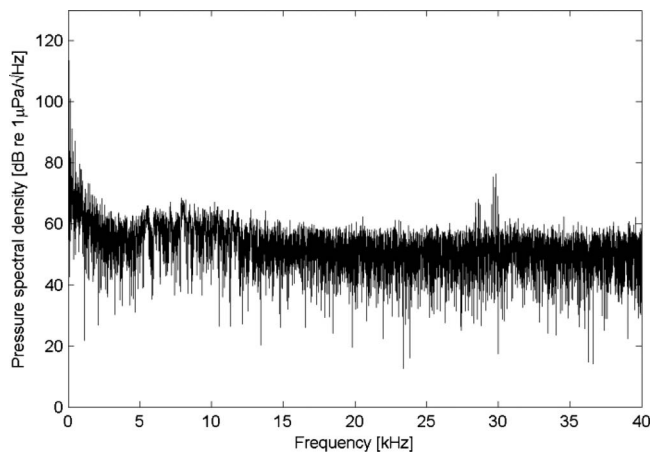


FIG. 3. Background noise level (plotted as pressure spectral density) recorded in the research pool at F&B during quiet conditions. (Analysis carried out on a 2.62 s sequence sampled at 400 kS/s using a Hanning window. The 0.38 Hz FFT bin size was then normalized to a 1 Hz power spectral density band.)

tion depth of factor 1. A custom-made software application was used to program all acoustic stimuli transmitted to elicit the AEPs during the hearing threshold tests. The signal generation system consisted of a data acquisition card (National Instruments DAQ 6062 E) and two function generators (Thurlby Thandar TG 230 and Agilent 33220A—with the first triggering the latter). At frequencies between 4 and 8 kHz all signals were amplified by a power amplifier PA 100E (Ling Dynamic Systems Ltd., Royston, UK) and transmitted via an underwater transducer USRD J-9. At higher frequencies a power amplifier Brüel&Kjaer 2713 was used to amplify the signals. Due to differences in their transmit response and the geometry of the pool, five different sound transducers had to be used to transmit the acoustic stimuli during the AEP tests: Signals at 4 and 8 kHz were transmitted via an underwater transducer USRD J-9, at 16 and 80 kHz via a Reson TC 4033, at 22.4 kHz via a SRD Ltd. 4 in. ball hydrophone, at 44.8 kHz via a SRD HS70, and all remaining frequencies were transmitted via a SRD HS150 hydrophone. All transmitted and received signals were constantly observed in real time at an oscilloscope and recorded for post-analysis via a monitoring hydrophone (Reson TC 4014) and a preamplifier (Etec B1501) for received level, signal quality, and undesired signal artifacts using software packages SEAPRODAQ (Pavan *et al.*, 2001) and custom software LU-DAQ. The evoked potentials were fed into a custom-built input station consisting of an amplifier (20 dB gain) and an optical separation unit (including 20 dB gain). Additionally, the signals were band-pass filtered (high-pass frequency: 300 Hz, low-pass frequency: 10 kHz, NF Electronic Instruments FV-665) to avoid artifacts. Each sequence of 500 successive potentials was averaged and displayed online as well as stored for post-hoc analysis.

The background noise in Kerteminde harbor is dominated by shipping noise from a variety of boat traffic ranging from recreational and small fishing boats passing the enclosure to fishing boats turning into the unloading area on the opposite side of the harbor and supply vessels for a nearby island (see comparison: Figs. 3 and 4). The background noise

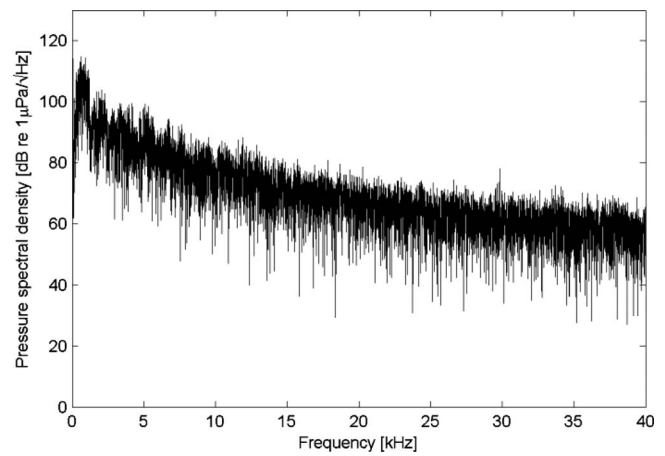


FIG. 4. Background noise level (plotted as pressure spectral density) recorded in the research pool at F&B recorded at the same position as in Fig. 2 during noisy conditions. (Analysis carried out on a 2.62 s sequence sampled at 400 kS/s using a Hanning window. The 0.38 Hz FFT bin size was then normalized to a 1 Hz power spectral density band.)

was thus dominated by low-frequency noise at varying levels and frequencies, depending on the size, speed, and activity of the respective boats.

E. Sound exposure procedure

A TS was defined as a difference of twice the standard deviation from the average hearing threshold at the particular frequency applied. The TTS criterion of 6 dB as proposed by Southall *et al.* (2007) was used as a second, frequency-independent criterion in this study. The tolerance of the animal's auditory system was then tested by first exposing the animal to a sound impulse as a fatiguing stimulus and then immediately re-measuring the hearing threshold. Any reduction in the animal's hearing sensitivity exceeding the preset TTS criteria would be regarded as evidence of an actual TS. Subsequent measurements of the animal's hearing threshold at the affected frequency would provide information about the recovery function of the auditory system.

The animal's hearing sensitivity was tested at three frequencies (4, 32, and 100 kHz) separately for TTS at a given exposure level of the fatiguing stimulus; i.e., only one hearing frequency was tested after each exposure. As long as the hearing threshold was shown to remain within its normal variation at all three frequencies, the subsequent exposure level of the fatiguing stimulus would be elevated and this procedure repeated until a TS is detected. This precautionary approach was chosen to avoid any risk of permanent hearing loss.

Various metrics have been used for both peak and energy amplitude, hearing threshold, spectral level, and spectral density, many discussed by Madsen (2005). A summary of calculation methodology is given below. Where possible, reported units are provided in formats used in other relevant studies to allow comparison with previous results.

For a specific pulse, the peak-to-peak pressure (P_{pk-pk}) was calculated. Since the peak may have a negative pressure, the peak-to-peak pressure is equivalent to the sum of the magnitudes of the peak positive and peak negative pressures.

Peak pressure is defined as the maximum magnitude of peak positive or peak negative pressure. The value is expressed as the peak-to-peak sound pressure level (SPL) in dB re 1 μPa . This is calculated from

$$\text{SPL}_{\text{pk-pk}} = 20 \log \left[\frac{P_{\text{pk-pk}}}{P_0} \right]$$

where P_0 is the reference pressure of 1 μPa (peak-to-peak).

The SEL for a single pulse is the integral of the square of the pressure waveform over the duration of the pulse using a 90% energy criterion. The duration of the pulse is defined as the region of the waveform containing the central 90% of the energy of the pulse. Given by

$$E_{90} = \int_{t_5}^{t_{95}} p^2(t) dt$$

The value is then expressed in dB re 1 $\mu\text{Pa}^2 \text{ s}$ and is calculated from

$$\text{SEL} = 10 \log \left[\frac{E_{90}}{E_0} \right]$$

where E_0 is the reference value of 1 $\mu\text{Pa}^2 \text{ s}$, t_5 is the time of a 5% increase in energy for the total pulse energy, and t_{95} is the time of 95% of the total energy of the pulse. The pulse duration is therefore defined as the time taken from 5% to 95% of the total pulse energy.

The root mean square (rms) pressure was calculated by taking the square root of the average of the square of the pressure waveform over the duration of the pulse, again using a 90% energy criteria, with the pulse duration defined as above. This is given as

$$P_{\text{rms}} = \sqrt{\frac{1}{T_{90}} \int_{t_5}^{t_{95}} P^2(t) dt}$$

F. Sound source for the fatiguing stimulus

A small sleeve airgun (20 in³) was used as sound source to produce the fatiguing sound stimuli during the second module. This device was pressurized with nitrogen at a pressure of 137 bar (2000 psi) and was operated at a depth of 2 m (i.e., in mid-water) from a small inflatable boat (source boat) in Kerteminde harbor at varying positions between the F&B and the eastern exit of the harbor area. The exact position of the source boat was determined by GPS, and this information, along with time, weather conditions, and other relevant information on the sound source, was documented for further analysis. An intensive calibration of the airgun had been conducted prior to the study using calibrated hydrophones at the receiving position at the F&B to predict the received levels of the airgun stimuli as a function of its distance to the receiving position in the main pool at the F&B.

The sudden release of pressure from the airgun during a “shot” results in an oscillating air bubble, which projects a short (less than 50 ms), intense impulse (Fig. 5) into the water and across adjacent boundaries (ground wave; audibility of airgun shot in air). The main acoustic energy of this

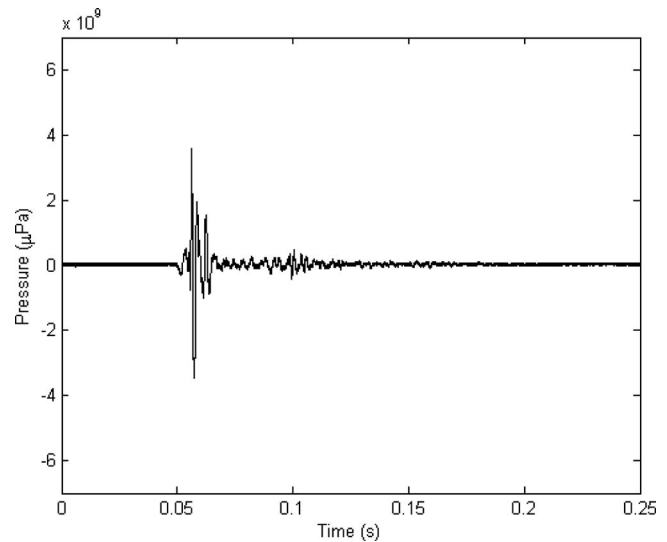


FIG. 5. Time domain representation of an airgun impulse. The airgun was fired at 2 m water depth in Kerteminde harbor and the impulse was recorded at a distance of 14 m to the receiving hydrophone.

impulse is centered below 500 Hz but considerable energy can also be detected up to above 20 kHz, well above background noise in quieter periods (Fig. 6).

Both high- and low-frequency components of the airgun emissions are likely to be attenuated over greater distance in a waveguide. The pulse recorded at the closest range from the airgun to the receiving position used in this study (shown in Fig. 6) therefore has the broadest observed spectrum and was felt to represent the worst case with regard to the potential auditory effects.

Prior to each airgun shot, the two female harbor porpoises were separated into the sound-insulated floating pen. Their general behavior and breathing rates were observed for the period of the sound exposure and compared with baseline data previously obtained under normal conditions. Eigil remained in the main pool. A receiving hydrophone was positioned at 1.5 m water depth at a position at the narrow end of

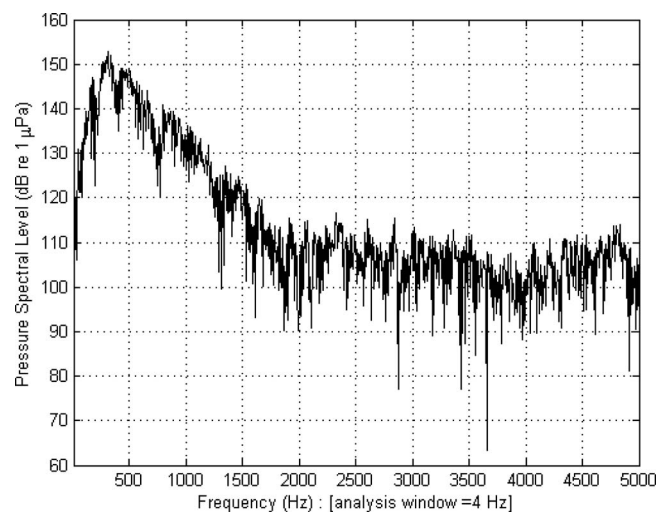


FIG. 6. Frequency spectrum analysis of the recorded airgun impulse (Fig. 4) showing the pressure spectral level (dB re 1 μPa). The frequency spectrum is plotted in hertz, and the spectrum levels are based on a 4 Hz analysis band.

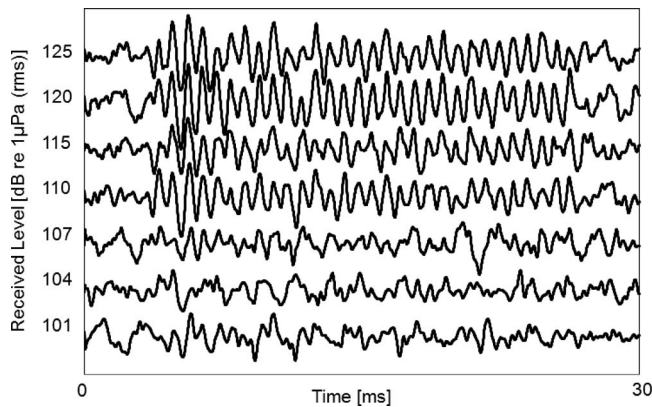


FIG. 7. Examples of EFRs in a harbor porpoise in response to acoustic stimulation with AM signals (averaged over 500 presentations); sampling duration was 30 ms, carrier frequency was 100 kHz, modulation rate was 1.2 kHz, and modulation depth was factor 1. Received levels descended from 125 dB re 1 μ Pa (rms) in 5-dB steps to 110 dB re 1 μ Pa (rms) and then in 3-dB steps to 101 dB re 1 μ Pa (rms).

the pool facing the eastern exit of Kerteminde harbor. This position had proven to receive the most intense signals during the airgun calibration. The airgun was triggered as soon as Eigil was within approximately 1 m of the receiving hydrophone with his body fully underwater. Control experiments were repeatedly made by conducting the complete procedure except for the exposure to the fatiguing stimulus. The animal's behavior was monitored and video recorded for further analysis. Immediately after each exposure to the fatiguing stimulus, the animal was then led into the research pool where the AEP setup was located. The post-exposure AEP measurements began less than 4 min after the exposure and typically were concluded within 12 min. Within this period his hearing sensitivity could be determined at a single frequency. During this second module, Eigil's hearing sensitivity was tested at 4, 32, and 100 kHz. These frequencies were chosen as representative frequencies for the low, mid-, and high ranges of the animal's functional hearing spectrum.

III. RESULTS

A. Hearing threshold

Eigil's baseline audiogram was determined based on the AEP measurements (Fig. 7) at frequencies between 4 and 140 kHz. At the highest frequency tested, 160 kHz, no AEP responses were detected. The measurements of Eigil's auditory sensitivity at the remaining frequencies resulted in elevated thresholds compared to hearing data published for other harbor porpoises (Fig. 8).

The shape of Eigil's hearing curve with its two minima at the mid- and high-frequency ranges is in good accordance with the previously published data. However, a clear rise in threshold was measured compared to data obtained by Kastelein *et al.* (2002) in a behavioral hearing study, with the maximum difference at 80 kHz. At the higher frequencies Eigil's threshold values are still elevated by 10–20 dB, but the difference is not as pronounced compared to the thresholds obtained by Andersen (1970). Compared to the results from the AEP study by Popov and Supin (1990), Eigil's thresholds are elevated by roughly 10 dB. The mean hearing

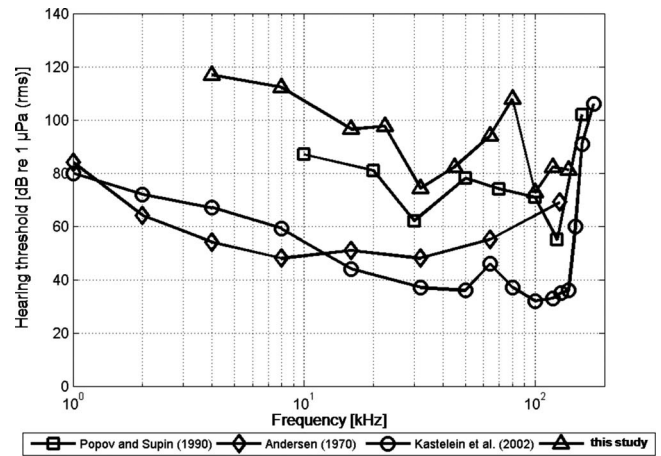


FIG. 8. Harbor porpoise hearing threshold data from different studies. The triangles represent the threshold values achieved in this study. Data from another AEP study (Popov and Supin, 1990) as well as from two behavioral auditory studies (Andersen, 1970; Kastelein *et al.*, 2002) are given for comparison.

thresholds at 4, 32, and 100 kHz, respectively, were at 116.9, 74.2, and 72.7 dB re 1 μ Pa (rms). Based on the variation of the hearing thresholds measured during the first module, the TTS criteria were defined as 122.9 dB re 1 μ Pa (rms) at 4 kHz, 79.0 dB re 1 μ Pa (rms) at 32 kHz, and 85.7 dB re 1 μ Pa (rms) at 100 kHz.

B. TTS tests

Over a period of 4.5 months, Eigil was exposed to a total of 24 airgun impulses. The received peak pressure of the pulses ranged from 161.2 dB_{pk-pk} re 1 μ Pa to 202.2 dB_{pk-pk} re 1 μ Pa, with an acoustic energy (SEL) ranging from 140.5 dB re 1 μ Pa² s to 167.2 dB re 1 μ Pa² s. These levels were achieved using source ranges between 150 and 14 m from the animal's position during the exposure.

1. Threshold shifts

A TTS was first measured after Eigil had been exposed to an airgun impulse at a peak pressure of 200.2 dB_{pk-pk} re 1 μ Pa with corresponding SEL of 164.5 dB re 1 μ Pa² s. The TS was measured when the animal hearing was tested after the exposure for its sensitivity at 4 kHz. Since this TS was only 1.8 dB above the predefined TTS criterion, the exposure was repeated several days later with a received peak pressure level of 202.1 dB_{pk-pk} and a SEL of 165.5 dB re 1 μ Pa² s. The resulting TS at 4 kHz was 9.1 dB above the TTS criterion and hence a clear support of TTS. Another verification of this effect was achieved 2 days later, after an exposure at a peak pressure level of 201.9 dB_{pk-pk} re 1 μ Pa with a SEL of 165.8 dB re 1 μ Pa² s, when Eigil's hearing revealed a TS at 4 kHz of 15 dB (Fig. 9). No significant elevation of hearing threshold at 32 kHz was observed at a comparable exposure level to the 4 kHz test case. The received energy was similar to the 4 kHz case, but a slightly lower received peak-to-peak pressure was observed (Fig. 10). No statistical change in hearing sensitivity was observed after an exposure to similar source levels

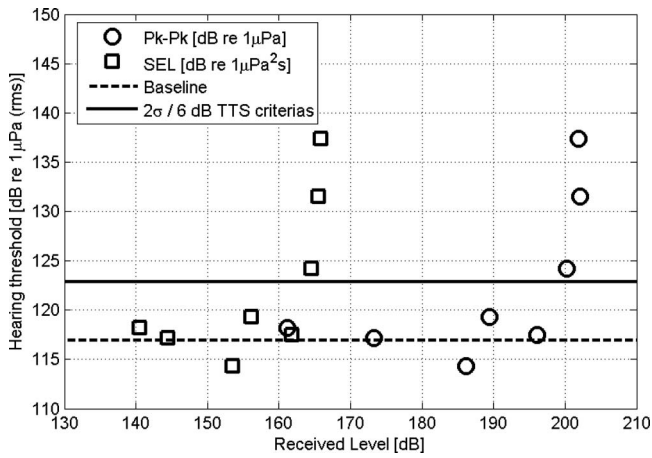


FIG. 9. Hearing threshold at 4 kHz for a harbor porpoise after exposure to airgun stimuli (i.e., post-exposure) at different received levels plotted in relation to the animal's pre-exposure hearing sensitivity. Each post-exposure hearing threshold is plotted twice—circles indicating the received peak-to-peak pressure of the fatiguing stimuli and squares indicating the equivalent received SELs of the same exposure impulses. The dashed line represents the normal hearing threshold and the solid line the two TTS criteria used for comparison (which are identical at 4 kHz). Symbols above the solid line indicate a TS of hearing threshold.

for the 100 kHz test case—as regards both received peak pressure and energy (Fig. 11). It should be noted that the airgun source itself creates less energy at the mid- and high-frequency ranges than at 4 kHz.

2. Recovery

An important factor for the assessment of this noise-induced effect is the recovery of the animal's auditory system. After the first clear TS had been measured, a series of AEP measurements was conducted over the following days to follow the further development of Eigel's hearing sensitivity at the affected frequency. 178 min after the initial exposure his hearing had recovered only partially from its TS. It

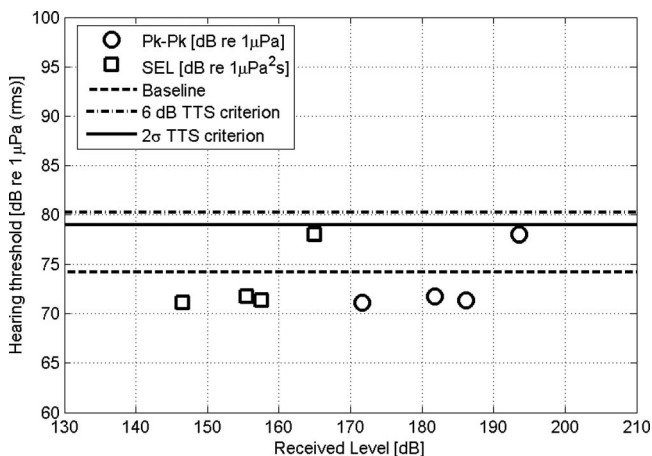


FIG. 10. Hearing threshold at 32 kHz for a harbor porpoise after exposure to airgun stimuli (i.e., post-exposure) at different received levels plotted in relation to the animal's pre-exposure hearing sensitivity. Each post-exposure hearing threshold is plotted twice—circles indicating the received peak-to-peak pressure of the fatiguing stimuli and squares indicating the equivalent received SELs of the same exposure impulses. The dashed line represents the normal hearing threshold. The other lines indicate the two different TTS criteria used for comparison.

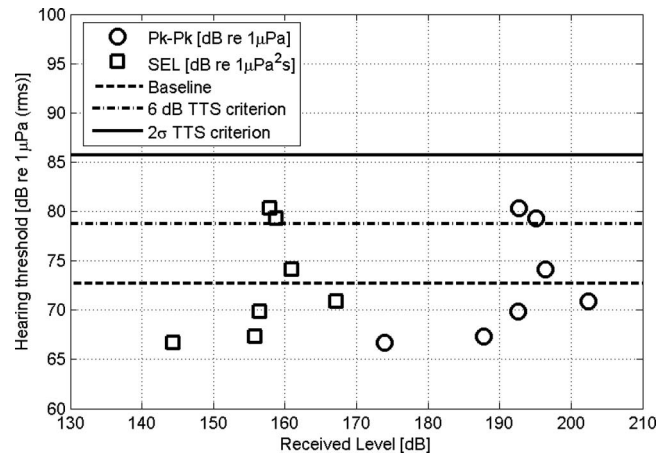


FIG. 11. Hearing threshold at 100 kHz for a harbor porpoise after exposure to airgun stimuli (i.e., post-exposure) at different received levels plotted in relation to the animal's pre-exposure hearing sensitivity. Each post-exposure hearing threshold is plotted twice—circles indicating the received peak-to-peak pressure of the fatiguing stimuli and squares indicating the equivalent received SELs of the same exposure impulses. The dashed line represents the normal hearing threshold. The other lines (dotted-dashed line and solid line) indicate the two different TTS criteria used for comparison; symbols above both these lines indicate a temporary shift of hearing threshold.

was reduced by 2.9 dB but still being elevated above the TTS criterion. Eigel's sensitivity at 4 kHz improved by 3.5 dB, 269 min post-exposure but only by another 1.4 dB, 29 h post-exposure (Fig. 12).

Assuming a linear recovery from TTS, the animal's hearing sensitivity would have reached the TTS criterion level again in 12 h for the 202.1 dB exposure. However, a log-fitted curve provides a better fit to the data (i.e., the highest regression coefficient) for calculating Eigel's auditory recovery function. By applying this function the animal's hearing sensitivity would have recovered back to the level of the TTS criterion in 55.0 h.

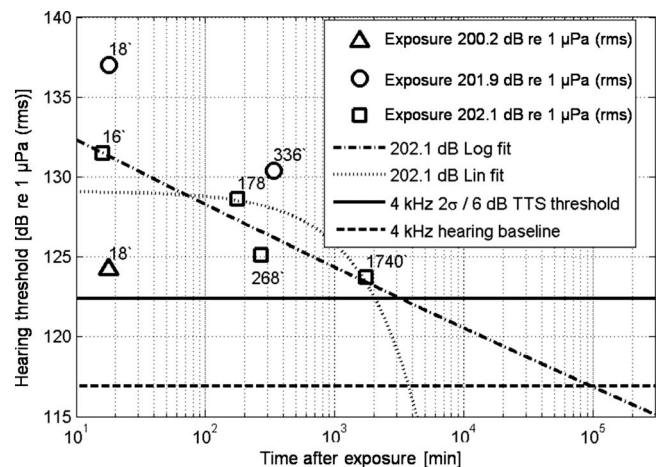


FIG. 12. Recovery function of a harbor porpoise's hearing threshold at 4 kHz after sound-induced TSs. Hearing thresholds measured subsequent to the exposures after different times (given in minutes next to the symbols) are indicated by different shapes for every exposure to the fatiguing stimulus. The recovery function for the exposure at 202.1 dB re 1 μ Pa (rms) is indicated by the diagonal and curved lines. The dashed line represents the normal hearing threshold and the solid line the two TTS criteria used for comparison (which are identical at 4 kHz).

3. Behavioral reactions

Eigil showed no behavioral reaction during the first exposures when he was exposed to a received pressure level of less than $174 \text{ dB}_{\text{pk-pk}}$ re $1 \mu\text{Pa}$ or a SEL of 145 dB re $1 \mu\text{Pa}^2 \text{ s}$. At higher received levels, the animal showed repeatedly a typical aversive reaction at the time of the sound exposure and behavioral avoidance in the direction of the location of the source. Subsequently the animal avoided approaching the exposure station prior to further exposures as well as during control experiments. It should be noted that the exposure station was deliberately placed at a point of maximum received level within the total available enclosure. After a TTS had been documented and confirmed, the received levels were not raised any higher and no further trials were conducted.

Because one of the female harbor porpoises was pregnant during the exposure period, special measures were taken to protect her and the other animals from unnecessary sound exposures. Both females were kept in a sound-insulated pool and their behavior was continuously monitored during the sound exposures. None of them showed any obvious behavioral reactions during the airgun experiments. The attenuation of the airgun impulses inside their pool was at the order of 30–40 dB lower than at the exposure station. Correspondingly, the two females were never exposed to peak-to-peak pressure levels of more than 160 dB re $1 \mu\text{Pa}$.

IV. DISCUSSION

The TSs documented in this study represent the first data of its kind for harbor porpoises. Up to now all assessments of potential effects of anthropogenic sounds on harbor porpoises had to be made based on data from other odontocete species, or even terrestrial animals. Thus, the results of this study provide the first reliable information for the harbor porpoise for airgun (or impulse) exposures. These data, and more from future studies, could serve as a basis not only for defining noise exposure criteria for this species but also for deriving group-specific noise exposure criteria for all high-frequency cetaceans. The TS levels for the harbor porpoise differ strongly from data on the bottlenose dolphin or the beluga. This study provides more empirical data for high-frequency echolocating species than was available for Southall *et al.* (2007). Thus, the authors suggest that the proposed thresholds should be adapted accordingly.

The analysis of the animal's observed behavioral reactions to the fatiguing stimuli for the first time provides quantitative clues of a behavioral threshold in harbor porpoises. The fact that Eigil was swimming away from the location of the sound source after exposure to the airgun stimulus but not in control experiments infers avoidance or flight behavior. In a free-ranging animal this reaction might have lasted over a longer period of time than observed in Eigil, who calmed down and was back under behavioral control of the trainers after a few seconds when he was sent to subsequent hearing tests. It also remains questionable whether or not the level of $174 \text{ dB}_{\text{pk-pk}}$ re $1 \mu\text{Pa}$ pressure or a SEL of 145 dB re $1 \mu\text{Pa}^2 \text{ s}$ can be applied as threshold limit for behavioral reactions to impulsive sounds in harbor porpoises

in general as Eigil was rewarded for tolerating the intense sound exposures and reactions might occur even at lower levels. It seems more likely that this limit varies individually and may be context-specific. So far, the only available data on behavioral reactions of harbor porpoises to impulsive sound have come from observations during the construction of wind turbines at Horns Rev, Denmark (Tougaard *et al.*, 2003) where at a distance of up to 15 km a movement directed away from the sound source was observed in the animals. In the BROMMAD study (Gordon *et al.*, 2000), by contrast, no obvious behavioral reactions were observed in free-ranging harbor porpoises in response to airgun exposures at an estimated received level of $176 \text{ dB}_{0\text{-pk}}$ re $1 \mu\text{Pa}$. In this context, the results of the present study constitute the first behavioral threshold in harbor porpoises that was measured under controlled acoustic conditions. The resulting data may be used as a first indication of a threshold range for behavioral reactions of harbor porpoises. The disturbing nature of this sound to harbor porpoises at the given intensities is emphasized by the avoidance behavior observed in Eigil prior to exposures after the exposure level had passed his behavioral threshold for the first time. The fact that Eigil was actively avoiding the monitoring hydrophone showed that he was sensitized. It was a lasting effect as he showed no signs of habituation during the remaining exposures.

The rate of recovery from TTS slowed during recovery period, suggesting a log-correlation in the recovery function. These first data would suggest that recovery rates are different between harbor porpoises and the previously tested mid-frequency cetaceans. The latter usually recover within minutes or, at a maximum, within 2 h from a comparable amount of TS (Finneran *et al.*, 2002; Nachtigall *et al.*, 2003, 2004). Such a slow recovery of the harbor porpoise's hearing sensitivity would also indicate that the third exposure to the airgun stimulus at levels over 200 dB re $1 \mu\text{Pa}$ (received level, RL of 201.9 dB re $1 \mu\text{Pa}$) may have been premature as the TS was not yet fully recovered. The documented shift of 15 dB above the TTS criterion therefore could then be considered as a cumulative effect from the two consecutive exposures. The level for onset of TTS should accordingly be calculated based on the first two TS values, i.e., a peak-to-peak pressure of $199.7 \text{ dB}_{\text{pk-pk}}$ re $1 \mu\text{Pa}$ and a SEL of 164.3 dB re $1 \mu\text{Pa}^2 \text{ s}$. These levels depend of course on the TTS criterion chosen and would be altered accordingly. Nevertheless, due to the comparatively strong variability within the experimental conditions, a frequency-specific definition of the TTS criterion for this type of fatiguing stimuli seems most appropriate.

The AEP method is the only available method to conduct comparable studies on wild animals. Those studies are relevant to validate the results from a single captive animal in a larger number of animals at a later stage. The results of this study show, on the one hand, that the AEP method can be successfully applied for auditory studies on harbor porpoises even if the animals are unrestrained like Eigil, who was actively swimming and free to leave the experiments at any time. His constant movement during the experiments, on the other hand, caused strong myogenic potentials, which

were recorded along with the auditory potentials during the experiments. These myogenic potentials are strong enough to raise the overall neuronal noise level of the recorded potentials. Any masking of the lowest levels of the auditory potentials by other electrophysiological signals, such as the myogenic potentials, could obscure the real lower end of the regression line, hence leading to a zero-crossing of the regression at a higher threshold value. Consequently the resulting hearing threshold would be elevated.

Probably the most prominent factor that may have influenced the hearing thresholds is the level of background noise in Kerteminde harbor. It is most likely that this broadband noise masked perception of the AEP stimuli by Eigil. A similar effect has been found in auditory studies in humans (Parker *et al.*, 1976) and also in harbor porpoises (Lucke *et al.*, 2007). Acoustic events, such as boats passing at close distance to the research station, were avoided during the experiments by pausing the session. Nevertheless, it was impossible to conduct the experiments at a consistently low level of background noise. As these conditions varied within each research session, and with extreme noise events excluded, one may assume that roughly the same overall noise conditions applied for all sessions.

Despite these physical factors affecting the baseline hearing thresholds, the results may also reflect a genuine hearing deficit that Eigil either developed due to an unnoticed infection of his auditory system or as a result of previously unmonitored exposure to intense sound or a long-term exposure to sounds, e.g., from the nearby harbor. However, it can be ruled out that the elevated thresholds are the result of ototoxic drugs as Eigil is known to have never received such treatments. An age-related hearing deficit is also unlikely as it usually only occurs at high frequencies. The elevated baseline hearing thresholds stretch over both the high and low frequencies. Further aspects leading to error in estimation of Eigil's hearing threshold are the comparatively conservative statistical analysis of the resulting EFR data (F-test) and the use of AEP stimuli, which are likely to be shorter than the auditory integration time of the animal's hearing system.

As a consequence of this physiological and physical masking, the measured baseline hearing thresholds cannot be regarded as absolute but should be defined as masked thresholds, and, accordingly, the documented TSs have to be regarded as masked temporary threshold shifts. The presence of masking noise may have reduced the amount of TTS measured, as indicated by TTS studies on humans (Humes, 1980) and chinchillas (Ades *et al.*, 1974), simulating a pre-exposure reduction in hearing sensitivity. Nevertheless, the onset level of TTS itself, as defined in this study, is likely to be unaffected by the masking noise (Finneran *et al.*, 2005; Southall *et al.*, 2007), presumably due to its comparatively low acoustic energy in comparison to the intense airgun stimuli.

Whether the differences in TTS levels between harbor porpoises and the marine mammal species tested so far are species-specific or representative of the functional hearing groups, as defined by Southall *et al.* (2007), remains unclear. More harbor porpoises, as well as other high-frequency toothed whale species, need to be tested to elucidate this

correlation. As for terrestrial animals (Henderson, 2008), the large difference in acoustic tolerance in toothed whales is likely to be attributable to the physical differences in the conductive apparatus rather than to systematic differences in the inner ear. Anatomical differences in the fine structure of the inner ear (Wartzok and Ketten, 1999; Ketten, 2000) and correlated differences in stiffness of the basilar membrane could account for a lower acoustic tolerance to intense sounds in harbor porpoises compared to the toothed whale species tested so far. Moreover, differences in metabolic processes in the inner ear could potentially mediate the high TTS growth rate as well as the long recovery time in harbor porpoises. In the absence of more detailed information it may be valid to generalize and describe this correlation best by means of a mass dependency in the dose-response function for acoustic effects in toothed whales, as documented by Ketten (2006) for the effects of blast impacts.

The TTS data defined in this study are applicable as baseline for the assessment of all activities that go along with the emission of short, impulsive sounds with regard to harbor porpoises. This includes seismic surveys as well as piling construction, both of which show strong acoustic commonalities despite the complexity of their sound emissions. Underwater explosions, however, should be treated separately in this context due to their specific acoustic characteristics of the shock wave, which may yield strong auditory effects irrespective of the peak pressure or energy of the impulse.

Seismic surveys, piling operations, and several other anthropogenic activities at sea involve the repeated emission of intense impulses at varying repetition rates (e.g., 10–15 s interval for seismic surveys and 2–30 s interval for piling). Marine mammals in the vicinity of these operations will consequently be exposed to multiple impulses. While the TTS values determined in this study apply only to a single exposure to a pulsed signal, the auditory effects will accumulate with repeated exposures to such signals if the interval between subsequent exposures is shorter than the recovery time of the hearing system. So far there is no information available on the underlying summation procedure for marine mammals. For harbor porpoises it seems unlikely that they will stay in the area of such intense sound emissions. Nevertheless, if these operations are started without sufficient time for animals to leave the area where received levels will be above or near the TTS levels (as determined in this study), there is an increased risk of TTS or even PTS. The comparatively high TTS growth factor, in combination with the slow recovery rate, worsens this scenario drastically for harbor porpoises compared to mid-frequency odontocetes.

The results emphasize the need for dedicated studies on the cumulative effects of multiple exposures.

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