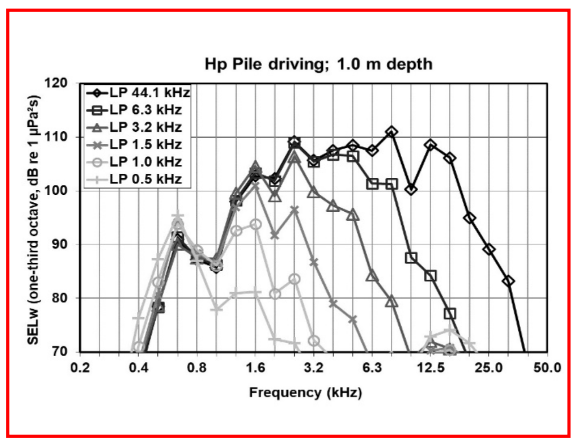
Behavioral Responses of a Harbor Porpoise (*Phocoena phocoena*) Depend on the Frequency Content of Playbacks of Pile-driving Sounds

SEAMARCO final report 2021-03
7 June 2021



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Depend on the Frequency Content of Playbacks of Pile-driving Sounds

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Nederlandse samenvatting

Gedragsreacties van een bruinvis (*Phocoena phocoena*) zijn gerelateerd aan de frequentie-inhoud van heigeluid

De komende decennia zullen er wereldwijd veel offshore windmolenparken worden gebouwd. Zo ook in de Noordzee door vrijwel alle omliggende landen. De tot nu meest gebruikte methode om windmolens te verankeren in de zeebodem is d.m.v. het heien van zogenaamde mono-piles. Daarbij worden hoge geluidsniveaus geproduceerd onderwater. Deze niveaus kunnen negatieve gevolgen hebben voor het gedrag en gehoor van zeedieren.

Bezorgdheid over de effecten van antropogeen geluid op zeezoogdieren heeft geleid tot pogingen om veiligheidscriteria vast te stellen voor toelaatbare niveaus van onderwatergeluid. Deze criteria zijn opgesteld voor gedragseffecten (bijv. Dosis-responsrelaties) en effecten op het gehoor [Tijdelijke gehoordrempelverschuiving (TTS) en permanente gehoordrempelverschuiving (PTS)].

Antropogene geluiden zoals onderwater offshore heigeluiden zijn breedbandig, maar het gehoor van zoogdieren is niet voor alle frequenties even gevoelig. Bij het opstellen van de huidige regelgeving voor wind op zee (Kader Ecologie en Cumulatie) kon nog geen rekening worden gehouden met die frequentiegevoeligheid, vanwege het ontbreken van frequentiegewogen dosis-responsrelaties.

Om de frequentieafhankelijke gevoeligheid voor gehoorschade (TTS en PTS) in te schatten bij zeezoogdieren, zijn inmiddels frequentiegewogen drempelwaarden voor het optreden van PTS voorgesteld voor een aantal zeezoogdiergroepen (Southall et al, 2019). De afgelopen jaren heeft SEAMARCO voor het Living Marine Research programma van de Amerikaanse Overheid en het Wind op Zee programma van de Nederlandse Overheid (WOZEP) TTS onderzoeken uitgevoerd met bruinvissen en gewone zeehonden om frequentiegewogen drempelwaarden voor het optreden van TTS en PTS voor deze soorten te kunnen maken.

Echter, het is niet vanzelfsprekend dat dezelfde frequentieweging kan worden gebruikt om de geluidsniveaus te voorspellen waarop gedragsreacties optreden bij deze diersoorten. Daarom heeft SEAMARCO samen met TNO in het kader van WOZEP een gedragsonderzoek uitgevoerd met een bruinvis, met als doel om de invloed van de frequentie-inhoud van heigeluid op de gedragsrespons bij bruinvissen te testen. De bruinvis werd daarvoor blootgesteld aan 6 hei-geluiden die allemaal hetzelfde breedbandige geluidsniveau hadden, maar die verschilden in de energie in het hoogfrequente deel van het spectrum.

De resultaten van de studie toonden aan dat bruinvissen vooral reageren op het hoogfrequente deel van het heigeluid spectrum, en minder op het laagfrequente deel, waar juist de meeste energie zit. Daarom is de conclusie dat het zinvol is om voor het inschatten van het effect van heigeluid op het gedrag van bruinvissen het geluid te wegen met een soort-specifieke weegcurve, zodat energie in het laagfrequente deel van het spectrum minder meetelt in de door de overheid op te stellen geluidnorm voor impulsief (hei) geluid om het effect op bruinvisgedrag te beperken.

Behavioral Responses of a Harbor Porpoise (*Phocoena phocoena*) Depend on the Frequency Content of Playbacks of Pile-driving Sounds

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Abstract

The loud, impulsive, broadband under-water sounds produced during offshore pile driving are known to have auditory and behavioral effects on harbor porpoises in the areas around piling sites. Southall et al. (2019) proposed criteria to prevent auditory effects in marine mammals, expressed as allowable thresholds for frequency-weighted sound exposure levels and unweighted peak sound pressure levels. Thresholds to prevent behavioral effects have not yet been set, and it is not clear whether or not weighting of piling sounds is useful for predicting behavioral responses and therefore required to set safety criteria and develop mitigation measures. A harbor porpoise in a pool with low background noise was exposed to playbacks of piling sounds for test periods of 15 min. Her behavioral responses (distance from the transducer, respiration rate, swimming speed and number of jumps from the water) were quantified in comparison to baseline periods without piling sound. The full-spectrum playback piling sound (sound 1) was recorded in the North Sea at 100 m from a piling site for a wind turbine. For comparison, five low-pass filtered (6.3, 3.2, 1.5, 1.0, and 0.5 kHz) versions of the sound, in which the bandwidth decreased with increasing number (reduced-spectrum sounds 2-6), were played back at the same duty cycle (46 strikes/min) and at very similar single-strike sound exposure levels (power average in the pool: 135 dB re 1 $\mu Pa^2 s$, $t_{90:}$ 90-100 ms). In test periods, the harbor porpoise responded to sounds 1-3 by moving away from the transducer and increasing her respiration rate, and to sounds 4-6 only by moving away from the transducer. In every test period with sounds 1 and 2, the porpoise's swimming speed was higher than in the associated baseline period, and she increased her swimming speed in seven of the 15 test periods with sound 3. She responded to sounds 1 and 2 by jumping occasionally. As the bandwidth of the piling sounds decreased (from the full-spectrum sound 1 to the most narrow-band reduced-spectrum mostly low-frequency sound 6), the porpoise's behavioral response became weaker. This indicates that harbor porpoises respond most strongly to the higher frequencies in piling sounds. Therefore, auditory weighting of the sound exposure level will improve prediction of behavioral responses, and behavioral response threshold levels for criteria should also be expressed as weighted sound exposure levels. Mitigation of the effects of piling sounds on harbor porpoise behavior should be focused on reducing the high-frequency part of the spectrum.

Keywords*:* Acoustics, auditory frequency weighting, behavior, coastal waters, conservation, disturbance, habitat, mammals, marine ecology, noise, odontocete, offshore, offshore wind farms, wind energy.

Introduction

Sound is important for odontocetes (toothed whales) as a means of orientation and communication, and to locate prey, conspecifics, and predators (Richardson et al., 1995; Nowacek et al., 2007; Wright et al., 2007). Therefore, odontocetes are likely to be disturbed by extraneous noise in their environment. In addition to natural sounds, human activities increasingly add noise to the environment, which may have negative effects on odontocetes by causing auditory masking, temporary or permanent hearing threshold shifts, or behavioral effects (NRC report, 2003).

Coastal waters support high densities of odontocetes and are heavily used by humans producing noise through, for example, construction of harbors, oil and gas industry operations, and construction of offshore wind farms. Although alternative methods of attaching wind turbines to the sea floor are being investigated, installation commonly involves percussion pile drivers, which produce high-amplitude, impulsive sounds. The duration, sound exposure level (SEL) and peak level of the sounds depend on the source level, the distance from the pile-driving site at which the sound is measured, and local water depth and sediment properties (Bellmann et al., 2020; Martin et al., 2020).

The effects of pile-driving sounds are of particular interest in relation to the harbor porpoise (*Phocoena phocoena*), because it has a wide distribution area in the coastal waters of the Northern Hemisphere, acute hearing, and functional hearing over a wide frequency range (Kastelein et al., 2017). Piling sound can reduce the ability of harbor porpoises to catch fish (Kastelein et al., 2019a) and cause them to flee from areas around piling sites (Tougaard et al., 2009; Dähne et al 2013). Kastelein et al. (2013c) conducted a dose-response study by exposing a harbor porpoise in a pool to recordings of pile-driving sounds made 100 m from a piling site. Above 0.63 kHz, the spectrum recorded at sea could be mimicked in the pool. Calculations based on a broadband SEL threshold showed that harbor porpoises would avoid piling noise up to a distance of 30 km away from a piling site (Kastelein et al., 2013c). This distance is at the high end of distances over which harbor porpoise have been observed to avoid piling sounds (Tougaard et al., 2009; Bailey et al., 2010; Brandt et al., 2011, 2018; Dähne et al., 2013; Haelters et al., 2014; Graham et al., 2019). At sea, as the distance from a piling sound source increases, the energy in the high-frequency part of the piling sound's spectrum is reduced, because water acts as a low-pass filter. Noise abatement measures such as air bubble screens also are more effective in reducing the high frequencies. Hearing sensitivity in harbor porpoises increases sharply between 0.1 kHz and 20 kHz (Kastelein et al., 2017), so that, depending on the level, the high-frequency part of a sound's spectrum may determine both the audibility of the sound (Kastelein et al., 2011a) and the severity of the behavioral response to it (Kastelein et al., 2012, 2013a, 2014, 2015, 2019b, Dyndo et al., 2015). The spectrum, level and duration of pile-driving sounds depend on properties of the pile (diameter, length, shape, wall thickness, depth in the sediment, etc.), the hammer size, the use of noise abatement methods, the environment (substrate, water depth, etc.), the propagation conditions, and the measurement distance from the sound source.

At present, unweighted noise levels are used to assess the impact of pile-driving sound on harbor porpoise behavior. The aim in the present study was to compare the response of a harbor porpoise to playbacks of piling sounds, at a duty cycle commonly used when driving monopiles for offshore wind turbines, with six different spectra due to differing degrees of low-pass filtering, but with the same broadband unweighted SEL. The ultimate goal was to improve predictions of behavioral responses of harbor porpoises to pile-driving sounds. As suggested by Tougaard et al. (2015), frequency weighting with a function approximating the inversed porpoise audiogram might be appropriate when assessing impact of impulsive (piling) sounds. We address the following question: is behavioral response in the harbor porpoise better explained by frequency-weighted metrics (Southall et al., 2019) or by unweighted metrics?

Methods

Study Animal and Facility

The female harbor porpoise (F05) used in this study was 9-10 years old, her body weight was ~ 44 kg, her body length 155 cm, and her girth at axilla ~ 80 cm. Her hearing was assumed to be representative, as it was similar to that of four other harbor porpoises (Kastelein et al., 2017). She received four meals of fish per day.

The study animal was kept at the SEAMARCO Research Institute, the Netherlands, in a pool complex specifically designed and built for acoustic research, consisting of an indoor pool (described in detail by Kastelein et al., 2010), and an outdoor pool (12 × 8 m, 2 m deep) in which the present study was conducted (**Figure 1**). The walls of the outdoor pool were made of plywood covered with nets on which aquatic vegetation grew (reducing high-frequency reflections). The bottom was covered with sloping sand. The water circulation system and the aeration system for the bio-filter were made to be as quiet as possible, and the pumps were switched off before sessions and kept off during sessions, so that there was no current in the outdoor pool. The equipment used to produce the sound stimuli was housed out of sight of the study animal, in a research cabin next to the pool (**Figure 1**).

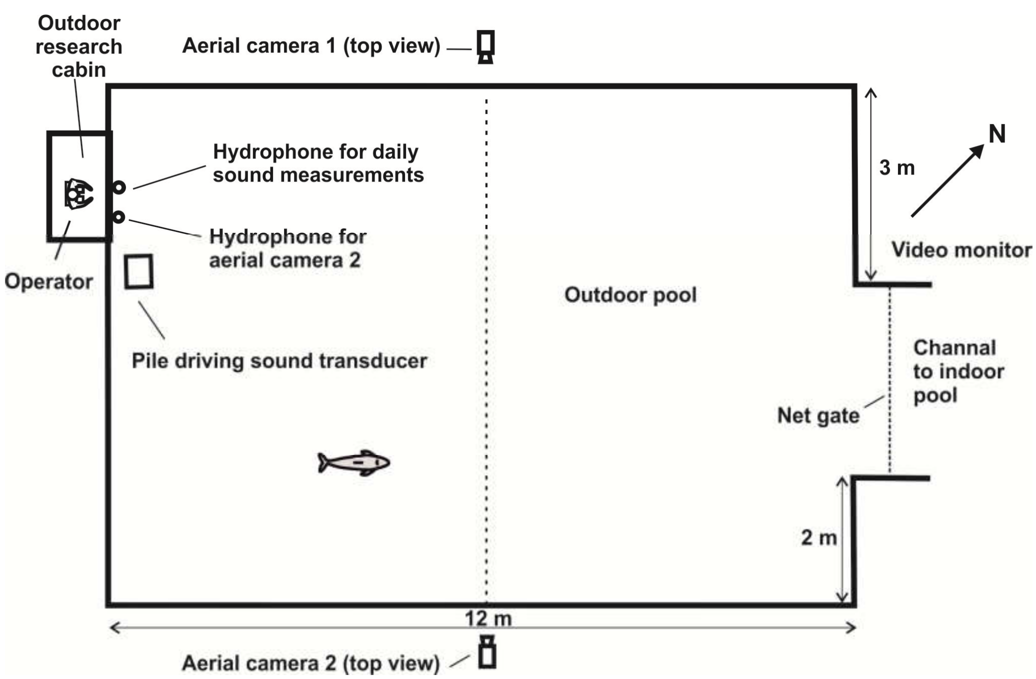

Figure 1. Top scale view of the outdoor study facility, showing the female harbor porpoise and the locations of aerial camera 1 (8 m above the water level), aerial camera 2 (5 m above the water level), the underwater transducer emitting the piling sounds at the bottom of the pool, the hydrophone (used to listen to the piling sounds and background noise), and the research cabin, which housed the video and audio equipment, the operator and the data collector. The pool was 2 m deep. The central dashed line shows the division of the pool into two halves (see **Tables 1 and 2**).

Acoustics

Background Noise - The background noise in the outdoor pool was measured twice during the study, between 0.025 kHz and 160 kHz, under conditions that were typical for the sessions (circulation pumps switched off, no rain; wind force Beaufort ≤ 4). The background noise level was low (**Fig. 2**). Above 3.2 kHz, the recorded level was so low that it was mainly determined by the self-noise of the recording equipment.

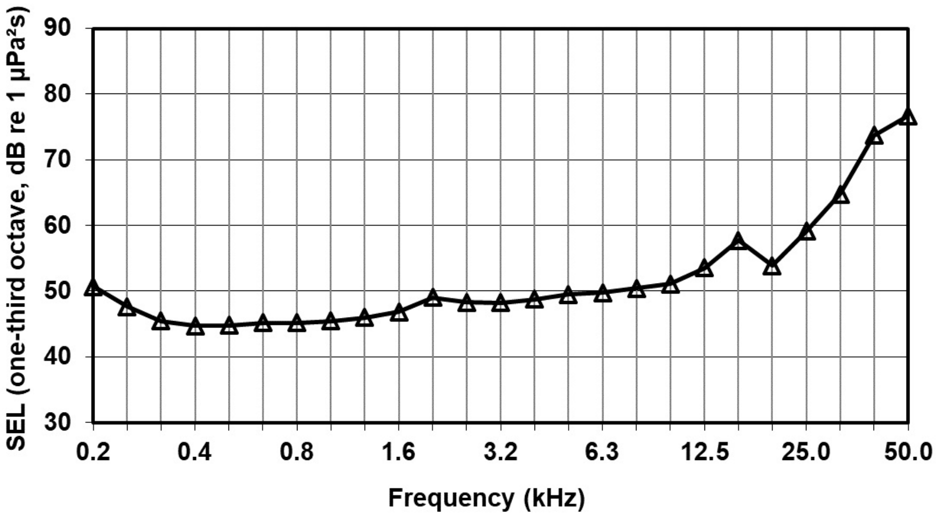

Figure 2. The mean background noise in the outdoor pool, represented in one-third octave (base-10) bands. Each mean was calculated from measurements at three depths, and the sound pressure level was averaged over 10 s and converted to sound exposure level (SEL) for a 100 ms time period by adding $10\log_{10}(0.1)$. The level is very low; for most of the spectrum, it is below the level measured during Sea State 1 in the open sea. Above 3.2 kHz, the background noise level is dominated by the self-noise of the recording system.

Test Stimuli - The effect of the frequency content of the sound to which a porpoise is exposed on its behavioral response was tested by playing back filtered pile-driving sounds. Offshore pile-driving sounds were recorded at 100 m from a foundation pile that was being driven into the seabed for a wind turbine in the Dutch offshore wind farm 'Egmond aan Zee'. A WAV file was made of a series of five consecutive pile-driving strike sounds with a strike rate of 46 strikes/min. The recording was sampled at 88.2 kHz sample frequency and high-pass filtered (2nd order Butterworth) at 0.5 kHz, because lower frequencies could not be reproduced efficiently due to the characteristics of the transducer and, to some extent, due to the limited water depth in the pool (2 m; Kastelein et al, 2013a). This playback sound, which has been used in previous pool studies (Kastelein et al., 2013b, 2013c), is referred to as sound 1, or the 'full-spectrum' piling sound.

The full-spectrum piling sound was further modified by means of low-pass filtering (2nd order Butterworth). Five filter frequencies were selected, at center frequencies of one-third octave (base-10) bands between 0.5 kHz and 20 kHz. The amplitude of the five reduced-spectrum piling sounds was adjusted to keep the unweighted broadband SEL as consistent as possible for all six piling sounds (**Table 1**), and the six piling sounds were played back at the same duty cycle. Single-pulse SEL was selected as the appropriate metric to describe the magnitude of exposure, in order to maintain consistency with previous studies and with legislation in some countries bordering the geographic range of the harbor porpoise, such as Germany.

Table 1. The sound exposure level (SEL) in the outdoor pool for each of the six playback piling sounds. Statistics (power mean, dB mean ± standard deviation) are presented for the entire pool, and separately for each half of the pool: locations ≤ 6 m and > 6 m from the south-western end of the pool, where the transducer was (see **Figure 1**). Sound 1 is the full-spectrum playback piling sound; sounds 2-6 are reduced-spectrum piling sounds.

Sound	Low-pass filter frequency	SEL (entire pool) (n = 231)		SEL (distance ≤ 6 m) (n = 126)		SEL (distance > 6 m) (n = 105)	
	kHz	Power mean	dB mean ± SD	Power mean	dB mean ± SD	Power mean	dB mean ± SD
1 (full spectrum)	44.1	135	134 ± 3	137	136 ± 3	132	131 ± 1
2	6.3	136	134 ± 3	138	137 ± 3	132	132 ± 1
3	3.2	135	134 ± 3	138	137 ± 3	132	132 ± 1
4	1.5	135	133 ± 3	137	136 ± 3	132	131 ± 2
5	1.0	135	133 ± 4	137	135 ± 4	131	131 ± 2
6	0.5	133	131 ± 4	135	134 ± 4	129	128 ± 3

Playback Equipment - The digitized sequences (WAV files; sample frequency 88.2 kHz, 16-bit) were played back in a loop by a laptop computer (Acer Aspire - 5750) with a program written in LabVIEW, to an external data acquisition card (National Instrument - USB6259); the output was digitally controlled in 1 dB steps with the LabVIEW program. The output of the data acquisition card went through a custom-built buffer to a power amplifier (East & West - LS5002) which drove the transducer (Lubell - LL1424HP) through an isolation transformer (Lubell - AC1424HP). The transducer was placed on the pool floor, parallel to the bottom, at the south-western end of the outdoor pool (3 m from the western corner; **Fig. 1**).

Before each session, a 1.5 kHz FM signal was used to monitor the output of the sound system to the transducer via an oscilloscope (Tektronix - 2201), a voltmeter (GW Instek-GDM-8255A), and the underwater sound was monitored with a custom-built hydrophone connected via a spectrum analyzer (Velleman - PCSU1000) to a laptop computer (Samsung – NP-N145). The attenuation system was linear over the sound pressure level range used in the study.

The audible background noise and the piling sounds were monitored via a hydrophone (Labforce - 90.02.01) and a conditioned charge pre-amplifier (SEAMARCO - CCAMS1000-3). The output of the pre-amplifier was digitized via the analog-to-digital converter (König-grabber - CMP-USBR60) and recorded on the computer (Acer Aspire - 5750G) in synchrony with the video images. The output of the pre-amplifier was also fed to an amplified loudspeaker (Medion - MD5432), so that the operator in the research cabin could monitor the human-audible part of the background noise during sessions.

Recording Equipment for Sound in the Pool - The SEL distribution of the piling sounds and the background noise in the outdoor pool were measured while the porpoise was not present. The recording and analysis equipment consisted of three hydrophones (Brüel & Kjaer (B&K) - 8106), a multichannel high-frequency analyzer (B&K - PULSE-3560 C), and a laptop computer with B&K PULSE software (Labshop version 12.1). The system was calibrated with a pistonphone (B&K - 4223). The recordings were made with a 0.01 kHz high-pass filter and at a sample rate of 512 kHz.

Determination of the Sound Exposure Level used During Playback - During a pilot study, the received SEL of the full-spectrum playback piling sound (sound 1) was gradually increased until it caused the porpoise to respond by increasing her distance to the transducer and respiration rate. At 2 m horizontal offset from the transducer, this unweighted SEL was 140.4 ± 1.4 dB re 1 μPa^2s (mean ± standard deviation; measured at three depths, n = 3). The playback piling sound was not distorted, and this SEL was selected for all six piling sounds and was used in all sessions.

Acoustic Characterization of Piling Sound Sequences - The six piling sounds were characterized in terms of the measured SEL in dB re 1 μPa^2s over their duration (t_{90} in s): the time interval between the points when the cumulative SEL (the integrated broadband sound pressure level squared) reached 5% and 95% of the total exposure. Thus, the duration contained 90% of the total energy in the sound (Madsen, 2005). The piling sounds were recorded in the pool. The one-third octave band spectrum of the unweighted SEL of each of the six piling sounds, measured at 1 m depth and 2 m from the transducer, is shown in **Figure 3a**. Compared to the full-spectrum sound 1, the reduced-spectrum sounds 2-6 had less energy in the high-frequency part of the spectrum.

The one-third octave band spectrum of the vhf-weighted SEL (SELw; Southall et al., 2019) of each of the six piling sounds, measured at 1 m depth, 2 m from the transducer, is shown in **Figure 3b.** The vhf weighting removed much of the energy in the low-frequency part of the spectrum.

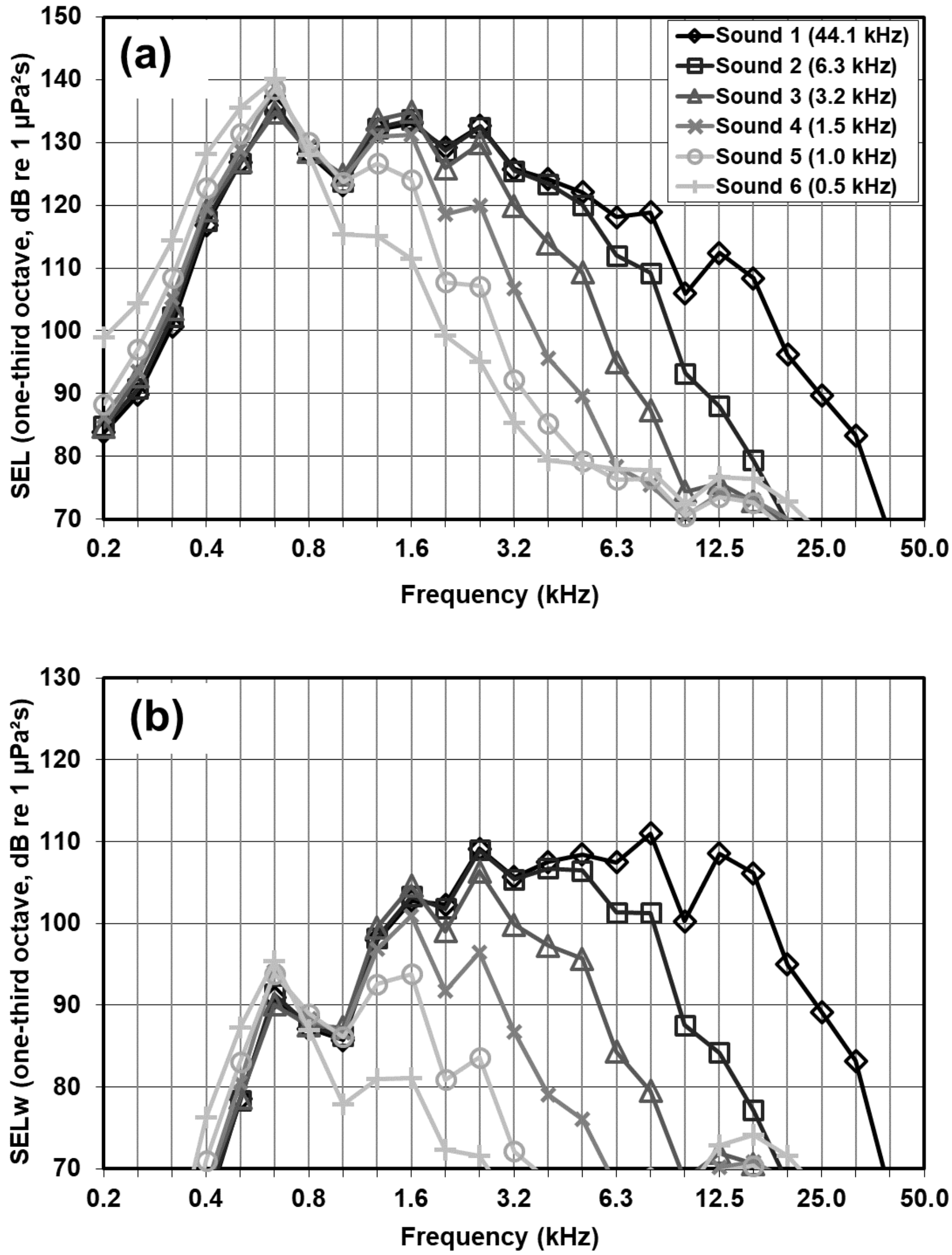

Figure 3. The one-third octave band spectra of the unweighted sound exposure levels (SEL; a) and vhf-weighted sound exposure levels (SELw; b; Southall et al., 2019) of each of the six playback piling sounds at six different low-pass filter settings (sound 1 is the full-spectrum playback piling sound; see legend for low-pass frequencies). The sounds were measured at 1 m depth, 2 m from the transducer.

Sound Exposure Levels in the Pool During Playback - To determine the sound distribution in the pool, the SEL of each of the six piling sounds was measured at 77 locations at three depths (0.5, 1.0, and 1.5 m), for one signal per sequence, per piling sound, per location. The distribution of the received unweighted SELs at the 231 positions in the pool are shown in **Figure 5**. The distribution of the received SELw (Southall et al., 2019) are shown in **Figure**

6; the five reduced-spectrum piling sounds showed a decreasing SELw (Southall et al., 2019). **Tables 1 and 2** show that the variation in the levels increases with decreasing bandwidth (lower filter frequency), so that the SELw at distances ≤ 6 m from the south-western end of the pool, where the transducer was, are significantly higher than those at distances > 6 m. The unweighted SEL remains approximately constant (**Table 1**), but the SELw decreases with decreasing bandwidth (lower filter frequency; **Table 2**).

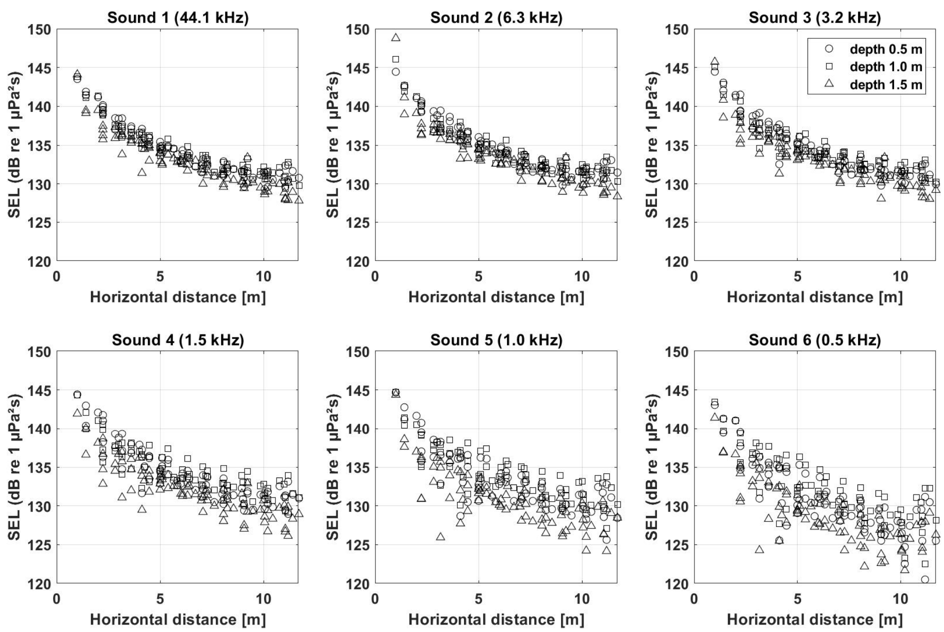

Figure 5. The one-third octave unweighted sound exposure level (SEL) distribution in the pool for each of the six playback piling sounds with different low-pass filtering levels, as a function of the horizontal distance to the transducer, measured at three depths (0.5m: ○, 1.0 m: □ and 1.5 m: Δ; n = 77 measurements per depth). Sound 1 is the full-spectrum piling sound; sounds 2-6 are reduced-spectrum piling sounds. The variation in SEL increases as the bandwidth is reduced (so that the high-frequency part of the spectrum contains less energy). Most of the unweighted SEL is determined by the peak in the low-frequency part of the spectrum (~0.6 kHz; **Figure 3a**).

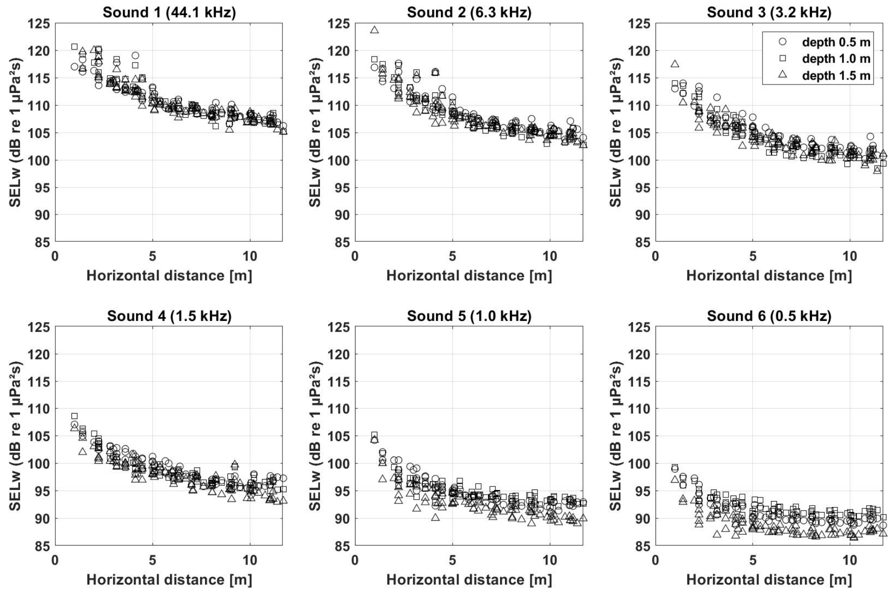

Figure 6. The one-third octave vhf-weighted sound exposure level (SELw; Southall et al., 2019) distribution in the pool for each of the six playback piling sounds with different low-pass filtering levels, as a function of the distance to the transducer, measured at three depths (0.5m: ○, 1.0 m: □ and 1.5 m: Δ; n = 77 measurements per depth). Sound 1 is the full-spectrum piling sound; sounds 2-6 are reduced-spectrum piling sounds. The weighted broadband SELw is lower than the unweighted SEL (**Figure 5**), as much of the energy in piling sounds is in the low-frequency part of the spectrum, and this energy is removed by the vhf-weighting.

Table 2. The sound exposure level in the pool measured with vhf weighting (SELw; Southall et al., 2019) for the full-spectrum sound 1 (unweighted and weighted) and for each of the five reduced-spectrum sounds 2-6 (weighted). Statistics (power mean, dB mean and standard deviation) are presented for the entire pool, and separately for each half of the pool: locations ≤ 6 m and > 6 m from the south-western end of the pool, where the transducer was (**Figure 1**). Sound 1 is the full-spectrum playback piling sound; sounds 2-6 are reduced-spectrum piling sounds.

Sound	Low-pass filter frequency	SELw (entire pool) (n = 231)		SELw (distance ≤ 6 m) (n = 126)		SELw (distance > 6 m) (n = 105)	
	kHz	Power mean	dB mean ± SD	Power mean	dB mean ± SD	Power mean	dB mean ± SD
1 (unweighted full spectrum)	44.1	135	134±3	137	136 ± 3	132	131 ± 1
1 (weighted full spectrum)	44.1	113	111 ± 3	115	114 ±3	108	108 ± 1
2	6.3	110	108 ± 4	113	111 ± 3	106	105 ± 1
3	3.2	106	104 ±3	108	107 ±3	102	102 ± 1
4	1.5	99	98 ±3	101	101 ± 2	97	96 ± 1
5	1.0	95	94 ±3	97	96 ± 3	93	92 ± 1
6	0.5	91	90 ±2	93	92 ± 3	90	89 ± 2

Video Recording

The harbor porpoise's behavior was filmed from above by a waterproof aerial camera (aerial camera 1; Conrad - 750940) with a wide-angle lens and a polarizing filter to prevent saturation of the video image by glare from the water surface. Aerial camera 1 was placed on a pole 8 m above the water surface on the north-western side of the outdoor pool (**Figure 1**). The entire surface of the pool was captured on the video image. The image was visible to the operator and was digitized by an analog-to-digital converter (König – grabber-CMP-USBR60) and stored on a laptop computer (Acer Aspire - 5750G). The porpoise was also filmed by an action camera (aerial camera 2) on a pole 5 m above the water surface. The recordings were analyzed after the sessions were conducted.

Experimental Procedure

The transducer producing the piling sounds was positioned in the water at the south-western end of the pool at the start of each day (**Figure 1**). A session consisted of a baseline period (no sound transmitted) or a test period (playback piling sound transmitted), followed by a pause of random length (1-5 h) in which no sound was emitted, followed by either a test or a baseline period. All test and baseline periods lasted 15 minutes. In each session, one baseline and one test period were conducted, in random order. One session was conducted per day, five to seven days per week, beginning between 08.30 and 16.00 h, and with random timing relative to the feeding moments. During the sessions, only the operator and data collector were allowed in the vicinity of the outdoor pool; they sat very still in the research cabin.

In each test period, one of the six piling sounds was transmitted. Each sound was tested in 15 sessions, resulting in 90 sessions (22.5 h of baseline periods and 22.5 h of test periods in all). The six piling sounds were tested in random order within the 90 sessions. To prevent masking of the sounds by background noise and reduce the influence of the weather on the behavior of the porpoise, tests were not carried out during rain or when the wind force was Beaufort 5 or above. The study was conducted between April and October 2020.

Response parameters and behavioral data recording

For each of the six piling sounds, four response parameters were quantified and compared for the paired baseline and test periods within each session.

Firstly, the porpoise's distance from the transducer was quantified as follows, to determine whether she responded to the sounds by swimming away from the transducer. From the video camera 2 recordings, the locations where the porpoise surfaced during the baseline and test periods were recorded on a grid superimposed on the computer screen. The grid corresponded to a pool grid of 1 × 1 m, and was made by connecting lines between 1 m markers on the pool's sides. The grid square in which the porpoise surfaced was determined, and the center point of the grid square was used to calculate the distance between the porpoise's surfacing location and the transducer, via triangulation (ignoring depth). The water was always clear, and when light conditions (which depended on the weather and the time of day) were such that the bottom of the pool was visible and the porpoise could be seen well below the water surface, it was clear that the surfacing locations were a good indication of the porpoise's general swimming area.

Secondly, the porpoise's respiration rate (number of breaths in 15 minutes) in each baseline period was compared to the number during the test period in the same session.

Thirdly, the porpoise's relative swimming speed in the test period, relative to the baseline period of that session, was recorded (-1 = slower than the baseline, 0 = similar to the baseline, and 1 = faster than the baseline).

Fourthly, although the porpoise rarely jumped out of the water, all jumps during baseline and test periods were recorded.

Analysis

To investigate in detail the porpoise's response to the six piling sounds, paired t-tests were used to compare her distance from the transducer and respiration rate in baseline periods and associated test periods. For all analyses, assumptions of the tests were conformed to, and the level of significance was 5% (Zar, 1999). Paired t-tests on the same dependent variable (distance from the transducer and respiration rate) were not considered to be independent, so *P*-values were adjusted according to the Holm–Bonferroni method (Quinn and Keough, 2002). Swimming speed and number of jumps were compared without statistical analysis due to the small number of occurrences.

Results

During baseline periods, the harbor porpoise usually swam large ovals in the outdoor pool. The distance between her surfacing locations and the transducer (mean ± standard deviation: 5.2 ± 0.2 m) and her respiration rate (53 ± 0.6 breaths in 15 minutes) were similar in all 90 baseline periods, and she never jumped.

In test periods, the harbor porpoise responded to piling sounds 1-3 by moving away from the transducer and increasing her respiration rate, and to piling sounds 4-6 only by moving away from the transducer (**Table 3, Figure 7**). She responded to piling sounds 1 and 2 by jumping occasionally (**Table 3**). In every test period with sounds 1 and 2, the porpoise's swimming speed was higher than in the associated baseline period, and she increased her swimming speed in seven of the 15 test periods with sound 3 (**Table 3**).

As the bandwidth of the piling sound decreased (from the full-spectrum sound 1 to the most narrow-band reduced-spectrum sound 6), the harbor porpoise's behavioral response became weaker. In response to sounds 4-6, she only moved slightly away from the transducer. Her mean displacement distance, relative to the mean distance to the transducer in the baseline periods, was 4.4 m (± 2.0 m) in response to sound 1; it decreased to 1.4 m (± 2.0 m) for sound 6 (**Figure 7a**).

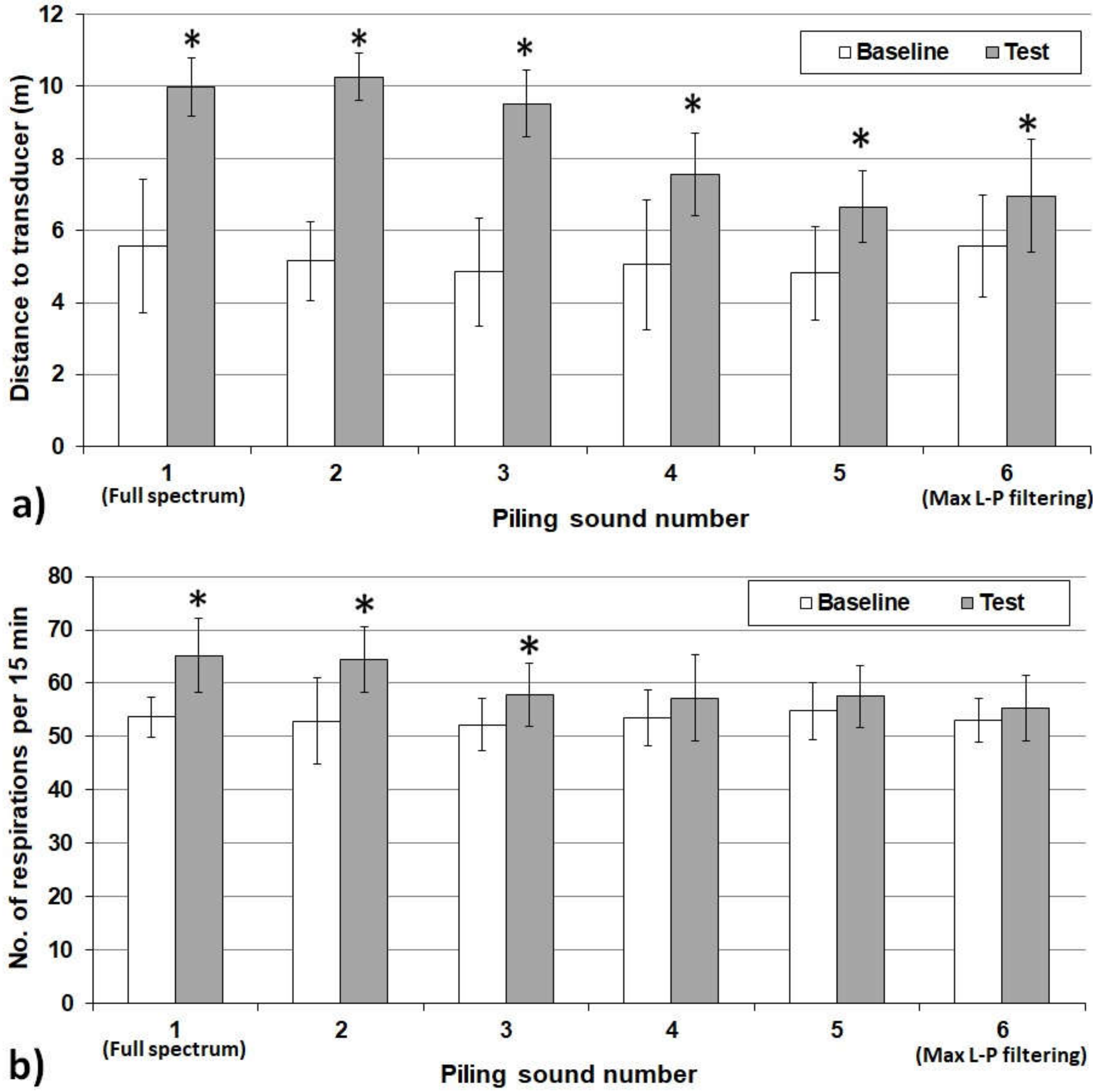

Figure 7. The behavior of the female harbor porpoise during baseline periods without piling sound, and in test periods with six playback piling sounds (each at an unweighted mean single-strike sound exposure level of ~135 dB re 1 μPa^{2}s): a) the distance from the transducer (12 m is the length of the outdoor pool), b) the number of respirations per 15 min. Each bar indicates mean ± one standard deviation (n = 15), an * indicates a significant difference between baseline and test periods (paired t-tests; see **Table 3**). Sound 1 is the full-spectrum piling sound; sounds 2-6 are reduced-spectrum piling sounds, and sound 6 has maximum low-pass filtering.

Table 3. Results of paired t-tests to compare the porpoise's distance from the transducer and respiration rate in baseline and associated test periods, for six playback piling sounds; see also **Figure 7**. The sample size for each test is 15. T-values and adjusted *P*-values (Holm–Bonferroni method; Quinn and Keough, 2002) are shown; NS = not significant. In all cases, the mean value for the test period was greater than that for the baseline period. The porpoise responded to piling sounds 1-3 by moving away from the transducer and increasing her respiration rate, and to piling sounds 4-6 by moving away from the transducer. No jumps occurred in baseline periods; the total number of jumps recorded in all test periods is shown for each piling sound. The porpoise responded to piling sounds 1 and 2 by jumping occasionally. In every test period with sounds 1 and 2, the porpoise's swimming speed was higher than in the associated baseline period. Of the 15 test periods with sound 3, the porpoise increased her swimming speed in seven.

Piling sound	Low-pass filter freq.	Distance from transducer	Respiration rate	Relative swimming speed	Number of jumps
	kHz	m; test minus baseline	Breaths/15 min period; test minus baseline	Baseline swimming speed = zero	In all 15 test periods for each piling sound combined; zero jumps occurred in baseline periods
1 (full spectrum)	44.1	T=8.68, *P*=0.000	T=6.71, *P*=0.000	Increased in all test periods	6, spread over four test periods
2	6.3	T=21.37, *P*=0.000	T=5.71, *P*=0.000	Increased in all test periods	5, in one test period
3	3.2	T=8.84, *P*=0.000	T=3.30, *P*=0.020	Increased in 7 of 15 test periods	0
4	1.5	T=4.95, *P*=0.000	T=1.56, *P*=0.423 NS	Unchanged in all test periods	0
5	1.0	T=4.02, *P*=0.002	T=1.49, *P*=0.316 NS	Unchanged in all test periods	0
6	0.5	T=2.73, *P*=0.016	T=1.47, *P*=0.164 NS	Unchanged in all test periods	0

Discussion and Conclusions

Evaluation of Study Animal and Playback Piling Sounds

The hearing of the study animal was similar to that of four young male harbor porpoises of similar age (Kastelein et al., 2017), and was thus probably representative of the hearing of harbor porpoises of her age, suggesting that she perceived the sounds as most harbor porpoises would. The effect of a sound on behavior can vary between individuals and may be context-dependent, but the aim of the present study was to compare the effects of six piling sounds on one individual. The differences in response that were observed are valid, because the sessions occurred under very low and, more importantly, constant background noise conditions.

After each test period in which the porpoise responded to the sound, her behavior was observed to return to normal immediately; being exposed to the piling sounds at the levels used in this study for 15 minutes had no lasting effect on the porpoise's behavior. A quick return to baseline behavior had been seen in previous acoustic alarm (pinger) studies with harbor porpoises (Kastelein et al., 2000, 2001, 2006 and 2008a, b) and was the reason for not including a post-test observation period, as was done in a previous pinger study (Kastelein et al., 2000).

Harbor porpoises at sea do not return to piling sites soon after pile driving has stopped: Brandt et al. (2011) observed reduced porpoise acoustic activity within a 2.6 km range from a piling site 24-72 h after sounds stopped, but shorter return times (~6 h) occurred where noise abatement methods such as air bubble screens were employed (Dähne et al. 2017; Brandt et al. 2018). The observed difference may relate to the SEL experienced by the porpoises, which, in the case of porpoises at sea, depends on their distance to the site when piling starts. The SELs in the present study were much lower than those experienced by harbor porpoises in the vicinity of offshore construction sites (~170-180 dB re 1 $\mu Pa^2 s$ at 750 m, for piling without noise abatement methods; Brandt et al., 2018).

The reduced energy in the very low end of the frequency spectrum of playback piling sounds relative to real piling sounds at sea was probably irrelevant for the harbor porpoise in the present study, as the hearing sensitivity of harbor porpoises is low for sounds below 1 kHz (Kastelein et al., 2017; Southall et al., 2019), and they respond predominantly to energy above 1 kHz (Dyndo et al., 2015). The playback piling sounds used in the present study served as examples.

The four response parameters may have been related to one another. Faster swimming requires a greater oxygen uptake via an increased respiration rate. At higher swimming speeds, porpoises can save energy by leaping clear of the water (Weihs, 2002), and while airborne they are not subjected to underwater noise. Even in response to the most reduced-spectrum sounds, the harbor porpoise was displaced from its usual swimming pathway. Displacement, followed by the faster swimming, increased respiration rate and jumps, suggests that the behavioral responses of the porpoise were cumulative. Compared to pile driving at sea, the experimental conditions involved lower sound levels and less space, so extrapolation of the results directly to wild harbor porpoises should be done with caution..

Predicting Behavioral Responses of Harbor Porpoises to Pile-driving Sounds

Exposure to the full-spectrum playback piling sound (sound 1) at an average unweighted broadband SEL in the pool of 135 dB re 1 $\mu Pa^2 s$ resulted in significant increases in the porpoise's distance from the transducer and respiration rate. However, it may be unrealistic to use playback studies to derive an SEL threshold for behavioral responses to unweighted broadband SEL values measured during pile driving at sea (Kastelein et al., 2013c). The observed reduction in the porpoise's responses to sounds played back at almost equal unweighted broadband SELs, but with decreasing frequency bandwidth (sounds 1-6), demonstrates that the frequency content of sounds is an important factor determining the response of harbor porpoises. The decreasing response was aligned with decreasing values of SELw, measured as proposed by Southall et al. (2019). Exposure to pile-driving sound with average SELw values increasing from 90 dB to 111 dB re 1 $\mu Pa^2 s$ in the present study resulted in increasing avoidance of the area close to the transducer, and a significantly increased respiration rate was measured at SELw values of ~100 dB re 1 $\mu Pa^2 s$ and above. This suggests that it is worth investigating whether the observed relationship between weighted SELw and avoidance behavior of harbor porpoises can be confirmed in field research (see Brandt et al., 2018), and whether a generalized, frequency-weighted response threshold can be established, as conjectured by Tougaard et al. (2015). It is clear that, in order to improve current predictions of behavioral responses of harbor porpoises to pile-driving sounds, unweighted metrics should be replaced by metrics that include an auditory weighting (Southall et al., 2019).

Mitigation
The present study shows that the high-frequency part of the spectrum of impulsive pile-driving sounds has a relatively large effect on the behavior of harbor porpoises, confirming findings from previous studies with impulsive and non-impulsive sounds. When the same study animal was exposed to impulsive airgun sounds produced behind an air bubble screen, the screen removed the high-frequency part of the spectrum and thus reduced the porpoise's response to the sounds (Kastelein et al., 2019b). The startle responses of harbor porpoises to 1-2 kHz and 6-7 kHz sweeps with high-frequency harmonics are also stronger than their responses to the same signals without harmonics (Kastelein et al., 2012). Therefore, in order to reduce the impact of impulsive sounds (and other broadband sounds) on harbor porpoises in the wild, reduction of the energy in the high-frequency part of the spectrum should be the priority. Removal of only this high-frequency energy in mitigation would make mitigation more feasible and affordable. Bubble screens are already in use in some countries in which offshore pile driving occurs, and have proved to be very effective in reducing radiated noise above 1 kHz (Dähne et al., 2017; Tougaard and Dähne, 2017).

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Literature Cited

Bailey, H., Senior, B., Simmons, D., Rusin, J., Picken, G., & Thompson, P.M., (2010). Assessing underwater noise levels during pile-driving at an offshore windfarm and its potential effects on marine mammals. *Marine Pollution Bulletin 60*, 888-897.

Bellmann M. A., Brinkmann J., May A., Wendt T., Gerlach S. & Remmers, P. (2020). Underwater noise during the impulse pile-driving procedure: Influencing factors on pile-driving noise and technical possibilities to comply with noise mitigation values.

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Brandt, M. J., Diederichs, A., Betke, K., & Nehls, G. (2011). Responses of harbour porpoises to pile driving at the Horns Rev II offshore wind farm in the Danish North Sea. *Marine Ecological Progress Series 421*, 205-216.

Brandt, M.J., Dragon, A.C., Diederichs, A, Bellmann, M.A., Wahl, V., Piper, W., Nabe-Nielsen, J., & Nehls, G. (2018). Disturbance of harbour porpoises during construction of the first seven offshore wind farms in Germany. *Marine Ecological Progress Series, 596*, 213–232. (doi:10.3354/meps12560

Dähne, M., Gilles, A., Lucke, K., Peschko, V., Adles, S., Krügel, K., Sundermeyer, J., & Siebert, U., (2013). Effects of pile-driving on harbour porpoises (*Phocoena phocoena*) at the first offshore windfarm in Germany. *Environmental Research Letters 8*, 1-16.

Dähne, M., Tougaard, J., Cartensen, J., Rose, A., & Nabe-Nielsen, J. (2017). Bubble curtains attenuate noise from offshore wind farm construction and reduce temporary habitat loss for harbour porpoises. *Marine Ecological Progress Series 580*, 221–237. https://doi.org/10.3354/meps12257

Dyndo, M., Wiśniewska, D. M., Rojano-Doñate, L. & Madsen, P. T. (2015). Harbour porpoises react to low levels of high frequency vessel noise. *Scientific Reports, 5*, 11083.

Graham, I. M., Merchant, N. D., Farcas, A., Barton, T.R., Cheney, B., Bono, S., & Thompson, P.M. (2019). Harbour porpoise responses to pile-driving diminish over time. *Royal Society Open Science 6,* 190335. http://dx.doi.org/10.1098/rsos.190335

Haelters, J., Dulière, V., Vigin, L., & Degraer, S. (2014). Towards a numerical model to simulate the observed displacement of harbour porpoises *Phocoena phocoena* due to pile driving in Belgian waters. *Hydrobiologia, 756*, 105-116. DOI 10.1007/s10750-014-2138-4

Kastelein, R.A., Rippe, H.T., Vaughan, N., Schooneman, N.M., Verboom, W.C., & de Haan, D., (2000). The effect of acoustic alarms on the behavior of harbor porpoises (*Phocoena phocoena*) in a floating pen. *Marine Mammal Science, 16*, 46-64.

Kastelein, R.A., de Haan, D., Vaughan, N., Staal, C., & Schooneman, N.M., (2001). The influence of three acoustic alarms on the behaviour of harbour porpoises (*Phocoena phocoena*) in a floating pen. *Marine Environmental Research, 52*, 351-371.

Kastelein, R.A., Jennings, N., Verboom, W.C., de Haan, D., & Schooneman, N.M. (2006). Differences in the response of a striped dolphin (*Stenella coeruleoalba*) and a harbour porpoise (*Phocoena phocoena*) to an acoustic alarm. *Marine Environmental Research, 61*, 363-378.

Kastelein, R.A., Verboom, W.C., Jennings, N., & de Haan, D. (2008a). Behavioral avoidance threshold level of a harbor porpoise (*Phocoena phocoena*) for a continuous 50 kHz pure tone (L). *Journal of the Acoustical Society of America, 123*, 1858-1861.

Kastelein, R.A., Verboom, W.C., Jennings, N., de Haan, D., & van der Heul, S. (2008b). The influence of 70 and 120 kHz tonal signals on the behavior of harbor porpoises (*Phocoena phocoena*) in a floating pen. *Marine Environmental Research, 66*, 319-326.

Kastelein, R.A., Hoek, L., de Jong, C.A.F., & Wensveen, P.J., (2010). The effect of signal duration on the underwater detection thresholds of a harbor porpoise (*Phocoena phocoena*) for single frequency-modulated tonal signals between 0.25 and160 kHz. *Journal of the Acoustical Society of America, 128*, 3211-3222.

Kastelein, R. A., Hoek, L., & de Jong, C. A. F. (2011a). Hearing thresholds of a harbor porpoise (*Phocoena phocoena*) for sweeps (1-2 kHz and 6-7 kHz bands) mimicking naval sonar signals. *Journal of the Acoustical Society of America, 129*, 3393-3399. http://dx.doi.org/10.1121/1.3569739.

Kastelein, R.A., Steen, N., Gransier, R., Wensveen, P.J., & de Jong, C.A.F. (2012). Threshold received sound pressure levels of single 1-2 kHz and 6-7 kHz up-sweeps and down-sweeps causing startle responses in a harbor porpoise (*Phocoena phocoena*). *Journal of the Acoustical Society of America, 131*, 2325-2333.

Kastelein, R.A., Gransier, R., van den Hoogen, Marjan, & Hoek, L. (**2013a**). Brief behavioral response threshold levels of a harbor porpoise (*Phocoena phocoena*) to five helicopter dipping sonar signals (1.33 to 1.43 kHz). *Aquatic Mammals, 39*, 162-173, DOI 10.1578/AM.39.2.2013.162.

Kastelein, R.A., Hoek, L., Gransier, R., & de Jong, C.A.F., (2013b). Hearing thresholds of a harbor porpoise (*Phocoena phocoena*) for playbacks of multiple pile driving strike sounds. *Journal of the Acoustical Society of America, 134*, 2302-2306.

Kastelein, R. A., van Heerden, D., Gransier, R., & Hoek, L (2013c). Behavioral responses of harbor porpoise (*Phocoena phocoena*) to playbacks of broadband pile driving sounds. *Marine Environmental Research, 92*, 206-214, DOI: 10.1016/j.marenvres.2013.09.020

Kastelein, R. A., Schop, J., Gransier, R., Steen, N., & Jennings, N. (2014). Effect of series of 1-2 kHz and 6-7 kHz up-sweeps and down-sweeps on the behavior of a harbor porpoise (*Phocoena phocoena*). *Aquatic Mammals 40(3)*, 232-242, DOI 10.1578/AM.40.3.2014.232.

Kastelein, R. A., van den Belt, I., Gransier, R, & Johansson, T. (2015). Behavioral responses of a harbor porpoise (*Phocoena phocoena*) to 24.5-25.5 kHz sonar down-sweeps with and without side bands. *Aquatic Mammals, 41*, 400-411. DOI 10.1578/AM.41.4.2015.400

Kastelein, R.A., Helder-Hoek, L. & Van de Voorde, S. (2017). Hearing thresholds of a male and a female harbor porpoise (*Phocoena phocoena*). *Journal of the Acoustical Society of America, 142*, 1006–1010. DOI: http://dx.doi.org/10.1121/1.4997907.

Kastelein, R.A., Huijser, L. A. E., Cornelisse, S., Helder-Hoek, L., Jennings, N., & de Jong, C. A.F. (2019a). Effect of pile-driving playback sound level on fish-catching efficiency in harbor porpoises (*Phocoena phocoena*). *Aquatic Mammals, 45(4)*, 398-410, DOI 10.1578/AM.45.4.2019. 398

Kastelein, R.A., von Benda-Beckmann, A.M., Lam, F-P. A., Jansen, E, & de Jong, C.A.F. (2019b). Effect of a bubble screen on the behavioral responses of captive harbor porpoises (*Phocoena phocoena*) exposed to airgun sounds. *Aquatic Mammals, 45(6)*, 706-716, DOI 10.1578/AM.45.6.2019.706

Madsen, P. T. (2005). Marine mammals and noise: Problems with root mean square sound pressure levels for transients. *Journal of the Acoustical Society of America, 117*, 3952-3957.

Martin, S.B., Lucke, K., & Barclay, D.R. (2020). Techniques for distinguishing between impulsive and non-impulsive sound in the context of regulating sound exposure for marine mammals. *Journal of the Acoustical Society of America 147 (4)*, 2159–2176

Nowacek, D.P., Thorne, L.H., Johnston, D.W., & Tyack, P.L. (2007). Responses of cetaceans to anthropogenic noise. *Mammal Review, 37*, 81–115.

Quinn, G.P., & Keough, M.J., (2002). Experimental Design and Data Analysis for Biologists. Cambridge, Cambridge University Press, p. 537.

Richardson, W.J., Greene, C.R., Malme, C.I., & Thomson, D.H., (1995). Marine Mammals and Noise. Academic Press, London, p. 576.

Southall, B.L., Finneran, J. J., Reichmuth, C., Nachtigall, P. E., Ketten, D. R., Bowles, A. E.,

(….), & Tyack, P. L. (2019). Marine mammal noise exposure criteria: updated scientific recommendations for residual hearing effects. *Aquatic Mammals 45 (2)*, 125-232. DOI 10.1578./AM.45.2.2019.125

Tougaard, J., Carstensen, J., Teilmann, J., Skov, H., & Rasmussen, P. (2009). Pile driving zone of responsiveness extends beyond 20 km for harbor porpoises (*Phocoena phocoena*) (L.). *Journal of the Acoustical Society of America, 126*, 11-14.

Tougaard, J., Wright, A.J. & Madsen, P.T. (2015). Cetacean noise criteria revisited in the light of proposed exposure limits for harbour porpoises. *Marine Pollution Bulletin, 90*, 196–208. DOI: 10.1016/j.marpolbul.2014.10.051.

Tougaard, J. & Dähne, M. (2017). Why is auditory frequency weighting so important in regulation of underwater noise? *Journal of the Acoustical Society of America 142*, EL415-EL420.

Weihs, D. (2002). Dynamics of dolphin porpoising revisited. *Integrated and Comparative Biology, 42*, 1071-1078. doi: 10.1093/icb/42.5.1071

Wright, A.J., Soto, N.A., Baldwin, A.L., Bateson, M., Beale, C.M., Clark, C., Deak, T., Edwards, E.F., Fernández, A., Godinho, A., Hatch, L.T., Kakuschke, A., Lusseau, D., Martineau, D., Romero, M.L., Weilgart, L.S., Wintle, B.A., Notarbartolo-di-Sciara, G., & Martin, V., (2007). Do marine mammals experience stress related to anthropogenic noise? *International Journal of Comparative Psychology, 20*, 274–316.

Zar, J.H. (1999). Biostatistical Analysis, fourth ed*.* New York, Prentice Hall, p. 718.