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Behavioral Responses of a Harbor Porpoise (*Phocoena phocoena*) to 25-kHz FM Sonar Signals

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Abstract

The effects of three sonar sound types (peak frequency *ca.* 25 kHz with high-frequency side bands at 71 and 121 kHz) on the behavior of a harbor porpoise (*Phocoena phocoena*) were quantified in a quiet pool. Sequences (with different pulse intervals, resulting in different duty cycles) of 50-ms frequency modulated (FM) signals, 600-ms continuous waves (CW), and 900-ms combinations of FM and CW signals (Combo) were transmitted at four average received broadband sound pressure levels ($SPLs_{av.re.}$) to determine the dose-response relationship. Effects ranged from just no change in the harbor porpoise's respiration rate to increases of 53% (FM), 38% (CW), and 63% (Combo). The animal's agitation was evidenced by increased jumps, and his response increased with SPL. $SPLs_{av.re.}$ causing responses were 125 to 148 dB re 1 µPa (FM) and 118 to 153 dB re 1 µPa (CW and Combo). At the same $SPLs_{av.re.}$, the greatest response was to the Combo signal (at the duty cycles used in this study). At sea, harbor porpoise response distances will vary with context such as, for example, social situation, sound propagation, and background noise levels.

Key Words: behavior, coastal waters, conservation, disturbance, habitat, marine mammals, marine ecology, noise, ultrasonic sonar, odontocete, harbor porpoise, *Phocoena phocoena*

Introduction

Sound is important for marine animals 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, marine animals are likely to be disturbed by noise in their environment. In addition to natural sound, human activities increasingly add noise to the environment that may have negative behavioral, physiological, and auditory effects (i.e., masking effects, and temporary and permanent hearing threshold shifts) on marine fauna.

Anthropogenic noise in the oceans has increased during the last century, mostly due to increased shipping. Navies worldwide contribute to the ambient noise by means of explosions during exercises, removal of ammunition, and by using various types of sonar systems. Active sonar systems used in anti-submarine warfare produce intermittent underwater noises of various types, including frequency swept signals (sweeps). Long-range fish detection sonars such as SIMRAD SX90 and SU90 use similar signals: high-power, 20- to 30-kHz narrow-band FM chirps (Simrad, 2015a, 2015b).

The effects of ultrasonic sonar sounds on the harbor porpoise (*Phocoena phocoena*) are of particular interest because it has a wide distribution area in the Northern Hemisphere, acute hearing, and functional hearing over a very wide frequency range (Kastelein et al., 2002, 2009, 2010). Harbor porpoises are relatively easily deterred by anthropogenic underwater noises such as those produced by ships (Amundin & Amundin, 1973; Polacheck & Thorpe, 1990), acoustic alarms to prevent unwanted bycatch in gillnet fisheries (Kastelein et al., 2000, 2001, 2006; Culik et al., 2001; Johnston, 2002; Olesiuk et al., 2002; Teilmann et al., 2006), offshore wind turbines (Koschinski et al., 2003), and underwater data communication systems (Kastelein et al., 2005a). Behavioral response threshold SPLs of harbor porpoises have been determined for noise bands and tonal signals around 12 kHz (Kastelein et al., 2005a), a continuous 50 kHz tone, and continuous and pulsed 70 and 120 kHz tones (Kastelein et al., 2008a, 2008b). Both the spectrum and the received level of an underwater noise, in combination with signal duration and the duty cycle (i.e., presentation rate), appear to determine the effect the sound has on the behavior of harbor porpoises.

Depending on their goal, active naval sonar systems use signals with different frequencies and duty cycles. Many navies use mid-frequency active sonar systems (MFAS, with sweeps in the 5 to 10 kHz band; Funnell, 2009). The sweeps are usually of short duration (up to 1.2 s), and the sweep interval (usually between 10 and 30 s) depends on the expected distance of target submarines (Funnell, 2009). The effect of these active sonar system sounds on marine mammal behavior has been studied (Kastelein et al., 2012; Miller et al., 2012). However, the Baltic Sea is relatively shallow at an average depth of 52 m (increasing the occurrence of reverberations) and has low salinity (leading to less sound absorption than in the oceans). Consequently, anti-submarine warfare active sonar systems tailored to the Baltic Sea typically operate at higher frequencies (~25 kHz) than those of most NATO navies (Pihl & Ivansson, 2009).

The goal of the present study was to quantify the behavioral responses of a harbor porpoise to several ultrasonic sonar signals at duty cycles that are representative to those commonly used during operations. With this behavioral response information, combined with information on the source level of sonar sounds, the background noise, and local propagation conditions, the extent of the area around a sonar source in which porpoise behavior is likely to be influenced can be estimated.

Methods

Study Animal

The male harbor porpoise (ID No. 02) was 7 y old at the time of the study. His body weight during the study was around 40 kg, his body length 146 cm, and his girth at axilla was around 73 cm. His hearing was assumed to be representative for animals his age of the same species; it was similar to that of two other young harbor porpoises (Kastelein et al., 2002, 2009, 2010). His hearing was tested after the present study and was found to have remained stable since 2010. The animal had participated in other behavioral response studies (Kastelein et al., 2011a, 2012). He received four meals of fish per day.

Study Area

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

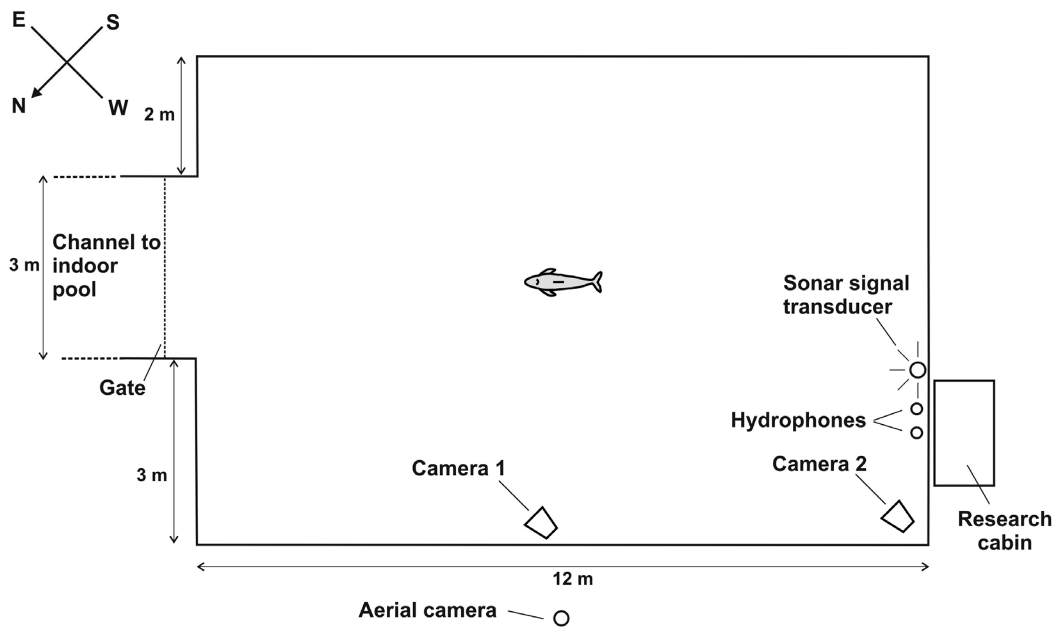

Figure 1. Top scale view of the study facility, showing the study animal, the location of the aerial camera, the two cameras at the edge of the pool, the underwater transducer emitting the sonar signals, and the hydrophones (only used to listen to the signals and ambient noise). Also shown is the research cabin that housed the video and audio equipment and the operator.

in detail by Kastelein et al., 2010) and an outdoor pool (12 m × 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 polyester and 3-cm thick coconut mats with their fibers embedded in 4-mm thick rubber (reducing reflections mainly above 25 kHz). The bottom was covered with sand. The water circulation system and the aeration system for the biofilter were made to be as quiet as possible and were switched off 30 min before research sessions and kept off during sessions so that there was no current in the pool during sessions. The equipment used to produce the sound stimuli was housed out of sight of the study animal in a research cabin adjacent to the pool (Figure 1). For more details about the study area, see Kastelein et al. (2012).

Acoustic Measurements

The background noise and the distribution of the test signal sequence sound in the pool was measured while the animal was not present. The recording and analysis equipment consisted of two hydrophones (Brüel & Kjær [B&K] – 8106 [10 Hz to 140 kHz]); one at 0.75 m depth and one at 1.5 m depth) with a multichannel high-frequency analyzer (B&K – PULSE 3560 D) and a laptop computer with *Labshop*, Version 12.1 (B&K – PULSE). The frequency response of the recording system is related to the sample rate, which was 524,288 Hz. The system was calibrated with a pistonphone (B&K – 4223).

Acoustic Characterization of the Sequence

The sequences were characterized in terms of their sound pressure level (SPL in dB re 1 µPa, root mean square [RMS]; all SPLs mentioned in this paper are RMS). The duration of a signal (t_{90} in s) was determined as the time interval between the points when the cumulative sound exposure reached 5 and 95% of the total exposure (i.e., the duration contained 90% of the total energy in the signal) (Madsen, 2005). The SPL was determined from the power sum of 1/3-octave bands from 1 to 160 kHz (broadband), taking into account the frequency-dependent sensitivity of the hydrophones.

Underwater Background Noise and Test Stimuli

The background noise in the pool between 25 Hz and 160 kHz was measured twice during the study under conditions that were typical of the research sessions (no rain; wind force Beaufort 4 [5.8 to 8.3 m/s] or below). The background noise level was low (Figure 2). Above 4 kHz, the level was mainly determined by the self-noise of the recording equipment.

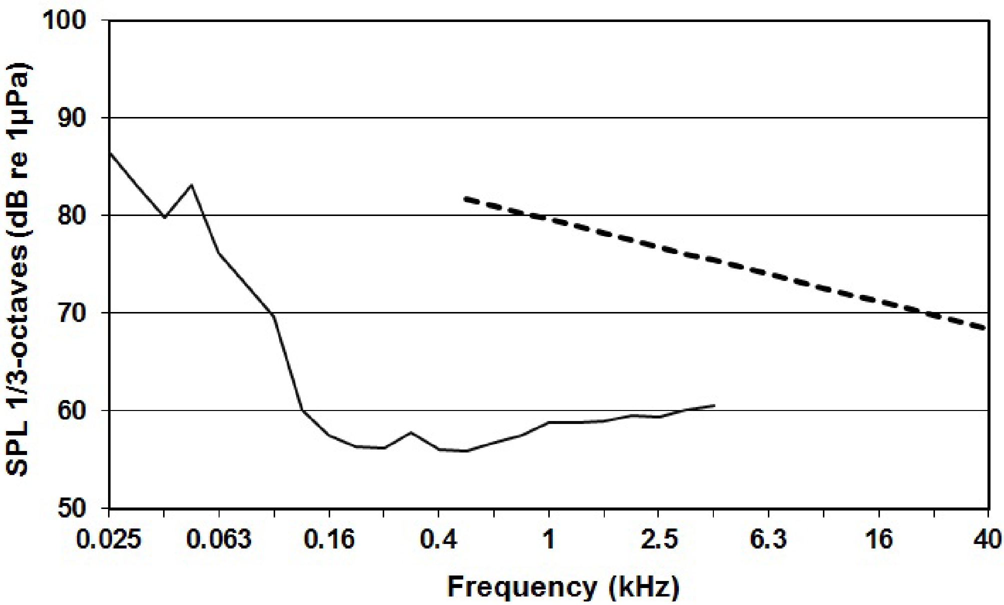

Figure 2. The background noise in the pool represented in 1/3-octave bands (SPL in dB re 1 µPa) averaged over 10 s. The level is very low; for most of the spectrum, it is below that measured during Beaufort sea state 1 (dashed line; Knudsen et al., 1948). Above 4 kHz, the measured background noise level is dominated by the self-noise of the recording equipment and is therefore not shown.

The signals used in this study were not intended to mimic specific naval sonar signals as the signals' spectrum can be varied by the sonar operator depending on the situation and depends on the transmission source level (i.e., the harmonic content depends on the source level due to the non-linearity of the systems). Instead, the signals used here should be considered as generic signals in the general fundamental frequency band of naval sonars and long-range fish detection sonars. The following three sound types were tested: (1) a hyperbolic frequency-modulated (down-sweep) signal (FM), (2) an amplitude-modulated continuous wave (CW), and (3) a combination of FM and CW signals (Combo). The signals were presented at different duty cycles (Figure 3; Table 1) appropriate for operational use. Apart from the fundamental frequency (around 25 kHz), high-frequency side bands were generated (at 71 and 121 kHz) in all three sound types.

Audio and Video Equipment

The digitized sequences (WAV files; sample frequency 96 kHz, 16-bit) were played back by laptop computer 1 (Acer Aspire – 5750) with a program written in *LabVIEW*, Version 2010, to an external data acquisition card (NI – USB6259); the output was digitally controlled in 1 dB steps. The sounds were projected underwater via a toroidal beam transducer (EDO Western – 337; frequency response linear up to 40 kHz). The transducer was suspended 1 m below the water surface at the southwestern end of the pool (Figure 1). The side bands were not in the WAV file but were generated by the electronic sound generating system. The output of the sound system to the transducer was monitored by means of an oscilloscope (Tektronix – 2201), a voltmeter (Agilent – 34401A), and a spectrum analyzer (Velleman – PCSU1000). The spectrum of sound produced in the pool is shown in Figure 4. The sound generating system was linear over the entire SPL range used in the study.

The animal's behavior was filmed from above by a waterproof camera (Conrad – 750940) with a wide-angle lens; a polarized filter prevented saturation of the video image by glare from the water surface. The camera was placed on a pole 9 m above the water surface on the northwestern side of the pool (Figure 1). The entire surface of the pool was captured on the video image. The output of the camera was fed through a video multiplexer (MX-8 – CSX) which added the time and date to the images. Thereafter, the output was digitized by an analog-to-digital converter (König – Grabber) and stored on laptop computer 2 (Medion – MD96780). The animal was also filmed by two black and white video cameras (Ocean Systems Inc. – Delta Vision) on the northwestern side of the pool, just above the water surface (Figure 1). The images from the cameras were visible to the operator on two monitors in the research cabin.

The audio part of the background noise was monitored aurally via a custom-made hydrophone (frequency response up to 140 kHz) 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) and recorded on laptop computer 2 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 background noise during sessions.

The test sound sequences were recorded via another custom-built hydrophone and a custom-built ultrasound detector. The output of the ultrasound detector was fed into the other audio channel of the analog-to-digital converter (König – Grabber) and was thus also recorded in synchrony with the video images. The operator could check the transmission of the test sounds via the loudspeaker of the ultrasound detector.

Determination of the SPL of the Playback Sequences

During a pilot study before the main experiment, the average received SPL of the playback of each of the three sonar signal sequences was gradually increased up to a level just below that at which the harbor porpoise's behavior was seen to change, and then up to a level at which it was judged that higher levels would compromise the animal's welfare (he showed an increased respiration rate, swam very fast, and jumped). The lowest $SPL_{av.re.}$ used was 77 dB re 1 µPa for the FM and 76 dB re 1 µPa for the CW and Combo, and the maximum $SPL_{av.re.}$ used was 148 dB re 1 µPa for the FM and 153 dB re 1 µPa for the CW and Combo.

SPL Distribution in the Pool

To determine the sound distribution in the pool, the SPL for each sound type was measured at 77 locations at two depths (0.75 and 1.5 m). The reported SPLs were from one signal per sequence per location. The measured distribution of the received SPL at the 154 positions in the pool is shown in Figure 5 (at an average received broadband SPL [$SPL_{av.re.}$] of 153 dB re 1 µPa), and the $SPLs_{av.re.}$ per sequence are shown in Table 2. The SPLs were generally higher at 1.5 m deep than at 0.75 m deep; and at each depth, these levels decreased with increasing distance from the transducer. The t_{90} varied depending on the location in the pool (means ± SD are shown in Table 1). In an effort to characterize the reverberation time in the pool, the T-60, the time taken for the pool's impulse response to decay to 60 dB below its maximum, was

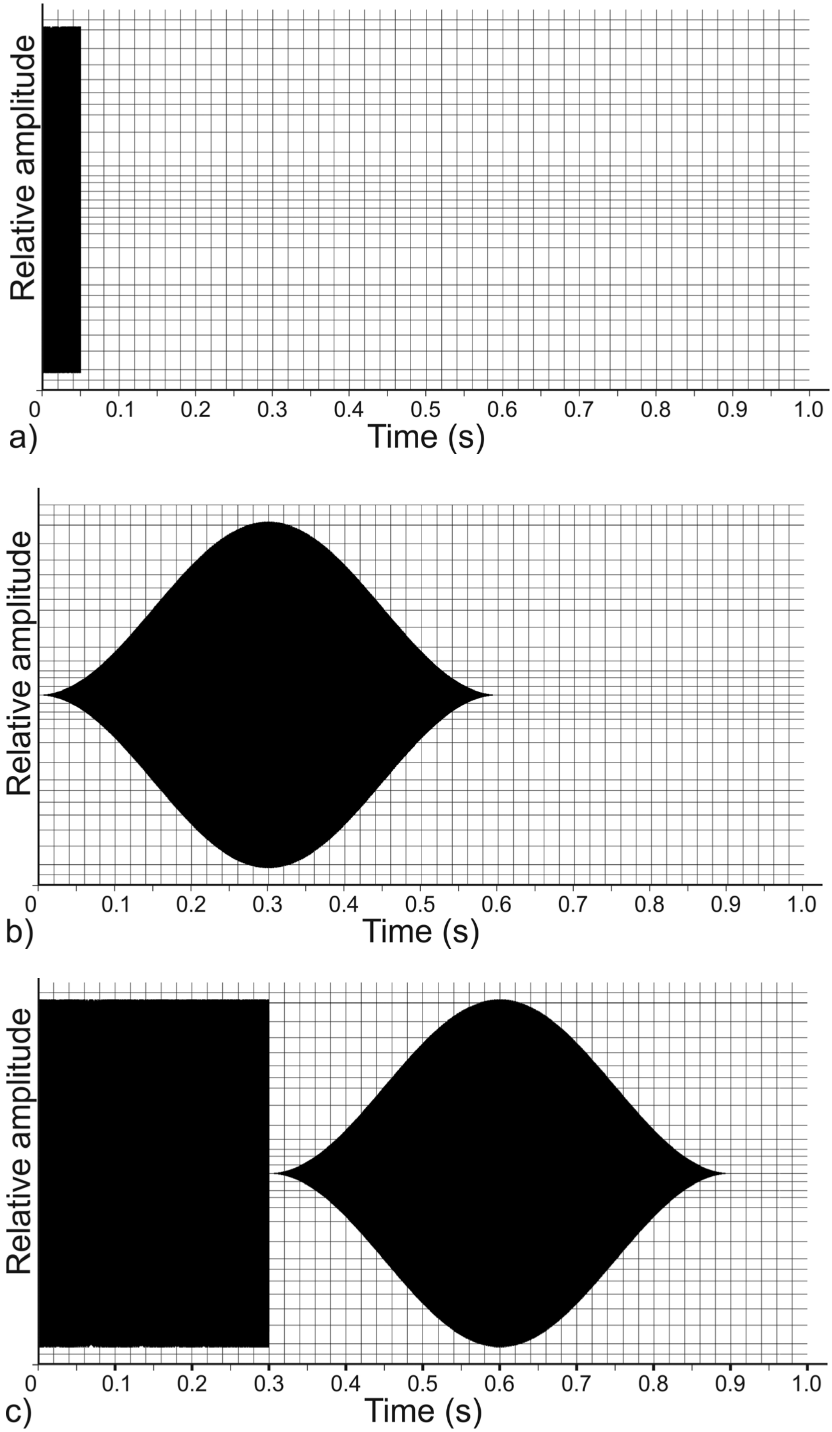

Figure 3. The waveforms of the three sonar sound types with peak frequency around 25 kHz: (a) the 50-ms FM, (b) the 600-ms amplitude modulated CW, and (c) the Combo (300-ms FM followed by 600-ms CW). The durations shown here deviate from the reported t_{90} in Table 1 because t_{90} is the time interval between the points when the cumulative sound exposure reached 5 and 95% of the total exposure (i.e., the duration containing 90% of the total energy in the signal) (Table 1; Madsen, 2005).

Table 1. Details of the three sonar sound types (frequency modulated = FM, continuous wave = CW, and combination = Combo) as recorded in the pool; N = 154 measurement positions in the pool (see "SPL Distribution in the Pool" section). *The t_{90} varied depending on the location in the pool; therefore, means ± standard deviations (SD) are given.

	Sonar sound type		
	FM	CW	Combo
Description	Hyperbolic down-sweep	Amplitude modulated tone, bell curve	Combination of FM and CW
Frequency band fundamental (kHz)	25.5-24.5	25	25.5-24.5
Frequency side bands (kHz)	71 and 121	71 and 121	71 and 121
Signal duration (t_{90} ms)*	43 ± 10	280 ± 20	667 ± 25
Pulse interval (s)	2	10	10
Duty cycle (%)	2.4	5.6	8.3

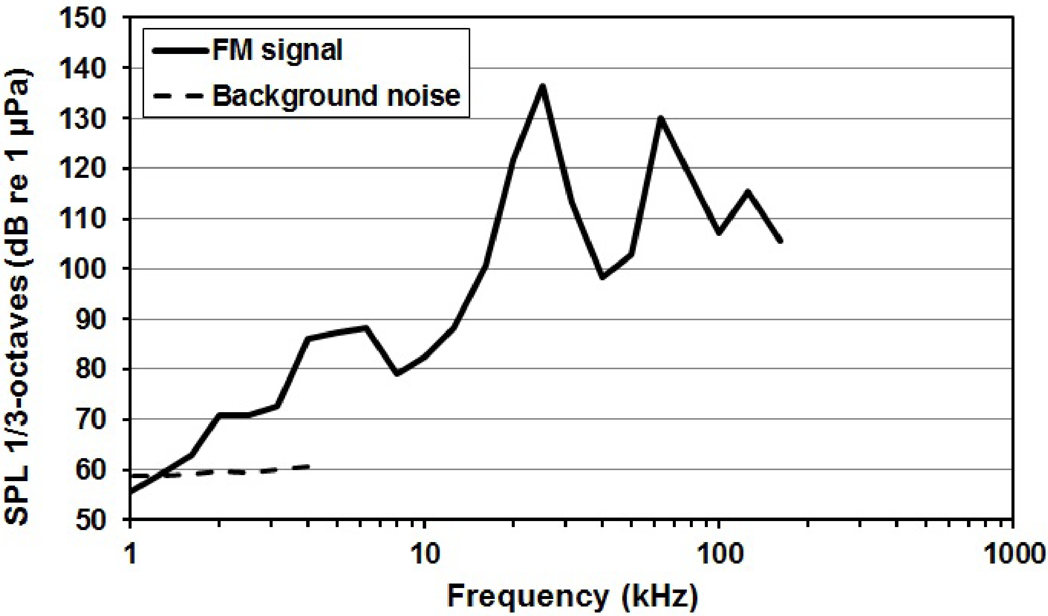

Figure 4. The ⅓-octave spectrum (SPL in re 1 µPa) of the recorded FM signal in the pool at 2 m from the transducer (broadband average SPL in pool: 148 dB re 1 µPa). Energy peaks occurred at 25, 71, and 121 kHz. The spectra of the two other sound types (CW and Combo) were almost identical to that of the FM signal. Also shown is the background noise level in the pool up to 4 kHz.

estimated. The mean T-60 was 83 ms (range 80 to 88 ms, depending on receiver location).

Experimental Procedure

The transducer producing the playback sequences was positioned in the water at the southwestern end of the pool 10 min before the first session of each day (Figure 1). Sessions consisted of a 30-min baseline period (no sound emission), followed by a 30-min test period (emission of sound sequences). Usually one or two sessions were conducted per day, 5 d/wk, beginning between 0900 and 1500 h. During the research sessions, only the operator in the research cabin was allowed within 10 m of the pool.

During each session, one of the three sound types was tested at one of the four source levels; each combination was tested six times, resulting in a total of 72 sessions. The three sound types (FM, CW, and Combo) and four SPLs were tested in random order. In total, during each 30-min test period, 878 FM signals, 169 CW signals, and 165 Combo signals were produced.

To prevent masking of the signals by background noise, tests were not carried out during rainfall or when wind speeds were above

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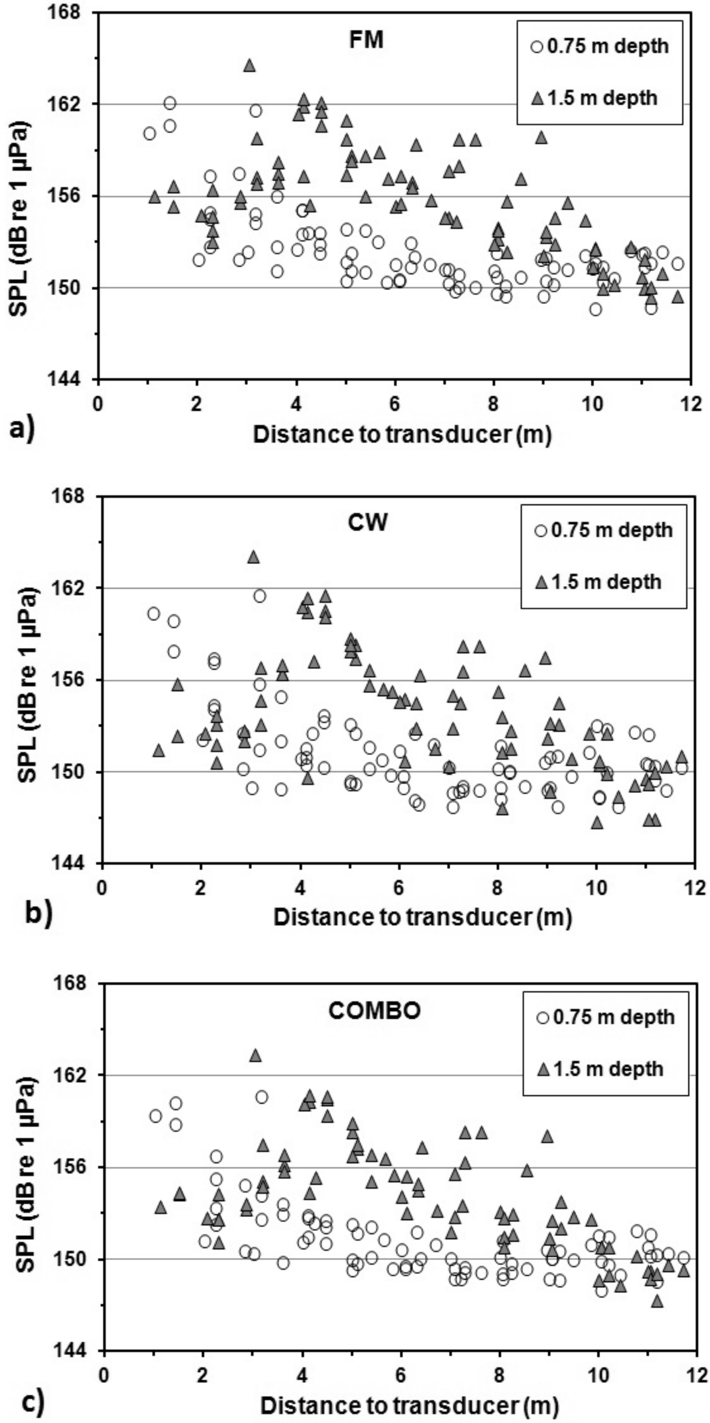

Figure 5. The broadband SPL (the SPL determined from the power sum of 1/3-octave bands from 1 to 160 kHz) distribution in the pool as a function of the distance to the transducer at two depths (0.75 m: ○ and 1.5 m: ▲) for the three sonar signals (n = 77 measurements/depth) at an average received broadband SPL (over all 154 measurement locations) of 153 dB re 1 µPa; (a) FM, (b) CW, and (c) Combo. The SPLs of the three sonar sounds were similar per location in the pool.

Beaufort 4 (5.5 to 7.9 m/s). The study was conducted between January and April 2012.

Response Parameters and Behavioral Data Recording

Three objective behavioral parameters were used to quantify the harbor porpoise's responses to the sound sequences: his respiration rate, his distance from the transducer (recorded as his surfacing location in the pool relative to the transducer), and the number of times he jumped out of the water. These parameters were quantified and compared for baseline and test periods. To determine if any changes in behavior, such as habituation or sensitization, occurred during the 30-min test periods, the data were analyzed separately for three 10-min sections of the test periods.

The study animal's distance from the transducer was quantified as follows to determine whether he responded to the sounds by swimming away from the sound source: from video camera recordings, all the locations where the harbor porpoise surfaced during the 30-min baseline and 30-min test periods were recorded on a grid superimposed on the screen of laptop computer 2. 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. 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, the porpoise could be seen well below the water surface. He did not swim far away from the surfacing locations. Hence, the surfacing locations were a good indication of the porpoise's general swimming area. To determine whether the porpoise responded to the sounds by increasing his respiration rate, the number of respirations in the baseline periods was compared to the number during the test periods.

In addition to the objective behavioral parameters (distance from the transducers, respiration rate, and number of jumps), a subjective behavioral parameter was recorded for each of the three 10-min sections of each test period: the harbor porpoise's swimming speed relative to the preceding baseline period (classed as 0: no difference in swimming speed, 1: increased swimming speed, or -1: decreased swimming speed). The average of the six sessions (for each sound type–level combination) per 10-min section of the test period was used to evaluate the change in swimming speed relative to the 30-min baseline period (maximum average: 1).

Analysis

ANOVAs were used to evaluate changes in the harbor porpoise's distance from the transducer and respiration rate in the 10-min sections of each test period, taking into account the level (included as a factor) and the session number (included as a covariate). Paired *t*-tests were used to compare in detail these parameters and the swimming speed in baselines and associated 30-min test periods. In order to compare the effects of the three sound types (at the duty cycles used in the present study) with one another, a further ANOVA was conducted on differences in respiration rate only, with sound type as an additional factor (alongside level and 10-min section as factors and session number as a covariate). Levels which had been selected in the pilot study to have just no effect were excluded from this analysis. For all analyses, assumptions of the tests were conformed to (for ANOVA normality of residuals and homogeneity of variances, and for paired *t*-tests normality of data), and the level of significance was 5% (Zar, 1999).

Results

During baseline periods, the harbor porpoise usually swam large clock-wise ovals in the pool. The mean distance between the animal's surfacing locations and the transducer was 6.1 ± 1.4 m (± SD), his mean respiration rate was 98 ± 11 breaths in 30 min, and the porpoise only jumped three times in total, in two sessions (out of 72 sessions).

FM Sound Type

An ANOVA showed that the $SPL_{av.re.}$ of the FM sound type had no effect on the harbor porpoise's distance from the transducer, and the sound caused no habituation during test periods (there was no effect of the 10-min sections). The $SPL_{av.re.}$ had a significant effect on the respiration rate (Tables 2 & 3; Figure 6a). Detailed comparison of the objective behavioral parameters (respiration rate and distance to the transducer) and the relative swimming speed in the baseline and associated test periods by means of paired *t*-tests showed that the harbor porpoise did not respond to the lowest $SPL_{av.re.}$ (77 dB re 1 μPa) of the FM sound type. At and above $SPLs_{av.re.}$ of 125 dB re 1 μPa, significantly higher respiration rate and faster swimming occurred, but no significant displacement, except at the $SPL_{av.re.}$ of 137 dB re 1 μPa when the animal swam on average 1 m closer to the sound source than during the baseline periods (Table 4; Figure 7a). The animal began to jump during test periods when the $SPL_{av.re.}$ was 125 dB re 1 μPa, and the number of jumps was increased greatly at the highest $SPL_{av.re.}$ (148 dB re 1 μPa; Table 4). The animal's behavior was not quantified in the period after sound exposure ended; however, in all cases, it was observed to return to normal immediately.

CW Sound Type

An ANOVA showed that the SPL of the CW sound type had no effect on distance from the transducer. Habituation during test periods was evidenced by the effect of the 10-min sections on the respiration rate (significantly more breaths occurred in section 1 than in sections 2 & 3). The SPL had an effect on the respiration rate as expected (Tables 2 & 3; Figure 6b). Detailed comparison of the objective behavioral parameters and the relative swimming speed in the baseline and associated test periods by means of paired *t*-tests showed that the harbor porpoise did not respond to the lowest $SPL_{av.re.}$ (76 dB re 1 µPa) of the CW sound type. At and above $SPLs_{av.re.}$ of 118 dB re 1 µPa, significantly more respirations occurred; and at and above $SPLs_{av.re.}$ of 136 dB re 1 µPa, faster swimming occurred. No significant displacement was recorded at any $SPL_{av.re.}$ (Table 4; Figure 7b). The animal began to jump during test periods when the $SPL_{av.re.}$ was 118 dB re 1 µPa, and the number of jumps increased at higher levels (Table 4). The animal's behavior was not quantified in the period after sound exposure ended; however, in all cases, it was observed to return to normal immediately.

Combo Sound Type

An ANOVA showed that the SPL of the Combo sound type had no effect on distance from the transducer. Habituation during test periods was evidenced by the effect of the 10-min sections on the respiration rate (significantly more breaths occurred in sections 1 & 2 than in section 3). The SPL had an effect on the respiration rate as expected (Tables 2 & 3; Figure 6c). Detailed comparison of the objective behavioral parameters and the relative swimming speed in the baseline and

Table 2. The four average received broadband SPLs ± SD (dB re 1 µPa; $N = 154$ measurement locations in the pool) of the three sonar sound types (FM, CW, and Combo) during the sessions, and their effect on the harbor porpoise's respiration rate. The broadband SPL is determined from the power sum of 1/3-octave bands from 1 to 160 kHz.

FM		CW		Combo	
$SPL_{av.re.}$ ± SD (dB re 1 µPa)	Increase in respiration rate relative to baseline period (%)	$SPL_{av.re.}$ ± SD (dB re 1 µPa)	Increase in respiration rate relative to baseline period (%)	$SPL_{av.re.}$ ± SD (dB re 1 µPa)	Increase in respiration rate relative to baseline period (%)
77 ± 3.5	2	76 ± 3.7	6	76 ± 3.4	-3
125 ± 3.5	32	118 ± 3.7	24	118 ± 3.4	37
137 ± 3.5	38	136 ± 3.7	35	136 ± 3.4	51
148 ± 3.5	53	153 ± 3.7	38	153 ± 3.4	63

Table 3. Results of three ANOVAs to evaluate changes in the harbor porpoise's (*Phocoena phocoena*) respiration rate in the 10-min sections of each test period, taking into account the SPL (included as a factor) and the session number (included as a covariate). *df* = degrees of freedom, Adj. MS = adjusted mean square, and NS = not significant.

Source of variation	*df*	Adj. MS	F	*p*
FM				
Session number	1	127.88	5.14	0.027
10-min section	2	78.18	3.14	NS
Level	3	929.12	37.32	< 0.001
Error	65	24.89		
CW				
Session number	1	132.46	8.09	0.006
10-min section	2	203.93	12.46	< 0.001
Level	3	453.70	27.72	< 0.001
Error	65	16.37		
Combo				
Session number	1	35.91	1.62	NS
10-min section	2	154.54	6.99	0.002
Level	3	1542.45	69.77	< 0.001
Error	65	22.11		

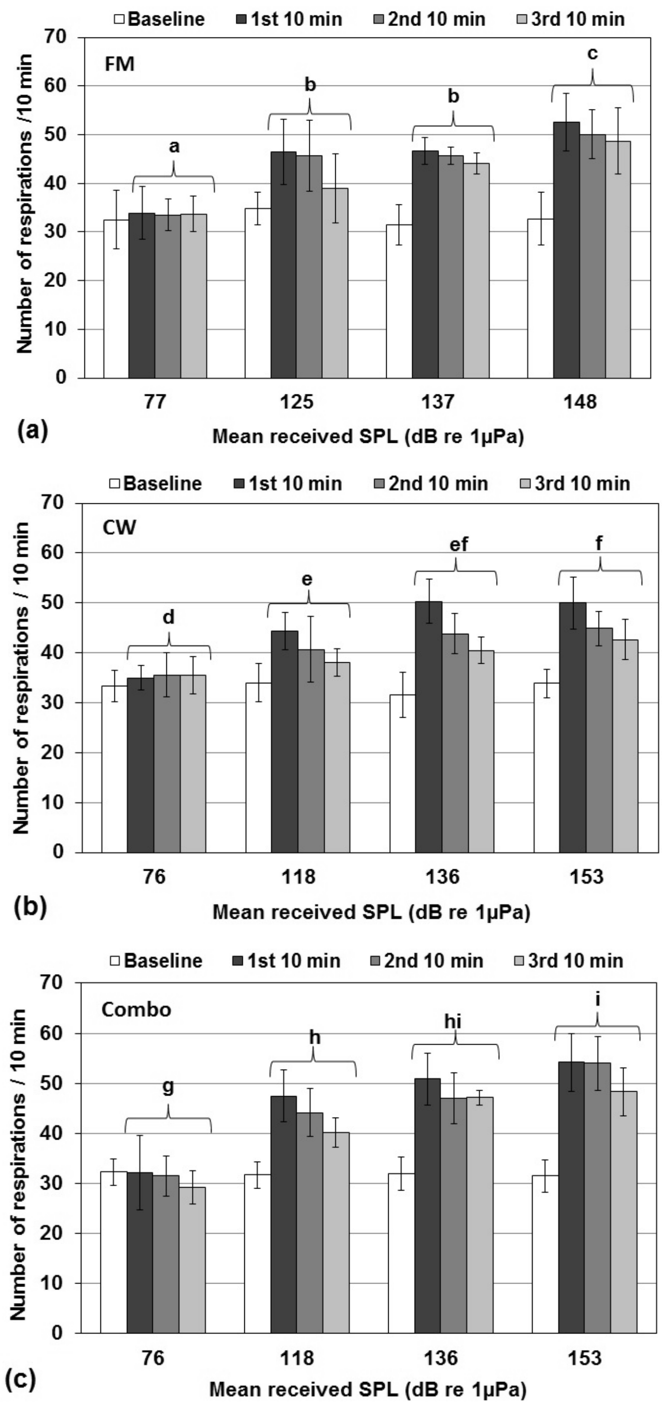

Figure 6. The behavioral response of the harbor porpoise to (a) the FM sonar sound type, (b) the CW sound type, and (c) the Combo sound type, showing the mean respiration rate per 10 min in the baseline and per 10-min section of the test period for each of the four average received broadband SPLs. Within graphs (a), (b), and (c), the same lower case letters indicate average received levels between which *post-hoc* tests showed no significant difference in the respiration rate (see Table 3). Each error bar indicates ± 1 SD ($n = 6$).

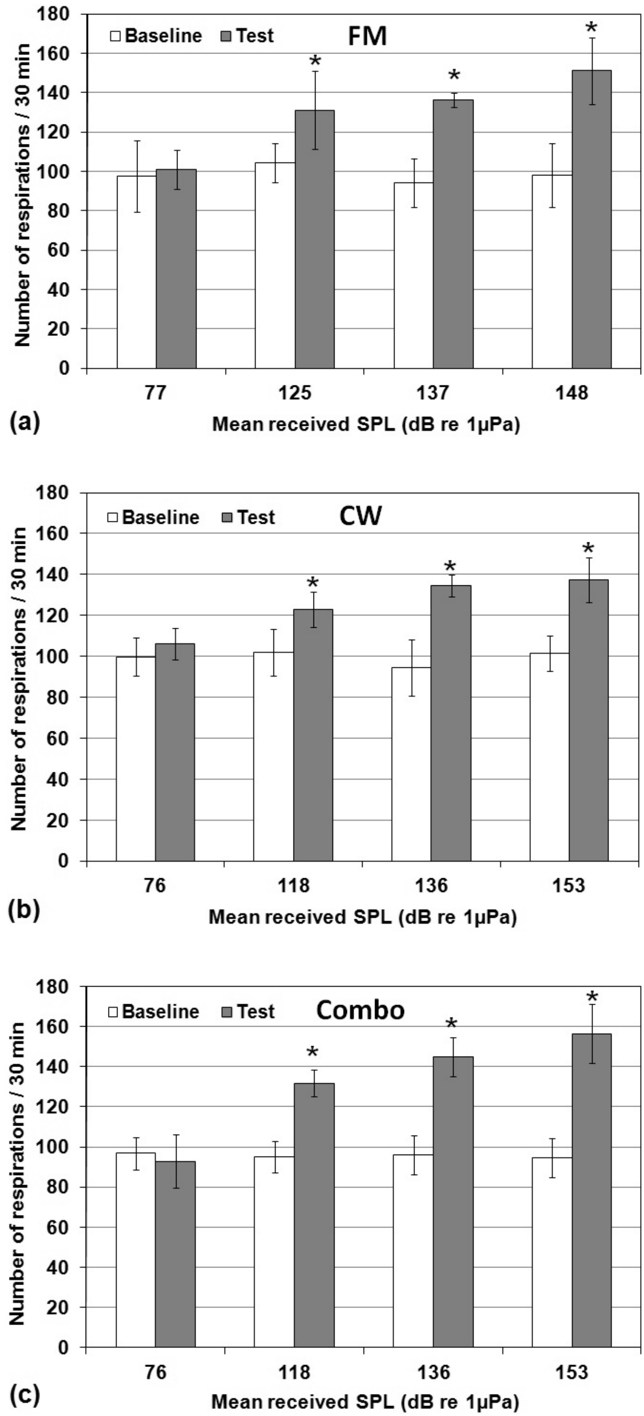

Figure 7. The mean respiration rate of the harbor porpoise during 30-min baseline periods and 30-min test periods in response to four mean received broadband SPLs of the sound types FM (a), CW (b), and Combo (c). Each error bar indicates ± 1 SD ($n = 6$), and * indicates a significant difference ($p < 0.05$) between baseline and test periods (paired *t*-tests; see Table 4).

Table 4. Results of paired *t*-tests to compare the harbor porpoise's distance from the transducer, respiration rate, and swimming speed in baseline and associated test periods at four average received broadband SPLs (dB re 1 µPa) for each sound type (FM, CW, and Combo; *n* = 6; see also Figure 7). Exact *p* values are shown where significant; NS = not significant. Where the test was significant, the value for the test period was greater than that for the baseline period, with the exception of *, for which the animal swam on average 1 m closer to the transducer in test periods than during baseline periods.

FM					CW					Combo				
Average rec. SPL	Distance	Respiration rate	Total jumps (test-baseline)	Speed	Average rec. SPL	Distance	Respiration rate	Total jumps (test-baseline)	Speed	Average rec. SPL	Distance	Respiration rate	Total jumps (test-baseline)	Speed
77	NS	NS	0	NS	76	NS	NS	0	NS	76	NS	NS	0	NS
125	NS	0.013	11	0.010	118	NS	0.018	1	NS	118	NS	0.000	6	0.011
137	0.040*	0.000	5	0.007	136	NS	0.001	7	0.006	136	NS	0.001	11	0.001
148	NS	0.007	52	< 0.001	153	NS	0.001	9	0.001	153	NS	0.000	21	< 0.001

associated test periods by means of paired *t*-tests showed that the harbor porpoise did not respond to the lowest $SPL_{av.re.}$ (76 dB re 1 µPa) of the Combo sound type. At and above $SPLs_{av.re.}$ of 118 dB re 1 µPa, significantly more respirations and faster swimming occurred, but no significant displacement was recorded at any level (Table 4; Figure 7c). The animal began to jump during test periods when the $SPL_{av.re.}$ was 118 dB re 1 µPa, and the number of jumps increased at higher SPLs (Table 4). The animal's behavior was not quantified in the period after sound exposure ended; however, in all cases, it was observed to return to normal immediately.

Effect of the Three Sound Types on Respiration Rate

An ANOVA on respiration rate only, with sound type as a factor and from which SPLs with just no effect were excluded, showed that the effects of sound types (at the duty cycles used in the present study) could be ordered as CW < FM < Combo. The harbor porpoise's respiration rate was significantly lower in response to CW sounds than in response to Combo sounds, but his response to FM sounds did not significantly differ from his response to CW or Combo sounds.

Discussion

Evaluation

The hearing of the harbor porpoise in the present study was similar to that of two other young male porpoises of similar age (Kastelein et al., 2002, 2009, 2010) and, thus, was probably representative of the hearing of harbor porpoises of his age. However, the response of this animal to the sonar signals may not have been representative for its species. The study should be repeated with other porpoises as responses may vary between individual porpoises (Kastelein et al., 2000, 2001, 2008b), but this is unlikely to be possible in the near future. Worldwide, only a few harbor porpoises are kept in captivity, and the facilities that keep this species are not designed for acoustic behavioral response studies (the ambient noise level cannot be controlled sufficiently).

Behavioral responses to sounds are context-dependent (they vary with location, time of day, season, social setting, ongoing behavior such as foraging or migrating, etc.) and depend on the background noise level. The effects observed in the present study occurred under very low background noise and in unmasked conditions. Under higher background noise conditions, the effect may be less severe as was observed in the same porpoise for 6 to 7 kHz up-sweeps transmitted under controlled ambient noise conditions resembling those of various sea states (Kastelein et al., 2011a).

Prior to the present study, the harbor porpoise had participated in other behavioral response studies. It is possible that his responses in the present study were influenced by his previous experiences. However, in the previous studies, the signal frequencies were much lower (1 to 2 kHz sweeps and 6 to 7 kHz sweeps) than those used in the present study (25 kHz).

The effect of the CW and Combo sound types on the animal's respiration rate diminished slightly by habituation during the 30-min test periods as evidenced by the effect of the 10-min sections (Figure 6). The results of the present study suggest that in the wild, harbor porpoises which are exposed to these signals at broadband $SPLs_{av.re.}$ of above ~110 dB re 1 µPa will show behavioral responses to the sonar signals.

After each session, the animal's behavior immediately returned to normal. Being exposed to sequences of the three sonar signals at the levels used in this study for 30 min had no lasting effect on his 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, 2008a, 2008b) and was the reason for not including a posttest observation period as was done in a previous pinger study (Kastelein et al., 2000). In nature, short-term disturbances probably do not displace harbor porpoises for long. For example, at a fish aquaculture cage site, the presence of the cages and workers did not appear to displace harbor porpoises from the area except during short intervals when high disturbance activities, such as a food delivery by barge or cage cleaning using high pressure hoses, were occurring. After these activities ended, harbor porpoises were typically observed in the vicinity of cages within 5 to 10 min (Haarr et al., 2009).

Conducting the behavioral response study in a pool had advantages. The background noise could be controlled and was very low, and the animal's behavior could be filmed. The main disadvantage was that, though the walls of the pool were designed to reduce reflections in particular of sounds above 25 kHz, some reverberation still occurred. If the reverberation time had been significantly longer than the duration of the signals, the sound level in the pool may have remained high after each signal was supposed to have ended at the end of the sound transmission. This could have led to behavioral responses distinct from those that would be observed in an anechoic pool (or in a free field). The mean T-60 in the pool was estimated to be 83 ms (range 80 to 88 ms, depending on receiver location). This is longer than the FM signal, but at 60 dB, the acoustic energy levels had decreased 1,000,000 times. The decay was approximately exponential after the first 10 ms, so a 20 dB decrease had occurred within 30 ms. Consequently, the levels had dropped significantly within 30 ms of the end of each sound transmission.

Another disadvantage of conducting the study in a pool was that only a small SPL gradient could be achieved (Figure 5). Therefore, instead of one received level, sounds at four received levels were presented to the animal, thus allowing a gradient in the effect on respiration rate to be demonstrated. The responses observed in the present study at the highest average received levels, if observed in free-ranging animals, would be classed as 4 to 5 on the severity scale for ranking observed behavioral responses (range 0 to 9) presented by Southall et al. (2007).

The hearing sensitivity of harbor porpoises for a sound depends on the direction from which it comes, and the directionality of porpoise hearing depends on the frequency content of the signal; an increase in frequency results in an increase in the directivity index (Kastelein et al., 2005b). For the high-frequency signals (centered around 25 kHz) used in the present study, the level perceived by the porpoise could vary by up to about 12 dB from the received level, depending on the animal's orientation relative to the sound source (if no reflection had occurred in the pool). This means that in the wild, harbor porpoises can locate high-frequency sonar sound sources fairly well and can reduce potentially annoying received levels by orienting themselves away from the sound source or by swimming away. Navy sonars typically feature horizontal and/or vertical directionality, which focuses the sonar beam into a sector and limits the ensonified area, thus reducing the number of animals that may be ensonified.

Comparison of the Effect of the Three Sound Types

The duty cycles of the three sound types varied, due to differences in both signal durations and pulse intervals. Comparison of the change in the harbor porpoise's respiration rate (in test periods relative to baseline periods) with the average received SPL during test periods for the three sonar sound types shows that the effect increased with increasing SPL and that the order of effects for higher received levels is CW < FM < Combo (Figure 8). Statistical analyses also showed that the change in respiration rate was significantly lower in response to CW sounds than in response to Combo sounds, but the response to FM sounds was not significantly different from the response to CW or Combo sounds. This suggests that the duty cycle, without consideration of the pulse interval, cannot be used to predict how exciting or frightening a sound is. In this study, the duty cycle provides little information as the signals differed greatly in duration. The Combo had the highest duty cycle (8.3%) and signal duration (667 ms), followed by the CW (5.6%; 280 ms) and the FM (2.4%; 43 ms). Therefore, the magnitude of the effect of a sound on the harbor porpoise cannot be predicted from the energy per unit time, from its spectrum, or from its signal duration; it must relate to a combination of these factors and possibly to other factors, perhaps psychological in nature.

Implications for Sonar Use

Among the sonar sounds tested, the CW and FM sound types were found to affect the harbor porpoise the least (when transmitted at the signal durations and duty cycles tested in the present study). Figure 8 facilitates the definition of behavioral disturbance thresholds. An acceptable increase in respiration rate can be related to a received SPL threshold that can be integrated into a marine mammal risk assessment tool, such as Environmental Risk Management Capability (BAE Systems, UK) or SAKAMATA (TNO, the Netherlands), to allow biologically relevant active sonar risk management. The fitted lines in Figure 8 show that at respiration rate increases of over

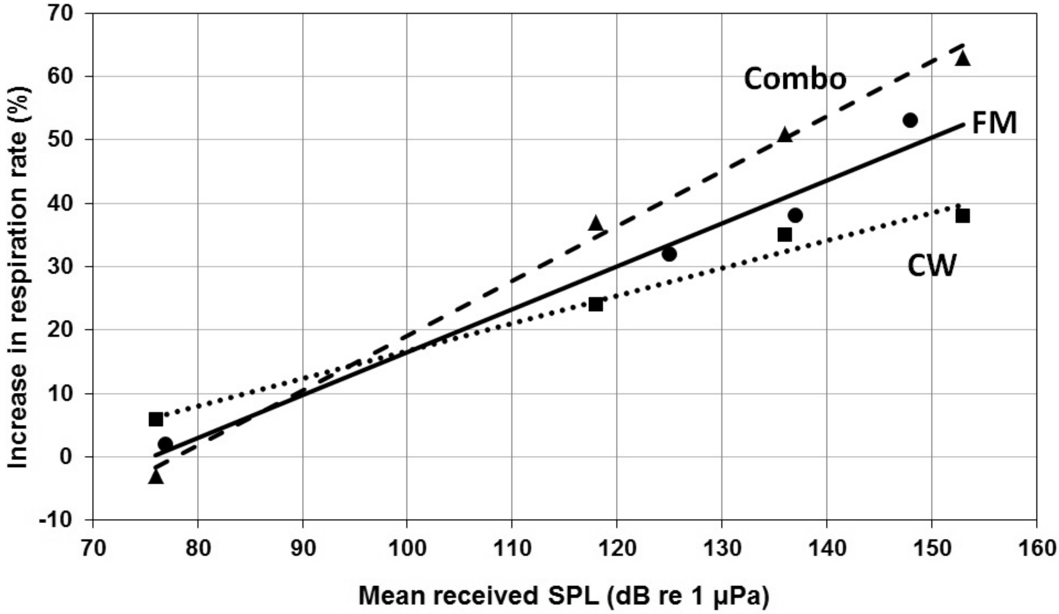

Figure 8. The increase in respiration rate (relative to the baseline period) in relation to the average broadband SPL received by the harbor porpoise during test periods with the three sound types: FM (solid line ●; linear regression: y = 0.6765x - 51.109; $R^2 = 0.98$); CW (dotted line ■; y = 0.434x - 26.657; $R^2 = 0.98$), and Combo (dashed line ▲; y = 0.8657x - 67.538; $R^2 = 0.99$). The order of effects for higher received SPLs is CW < FM < Combo. Variation in effect may be due to both the sound type and the duty cycle (which was different for each sound type) and may relate to other factors, perhaps psychological in nature.

30%, there are differences of over 18 dB between the $SPLs_{av.re.}$ that correspond to a certain respiration rate increase for the sound types used in this study. The fitted $SPLs_{av.re.}$ for the FM and CW sound types at 40% increased respiration rate are 135 and 153 dB re 1 µPa, respectively. The corresponding value for the Combo sound type is 124 dB re 1 µPa. These are large differences. Assuming a transmission loss of $17\log_{10}$ (distance), a 29 dB difference in response threshold SPL corresponds to a more than 50-fold difference in distance. The severe responses observed in the present study took place under very quiet conditions. If the ambient noise level increased, and thus the signal to noise ratio decreased, the responses would be expected to decrease as was observed in a study with 6 to 7 kHz sonar signals (Kastelein et al., 2011a).

Suggestions for Research

It is not clear how the high-frequency side bands (71 and 121 kHz) affected the behavioral response of the signals used in the present study. It cannot be determined whether the differences between responses to signals were due to the differences in high-frequency content or the differences between the fundamental signals. It would be of interest to test the effect of signals with the same fundamental frequency (25 kHz), with and without high-frequency side bands. If the side bands play a role, a potential mitigation method would be to reduce the energy in side bands or harmonics of similar sounds used in sonar applications. Harmonics in LFAS and MFAS sonar signals have been shown to play an important role in the ability of a harbor porpoise to detect the sounds (Kastelein et al., 2011b) and in the signals' effect on behavior (Kastelein et al., 2012, 2014).

The present study was conducted under very quiet conditions. At sea, ambient noise levels vary but are often higher than those in the present study. It would be of interest to study the effect of sonar signals in ambient noise conditions carefully controlled to correspond with several sea states. The effect of sonar signals on harbor porpoises is expected to decrease as the sea state increases as was seen with 6 to 7 kHz sonar sweeps (Kastelein et al., 2011a). Therefore, the received SPLs corresponding to a given increase in respiration rate are likely to be higher under more realistic conditions (i.e., conditions with higher ambient noise), so it is possible, under certain conditions, that sounds with higher SPLs than the response threshold SPLs reported in the present study could safely be used in sonar applications.

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