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Perception of Low-Frequency Acoustic Signals by a Harbour Porpoise (*Phocoena phocoena*) in the Presence of Simulated Offshore Wind Turbine Noise

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Abstract

Using auditory evoked potential (AEP) methods, a study was conducted on a harbour porpoise (*Phocoena phocoena*) at the Dolfinarium Harderwijk in The Netherlands. The study measured the audible range of wind turbine sounds and their potential masking effects on the acoustic perception of the animal. AEPs were evoked with two types of acoustic stimuli: (1) click-type signals and (2) amplitude-modulated signals. The masking noise resembling the underwater sound emissions of an operational wind turbine was simulated. At first, the animal's hearing threshold was measured at frequencies between 0.7 and 16 kHz. Subsequently, these measurements were repeated at frequencies between 0.7 and 2.8 kHz in the presence of two different levels of masking noise. The resulting data show a masking effect of the simulated wind turbine sound at 128 dB re 1 μ Pa at 0.7, 1.0, and 2.0 kHz. This masking effect varied between 4.8 and 7.3 dB at those frequencies. No significant masking was measured at a masking level of 115 dB re 1 μ Pa. The available data indicate that the potential masking effect would be limited to short ranges in the open sea, but limitations exist to this conclusion and all estimates are based on existing turbine types, not taking into account future developments of larger and potentially noisier turbine types.

Key Words: harbour porpoise, *Phocoena phocoena*, acoustic perception, masking, auditory evoked potentials, North Sea, offshore wind turbines

Introduction

The harbour porpoise (*Phocoena phocoena*) has become a subject for intense studies over the last

decade because of numerous threats for this species and the lack of knowledge in many aspects of its natural history. Many of these threats are of an acoustic nature. Since harbour porpoises were shown to be very sensitive to different types of acoustic signals (Thompson, 2000; Cox et al., 2001; Bain & Williams, 2006), there is growing concern about the behavioural and physical effects as well as their implications for the individuals, respectively, and the porpoise populations in ensonified areas. Recently, an additional source for acoustic emissions is being introduced into their environment by the planned installation of wind turbines in offshore areas. Many projects are currently under consideration in several European countries to build units in the offshore areas of the North and Baltic Seas, hence spatially overlapping with the distribution of harbour porpoises. To date, these plans comprise the installation of several thousand wind turbines. During the operational phase, the wind turbines emit low-frequency, continuous noise via vibrations of the pilings.

A principal key for assessing the impact of these noise emissions on the harbour porpoises is the understanding of the auditory sensitivity and perception capabilities of this species. In harbour porpoises, like in several other cetacean species, the auditory sense evolved to be the primary sensory modality. This is not only represented by the sophisticated sound production mechanism, as seen especially in odontocete cetaceans (Amundin, 1991), but also by the auditory capabilities of these animals. Harbour porpoises are proven to actively use underwater sound by means of echolocation (Busnel et al., 1965; Møhl & Andersen, 1973; Akamatsu et al., 1994) to obtain considerable information about their environment for a number of different purposes (e.g., detection of food, obstacles, predator avoidance, and navigation). Also, the auditory sensitivity of the harbour

porpoises has been studied by Andersen (1970) and Kastelein et al. (2002), each obtaining behavioural audiograms of a single captive harbour porpoise. Bibikov (1992) obtained electrophysiological audiograms from three harbour porpoises by measuring the auditory evoked potentials (AEPs). Harbour porpoises hear at least between 250 Hz to 160 kHz. Their most sensitive hearing range (32 dB re 1 μ Pa is at 100 to 140 kHz) (Kastelein et al., 2002) overlaps the frequency content of their echolocation clicks (i.e., between 125 kHz and 148 kHz) (Møhl & Andersen, 1973; Hatakeyama & Soeda, 1990; Goodson et al., 1995). Towards the lower frequencies, their acoustic sensitivity drops consistently, reaching 92 dB re 1 μ Pa at 0.5 kHz.

The AEP method is one of the methods commonly used to collect audiometric data from humans, as well as animals, and is increasingly used also for studying the auditory system of marine mammals. This electrophysiological method allows measuring hearing in a comparatively short period of time. The presentation of acoustic stimuli generates neuronal potentials in the auditory system (i.e., AEPs) upon perception of these stimuli. These potentials are generated within neuronal nuclei at different positions in the auditory system thereby forming an electrical field, which can be detected and recorded even on the skin surface—the electrical far-field. This so-called electrical far-field of the potentials is not evenly distributed on the body surface. The strongest potentials and thereby the ideal position for the active electrode is species-specific and has to be established prior to the auditory measurements.

The typical AEP of a harbour porpoise, similar to that of a bottlenose dolphin (*Tursiops truncatus*), has three positive and two negative peaks with increasing amplitudes, but the harbour porpoise waves have longer latencies than those in bottlenose dolphins (Bullock et al., 1968; Ridgway et al., 1981; Bibikov, 1992; Supin et al., 2001).

A refined methodological approach is based on the use of rhythmic sound modulations. By sinusoidally modulating the amplitude of a carrier tone or sound pulse sequence, it is possible to elicit a neuronal response, which includes a specific frequency component that is equivalent to the modulation frequency of the stimulus, an effect called envelope-following response (EFR). By applying a fast Fourier transformation (FFT) analysis, the modulation frequency component can be identified and quantified. The resulting amplitude of the EFR represents the energy content of the neuronal response at the given modulation frequency. Nevertheless, the strength of this EFR response can simultaneously be taken as a relative measure

for the perception of the carrier frequency of the amplitude-modulated (AM) signal because no EFR response could be elicited if the signal was not within the functional and dynamic range of the auditory system. Usually, the EFR begins with a delay of a few milliseconds after the stimulus onset and lasts for at least 10 ms. In general, a period of 16 ms (e.g., between 7 and 23 ms) was analysed (cf. Supin et al., 2001).

The advantage of this AM approach is its higher-frequency specificity. While EFR can be attributed to a specific frequency, short pulsed signals have a broader frequency spectrum in general and thus represent a measurement of the auditory sensitivity over a wider frequency range. On the other hand, a lower frequency limit exists for the use of AM signals. Auditory measurements at low frequencies therefore are preferably conducted using short pulsed signals.

The perception of a noisy sound by a harbour porpoise might simply be ignored by the animal. With an increase in the received sound pressure levels (SPLs) of noise, the perception might increase stress for the animal and result in a behavioural reaction or interfere with the perception of biologically important sounds. The detection of a signal by a marine mammal's ear, in general, can be affected by interference from noise in frequency bands near that signal (e.g., Erbe & Farmer, 1998; Finneran et al., 2002). This effect is called masking, and whether or not such masking occurs depends on the acoustic characteristics of a sound, its received level, and the acoustic sensitivity and characteristics of the receiver's hearing system.

Sounds associated with wind turbine operation have their main acoustic energy in a low-frequency band and show strong tonal components at type-specific frequencies below 1 kHz, thereby overlapping with the hearing range of the harbour porpoise. The aim of the present study was to assess the range of audibility of wind turbine signals for the harbour porpoise, as well as the amount of masking effect for these animals—that is, the impaired or impeded perception of signals in the presence of wind turbine acoustic emissions.

Materials and Methods

Subject and Facility

The auditory measurements were conducted on a stranded male harbour porpoise (code PpSH047) kept at the Netherlands Cetacean Research and Rehabilitation Centre (SOS Dolfijn) at the Dolphinarium Harderwijk, Harderwijk, The Netherlands. This animal ("Daan") stranded in 1997 and was approximately 7- to 8-y-old at the time of the research. His body length was 136 cm

and his weight was 38 kg. Daan was previously trained to participate in psychophysical studies and was also the study animal for the measurement of the behavioural audiogram by Kastelein et al. (2002).

Daan was kept in an oval-shaped concrete indoor pool at the Dolfinarium Harderwijk. The dimensions of the research pool were 8.6 m by 6.3 m, with an average water depth of 1.5 m. During the research, the water level was kept constant (± 5 cm) and there was no strong current in the water during the measurements because all pumps were switched off before the start of any session.

Set-up and Behavioural Procedures

The underwater station at which the animal was trained to station for the audiometric measurements was placed at a depth of 0.75 m (mid-water) in the research pool. This station was made of a plastic ball-shaped mesh (3-cm diameter) attached to a PVC pipe hanging vertically from a pole lying across the pool. The three transducers used for transmitting the AEP stimuli were positioned at a fixed distance of 1 m to the animal's rostrum of 1 m at a depth of 0.75 m.

Due to the small size of the research pool and its shape, every acoustic stimulus emitted into the research pool was subject to constructive and destructive interferences due to multi-path reflections from any boundary within less than a

millisecond. Two acoustic-reflecting baffle-boards were installed 0.4 m from the hydrophones (Figure 1) to prevent the animal from receiving the direct sound reflections off the water surface and pool bottom. Both baffle-boards (width: 1.2 m; effective height in the water: 0.3 m each) consisted of cork-loaded neoprene tiles attached to a wooden plate. One baffle-board was positioned at the bottom of the pool, the other one was positioned at the water surface, and both were perpendicular to the direct sound path. A monitoring hydrophone was placed in front of the underwater station of the animal. Additionally, all sessions were visually monitored via a digital video camera installed overhead, and the video signals were monitored on a video screen near the primary researcher.

Any external noise which could potentially mask the stimulus was reduced as far as possible by interrupting all noise-related activities and switching off all machines in the rehabilitation centre during the conduct of the measurements.

Daan was trained to voluntarily participate in the measurements (i.e., to accept being equipped with the sensors and to subsequently swim to an underwater station where he positioned himself and was exposed to the acoustic stimuli). The animal had to continuously station there for at least 25 s as motionless as possible. His body axis was positioned in a direct line with the transmitting source. Upon the presentation of a

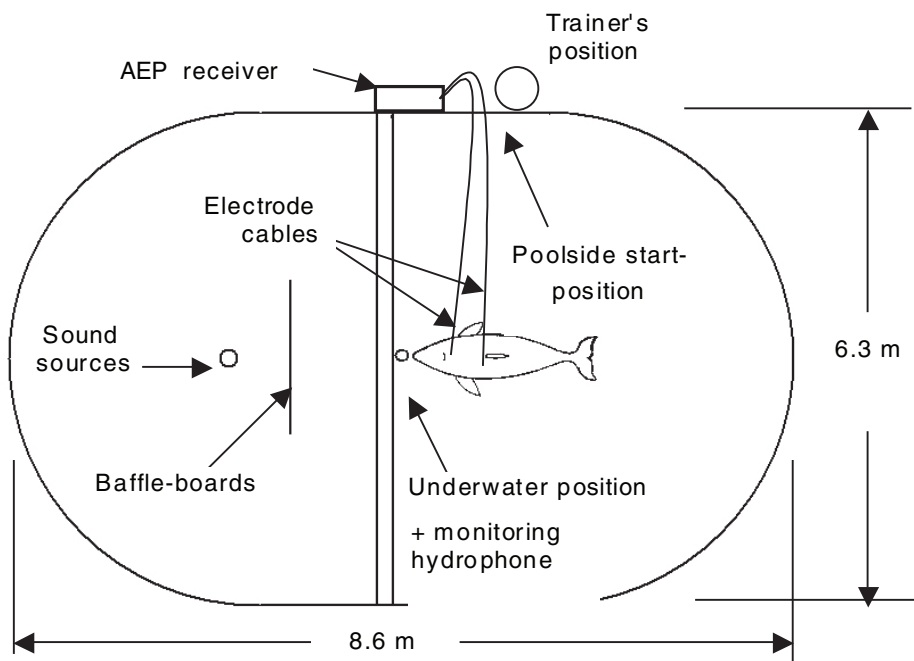


Figure 1. Schematic overhead view onto research pool and set-up with the animal's position at its underwater station indicated

bridging stimulus, Daan returned to the poolside start position to receive reinforcement (fish) and paused before repeating the procedure. Each of these behavioural sequences was called a trial, and a full research session consisted of one up to five trials.

Experimental Procedures

Methods—Measurements were conducted using the AEP method. Two surface electrodes (active and reference electrode) were placed on the animal's skin using suction cups—one near the blowhole and the other near the dorsal fin—to record the neural responses evoked by the auditory system.

Stimuli and Frequencies—The hearing threshold measurements were conducted at frequencies ranging from 0.7 kHz to 16.0 kHz, thus covering a range in which masking from the operational noise of the wind turbines could theoretically occur. Subsequently, measurements were repeated in the presence of the simulated wind turbine sounds emitted at two intensities. Starting from 0.7 kHz, this procedure was repeated at all higher frequencies in an ascending order until no masking was detected. Based on statistical considerations, these measurements had to be repeated several times at each of these five frequencies and at two masking sound intensities.

At frequencies between 2.0 kHz and 16.0 kHz, AM signals were used; while at < 2.0 kHz, a short pulsed signal (tone pip) was taken as the stimulus (thus providing a frequency overlap at 2.0 kHz for comparison). This stimulus (Figure 2) lasted for 1.5 cycles of the test frequency and was cosine-gated over 0.1 ms. All AM signals were fully modulated (modulation depth = 100%) and had a duration of

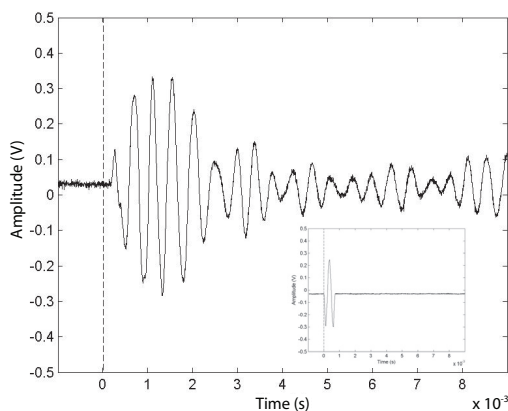


Figure 2. Waveform of the received signal of a 2-kHz Ricker type pulse at 1-m range; the small inserted figure shows the 2-kHz tone pip as an outgoing drive signal in comparison.

25 ms with a rise/fall time of 0.5 ms. All signals were transmitted at varying levels, starting at clearly audible levels and subsequently decreasing SPLs at 5-dB intervals in a series of descending runs. All signals were alternated in phase by 180° between successive stimuli.

Masking Sound—Meanwhile, several underwater acoustic measurements of sounds emitted from a wind turbine were sampled in Sweden, Denmark, and Germany (Westerberg, 1994; Degn, 2000; Fristedt et al., 2001; Ingemansson Technology AB, 2003; Betke et al., 2004; see also Wahlberg & Westerberg, 2005, and Madsen et al., 2006, for reviews). They all differed with regard to the type and size of wind turbine, its foundation, the bottom sediment, and water depth, as well as the weather and wind conditions. Nevertheless, in general, sounds associated with wind turbine operation have their main acoustic energy in a low-frequency band and show strong tonal components at type-specific frequencies below 1 kHz. Therefore, a masking sound was simulated resembling the noise of an offshore wind turbine over a frequency spectrum from 16 Hz up to > 1 kHz, with strong tonal components at 200 Hz and 500 Hz.

The simulated operational noise was continuously transmitted during the masking experiment at two varying levels: moderate (m_1) and high (m_2) with the high level reaching a maximum received level at 200 Hz of 128 dB re $1 \mu\text{Pa}$ at 1 m (see Figure 3) and the moderate masking level at a maximum level of ~ 115 dB re $1 \mu\text{Pa}$ at 1 m.

Stimulus Generation and Presentation

All AEP stimuli up to 16 kHz were generated by using the software *SigGen* (Tucker-Davis Technologies [TDT], Alachua, FL, USA). The

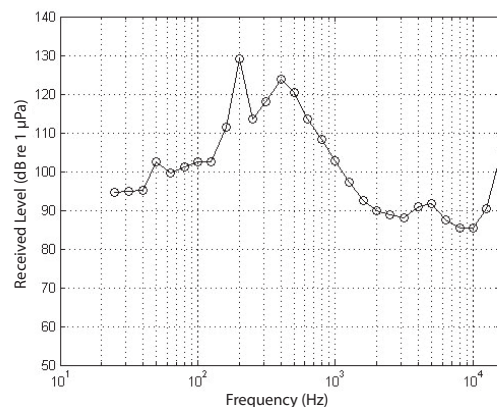


Figure 3. Frequency spectrum of simulated operational sound of an offshore wind turbine; the received levels (third-octave analysis) represent the high masking noise level used during the masking experiment.

stimuli were then fed into a TDT Workstation, System 3, at a digitisation rate of 50 kHz, subsequently amplified by a Brüel & Kjær amplifier 2713 and transmitted via a hydrophone into the water (0.7 kHz to 2.0 kHz: SRD ball hydrophone [10.16-cm diameter], 2.8 kHz to 16.0 kHz: Reson TC 4033). The emitted signals were constantly monitored via a hydrophone (Reson TC 4014) installed in front of the animal, amplified by a custom-made pre-amplifier (etec B1501). All signals were recorded onto a hard disk drive.

During the experiment, the masking sound was constantly played from a laptop computer, with a power amplifier (PA 100E, Ling Dynamic Systems Ltd., Royston, UK) and transmitted via an underwater transducer (USRD J-9). All AEP stimuli were presented to the animal at a rate of 20 signals/s and were monitored on an oscilloscope simultaneously. The hydrophones were calibrated before, during, and after the study. Furthermore, the calibration factor of all other devices used in the experiment was checked thoroughly and taken into account for the analysis in the frequency range from 0.7 kHz to 160.0 kHz.

Impulsive Sounds (0.7 kHz to 2.0 kHz)—The analysis of the recorded low-frequency sound stimuli showed a strong tonal component close to the natural resonance of the transducer (SRD ball hydrophone) at 2.0 kHz. The extension of the signal shown in Figure 4 is due to the transducer being driven at a frequency close to its natural resonance causing the transducer to ring. This stretching of the pulse has resulted in the generation of a strong relatively narrowband tonal around 2.0 kHz shown in Figures 5 and 6. Analysis of similar signals of 1.5 cycles at 1.4 kHz, 1.0 kHz, and 0.7 kHz generated strong resonance effects at the transducer natural frequency. These effects were seen as impulsive transients with an energy of 5 dB below the maximum value. Such impulsive signals at the beginning and end of the pulse form a relatively broadband spectral response. The impulses are then joined with multi-path arrivals at the receiver, resulting in an extended relatively broadband (1.0 to 8.5 kHz) signal generation at lower-received levels than centre frequency.

Amplitude-Modulated Stimuli (2.0 kHz to 16.0 kHz)—The transmitted AM signals (e.g., 8.0 kHz, see Figures 7 & 8) showed no on- or off-set response of the hydrophone. Due to their duration of 25 ms, they were subject to constructive and destructive interference from multi-path signals after < 1 ms. As the degree of distortion varied over the period of each trial between successive signals, the averaged received stimuli were less affected and retained their acoustic characteristics. Sidebands were observed as tonal components at frequencies 1.2 kHz above and below the carrier

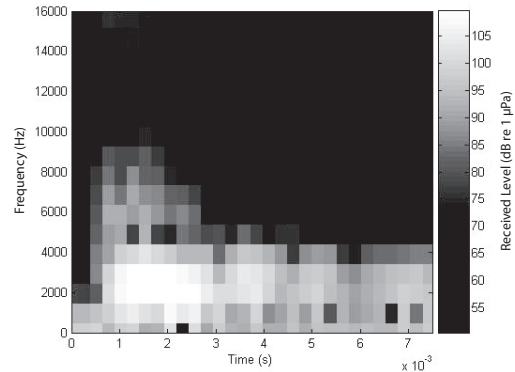


Figure 4. Spectrogram of the 2-kHz Ricker type pulse; strong tonal component due to transducer natural resonance effects.

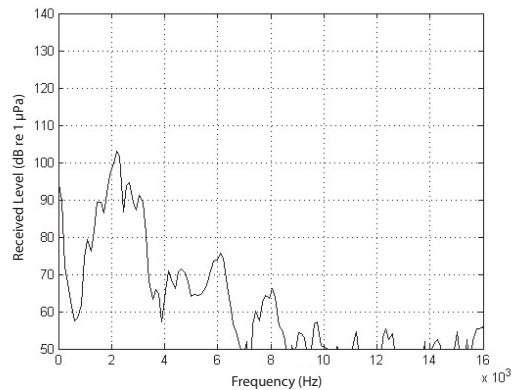


Figure 5. Spectral response for 8.2-ms analysis window, including multi-path signals; spectrum levels based on a 125-Hz analysis band.

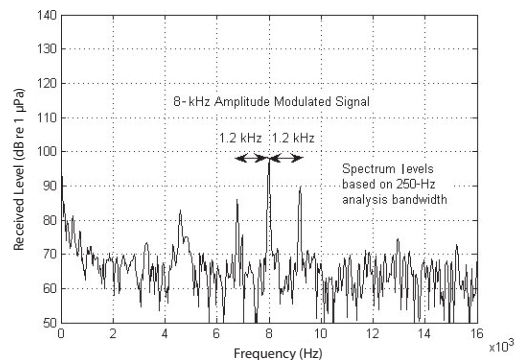


Figure 6. Frequency spectrum of the 8-kHz amplitude-modulated stimulus; two distinct signal components exist at 1.2 kHz below and above the stimulus, representing the hydrophone response to the stimulus modulation at a frequency of 1.2 kHz. The additional dominant component at 4.5 kHz is a result of internal noise and was not transmitted into the water.

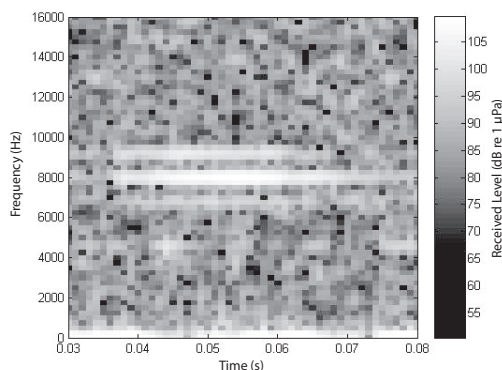


Figure 7. Spectrogram of the 8-kHz amplitude-modulated stimulus over a period of 50 ms; pulse duration is 25 ms. The received levels are colour-coded on a gray scale, with white being the most intense and black the least intense. Two signal components can be identified at 6.8 kHz and 9.2 kHz, representing the hydrophone response to the stimulus modulation at 1.2 kHz. Further dominant signal components are related to the receiving system and have not been transmitted into the water.

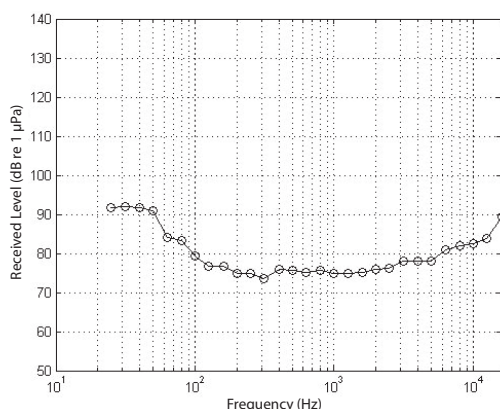


Figure 8. Background noise spectrum analysed in third-octave steps

frequency, thus representing the frequency difference between carrier and amplitude-modulation frequency (correspondingly, the frequency difference was 0.7 kHz at a carrier frequency of 2.0 kHz). These artefacts occurred at levels 10 dB below the carrier frequency signal on average, however.

Background Noise

The background noise in the research pool was recorded while no animal was in the water. The average background noise level in the research pool was about 55 dB re 1 $\mu\text{Pa}^2/\text{Hz}$. Only at 50 Hz is a high-energy amplitude present, which can be attributed to either internal electric noise in the recording system or transmission of this signal into the water.

Response Acquisition

The electrode responses (active and reference) served as input to a low noise differential amplifier (TDT RA4L; 20-dB gain). The amplified analogue signal was then passed through an anti-aliasing filter and led to an A/D converter (TDT RA16). The response (digitisation rate: 25 kHz) was digitally filtered, written to a memory buffer, tested for the presence of unwanted signal artifacts (artifact reject level: 80%), and added to the buffer containing the responses to the previous n stimulus presentations. The evoked responses were recorded and averaged over 500 recordings to acquire one data set. On average, five of these data sets were acquired at each intensity level for each frequency. All responses were bandpass filtered between 500.0 Hz and 2.0 kHz. Examples of the acquired AEP evoked by using tone pips as well as the AM stimuli are presented in Figures 9 and 10.

Mapping the Porpoise Head—Prior to the audiometric measurements, the optimum position of the active electrode was established by changing its position along the dorsal bodyline of the porpoise. A clearly audible AM stimulus was used at a constant, supra-threshold SPL to elicit neuronal responses. These tests were done at a distance of 3.0, 6.0, 7.5, 9.0, and 12.0 cm behind the blowhole. The strongest EFRs resulted from measurements with the active electrode placed at a distance of 7.5 cm behind the animal's blowhole (Figure 11). Consequently, this position was chosen as the ideal position for the suction cup. The reference electrode was placed near the dorsal fin, approximately 40 cm from the blowhole.

Modulation Rate Transfer Function (MRTF)—The auditory system showed qualitative differences in its ability to follow the envelope of a sinusoid. To identify the best frequency to modulate, the amplitude of the carrier signal, the so-called modulation rate transfer function (MRTF) was established prior to the auditory measurements. An MRTF was acquired using different frequencies to modify a carrier signal of 20 kHz. The resulting MRTF revealed three modulation frequencies with almost identical response amplitudes for the EFR: at 0.7, 0.9, and 1.2 kHz (Figure 12). All three frequencies could be used to modulate the amplitude of the carrier signal and would result in the highest achievable EFR amplitudes. Thereby, the lower (0.7 kHz) allowed the lowest possible carrier frequency to be tested, and the highest (1.2 kHz) provided the best signal-to-noise ratio of the EFR responses. For the latter reason, 1.2 kHz was chosen as the best modulation frequency for the AM stimuli in this study, except for 2.0 kHz where 0.7 kHz was used as the modulation frequency instead.

Data Analyses

Pulsed Stimuli—Useful data sets (i.e., curves of 500 AEP responses) were identified by first obtaining an idealised response curve for every frequency at every masking level. Those were achieved by cross-correlating the data sets with a best-fit curve (visually selected from data sets measured under optimum conditions, e.g., with regard to the animal's behaviour and lowest level of disturbing external factors), thereby determining their cross-correlation coefficient and the lag at which it occurred. Subsequently, the measured curve was considered as a response if its cross-correlation coefficient was in excess of a certain threshold and its lag was smaller than a given threshold. Corresponding thresholds were determined by cross-correlating the idealised response curves with controls (i.e., "response" curves measured without prior stimulation) and then cutting off the smallest 60% (corresponding to the lowest correlation coefficient of the best-fit curves) of the obtained cross-correlation coefficients and the largest 95% of the absolute lags. The remaining curves were further analysed by measuring the peak-to-peak amplitude of the most prominent and most consistently detectable potential and applying a linear regression to the resulting data of amplitude response as a function of stimulus level. The zero-crossing of the regression function represented the hearing threshold for the given frequency. To associate 95% confidence limits with the determined hearing thresholds, these data were subsequently bootstrapped with 1,000 samples. The data were tested for significance to verify the relationship between the received level of the acoustic stimuli and the evoked neuronal response. This analysis was applied to the data sets achieved by using tone pips between 0.7 kHz and 2.0 kHz as acoustic stimuli and under the different conditions (no masking, moderate, and full masking). This complex statistical analysis allows the declarative strength of the calculated threshold values to be assessed and was done for all tested frequencies irrespective of the masking condition.

Amplitude Modulated Stimuli—An FFT was applied to all data sets achieved by using AM signals as acoustic stimuli (i.e., between 2.0 kHz and 16.0 kHz). The magnitude of the EFR was assessed in the frequency domain by observing the amplitude of the spectra at the frequency of the amplitude modulation. By applying an F-Test to the resulting FFT data, those EFR responses were identified, which were significantly different from any electrophysiological background noise being present in the AEP recordings. Two methods exist for determining the hearing threshold value from the remaining EFR data. One is

a regression analysis on all resulting EFR amplitudes to identify the zero-crossing and hence the threshold value. A second method (cf. Supin et al., 2001) includes a visual analysis of data prior to a regression analysis. This method is based on the fact that the EFR values at the frequency of amplitude modulation ideally decrease clearly from high values at supra-threshold stimuli levels to medium values at lower intensities. The values would remain constant over a certain range of stimulus intensities, thus forming a plateau if plotted graphically before they finally decreased at stimulus intensities near the threshold. Only the EFR values of the latter intensity range were analysed in this study by using a regression analysis and thus provided a very precise estimate of the hearing threshold. This approach required EFR data of high signal-to-noise ratio, however. As soon as the recorded AEP responses are disturbed by other neuronal activity, a clear distinction of the final phase becomes increasingly difficult. Both types of analysis were applied to the available data in this study, where applicable. For both methods, the 95% confidence limits of the resulting threshold values were calculated by bootstrapping over 1,000 samples.

Results

Impulsive Sound Stimuli

At 2.0 kHz and below, tone pips were used as acoustic stimuli for measuring Daan's auditory sensitivity and the potential masking effect of wind turbine-related operational noise. The threshold values and 95% confidence limits as listed in Table 1 were determined after a statistical analysis and filtering of these data.

Data for the measurements at 1.4 kHz in the presence of high masking levels revealed an insignificant and nonrealistic threshold value, as well as confidence limits. All other threshold values were significant ($p < 0.01$). Nevertheless, the resulting threshold values varied considerably between 25.2 dB at 1.0 kHz and 78.7 dB at 2.0 kHz. At 0.7 and 1.0 kHz, the threshold values increased with increasing masking level, while at 1.4 and 2.0 kHz, the threshold values at moderate masking intensity were below the unmasked threshold. At 2.0 kHz, however, the high masking level led to a substantial increase of the threshold as compared to the unmasked situation. Most importantly, the threshold value at moderate masking conditions was within the confidence limits for all four frequencies. In contrast, the threshold values measured in the presence of high levels of masking sound were outside the confidence limits for 0.7, 1.0, and 2.0 kHz. The threshold value achieved at a high level of masking noise was 6.8 dB above the

Table 1. Hearing threshold values of a harbour porpoise and statistical results for three frequencies tested with tone pips as acoustic stimuli at three different levels of masking noise (m_0 = no masking, m_1 = moderate masking, and m_2 = high masking level); the threshold values are given along with 95% confidence limits, information on significance (** = highly significant), and further statistical information. Significance reflects the relationship between the received level of the acoustic stimuli and the evoked neuronal responses. The number of data points included in the analysis is given for every frequency. Numerator DF are always 1.

Frequency	Masking level	Lower confidence limit	Threshold	Upper confidence limit	F	Error DF	Significance	Number of values	
[kHz]	[m ₀ -m ₂]	[dB re 1 μPa]			p				
0.7	0	50.8	60.8	65.3	52.81	46	0	**	48
0.7	1	60.5	64.8	68.1	82.29	24	0	**	26
0.7	2	69.1	72.1	74.8	82.20	25	0	**	27
1.0	0	-9.4	25.2	43.0	9.76	46	0.003	**	48
1.0	1	10.0	39.7	54.0	12.58	48	0.001	**	50
1.0	2	33.8	47.8	56.6	21.05	36	0	**	38
2.0	0	62.1	69.9	71.3	49.62	34	0	**	36
2.0	1	46.8	64.8	74.7	15.24	17	0.001	**	19
2.0	2	74.5	78.6	81.1	55.85	19	0	**	21

upper confidence limit of the unmasked threshold values at 0.7 kHz, 4.8 dB above at 1.0 kHz, and 7.3 dB above at 2.0 kHz.

Amplitude-Modulated Stimuli

Threshold and Masking Analysis—The analysis, as used for example, by Supin et al. (2001), included only those data which were visually identified as being close to the threshold. The results for the masking experiment at 2.0 and 2.8 kHz are listed in Table 2(a) for all three masking conditions. Threshold values were also calculated for the remaining frequencies for an unmasked condition; the results for all unmasked results are listed in Table 2(b).

All data sets included in the regression analysis revealed significant ($p < 0.05$) results. At 2.0

kHz, the threshold differed only slightly between the three masking levels, with the lowest level analysed for moderate masking. The thresholds achieved in the unmasked and masked conditions were almost identical. At 2.8 kHz, the threshold slightly increased from the unmasked to the moderate condition, while the threshold established for the high masking level was almost 10 dB below the unmasked threshold value.

The threshold values achieved by analysing only the visually selected data sets in an unmasked condition between 2.0 and 16.0 kHz showed only a moderate variation, ranging from 59.4 dB_{rms} re 1 μ Pa at 5.6 kHz up to 85.0 dB_{rms} re 1 μ Pa at 16.0 kHz—a range of 25.6 dB. With an exception at 2.0 kHz, the confidence intervals are comparatively narrow.

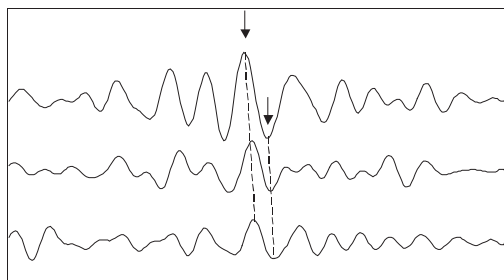


Figure 9. Examples of potentials evoked with tone pip in the harbour porpoise; sampling duration was 10 ms, and centre frequency of the stimulus was 2 kHz. Received level descended from 83 dB re 1 μ Pa (upper trace) in 5-dB steps to 73 dB (lower trace). Arrows indicate the positive and negative peak amplitudes used for threshold analysis. Dashed lines indicate equivalent peaks in the different traces.

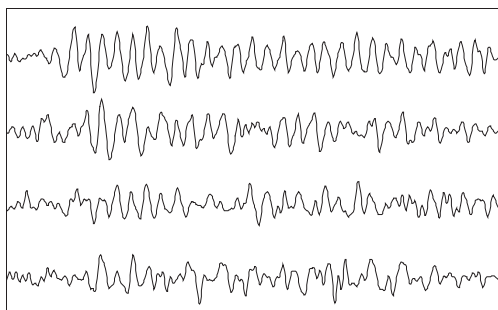


Figure 10. Examples of EFR in a harbour porpoise to AM sound stimuli; sampling duration was 30 ms, carrier frequency was 22.4 kHz, modulation rate was 1.1 kHz, and modulation depth was 100%. Received level descended from 76 dB re 1 μ Pa (upper trace) in 3-dB steps to 67 dB re 1 μ Pa (lower trace).

A comparison of the resulting thresholds with comparable data published from other auditory studies on harbour porpoises are given in Figure 11. Between 2.0 and 5.6 kHz, the resulting sensitivity is in good accordance with other audiometric data published for harbour porpoises so far. In relation to the audiogram measured by Kastelein et al. (2002) on the same animal using a behavioural technique, the ABR data revealed even higher sensitivities. At frequencies of 8 kHz and above, the threshold rose—that is, the animal seems to be less sensitive.

Discussion

Two different types of acoustic stimuli were used in this electrophysiological study to evoke neuronal responses and acquire auditory data from a harbour porpoise. This technique has been successfully used before (*cf.* Supin et al., 2001, for review) and provides in principle the opportunity to collect data in a relatively short period of time. The acoustic conditions of the research environment in which this study was conducted demanded an intermediate approach, which included behavioural training and active participation of the study animal. The advantage of this was the possibility to conduct tests at low frequencies, the main focus of this study. By having to conduct the AEP measurements on a free-moving animal, however, the signal-to-noise ratio and hence the quality of the resulting AEP data was likely to be compromised.

The intensity and acoustic characteristics of sounds associated with offshore wind turbines may vary due to the wind speed, type of wind turbine, bottom substrate, water depth, and weather, as well as oceanographic conditions and other minor parameters (e.g., direction to the wind turbine at which a signal is perceived). Based on the information gathered from recordings made at several

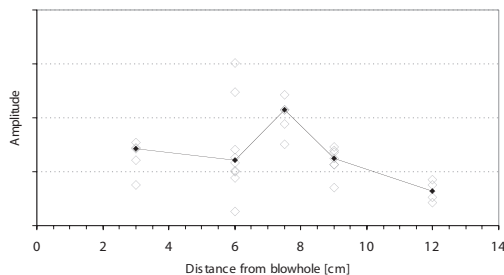


Figure 11. Amplitude dependence of EFR (grey symbols) on the positioning of the active electrode along the longitudinal axis behind the blowhole; AM signals (carrier frequency of 22.4 kHz; modulation frequency of 1.1 kHz) at a received level of 94 dB_{rms} re 1 μ Pa were used as stimuli. The median values are plotted as black symbols.

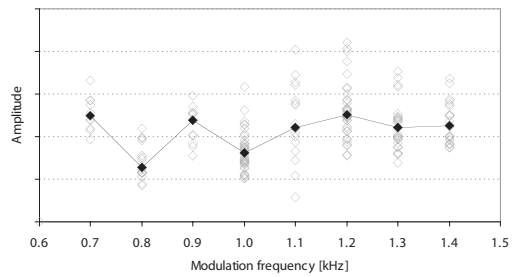


Figure 12. Amplitude of the EFR (grey symbols) as a function of the modulation rate of a carrier signal (carrier frequency of 22.4 kHz; active electrode placed 7.5 cm behind blowhole; received level of 94 dB_{rms} re 1 μ Pa). The median values are plotted as black symbols.

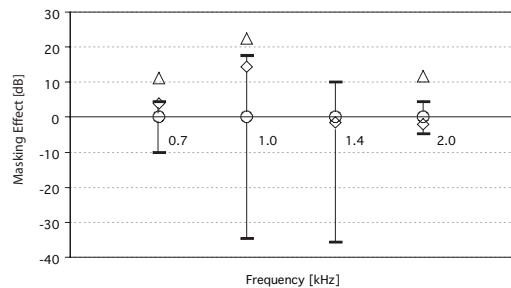


Figure 13. Measured masking effect (in dB) of simulated operational noise of a wind turbine on the hearing threshold of a harbour porpoise at low frequencies; threshold values were achieved by using tone pips at four different frequencies. Circles represent the (relative) hearing threshold with no masking noise present; error bars indicate the 95% confidence intervals. Diamonds show threshold values at moderate masking levels, Triangles represent the threshold values at high levels of masking noise both are relative to unmasked thresholds.

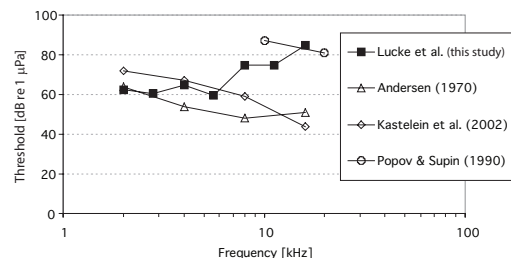


Figure 14. Harbour porpoise hearing threshold data from different studies; the filled symbols represent the threshold values achieved in this study. Data from another AEP study (Popov & Supin, 1990) as well as from two behavioural auditory studies (Andersen, 1970; Kastelein et al., 2002) are given for comparison.

Table 2. (a) hearing threshold values of a harbour porpoise and statistical results for two frequencies tested with AM stimuli at three different levels of masking noise (m_0 = no masking, m_1 = moderate masking, and m_2 = high masking level); (b) without masking sound present. The threshold values are given with 95% confidence limits, information on significance (* = significant; ** = highly significant), and further statistical information. Significance reflects the relationship between the received level of the acoustic stimuli and the evoked neuronal responses. The data sets included into the threshold analysis were visually selected; the number of data points included in the analysis is given for every frequency and masking condition. Numerator DF are always 1.

(a)

Frequency	Masking level	Lower confidence limit	Threshold	Upper confidence limit	F	Error DF	Significance	Number of values	
[kHz]	[m ₀ -m ₂]	[dB re 1 μPa _{rms}]			p				
2	0	20.0	62.5	68.1	4.76	31	0.037	*	33
2	1	44.3	57.8	62.4	44.78	38	0	**	40
2	2	56.2	62.6	66.6	22.62	29	0	**	31
2.8	0	49.2	60.5	64.6	6.47	29	0.017	*	31
2.8	1	59.0	61.8	64.0	37.8	33	0	**	35
2.8	2	18.3	51.0	59.5	14.13	48	0	**	50

(b)

Frequency	Masking level	Lower confidence limit	Threshold	Upper confidence limit	F	Error DF	Significance	Number of values	
[kHz]	[m ₀ -m ₂]	[dB re 1 μPa _{rms}]			p				
2	0	20.0	62.5	68.1	4.76	31	0.037	*	33
2.8	0	49.2	60.5	64.6	6.47	29	0.017	*	31
4	0	60.5	64.7	67.1	26.92	53	0	**	55
5.6	0	55.0	59.4	63.2	75.28	54	0	**	56
8	0	49.5	74.5	79.4	6.45	15	0.023	*	17
11.2	0	64.1	74.7	80.7	6.56	57	0.013	*	59
16	0	82.3	85.0	87.2	97.63	52	0	**	54

wind farms so far (see Wahlberg & Westerberg, 2005, and Madsen et al., 2006, for reviews), the simulated operational sound of an offshore wind turbine used in this study matched those real sound emissions in general. It contained white noise filtered to reduce frequencies above 1.0 kHz with the addition of strong tonal components resembling the major acoustic emissions of the wind turbines. Because there is some variation in the reported noise levels from operating wind turbines, ranging to source levels of up to a maximum of 145 dB_{rms} re 1 μ Pa (back calculated from reported measurements; Wahlberg & Westerberg, 2005) for the low frequency tonal components, the masking level was chosen to be as high as possible. The maximum-received level of 128 dB_{rms} re 1 μ Pa should be clearly audible to the harbour porpoise whose hearing threshold was previously measured to be 115 dB_{rms} re 1 μ Pa at 0.25 kHz and 92 dB_{rms} re 1 μ Pa at 0.5 kHz (i.e., at or near the frequencies of the tonal

components of the simulated sound) (Kastelein et al., 2002).

As revealed by the threshold analysis at the low frequencies (0.7 to 2.8 kHz), this sound had a masking effect on the perception of auditory stimuli by the harbour porpoise. Such a masking effect has been identified from the experiments with tone pips as acoustic stimuli at 0.7, 1.0, and 2.0 kHz. While these data could not be analysed for the 1.4 kHz data sets, the results from the masking experiment with AM signals as acoustic stimuli revealed no masking effect at 2.0 and 2.8 kHz. Masking occurs if the threshold value analysed for a moderate or high level of masking noise is above the upper limit of the confidence interval of the threshold value achieved at the frequency in an unmasked condition. Thus, masking occurred at levels between 4.8 dB (at 1.0 kHz) and 7.3 dB (2 kHz), with an intermediate masking effect of 6.8 dB at 0.7 kHz.

The observed variation in threshold values at different levels of masking, as well as the comparatively large confidence intervals can be attributed to the reduced signal-to-noise ratio of the measured AEP signals in this study. Since the animal was unrestrained during the measurements and had to actively position itself constantly in front of its station, the resulting myogenic potentials are likely to have raised the level of the overall neuronal potentials. This elevated level might have especially masked the auditory potentials, which were measured at received acoustic levels near the auditory threshold. This electrophysiological masking effect leads to a higher degree of variation in the analysis of the auditory thresholds as the near-threshold AEPs are influencing the definition of this threshold. In addition, the amplitude of the EFR has been negatively influenced by a reduction in the modulation depth of the AM stimuli due to interference from reflections in the pool. Even though the research conditions were kept as constant as possible, this effect occurred at varying levels at different frequencies and could not be eliminated by the baffles. Moreover, it was also likely to be influenced by other external factors (e.g., water level and wave action). Nevertheless, the observed difficulties in defining the hearing threshold were compensated by means of a complex statistical analysis.

It has to be taken into consideration that the acoustic stimuli used during this part of the experiment was broadband with a spectrum ranging up to a maximum 8.5 kHz with an energy of 5 dB below the maximum value. This acoustic artefact occurred due to the response characteristics of the transmitting hydrophone in relation to the stimulus. Taking into account that the threshold levels achieved with the tone pips as stimuli can not be regarded as absolute hearing thresholds, the data clearly showed a masking effect. Due to the broadband spectrum, the test stimuli have certainly acoustically stimulated higher-frequency bands on the basilar membrane, rather than being limited to the anticipated frequency bands. At those higher frequencies, the auditory sensitivity should be higher, thus making a perception even more likely. With increasing frequency, those higher-frequency bands should be increasingly less affected by the masking sounds. Thus, with regard to the observed masking, the frequency spread indicated that the actual masking effect of the wind turbine-related sounds could be larger at the frequencies targeted initially. Hence, a narrower test signal would be very likely to reveal a more pronounced masking effect.

If the received level of the operational sounds on average dropped below 120 dB within a range of 100 m from a wind turbine (Madsen et al.,

2006), the level of the masking sound used in this study (high masking level: 128 dB re 1 μ Pa) would have been received only at a short distance from an average type of offshore wind turbine (several tens of meters). The difference between the effective masking intensity at the high masking level and the non-effective moderate masking level was approximately 13 dB. Thus, the effective range of the observed masking would be comparatively small as the operational sound of an offshore wind turbine would be attenuated by 13 dB in shallow water within 20 m from the sound source (assuming spreading with a loss of $10 \log r$ [r = distance in m]) and at less than 10 m distance from the sound source in deep waters (assuming spherical spreading with a loss of $20 \log r$). Due to oceanographic or geological features, the spreading loss can reach even higher levels, thus decreasing the effective masking range of the wind turbine sounds. The actual sound measurements have been carried out at comparatively small wind turbines, however. Several offshore wind farms are currently planned to consist of turbines of up to 5 MW. It is unclear to what extent the sound emissions of these turbines will be elevated with the increased turbine size. So far, these emissions have only been modelled (DEWI, 2004) but should be measured upon construction of the turbines.

Nevertheless, with regard to their regulatory implications, these results indicated that the masking effect of the operational sound emissions of wind turbines is small. The perception of sounds which might be biologically meaningful to the harbour porpoises would be constantly reduced, but the masking range is limited to several tens of meters around the wind turbine (i.e., the harbour porpoise will encounter an impaired perception of low- to mid-frequency signals only in the vicinity of the wind turbines). As the distance between turbines will be several hundred meters, the potential masking zone of neighbouring wind turbines will not overlap. Therefore, from an ecological point-of-view, the results indicated that the overall effect of the operational sound emissions of the planned wind turbines is very likely to be small for the individual harbour porpoise and negligible on a population level.

Studies on the behavioural reactions of harbour porpoises to continuous sound from offshore wind turbines (Koschinski et al., 2003) indicated that displacement occurs over slightly longer distances than the masking effect. As the overall noise regime in this study differed markedly from the North Sea noise conditions, and due to context-specific variation and the strong individual differences in reactions of the animals, it remains difficult to assess the effective range of behavioural

effects from wind turbine sound as compared to the masking.

The conclusions that can be drawn from this study are naturally limited by the sample size of $n = 1$. The normal individual variation in hearing sensitivity could theoretically account for a higher auditory sensitivity of several dB. The audiometric data published for harbour porpoises so far, as well as the results from this study indicate that at least at the lower frequencies (< 5.6 kHz), this variation is confined to 13 dB, whereas the thresholds measured in this study are at low or intermediate levels within this range. Age-related hearing loss (e.g., Moore & Finneran, 2004) that could reduce the masking effect of wind turbine-related sound can be ruled-out for this study since at these low frequencies the animal's measured hearing threshold is in good accordance with the levels measured on the same animal when it was young. Moreover, in other odontocetes, age-related hearing loss occurred at much higher frequencies.

The echolocation clicks of harbour porpoises are short, transient signals with a high acoustic energy at frequencies centred around 130 kHz. Apart from these clicks, no other sounds have been recorded so far which can be attributed to serve for communication purposes for these animals. In this context, it has been hypothesized that the low-frequency portion of the click, which has been documented by Verboom & Kastelein (1995) could code some information for the harbour porpoise (Schevill et al., 1969). Within the scope of this study, a low-frequency component could not be documented for either of the two harbour porpoises in the research pool. This does not imply that the low-frequency component does not exist in the porpoise click. The low-frequency component was characterized to have a source level of approximately 100 dB re 1 μ Pa and a frequency spectrum of 1.4 to 2.5 kHz. The range of such a quiet signal is limited by the natural background noise in the sea anyway. Any additional sound source in a similar or adjacent frequency range would be likely to mask the perception of these sounds and, thus, reduce the effective range of this low-frequency component. As the masking effect has been documented for a broadband test stimulus, a masking effect is even more likely to occur for this low-frequency part of the echolocation signal based on the existing data.

The threshold results achieved with AM signals can be considered as absolute hearing threshold values at frequencies between 2.0 and 5.6 kHz. At these frequencies, the resulting sensitivity is in good accordance with other audiometric data published for harbour porpoises so far. In relation to the audiogram measured by Kastelein et al. (2002) on the same animal using a behavioural technique, the AEP data revealed even lower threshold values.

At frequencies of 8.0 kHz and above, however, the threshold seems to be less sensitive. At these higher frequencies, the confidence intervals for the threshold values are comparatively small, thus indicating a relatively high declarative strength of the data. The rise in threshold can either be attributed to the fact that Daan lost some of its auditory sensitivity at these frequencies over the last five years or the data reflect the presence of ambient masking noise which is unrelated to this study.

The system set-up was designed to enhance the acoustic situation as much as possible. An important factor for a masking study is the existing background noise in the research environment. In the research pool, the noise floor seemed to be relatively low at relevant frequencies. Looking at the $\frac{1}{3}$ -oct analysis of the background noise, however, it is not surprising to see a rise in the energy content with increasing frequency as the effective range of each $\frac{1}{3}$ -oct interval becomes wider with increasing frequency. Given the assumption that the ear of marine mammals can be modelled as a series of bandpass filters, it strongly depends on the width of these filters as to how much the background noise influences the perception of acoustic stimuli. If the animal's auditory filters were also a $\frac{1}{3}$ -oct bandwidth, the sound energy integrated in these filters would also rise with increasing frequency for a uniformly distributed noise floor. In the open ocean, the background noise drops from high levels at low frequencies with increasing frequency to very low levels, and it only rises up again to substantial levels at frequencies above the frequency spectrum of the harbour porpoises (Wenz, 1962). The background noise recorded in the research under relatively undisturbed conditions nevertheless includes a considerable amount of acoustic energy even at higher frequencies so that at frequencies above 10.0 kHz, the $\frac{1}{3}$ -oct level rises to over 90 dB re 1 μ Pa. At higher frequencies (≥ 22.5 kHz), however, the auditory filters of harbour porpoises are not of constant quality (ratio of centre frequency of the auditory filter to the passband bandwidth), but of constant bandwidth as shown by Popov et al. (2006). Assuming that this auditory property is constant over the animal's entire hearing range, the constant noise floor in the pool might have had some masking effect, but this would not solely explain the increase in Daan's hearing threshold at and above 8.0 kHz. As systematic causes for elevated hearing thresholds were attempted to be avoided, they can nevertheless not be ruled-out completely for a study in such an acoustically very challenging environment. The documented difference theoretically also could be attributed to the fact that behavioural audiograms can be affected by the attention of the animal and/or whether it was trained to be conservative

or liberal in responses. Ultimately, the measured difference in threshold between the behavioural and the electrophysiological methods on the same animal in the same pool could reflect an actual hearing loss.

Absolute hearing thresholds are basic for predicting the perception of acoustic stimuli, but they do not provide sufficient information on whether a signal will be masked or not. Over the last decades, the amount of sound emitted into the water has increased substantially (*cf.* Hildebrand, 2004). In this context, it seems necessary to collect more information on masking thresholds and masking-related parameters. Numerous research activities have already focused on the potential effects of sounds on the marine environment; this study is one of them.

Acknowledgments

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