# AQUO

**Achieve QUieter Oceans by shipping noise footprint reduction**

**FP7 - Collaborative Project n°314227**

**WP 4.2: Noise sensitivity of marine life**
**SubTask 4.2.2. Masking effects of shipping noise on harbour porpoises**

# Masking effect of white noise and simulated shipping sound on low-frequency auditory perception in harbour porpoises (*Phocoena phocoena*)

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# SUMMARY

This study has been realized in the scope of AQUO, a collaborative research project supported by the 7th Framework Programme through Grand Agreement N°314227, whose final goal is to provide to policy makers practical guidelines to mitigate underwater noise footprint due to shipping, in order to prevent adverse consequences to marine life. The present document is the deliverable D4.3 “Masking effects of shipping noise on harbour porpoises”. This study has been realized in the scope of the work package N°4, which is dedicated to bioacoutics of marine fauna in relation with shipping underwater noise. This work package aims contributing to a better knowledge of the impact of underwater sound on marine fauna (marine mammals, fish, and invertebrates), and the present study is focused on harbour porpoise, which is a representative species for marine mammals in European waters.

Sound is a primary means for harbour porpoises (*Phocoena phocoena*) to sense their aquatic environment. Like other toothed whales, they use echolocation to probe their marine environment and rely on the detection of echoes reflected from objects in their surroundings for orientation, navigation, foraging and predator avoidance. Anthropogenic sound could reduce a harbour porpoise’s ability to detect echoes. Broadband sound is generated as a by-product by the machinery of ships or emitted due to propeller cavitation into the marine environment. This acoustic energy of ship sound is centred below 100 Hz, but extends under normal conditions well into the high (ultrasonic) frequency range where harbour porpoises have their best hearing sensitivity. A reduced efficiency in their acoustic perception due to acoustic masking through ship noise could have deleterious effects on these animals. This study on ‘the masking effect of ship noise on hearing in harbour porpoises’ consisted of a series of auditory measurements in four harbour porpoises held at two facilities in the Netherlands. Their hearing sensitivity was first tested in an un-masked situation by measuring auditory brainstem responses (ABR) to repeated acoustic stimulation with tone-pips at frequencies between 0.5-16 kHz. Subsequently, these measurements were repeated in the presence of red noise, resembling the acoustic underwater signature of ships as well as Gaussian white noise which is the standard type of masking sound used in laboratory masking studies.

The results show that ship noise has the potential to mask the auditory perception of harbour porpoises over the entire frequency range tested in this study. Modelling a ship’s masking range is a complex task as it depends on the sound source, the sound propagation, ambient noise level and the receiver. Determining the exact amount of masking is equally difficult, as numerous (mainly physiological) factors contributing to masking exist as well as masking-release mechanisms and strategies. Nevertheless, the detection ratios (Signal-to-Masker Ratio, SMR) determined in this study prove that masking through ship noise occurs if an animal is close enough to a ship. Furthermore, they allow assessment of the scale of the masking. The masking effect of ship noise on the hearing in harbour porpoises has been proven to be frequency dependant. The median SMR in the presence of ship-like (red) noise and white noise reach respective maxima of 28 dB at 5.6 kHz and 31 dB at 16 kHz. As a general trend, the SMR are relatively stable at low levels of <14 dB, and at frequencies ≤1.4 kHz (red noise) and ≤4 kHz (white noise). With increasing frequency, the median SMR increased.

Masking through ship noise changes the entire auditory scene for harbour porpoises and the range over which they can operate acoustically. This, in turn, can negatively affect the animals' energy budget and ultimately their fitness and population dynamics. Ideally, masking should be considered as a significant conservation issue when assessing existing or new anthropogenic sound sources/activities at sea. Contrary to the common understanding that only the low frequency part of ship noise has to be considered with regards to marine fauna, this study provides further evidence that, despite the decrease in acoustic energy contained in the ship signature at the higher frequencies, the actual masking effect increases. This needs to be taken into account in assessing the potential impact zones and durations, but also in defining the noise monitoring parameters as in regulatory frameworks.

# 1. INTRODUCTION

Marine mammals evolved in the marine environment where light and other forms of energy attenuate rapidly, only sound propagates well. Because of the ease with which acoustic information is transmitted in water over large distances acoustic signals evolved to be the principal mode of information transmission for marine mammals. Specifically, the toothed whales use sound as a primary means for sensing their aquatic environment [1]. They have developed sophisticated bio-sonar capabilities and the production, perception, and processing of sound is critical for several of their life functions including communication, foraging, navigation, and predator-avoidance [2].

Habitats of marine mammals overlap with areas of intense anthropogenic activities. With the rapid increase in the worldwide ship traffic over the past decades [3][4] vessel noise - emitted into the oceans as a mere by-product - increased significantly and is now considered the dominant anthropogenic underwater noise source in the low frequency range [5]. The European Union recognises underwater sound as a form of pollution in their Marine Strategy Framework Directive (MSFD [6]). Within descriptor 11, the 1/3$^{rd}$ octave frequency bands centred at 63 and 125 Hz are specifically considered to be relevant proxies for underwater ship noise [7][8]. However, it is questionable if these two frequency bands are suitable indicators as the acoustic signature of different ship types varies [3][9]. Mid-to-high frequency noise levels have been shown to increase with higher ship speed [10][11][12]. These higher frequency bands are more relevant for toothed whales as they have acute hearing sensitivity (SPL <60 dB re 1 µPa) at higher frequencies (10-140 kHz) [13] (corrected by [14]).

To investigate potential impacts of shipping noise on marine mammals, this project focusses on one species, the harbour porpoise (*Phocoena phocoena*). The harbour porpoises range widely throughout coastal waters in the northern hemisphere and is the most abundant toothed whale in the North Sea [15], where shipping intensity is amongst the greatest in the world. Harbour porpoises produce highly directional, short sound signals (‘clicks’) centred around 130 kHz [16] to probe their marine environment, They rely on the detection of echoes reflected from objects in their surroundings to find prey, orientate themselves and avoid obstacles or predators (‘echolocation’) [2][17][18]. Avoidance behaviour of harbour porpoises in response to noise emissions from distant ships has been documented [19][20]. Dyndo et al. [21] measured a substantial increase in underwater sound in the mid- to high frequency range during the passage of vessels at ranges of >1000 meters in shallow waters and documented simultaneous strong behavioural responses in harbour porpoises. As low frequency sound does not propagate well in shallow water [22] this suggests that the animals can hear and reacted to the mid- to high frequencies components of the sound. Hermannsen et al. [8] provide over 20 different recordings of ship noise which was substantially above ambient noise across a broad frequency range from 25 Hz up to 160 kHz. These sounds would be within the range of most sensitive hearing of the harbour porpoises. They argue that for harbour porpoises living in areas of high shipping intensity “this could have considerable effects on the behaviour and acoustic umwelt of these small toothed whales in shallow water.” [8].

Besides behavioural effects the main risk of increased vessel noise would be masking of signals that are biologically relevant for harbour porpoises. Masking is a reduction in the animal’s ability to detect relevant sounds in the presence of other sounds. Quantitatively, masking refers to the amount in decibels by which an auditory detection threshold is raised in the presence of noise [23]. Masking occurs at the receiver and as the sound changes on its propagation path from the source to the receiver, its masking potential changes accordingly.

Moreover, masking in itself is a complex phenomenon involving both the auditory periphery and the central nervous system of the receiving animal. In the absence of sufficient data, a comprehensive assessment of masking effects is as yet not possible for marine mammals. Models have been developed to predict masking levels for particular combinations of sender, environment, and receiver characteristics. Such models used to estimate auditory masking require species-specific knowledge of auditory sensitivity and frequency tuning, as well as information about signal and noise characteristics at the location of the listener (e.g., [24][25][26][27][28][12][29]). The acoustic characteristics of the signal, the masker and the ambient environment affect the potential for and the amount of masking. The potential for masking is greatest if the masker occupies the same frequency band(s) as the signal, if the signal-to-noise ratio is low and if signal and masker coincide, i.e. arrive at the same time, at the receiver (see also chapter 3. Masking).

In order to determine the potential for masking of a (e.g. biologically relevant) signal by ship noise, the following characteristics need to be considered:

- Signal level (spectrum level)
- Masker level (spectrum level)
- Timing and temporal variability of signal and masker (duration, duty, cycle, gaps, pulse length etc.)
- Sound propagation
- Ambient noise
- Characteristics of receiver's auditory system

The hearing abilities of an animal/species under consideration are crucial in determining the potential for masking. Typically, the hearing sensitivity is measured using pure tones and is graphically displayed in an audiogram as a function of hearing threshold versus frequency (Figure 1). Mammalian audiograms exhibit a characteristic U-shape, with a centre region of best sensitivity and decreased sensitivity towards both lower and higher frequencies [30].

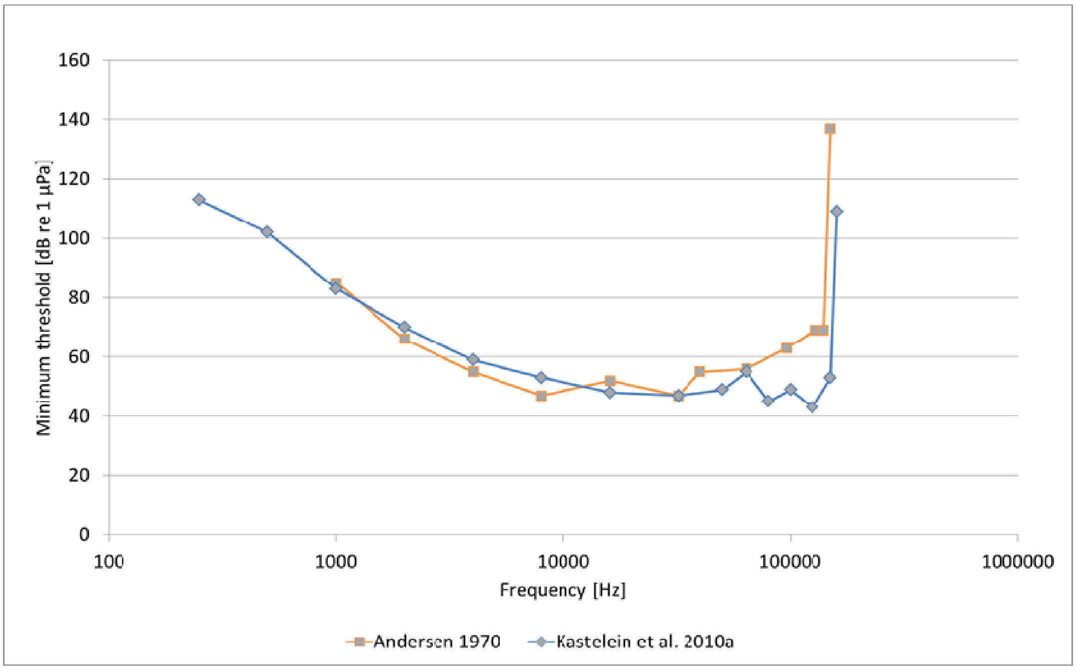



*Figure 1. Audiograms of two harbour porpoises showing their hearing thresholds over a wide range of frequencies [31][14]. Minimum threshold levels are displayed in [$dB_{rms}$ re 1 µPa] as a function of frequency [Hz] on a logarithmic scale.*

Andersen [31] and Kastelein et al. [14] showed that harbour porpoises have their best hearing sensitivity between 16 and 140 kHz with a lower/upper limit in their functional hearing (equivalent to ≥60 dB above best sensitivity) of <500 Hz and >160 kHz, respectively. Audiograms can be measured behaviourally in captive, trained animals as in the studies by Andersen [31] and Kastelein et al. [14], or physiologically by measuring auditory evoked potentials (AEPs) in captive or temporarily restrained, wild animals. Behavioural audiograms provide the most reliable results about the actual perception of sounds, but are more time- and resource consuming than physiological audiograms. The latter ones provide good estimates of hearing sensitivity (e.g., [32][33][34][35][36][37]) and are ideal for comparative studies. The main advantage of this method is that measurements can be conducted much faster and usually do not require training of the subjects. AEPs are neuronal signals generated by the auditory periphery (inner ear, eight nerve and lower auditory nuclei of the brainstem) upon stimulation by a sound. These signals can be measured non-invasively at the surface of a subject's skin (see 4.4 Response acquisition). While hearing thresholds obtained using the two methods (behavioural and physiological) are not interchangeable, both provide information of frequency-specific hearing sensitivity [38].

This research on the masking effect of shipping noise on harbour porpoises is part of the collaborative research project 'Achieve QUieter Oceans by shipping noise footprint reduction' (AQUO) which aims to identify and if possible reduce the noise footprint of shipping activities in the world's oceans. Within the AQUO framework, this study is designed to test the masking effect of shipping noise on harbour porpoises. The measurements necessary to achieve the relevant information had to be conducted in a controlled acoustic environment. Suitable access to animals was available at two facilities in the Netherlands.

The classical approach to quantify masking effects is based on testing the perception of a pure tone in the presence of Gaussian white noise which allows the Critical Ratio (CR) and Critical bandwidth (CB) of a subject's hearing system to be derived. Previous masking experiments in harbour porpoises provide excellent data on the performance of their hearing system in the presence of pure tones as masker. Popov et al. [39] showed that the bandwidth of the porpoise hearing system is almost constant, but unfortunately they did not test frequencies below 22.5 kHz. Kastelein et al. [40] tested the masking effect of pure tones between 0.315 and 150 kHz and found a constant CR of 18 dB up to 4 kHz and increasing CR (by 3.3 dB/octave) up to 39 dB for frequencies above.

Their approach, however, does not provide results that are easily applicable to a real-world scenario such as assessing the potential masking effect of ship noise on harbour porpoise hearing. This is because signals which may be biologically relevant for harbour porpoises are not of purely tonal nature (most biological sounds are more or less broadband), nor is the masking noise these animals may encounter Gaussian white noise. The approach used in laboratory (human) research is therefore not fully applicable for the recent study. Instead, red noise was used in this study as the best proxy for ship signature to simulate the masking situation the animals may encounter as closely as possible.

# 2. Shipping sound

Ships emit sound into the marine environment when operating with most of the acoustic energy concentrated in the low frequency range [4]. The amount of low frequency ship noise radiated from a ship is correlated with size and speed of the vessel [41][10][9] (Figure 2). However, increased ship speeds can cause increased noise levels at medium-to-high frequencies [11][12][8]. Propeller cavitation is causing a broadband noise which can become even more dominant than the low frequency noise radiated by ships [3].

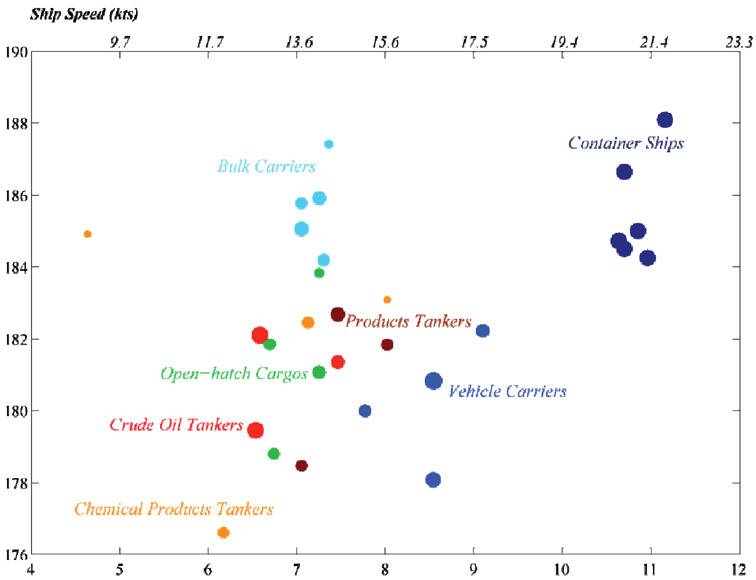



*Figure 2. Broadband ship source level vs. speed for measured ships measured by McKenna et al. (2012); bubble colour signifies ship-type (AIS), bubble size represents the relative size of the ship [9].*

In waters as shallow as the North Sea, however, the low frequency component of the vessel noise is not propagated [22] while mid- and high frequency components between 10 and 160 kHz are clearly detectable above the ambient noise level [21][8].

Figure 3 shows the spectrogram measured from a vessel passing by an underwater sound recorder. Due to propeller cavitation, the noise emitted by this vessel is broadband from 30 Hz to >20 kHz and continuous. With decreasing distance to the noise logger the energy of the ship's sound increases, represented by colours changing from blue to red (highest intensity). The spectral analysis shows substantial acoustic energy (green to red) above ambient noise (blue) over the entire frequency range of the recording (24 kHz). The curved pattern of the lines is known as a Lloyd's mirror interference pattern, where the vessel's closest point of approach is at the local minimum. As a function of range, some frequencies cancel out destructively while others add constructively. This effect is based on the constructive and destructive interference of the direct path arrival of the signal and the destructive (out-of-phase) arrival of the signal after being reflected at the surface [42]. The result, when displayed as power spectral density plot over time, is a U-shaped interference pattern. This specific propagation phenomenon is relevant for the assessment of potential masking effects and will be referred to in the discussion.

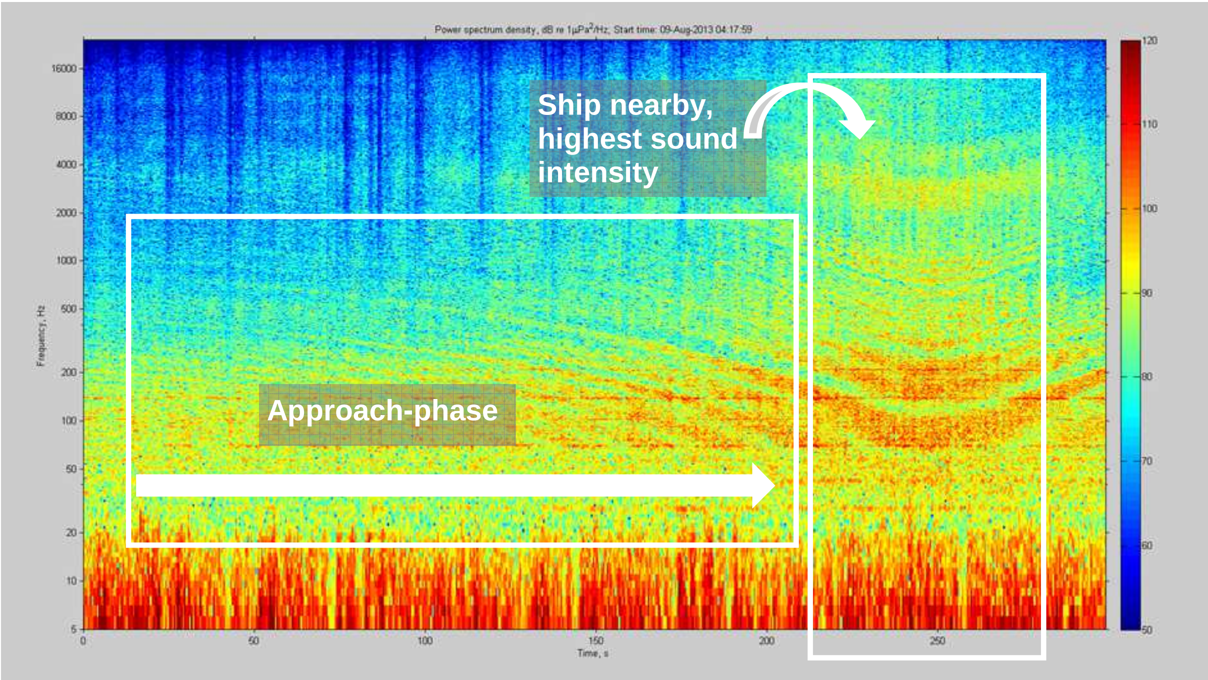



*Figure 3. Spectrogram of a ship passage recorded by a hydrophone/ noise logger deployed at 34 m water depth. Time is displayed on the x-axis, the frequency on a logarithmic scale [Hz] on the y-axis. The power spectral density (colour-coded, see legend on the right) is integrated over 1 s and displayed in dB re 1µPa$^2$/Hz.*

The acoustic signature of ships varies depending on size, speed, propulsion type (blades vs. jet-propulsion), and load of the vessel [9] (Figure 4). It is difficult to identify which factor is most relevant as they are often inter-related. So far, useful measurements are available for some vessel types (e.g. merchant ships, tug boats), but the situation is data-poor or measurements are non-existent for many other categories (e.g. naval, oceanographic, fishing, small craft, ferries).

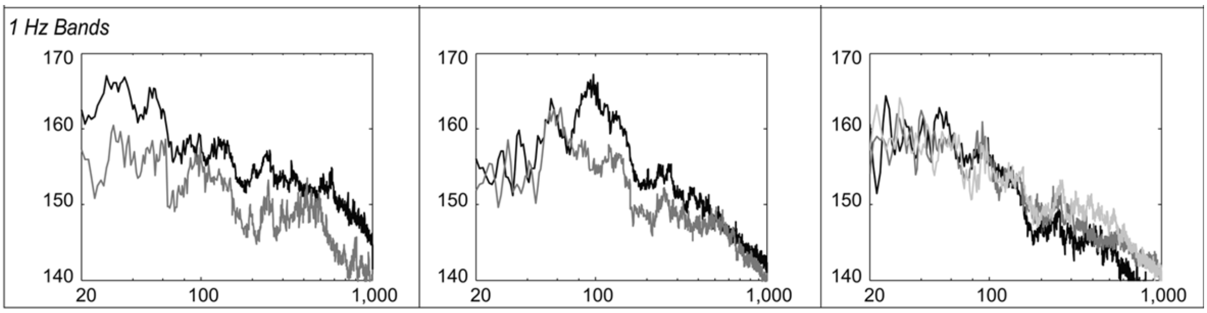



*Figure 4. Source levels for (a) container ships and vehicle carriers, (b) bulk carriers and open hatch cargo vessels, and (c) three types of tankers displayed in 1 Hz band levels [9]. Source level [dB re 1 µPa$^2$ in 1 m] is displayed as function of frequency on a logarithmic scale [Hz].*

To compensate for the variability of vessel signatures several models have been developed predicting underwater radiated noise from ships (such as [43]). In addition, a parametric explicit model was developed within AQUO (WP 2) which has greatly improved the predictive power with regards to various vessel types as a function of frequency, size and speed. Nevertheless, the complexity of factors attributing to the sound radiated from ships in concert with sound propagation effects which change the acoustic characteristics of the emitted sound still results in a multitude of vessel signatures. No single, typical signature emerges as the most appropriate to use in a masking study.

In lieu of there being a single appropriate sound source to represent shipping sound, in this study red-noise with a spectral peak at 100 Hz has been chosen as a proxy for ship noise. This resembles the average spectral content of various ship signatures. Red (or Brownian) noise is statistical noise with a maximum spectral density at lower frequencies and a decrease in power of 6 dB per octave (20 dB per decade).

All data presented in this report are acoustically unweighted and can be converted to other metrics (such as: sound pressure level (SPL) for continuous sound, sound exposure level (SEL) also for transient sounds and/or zero to peak sound pressure level (Lz-p) for transient sounds) (see [44][45]).

# 3. Masking

The acoustic interference caused by an acoustic masker may decrease the perceived loudness of the signal, may make a given change in the signal less discriminable, or may make the signal inaudible for the listening/receiving animal. While discrimination and recognition of signals are important in the ecological context, they are extremely difficult to determine in animal studies. As a consequence, most masking studies in marine mammals as well as for most terrestrial animals have focussed on the detectability of sounds in the presence of a masker. The ear receives acoustic signals plus acoustic noise (the masker). There is then a succession of mechanical (middle ear to cochlea), neurological (inner ear to cortex), and psychological (cortex) processing of the combined signal and noise to determine whether or not a signal is present.

The concept of masking is based on the assumption that in this process the cochlea is acting as a bank of overlapping band-pass filters of a certain bandwidth; in humans the auditory filter is approximately 1/3$^{rd}$ octave over a broad range of frequencies, hence the bandwidth is proportionally getting wider with increasing frequency. The relevant information on masking available for marine mammals indicates similar patterns to those observed in terrestrial mammals [46][47][48]. For some species, such as the harbour porpoise, the bandwidth of their auditory filters may fall below 1/12$^{th}$ octave (derived from CR measurements; [49][39]) suggesting the possibility of an enhanced capability to detect signals in noisy environments [50]. Moreover, Kastelein et al. [40] found that in the harbour porpoise, the auditory filter varies with frequency – while at low frequencies the filters seems to be of constant bandwidth, their bandwidth increases at higher frequencies.

A criterion inter-related with the concept of auditory filters is the Critical Ratio (CR), the lowest signal-to-noise ratio at which an animal can detect a tonal signal in the presence of a broadband Gaussian white noise [51][52]. The lower an animal's CR, the better its ability to detect a signal in noise. The CR is easier to measure and is calculated as the difference between the root-mean-square sound pressure level $SPL_{rms}$ of a just-audible tonal signal (SPL in dB re 1 µPa) and the power spectral density level (PSD in dB re 1 $\mu Pa^2/Hz$) of the masking noise at the frequency of the signal (Figure 5). Typically, the CR is expressed in Decibels, as 10 times the logarithm of the tone power divided by the noise power density (PD): $CR = 10 \cdot \log10 (PD_{tone}/PD_{noise})$. The CR is an easy means to calculate the detection threshold level of a signal under masked conditions [53]. However, it is cautioned that neuronal processing at the higher cortical levels reduces the masking effect (see below: 'Masking release mechanisms'). Also, certain behavioural strategies can be used to lower the interference from a masker.

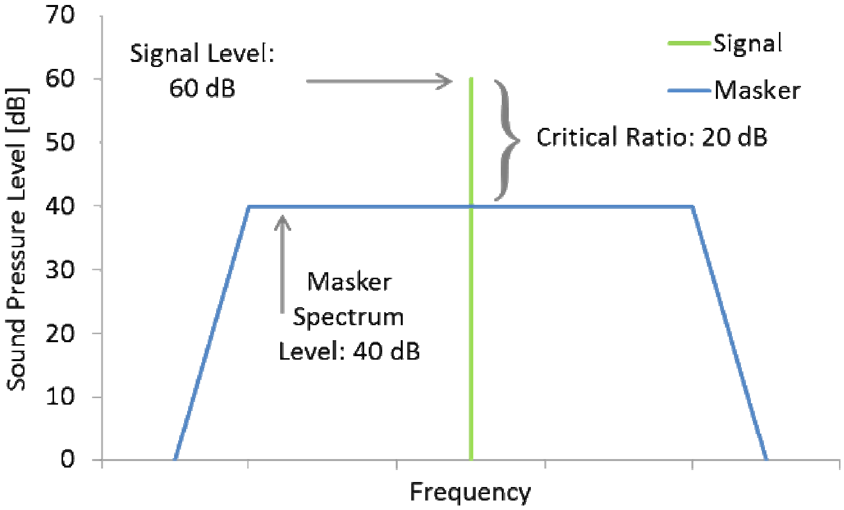



*Figure 5. Schematic diagram depicting the relationship between signal, masker and critical ratio; Sound pressure level [dB] is displayed as function of frequency (no scale) (after [25]).*

The only masking experiment in harbour porpoises involving a masker resembling a man-made sound instead of a tonal stimulus was conducted by Lucke et al. [54]. Using the auditory brainstem response (ABR) method, they measured an animal's hearing sensitivity for signals with test frequencies of 0.7–16 kHz in the presence of simulated offshore wind turbine noise as a masker. Their results indicated that the masking effect of this type of noise would be limited within a few hundred meters around a wind turbine.

## 3.1. Masking release mechanisms

Harbour porpoises are likely to have auditory mechanisms that increase their detection capabilities in noisy situations ('masking release') as has been shown for other toothed whale species: temporal- masking release [55][56][57], spatial masking release [58], co-modulation masking release ([59] for humans; [60] for bottlenose dolphins, *Tursiops truncatus*) and within-valley listening [61].

Temporal release from masking occurs when the masking noise is not constant and the signal can be detected in the 'silent' intervals. Spatial release from masking is possible when signal and noise are directional and received from different directions. Harbour porpoises have good directional sensitivity for frontal-oriented signals and poor sensitivity for signals coming from behind [58]. Co-modulation masking release applies if the masking noise and the signal exhibit similar amplitude modulation patterns (hence coherently modulated) across a broader range of frequencies than the signal, while 'within-valley listening' is based on the gaps in the spectrum of sounds with a non-constant frequency spectrum. Signals with acoustic energy in these gaps can possibly be detected even if the masking level is too high otherwise. If effective, these mechanisms would contribute by improving the detection of sounds in the presence of ship noise.

## 3.2. Biological significance of masking

Harbour porpoises use sound actively for communication [62] as well as echolocation [63] to estimate the location, range and direction of an object [64]. Masking can negatively affect both functions with acute and long-term consequences for the animal. It is difficult to study masking effects in free-ranging animals as masking does not necessarily trigger a behavioural response in an animal, but rather cause the absence of a behavioural response. Failing to detect and appropriately react to the presence of a threat (a predator) or a conspecific may have serious impacts on fitness of the animal [8]. Even if harbour porpoises possess auditory mechanisms that improve their detection capabilities, their auditory scene and the range over which an animal can operate acoustically are altered, when they are exposed to additional noise [12].

# 4. Methods

## 4.1. Ethics statement

The measurements were performed in accordance with Directive 2010/63/EU. All experiments were performed according to the Australian Code of Practice for the care and use of animals for scientific purposes under an approval from the Curtin University Animal Ethics Committee (AEC_2014_23). The experiments were conducted also under permit IMARES 12-02 issued by the Dutch Royal Academy of Sciences under article 11 and 16 of the Dutch law for animal welfare. All measurements were conducted under the supervision of the responsible veterinarian.

## 4.2. Subjects and facilities

The tests were conducted in outdoor pools on four trained harbour porpoise at the Dolfinarium Harderwijk and Ecomare, both located in the Netherlands. Two animals were initially stranded and rehabilitated at Stichting SOS Dolfijn (Harderwijk, The Netherlands), but declared non-releasable due to chronic health issues, the other two animals were born in captivity (see Table 1).

*Table 1. List of harbour porpoises used for auditory measurements*

| Animal ID | Age | Sex | Origin |
|---|---|---|---|
| PpSH 163 | 4-5* | Female | Stranded, declared non-releasable |
| PpZH 003 | 2 | Female | Captive born, non-releasable |
| PpZH 004 | 2 | Female | Captive born, non-releasable |
| PpSH 174 | 3 | Male | Stranded, declared non-releasable |

The pool at the Dolfinarium Harderwijk is kidney shaped (dimensions: 16 x 9 m, depth: 3 m) with a slightly rounded and slanted bottom profile and an effective width (as used in this study for propagating the masking sound) of 9 m. At one side a small inlet of 1.5 x 1.5 m and a water depth of 1 m is formed. At Ecomare the measurements were conducted in an oval pool (dimensions: 6 x 4 m, depth: 1.5 m) with a slightly rounded and slanted bottom profile.


*Figure 6. Photos of the research pools at Ecomare (left) and Dolfinarium Harderijk (right); white arrows indicating the animal's location during the measurements.*

Prior to the auditory measurements, all animals were trained for husbandry behaviours and accustomed to being caught by trainers and restrained for periods of over one hour. During the auditory measurements, the animals were positioned in a floating stretcher at the water surface and held at a constant position (indicated by subsurface markers) by two trainers. At the Dolfinarium Harderwijk, the animal was separated from the other animals, caught by two trainers and placed in the stretcher before being moved into its position in the inlet. At Ecomare, the animal was separated and put into the stretcher directly in the final position for the measurements. At both sites, the animals were positioned with their head facing the open pool (Figure 7).

*Figure 7. Catching and placing the harbour porpoise in a floating stretcher (left) and stable positioning at the water surface during the auditory measurements (right).*

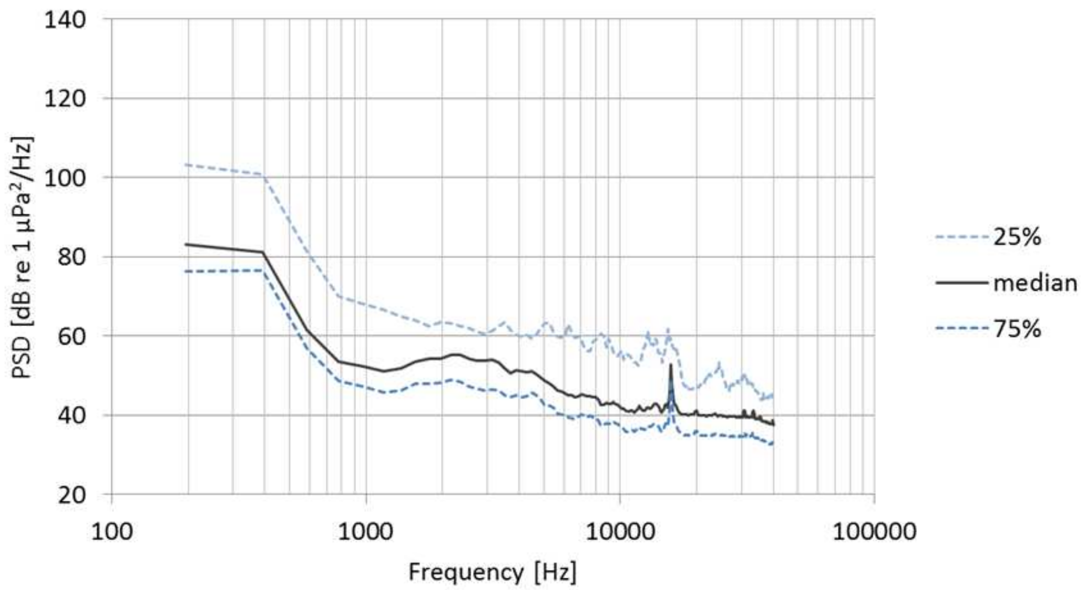
Any abnormal behaviour shown by the animal was directly reported and noted by the principal investigator. Breathing rates were recorded (as a total) over a 5 minute interval at the beginning and end of each session, or – during longer sessions – repeated after 30 minutes.
During the measurements at both facilities, the other harbour porpoises were kept separate in an adjacent pool where they were effectively decoupled from the acoustic situation in the test pool; their acoustic emissions and behaviour did not cause any measurable changes in the background noise (as confirmed by acoustic measurements) at the test site and did not affect the animals' behaviour (as determined visually by the trainers).

## *4.3. Acoustics – setup, stimuli, calibration*

Two types of acoustic signals were employed in this study; generated sound: the acoustic signal for the auditory test ('stimulus') and masking sound ('masker'). While the sound source for the stimuli was placed at a position slightly below the animal's position at a distance of 0.9 m, the masking sound source was placed in both locations at mid-water on the side of the pool opposite to the animal's position.

### 4.3.1. Ambient noise

The pumps of the filtration system were switched off at both facilities during the measurements to reduce the ambient noise in the pools. Background noise as well as the white and red noise masking sounds at the approximate location where the animals were to be positioned, were repeatedly measured prior to a session using hydrophones ITC 1001 and ITC 1042 (International Transducer Corporation, USA). The recordings were analysed using a custom MatLab (The MathWorks Inc., USA) code to calculate the median power spectral density (PSD) as well as the 25% and 75% percentiles based on several recordings at each site (Figure 8 - Figure 10).



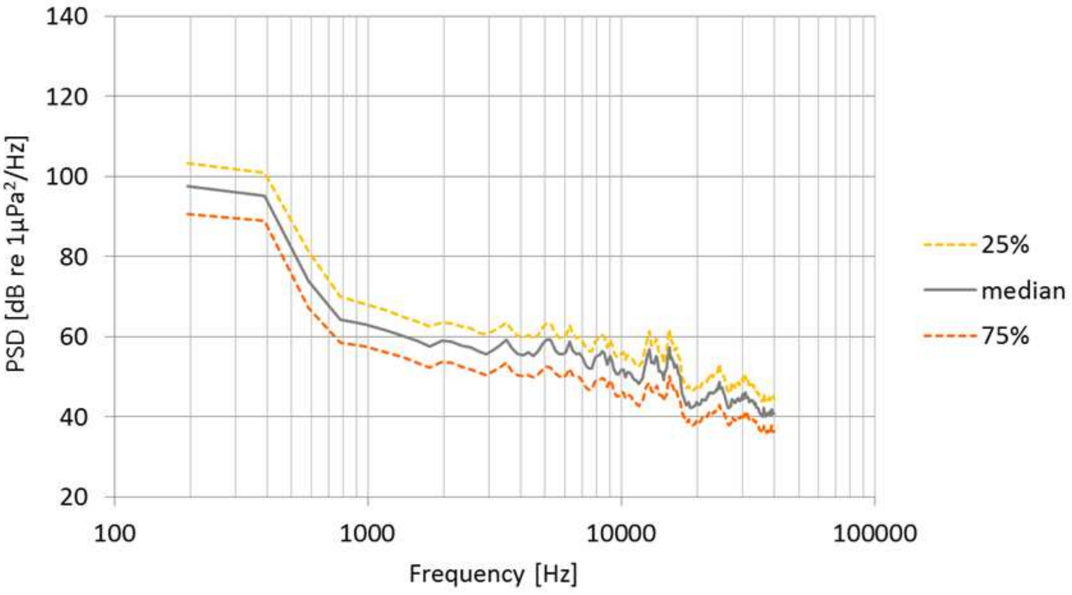




*Figure 8. Percentile power spectral density levels for the ambient noise at the animals' position in the pool at the Dolfinarium Harderwijk (upper graph) and Ecomare (lower graph). Power spectral density [dB re 1 µPa2/Hz] is displayed as function of frequency [Hz] on a logarithmic scale). The 25$^{th}$, 50$^{th}$, and 75$^{th}$ were plotted. The 75$^{th}$ percentile curve describes the frequency dependent levels exceeded by 75% of the samples. Equivalently, 25% of the levels are below the 25$^{th}$ percentile curve. The 50% percentile is the median.*

### 4.3.2. Acoustic stimuli and masker

The stimuli for the auditory measurements as well as AEP recordings were generated and performed using the EVREST system [65]. The stimuli (at 0.5-16 kHz in half octave steps) consisted of short tone bursts (‘tone pips’) of varying duration and number of cycles (Table 2). Stimuli for threshold measurements at frequencies between 10-160 kHz consisted of sinusoidal amplitude modulated (SAM) tones. This type of signal elicits a so-called envelope following response (EFR)[1], a harmonic evoked potential with a fundamental frequency related to the SAM tone [66][67]. This allows a more frequency-specific analysis of the hearing sensitivity than responses evoked by the tone pips, but is not applicable at frequencies below 2.8 kHz [54]. SAM stimuli were fully modulated (modulation depth: 100 %) at a modulation frequency of 1.203 kHz. All SAM stimuli were cosine gated (rise/fall: 1ms) with a total stimulus duration of 32 ms.

All stimuli were digitally generated, converted to analogue with a 1 MHz update rate and 16-bit resolution, low-pass filtered at 200 kHz (8-pole Butterworth, 3C module, Krohn-Hite Corporation, USA), and attenuated before being applied to the sound source.

*Table 2. Type, duration and number of cycles (rise-plateau-fall) of the tone bursts (‘tone-pips’) used as acoustic stimuli for the ABR measurements.*

| Frequency<br>*[Hz]* | Duration one cycle<br>*[ms]* | Type of tone-pip<br>*(no. of cycles during rise-plateau-fall)* | Total no. of cycles |
|---|---|---|---|
| 500 | 2 | 1-0-1 | 2 |
| 700 | 1.429 | 1-1-1 | 3 |
| 1000 | 1 | 1-1-1 | 3 |
| 1400 | 0.714 | 1-1-1 | 3 |
| 2000 | 0.5 | 2-1-2 | 5 |
| 2800 | 0.357 | 2-1-2 | 5 |
| 4000 | 0.25 | 2-1-2 | 5 |
| 5600 | 0.179 | 2-1-2 | 5 |
| 8000 | 0.125 | 2-1-2 | 5 |
| 11200 | 0.089 | 2-1-2 | 5 |
| 16000 | 0.063 | 2-1-2 | 5 |

The cochlea is more or less scaled logarithmically [69]. In order to stimulate a constant proportion of the cochlear partition, a stimulus with a constant number of cycles (2-1-2 cycle rise-plateau-fall time; producing a constant number of octaves) were chosen. Frequencies at ≤1.4 kHz had to be tested with a shorter signal (lower no. of cycles) to avoid possible overlap of stimulus artefact with the onset of the neuronal response (latency of 3-4 ms after stimulation).

In addition to using red noise as a proxy for shipping noise, to allow for a comparison with previously published data on masking criteria in harbour porpoises [40][39] Gaussian white noise (with a ‘flat’ spectrum; see Glossary for further definition) was used as alternative masker. Both types of sound were generated by a custom LabView (National Instruments,

[1] This response is also called Auditory Steady-State Response (ASSR) [68]

USA) code (written by P.A. Lepper, Loughborough University, UK) compensating for the transmitting voltage response of the transducer.

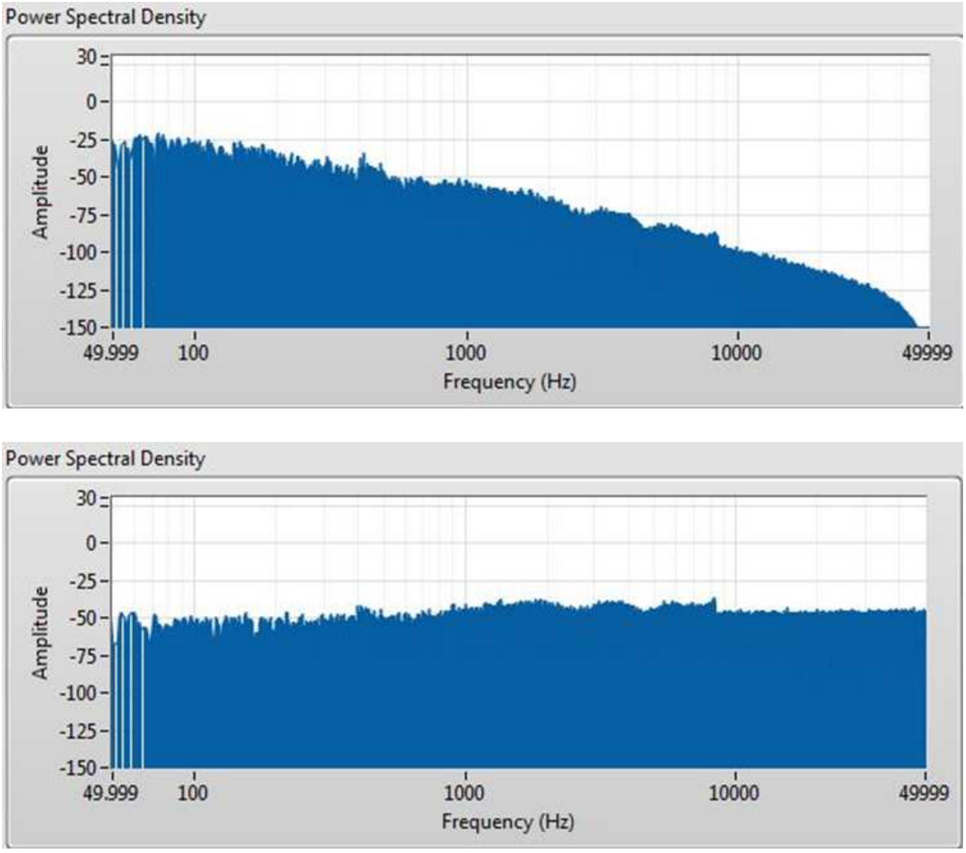
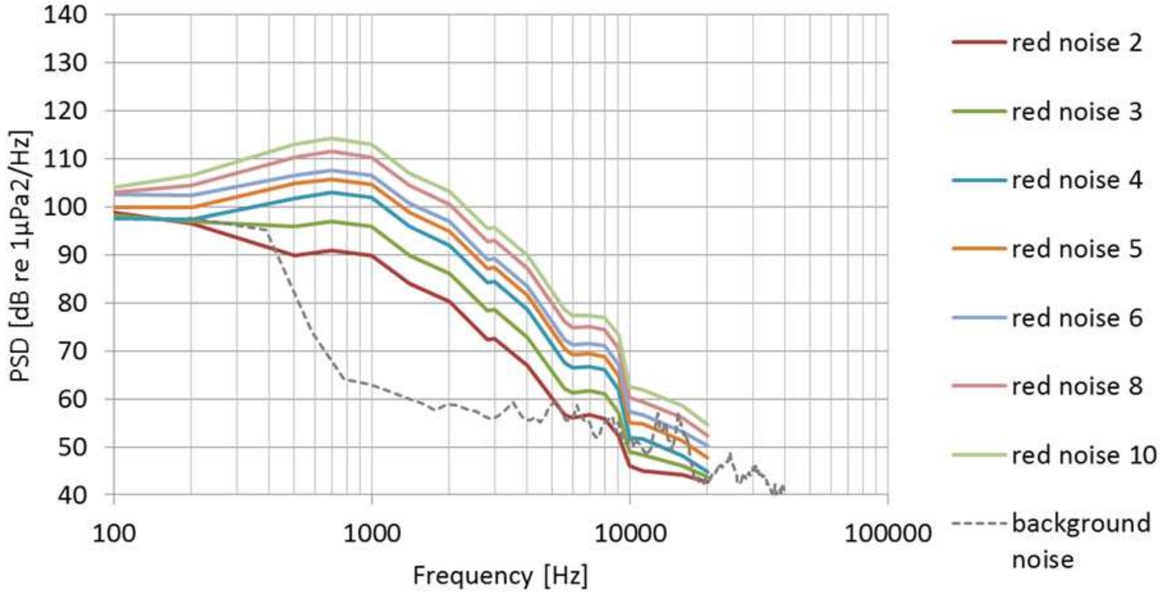



*Figure 9. Power Spectral Density (PSD) plot of Brownian noise ('red noise; upper graph) and Gaussian white noise (lower graph). Amplitude [dB, normalised to maximum output of the sound producing system] is displayed as function of frequency [Hz] on a logarithmic scale.*

The masking sound was transmitted into the water via a high-power broadband piezoelectric underwater transducer (Lubell LL-1424HP, Lubell Labs Inc., USA) fitted with a power amplifier (Crown CDi 2000, Crown International, USA). The transducer was suspended at mid water depth at the side of the pool opposite to the animal's station.



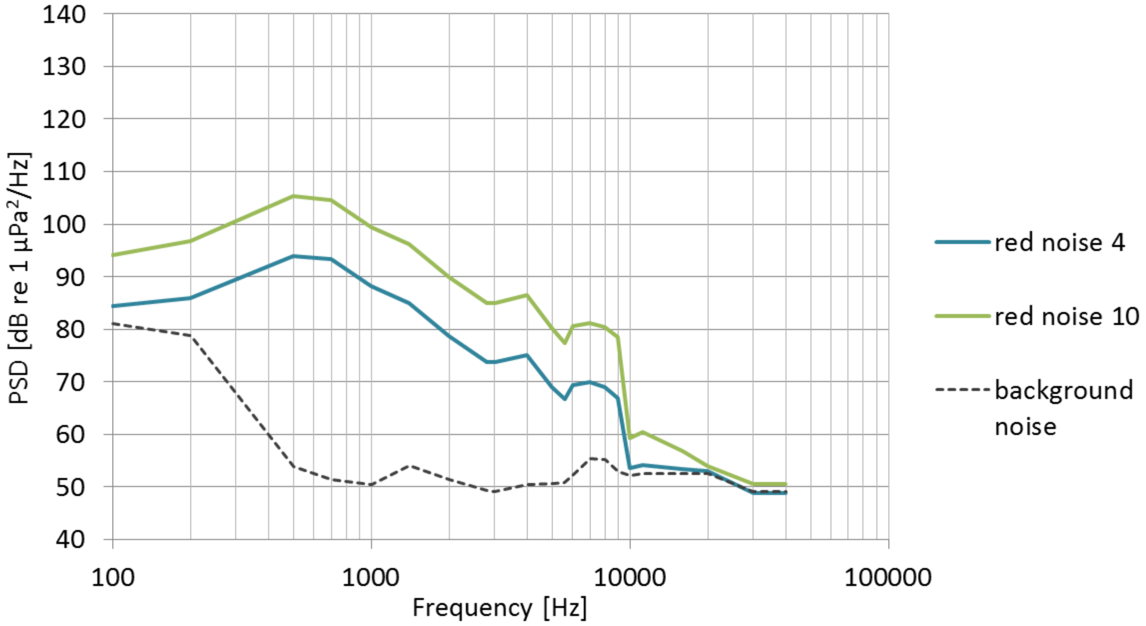




*Figure 10. Power spectral density (PSD) levels for red noise masking sound at the animals' position in the pool at the Dolfinarium Harderwijk (upper graph) and Ecomare (lower graph). The power spectral density [dB re 1 µPa$^2$/Hz] is displayed as a function of frequency [Hz] on a logarithmic scale. The PSD of the median background noise is displayed as dotted grey line.*

The masking effect of white noise was only measured in the three harbour porpoises tested at the Dolfinarium Harderwijk, the power spectral density of the masking sound during these measurements is shown in Figure 11.

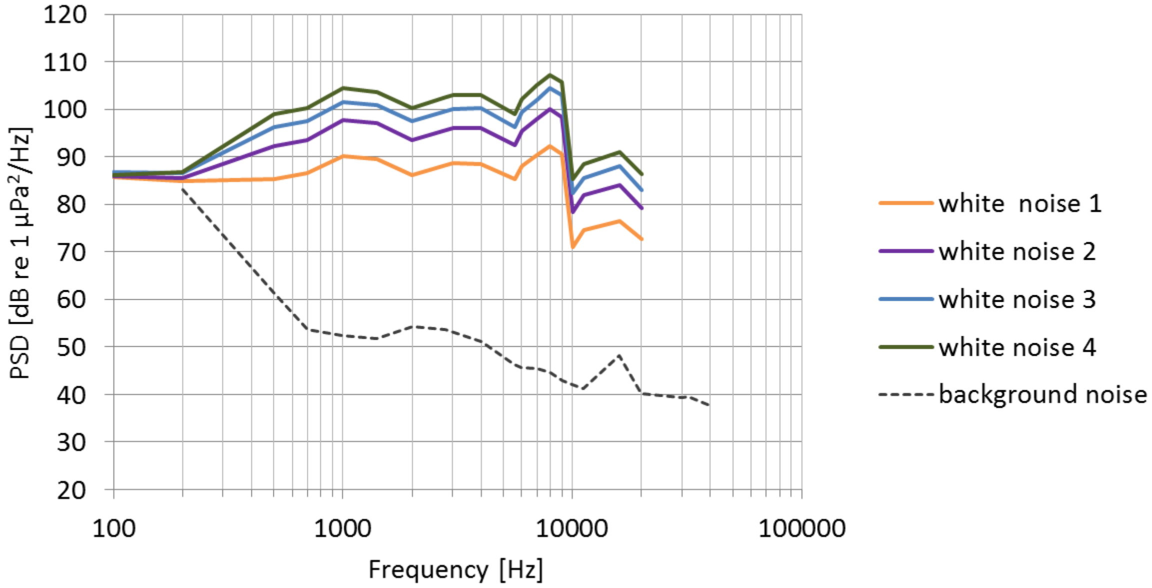



*Figure 11. Power spectral density (PSD) levels for Gaussian ('white') noise masking sound (colour-coded) at the animals' position in the pool at the Dolfinarium Harderwijk. The power spectral density [dB re 1 µPa$^2$/Hz] is displayed as function of frequency [Hz] on a logarithmic scale. The PSD of the median background noise is displayed as dotted grey line.*

### 4.3.3. Stimulus presentation

Auditory test stimuli were presented to the animals in a series of attenuating amplitudes, starting at levels that clearly produced an AEP and reducing to the point that the AEP was no longer detectable in the electrophysiological recordings. Hearing thresholds were repeatedly measured at frequencies 1 to 16 kHz in half octave steps. In addition, measurements were conducted at 0.5 and 0.7 kHz, and in 10 kHz steps between 10 and 180 kHz (Figure 9). Tone pips were presented at a rate of 43/s, SAM tones at a rate of 24.8/s.

The auditory stimuli were emitted from a transducer ITC 1001 (International Transducer Corporation, USA) for frequencies between 0.5-16 kHz (for tone-pips) and ITC 1042 (for SAM stimuli) at frequencies between 10-160 kHz. The transducers were placed, approximately 0.9 m in front of the lower jaw of the animal (i.e. in the pan region, the most sensitive region for acoustic stimulation in toothed whales) at 0.6 m water depth. The porpoise was stationed in a floating stretcher at the water surface such that its pan region was completely submerged but not covered by the floating stretcher and its dorsal surface was above the water. All sounds were monitored via a transducer ITC 1042 for the frequencies between 0.5-16 kHz (tone-pips) and additionally via a TC 4033 (Teledyne RESON A/S, DK) at frequencies between 10-160 kHz (SAM stimuli). All stimuli and masker signals were recorded at the approximate location of the animal's ears (at the animal's pan region, i.e. the posterior end of the lower jaw) while positioned in the stretcher, but without the animal or trainers present in the water. The variation in the acoustic field was relatively low (± 3 dB) around the animal's head. All hydrophones/transducers were calibrated before and after the measurements, some critical components were also repeatedly functionally monitored and calibrated during the study period.

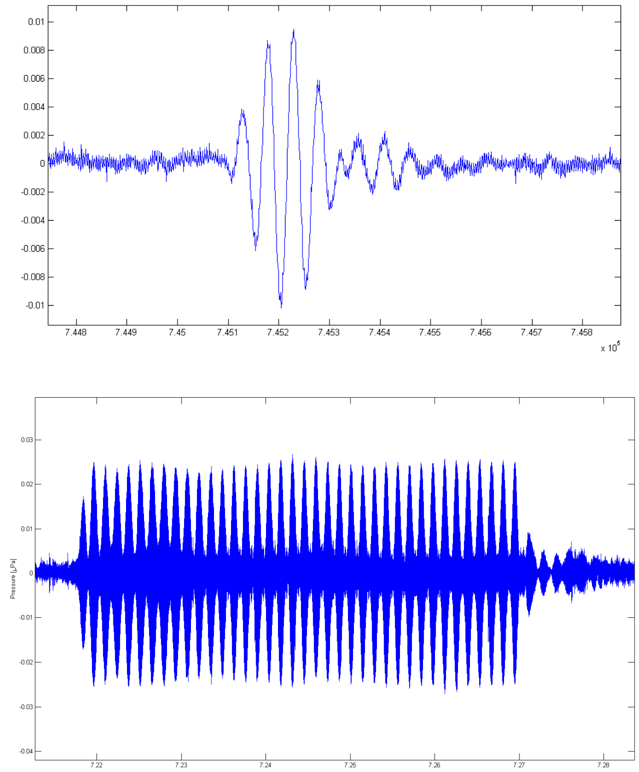



*Figure 12. Exemplary representation of the waveform of a tone pip (2 kHz, upper graph) and sinusoidal amplitude modulated (SAM) tone (carrier frequency: 100 kHz, modulation frequency: 1.203 kHz; lower graph) recorded at the animal's position, but without the animal or trainers present in the water.*

## 4.4. Response acquisition

The auditory measurements were conducted using the electrophysiological method of measuring auditory evoked potentials (AEP). The AEP method is non-invasive and enables rapid measurements of auditory sensitivity even in situations in which the subject is unwilling or unable to participate in common behavioural testing. It is based on the presentation of acoustic stimuli which will generate a synchronous discharge of multiple neurons in the auditory complex upon perception of these stimuli. These AEPs form an electric field in the body and can be measured as potentials from electrodes placed on the surface of the subject's skin [70]. To detect these relatively small potentials reliably and within the multitude of non-auditory neuronal signals in a subject's body, the acoustic stimuli are repeatedly presented to the subject and the measured potentials are averaged coherently. Thereby, all non-acoustic or incoherent neuronal potentials are reduced or completely eliminated. The entire duration of an AEP is several hundred milliseconds, but only the first ten milliseconds contain the response needed to assess the actual detection of an acoustic stimulus. This early response originates in the auditory periphery and is generated in the auditory (eighth) nerve and auditory nuclei of the brainstem [70]. As the focus of this study is to determine the detection threshold for acoustic stimuli, it is most appropriate to analyse these early neuronal responses, termed Auditory Brainstem Response (ABR). The amplitude of these responses is usually within the nano-Volt [nV] range and individual waves can be identified and analysed quantitatively. The peaks and troughs in the waveforms of these early responses are labelled according to the nomenclature described by [71] (see Figure 13).

Three surface electrodes (active, reference and ground) were placed along the dorsal midline of the harbour porpoise. The active electrode was placed 7 cm behind the animal's blowhole [72][54] the reference electrode was attached slightly lateral below the dorsal fin, and the ground electrode halfway between the two. All electrodes were 10 mm gold-cup electrodes mounted into standard suction cups. Electrodes were coupled to the skin of the whales with conductive gel. The electrode responses were filtered (high pass cut-off: 300 Hz, low pass cut-off: 3 kHz) and amplified 100,000 times by a low noise, differential biopotential amplifier (IP511; Grass Technologies, USA) before being fed into a A/D conversion card (NI-DAQ USB-6251, National Instruments Corporation, USA) at a digitization rate of 50 kHz with 16-bit resolution over a 23 ms sweep duration and stored on a laptop computer. The differential electrode signal was synchronously averaged, using a weighted averaging method [73], with a total of up to 2048 ABR responses to the identical type and intensity of stimulus. All ABR responses were recorded and analysed in real-time using EVREST. The analysis period for the neuronal responses evoked by the tone-pips (0.5-16 kHz) were analysed over a 3-4 ms window within the first 10 ms, the neuronal responses evoked by stimulation with SAM tones over a window 5-35 ms after onset of the stimulation. The responses recorded after tone-pip stimulation were subject to a Single-Point F test ($F_{sp}$; [74]) to objectively identify the detection of a neuronal response, the SAM-tone evoked responses were tested using a Magnitude-Squared Coherence test (MSC; [75]).

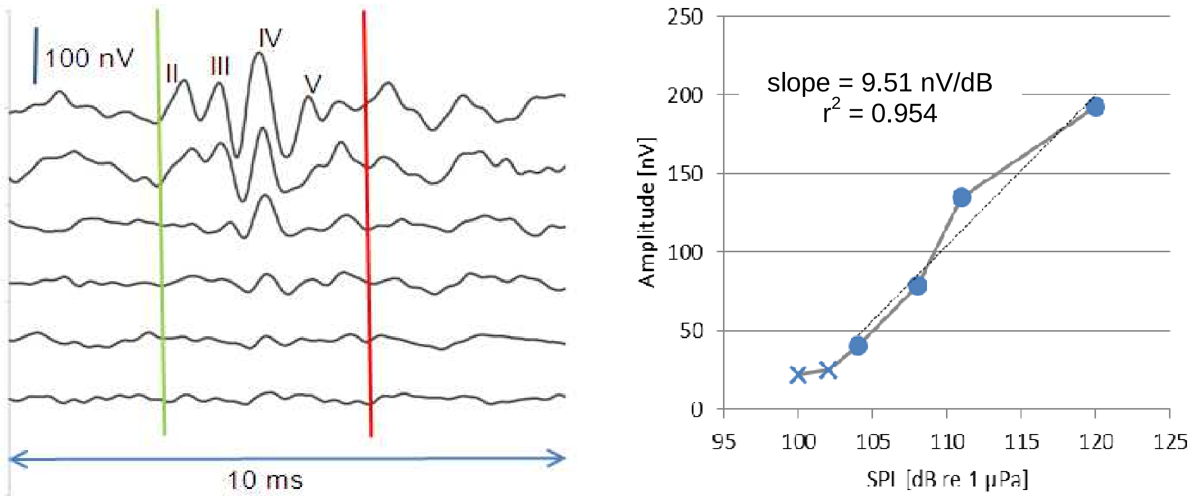



*Figure 13. Left graph: Evoked potentials (Auditory Brainstem Responses, ABR) evoked in a harbour porpoise (PpSH 174) by stimulation with tone pips at 1.4 kHz at various sound pressure levels (SPLs); neuronal responses are filtered between 300 Hz-3 kHz. The vertical green and red lines indicate the beginning/end of the analysis window. Right graph: Corresponding input/output function for the measured neuronal responses displayed as amplitude of repeatedly identified neuronal wave IV over SPL of the stimuli. Filled circles indicate a response detection (slope and correlation coefficient of the trend-line for response detections are given), a cross indicates that no response was detected after stimulation at this SPL.*

## 4.5. Threshold determination

Processing and analysis of auditory threshold involved two stages. Firstly, during the measurements an adaptive staircase ABR threshold technique was used in EVREST to allow for real-time detection of the hearing thresholds. EVREST adjusted automatically the stimulus SPL from one measurement to the next based on whether an ABR was detected during the previous trial. If a response was detected (a 'hit'), the stimulus SPL was reduced. If the response was not detected (a 'miss'), the stimulus SPL was increased. At the transitions from detections to non-detections and vice-versa, the amount in decibels the SPL was increased or decreased by a ratio of 0.5. The measurement was complete when the step size was reduced to below 5 dB. The hearing threshold is defined in this study as the mean of the SPLs corresponding to the lowest hit and the next highest miss [65].

For the measurements conducted using tone pips at frequencies between 0.5-16 kHz a $F_{sp}$ test was used as an objective response detection (ORD) technique to analyse the recorded neuronal responses on-site, in real-time, for the presence or absence of ABRs. However, the rate of false-positive identification of a neuronal response in the time domain signal (waveform) was considered too high and not sufficient control data quality. Therefore, all recorded responses for all frequencies tested were re-evaluated by reviewing the information stored in the .aep files using EVREST by an audiologist experienced in marine mammal ABRs. An MSC test was used as the ORD technique for the neuronal responses elicited by the SAM tones at frequencies between 20-160 kHz. This method analyses the response in the frequency domain and has a low false detection rate, i.e. it provides an unambiguous criterion for the detection of a neuronal response (hit) or the lack of it (miss). Additionally, the recorded neuronal responses were all reviewed visually, to ensure high data quality (i.e. no artefact or other data corruption had occurred).

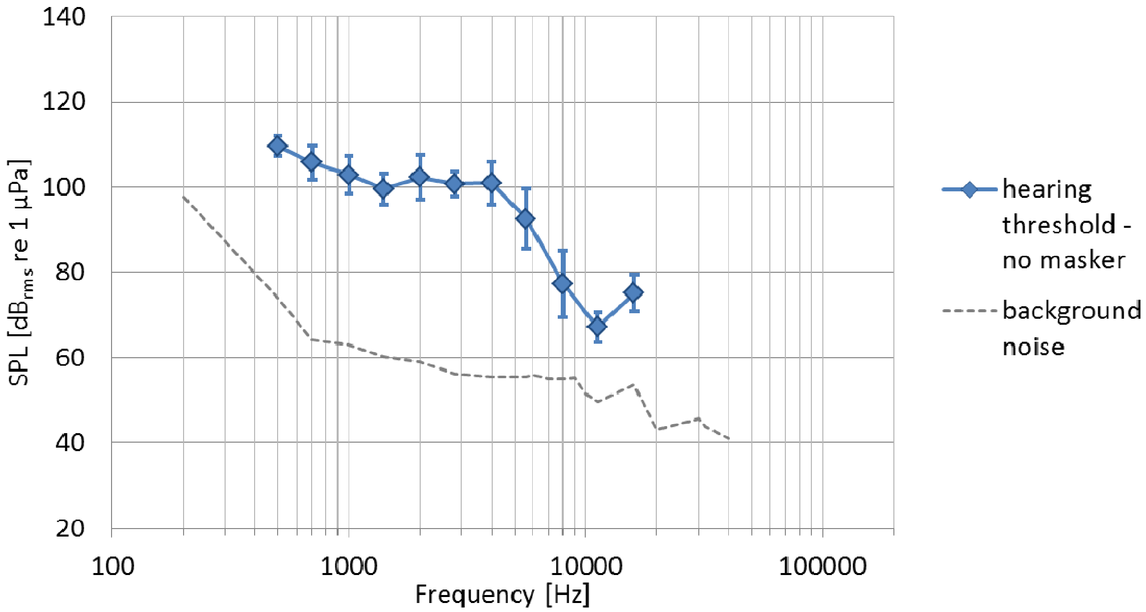
## *4.6. Analysis of sound recordings*

A Matlab script (The MathWorks, Inc.) was used to analyse the sound recordings of the stimuli, masker and background noise from both sites. The $SPL_{rms}$ of each tone-pip signal was computed over the duration of each tone-pip. The PSD of the noise was averaged over a $1/3^{rd}$ octave band surrounding the tone frequency. The resulting levels ($SPL_{rms}$ for the stimuli and masker, power spectral density (PSD) for the background noise) were corrected for the frequency response of the acoustic recording system and hydrophone sensitivity. The detection threshold levels determined using EVREST were corrected according to the resulting levels and analysed in comparison to the received masker levels.

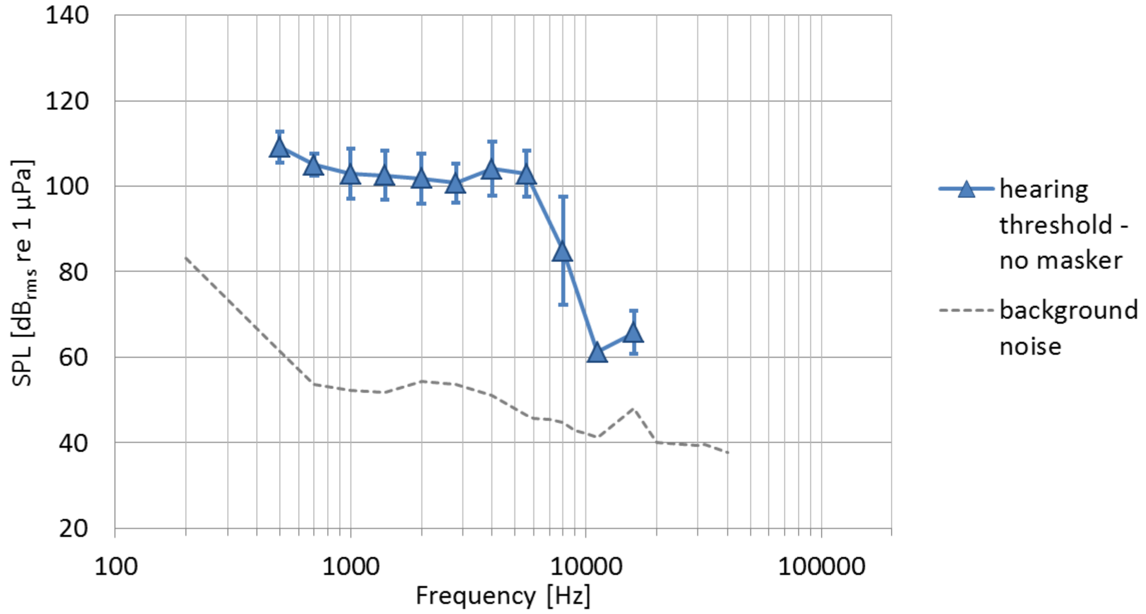
# 5. Results

## 5.1. Hearing threshold (no masker)

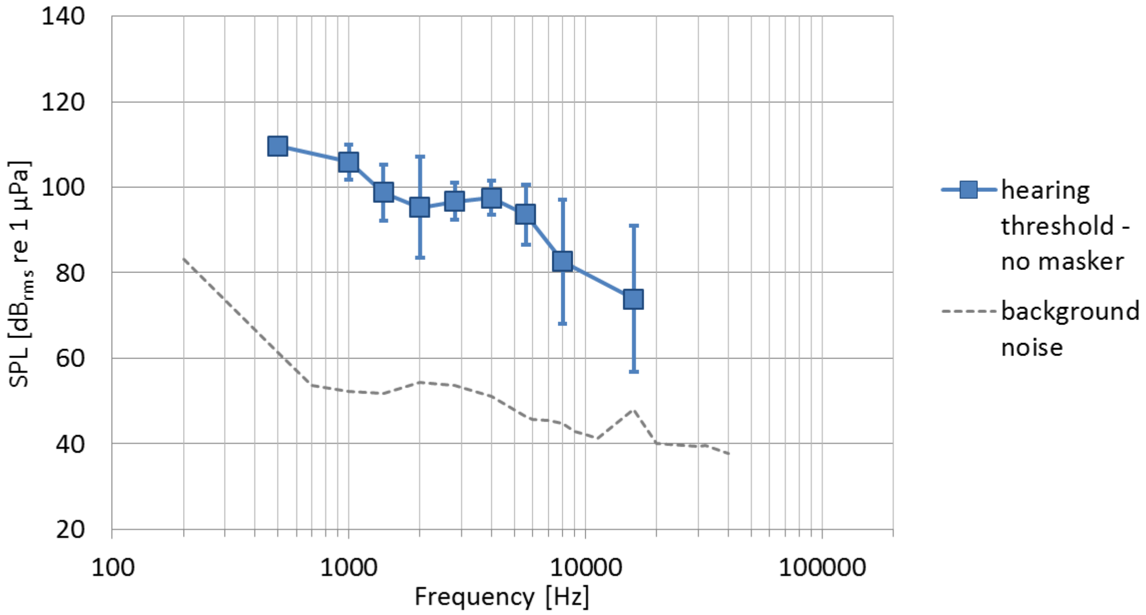
The hearing sensitivity of all four harbour porpoises was measured in the absence of masking sound (**Erreur ! Source du renvoi introuvable.**, **Erreur ! Source du renvoi introuvable.**).



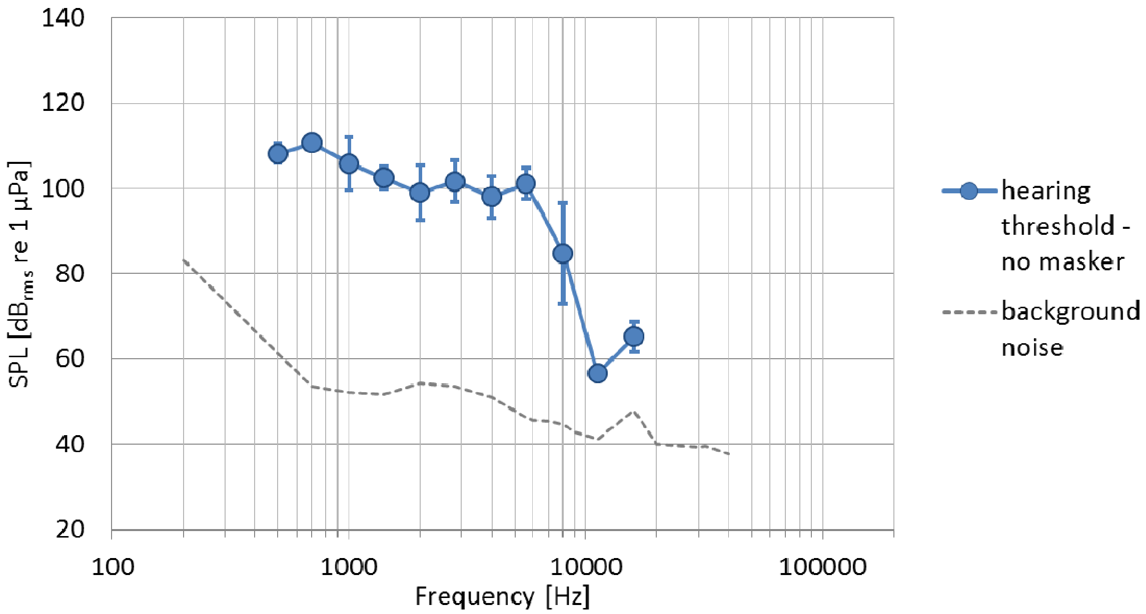

*PpSH174*




*PpZH003*




*PpSH163*




*PpZH04*

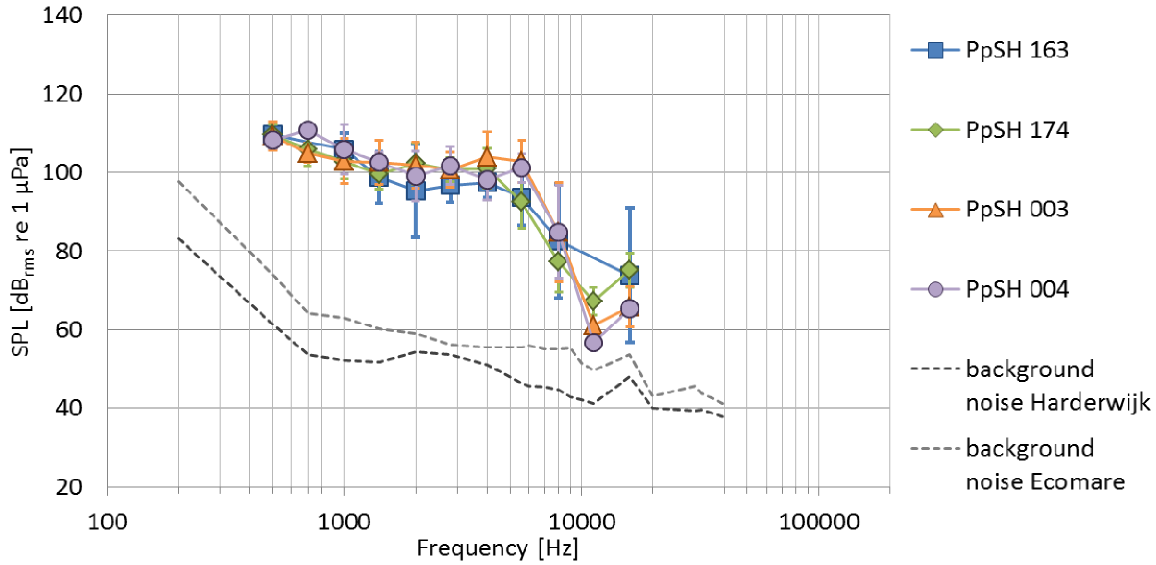



*Figure 14. Hearing threshold of four harbour porpoises measured using auditory evoked responses to tone-pips at frequencies between 0.5 – 16 kHz. Thresholds are displayed for each animal separately and then combined in the bottom graph. The SPL is displayed as a function of frequency [Hz] on a logarithmic scale. Error bars indicate standard deviation. The hatched lines show the power spectral density (PSD, in [dB re 1 µPa$^2$/Hz]) of the background noise at the two facilities during these auditory measurements.*

*Table 3. Median hearing thresholds (with standard deviations) of four harbour porpoises measured at two facilities using auditory evoked responses to tone-pips at frequencies between 0.5 – 16 kHz. Thresholds are listed separately (animal identity: PpSH x ) and power spectral density of the background noise at each facility are provided.*

| | Ecomare | hearing threshold | | Harderwijk | hearing threshold | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Frequency | background noise | PpSH 174 | sd. | background noise | PpZH 003 | sd. | PpSH 163 | sd. | PpZH 004 | sd. |
| [Hz] | [dB re 1 µPa$^2$/Hz] | [dB$_{rms}$ re 1 µPa] | [dB] | [dB re 1 µPa$^2$/Hz] | [dB$_{rms}$ re 1 µPa] | [dB] | [dB$_{rms}$ re 1 µPa] | [dB] | [dB$_{rms}$ re 1 µPa] | [dB] |
| 500 | 74 | 110 | 2.4 | 61 | 109 | 3.6 | 110 | | 108 | 2 |
| 700 | 64 | 106 | 4.0 | 54 | 105 | 2.5 | | | 111 | |
| 1000 | 63 | 103 | 4.5 | 52 | 103 | 5.8 | 106 | 4 | 106 | 6 |
| 1400 | 60 | 100 | 3.6 | 52 | 103 | 5.7 | 99 | 7 | 103 | 3 |
| 2000 | 59 | 102 | 5.3 | 54 | 102 | 5.8 | 95 | 12 | 99 | 6 |
| 2800 | 56 | 101 | 3.0 | 54 | 101 | 4.5 | 97 | 4 | 102 | 5 |
| 4000 | 55 | 101 | 5.0 | 51 | 104 | 6.3 | 97 | 4 | 98 | 5 |
| 5600 | 56 | 93 | 6.9 | 46 | 103 | 5.3 | 94 | 7 | 101 | 4 |
| 8000 | 56 | 77 | 7.8 | 46 | 85 | 12.6 | 83 | 15 | 85 | 12 |
| 11200 | 55 | 67 | 3.5 | 45 | 61 | | | | 57 | |
| 16000 | 55 | 75 | 4.3 | 45 | 66 | 5.1 | 74 | 17 | 65 | |

The hearing sensitivity at medium to high frequencies was tested systematically in PpSH 174 and at selected frequencies in the other three animals to test for systemic hearing pathologies (Figure 1, Table 1).

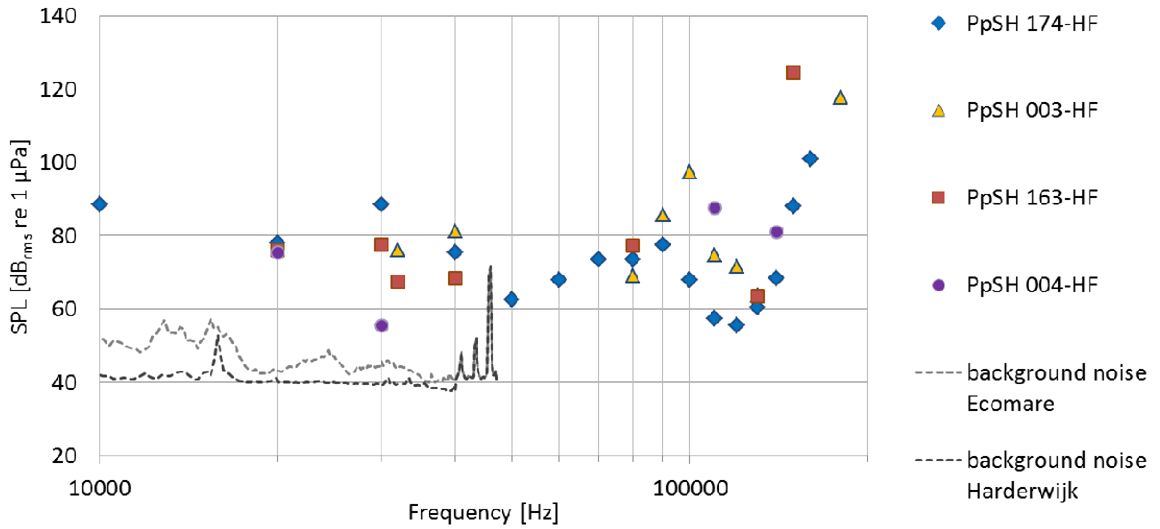



*Figure 1. Hearing threshold of four harbour porpoises measured using auditory evoked responses to sinusoidal amplitude modulated (SAM) tones between 10-180 kHz; SAM tones were modulated at 1,203 Hz (see Annex 1 for details), modulation depth: 100%. The SPL is displayed as function of frequency [Hz] on a logarithmic scale. The grey lines show the power spectral density (PSD, in [dB$_{rms}$ re 1 µPa$^2$/Hz]) of the background noise at the two facilities during these auditory measurements.*

*Table 1. Hearing threshold of four harbour porpoises measured at two facilities using auditory evoked responses to sinusoidal amplitude modulated tones between 10-180 kHz. Thresholds are listed separately for the four animals tested (animal identity: PpSH x ) as well as the median and standard deviation (sd.).*

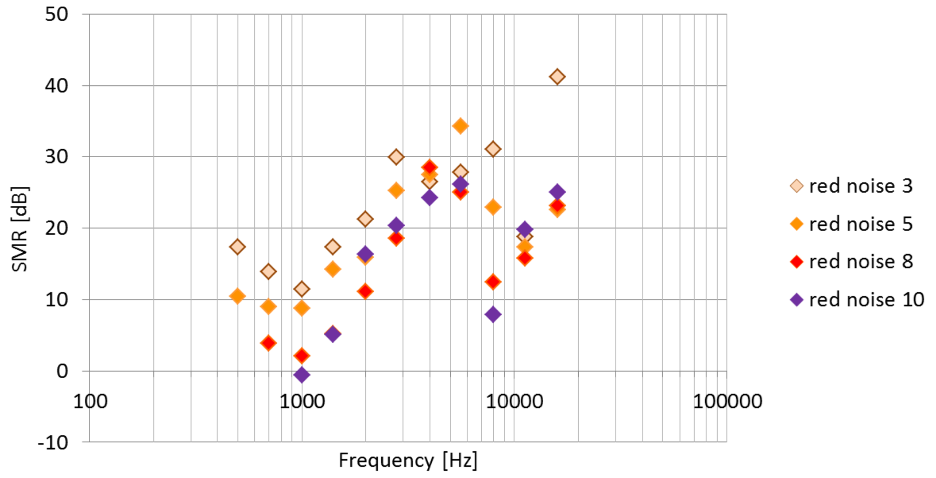
| | Ecomare | hearing threshold | | Harderwijk | hearing threshold | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Frequency | background noise | PpSH 174 | sd. | background noise | PpZH 003 | sd. | PpSH 163 | sd. | PpZH 004 | sd. |
| [Hz] | [dB re 1 µPa$^2$/Hz] | [dB$_{rms}$ re 1 µPa] | [dB] | [dB re 1 µPa$^2$/Hz] | [dB$_{rms}$ re 1 µPa] | [dB] | [dB$_{rms}$ re 1 µPa] | [dB] | [dB$_{rms}$ re 1 µPa] | [dB] |
| 10000 | 51 | 89 | | | | | | | | |
| 20000 | 43 | 78 | | | | | 76 | 13 | 75 | 4 |
| 30000 | 46 | 89 | | | | | 78 | | 56 | |
| 32000 | 44 | | | | 76 | | 68 | | | |
| 40000 | 41 | 76 | | | 81 | 7 | 69 | 7 | | |
| 50000 | | 63 | | | | | | | | |
| 60000 | | 68 | | | | | | | | |
| 70000 | | 74 | | | | | | | | |
| 80000 | | 74 | | | 69 | | 77 | 5 | | |
| 90000 | | 78 | | | 86 | | | | | |
| 100000 | | 68 | | | 98 | 3 | | | | |
| 110000 | | 58 | | | 75 | | | | 88 | |
| 120000 | | 56 | | | 72 | 12 | | | | |
| 130000 | | 61 | | | 64 | 8 | 64 | 11 | | |
| 140000 | | 69 | | | | | | | 81 | 7 |
| 150000 | | 88 | | | | | 125 | | | |
| 160000 | | 101 | | | | | | | | |
| 180000 | | | | | 118 | | | | | |

## 5.2. Masking effect

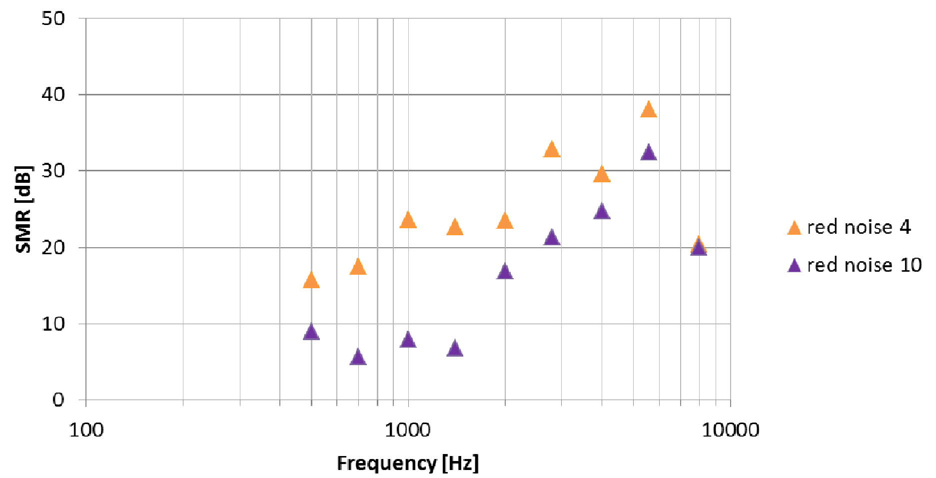
The effect of the masking noise on the hearing sensitivity of the harbour porpoises is calculated and displayed as the ratio (in dB) between the hearing threshold in the absence and presence of the masker during the auditory measurements. As the test signals (ABR stimuli) were not pure tones, the resulting masking effect is expressed as Signal-to-Masker Ratio (SMR), representing The masking effect is calculated as the ratio (in dB) of an animal's tone detection threshold at a given frequency and the power spectral density of the masker at this frequency (SMR).

### 5.2.1. Red noise

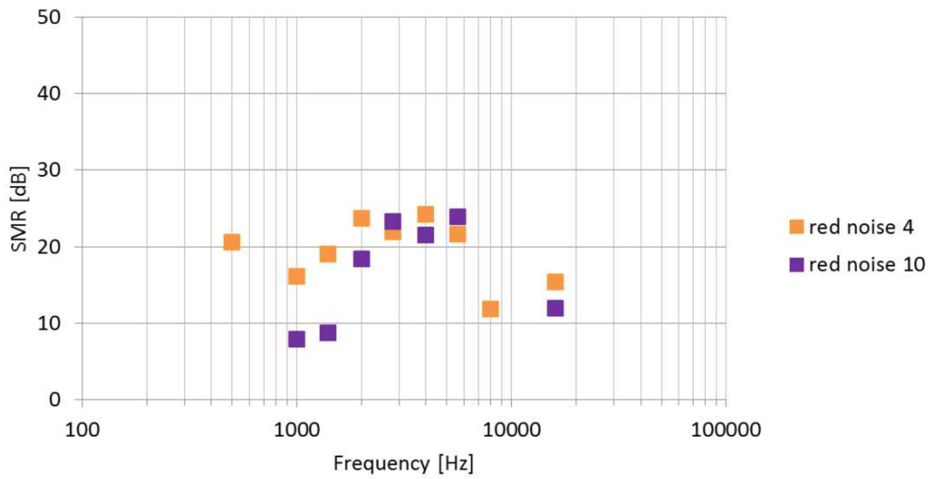
The masking effect of red noise was measured in all four harbour porpoises at five levels (*Figure 2, Table 2 & Table 3*).



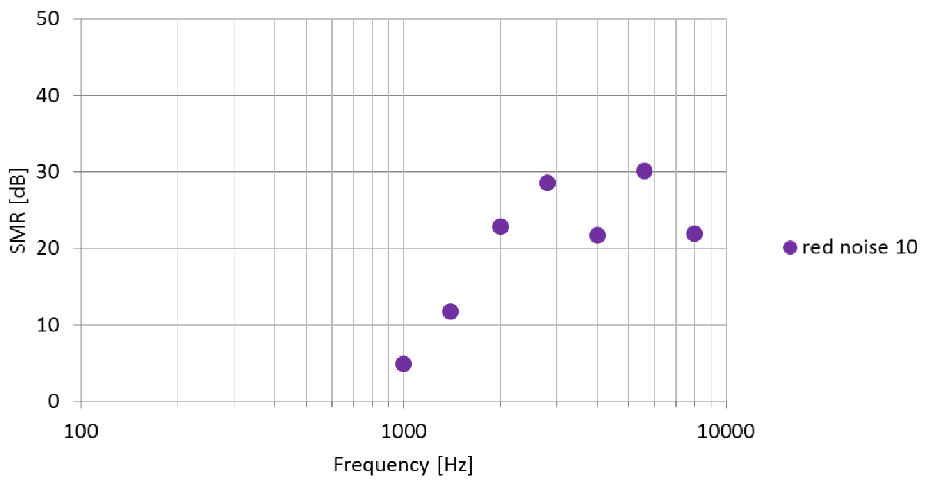

*PpSH 174*




*PpZH 003*




*PpSH 163*




PpZH 004

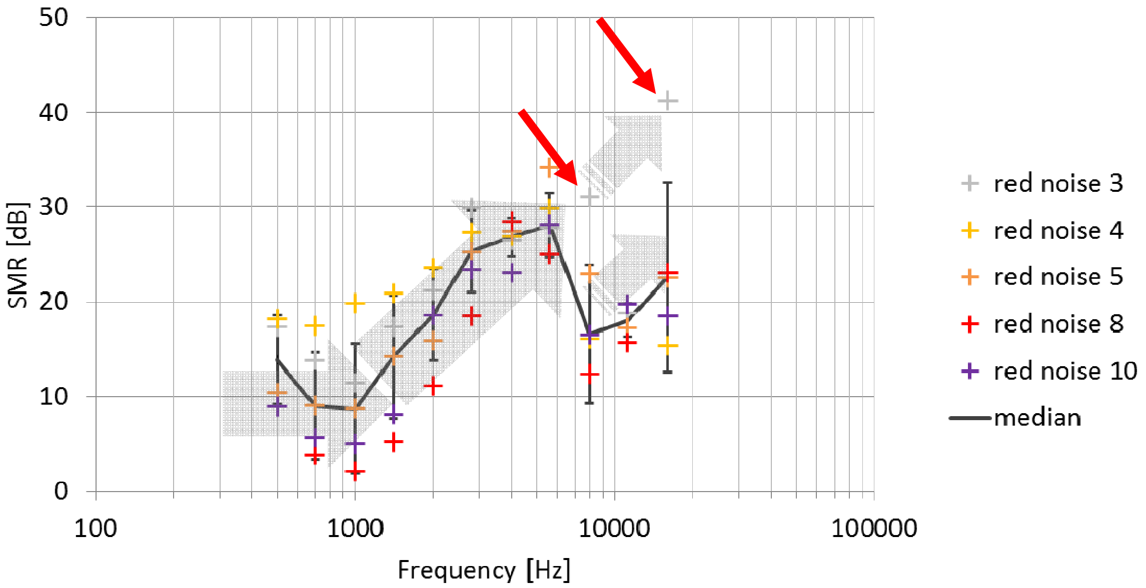



*Figure 2. Masking effect of red noise at various received sound pressure levels on the hearing sensitivity of four harbour porpoises displayed separately (previous two pages, animal identity: PpSH x) and combined (this page). The signal to masker ratio (SMR) is displayed as a function of frequency [Hz] on a logarithmic scale. Error bars indicate standard deviation based on this sample of data. Grey shaded arrows indicate overall trends. Red arrows indicate two values discussed separately in the discussion.*

*Table 2. Masking effect of red noise on the hearing sensitivity of four harbour porpoises (animal identity: PpSH x) at Ecomare and Dolfinarium Harderwijk listed for each animal separately. The masking effect is calculated as the ratio (in dB) of an animal's tone detection threshold at a given frequency and the power spectral density of the masker at this frequency (SMR). Data are listed for all tested received sound pressure levels (3-10).*

| | Ecomare | Masking effect (SMR) | | Harderwijk | Masking effect (SMR) | | | Ecomare | Masking effect (SMR) |
|---|---|---|---|---|---|---|---|---|---|
| Frequency | Red noise - level 3 | PpSH 174 | | Red noise - level 4 | PpZH 003 | PpSH 163 | | Red noise - level 5 | PpSH 174 |
| [Hz] | [dB re 1 µPa$^2$/Hz] | [dB] | | [dB re 1 µPa$^2$/Hz] | [dB] | [dB] | | [dB re 1 µPa$^2$/Hz] | [dB] |
| 500 | 96 | 17 | | 94 | 16 | 16 | | 105 | 10 |
| 700 | 97 | 14 | | 93 | 17 | 19 | | 106 | 9 |
| 1000 | 96 | 11 | | 88 | 24 | 24 | | 105 | 9 |
| 1400 | 90 | 17 | | 85 | 23 | 22 | | 99 | 14 |
| 2000 | 86 | 21 | | 79 | 23 | 24 | | 95 | 16 |
| 2800 | 78 | 30 | | 74 | 33 | 22 | | 87 | 25 |
| 4000 | 78 | 27 | | 75 | 30 | 12 | | 82 | 27 |
| 5600 | 78 | 28 | | 67 | 38 | 15 | | 70 | 34 |
| 8000 | 61 | 31 | | 69 | 20 | 16 | | 69 | 23 |
| 11200 | 48 | 19 | | 94 | 16 | 19 | | 55 | 17 |
| 16000 | 46 | 41 | | 93 | 17 | 24 | | 51 | 23 |

| | Ecomare | Masking effect (SMR) | | Ecomare | Masking effect (SMR) | Harderwijk | Masking effect (SMR) | | |
|---|---|---|---|---|---|---|---|---|---|
| Frequency | Red noise - level 8 | PpSH 174 | | Red noise - level 10 | PpSH 174 | Red noise - level 10 | PpZH 003 | PpSH 163 | PpZH 004 |
| [Hz] | [dB re 1 µPa$^2$/Hz] | [dB] | | [dB re 1 µPa$^2$/Hz] | [dB] | [dB re 1 µPa$^2$/Hz] | [dB] | [dB] | [dB] |
| 500 | | | | | | 105 | 9 | 8 | |
| 700 | 111 | 4 | | | | 105 | 6 | 9 | |
| 1000 | 110 | 2 | | 113 | -1 | 99 | 8 | 18 | 5 |
| 1400 | 104 | 5 | | 107 | 5 | 96 | 7 | 23 | 12 |
| 2000 | 100 | 11 | | 103 | 16 | 90 | 17 | 22 | 23 |
| 2800 | 93 | 19 | | 95 | 20 | 85 | 21 | 24 | 29 |
| 4000 | 87 | 28 | | 90 | 24 | 86 | 25 | | 22 |
| 5600 | 76 | 25 | | 78 | 26 | 77 | 32 | | 30 |
| 8000 | 74 | 12 | | 77 | 8 | 80 | 20 | | 22 |
| 11200 | 59 | 16 | | 62 | 20 | | | | |
| 16000 | 56 | 23 | | 59 | 25 | 69 | | 12 | |

*Table 3. Masking effect of red noise on the hearing sensitivity of four harbour porpoises (Identity: PpSH x) at Ecomare and Dolfinarium Harderwijk – all data combined. The masking effect is calculated as the ratio (in dB) of an animal's tone detection threshold at a given frequency and the power spectral density of the masker at this frequency (SMR). Data are listed for all tested received sound pressure levels (3-10) as well as the median, standard deviation (sd.) and the number of data points per frequency (count).*

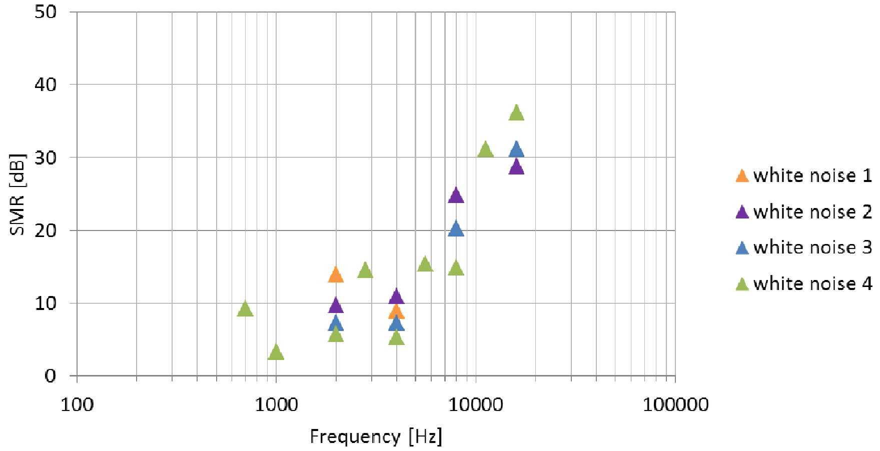
| | Masking effect (SMR) at red noise level | | | | | Masking effect (SMR) | | |
|---|---|---|---|---|---|---|---|---|
| Frequency | 3 | 4 | 5 | 8 | 10 | Median / sd./ count | | |
| [Hz] | [dB] | [dB] | [dB] | [dB] | [dB] | [dB] | [dB] | no. |
| 500 | 17.4 | 18.2 | 10.4 | | 8.9 | 13.9 | 4.7 | 4 |
| 700 | 13.9 | 17.5 | 9.0 | 3.8 | 5.7 | 9.0 | 5.7 | 5 |
| 1000 | 11.5 | 19.9 | 8.7 | 2.1 | 5.0 | 8.7 | 6.8 | 5 |
| 1400 | 17.4 | 20.9 | 14.2 | 5.2 | 8.1 | 14.2 | 6.5 | 5 |
| 2000 | 21.2 | 23.6 | 15.9 | 11.1 | 18.6 | 18.6 | 4.8 | 5 |
| 2800 | 29.9 | 27.4 | 25.3 | 18.5 | 23.4 | 25.3 | 4.3 | 5 |
| 4000 | 26.5 | 26.9 | 27.4 | 28.5 | 23.1 | 26.9 | 2.0 | 5 |
| 5600 | 27.9 | 29.9 | 34.2 | 25.1 | 28.1 | 28.1 | 3.4 | 5 |
| 8000 | 31.1 | 16.1 | 22.9 | 12.4 | 16.6 | 16.6 | 7.3 | 5 |
| 11200 | 18.8 | | 17.3 | 15.7 | 19.7 | 18.1 | 1.8 | 4 |
| 16000 | 41.2 | 15.4 | 22.6 | 23.1 | 18.5 | 22.6 | 10.0 | 5 |

The animals' hearing sensitivity is differentially reduced by the presence of red noise. When analysing the results of all levels of received red noise, the masking effect increases from 1 to 5.6 kHz by 8 dB/octave. This increase in masking is strongest in the lowest frequency range (10.5 dB/octave between 1-2.8 kHz) while it is lower at the higher range (5.5 dB/octave between 2.8-5.6 kHz). The masking effect is sharply reduced between 5.6-8 kHz (>20 dB/octave), but the SMR remains above 15 dB at the highest frequencies tested (8-16 kHz, with an increase of 6dB/octave over this frequency range). The absolute masking effect at each frequency varies between the different masker levels from 1.8 dB to 10 dB (at 11.2 and 16 kHz, respectively).

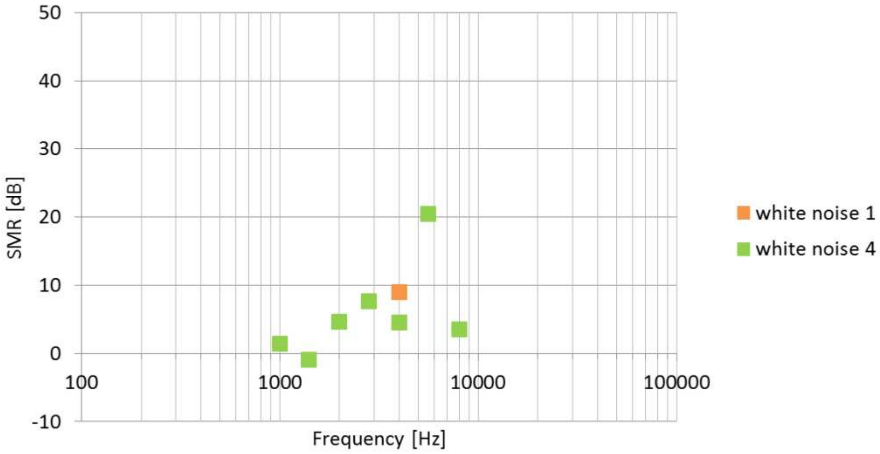
### 5.2.2. White noise

The masking effects of two levels of white noise were measured in three harbour porpoises at the Dolfinarium Harderwijk, a further two levels were measured on one of the porpoises (*Figure 3, Table 4 &*

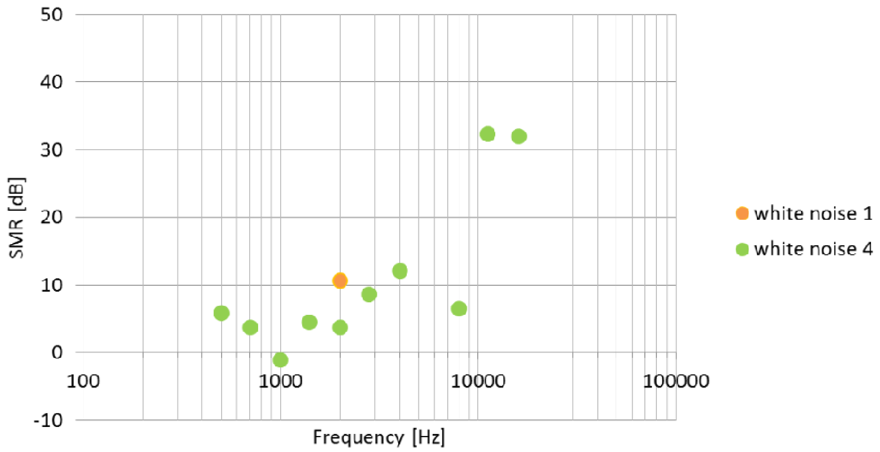
*Table* 5).




*PpZH 003*




*PpSH 163*




*PpZH 004*

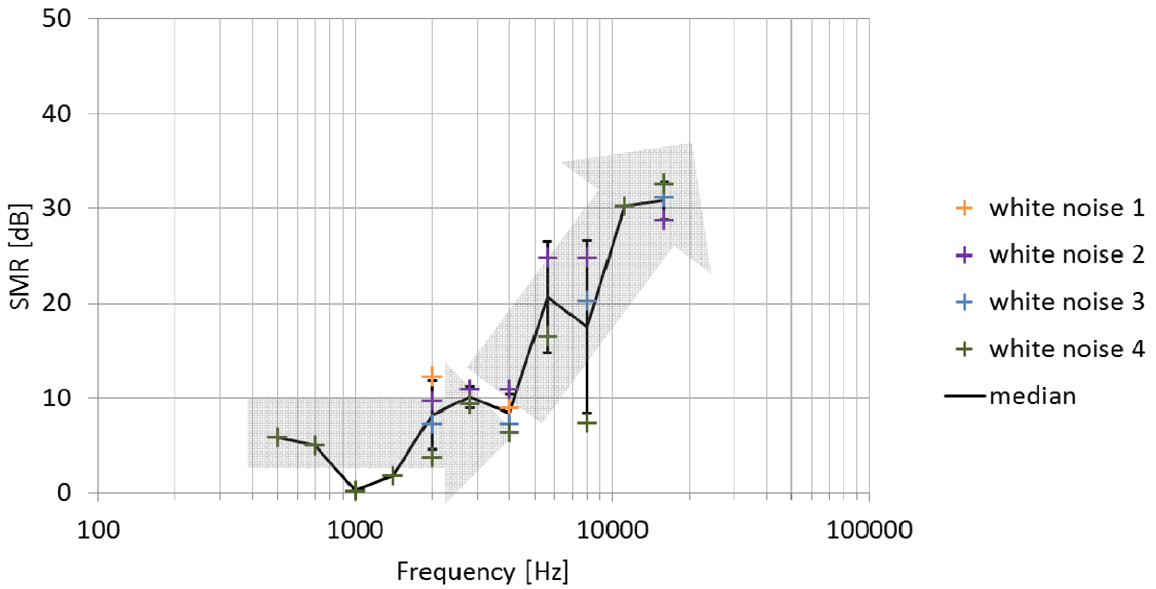



*Figure 3. Masking effect of Gaussian ('white') noise at four received sound pressure levels on the hearing sensitivity of three harbour porpoises displayed separately and combined (bottom graph). The signal to masker ratio (SMR) is displayed as a function of frequency [Hz], which is on a logarithmic scale. Error bars indicate standard deviation based on this sample of data. The grey shaded arrows indicate overall trends.*

*Table 4. Masking effect of white noise on the hearing sensitivity of three harbour porpoises (Identity: PpSH x) at Dolfinarium Harderwijk listed for each animal separately. The masking effect is calculated as the ratio (in dB) of an animal's tone detection threshold at a given frequency and the power spectral density of the masker at this frequency (SMR).*

| | Harderwijk | Masking effect (SMR) | | | Harderwijk | Masking effect (SMR) |
|---|---|---|---|---|---|---|
| Frequency | White noise - level 1 | PpZH 003 | PpSH 163 | PpZH 004 | White noise - level 2 | PpZH 003 |
| [Hz] | [dB re 1 µPa$^2$/Hz] | [dB] | [dB] | [dB] | [dB re 1 µPa$^2$/Hz] | [dB] |
| 500 | | | | | | |
| 700 | | | | | | |
| 1000 | | | | | | |
| 1400 | | | | | | |
| 2000 | 86 | 14 | | 11 | 94 | 10 |
| 2800 | | | | | | |
| 4000 | 89 | 9 | 9 | | 96 | 11 |
| 5600 | | | | | | |
| 8000 | | | | | 100 | 25 |
| 11200 | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 16000 | | | | | 84 | 29 |

| | Harderwijk | Masking effect (SMR) | Harderwijk | Masking effect (SMR) | | |
|---|---|---|---|---|---|---|
| Frequency | White noise - level 3 | PpZH 003 | White noise - level 4 | PpZH 003 | PpSH 163 | PpZH 004 |
| [Hz] | [dB re 1 µPa$^2$/Hz] | [dB] | [dB re 1 µPa$^2$/Hz] | [dB] | [dB] | [dB] |
| 500 | | | 99 | | | 6 |
| 700 | | | 100 | 6 | 0 | 4 |
| 1000 | | | 104 | 1 | -1 | -1 |
| 1400 | | | 104 | | | 4 |
| 2000 | 98 | 7 | 100 | 3 | 5 | 4 |
| 2800 | | | 102 | 12 | 8 | 9 |
| 4000 | 100 | 7 | 103 | 3 | 5 | 12 |
| 5600 | | | 99 | 12 | 20 | |
| 8000 | 104 | 20 | 107 | 12 | 4 | 7 |
| 11200 | | | 89 | 28 | | 32 |
| 16000 | 88 | 31 | 91 | 33 | | 32 |

*Table 5. Masking effect of white noise on the hearing sensitivity of three harbour porpoises (Identity: PpSH x) at Dolfinarium Harderwijk – all data combined. The masking effect is calculated as the ratio (in dB) of an animal's tone detection threshold at a given frequency and the power spectral density of the masker at this frequency (SMR). Data are listed for all tested received sound pressure levels (3-8) as well as the median, standard deviation (sd.) and the number of data points per frequency (count).*

| | Masking effect (SMR) at white noise level | | | | Masking effect (SMR) | | |
|---|---|---|---|---|---|---|---|
| Frequency | 1 | 2 | 3 | 4 | Median / sd./ count | | |
| [Hz] | | | | | | | |
| 500 | | | | 6 | 6 | | 1 |
| 700 | | | | 5 | 5 | | 1 |
| 1000 | | | | 0 | 0 | | 1 |
| 1400 | | | | 2 | 2 | | 1 |
| 2000 | 12 | 10 | 7 | 4 | 8 | 4 | 4 |
| 2800 | | 11 | | 9 | 10 | 1 | 2 |
| 4000 | 9 | 11 | 7 | 6 | 8 | 2 | 4 |
| 5600 | | 25 | | 15 | 21 | 6 | 2 |
| 8000 | | 25 | 20 | 7 | 18 | 9 | 3 |
| 11200 | | | | 30 | 30 | | 1 |
| 16000 | | 29 | 31 | 33 | 31 | 2 | 3 |

The presence of white noise causes an overall increase in hearing sensitivity in the harbour porpoises of 9 dB/octave between 1 and 16 kHz. This increase in masking is not steady over the frequency range tested, but varies between 0.1 (2-4 kHz) and 13.4 dB/octave (8-16 kHz). There is no systematic correlation between the increasing SPL of the masking noise (with increasing masker levels 1-4).

# 6. Discussion

The acoustic masking effect of a sound resembling ship noise on the hearing sensitivity of harbour porpoises has been demonstrated in this study. All four harbour porpoises had a healthy hearing system, their hearing thresholds in the absence of any of the two types of masking noise were within the limits of normal hearing and no pathological changes were found at any frequency range. Except for hearing thresholds measured at 11.2 kHz, hearing sensitivity was clearly elevated compared to the background noise in the pools, indicating that the measurements were not masked by the existing noise floor in the pools.

This study aimed at investigating the masking effect of ship noise on harbour porpoise hearing. Masking is quantified in this study as the ratio (in dB) of an animal's tone detection threshold at a given frequency and the power spectral density of the masker at that frequency. The observed masking effect reaches a maximum of 28 dB at 5.6 kHz for ship-like (red) noise and 31 dB at 16 kHz in the presence of white noise. The SMR values achieved vary between the four harbour porpoises, the two types of masking sound, as well as the different levels presented within each masking sound. Most likely, this variation can be related to individual differences in hearing sensitivity between the animals as well as the analytical resolution of the ABR data. As a general trend, however, the median SMR were relatively stable at low levels of <14 dB and frequencies of ≤1.4 kHz (red noise) and ≤4 kHz (white noise). With increasing frequency, the median SMR for red noise increased by 8 dB/octave to a maximum of 28 dB while in the presence of white noise median SMR values increased by 9 dB/octave to a maximum of 32 dB at 16 kHz. This general trend is similar to the results measured by [40], although the overall levels in the present study were lower at the low frequencies, and increased at a higher rate (dB/octave) as compared to Kastelein et al. [40].

The masking results achieved in this study at the lowest masking level tested (red noise 3) indicate a continued, constant increase in SMR to a maximum of 41 dB (*Figure 2*). At higher levels of red masking noise (red noise 5-10), however, the SMR drops by 12 dB between 5.6 and 8 kHz before increasing again at frequencies above 8 kHz. These results are inconsistent in comparison to the results measured in the presence of white noise and data by [40]. This drop may be related to the decreasing sound energy in the red masker noise (as compared to the constant or mildly increasing white noise spectrum at these frequencies) in relation to the width of the animals' auditory filter in this frequency band. Also note that the SMR drop of 12 dB from 5.6 to 8 kHz coincides with a dramatic increase in hearing sensitivity of the animals by 40 dB over the same frequency range in the absence of noise, and might indicate not just enhanced sensitivity but also enhance signal detection capabilities in this frequency range. The fact that this drop in SMR was only seen in red noise and not in white noise might relate to the fact that the red noise spectrum was decreasing while the white noise spectrum was mildly increasing in this frequency band, and the shape and width of the auditory filters determines the amount of noise present in this filter (note that a 1/3$^{rd}$ octave filter was used to compute the power spectral density of the noise in both cases). The difference between the red and white noise conditions can also be caused by acoustic interference in the pool (standing waves) at the time and location of the measurements. Note that the received spectra of the masking noise were measured at the position of the animal's head in the absence of the animal and the trainers. However, during animal testing, i.e. in the presence of animal and trainers inside the pool, the acoustic field might have differed from when the red noise was tested to when the white noise was tested. Finally, physiological processes at high levels of masking noise might have differed from low-level conditions. None of these hypotheses can be tested at this stage, but should be revisited later.

The main concern with regards to the masking effect of ship noise on the hearing in harbour porpoises is focussed on the low frequencies as the main spectral (acoustic) energy of ship sound is usually centred at frequencies near or below 100 Hz [9]. The maximum of the masking noise used in this study had its peak acoustic energy density between 100 and 1000 Hz, depending on noise type and level. However, in order for such sounds to be transmitted, the water depth must be at least equivalent to >1/4$^{th}$ of the wavelength of the signal. With pools used in this study being <3 m deep, only frequencies >300 Hz could be generated and propagated (as measured). This resulted in a maximum received power spectral density of the masking sound at 500 and 600 Hz (Ecomare and Dolfinarium Harderwijk, respectively). Moreover, the ABR method also has limitations in the low frequency range as it is difficult to elicit the neuronal response with low frequency stimuli (usually <1 kHz). These practical limitations resulted in shifting the frequency range tested in this study to 500 Hz and above. Also, the hearing sensitivity of harbour porpoises at low frequencies is poor [14] and probably reaches the limits of its 'functional' hearing range at 250 Hz which should reduce the risk of masking through ship noise and hence the need to test at these low frequencies.

Ships, however, also emit considerable acoustic energy at high frequencies (as shown by [76][77][8][21]) (see also Figure 3), in some cases up to 160 kHz, and thereby well into the range of good hearing sensitivity in the harbour porpoise (>10 kHz). As high-frequency underwater sound is absorbed more rapidly than low-frequency sound, the high frequencies from ship noise (possibly caused by cavitation rather than by the ships' machinery) will transmit over a shorter distance, and thus will have a smaller range of masking. The ideal frequency range to be tested was therefore chosen between 1-16 kHz with additional measurements at 500 Hz and 700 Hz. The ship-like (red) noise and white noise used were limited to 10 kHz by the acoustic properties of the sound source for the masking noise.

Masking by shipping noise is likely to influence different hearing requirements in different ways. Harbour porpoise have three main requirements of their hearing: to socialise with conspecifics, detect predators that also produce sound, and to receive echoes from sounds they produce themselves – to locate prey and potential obstacles. Probably the biologically most relevant sounds for a harbour porpoise are the (highly directional) clicks emitted by its conspecifics. The high frequency content of the clicks will quickly be absorbed, but the medium to low frequencies (not or less affected by absorption) may be detectable by a porpoise under 'normal' background noise conditions over a range of 1200 m ("*assuming the animals are facing each other'*, [62]). Killer whale (*Orcinus orca*), Grey seal (*Halichoerus grypus*) and bottlenose dolphin (*Tursiops truncatus*) vocalisations would be another category of biologically significant signals for harbour porpoises as these species are known to predate on this species (killer whales: [78][79] and grey seals: [80]) or physically injure them (bottlenose dolphins: [81]). While grey seals have not been shown to vocalise outside social interactions, killer whales and bottlenose dolphins emit in a frequency band between 1-20 kHz [82][83]. The range over which conspecific animals would be able to detect an animal's communication sound is called the 'active communication space' [84][8].

The mere perception of a sound is the most fundamental part of communication and requires the lowest ratio between the signal and the ambient noise. Other tasks, such as discrimination recognition or comfortable communication may require an additional 2-15 dB in signal strength [85][25]. Under natural conditions, the communication range would be limited by the prevailing 'ambient' noise, generated by physical and biological sources ('noise limited') or by the hearing threshold ('threshold limited'). The thresholds measured in this study in the absence of the masker ('control') provide an estimate of the detectability of signals in such an acoustically undisturbed, threshold limited situation. This detection function changes as soon as additional noise is introduced. When the red (or white) noise is emitted, the detection becomes noise limited. These SMR represent the true detection ratios while in the control situation the ratio between the detection threshold for the stimuli and the background noise is unrelated (threshold limited).

The results of this study show that red noise (a proxy for ship noise) has an increasing masking effect (SMR) from 9 dB at 1 kHz to its highest masking effect of 28 dB at 5.6 kHz. The masking continues into the higher frequencies with levels between 17 and 23 dB at 11.2 kHz and 16 kHz, respectively. The SMR of 28 dB (measured at 5.6 kHz) would mean that – assuming that the SMR for sounds equals the SMR for stimuli as used in this study and absorption can be ignored for the at the frequencies considered – the sound pressure level of any (narrowband) biological meaningful signal would have to be 28 dB higher in sound level than the broadband ship noise PSD to allow for detection in this frequency range.

In order to be detectable, the received level of a biologically relevant signal ($RL_{biol}$) would need to exceed the detection threshold (DT), ambient noise or ship noise.

(1) $RL_{biol} > DT$

In a quiet deep-water scenario the ambient noise could be estimated to have a power spectral density at 1 kHz of 55 dB re 1µPa$^2$/Hz (Wenz, 1962; Sea-state 1). In this case, the detection would be limited by hearing threshold of the harbour porpoises (threshold at 1 kHz determined by [14]: 82 $dB_{rms}$ re 1 µPa).

As soon as the PSD of the ambient noise ($PSD_{amb}$) exceeds DT, the equation changes to:

(2) $RL_{biol} > PSD_{amb} + SMR$

As soon as the PSD of a ship's acoustic emission ($PSD_{ship}$) exceeds the prevailing ambient noise (and the detection threshold) the equation changes to:

(3) $RL_{biol} > PSD_{ship} + SMR$

The range over which ship noise will mask the auditory perception of signals for harbour porpoises can only be modelled/calculated if the source level (SL) as well as the spectral and temporal characteristics of the ship's acoustic signature are known and the sound propagation can be modelled for the area.

In a deep water environment as described by [76] the spherical spreading loss of 20*$\log_{10}$(range) is applicable. In a shallow water regime as in most areas in the North Sea, the transmission loss is likely closer to 16 $\log_{10}$(range) [86]. This would increase the acoustic footprint of the ship, but at the same time reduce the transmission loss for any biologically relevant signal would be equally reduced, thereby compensating for the increased masking effect of the higher received ship level at least to some extent.

Ship noise is almost omnidirectional, therefore spatial release from masking is not effective; temporal release from masking is not efficient either as ship noise is quasi continuous; co-modulation and in-valley listening could be efficient mechanism at reducing the masking effect of ship noise, as the spectrum of ship noise creates a nearly pulsed pattern due to propeller cavitation and shows gaps in its frequency spectrum due to the Lloyd's mirror effect. The scale of any such masking release, however, is not quantifiable yet for harbour

porpoises (as well as marine mammals in general) and would also depend on situation- and location-specific acoustic signature of the ship and the sound propagation conditions.
Harbour porpoises evolved in an environment full of natural sound sources such as other animals' vocalisations, currents, waves and rain. Each of the biological, geological or oceanographic sound sources contributes to the natural 'acoustic scene' (the acoustic signature of the sum of all sounds) over a certain distance. A harbour porpoise has to deal with this naturally variable acoustic environment. Anthropogenic sound sources like ships add to this natural acoustic environment. Even though the acoustic masking 'footprint' around each ship may be relatively small (a couple of hundred to a few thousand meters), each additional masking event an animal is exposed to may accumulate and have long-term implications on the animal's fitness. The animal may have to spend slightly more effort to find prey each time its perception is masked. If exposed to additional noise repeatedly this will negatively affect its energy budget [8]. Masking can also reduce an animal's attentiveness which may eventually result in failing to detect a predator or a gill-net, with potentially fatal consequences for the animal. Thus each masking event may seem negligible, but in the long-term and concert with other environmental stressors, it can have impacts on the individual's fitness as well as population dynamics [5][87].

Potentially, the most efficient mitigation method to impacts of masking by ship noise is to achieve quieter ships. Outcomes of other mitigation measures are likely to be complicated. For example, reducing ship speeds could reduce the sound levels it produces at a given time, but extends the time over which it produces sound in a given area. Is it better to have a fast ship with a high source level in the area for a short amount of time (speeding up), or to have a slower ship in area for longer (slowing down)? With regards to the potential physical and behavioural effects of sound on marine animals, the maximum reduction in radiated sound energy may be most relevant in this context. As shown by [9] there is a trade-off between traveling slower (which leads to a decrease in SL) and spending more time in the area (increasing the exposure time). They calculated that the reduction in cumulative noise from a single ship passage is strongest at an operational speed of 35% (equivalent to 7.7 knots) relative to the optimal speed for which the ship is designed. However, except for protected areas, this approach may not provide sufficient conservation value to be implemented. Reducing the noise footprint of each ship in the design phase seems the most viable solution to reducing overall shipping noise.

# 7. Conclusions

The masking effect of ship noise on the hearing of harbour porpoises has been proven to be frequency dependant. Contrary to the common understanding that only the low frequency part of ship noise has to be considered with regards to marine fauna, this study provides further evidence that the actual masking effect increases at higher frequencies, despite the decrease in acoustic energy contained in the ship signature at the higher frequencies. This needs to be taken into account in assessing the potential impact zones and durations, but also in defining the noise monitoring parameters, as in the MSFD descriptor 11. So far, two low frequency (63 and 125 Hz) third-octave bands are proposed in the MSFD in relation to mitigation of effects on species that hear in medium-to-high frequency ranges, a limitation which is poorly related to the acoustic reality of harbour porpoises. Moreover, by focussing on these low frequencies, any monitoring and/or mitigation measure will be falsely directed with important and rather detrimental implications for a high-frequency specialist such as the harbour porpoise.

So far, no national or international regulation of underwater sound considers explicitly the effect of masking on the marine environment. Masking through ship noise, however, changes the entire auditory scene for harbour porpoises (as for many other receiving and listening marine animals) and the range over which they can operate acoustically. This, in turn, can negatively affect the animals' energy budget and ultimately individual fitness and population dynamics. Ideally, masking should be considered as a significant conservation issue when assessing existing or designing new anthropogenic sound sources/activities at sea. The large variation in the auditory systems of cetaceans points to a need for determining masking level indicators fitted to categories of species that have different hearing capabilities (see [88][89]). In this regard, the outcome of this study can be used as a baseline for the harbour porpoise, an abundant and wide-spread, small toothed cetacean, and one of the top predators in European waters.

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# ANNEXES

## *A.1 Glossary*

*Units*

| | |
|---|---|
| dB | decibel |
| Hz | Hertz |
| kHz | kilohertz |
| Pa | Pascal |
| µPa | micro-Pascal |
| dB re 1 µPa | decibels referenced to 1 micro-Pascal |
| dB re 1 $\mu Pa^2 \cdot s$ | decibels referenced to 1 micro-Pascal-squared*seconds |

*Terms*

| **Terms** | **Definition** |
|---|---|
| ABR | See: Auditory Brainstem Response |
| Acoustic Pressure | The force per unit area exerted by a sound wave above and below the ambient or static equilibrium pressure is called the acoustic pressure or sound pressure. The units of pressure are pounds per square inch (psi) or, in the SI system of units, Pascal [Pa]. |
| AEP | See: Auditory Evoked Potential |
| Ambient noise | The background din of underwater noise in which no single signals can be identified; contributors include distant shipping, wind, and biological choruses |
| Amplitude | The maximum positive and negative deviation of a wave, e.g. a sound wave. |
| Anthropogenic Effects | Processes, objects, energy, or materials that are derived from human activities, as opposed to those occurring naturally. |
| Anthropogenic noise | Collective for all human produced sources of unwanted sound. |
| Audiogram | Graphical representation of audibility thresholds as a function of tone frequency |

| Terms | Definition |
|---|---|
| Auditory Brainstem Response (ABR) | Neuronal response measured after stimulation of the hearing system with an acoustic signal; refers to the early neuronal responses of the auditory periphery (hearing nerve–to–auditory nuclei in the brainstem, duration 0-15 ms). |
| Auditory Evoked Potentials (AEP) | Neuronal response measured after stimulation of the hearing system with an acoustic signal; refers to the entire sequence of auditory evoked neuronal activity in the auditory periphery and cortical areas (duration 0-500 ms). |
| Auditory integration time | the time over which temporal auditory summation occurs, i.e., the time over which the auditory system integrates acoustic energy. |
| Co-modulation masking release (CMR) | A release from masking that occurs for coherently modulated sound, i.e., sound with amplitude fluctuations that are consistent across a range of frequencies. |
| Critical Band (CB) | Considering the auditory system a series of bandpass filters, mostly noise energy in the filter of width CB surrounding the signal is effective at masking. Under Fletcher's (1940) equal-power and rectangular filter assumptions, in the case of a tone being masked by white noise, at detection threshold, Pt = $PSD_n$ x CB, therefore the CB can be expressed in terms of CR, CB = 10*CR/10. |
| Critical ratio (CR) | Considering broadband white noise masking a pure tone signal, CR = 10 log10 ($P_t/PSD_n$), where $P_t$ is the tone power at its detection threshold in the noise, and $PSD_n$ is the power spectral density of the noise |
| Decibel [dB] | A logarithmic scale for describing differences in e.g. sound pressure relative to a reference pressure. The standard reference for in-air sound is 20 micro-Pascal (µPa), for underwater sound pressure 1 µPa. The dB symbol is followed by a second symbol identifying the specific reference value (i.e., re 1 µPa). Decibel is a dimensionless ratio term that can be applied to any two values. Decibels are expressed as 10 times the logarithm of the ratio of a value (V) to its reference value (Vref), or: N decibels (dB) = 10*log (V/Vref). Decibels should always be accompanied by a reference value that defines the ratio being expressed unless clearly specified in the beginning. In this report all references to dB that are not accompanied by a specific reference value are dB of sound pressure level (SPL), referenced to 1 micro Pascal of pressure. |
| Emission *vs.* Immission | With regards to exposure to sound, "emission" refers to sound from the source and "immission" refers to sound received by a person or animal. |

| Terms | Definition |
|---|---|
| Ensonification | The words, "insonify" and "ensonify," are often used as synonyms but, they have subtle but different meanings. "Sonify" is a verb that simply means, "to add sound." Likewise "insonify" means "to add sound into." |
| Frequency bandwidth | The range of frequencies over which a sound is produced or received. |
| Frequency spectrum | See Spectrum. |
| Hertz [Hz] | The units of frequency where 1 Hertz = 1 cycle per second. The abbreviation for hertz is "Hz." |
| Gaussian white noise | Statistical noise in which the values at any pair of times are identically distributed (hence appear to have a 'flat' spectrum) and statistically independent (and hence uncorrelated). |
| Immission | See: 'Emission vs. Immission'. |
| Impulsive sound | Transient sound produced by a rapid release of energy, usually electrical, mechanical or chemical such as circuit breakers, airguns or explosives. There are no clear boundaries between impulse sounds and tonal ("continuous") sounds, but generally speaking impulse sounds are 1) of short duration (less than 1 second, and usually much shorter), and 2) have an irregular waveform, rather than the smooth sinusoidal waveform generated by most sonars or speech, for example. |
| Infrasound | Sound at frequencies below the hearing range of humans. These sounds have frequencies below about 20 Hz. |
| MSFD | Marine Strategy Framework Directive |
| Masking | The process or the amount (M) by which the threshold of hearing for one sound is raised by the presence of another (masking) sound; M [dB] = $DT_n$ - $DT_o$, where DT is the detection threshold of the signal in the absence (o) or presence (n) of noise. |
| Masking release | Masking release occurs when the detection threshold for a masked sound decreases, usually as mediated by a specific mechanism, e.g., temporal or spatial masking release. |
| Noise | A sound that has the potential to interfere with the life functions of marine mammals; with regards to acoustic masking, noise is the masking sound. |
| Octave | The interval between one musical pitch and another with half or double its frequency. It is defined as the unit of frequency level when the base of the logarithm is two. |
| $1/3^{rd}$ octave band level | Power spectral density integrated into bands that are $1/3^{rd}$ of an octave wide. |

| Terms | Definition |
|---|---|
| 1/3rd octave bands | A series of adjacent frequency bands, that are 1/3rd of an octave wide; in the absence of CB data for several marine mammal species, 1/3rd octaves are commonly used as surrogates. |
| Peak pressure | The highest pressure above or below ambient that is associated with a sound wave. |
| Permanent Threshold Shift | The permanent (irreversible) reduction in hearing sensitivity (‘hearing loss’) resulting from exposure to intense impulse or continuous sound |
| Power spectral density | PSD; describes how the power of a signal is distributed with frequency; typically computed as mean square pressure spectral density levels [dB re 1 µPa$^2$/Hz] |
| Pressure | Acoustic pressure is a deviation from the ambient hydrostatic pressure caused by a sound wave. |
| Propagation loss | Transmission losses of sound over distance through a medium (air, seawater etc.). The propagation losses of sound are frequency-depended and also depend on complex number of factors (bottom structure, sediment, etc.) and are mostly irregular in coastal waters. In the far-field of a sound source the rate of decrease is proportional to the distance 1/r. In an unbounded, homogenous medium, propagation loss will be on the order of 6 dB for every doubling of the distance. |
| PSD | See Power spectral density. |
| Received level (RL) | The received level of sound, typically in terms of $SPL_{rms}$, at the position of the receiver. |
| Red noise (Brownian noise) | Statistical noise with its maximum in spectral density at lower frequencies. It decreases in power by 6 dB per octave (20 dB per decade). |
| Rise time | The interval of time required for a signal to go from zero, or its lowest value, to its maximum value. Frequency spreading and environmental scattering would tend to "smear" the rise time as the sound propagated away from the source. |
| RMS | See: Root-mean-square sound pressure level |
| Root-mean-square sound pressure level ($SPL_{rms}$) | 20 times the logarithm to base 10 of the root of the average (over some duration T) of the squared pressure time series, where $P_{ref}$ = 1 µPa. These amplitudes include an averaging of the pressure wave signal over a certain time window. For sinusoidal signals, the rms-pressure is usually about 9 dB lower than the peak-to-peak pressure. |
| Signal-to-masker ratio (SMR) | Ratio of mean-square pressures of signal and noise; SMR = $SPL_{rms}$(signal) – $SPL_{rms}$(noise). |
| Sound attenuation | Reduction of the level of sound pressure. Sound attenuation occurs naturally as a wave travels in a fluid or solid through dissipative processes (e.g., friction) that convert mechanical energy into thermal energy and chemical energy. |

| Terms | Definition |
|---|---|
| Sound exposure level (SEL) | The constant sound level acting for one second, which has the same amount of acoustic energy, as indicated by the square of the sound pressure, as the original sound. It is the time-integrated, sound-pressure-squared level. SEL is typically used to compare transient sound events having different time durations, pressure levels, and temporal characteristics (in dB re 1 $\mu Pa^2 \cdot s$). |
| Sound Pressure Level | Pressure level of a sound source measured at a certain distance from a sound source and commonly referred to a reference pressure level of 1 μPa and expressed in dB re 1 μPa. |
| Soundscape | Characterization of a sound-field in terms of its spatial, temporal and frequency dependence, and the sources responsible for the sound (ISO/DIS 18405); this refers to only the physical component of the soundscape due to lack of understanding of perceptual component in aquatic animals. |
| Spatial release from masking | A release from masking that occurs when signal and noise sources are located at different points in space. |
| Spectrogram | A graph, which displays acoustic energy as a function of frequency allowing frequency patterns to be visualised, and reverberations to be depicted. |
| Spectrum | A graphical display of the contribution of each frequency component contained in a sound. |
| SPL | Sound pressure level |
| Temporary Threshold Shift | the reversible reduction in hearing sensitivity resulting from exposure to intense impulse or continuous sound |
| Threshold | The threshold generally represents the lowest signal level an animal will detect in some statistically predetermined percent of presentations of a signal. |
| Transducer | A device (hydrophone e.g.) to convert underwater sound into electrical voltage or vice-versa. |
| Ultrasound | Sound at frequencies above the hearing range of humans. These sounds have frequencies above about 20 Hz. |
| Within-valley listening | A release from masking that occurs when the listener focuses on quieter gaps within the noise. |

## A.2 Modulation Rate Transfer Function (MRFT)

The signal to noise ratio of the neuronal responses evoked with SAM tones (hence the resolution of the results) depends on the modulation frequency used to modify the carrier frequency of the signal (see **Erreur ! Source du renvoi introuvable.**). In order to determine the best modulation frequency, a single carrier (130 kHz) was tested under identical conditions (acoustically and in terms of measurement setup) on animal PpZH 004. Supin et al. [72] found the best response for ABR measurements in a bottlenose dolphin at a modulation frequency of 1 kHz, Lucke et al. [54] determined the highest SMR in a harbour porpoise at 1.1 kHz. At a step size of 49 Hz, a total of 34 modulation frequencies were tested.

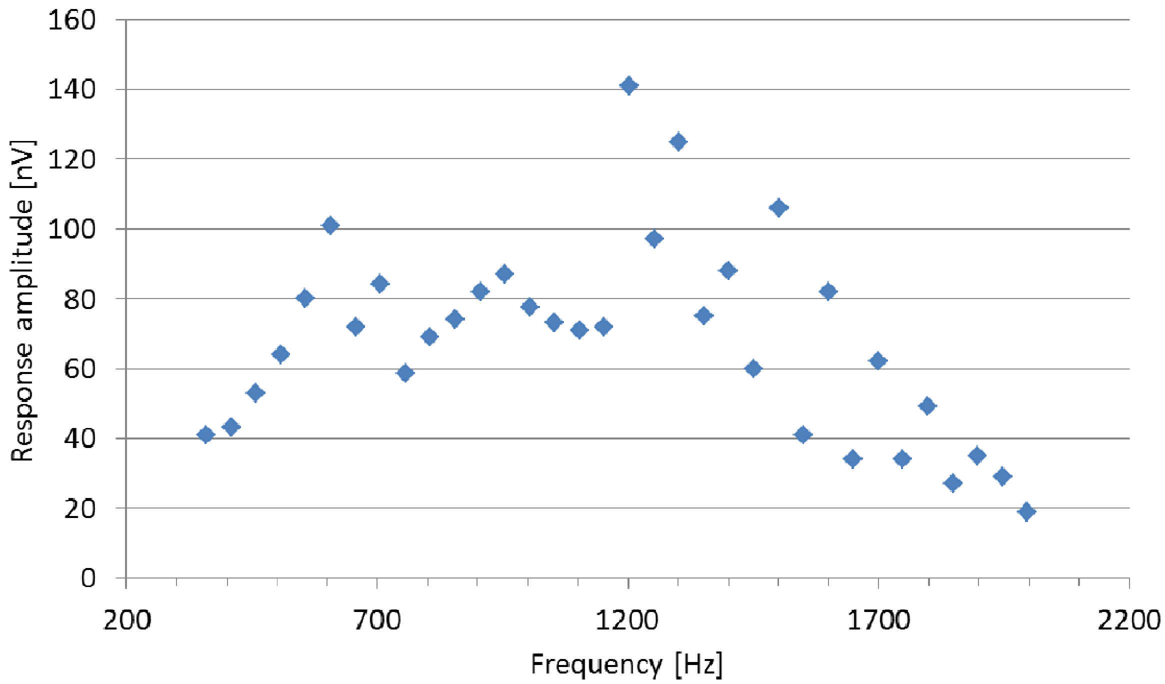


*Figure 4. Modulation rate transfer function of a harbour porpoise (PpZH 004) measured at the Dolfinarium Harderwijk. The responses were measured after stimulation with a sinusoidal amplitude modulated signal (SAM) at 130 kHz at varying modulation frequencies. The response amplitude (in nV) is plotted as function of the centre of the frequency bin tested.*

The maximum response (141 nV) was measured at 1,203 Hz which was used as the modulation frequency in all hearing threshold measurements using SAM tones at carrier frequencies between 10-180 kHz.