ACOUSTIC MITIGATION DEVICES (AMDs)
TO DETER MARINE MAMMALS FROM PILE DRIVING AREAS AT SEA:
AUDIBILITY & BEHAVIOURAL RESPONSE OF A HARBOUR PORPOISE & HARBOUR SEALS

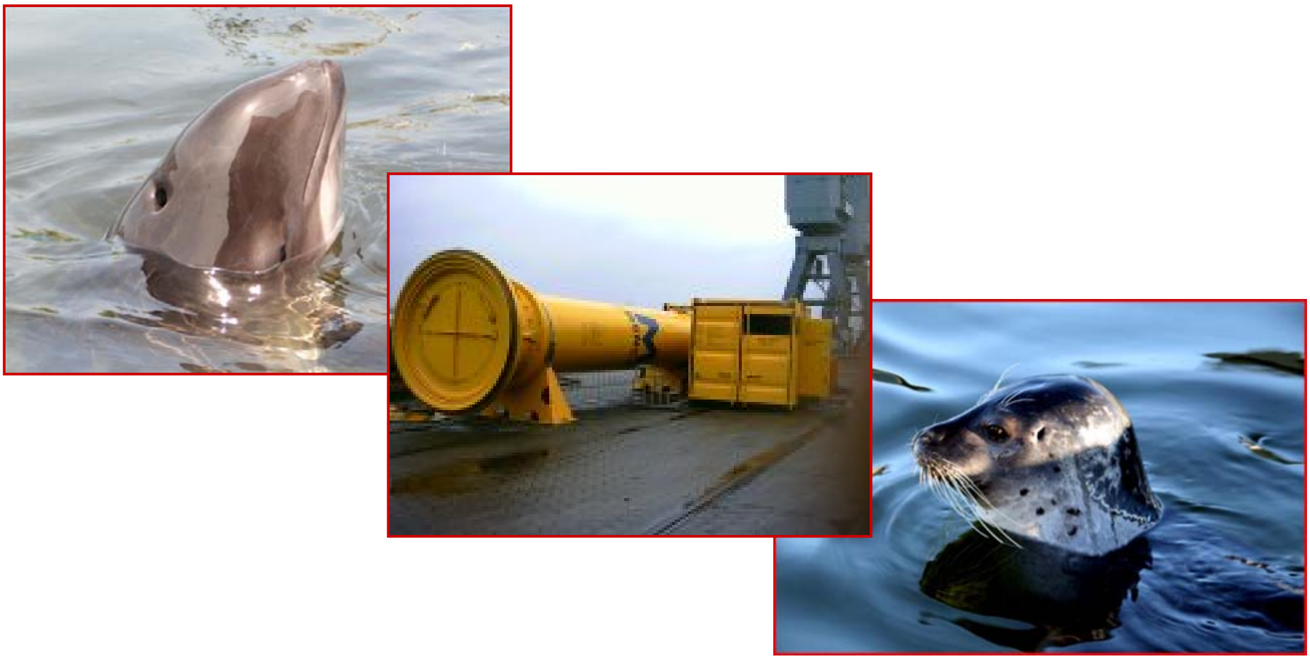

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Table of Contents

Figures and Tables

Figures

Figure 34. 1/3-octave spectra of the Sensation Level (‘Weighted SPL’) of the original recordings by Subacoustech and of the reproduced sounds of the three AMD devices, both scaled to the same broadband level at the observed hearing threshold [Sensation Level = the SPL of the AMD signals averaged over the 90% energy duration of the signals in 1/3-octave bands, minus the harbour seals’ 50% hearing threshold levels for 1000 ms tonal signals (Kastelein *et al*., 2009,a,b) at the 1/3-octave centre frequencies]. Note: the time averaged spectra for the three different signals of the AquaMark 100 are identical.

Figure 35. One of the two seals hauled out frequently on the floating platform when the AMD sounds were projected under water at the highest levels.

Tables

Table 1. The acoustic characteristics of sounds produced by the three AMDs, from original recordings by Subacoustech.

Table 2. The three mean received SPLs ± SD (dB re 1 µPa; n = 231) at which each AMD was tested in the experiments.

Table 3. The three mean received SPLs ± SD (dB re 1 µPa; n = 99 measurements in the pool) at which each AMD was tested in the experiments.

Table 4. The mean 50% hearing threshold amplitudes (± SD, n = 6 sessions; based on broadband SPL (average over the 90% energy signal duration) and prestimulus response levels (for both signal-present and signal-absent trials) of the 4.5-year-old male harbour porpoise, for the five reproduced AMD (Acoustic Mitigation Device) sounds (the AquaMark 100 produced 3 different sounds). Also shown are the broadband hearing threshold levels for the AMD signals, estimated from the harbour porpoise’s audiogram for tonal signals of 1500 ms duration (Kastelein *et al*., 2010).

Table 5. Results of paired t-tests to compare the objective parameters, and sign tests to compare the subjective parameters, in baseline and test sessions, for each AMD/level combination. The sample size for each combination is six, and exact P-values are shown in bold if significant.

Table 6. The mean 50% hearing threshold amplitudes (± SD, n = 6 sessions) and prestimulus response levels (for both signal-present and signal-absent trials) of the two 4-year-old female harbour seals for the five reproduced sounds of the three AMDs (Acoustic Mitigation Devices; the AquaMark 100 produced 3 different sounds). Also shown are the broadband hearing threshold levels for the AMD signals, estimated from the harbour seal audiogram for tonal signals of 1000 ms duration (Kastelein *et al*., 2009a).

Table 7. Results of paired t-tests to compare behavioural scores in baseline and test sessions, for each combination of AMD and level. The sample size for each combination is five, and exact P-values are shown in bold if significant (P ≤ 0.05).

Table 8. Estimated distances at which the three AMDs are audible to harbour porpoises under two background noise level conditions (Sea State 0 and Sea State 4) and based on two propagation models (10 Log R and 20 Log R propagation), for various levels of absorption (dB/m based on the frequency of the AMD sound).

Table 9. Estimated distances at which the three AMDs are audible to harbour seals under two background noise level conditions (Sea State 0 and Sea State 4), based on two propagation models (10 Log R and 20 Log R propagation), and for various levels of absorption (dB/m based on the frequency of the AMD sounds).

Table 10. The minimum distances at which sounds from the three AMDs are estimated to cause no behavioural response in harbour porpoises, calculated by means of two generic propagation loss models, for the mean received levels found in the present study just to cause no response.

Table 11. The distances at which sounds from the three AMDs are estimated to cause a small behavioural response in harbour porpoises, calculated by means of two generic propagation loss

models. The calculations are based on the received levels found in the present study to cause the porpoise to surface more frequently.

Table 12. The distances at which sounds from the three AMDs are estimated to cause porpoises to swim away from the sound source, calculated by means of two generic propagation loss models. The calculations are based on the received levels found in the present study to cause the porpoise to move away from the active transducers.

Table 13. The minimum distances at which sounds from the three AMDs are estimated to cause no behavioural response in harbour seals, as calculated for two propagation models.

Table 14. The distances at which sounds from the three AMDs are estimated to cause a small behavioural response in harbour seals, calculated for two propagation models. The calculations are based on the received levels found in the present study, which caused one seal to haul out occasionally.

Table 15. The distances at which sounds from the three AMDs are estimated to cause a strong behavioural response in harbour seals (probably deterring them at sea), calculated for two propagation models. The calculations are based on the received levels found in the present study, which caused one seal to haul frequently (10 % of the time).

Table 16. The objectives and results of this research.

1. Executive Summary

1.1 Introduction

For the sustainable development of the offshore renewable energy industry, it is necessary to reduce or avoid the damaging effects of noise (such as death or permanent hearing damage), from activities which produce high sound pressure levels, such as pile driving, on marine mammals. One way to achieve this is to ensure that marine mammals are not present in areas where loud noises are being produced, by deterring them by means of safe sounds produced by Acoustic Mitigation Devices (AMDs). Research is needed to determine whether AMDs can mitigate immediate vicinity impacts of loud sounds, which could give rise to temporary or permanent hearing damage, injury or death of marine mammals.

In the North Sea, the two most abundant marine mammal species are the harbour porpoise (*Phocoena phocoena*) and the harbour seal (*Phoca vitulina*). COWRIE commissioned SEAMARCO to carry out four studies on the audibility of sounds produced by three selected AMDs and their effect on the behaviour of harbour porpoises and harbour seals (playback experiments). In addition to achieving these aims, SEAMARCO estimated the distances at which sounds from AMDs are audible to, and elicit behavioural responses in, harbour porpoises and harbour seals.

1.2. AMD sounds

Most acoustic devices designed to deter marine mammals are either developed for pinnipeds (seals and sea lions) or for odontocetes (dolphins and porpoises). So far, no devices have been developed with the goal to deter both taxa.

For the current research, Subacoustech Ltd. was commissioned by COWRIE to measure the sound output of commercially available AMDs, and supply recorded AMD sounds to SEAMARCO. The AMDs were selected without input from SEAMARCO.

The following AMDs were selected: the AquaMark 100, designed to keep dolphins away from gill nets, the Ace Aquatec Seal Scrammer, and the Lofitech Seal Scarer, both designed to keep seals away from aquaculture sites. The AquaMark 100 produces structured chirps, with their main energy in the 40 to 50 kHz band. The Ace Aquatec produces a series of short complex pulses with varying frequency content, the main energy of which is concentrated in the 10 to 20 kHz range. The Lofitech produces a tonal pulse at 15 kHz, with weak harmonics.

AMDs are not acoustical devices developed for scientific research, but practical devices for use at sea. Even within AMDs from the same production series, some variation in output is likely. The margins applied by the manufacturers for the specifications of the AMDs are unknown, and wind park developers should check the output of AMDs before using them.

The AMDs operate at fixed acoustic source levels. To investigate the audibility of the sounds and the behavioural response of harbour porpoises and harbour seals to them, it was necessary to control the projected levels so that the signal levels experienced by the animals in the pool were representative of the levels received by at various distances from the AMDs at sea. Therefore, recordings of the AMD sounds were used, so that the level could be adjusted for the four studies.

1.3. Distance calculations

The distances at which sounds are audible by marine mammals and elicit behavioural responses in them are influenced by the ambient noise and the propagation conditions. Ambient noise can mask the AMD sounds. The ambient noise level depends for a large part on sea state (which depends on wind speed), the presence or absence of precipitation, and anthropogenic noise. Two extreme models of sound propagation are represented by cylindrical and spherical spreading. Cylindrical spreading is more likely to occur in shallow water or in temperature or salinity layers; spherical spreading occurs more often in deeper water.

1.4. Audibility of AMD sounds to a harbour porpoise

Hearing thresholds (stimulus levels resulting in a 50% detection rate) of a young adult male harbour porpoise for five sounds from three AMDs were quantified by using a behavioural psycho-acoustic technique (“go/no-go” response), in which signal amplitude was varied by the 1-up 1-down staircase method. The 50% hearing thresholds were very similar for all five test sounds (ca. 55 dB re 1 µPa), as predicted from their spectra and from the tonal audiogram of the porpoise. The actual AMDs have various source levels, so the audibility distances of AMDs for porpoises are predicted to vary. The distances at which harbour porpoises can hear the three AMDs were estimated for two extreme situations, by submitting information from the present study, the source levels of the AMDs, and two background noise levels to two generic propagation models. Under optimal conditions (quiet and with good propagation), the AquaMark 100 can be heard at 2 km, the Ace Aquatec at 91 km, and the Lofitech also at 91 km. Under poor conditions (noisy and with poor propagation), the AquaMark 100 can be heard at 0.2 km, the Ace Aquatec at 14 km, and the Lofitech at 18 km. More information is needed, in particular on the level at which pile driving sounds begin to have adverse effects on harbour porpoise hearing, before the effectiveness of the AMDs can be evaluated.

1.5. Behavioural response of a harbour porpoise to AMD sounds

The effect of three AMDs (with different spectra) on the behaviour of a young adult harbour porpoise was quantified in a large pool. The goal was to determine if the signals elicit behavioural responses in porpoises, and if so, at which level. The study animal was exposed to 30-minute series of AMD signals. The signal interval was randomised per signal type and was such that all series had a duty cycle (percentage of time sound is produced) of around 9%. Each AMD sound was tested at three source levels which were determined during pre-tests: a level which just did not cause a behavioural change, a level which caused a small change in the surfacing rate and swimming pattern, and a level which caused the porpoise to swim away from the transducers (i.e., exhibit displacement). The received broadband sound pressure levels, averaged over the signal duration, were, for the AquaMark 100 AMD: 70, 105, and 127 dB re 1 µPa; for the Ace Aquatec: 77, 117, and 139 dB re 1 µPa; and for the Lofitech: 91, 121, and 151 dB re 1 µPa. Because the source levels of the actual AMDs differ from these values, the distances at which they are expected to cause the three types of behavioural response in porpoises at sea vary. Information from the present study, along with the source levels of the AMDs and data on environmental properties, can be submitted to appropriate propagation models to estimate the distances at which the three AMDs elicit behavioural responses in harbour porpoises. As examples, distances for two situations (cylindrical and spherical spreading) were calculated for the three received levels (and corresponding behavioural responses). This showed that, though it was designed for dolphins, the AquaMark 100, at its normal source level, is unlikely to be effective at deterring porpoises. The Ace Aquatec and Lofitech AMDs, which were both designed for seals, do have the potential to deter harbour porpoises at useful distances (between 0.2 and 1.2 km); provided that there is no attraction (such as a food source) in the immediate area of the AMD, porpoises are expected to move away. Because of high levels of variability in response distances due to variation in environmental variables, distance ranges, rather than exact distances, at which each AMD elicits a response, should be considered when planning mitigation measures.

1.6. Audibility of AMD sounds to harbour seals

Hearing thresholds of two young adult female harbour seals were obtained by the same method as used for the porpoise. Seal 01's average hearing thresholds for the AMD signals were similar; AquaMark 100: 64 dB re 1 µPa, Ace Aquatec: 63 dB re 1 µPa, Lofitech: 66 dB re 1 µPa. Seal 02's thresholds were on average 3 dB lower (indicating higher hearing sensitivity) than those of seal 01. The similarity of the thresholds for the three AMD sounds is as predicted from the comparison of the spectra and the tonal audiograms of the study animals. The source levels of actual AMDs differ, so the distance at which they are audible to seals at sea also differs. The distances at which the three AMDs are audible to harbour seals were calculated, as examples, for two situations, by submitting information from the present study, the source levels of the AMDs, absorption data, and background noise levels to two propagation models. Under optimal conditions (quiet and with good propagation), the AquaMark 100 can be heard by harbour seals at 1.6 km, the Ace Aquatec at 91 km, and the Lofitech at 99 km. Under poor conditions (noisy and with poor propagation), the AquaMark 100 can be heard at 0.2 km, the Ace Aquatec at 14 km, and the Lofitech at 17 km. More information is needed, in particular on the level at which pile driving sounds begin to have adverse effects on seal hearing, before the effectiveness of the AMDs can be evaluated.

1.7. Behavioural response of harbour seals to AMD sounds

The effect of three AMDs (with different spectra) on the behaviour of harbour seals was quantified in a pool, to determine whether the signals elicit behavioural responses in seals; at which level behaviour starts to change; and what happens at higher levels. Two young adult female harbour seals were exposed to 30-minute series of AMD signals. The signal interval was random per AMD signal, and such that all series had a duty cycle of around 9%. Each AMD sound was tested five times (under low background noise conditions) at three source levels, determined during pre-tests, which (1) just did not cause a behavioural change, (2) caused a seal to haul out very occasionally, and (3) caused a seal to haul out approximately 10% of the time. The received broadband sound pressure levels, averaged over the signal duration were, for the AquaMark 100 AMD: 117, 127 and 137 dB re 1 µPa;for the Ace Aquatec: 109, 124, and 134 dB re 1 µPa; and for the Lofitech: 128, 133, and 138 dB re 1 µPa. Because the source levels of actual AMDs differ from these values, the distances at which they affect the behaviour of seals at sea vary. Information from the present study, along with the source levels of the AMDs and data on environmental properties, can be submitted to appropriate propagation models to estimate the distances at which the three AMDs elicit behavioural responses in harbour seals. As examples, distances for two situations (conditions with good propagation, and conditions with poor propagation) were calculated for the three received levels (and corresponding behavioural responses). This showed that the AquaMark 100, at its normal source level, is unlikely to be effective at deterring harbour seals. The Ace Aquatec and Lofitech AMDs, which were designed for seals, have the potential to deter harbour seals at useful distances (between 0.2 and 4.1 km); provided that there is no attraction (such as a food source) in the immediate area of the AMD, seals are expected to move away. Because of high levels of variability in response distances, due to variation in environmental variables, the distance ranges, rather than the exact distances, at which each AMD elicits a response, should be considered when planning mitigation measures.

1.8. Evaluation of results

In terms of robustness, the results fall into two categories. The hearing thresholds and received levels that cause particular behavioural impacts are scientifically robust data. However, the distances at which the porpoises and seals are estimated to be able to detect and respond to the AMD sounds at sea were calculated by means of propagation models, and are dependent on context and conditions. The estimated values are given only as examples. For each specific location and moment in time, a new estimation should be made.

The results were obtained from three animals with good hearing sensitivity, which was representative for young adult animals of each species, as was established during previous hearing studies. However, older animals probably have lower hearing sensitivity, as has been shown for many terrestrial mammals and a few marine mammal species. So, for older animals, the estimated detection distances for AMD sounds would be shorter than those calculated in this report. The hearing thresholds we found for AMD sounds, which are robust and repeatable, were measured for attentive animals listening for a familiar signal, in the direction of maximum hearing sensitivity (sound coming from in front of the animal). The hearing thresholds would probably be lower for inattentive porpoises and seals and for sounds coming from other directions. For each tested marine mammal species the hearing thresholds for the 3 AMD sounds were similar.

The behavioural response data, though they are scientifically robust, should be used with caution. Behaviour depends not only on hearing sensitivity, but also on many individual properties of animals (age, sex, experience, genetics, nature or disposition, etc.) and on the context (season, water depth, distance to shore, being alone or in a group, proximity to a feeding area, etc.). The present study showed that the two seals responded differently to the AMD sounds (one hauled out while the other did not). Other playback experiments with marine mammals also have revealed individual differences in responses to sounds. Thus, the behavioural response threshold levels are approximate, and will remain approximate even after many more studies, though testing the same sounds on a larger number of individuals would provide a better understanding of the range of received levels which cause the behavioural responses seen in the present study. However, for both the porpoise and the seals, the threshold levels causing a behavioural response we found were in the same range as levels causing similar behavioural responses in previous playback studies with these species.

Although habituation was not quantified, the effect of the AMD sounds did not appear to diminish during test sessions with the seals or the porpoise. After each session, the animals' behaviour returned to normal immediately. They co-operated in a psycho-acoustic tests only minutes after the AMD signals had ceased. So being exposed to the AMD signals (at the playback levels used in this study) for 30 minutes had no lasting effect on the animals' behaviour.

The results from the present study can be applied to young adult male and female porpoises and seals, as both species have limited sexual dimorphism. It is impossible to say whether the results from the harbour seals are also applicable to grey seals (*Halichoerus grypus*). The best way to find out would be to establish the audiogram of grey seals for tones. If the hearing thresholds of the grey seal are similar to those of the harbour seal, the detection ranges of grey seals for AMDs are probably similar to those of harbour seals. The effect of the AMDs on the behaviour of grey seals probably differs from that of harbour seals, as the general behaviour of grey seals is very different from that of harbour seals. In addition, the grey seal is highly sexually dimorphic, and the sexes differ greatly in their behaviour.

1.9. Conclusions

The following conclusions can be drawn from the AMD audibility studies:

1) The distance at which porpoises and seals were able to hear the AMD sounds varied greatly between the three AMDs. The AquaMark 100 can not be heard from as great a distance as the other two AMDs, because it has a lower source level and operates at higher frequencies, which are more readily attenuated than lower frequencies.
2) The audibility distance for a given AMD's sound may vary by a factor of 6 or more, depending on the propagation conditions and background noise.
3) The hearing threshold of both species for AMDs is so low, that detection is mainly dependent on the background noise level. For the harbour porpoise, the hearing thresholds at frequencies above 2.5 kHz are below the background noise level of Sea State 0; for the harbour seal, hearing thresholds in the 200 Hz-10 kHz range are below the background noise level of Sea State 0.

The following conclusions can be drawn from the AMD behavioural response studies:

1) The AquaMark 100, with its normal source level at least, is unlikely to be effective at deterring harbour porpoises and harbour seals. The two other AMDs (Ace Aquatec and Lofitech) have the potential to deter harbour porpoises and harbour seals at useful distances: provided that there is no attraction (such as a food source) in the immediate area of the AMD, porpoises and seals are expected to move away.
2) When AMDs are used at sea, variation in environmental variables leads to high levels of variability in response distances. For this reason, distance ranges, rather than exact distances, at which each AMD elicits a response, should be considered when planning mitigation measures.

1.10. Implications for environmental management

We calculated the distance ranges at which the three AMDs were deterring for harbour porpoises and harbour seals under various conditions. However, in order to determine if the AMDs tested in the present study can deter harbour porpoises and harbour seals far enough away from pile driving sites to prevent hearing damage (permanent hearing threshold shift; PTS) or temporary hearing loss (temporary hearing threshold shifts; TTS), and how they should be used, the following information is needed:

1) The received level and duration combinations of pile driving noises which cause TTS in harbour porpoises and harbour seals (SEAMARCO has been commissioned by the Dutch government to carry out research on this between September 2010 and April 2012 for harbour porpoises, and between July 2010 and April 2011 for harbour seals).
3) The received level and duration combinations of pile driving noises which cause PTS in harbour porpoises and harbour seals. This can be estimated from the TTS level/duration combination (by extrapolation from data on terrestrial mammals).
4) The required safety distance from pile driving activities at which harbour porpoises and harbour seals should be deterred, which can be calculated from the Source Level of a pile driving strike, the propagation conditions, the background noise level, and the estimated PTS level/duration combination for each of the two marine mammal species.
5) A comparison of the required safety zones for pile driving noise with the deterring zones of the AMDs (= the results from the present study).
6) The time porpoises and seals need, after the onset of AMD signals, to swim far enough away before the higher amplitude pile driving strikes begin.

7) The time-frame within which porpoise and seal behaviour returns to normal after pile driving ceases, so that, during lulls in pile driving activities, AMDs can be used if necessary to prevent porpoises and seals from entering the danger area.

Although we did not test the effect of sounds with various pulse intervals on seals and porpoises, we recommend that the inter pulse interval of AMDs should be shorter than 10 s, to dissuade porpoises and seals from approaching the AMDs during the inter pulse intervals.

Alongside the source level, the operational characteristics and use of AMDs are important factors in their efficacy. Harbour porpoises and harbour seals are unlikely to habituate to, and thus ignore, AMD sounds if they are presented for only an hour before the onset of pile driving. Wild harbour seals continuously exposed to acoustic harassment device (AHD) sounds for weeks or months did not react when an AHD (at a lower amplitude than usual) was activated. However, seals did not habituate to daily 45-minute presentations of high amplitude sounds over a period of 40 days.

2. Introduction

For the sustainable development of the offshore renewable energy industry, it is necessary to reduce or avoid the damaging effects of noise, from activities such as pile driving, on marine mammals. One way to achieve this is to ensure that marine mammals are not present in areas where loud noises are being produced, by deterring them by means of safe sounds produced by Acoustic Mitigation Devices (AMDs). Research is needed to determine whether AMDs can mitigate immediate vicinity impacts of loud sounds, which could give rise to the death or injury (including temporary or permanent damage to hearing) of marine mammals.

Sound is particularly important for marine mammals, as it may be used as a means of orientation, communication, and to locate prey, conspecifics and predators (Richardson *et al.*, 1995). Therefore, marine animals are likely to be disturbed by noise in their environment. Noise in the oceans may thus have negative physiological, auditory, and/or behavioural effects on marine fauna.

In the North Sea, the two most abundant marine mammal species are the harbour porpoise (*Phocoena phocoena*) and the harbour seal (*Phoca vitulina*). These species may be negatively influenced by pile driving sounds. Both have a wide distribution area in the coastal waters of the temperate zone of the northern hemisphere. Wind parks are often built on the continental shelf, because of the shallow water there and the relatively short distance between the wind generators and electricity users. AMDs may be useful for keeping harbour porpoises and harbour seals away from pile driving sites at sea. To be effective, AMD sound must be audible to the relevant marine mammals species, and they must deter them far enough away from the pile driving site. The four studies described here were commissioned in order to investigate whether AMD sounds are audible to harbour porpoises and harbour seals, and how they respond to the sounds behaviourally.

To determine the audibility and audibility zones of AMD sounds for harbour porpoises and harbour seals it is important to know the hearing threshold levels for the sounds. So far, the underwater hearing of harbour porpoises has been tested behaviourally for pure tones (Andersen, 1970) and narrowband FM signals (Kastelein *et al*., 2002; 2010), and with the Auditory Evoked Potential technique (Popov *et al*., 1986; Bibikov, 1992; Lucke *et al*., 2007). The underwater hearing of harbour seals has been quantified by measuring their responses to pure tones (Møhl, 1968; Terhune, 1988; Turnbull and Terhune, 1993; Kastak and Schusterman, 1998; Southall *et al*., 2005; Kastelein *et al*., 2010), narrow-band frequency-modulated (FM) signals (Kastelein *et al*., 2009a), and 1/3-octave noise bands (Kastelein *et al*., 2009b). Despite their differences, these test sounds are all relatively simple and have relatively narrow frequency bands. It is unknown what the hearing thresholds of harbour porpoises and harbour seals are for more complex and wider band signals, such as those produced by AMDs.

Therefore, the first goal of the two audibility studies was to determine the hearing thresholds of harbour porpoises and harbour seals for the sounds produced by three commercially available AMDs (one of which produces three different sounds). The second and third goals, which were extra to the original proposal, were to compare the hearing thresholds for the AMD sounds with the tonal thresholds (to determine whether hearing thresholds of broadband signals can be predicted from hearing thresholds for tonal sounds), and to estimate the distances at which the sounds made by the three AMDs can be heard by porpoises and seals at sea.

Assuming that the AMD sounds are audible, their effect on porpoise and seal behaviour needs to be known. Harbour porpoises are known to be relatively easily deterred by certain anthropogenic underwater noises, such as those produced by ships (Amundin and Amundin, 1973; Polacheck and Thorpe, 1990), acoustic alarms to prevent unwanted bycatch in gillnet fisheries (Laake *et al.,* 1998; Culik *et al*., 2001; Johnston, 2002; Olesiuk *et al*., 2002; Teilmann *et al*., 2006; Kastelein *et al*., 2008), offshore wind turbines (Koschinski *et al*., 2003), underwater data communication systems (Kastelein *et al*., 2005a), and naval sonar sweeps (Kastelein *et al.*, 2010, in prep). Avoidance threshold levels of harbour porpoises have been determined for: noise bands around 12 kHz, a continuous 50 kHz tone, and continuous and pulsed 70 and 120 kHz tones (Kastelein *et al*., 2005a, 2008a, 2008b). These studies show that both the spectrum and the received level of underwater sound play an important role in the effect the sound has on the behaviour of porpoises.

Harbour seals have acute hearing between 200 Hz and 40 kHz (Møhl, 1968; Kastelein *et al*., 2009 a, b; 2010), and are known to be deterred by certain anthropogenic underwater noises, such as those produced during seismic surveys (Harris *et al.,* 2001), by acoustic alarms to prevent unwanted predation on aquaculture farms (Taylor *et al*., 1997; Yurk and Trites, 2000), and by underwater data communication systems (Kastelein *et al*., 2006a). Avoidance threshold levels of harbour seals have been determined for noise bands around 12 kHz (Kastelein *et al*., 2006a) and for tonal signals between 8 and 45 kHz (Kastelein *et al.*, 2006b). The received level, spectrum, and familiarity (Jacobs and Terhune, 2002) of underwater sounds play an important role in determining the effect the sounds have on the behaviour of harbour seals.

The first goal of the two behavioural response studies was to determine whether the sounds made by three AMDs cause a behavioural response in harbour porpoises and harbour seals, and if so, to determine their effect on behaviour at three received levels. The second goal, which was extra to the original proposal, was to use the response data in models, to calculate examples of the distances at sea at which the AMDs may influence the behaviour of harbour porpoises and harbour seals.

The goals of the research can be summarised as follows:

- Determine the audibility (hearing thresholds) of AMD sounds for harbour porpoises and harbour seals
- Determine the behavioural response (avoidance thresholds) of harbour porpoises and harbour seals to AMD sounds
- Compare the hearing thresholds for the AMD sounds with the tonal thresholds (can hearing thresholds of broadband signals be predicted from hearing thresholds for tonal sounds?)
- Estimate the distance at which AMD sounds can be heard by harbour porpoises and harbour seals at sea
- Estimate the distance at which AMDs cause a behavioural response in harbour porpoises and harbour seals at sea.

The overall purpose of the research was to contribute towards the optimal use of AMDs to deter marine mammals from pile driving sites. The results will inform the selection of the optimum signal parameters of AMDs for deterring harbour porpoises and harbour seals sufficiently far from pile driving areas at sea before pile driving begins, so that wild porpoises' and seals' hearing will not be compromised.

3. Methodology

3.1 AMD sounds

Subacoustech Ltd. was commissioned by COWRIE to measure the sound output of commercially available AMDs and supply recorded AMD sounds to SEAMARCO (COWRIE update of progress with Acoustic Mitigation Device manufacturers by A. G. Brooker, S. A. H. Bryant, Subacoustech Report No.E238R010115, January 2010). The selection of the AMD systems was not under SEAMARCO's control. The following AMDs were selected: the AquaMark 100, designed to keep dolphins away from gill nets, the Ace Aquatec Seal Scrammer, and the Lofitech Seal Scarer, both designed to keep seals away from aquaculture sites. Subacoustech made the recordings of the AMD sounds in a lake (recording depth: 5 m, recording distance: 1 m, sample rate: 350800 samples per second). The acoustic parameters of the sounds are shown in Table 1. The broadband sound pressure levels (SPL) in **Table 1** were calculated by Subacoustech over an interval of 0.1 s.

The AquaMark 100 produces structured chirps, with their main energy in the 40 to 50 kHz band. The Ace Aquatec produces a series of short complex pulses with varying frequency content, the main energy of which is concentrated in the 10 to 20 kHz range. The Lofitech produces a tonal pulse at 15 kHz, with weak harmonics.

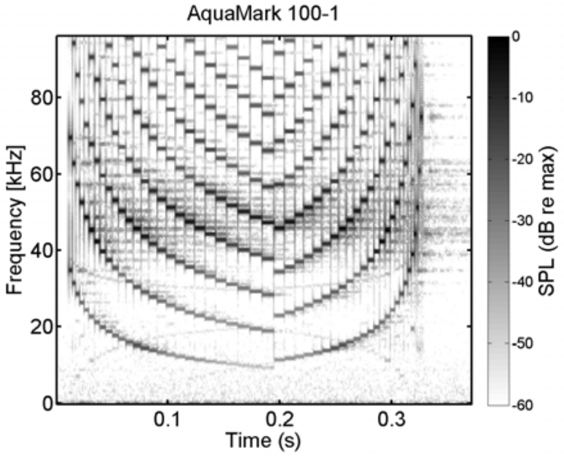
Table 1. The acoustic characteristics of sounds produced by the three AMDs, from original recordings by Subacoustech.

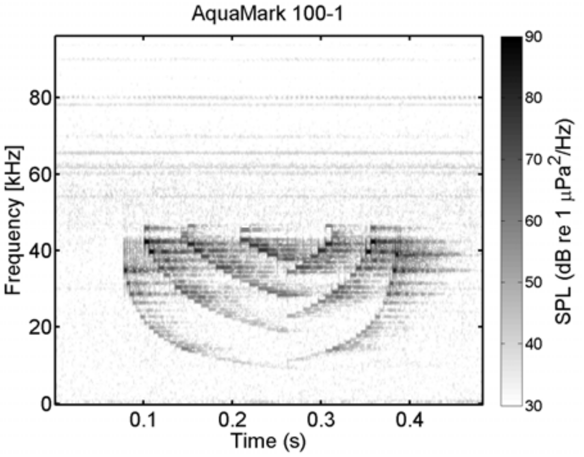
AMD	**SPL (RMS)**	**Peak-to-peak level**	**Original sound duration**	**Inter-pulse interval Type**	**Inter-pulse interval duration**
	dB re 1 µPa @ 1m	dB re 1 µPa @ 1m	(s)		(s)
AquaMark 100*	125	142	0.36	Random	5-30
Ace Aquatec	186	202	5.0#	Regular (adjustable) or triggered by animal	50-600
Lofitech	193	202	0.5	Random	1-30

* Produces three sounds in random order.
# A representative section of 0.5 s of the regular 5 s signal was used in the hearing tests.

The AMDs operate at fixed acoustic source levels. To investigate the audibility of the sounds and the behavioural response of harbour porpoises and harbour seals to them, it was necessary to control the projected levels so that the signal levels experienced by the animals in the pool were representative of the levels received by at various distances from the AMDs at sea. Therefore recordings of AMDs were used, so that the level could be adjusted for the four studies.

The original recordings provided by Subacoustec were re-sampled to 88.2 kHz, so that only the frequencies of the AMDs up to 44 kHz were reproduced, to conform to limitations of the sound card (Presonus Inspire 1394, maximum sample rate 96 kHz). Although the sound card only produced sounds up to 44 kHz, some energy was, fortunately, transmitted above this frequency, probably due to the resonance frequency of the transducers. The spectra of the original recordings were scaled so that the broadband levels of the unweighted original recordings and reproduced sounds were equal. The reproduced sounds used in the research were similar to the corresponding original recordings, especially within the dominant frequency bands (**Figs. 1 and 2**).


AquaMark 100-1
Frequency [kHz]
0
20
40
60
80
Time (s)
0.1
0.2
0.3
SPL (dB re max)
0
-10
-20
-30
-40
-50
-60


AquaMark 100-1
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40
60
80
Time (s)
0.1
0.2
0.3
0.4
SPL (dB re 1 μPa²/Hz)
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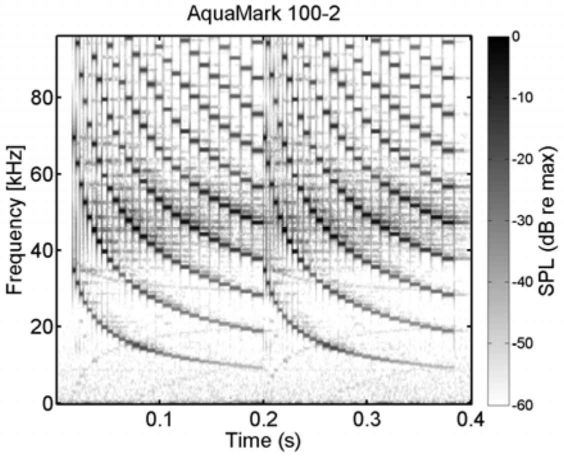
80
70
60
50
40
30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60
80
Time (s)
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0.3
0.4
SPL (dB re max)
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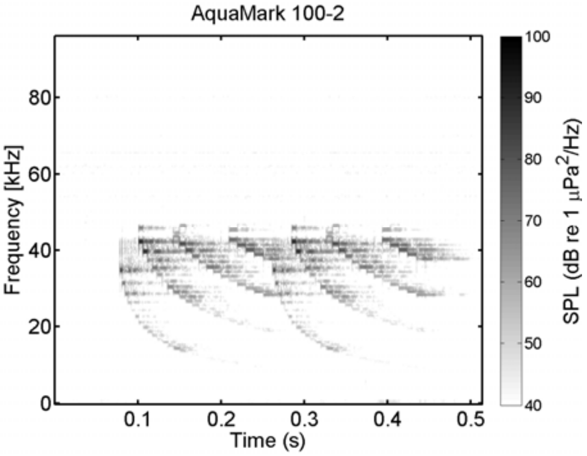
-20
-30
-40
-50
-60


AquaMark 100-2
Frequency [kHz]
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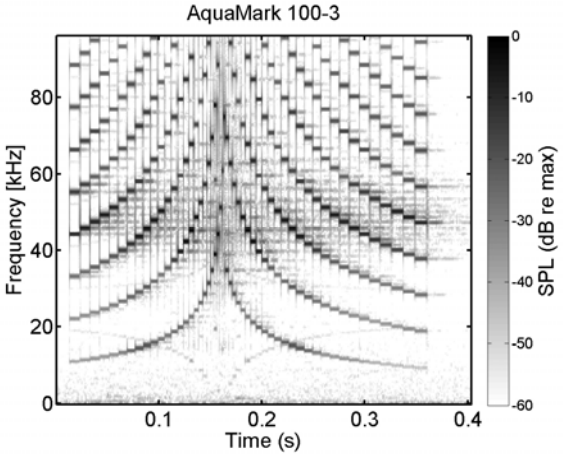
20
40
60
80
Time (s)
0.1
0.2
0.3
0.4
0.5
SPL (dB re 1 μPa²/Hz)
100
90
80
70
60
50
40


AquaMark 100-3
Frequency [kHz]
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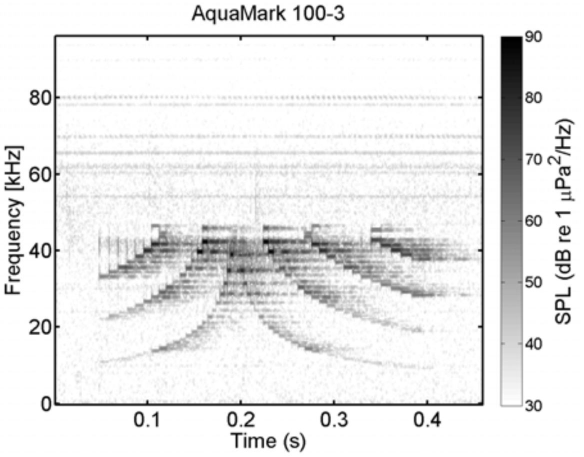
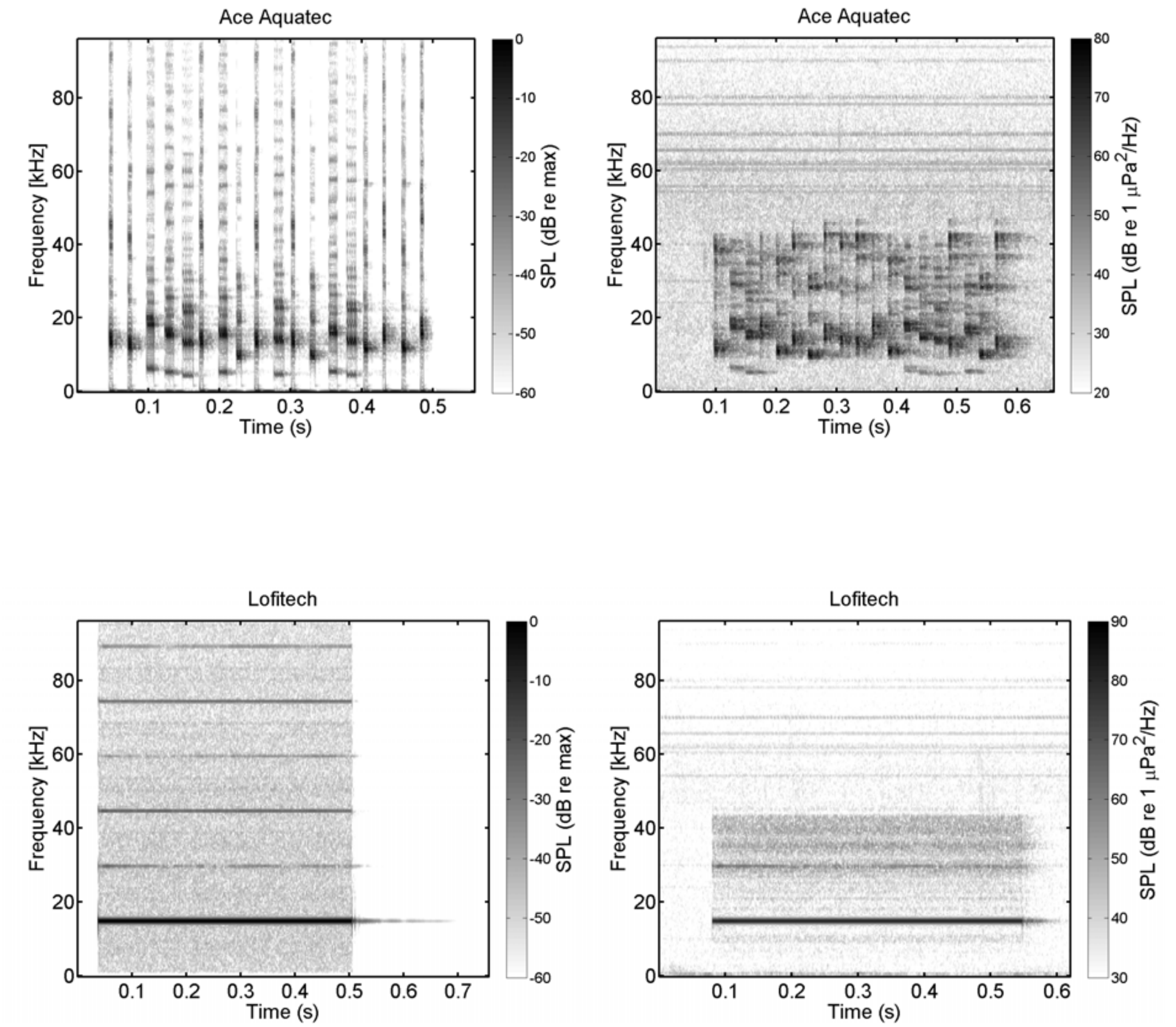
20
40
60
80
Time (s)
0.1
0.2
0.3
0.4
SPL (dB re max)
0
-10
-20
-30
-40
-50
-60


AquaMark 100-3
Frequency [kHz]
0
20
40
60
80
Time (s)
0.1
0.2
0.3
0.4
SPL (dB re 1 μPa²/Hz)
90
80
70
60
50
40
30



Ace Aquatec

Ace Aquatec

Lofitech

Lofitech

FIG. 1. Spectrograms of the five AMD sounds: original recordings (on the left), and reproduced sounds (re-sampled sound files to 88.2 kHz, so that only the frequencies up to 44 kHz were emitted; on the right), (Δ*f* = 375 Hz).

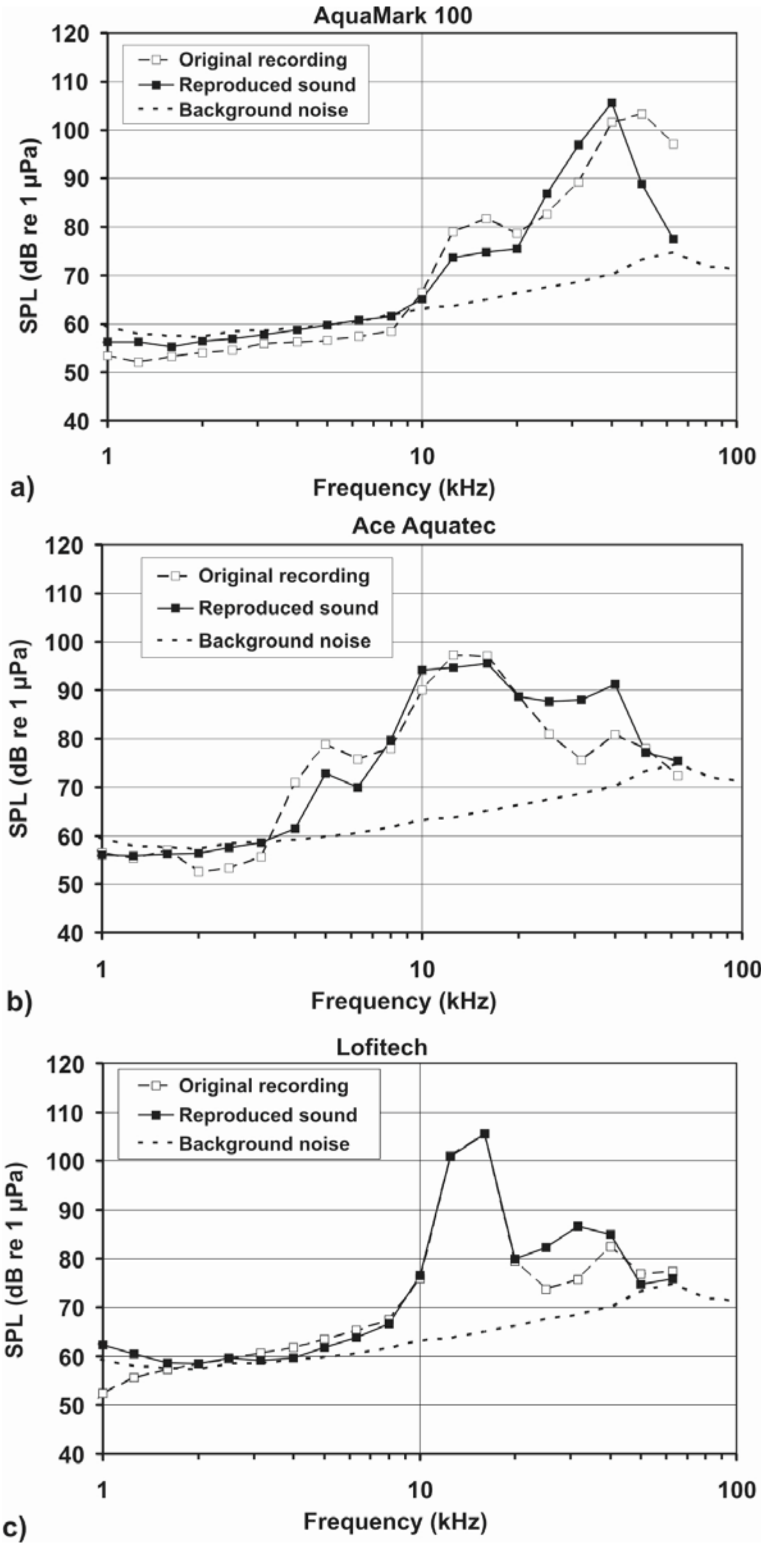


FIG. 2. The 1/3-octave band spectra (SPL averaged over the 90% energy duration of the signals) of the original recordings and reproduced sounds recorded in the pools. The spectra for the three different AquaMark 100 sounds are identical, so just one is shown. The spectra of the original recordings are scaled so that the broadband levels of the unweighted original recordings and reproduced sounds are equal. The reproduced sounds are similar to the corresponding original recordings, especially within the dominant frequency bands. For the AquaMark 100 sounds, the reproduced levels decline at frequencies above 44 kHz. The background noise is in 1/3-octave bands, as are the AMD sounds.

3.2 Audibility of AMD sounds to a harbour porpoise

3.2.1. Study animal

The male harbour porpoise used in this study (02) was stranded on the Dutch coast at the age of about 21 months and was then rehabilitated. He participated in a previous psychoacoustic study on the detection of tonal signals in noise (Kastelein *et al.*, 2009) and in a study on the effects of signal duration on detection threshold (Kastelein *et al.*, 2010). During the present study he was 4 years old, his body weight was around 38 kg, his body length was 142 cm, and his girth at axilla was approximately 75 cm.

The animal received between 2 and 3 kg of thawed fish (sprat, *Sprattus sprattus*, herring, *Clupea harengus*, mackerel, *Scomber scombrus*, capelin, *Mallotus villosus*, and blue whiting, *Micromesistius poutassou*) per day, equally divided over four meals.

3.2.2. Study area

The study was conducted at the SEAMARCO Research Institute, The Netherlands. Its location is remote and quiet, and was specifically selected for acoustic research. The animal was kept in a pool complex designed and built for acoustic research, consisting of an outdoor pool (12 x 8 m; 2 m deep) connected via a channel (4 x 3 m; 1.4 m deep) to an indoor pool (8 x 7 m; 2 m deep; **Fig. 3**), in which the study was conducted. The pool walls were made of plywood covered with polyester. To reduce sound reflections in the pool (mainly for signals above 25 kHz), the walls were covered with 3 cm thick coconut mats with fibres embedded in 4 mm thick rubber, and the bottom was covered with a 20 cm thick layer of sloping sand on which aquatic vegetation grew. The coconut mats extended to 10 cm above the water level, to reduce splashing sounds produced by waves.

Skimmers kept the water level constant, so that sound conditions were stable. Seawater was pumped directly from the nearby Oosterschelde, a lagoon of the North Sea, into the open system; 80% recirculation through biological and sand filters ensured year-round water clarity.

The water circulation system and the aeration system for the bio-filter were made as quiet as possible. This was done by choosing ‘whisper’ pumps, mounting the pumps on rubber mats, and connecting the pumps to the circulation pipes with very flexible hoses. The average monthly temperature varied during the year between 2 and 20 °C, and the salinity was around 3.4%. There was no current in the pool during the experiments, as all pumps were switched off 10 min before and during sessions. By the time a session started, no water flowed over the skimmers, so there was no flow noise during testing.

The equipment used to produce sound stimuli was housed out of sight of the study animal, in a research cabin 4 m away from the underwater listening station. The listening station was at the end of a 3 cm diameter water-filled polyvinylchloride tube (**Fig. 4**). This positioned the porpoise’s external auditory meatus 2 m from the sound source, 1 m below the water surface (**Fig. 3**).

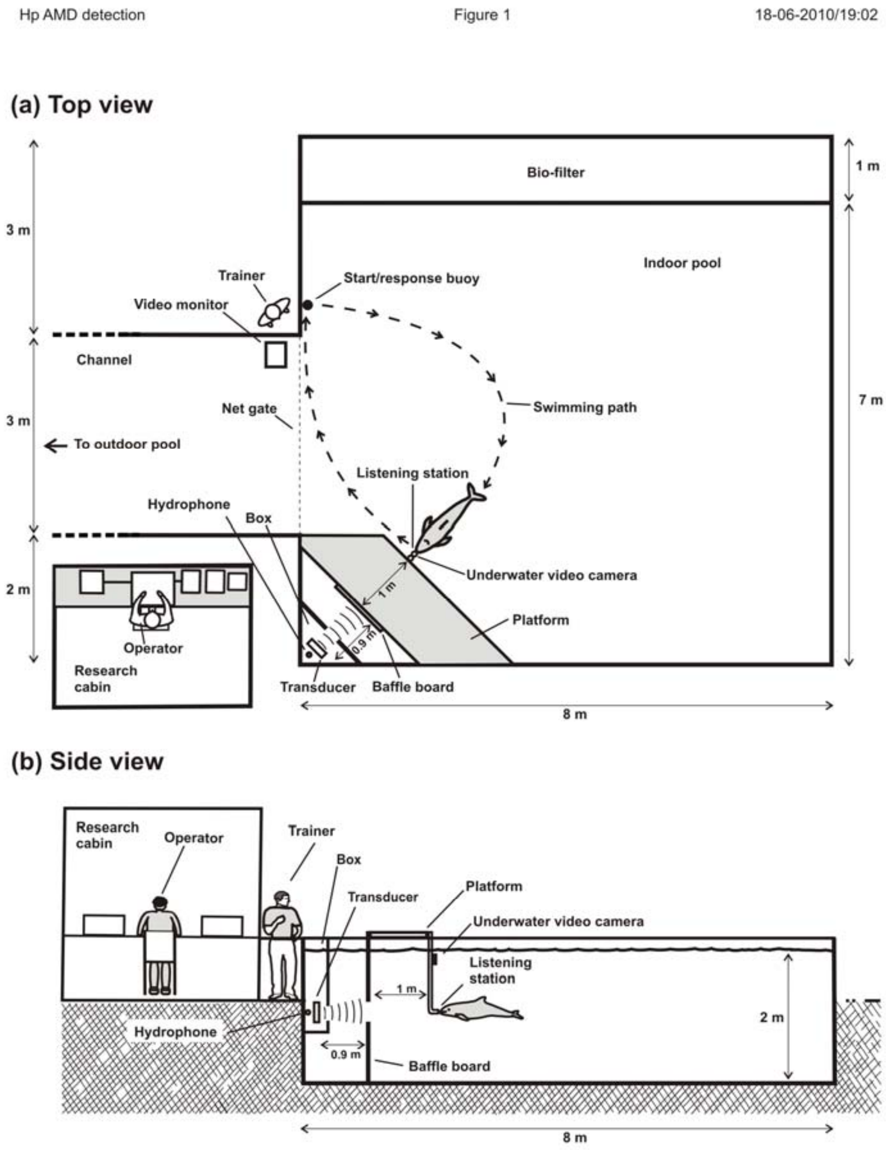


FIG. 3. The study facility, showing the harbour porpoise in position for testing at the listening station, a) top view and b) side view, both to scale. Also shown is the porpoise’s swimming path during trials.

FIG. 4. The harbour porpoise waiting at the listening station for an AMD sound. When he heard a sound, the animal would swim away from the station towards the trainer and the start/response buoy.

3.2.3. Equipment

Because of limitations of the available sound card (Presonus Inspire 1394, maximum sample rate 96 kHz), the original recordings provided by Subacoustech were re-sampled to 88.2 kHz, so that only the frequencies of the AMDs up to 44 kHz were reproduced. These reproduced sounds were used in the tests. Although porpoise hearing is sensitive up to around 140 kHz (Kastelein *et al*., 2010), and porpoises can hear the part of the AMD spectra above 44 kHz, the reduced spectrum of reproduced sounds used in the hearing tests probably had little effect on the hearing thresholds found in the present study, as most of the energy in the AMD sounds was below 44 kHz (**Fig. 2**).

The porpoise's hearing sensitivity was tested for five AMD sounds (three produced by the AquaMark 100, and one produced by each of the Ace Aquatec and Lofitech). Because the signal produced by the Ace Aquatec was much longer than that produced by the other two AMDs, a representative 0.5 s sample from the regular 5 s Ace Aquatec sound file was used.

A schematic of the equipment used to configure and emit outgoing signals is shown in **Fig. 5.** The digitised reproduced test sounds (WAV files; sample frequency 88.2 kHz) were played back on a laptop computer (Acer Aspire 5020) using Adobe Audition software (version 3.0). The output of the laptop passed through a FireWire interface (LogiLink, model 1394A), an external sound card (Presonus, model Inspire 1394), and a ground loop isolator, to a modified audiometer for testing human aerial hearing (Madsen Electronics, Midimate, model 622 with extended frequency range), which controlled the amplitude of sounds. The free field sound pressure level (SPL, dB re 1 μPa, RMS) at the porpoise's head while it was at the listening station could be varied in 2 dB increments. The reproduced sounds were projected underwater via two transducers in parallel: the spectrum up to 16 kHz via a balanced tonpilz piezoelectric acoustic transducer (Lubell, model LL 916) and the spectrum above 16 kHz via a piezoelectric HF transducer (Labforce). The combined output of the two transducers (resulting in the reproduced sounds) recreated the original recordings closely (**Fig. 2**).

Multi-path arrivals can introduce both temporal and spatial variations in the observed SPL at the listening station. To minimise this, the transducers were placed in a corner of the pool in a protective wooden box, which was lined with rubber with an irregular surface. The Lubell LL 916 transducer was suspended from four cords, and was placed just in front of the hole in the box. The Labforce transducer was hung from its own wire next to the Lubell transducer. The transducers were 1.9 m from the tip of the listening station (**Fig. 3**), and were positioned so that the acoustic axis of the projected sound beam pointed at the centre of the listening station (i.e., at the centre of the study animal's head while it was at the listening station). To reduce the reflections from the bottom of the pool and from the water surface reaching the listening station, a baffle board was placed between the transducers and the animal. The board consisted of 2.4 m high, 1.2 m wide, 4 cm thick plywood, covered with a 2 cm thick closed cell rubber mat on the side facing the transducer. A 30 cm diameter hole was made in the board with its centre at the same level as the porpoise's head and the transducers (1 m below the water surface).

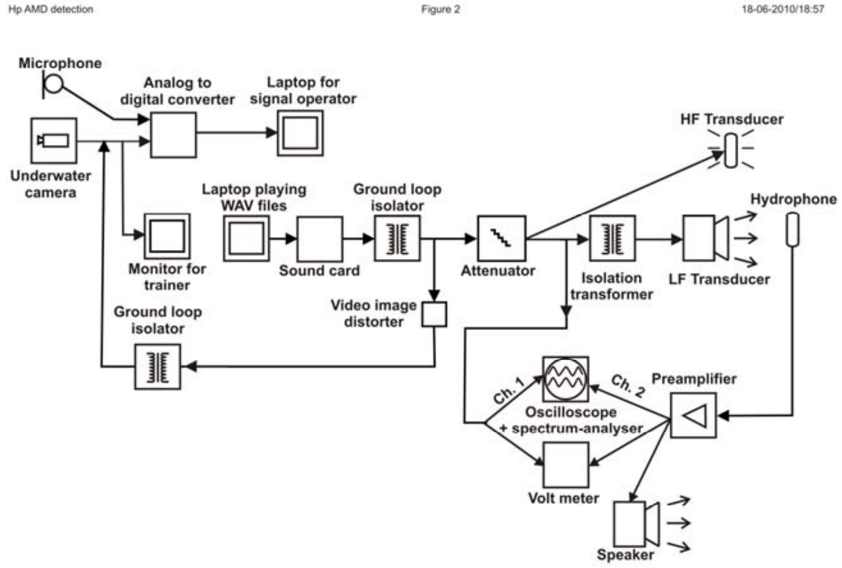


FIG. 5. Block diagram of the signal generation and listening systems.

3.2.4. Background noise and stimuli level calibration measurements

Great care was taken to make the porpoise's listening environment as quiet as possible. Nobody was allowed to move within 15 m of the pool during sessions. Underwater background noise levels were measured under the same weather conditions as during the test sessions (no rain, and wind speed Beaufort 4 or below).

The background noise and received levels of the reproduced sounds were measured by using recording and analysis equipment consisting of a B&K 8101 hydrophone with a TPD (custom-built) power supply, a B&K PULSE 3560 D multichannel high frequency analyser, and a laptop computer with B&K PULSE software (Labshop version 12.1). The system was calibrated with a B&K 4223 pistonphone. The sound pressure level of each pulse (frequency and duration) was derived from the received 90% energy flux density, divided by the corresponding 90% time duration (Madsen, 2005).

1/3-Octave band sound pressure level (SPL) spectra of the background noise were determined in the 25 Hz to 160 kHz bands (dB re 1 µPa). The background noise in the pool was very low (**Fig. 2)**; above 3 kHz it was close to the self-noise of the recording equipment.

The received SPL (dB *re* 1 µPa, rms) of each reproduced AMD sound was measured at the position of the porpoise's head during the hearing tests (**Figs. 3 and 4**). The calibration was conducted with two hydrophones, one at the location of each auditory meatus of the porpoise when it was positioned at the listening station. The SPL between the two locations varied by 0-2 dB, depending on the AMD sound. The average SPL of the two hydrophones was used to calculate the hearing thresholds. During trials, the porpoise's head position (at the listening station) was carefully monitored, and was consistent to within 2 cm for each external auditory meatus (a maximum of 2 degrees off the beam axis of the transducer). The received SPLs were calibrated at levels of around 20 dB above the threshold levels found in the present study. The linearity of the transmitter system was checked several times during the study; it was consistent to within 1 dB.

3.2.5. Experimental procedures

Before each session, the voltage output of the emitting system to the transducer, and the voltage output of the sound receiving system, were checked with an oscilloscope (Dynatek 8300), a voltmeter (Hewlett Packard 3478A), and a spectrum analyser (Velleman, PCSU1000), by producing a 1 kHz continuous signal on the laptop. If the values obtained were the same as during the SPL calibration session, a test session could begin (**Fig. 5**). Also, the background noise level was checked to make sure it was not too high for testing. Nearly every time they were checked, these conditions were met.

A trial began with the animal at the start/response buoy (**Fig. 3**). The signal operator had loaded the WAV file of a particular reproduced AMD sound and the received SPL for the first trial of the session (**Fig. 6**). The amplitude in the first trial of the session was approximately 6 dB above the hearing threshold determined during pre-tests. When the trainer gave a hand signal, the porpoise swam to the listening station (**Figs. 3, 4 and 7**).

The animal was filmed from above by means of an underwater video camera (Mariscope, model Micro) attached to the listening station (**Fig. 3**), so that his position at the listening station could be checked. The images were visible to the operator in the research cabin. The porpoise was trained to place the tip of its rostrum at the station and its body axis in line with the beam of the transducer. A maximum deviation of 3° from the beam axis was accepted in all directions. Trials were aborted when the animal was not in the correct position. The trainer indicated an incorrect position by knocking on the response buoy. When the porpoise returned to the start buoy, the trainer sent him straight back to the listening station.

Pre-tests showed that the animal did not need warm-up trials before the actual session began. After the trainer had sent the porpoise to the listening station, he or she moved out of the porpoise's view and watched the porpoise's position at the listening station on a monitor.

In signal-present trials, the porpoise stationed, then had to wait for a period of random duration between 6 and 12 s (established via a random number generator), before the signal operator produced the test signal. If the animal detected the sound, it was trained to leave the station ("go" response) at any time during the transmission of the signal and return to the start/response buoy (**Fig. 3a**). When the test signal was produced, a generator was activated that produced horizontal white lines on the video image. This helped the operator to determine visually whether or not the porpoise responded while the signal was being produced. If the animal responded, the signal operator told the trainer that the response was correct, after which the trainer gave the porpoise a fish reward. If the animal did not respond to the sound ("no-go" response), the signal operator signalled this to the trainer. The trainer then signalled to the animal (by tapping three times on the side of the pool) that the trial had ended, thus calling him back to the start/response buoy. No reward was given. If the animal responded before a signal was produced (a prestimulus response or false alarm), the signal operator indicated that the trainer should ignore the animal for about 10 seconds. After this short time, a new trial was started by calling the animal to the start buoy and then sending it to the listening station. When a prestimulus response was clearly triggered by an external sound which was also detected by the operator, data from the trial were not used. In such cases the trial was repeated as soon as the sound had stopped.

In signal-absent (control or catch) trials, the porpoise stationed, then the signal operator told the trainer after a time period of random duration (but between 6 and 12 s) to end the trial by blowing on a whistle. The animal returned to the start/response buoy and received a fish reward. If the porpoise left the station before the whistle was blown during signal-absent trials, this was regarded as a prestimulus response, and no reward was given. The same amount of fish was given as a reward for correct responses in signal-present and in signal-absent trials. After a trial with a correct response, the next trial would start immediately after the reward had been eaten by the porpoise.

In each session, one AMD sound was tested, but the signal amplitude was varied according to the 1-up 1-down adaptive staircase method. This conventional psychometric technique (Robinson and Watson, 1973) results in a 50% correct detection threshold (Levitt, 1971). If the animal heard a signal and responded to it (a hit), the signal presented in the next trial was 2 dB lower than that signal. If the animal did not hear a signal and remained at the station (a miss), the signal presented in the next trial was 2 dB higher. Prestimulus responses did not result in a change in signal amplitude. Each session consisted of ~25 trials and lasted for about 15 min. Sessions consisted of 2/3 signal-present and 1/3 signal-absent trials offered in random order. There were never more than three consecutive signal-present or signal-absent trials. In order to end with a positive event, the last trial was always one in which the animal responded correctly and received a reward. This methodology kept the study animal motivated throughout each session (he received a reward after approximately 75% of the trials). Each session, one of four data collection sheets was used; each sheet had a different random number series which was used to determine the random time between the animal stationing and signal presentation, and the random order of signal-present and signal-absent trials. The trainer never knew beforehand whether a trial was a signal-present trial or a signal-absent trial. When the porpoise left the station, the operator observed the animal's behaviour on a monitor in the cabin (**Fig. 3a**), told the trainer whether or not to reward the porpoise, and recorded the animal's responses. Other behaviours were solicited from the porpoise between trials to occupy him during occasional short periods of visible or audible disturbances outside the building.

Thresholds were determined for each of the five reproduced AMD sounds from the three AMDs. Each sound was tested until at least 68 reversal pairs had been obtained (in at least six sessions). Each session, one AMD sound was randomly selected from the five available sounds.

Three or four experimental sessions per day were conducted (at 0830, 1130, 1330 and 1600 h). Data were collected in April and May 2010.

FIG. 6. The signal operator and equipment used for the harbour porpoise AMD audibility study. Note the porpoise in the correct position at the listening station on the screen of a laptop (top), and the spectrum of one of the 3 AquaMark 100 sounds on the screen of a second laptop (bottom right).

FIG. 7. The porpoise near the start/response buoy, being sent towards the listening station by the trainer.

3.2.6. Determination of hearing thresholds

A switch from a test signal SPL that the porpoise responded to (a hit), to an SPL that it did not respond to (a miss), and *vice versa*, is called a reversal. The mean 50% hearing threshold for an AMD sound was determined by calculating the mean SPL of all reversal pairs in the 6 sessions for that AMD sound (range: 68 - 86 reversal pairs per AMD sound; the minimum number of reversal pairs was set at 68, but in some cases the last session needed to reach this number contained many reversal pairs).

3.2.7. Comparison of hearing thresholds with thresholds for tonal sounds

In order to investigate whether hearing threshold levels of broadband signals can be predicted based on their spectrum and level, the observed 50% hearing thresholds for the reproduced AMD sounds were compared with estimated thresholds based on the porpoise's audiogram for tonal noise. For this comparison, the tonal audiogram for signals of 1500 ms duration (Kastelein et al 2010), interpolated to 1/3-octave band centre frequencies, was subtracted from the recorded 1/3-octave band spectrum of the reproduced AMD sounds at the calibration level. The corresponding broadband 'sensation level', i.e., the frequency-integrated difference between the spectra of reproduced sounds and audiogram, was subtracted from the broadband calibration SPL of the reproduced sound to obtain an estimation of the hearing threshold, i.e., the broadband SPL at which the sensation level is equal to 0 dB. This estimation is based on the assumption that the hearing behaves as an energy detector and that waveform of sounds does not influence their audibility.

3.3 Behavioural response of a harbour porpoise to AMD sounds

3.3.1. Study animal

The study animal was the same male harbour porpoise (02) used in the audibility study (see section 3.2.1).

3.3.2. Study area

The animal was kept alone in a pool complex specifically designed and built for acoustic research, which consisted of an outdoor pool (12 x 8 m, 2 m deep) connected via a channel (4 x 3 m, 1.4 m deep) with an indoor pool (8 x 7 m, 2 m deep; **Fig. 8**). The study was conducted in the outdoor pool (**Fig. 9**). The pool walls were made of plywood covered with polyester. To reduce reflections of sound in the pool, the walls were covered with 3 cm thick coconut mats with their fibres embedded in 4 mm thick rubber (reducing reflections mainly above 25 kHz), and the bottom was covered with a 20 cm thick layer of sloping sand. The coconut mats reached up to 10 cm above the water level to reduce the splashing noise of waves.

The water level was kept constant with skimmers. The seawater was pumped directly from the nearby Oosterschelde, a lagoon of the North Sea, into the open system; 80% recirculation through sand filters ensured year-round water clarity.

The water circulation system and aeration system for the bio-filter were made as quiet as possible. This was done by choosing ‘whisper’ pumps, mounting the pumps on rubber mats, and connecting the pumps to the circulation pipes with very flexible hoses. The water temperature during the study period varied between 10 and 22 °C; the salinity was around 34‰. There was no current in the pool during the experiments, as the water circulation pump and air pump of the adjacent bio-filter were shut off 30 minutes before and during sessions. By the time a session started, no water flowed over the skimmers, so that there was little or no flow noise.

The equipment used to produce the sound stimuli was housed out of sight of the study animal, in a research cabin next to the pool (**Fig. 8**).

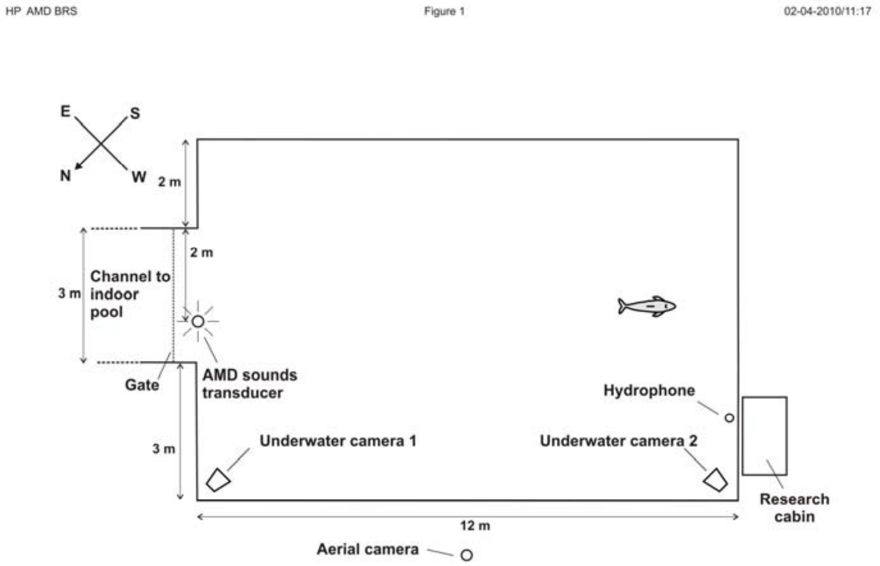


FIG. 8. Top scale view of the study facility, showing the study animal, the location of the aerial camera, the two underwater cameras, the underwater transducers emitting the AMD sounds, and the listening hydrophone. Also shown is the research cabin which housed the equipment and the operator.

FIG. 9. View of the outdoor pool in which the harbour porpoise AMD behavioural response study was conducted. Note the aerial camera, and the size of the porpoise’s head relative to the pool.

3.3.3. Video and audio equipment

A schematic diagram of the equipment used to configure and emit outgoing signals and record video and underwater sounds is shown in **Fig. 10** and the real-set up in **Fig. 11.** The digitised test sounds (WAV files; sample frequency 88.2 kHz) were played back by a laptop computer (Hewlett Packard, model Pavilion dv6) with Adobe Audition (version 3.0), via a Fire Wire interface to an external sound card (Presonus, model Inspire 1394) and an audio power amplifier (Velleman HQ VPA2200MBN), the output of which was controlled with two custom-built digitally controlled attenuators in series (SEAMARCO AS 2009-01 and SEAMARCO AS 2009-02; 1 dB steps). The sounds were projected underwater via two transducers in parallel: the spectrum up to 16 kHz by means of a balanced tonpilz piezoelectric acoustic transducer (Lubell, model LL 916) and the spectrum above 16 kHz by means of a piezoelectric HF transducer (Labforce). The combined output of the two transducers recreated the original spectrum of the AMDs closely. The transducers were suspended at 1 m below the water surface, at the northern end of the pool near the entrance of the channel to the indoor pool (**Fig. 8**). The output of the sound system to the transducers was monitored by means of an oscilloscope (Tektronix 2201), a voltmeter (Agilent 34401A), and a spectrum analyser (Velleman, PCSU1000).

The animal's behaviour was filmed from above by a waterproof camera (Conrad 750940) with a wide-angle lens and a Polaroid filter to prevent saturation of the video image by glare from the water surface. The camera was placed on a pole 9 m above the water surface on the north-western side of the pool (**Fig. 1**). The entire surface of the pool was captured on the video image. The output of the camera was fed through a video multiplexer (MX-8) which added the time and date to the images. Thereafter, the output was digitised by an analogue-to-digital converter (EZ Grabber, Vista version) and stored on a laptop computer (Medion, model MD96780). The animal was also filmed by two underwater video cameras (Ocean Systems Inc., model Delta Vision B/W) placed in two corners of the pool (**Fig. 10**). The images from the underwater cameras were visible to the operator on two monitors in the research cabin.

The audio part of the background noise and the test sounds were monitored via a hydrophone (Labforce model 90.02.01) and a conditioned charge pre-amplifier (SEAMARCO, model CCAMS1000-1). The output of the pre-amplifier was digitised via the analogue-to-digital converter and recorded on the computer in synchrony with the video images. The output was also fed to an amplified loudspeaker, so that the operator in the research cabin could monitor the background noise and the test sounds during sessions.

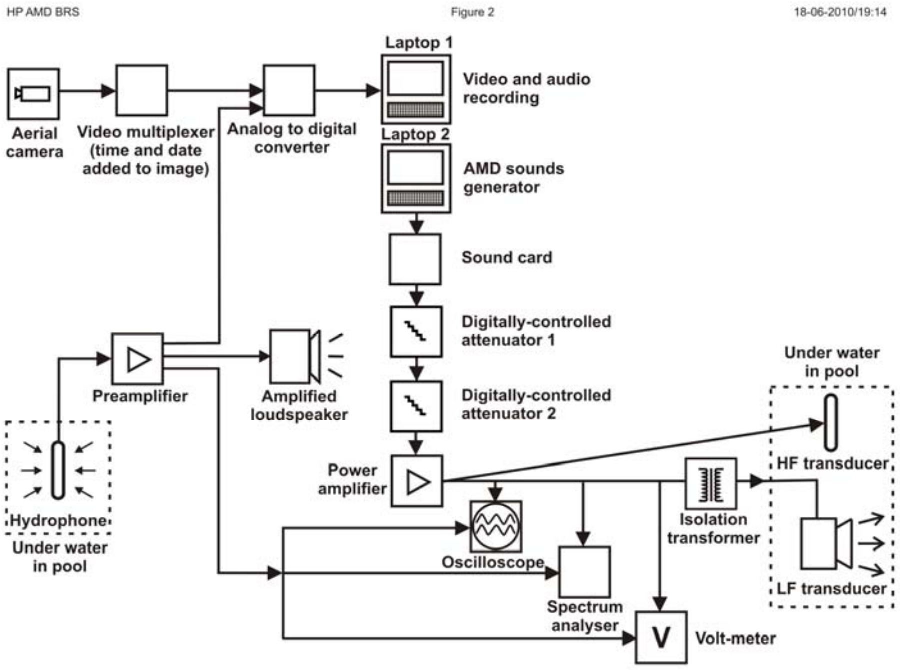


FIG. 10. Block diagram of the signal generation and control systems, and the listening and recording equipment used in the porpoise behavioural response study.

FIG. 11. The equipment used for the AMD behavioural response study.

3.3.4. Acoustics

3.3.4.1. Background noise

Methods for measuring underwater background are described in section 3.2.4.

3.3.4.2. Test stimuli (AMD sounds)

Test stimuli (AMD sounds) are described in section 3.1.

3.3.4.3. Sequence of AMD sounds

In normal operation, the duty cycles (the percentage of time sound is produced) of the three AMDs differ, and duty cycle is expected to influence the porpoise's response to sounds. Therefore, the sounds were manipulated so they could be transmitted in 30-minute sequences, all of which had a duty cycle of between 9 and 10 %. Random inter pulse intervals were generated using GraphPad Software (Quick Calcs Online Calculators for Scientists). For the AquaMark 100, with three different signals of 0.36 s, this meant 522 signal presentations per 30 min. and random inter pulse intervals between 1 and 6 s. For the Ace Aquatec, a 0.5 s representative sample was taken from the regular 5 s pulse (so that the pulse duration and inter pulse intervals resembled those of the other two AMDs). This resulted in 336 signal presentations per 30 min. and random inter pulse intervals between 1 and 5 s. For the Lofitech, with 0.5 s signals, this meant 372 signal presentations per 30 min. with random inter pulse intervals between 1 and 5 s.

3.3.4.4. Determination of the source level used in the tests

During pre-tests, the SL of each AMD signal was gradually increased until three levels were reached: 1) the maximum received level that caused just no behavioural changes, 2) the level which caused a small change in surfacing rate and distance to the transducer, and 3) the level which clearly deterred the porpoise from the sound source.

3.3.4.5. SPL distribution measurements

To determine the sound distribution in the pool, the SPL for each of the test sounds was measured at 77 locations (on a horizontal grid of 1 m x 1 m; **Fig. 12**). The SPL was measured at three depths per location on the grid (0.5, 1.0 and 1.5 m below the water surface). Thus, 231 measurements were made for each AMD signal. The reported SPLs were based on a recording of one signal per location. The Sound Pressure Level (dB re 1 μPa) of the signals was averaged over the duration of the signal. The analysis was done in the time domain. The cumulative Sound Exposure Level (SEL in dB re 1 $\mu Pa^2 s$) was determined from the sound pressure, by integration of the broadband pressure squared. The duration of the signals (T_{90} in s) was defined as the time between the moments when the cumulative sound exposure reached 5% and 95% of the total exposure, i.e., when it contained 90% of the total energy in the signal. The SPL was determined by subtracting 10 times the logarithm of the T_{90} duration from the cumulative SEL. The measured distribution of the received SPL at the 231 positions in the pool is shown in **Fig. 13,** and the spatially averaged exposure SPLs are shown in **Table 2**. The received levels show a decrease with increasing distance up to about 6 m; beyond this distance the field is relatively homogeneous. The received levels of the Lofitech show a much larger spread than those of the other two AMDs, because the Lofitech produces a tonal signal which is very sensitive to interference effects in the pool and to Doppler effects caused by motion of the water surface. This is illustrated in **Fig. 14,** which shows that the Lofitech signals exhibit an amplitude modulation in time, with a period in the order of 0.5 s. Such a modulation could be caused by a small Doppler shift in the frequency of the surface-reflected signal, which interferes with the direct signal.

FIG. 12. Measurement of the SPL distribution in the porpoise pool used for the AMD behavioural response study. Note the 1 m markers along the pool sides and on the aluminium pole. Via a pulley system the 3 hydrophones (0.5, 1, and 1.5 m deep) could be pulled along the pole.

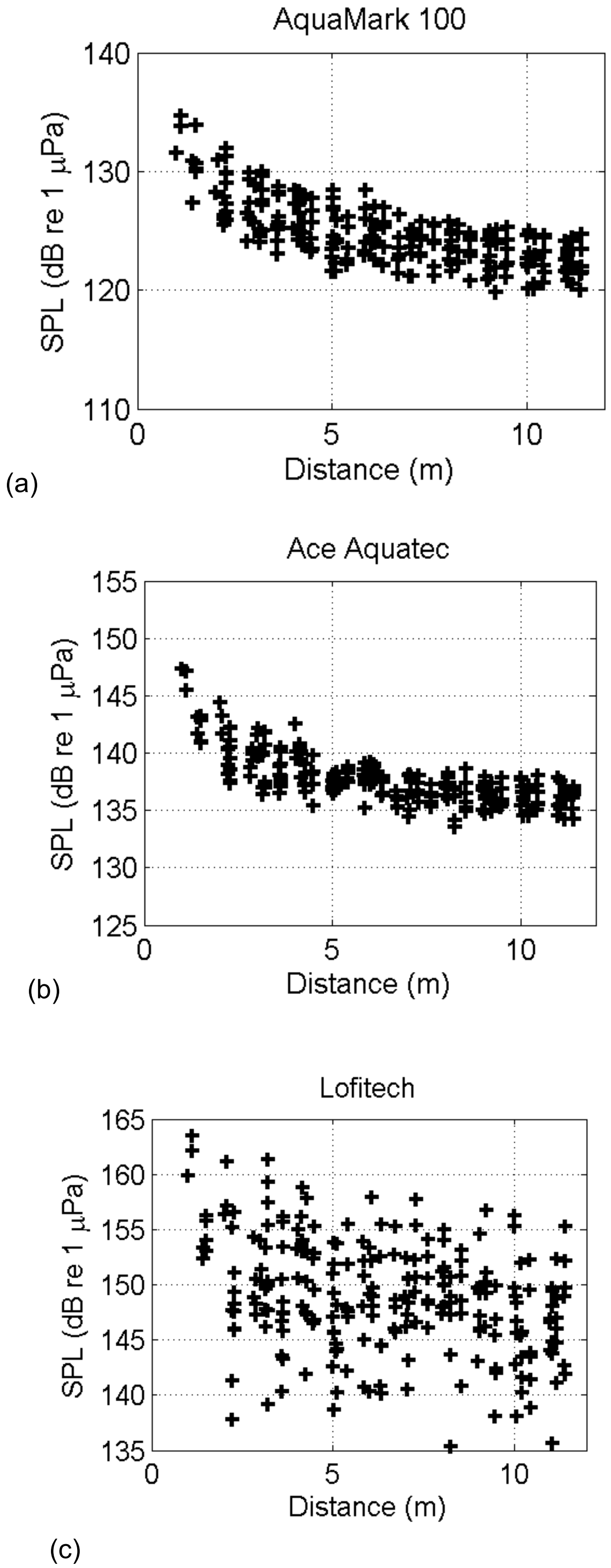


FIG. 13. The SPL distribution in the pool as a function of the distance to the transducer for the three AMD signals measured at three depths a) AquaMark 100, b) Ace Aquatec, and c) Lofitech.

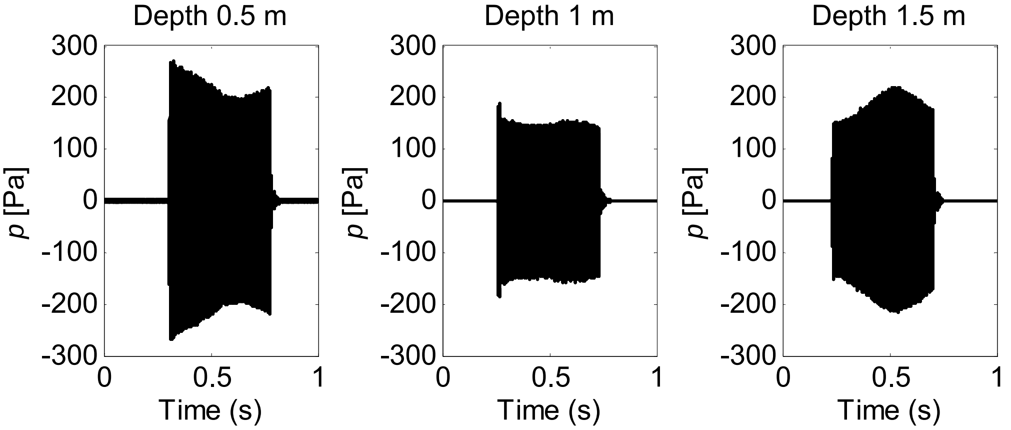


Fig. 14. The waveforms of the Lofitech signal, recorded at a horizontal distance of 1 m from the transducer, simultaneously at three hydrophone depths.

Table 2. The three mean received SPLs ± SD (dB re 1 µPa; n = 231) at which each AMD was tested in the experiments.

	Mean received SPL ± SD (dB re 1 µPa)		
Behaviour	**AquaMark 100**	**Ace Aquatec**	**Lofitech**
Maximum level not changing the porpoise's behaviour	70 ± 3	77 ± 2	91 ± 6
Causing a small change in surfacing rate and small displacement	105 ± 3	117 ± 2	121 ± 6
Causing clear displacement (movement away from the transducers)	127 ± 3	139 ± 2	151 ± 6

3.3.5. Experimental procedures

Thirty minutes before each session started, the transducers producing the AMD signals were positioned in the water at one end of the pool (**Fig. 8**). Sessions consisted of a 30-minute baseline period (no sound emission), followed immediately by a 30-minute test period (sound emission). Usually one, but occasionally two sessions were conducted per day, five to six days per week, beginning between 10.00 and 15.00 h. During the tests, personnel were not allowed within 10 m of the pool.

One AMD was tested at one of the three levels per session, and each AMD level was tested in six sessions, resulting in 54 sessions in all. The AMD type/level combinations were tested in random order. To prevent masking of the AMD sounds by background noise, tests were not carried out during rainfall or when wind speeds were above Beaufort 4. The study period was April to June 2010.

3.3.6. Response parameters and behavioural data recording

Two objective behavioural parameters were used to quantify the porpoise's responses to the AMD signals: his surfacing location in the pool relative to the transducers, and his number of surfacings (corresponding closely to his respiration rate). These parameters were quantified and compared for baseline and test periods.

The distance between the transducers and the location where the animal surfaced was quantified to determine whether the porpoise responded to the sounds by swimming away from the sound source. This was done as follows: from video camera recordings, the locations where the porpoise surfaced during the baseline and test periods were recorded on a grid superimposed on the computer screen (**Fig. 15**). The grid corresponded to a pool grid of 1 x 1 m, and was made by connecting lines between 1 m markers on the pool’s sides. The grid square in which the porpoise surfaced was determined, and the centre point of the grid square was used to calculate the distance between the porpoise’s surfacing location and the transducers, via triangulation. The water was always clear, and when light conditions (which depended on the weather and the time of day or angle of the sun) were such that the bottom of the pool was visible, the porpoise could be seen well below the water surface. Under such good visibility conditions he generally did not swim far away from the surfacing locations. Hence, the surfacing locations were a good indication of the porpoise’s general swimming area. To determine whether the porpoise responded to the sounds by increasing his surfacing rate, the number of surfacings in the baseline periods was compared to the number during the test periods.

In addition to the two objective behavioural parameters (distance from the transducers and number of surfacings), the following subjective behavioural data were recorded: the number of jumps made by the porpoise, his swimming speed, and his respiration force. For this, the test periods were divided into three 10-minute periods. To quantify swimming speed, every 10 minutes of the 30 minute test period, swimming speed in comparison to the baseline period was classed as a number between 0 and 1 (relatively very high speed was classed as 1; high speed was classed as 2/3; slightly elevated speed was classed as 1/3; no change in swimming speed was classed as 0). The average of the three numbers per test period was used to evaluate the change in swimming speed relative to the baseline period (maximum: 1). To quantify respiration force, every 10 minutes of the 30 minute test period, a 1 or 0 was allocated (breathing more forceful than during baseline = 1; similar to during baseline = 0). The average of the three numbers per test period was used to evaluate the respiration force relative to the baseline period (maximum: 1).

FIG. 15. View recorded with the aerial camera above the porpoise pool, used for the AMD behavioural response study. The grid drawn on the laptop screen indicates a 1x1 m grid. The arrow indicates the location of the porpoise. The underwater transducer was on the left side of the pool.

3.3.7. Analysis

All the video recordings were analysed by one person to ensure consistency. For distances from the transducers and numbers of surfacings, paired t-tests were used to compare the parameters in baselines and the associated test periods. The relative average swimming speed and respiration force of the animal was compared to zero (i.e., no change in test periods compared to associated baseline periods) by using sign tests. For all analyses the level of significance was 5% (Zar, 1984).

3.4 Audibility of AMD sounds to harbour seals

3.4.1. Study animals

The two female harbour seals (01 and 02) used in this study were 4 years old and each weighed approximately 50 kg. The seals had participated in three similar psychophysical hearing studies before the present study (Kastelein *et al*., 2009a,b; 2010), and so were well accustomed to the daily hearing test routine.

3.4.2. Study area

The study was conducted at the SEAMARCO Research Institute (Goes, The Netherlands), which is in a remote area specifically selected for acoustic research, in an outdoor pool (8 x 7 m, 2 m deep) with an adjacent haul-out platform (**Figs. 16 and 17**). The pool was constructed to be as quiet as possible and to reduce reflection of sounds above 25 kHz (see Kastelein *et al.*, 2009a).

During test sessions, the seals were tested alternately. The seal being tested positioned itself at the listening station (an L-shaped, 32 mm-diameter, water-filled polyvinylchloride tube with an end cap). The animal not being tested was trained to keep very still and quiet for 15 minutes in the water next to the haul-out platform (this was quieter than staying on land, where a scratch of a flipper nail could trigger a prestimulus response in the animal being tested). The operator and the equipment used to produce the stimuli were in a research cabin next to the pool, out of sight of both animals (**Figs. 16 and 17**).

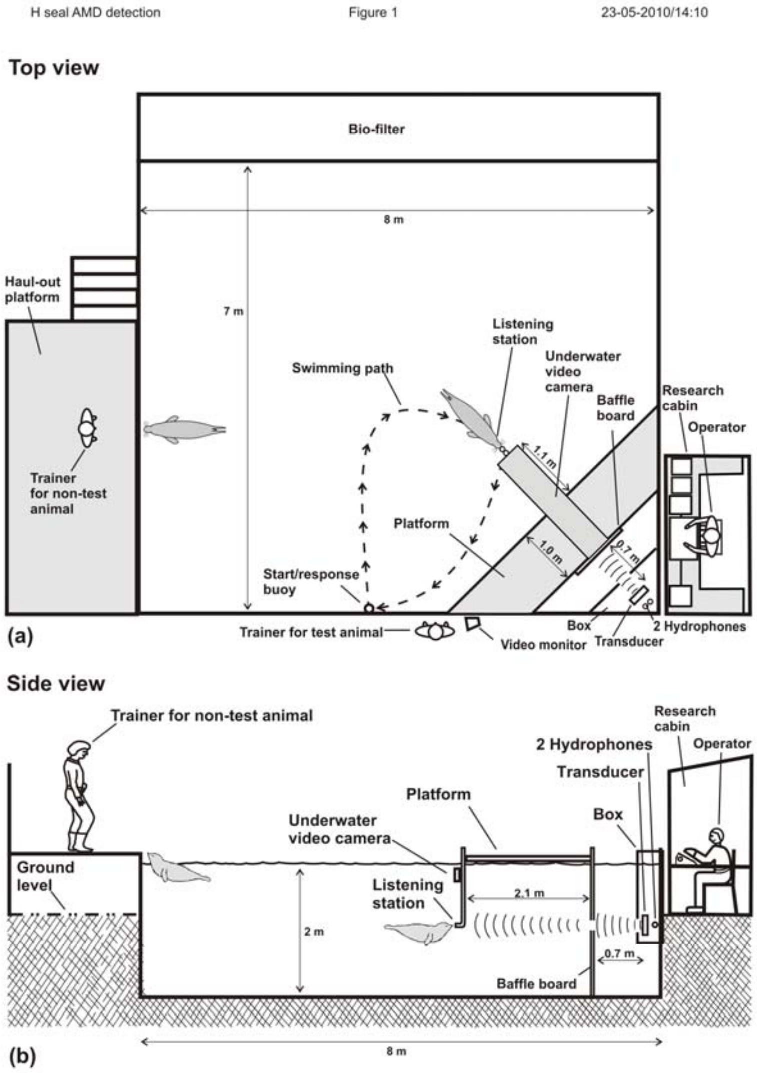


FIG. 16. The study area, showing one harbour seal in position for testing at the underwater listening station, and the animal not being tested waiting with its trainer; (a) top view and (b) side view, both to scale.

FIG. 17. Top view of the harbour seal pool in which both the AMD audibility and AMD behavioural response studies were carried out.

3.4.3. Equipment

The original recordings provided by Subacoustec were re-sampled to 88.2 kHz, so that only the frequencies of the AMDs up to 44 kHz were reproduced, to conform to limitations of the sound card (Presonus Inspire 1394, maximum sample rate 96 kHz). Sounds above 44 kHz were considered unlikely to be heard by harbour seals, as their range of best hearing is from 0.5 to 40 kHz (Kastelein *et al.*, 2009a, b). The reproduced sounds were used in the tests.

The seals' hearing sensitivity was tested for five AMD sounds (three produced by the AquaMark 100, and one produced by each of the Ace Aquatec and the Lofitech). Because the signal produced by the Ace Aquatec was much longer than that produced by the other two AMDs, a representative 0.5 s sample from the regular 5 s Ace Aquatec sound file was used.

A schematic of the equipment used to configure and emit the reproduced sounds is shown in **Fig. 18**. WAV files were played using Adobe Audition software on a laptop computer (Medion, model 96780), which, via a Fire Wire interface (Sweex FW022 Express Card), was connected to a sound card (Presonus Inspire 1394, maximum sample rate 96 kHz). A modified audiometer for testing human aerial hearing (Midimate, model 602 with extended frequency range, Taastrup, Denmark) was used to control the amplitude of the signals. The free field sound pressure level (SPL in dB re 1 µPa, rms) at the seal's head while it was at the listening station was varied in 2 dB increments. The sounds were projected underwater via two transducers in parallel: the spectrum up to 16 kHz via a balanced tonpilz piezoelectric acoustic transducer (Lubell, model LL 916) and the spectrum above 16 kHz via a piezoelectric HF transducer (Labforce). The combined output of the two transducers (resulting in the reproduced sounds) recreated the original recordings closely (**Fig. 3**).

The 1/3-octave band spectra (averaged over the 90% energy duration of the signals) of the original recordings and of the reproduced sounds recorded at the listening position of the seals are shown in **Fig. 3**. The spectra for the three different AquaMark 100 signals are identical, so just one is shown. The spectra are scaled so that the broadband levels of the unweighted original recordings and reproduced sounds are equal. The reproduced sounds in the pool are similar to those of the corresponding original recordings, especially in the dominant frequency bands.

Multi-path arrivals can introduce both temporal and spatial variations in the observed SPL at the listening station. To minimise these, the transducers were placed in a corner of the pool in a protective wooden box, lined with rubber with an irregular surface. The Lubell LL 916 transducer was hung with four nylon cords from the cover of the box and made no contact with the box. The Labforce transducer hung from its own wire next to the Lubell transducer. The transducers were 2.85 m from the tip of the listening station (**Fig. 16**), and were positioned so that the acoustic axis of the projected sound beam pointed at the centre of the listening station (i.e., at the centre of the study animal's head while it was at the listening station). To reduce reflections from the bottom of the pool and from the water surface reaching the listening station, a baffle board was placed between the transducer and the animal. The board consisted of 2.4 m high, 1.2 m wide, 4 cm thick plywood, covered with a 2 cm thick closed cell rubber mat on the side facing the transducer. A 30-cm diameter hole was made in the board with its centre at the same level as the seal's head and the transducer (1 m below the water surface).

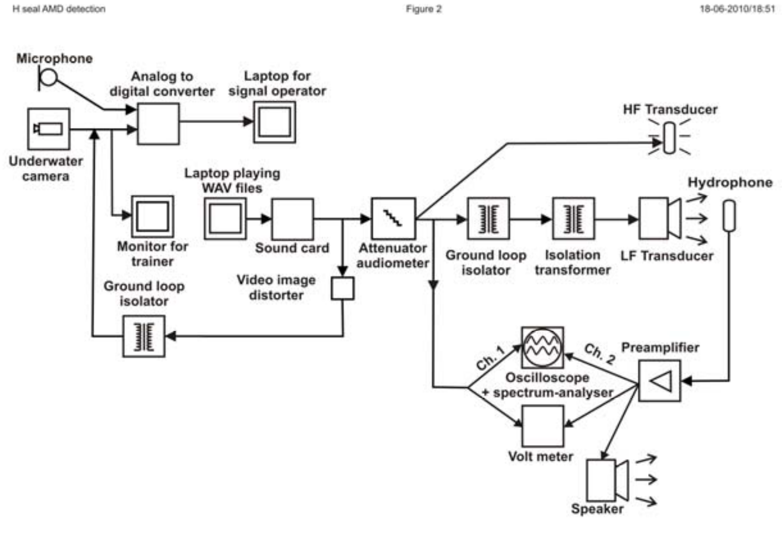


FIG. 18. Block diagram of the signal generation and listening systems.

3.4.4. Background noise and stimuli level calibration measurements

The background noise and received levels and spectra of the signals were measured regularly. The recording and analysis equipment consisted of a hydrophone (B&K 8106, Naerum, Denmark) with a multichannel high frequency analyser (B&K PULSE 3560 D), and a laptop computer with software (B&K PULSE Labshop version 12.1). The system was calibrated with a B&K 4223 pistonphone. The sound pressure level of each pulse (frequency and duration) was derived from the received 90% energy flux density, divided by the corresponding 90% time duration (Madsen, 2005).

Great care was taken to make the seal's listening environment as quiet as possible. Nobody was allowed to move within 15 m of the pool during sessions. Underwater background noise levels were measured twice during the study period, under the same weather conditions as during the test sessions (wind speed Beaufort 4 or below). 1/3-Octave band background noise sound pressure level (SPL) spectra were determined in the 25 Hz to 160 kHz bands (dB re 1 μPa). The mean background noise in the pool was very low (in the low frequency range it was below sea state 0; above 3 kHz it was close to the self-noise of the recording equipment; **Fig. 3**).

The received SPL (dB *re* 1 µPa, rms) of each reproduced AMD sound was measured where the seals' head would be during the hearing tests (**Fig. 16**). During trials, the seal's head position at the listening station was carefully monitored, and was consistent to within a few cm. The received SPLs were calibrated at levels of approximately 20 dB (depending on the AMD sound) above the hearing threshold levels found in the present study for each sound. The linearity of the transmitter system was checked several times during the study; it was consistent to within 1 dB.

3.4.5. Experimental procedures

Before each session, the acoustic equipment producing the stimuli was checked with an oscilloscope (Dynatec, model 8120), a voltmeter (Hewlett Packard, model 3478A), and a spectrum analyser (Velleman, PCSU1000) to ensure that it was functioning properly and that the stimuli were being produced accurately (**Fig. 18**). Also, the background underwater noise level was checked to make sure it was low enough.

The methodology of previous hearing studies with the same seals was used (Kastelein *et al*., 2009a,b; 2010). The seals were trained to respond ('go') in the presence of a signal, and to withhold the response ('no-go') in the absence of a signal. A trial began when the seal not being tested was near the platform with a trainer, and the seal being tested was positioned with its head at the start/response buoy at the edge of the pool, next to the test animal's trainer (**Figs. 16a and 19)**. When the trainer for the test animal gave a vocal command accompanied by a gesture (pointing downwards), the test animal descended to the listening station, so that its external auditory meatus was 200 cm from the sound source and 100 cm below the water surface (i.e., mid-water; **Figs. 16b and 20)**. Each animal was trained to position its nose against the listening station so that its head axis was in line with the projected beam axis of the transducer. The listening station was not connected to the sound box, so the animals were not able to use vibration via contact conduction to the nose to detect the signals. The animals' positions at the listening station could be viewed and checked from above by means of an underwater camera (Mariscope, Micro, Kiel, Germany) attached to the listening station. The images were visible to the trainer for the test animal (who was near the start/response buoy, out of the animal's view when it was at the listening station) and to the operator in the research cabin.

Two trial types were conducted during each experimental session: signal-present trials and signal-absent trials. In signal-present trials, the stimulus was presented unpredictably between 4 and 10 s after the animal was positioned correctly at the listening station. A minimum waiting time of 4 s was chosen because it took about 4 s for the waves, created by the animal's descent, to dissipate. If the animal detected the sound, it responded by leaving the listening station ('go' response) at any time during the signal's duration and returning to the start/response buoy (**Fig. 16a**). When the test signal was produced, a generator was activated that produced horizontal white lines on the video image. This helped the operator to determine visually whether or not the seal responded while the signal was being produced. If she did respond, the operator indicated to the trainer that the response was correct (a hit), after which the trainer gave a vocal signal and the seal received a fish reward. If the animal did not respond to the signal, the operator indicated to the trainer that the animal had failed to detect the signal (a miss). The trainer then indicated to the animal (by tapping softly on the side of the pool) that the trial had ended, thus calling her back to the start/response buoy. No reward was given following a miss. If the animal moved away from the listening station to the start/response buoy before a signal was produced (a prestimulus response), the operator indicated that the trainer should end the trial without rewarding the animal. After a prestimulus response, the animal was ignored for 8-10 s by the trainer.

In signal-absent, or catch, trials, the operator signalled to the trainer to end the trial after a predetermined random interval of 4-10 s from when the seal had stationed. The trial was terminated when the trainer blew very softly on a whistle. The tapping on the pool wall and whistle blowing were done softly, to minimise the difference in the level of sounds heard by the seals, in order to help them focus on very faint sounds throughout the sessions. If the animal being tested responded correctly by remaining at the listening station until the whistle was blown (a correct rejection), it then returned to the start/response buoy and received a fish reward. If the seal left the listening station before the whistle was blown (a prestimulus response), the operator indicated to the trainer to end the trial without rewarding the animal. The same amount of fish was given as a reward for correct responses in signal-present trials and in signal-absent trials. In both signal-present and signal-absent trials, the trainer was unaware of the trial type when she sent the animal to the listening station. After sending the animal to the listening station, the trainer stepped out of the seal's view.

A session generally consisted of 30 trials per animal and lasted for about 15 minutes per animal. Within sessions, the two seals were tested in random order. Sessions consisted of 70% signal-present and 30% signal-absent trials, presented in random order. There were never more than three consecutive signal-present or signal-absent trials. In order to end with a positive event, the last trial was always one in which the animal responded correctly and received a reward. For each session, one of four data collection sheets was used. Each sheet comprised a different random series of trial types and random times between the animal stationing and signal presentation. Each seal had its own set of four data collection sheets. In each session, one AMD sound was tested, but the signal amplitude was varied according to the 1-up 1-down adaptive staircase method. This conventional psychometric technique (Robinson and Watson, 1973) results in a 50% correct detection threshold (Levitt, 1971). During preliminary sessions, a rough threshold per test frequency was determined. During subsequent experimental sessions, the starting SPL of the signal was 10-15 dB above the estimated threshold for each AMD sound. Following each hit, the signal amplitude on the next signal-present trial was reduced by 2 dB. Following each miss, the signal level was increased on the next signal-present trial by 2 dB. Prestimulus responses did not lead to a change in signal amplitude for the next trial.

Thresholds were determined for each of the five reproduced AMD sounds from the three AMDs. To prevent the animals' learning process from affecting the threshold levels, a different randomly selected AMD sound was tested in each session. During a session, the two seals were presented with the same AMD signal. Usually three experimental sessions per day with each animal were conducted, five days per week (at 0900, 1200 and 1500 h). Data were collected between April and June 2010.

FIG. 19. The signal operator and equipment used for the harbour seal AMD audibility study.

FIG. 20. A harbour seal at the listening station waiting for an AMD sound. When she had heard a sound, the animal would swim away from the station towards the trainer and the start/response buoy.

3.4.6. Determination of hearing thresholds

Switches in the seal's response from a detected signal (a hit) to an undetected signal (a miss), or *vice versa*, called reversals, were used to calculate hearing thresholds. A detected level and the successive undetected level, or *vice versa,* are called a reversal pair. No warm-up trials were needed, as the thresholds were usually stable within sessions. Sessions with more than 20% prestimulus responses (i.e., in six or more of the 30 trials per session), which would have been omitted from the analysis, did not occur during the entire study period. Each sound was tested until at least 60 reversal pairs had been obtained (in at least six sessions). Although the minimum number of reversal pairs was set at 60, in some cases the last session needed to reach this number contained many reversal pairs, so that more reversal pairs were available and were used for analysis; the equipment settings remained the same in a session. Also, in each session, both seals were tested, so if one of the seals needed more reversals to reach the 60 reversal pair criterion, the other seal would have more reversal pairs.

3.4.7. Comparison of hearing thresholds with thresholds for tonal sounds

In order to investigate whether hearing threshold levels of broadband signals can be predicted based on their spectrum and level, the observed 50% hearing thresholds for the AMD sounds were compared with estimated thresholds based on the seals' audiogram for tonal noise. For this comparison, the tonal audiogram for signals of 1500 ms duration (Kastelein *et al*., 2010), interpolated to 1/3-octave band centre frequencies, was subtracted from the recorded 1/3-octave band spectrum of the reproduced AMD sounds at the calibration level. The corresponding broadband 'sensation level', *i.e*., the frequency-integrated difference between the spectra of reproduced sounds and audiogram, was subtracted from the broadband calibration SPL of the reproduced sound to obtain an estimation of the hearing threshold, *i.e.*, the broadband SPL at which the sensation level is equal to 0 dB. This estimation is based on the assumption that the hearing behaves as an energy detector, and that the waveform of sounds does not influence their audibility.

3.5 Behavioural response of harbour seals to AMD sounds

3.5.1. Study animals

The study animals were the two female harbour seals (01 and 02) used in the audibility study (see section 3.4.1).

3.5.2. Study area

The seal behavioural response study was conducted in the same pool as the seal audibility study (see section 3.4). The outdoor pool (8 x 7 m, 2 m deep) had three haul-out platforms (**Fig. 21 and 22**).

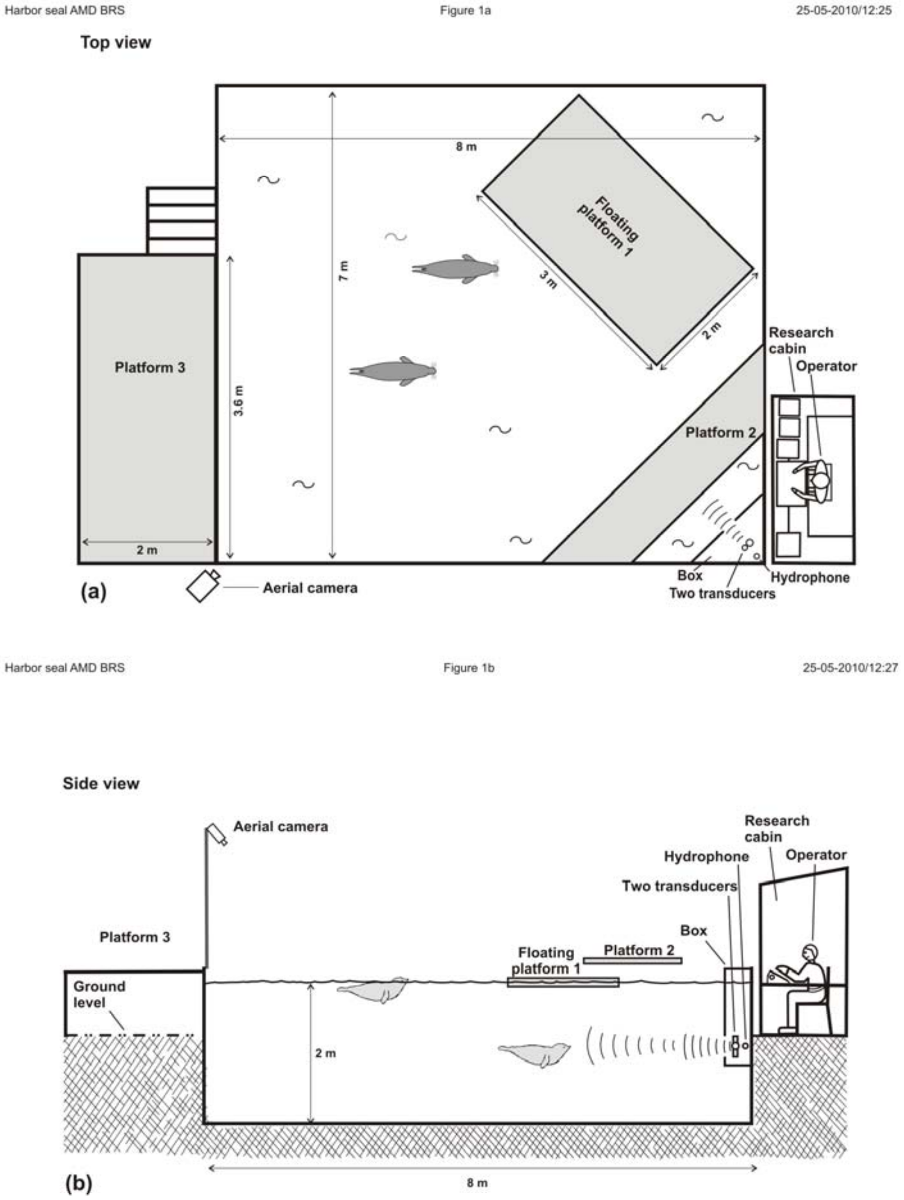


FIG. 21. Top and side scale view of the study facility, showing the study animals, the location of the aerial camera, the two underwater transducers emitting the AMD signals, the listening hydrophone and the three haul-out platforms. Also shown is the research cabin which housed the equipment and the operator.

FIG. 22. The pool in which the harbour seal AMD behavioural response study was conducted. Note the seal in the front, and the aerial wide-angle camera on the pole at the back of the picture.

3.5.3. Video and audio equipment

A schematic diagram of the equipment used to configure and emit outgoing signals and record video and underwater sounds is shown in **Fig. 23,** and the real set-up in **Fig. 24.** The digitised test sounds (WAV files; sample frequency 88.2 kHz) were played back using Adobe Audition (version 3.0) by a laptop computer (Medion, model MD96780) via a FireWire interface (Sweex FW022 Express Card) with an external sound card (Presonus, model Inspire 1394, maximum sample rate 96 kHz), to an audio power amplifier (JB Systems, model D2-1500), the output of which was controlled with a custom-built digitally-controlled attenuator (SEAMARCO AS 2008-03; 1 dB steps, 10 Hz-200 kHz). The sounds were projected underwater via two transducers in parallel: frequencies up to 16 kHz were projected by a balanced tonpilz piezoelectric acoustic transducer (Lubell LL916) with its isolation transformer, and frequencies above 16 kHz by a HF transducer (Labforce, model 90.02.01). The transducers were suspended 1 m below the water surface in a box in the eastern corner of the pool (**Fig. 21**). The output of the sound system to the transducer was monitored with an oscilloscope (Dynatec, model 8120), a voltmeter (Hewlett Packard, model 3478A), and a spectrum analyser (Velleman, PCSU1000).

The seals' behaviour was filmed from above by a waterproof camera (Conrad 750940) with a wide-angle lens. The camera was placed on a pole 6 m above the water surface on the southern corner of the pool (**Fig. 21**). The entire surface of the pool was captured on the video image. The output of the camera was digitised with an analogue-to-digital converter (Smart Group, model Zolid) and stored on a laptop computer (Medion, MD98110).

The audio part of the background noise and the test sounds were recorded via a hydrophone (Labforce, model 90.02.01) and a pre-amplifier (Bruel & Kjaer, model 2635). The output of the pre-amplifier was digitised via the analogue-to-digital converter and recorded on the laptop computer in synchrony with the video images. The output was also fed to an amplified loudspeaker, so that the operator in the research cabin could monitor the background noise and the test sounds during sessions. The signals were also fed from the hydrophone to an ultrasound detector (BatBox III, BatBox Ltd, UK). Via a microphone, the operator added the date, time of day, session number, and AMD being tested, to the video recording at the start of the session.

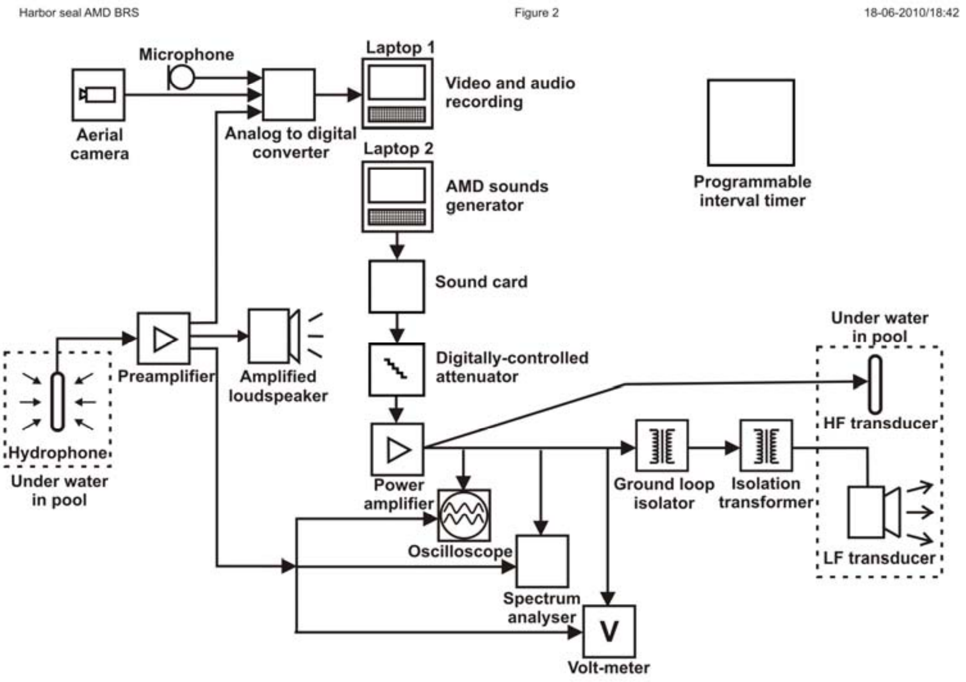


FIG. 23. Block diagram of the signal generation and control systems, and the listening and recording equipment, used in the seal behavioural response study.

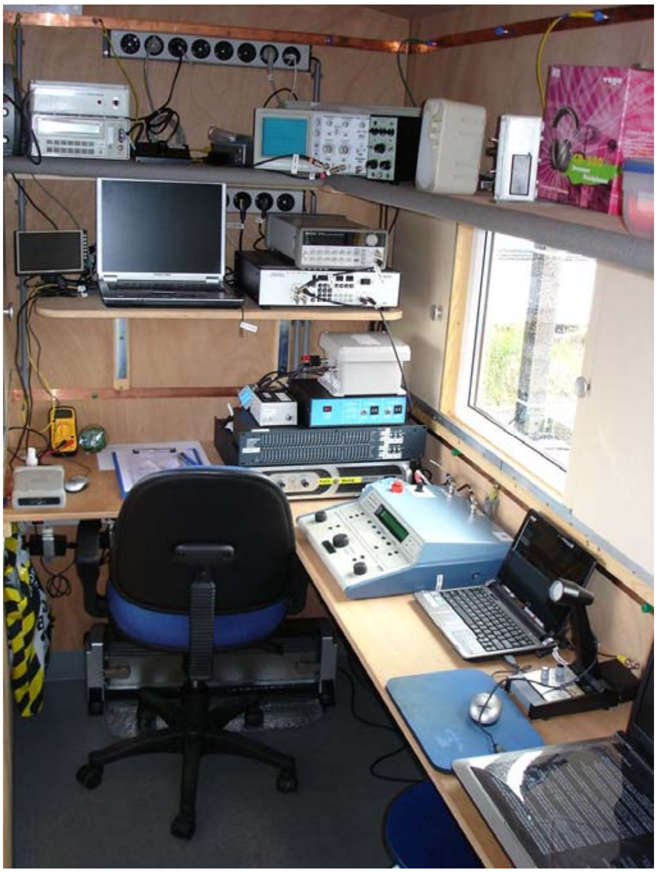


FIG. 24. The equipment used for the harbour seal behavioural response study.

3.5.4. Acoustics

3.5.4.1. Test stimuli (AMD sounds)

The AMD sounds are described in section 3.1.

3.5.4.2. Sequence of AMD sounds

The sound sequences used were the same as in the porpoise behavioural response study (see section 3.3.4.3).

3.5.4.3. Determination of the source levels used in the tests

During pre-tests in which the source level (SL) of each AMD signal was gradually increased, three levels were determined: (1) a received level which just did not cause a behavioural change, (2) a level which caused one of the seals to haul out (leave the water) occasionally, and (3) a level which caused one of the seals to haul out frequently. These three levels for each AMD were then tested (**Table 3**).

Table 3. The three mean received SPLs ± SD (dB re 1 μPa; n = 99 measurements in the pool) at which each AMD was tested in the experiments.

Behaviour	**Mean received SPL ± SD (dB re 1 μPa)**		
	AquaMark 100	**Ace Aquatec**	**Lofitech**
Maximum level not changing the seals' behaviour	117 ± 1	109 ± 1	128 ± 4
Causing a small change in the seals' behaviour	127 ± 1	124 ± 1	133 ± 4
Causing the seals to haul out frequently	137 ± 1	134 ± 1	138 ± 4

3.5.4.4. SPL distribution measurements

To determine the sound distribution in the pool, the SPL for each of the test sounds was measured at 33 locations (on a horizontal grid of 1 x 1.15 m; three locations could not be accessed because platforms were in the way; **Fig. 25**). The sound pressure level (SPL) was measured at three depths per location on the grid (0.5, 1.0 and 1.5 m below the water surface; **Fig. 26**). Thus, 99 measurements were made for each AMD signal. The reported SPLs were based on a recording of one signal per location.

The SPL (dB re 1 μPa) of the signals was averaged over the duration of each signal. The analysis was done in the time domain. The cumulative Sound exposure level (SEL in dB re 1 $\mu Pa^2 s$) was determined from the sound pressure, by integration of the broadband pressure squared. The duration of the signal (T_{90} in s) was defined as the time between the moments when the cumulative sound exposure reached 5% and 95% of the total exposure (i.e., when it contained 90% of the total energy in the signal). The SPL was determined by subtracting 10 times the logarithm of the T_{90} duration from the cumulative SEL. The distribution of the received SPL, as measured at 231 positions in the pool, is shown in **Fig. 27.** The SPL in the pool varied little (**Table 3**), but the received levels of the Lofitech varied more than those of the other two AMDs, because the Lofitech produced a tonal signal which is very sensitive to interference effects in the pool and to Doppler effects caused by motion of the water surface. This is illustrated in **Fig. 28**; the Lofitech signals exhibit a large variation over the depth, probably caused by the surface-reflected signal, which interferes with the direct signal. For further analysis the received SPL in the pool is considered to be homogenous.

FIG.25. Measurement of the SPL distribution in the harbour seal pool. Note the 1 m markings on the aluminium pole. The white floats support the cables of the 3 hydrophones.

FIG. 26. The 3 B&K 81010 hydrophones used to measure the SPL distribution, suspended at 0.5 m, 1 m, and 1.5 m depth.

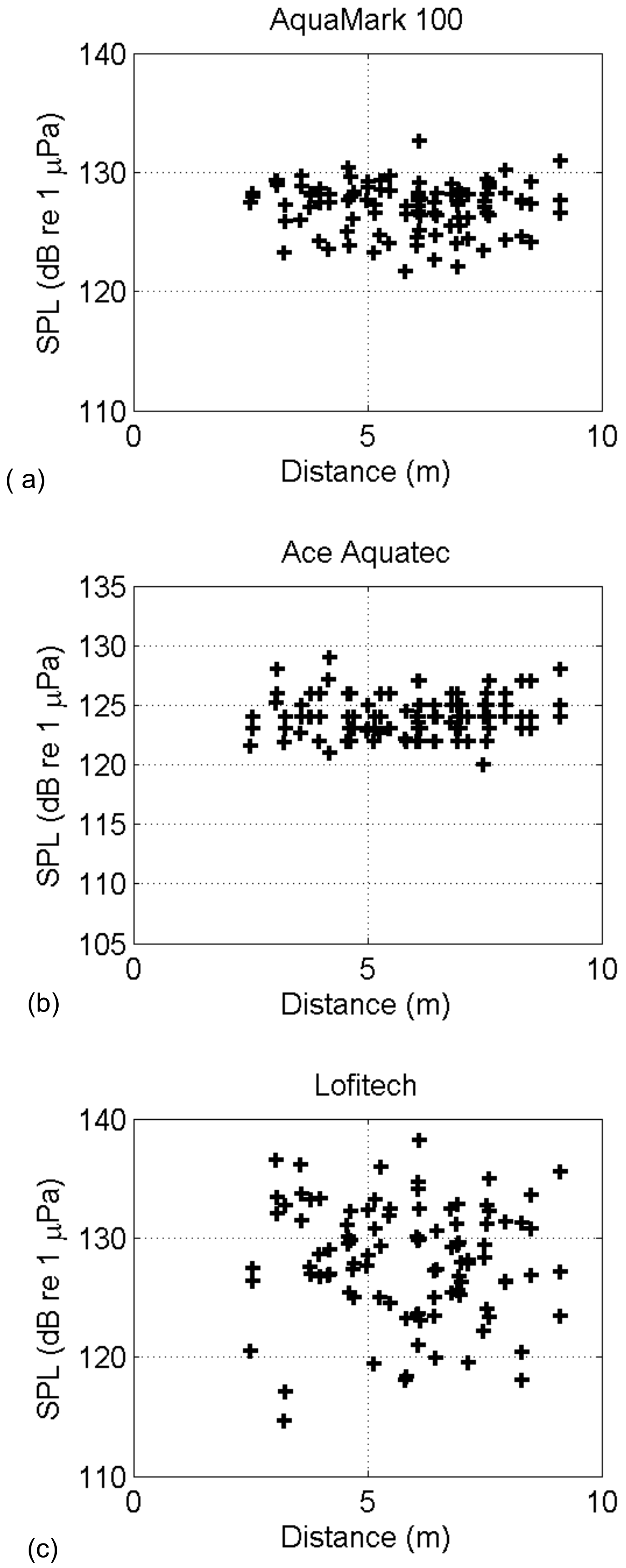


FIG. 27. The SPL distribution in the pool as a function of the distance to the transducer for the three AMD signals measured at three depths a) AquaMark 100, b) Ace Aquatec, and c) Lofitech.

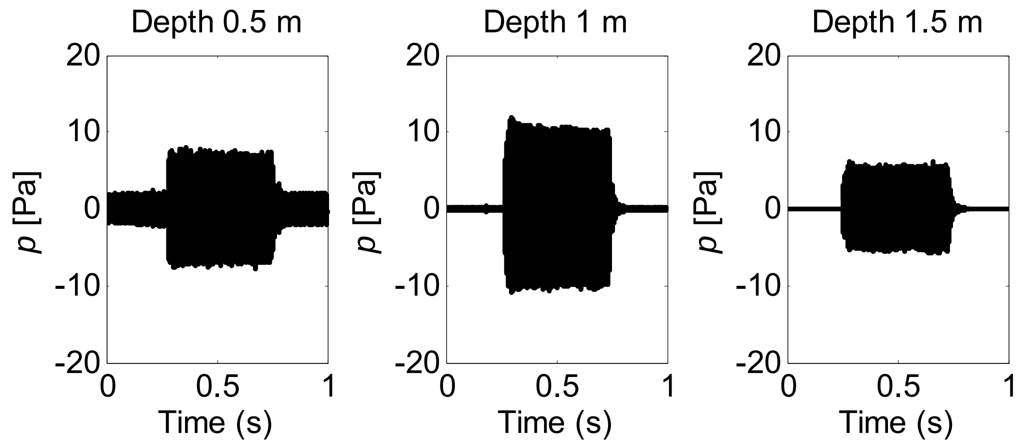


FIG. 28. The waveforms of the Lofitech signal, recorded at a horizontal distance of 2 m from the transducer, simultaneously at three hydrophone depths.

3.5.5. Experimental procedures

The transducers producing the AMD signals were in the pool throughout the study period (**Fig. 21**). Each session consisted of a 30-minute baseline period (no sound emission), followed immediately by a 30-minute test period (sound emission). Usually one, but occasionally 2, sessions were conducted per day, five to six days per week, beginning between 10.00 and 15.00 h. During the tests, personnel were not allowed within 10 m of the pool.

One AMD was tested at one of the three levels per session, and each of the three AMDs was tested in five sessions (45 sessions in all). The AMD type/level combinations were tested in random order. No people were allowed near the pool during the sessions (**Fig. 29**). Tests were not carried out during rainfall or when wind speeds were above Beaufort 4, as under these conditions the AMD sounds may have been masked by ambient noise. The study was conducted in April and May 2010.

FIG. 29. A researcher places a sign informing people that a behavioural response study is taking place, and asking them not to approach the pool.

3.5.6. Response parameters and behavioural data recording

The spot sampling method was used to record the behaviour of the two seals objectively: every 10 s the operator recorded whether each seal's head was under water or in the air, and if it was in the air, the location of the seal was recorded (grid location in the water, or on one of the three platforms; **Fig. 30**). From the video images on the laptop, the seals could not always be distinguished from each other (particularly while they were underwater or when only a head was visible). Therefore, all behavioural scores were of both seals together. When they were hauled out, the seals' identity could be established, but this information was not used in the analysis.

Four objective behavioural parameters were used to quantify the seals' responses to the AMD signals: 1) the distances between their surfacing locations in the pool and the transducers (at scoring moments), 2) the number of scores for which the animals were below the water surface, 3) the number of scores for which the animals were in the water but with their heads above the water surface, and 4) the number of scores for which the animals were hauled out on one of the three platforms.

These parameters were quantified for baseline and test periods. The surfacing locations were quantified to determine whether the seals responded to the sounds by swimming farther away from the transducers. This was done as follows: from the video camera images, the locations where the seals surfaced at a scoring moment during the baseline and test periods were marked on a grid superimposed (via a transparent sheet) on the computer screen. The grid was formed by lines between markers on the pool's sides.

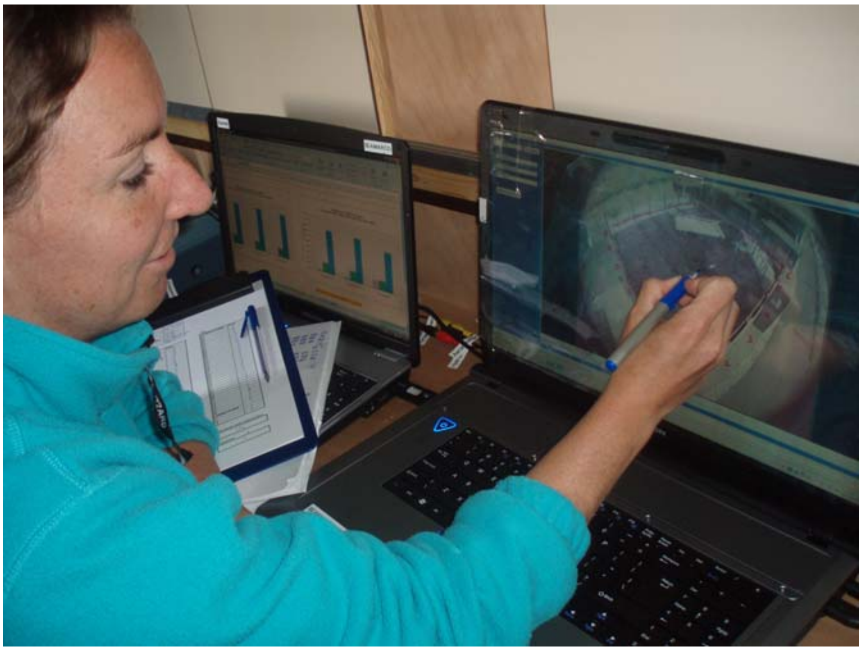


FIG. 30. Spot sampling: a researcher indicates the location of the seals on the screen of a laptop.

3.5.7. Analysis

All the video recordings were analysed by one person to ensure consistency. The distances between surfacing locations and the transducers, and the numbers of below-water scores, head above water scores, and hauled-out scores, were all quantified for the baseline and test period of each session (pooled for both animals). Paired t-tests were used to compare the parameters in baselines and the associated test periods. For all analyses, the level of significance was 5% (Zar, 1984).

3.6 Audibility distance calculations

Combining the 50% detection (hearing) thresholds (DT) from the present study with the source levels (SL) of the AMDs, an appropriate distance-dependent propagation loss (PL(*R*)) model, and the ambient noise level (NL), allows the detection distances (*R*) of the three AMDs for harbour porpoises in a given environment to be estimated from the equation:

SL - PL(R) - max(NL, DT) = 0.

All terms in this equation depend on the frequency and bandwidth of the signals. For broadband signals (AquaMark 100 and Ace Aquatec), NL is the broadband ambient noise level in the same bandwidth as the signals. For the tonal signal produced by the Lofitech AMD, the level of masking of the signal by broadband ambient noise also depends on the critical ratio of the porpoise's hearing (Kastelein *et al*., 2008; 2009) and seal's hearing (Turnbull and Terhune, 1990; Southall *et al*., 2000).

The frequency spectrum of the signal also influences the audibility distance, via an interaction with the detection of the bandwidth by the listener. For example, the Ace Aquatec produces a broadband signal (4 dB variation between 10 and 40 kHz; **Figs. 1 and 2**) while the Lofitech produces a tonal signal in which there is 25 dB variation between 10 and 20 kHz (**Figs. 1 and 2**). In consequence, the hearing threshold for the broadband signal depends on the level of the 1/3 octave band background noise, while that of the tonal signal depends on the spectrum level of the noise plus the critical ratio.

Actual audibility distances of sounds in the ocean depend on many variables, including the losses associated with spreading (which are influenced by water depth, bottom type, refraction associated with temperature stratification, etc.) and the masking effects of background noises (which are influenced by meteorological factors, ice cover, anthropogenic noises, etc.). As examples, the audibility distance of the AMDs by harbour porpoises were calculated for two extreme situations:

1. Under background noise conditions corresponding with Sea State 0 and cylindrical spreading (in a water depth H = 20 m) plus absorption attenuation (longest distance; 10 Log R + 10 Log H + αR).
2. Under background noise conditions corresponding with Sea State 4, and spherical spreading plus absorption attenuation (shortest distance; 20 Log R + αR).

The Sea State noise was estimated by means of the Knudsen curves (Knudsen *et al*., 1948). To calculate the frequency dependent absorption loss we used Thorp's expression (Thorp, 1967) as modified by Urick (1983). Calculations were carried out for all 1/3-octave bands between 4 kHz and 63 kHz. The audibility distance is defined as the distance at which the summed broadband difference between the received level (SL-PL) at that distance and the masked detection threshold (max(NL,DT)) equals zero.

3.7 Behavioural response distance calculations

Combining the response thresholds (RTs) from the present study with the source levels (SL) of the AMDs (**Table 1**) in an appropriate range-dependent propagation loss (PL(R)) model allows the distances (R) at which harbour porpoises and harbour seals are expected to respond to the three AMDs in a given environment to be estimated from the equation:

SL - PL(R) - RT = 0.

Masking by ambient noise is ignored in this estimation, because the observed response threshold levels are well above the levels of wind-generated surface noise up to Sea State 4 (Urick, 1983). All terms in this equation depend on the frequency and bandwidth of the signals. For example, the Ace Aquatec presents a broadband signal (4 dB variation between 10 and 40 kHz; **Figs. 1 and 2)** while the Lofitech presents a tonal signal in which there is 25 dB variation between 10 and 20 kHz (**Figs. 1 and 2)**. Actual response distances to sounds in the ocean depend on many variables, including losses associated with spreading (which are influenced by water depth, bottom type, refraction associated with temperature stratification, etc.). As examples, the distances at which harbour porpoises and harbour seals are expected to respond to the AMDs were calculated for two situations**:**

1) Cylindrical spreading (in a water depth H = 20 m) plus absorption attenuation (longest distance; 10 Log R + 10 Log H + αR).
2) Spherical spreading plus absorption attenuation (shortest distance; 20 Log R + αR).

The frequency-dependent absorption loss (αR) is estimated according to the expression due to Thorp as modified by Urick (Urick 1983). Calculations were carried out for all 1/3-octave bands between 4 kHz and 63 kHz. The detection distance is that at which the summed broadband difference between the received level (SL-PL) at that distance, and the response threshold (RT), equals zero. The distance estimates are approximate, and are accurate only to a factor of two.

4. Results

4.1 Audibility of AMD sounds to a harbour porpoise

4.1.1. Hearing thresholds

The prestimulus response rate (the percentage of signal-present and signal-absent trials in which the porpoise responded before hearing the AMD sound or whistle) varied between 2 and 6%, depending on the AMD sound (**Table 4**).

The 50% hearing threshold levels of the harbour porpoise for the five AMD sounds **(Table 4)** were almost identical at around 55 dB (re 1 µPa), in spite of the different frequency contents and waveforms of the sounds.

TABLE 4. The mean 50% hearing threshold amplitudes (± SD, n = 6 sessions; based on broadband SPL (average over the 90% energy signal duration) and prestimulus response levels (for both signal-present and signal-absent trials) of the 4.5-year-old male harbour porpoise, for the five reproduced AMD (Acoustic Mitigation Device) sounds (the AquaMark 100 produced 3 different sounds). Also shown are the broadband hearing threshold levels for the AMD signals, estimated from the harbour porpoise's audiogram for tonal signals of 1500 ms duration (Kastelein *et al*., 2010).

AMD Sound	Results			Hearing threshold estimated from audiogram (dB re 1 µPa)
	Mean 50% hearing threshold level (dB re 1 µPa, rms) ± S.D.	No. of rever-sal pairs	Pre-stimulus response rate (%)	
AquaMark 100/1	54 ± 1.6	68	3	48
AquaMark 100/2	55 ± 1.8	72	2	48
AquaMark 100/3	54 ± 1.7	77	6	48
Ace Aquatec	55 ± 1.5	80	2	49
Lofitech	55 ± 1.7	86	2	48

4.1.2. Comparison of hearing thresholds for AMD sounds with those for tonal signals

In the absence of ambient noise, the detection threshold for AMD sounds is determined by the hearing ability of the receiving animal. We assume that a broadband signal is detected when its 1/3 octave band levels reach or exceed the tonal hearing threshold levels in the corresponding frequency bands. The 1/3-octave spectra of the Sensation Level [SPL averaged over the 90% energy duration of the signals in 1/3-octave bands, minus the harbour porpoise's 1500 ms tonal 50% hearing threshold levels (Kastelein *et al*., 2010) at the 1/3-octave centre frequencies] of the original recordings by Subacoustech and of the reproduced sounds are shown in **Fig. 31**. Based on these graphs, the hearing threshold for all three AMD signals is predicted to be similar.

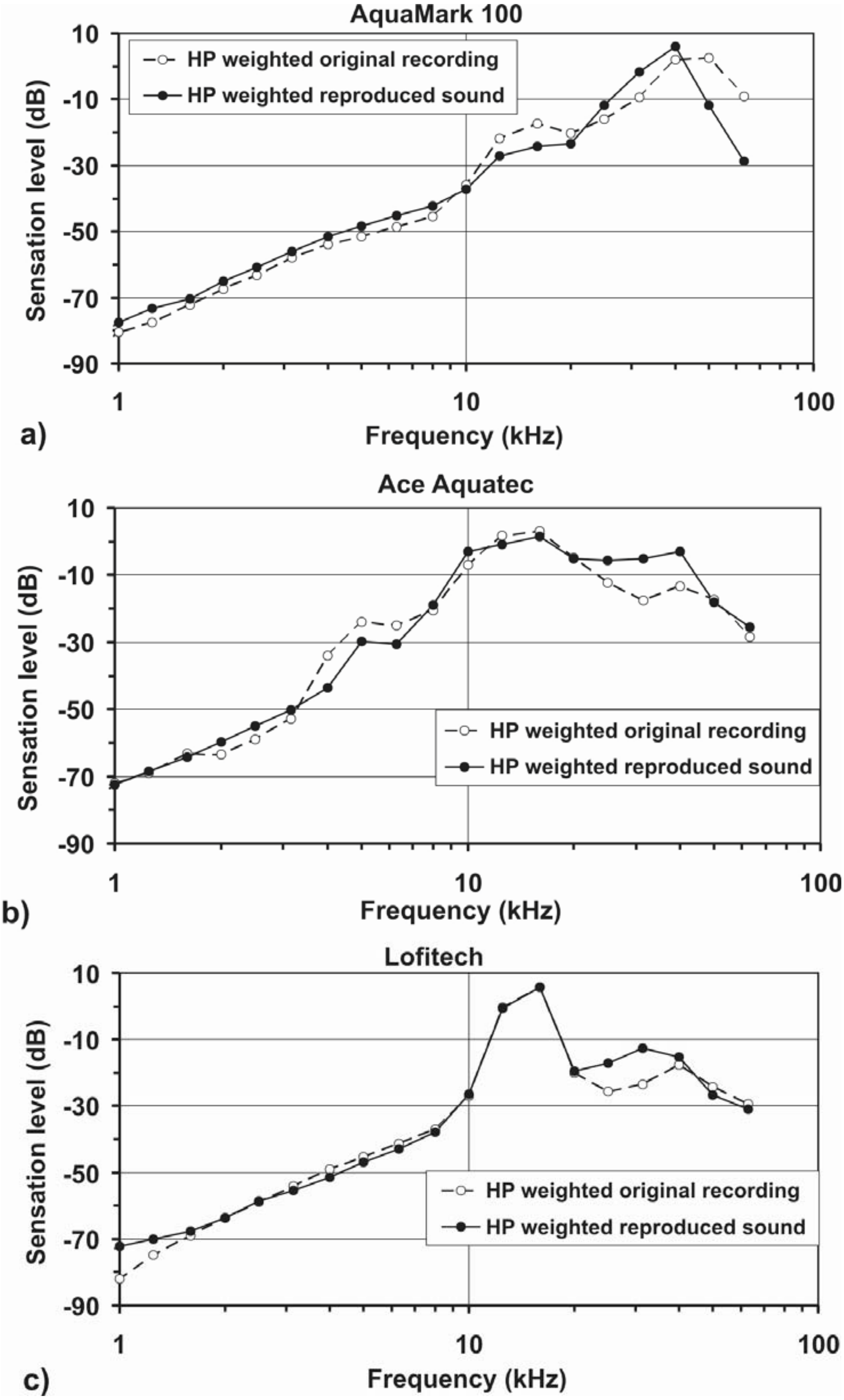


FIG. 31. 1/3-octave spectra of the Sensation Level (‘Weighted SPL’) of the original recordings by Subacoustech and of the reproduced sounds of the three AMD devices, both scaled to the same broadband level at the observed hearing threshold [Sensation Level = the SPL of the AMD signals averaged over the 90% energy duration of the signals in 1/3-octave bands, minus the harbour porpoise’s 50% hearing threshold levels for tonal signals of 1500 ms duration (Kastelein *et al*., 2010) at the 1/3-octave centre frequencies]. Note: the time averaged spectra for the three different signals of the AquaMark 100 are identical.

4.2 Behavioural response of a harbour porpoise to AMD sounds

During baseline periods, the porpoise usually swam large clock-wise ovals in the pool. The distance between the animal's surfacing locations and the transducers was similar in all 54 baseline periods (mean = 7.1 ± 0.8 m; **Fig. 32a**), and the porpoise jumped out of the water on only five occasions during baseline periods, compared to 130 times during test periods. He showed a regular baseline dive pattern consisting of long dives alternated with shorter dives.

Comparison of data from baseline and associated test periods showed that the animal did not react to the lowest sound levels (except for by moving away from the transducers in response to the AquaMark 100). As the mean received level increased, significant displacement occurred alongside significantly higher numbers of surfacings, swimming speed (**Fig. 33**) and respiration force in test periods, compared to associated baseline periods. Effects were stronger and more consistent at the highest level tested for each AMD (**Fig. 32 and Table 5).**

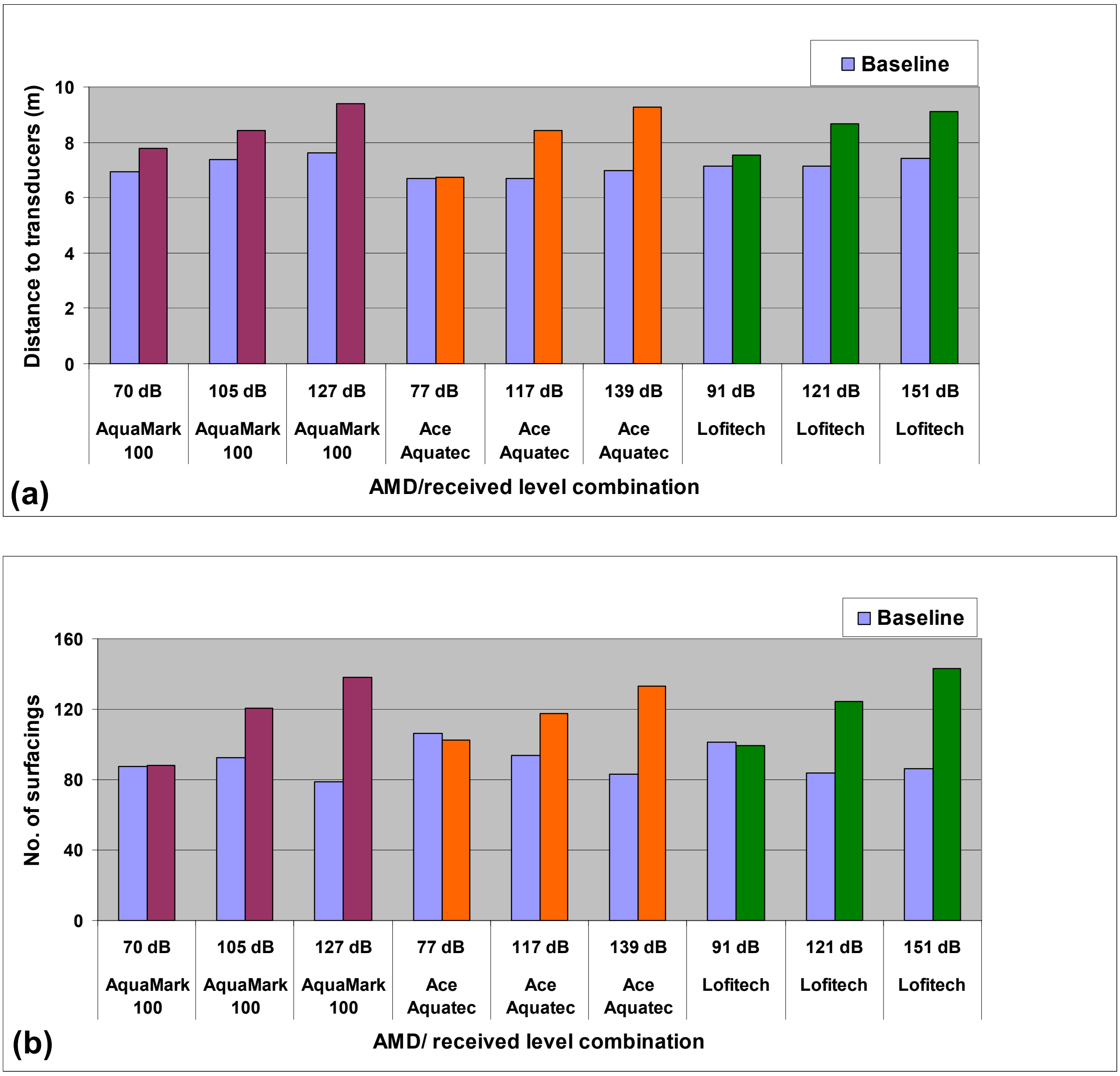


FIG. 32. The objectively measured behavioural responses of the harbour porpoise to three mean received levels for each of the three AMDs, showing, pooled for all sessions, a) the mean distance between porpoise and the transducers, and b) the mean number of surfacings during 30 min periods.

FIG.33. At the highest levels at which the AMDs were presented, the swimming speed of the porpoise increased.

Table 5. Results of paired t-tests to compare the objective parameters, and sign tests to compare the subjective parameters, in baseline and test sessions, for each AMD/level combination. The sample size for each combination is six, and exact P-values are shown in bold if significant.

AMD		**AquaMark 100**			**Ace Aquatec**			**Lofitech**		
Mean received SPL (dB re 1 µPa)		70	105	127	77	117	139	91	121	151
Objective parameters	**Number of surfacings**	0.907	**0.002**	**0.001**	0.299	**0.017**	**0.001**	0.701	**0.002**	**0.000**
	Distance to transducers	**0.032**	**0.002**	**0.001**	0.64	**0.001**	**0.004**	0.188	**0.018**	**0.003**
Subjective parameters	**Swimming speed**	1.000	**0.031**	**0.031**	1.000	**0.031**	**0.031**	1.000	**0.031**	**0.031**
	Respiration force	1.000	**0.031**	**0.031**	1.000	0.125	**0.031**	1.000	**0.031**	**0.031**

4.3 Audibility of AMD sounds to harbour seals

4.3.1. Hearing thresholds

The pre-stimulus response rates (the percentage of signal-present and signal-absent trials in which the seals responded before hearing the AMD sound or whistle) of seal 01 varied between 4 and 9% depending on the AMD sound, and those of seal 02 varied between 1 and 4% (**Table 6**). The hearing thresholds of seal 01 for the five AMD sounds varied between 65 and 69 dB re 1 µPa. The hearing thresholds of seal 02 were 2 – 5 dB (average 3.2 dB) lower than those of seal 01 (**Table 6**).

TABLE 6. The mean 50% hearing threshold amplitudes (± SD, n = 6 sessions) and prestimulus response levels (for both signal-present and signal-absent trials) of the two 4-year-old female harbour seals for the five reproduced sounds of the three AMDs (Acoustic Mitigation Devices; the AquaMark 100 produced 3 different sounds). Also shown are the broadband hearing threshold levels for the AMD signals, estimated from the harbour seal audiogram for tonal signals of 1000 ms duration (Kastelein *et al*., 2009a).

AMD sound	**Seal 01**			**Seal 02**			**Broadband hearing threshold, estimated from audiogram (dB re 1µPa)**
	Mean 50% hearing threshold level (dB re 1 µPa, rms) ± S.D.	**No. of rever-sal pairs**	**Pre-stimulus response rate (%)**	**Mean 50% hearing threshold level (dB re 1 µPa, rms) ± S.D.**	**No. of rever-sal pairs**	**Pre-stimulus response rate (%)**	
AquaMark 100/1	68 ± 1.5	68	6	65 ± 1.8	82	4	61
AquaMark 100/2	69 ± 1.7	79	4	64 ± 1.9	63	3	61
AquaMark 100/3	66 ± 1.6	70	6	63 ± 1.7	69	1	61
Ace Aquatec	65 ± 1.6	67	9	63 ± 1.7	77	3	61
Lofitech	69 ± 2.2	66	6	66 ± 2.1	68	2	62

4.3.2. Comparison of hearing thresholds for AMD sounds with those for tonal signals

In the absence of ambient noise, the detection threshold for AMD sounds is determined by the hearing ability of the receiving animal. We assume that a broadband signal is detected when its 1/3 octave band levels reach or exceed the tonal hearing threshold levels in the corresponding frequency bands. The 1/3-octave spectra of the Sensation Level [SPL averaged over the 90% energy duration of the signals in 1/3-octave bands, minus the harbour seal's 1000 ms tonal 50% hearing threshold levels (Kastelein *et al*., 2009) at the 1/3-octave centre frequencies] of the original recordings by Subacoustech and of the reproduced sounds are shown in **Fig. 34**. Based on these graphs, the hearing threshold for all three AMD signals is predicted to be similar.

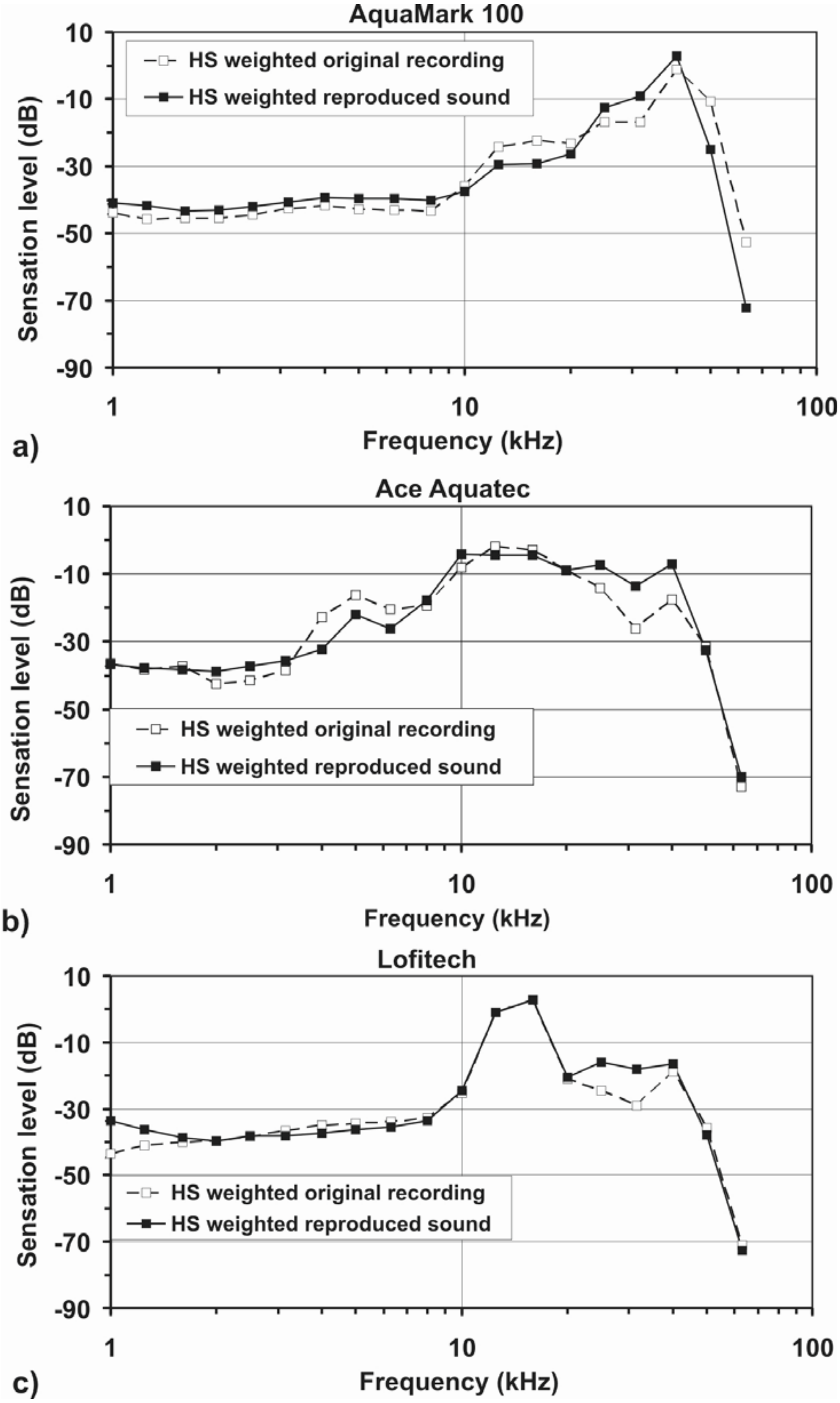


FIG. 34. 1/3-octave spectra of the Sensation Level ('Weighted SPL') of the original recordings by Subacoustech and of the reproduced sounds of the three AMD devices, both scaled to the same broadband level at the observed hearing threshold [Sensation Level = the SPL of the AMD signals averaged over the 90% energy duration of the signals in 1/3-octave bands, minus the harbour seals' 50% hearing threshold levels for 1000 ms tonal signals (Kastelein *et al*., 2009,a,b) at the 1/3-octave centre frequencies]. Note: the time averaged spectra for the three different signals of the AquaMark 100 are identical.

4.4 Behavioural response of harbour seals to AMD sounds

During the baseline periods, the seals surfaced on average 5.7 ± 0.12 m from the transducers. During test periods, pooled for all AMD/level combinations, the distance remained virtually the same (range: 5.7-6.0 m). This was as expected, because the SPL distribution in the pool was homogenous. During the baseline periods the seals spent on average (± SD) 82 ± 0.02 % of scored moments below water, 18 ± 0.02 % with their heads above water, and 0 ± 0.001 % hauled out.

Paired t-tests comparing the parameters in baseline and associated test periods showed that, during sessions with the lowest level sounds, the seals' behaviour was similar during test and baseline periods (**Table 7**). The animals rarely hauled out during the test periods.

The medium sound level of two of the AMDs caused seal 02 to haul out in 2-4% of the scored moments (overall the change was significant in paired t-tests for the AquaMark 100 and Ace Aquatec AMDs), and caused head above water scores to be significantly higher in test, than in associated baseline periods for the Ace Aquatec, and below water scores to be significantly lower in test, than in baseline periods for the Ace Aquatec and Lofitech AMDs (**Table 7**).

The highest received levels caused the seal to haul out in around 10% of scored moments (**Fig. 35**). Head above water scores were significantly higher in test, than in associated baseline sessions for the Ace Aquatec AMD. Below water scores were significantly lower in test, than in baseline sessions for all AMDs (**Table 7**).

FIG. 35. One of the two seals hauled out frequently on the floating platform when the AMD sounds were projected under water at the highest levels.

TABLE 7. Results of paired t-tests to compare behavioural scores in baseline and test sessions, for each combination of AMD and level. The sample size for each combination is five, and exact P-values are shown in bold if significant (P ≤ 0.05).

	AquaMark 100			**Ace Aquatec**			**Lofitech**		
Received level (dB)	117	127	137	109	124	134	128	133	138
Below water	0.282	0.074	**0.009**	0.757	**0.006**	**0.001**	0.480	**0.045**	**0.034**
Head above water	0.385	0.974	0.796	0.951	**0.038**	**0.035**	0.647	0.057	0.941
Hauled out	0.374	**0.028**	**0.003**	0.374	**0.005**	**0.003**	0.109	0.091	0.051

4.5 Estimated distances at which AMD sounds are audible

4.5.1. Estimated distances at which AMD sounds are audible to harbour porpoises at sea

The audibility distance estimates for harbour porpoises (**Table 8**) are approximate, and are accurate only to a factor of two. Under higher background noise levels, the detection distances would be lower. Under better propagation conditions (for instance: in shallow water, of if stratification due to temperature or salinity is present), the detection distances would be higher.

Table 8. Estimated distances at which the three AMDs are audible to harbour porpoises under two background noise level conditions (Sea State 0 and Sea State 4) and based on two propagation models (10 Log R and 20 Log R propagation), for various levels of absorption (dB/m based on the frequency of the AMD sound).

AMD	Audibility distance (km)	
	Background noise: Sea State 0 **Propagation model: 10 Log R**	**Background noise: Sea State 4** **Propagation model: 20 Log R**
AquaMark 100	2	0.2
Ace Aquatec	91	14
Lofitech	91	18

4.5.2. Estimated distances at which AMD sounds are audible to harbour seals at sea

The audibility distance estimates for harbour seals (**Table 9**) are approximate, and are accurate only to a factor of two. Under higher background noise levels, the detection distances would be lower. Under better propagation conditions (for instance: in shallow water, or if stratification due to temperature or salinity is present), the detection distances would be higher.

Signal duration and spectrum change over distance due to reverberations and absorption. Thus, the detection threshold for the AMD sounds used in the present study will also change also over distance.

Table 9. Estimated distances at which the three AMDs are audible to harbour seals under two background noise level conditions (Sea State 0 and Sea State 4), based on two propagation models (10 Log R and 20 Log R propagation), and for various levels of absorption (dB/m based on the frequency of the AMD sounds).

AMD	Audibility distance (km)	
	Background noise: Sea State 0 **Propagation model: 10 Log R**	**Background noise: Sea State 4** **Propagation model: 20 Log R**
AquaMark 100	1.6	0.2
Ace Aquatec	91	14
Lofitech	99	17

4.6 Estimated distances at which AMD sounds elicit behavioural responses

4.6.1. Estimated distances at which harbour porpoises respond to AMD sounds at sea

Based on the mean received SPLs which caused the three behavioural effect levels, the distances from the AMDs at which these effects are estimated to occur in harbour porpoises at sea. Because the conditions vary for each situation, here only two extreme situations are presented: the shortest distances R are based on propagation loss due to spherical spreading ($20 \log_{10} R + \alpha R$) with frequency-dependent absorption loss (αR) according to the expression due to Thorp as modified by Urick (Urick 1983). The longest distances are based on propagation loss due to cylindrical spreading ($10 \log_{10} R + 10 \log_{10} H + \alpha R$) in a water depth H of 20 m and Thorp absorption loss (**Tables 10, 11, and 12**).

The received SPL is calculated, as a function of distance to the AMD, for the two generic propagation loss models, in combination with the 1/3-octave source level spectra of the three AMDs. The source level spectra are obtained by scaling the measured spectra of the reproduced signals to the broadband source levels of the AMDs. The distances at which the behavioural effects are predicted to occur are those at which the calculated broadband received SPL corresponds with the threshold level observed in this study. The calculation only applies to the frequency range 4-63 kHz, because the measured spectra of the AMDs are dominated by background noise in the pool at lower frequencies.

The unmasked sensation levels associated with the behavioural broadband threshold levels (**Tables 10, 11, and 12**) were obtained from the difference between the 1/3-octave band spectrum of the received SPL and the tonal 50% hearing threshold of the harbour porpoise (from Kastelein *et al.*, 2010a) at the 1/3-octave centre frequencies.

TABLE 10. The minimum distances at which sounds from the three AMDs are estimated to cause no behavioural response in harbour porpoises, calculated by means of two generic propagation loss models, for the mean received levels found in the present study just to cause no response.

AMD	**Source Level**	**Mean received level just causing no behavioural response**	**Unmasked sensation level causing no response**	**Estimated minimum distances at which no behavioural effects are expected**	
	SPL@1 m, RMS	SPL, RMS	SPL, RMS	Cylindrical spreading	Spherical spreading
	dB re 1µPa	dB re 1µPa	dB	km	km
AquaMark 100	125	70	22	1	0.3
Ace Aquatec	186	77	20	57	17
Lofitech	193	91	40	24	10

TABLE 11. The distances at which sounds from the three AMDs are estimated to cause a small behavioural response in harbour porpoises, calculated by means of two generic propagation loss models. The calculations are based on the received levels found in the present study to cause the porpoise to surface more frequently.

AMD	**Source Level**	**Mean received level causing a small behavioural response**	**Unmasked sensation level causing small effect**	**Estimated distances at which some behavioural effects are expected**	
	SPL@1 m, RMS	SPL, RMS	SPL, RMS	Cylindrical spreading	Spherical spreading
	dB re 1µPa	dB re 1µPa	dB	km	km
AquaMark 100	125	105	57	<0.02	<0.02
Ace Aquatec	186	117	66	10	2
Lofitech	193	121	72	9	2

TABLE 12. The distances at which sounds from the three AMDs are estimated to cause porpoises to swim away from the sound source, calculated by means of two generic propagation loss models. The calculations are based on the received levels found in the present study to cause the porpoise to move away from the active transducers.

AMD	**Source Level**	**Mean received level clearly causing deterring effect**	**Unmasked sensation level causing clear deterring effect**	**Estimated distances at which porpoises are expected to be deterred**	
	SPL@1 m, RMS	SPL, RMS	SPL, RMS	Cylindrical spreading	Spherical spreading
	dB re 1µPa	dB re 1µPa	dB	km	km
AquaMark 100	125	127	79	<0.02	<0.02
Ace Aquatec	186	139	90	1.2	0.2
Lofitech	193	151	103	0.6	0.1

4.6.2. Estimated distances at which harbour seals respond to AMD sounds at sea

Based on the received SPLs which caused the three behavioural effect levels, the distances from the AMDs at which these effects on harbour seals are likely to occur at sea can be calculated.

Because the conditions vary for each situation, here only two situations are presented as examples: the longest distances *R* are based on propagation loss due to cylindrical spreading; the shortest distances on propagation loss due to spherical spreading (Tables 13, 14, and 15).

TABLE 13. The minimum distances at which sounds from the three AMDs are estimated to cause no behavioural response in harbour seals, as calculated for two propagation models.

AMD	**Source Level**	**Received level just causing no response**	**Unmasked sensation level causing no response**	**Estimated minimum distances at which no behavioural effects are expected**	
	SPL@1 m, RMS	SPL, RMS	SPL, RMS	Cylindrical spreading	Spherical spreading
	dB re 1μPa	dB re 1μPa	dB	km	Km
AquaMark 100	125	117	56	< 0.02	< 0.02
Ace Aquatec	186	128	67	4.2	0.6
Lofitech	193	109	48	13.8	4.5

TABLE 14. The distances at which sounds from the three AMDs are estimated to cause a small behavioural response in harbour seals, calculated for two propagation models. The calculations are based on the received levels found in the present study, which caused one seal to haul out occasionally.

AMD	**Source Level**	**Received level causing small behavioural effect**	**Unmasked sensation level causing small effect**	**Estimated distances at which some behavioural effects are expected**	
	SPL@1 m, RMS	SPL, RMS	SPL, RMS	Cylindrical spreading	Spherical spreading
	dB re 1μPa	dB re 1μPa	dB	km	Km
AquaMark 100	125	127	66	<0.02	<0.02
Ace Aquatec	186	133	72	2.6	0.4
Lofitech	193	124	63	7.5	1.7

TABLE 15. The distances at which sounds from the three AMDs are estimated to cause a strong behavioural response in harbour seals (probably deterring them at sea), calculated for two propagation models. The calculations are based on the received levels found in the present study, which caused one seal to haul frequently (10 % of the time).

AMD	**Source Level**	**Received level probably causing deterring effect**	**Unmasked sensation level probably causing deterring effect**	**Estimated distances at which seals are expected to be deterred**	
	SPL@1 m, RMS	SPL, RMS	SPL, RMS	Cylindrical spreading	Spherical spreading
	dB re 1μPa	dB re 1μPa	dB	km	km
AquaMark 100	125	137	76	<0.02	< 0.02
Ace Aquatec	186	138	77	1.4	0.2

Lofitech	193	134	73	4.1	0.7

5. Discussion

5.1. Evaluation of the harbour porpoise AMD audibility study

The AMD audibility study was conducted with only one porpoise. However, the masked hearing thresholds of the study animal were similar to those of another porpoise tested the year before the present study (Kastelein *et al*., 2009), and the study animal's basic audiogram for 1500 ms signals was the same as that of another porpoise when corrected for the use of FM signals (Kastelein *et al.,* 2002; 2010). This suggests that the present study animal had normal hearing. Therefore, the thresholds found in the present study for the AMD sounds are probably representative for young adult harbour porpoises.

The comparison of the 1/3-octave spectra of the original recordings and the Sensation Level of the reproduced sounds showed that the hearing threshold for all three AMD signals would be similar. However, for unknown reasons, the actual measured hearing threshold levels of the porpoise were 6-7 dB higher than the predicted levels. This suggests that the assumption that the porpoise's hearing behaves as an energy detector is only partially valid.

The 50% hearing threshold was measured for an attentive porpoise listening for a familiar signal, in the direction of maximum hearing sensitivity (sound coming from in front of the porpoise; Kastelein *et al*., 2005). The hearing distances would probably be lower for inattentive porpoises and for sounds coming from other directions.

5.2. Evaluation of the harbour porpoise AMD behavioural response study

Although habituation was not quantified, the effect of the AMD sounds did not appear to diminish during test sessions. After each session, the animal's behaviour returned to normal immediately. He co-operated in a psycho-acoustic test only minutes after the AMD signals had ceased. So being exposed to the AMD signals (at the playback levels used in this study) for 30 minutes had no lasting effect on the animal's behaviour. A quick return to baseline behaviour had been seen in previous acoustic alarm (pinger) studies with harbour porpoises (Kastelein *et al*., 2000, 2001, 2006 and 2008) and was our reason for not including a post-test observation period, as was done in a previous pinger study (Kastelein *et al*., 2000). Maybe if much higher received levels were used, the porpoise's behaviour would not have returned so quickly to the baseline level. In nature, short-term disturbances have also been observed not to displace harbour porpoises for long. At a fish aquaculture cage site, the presence of the cages and workers did not appear to displace harbour porpoises from the area except during short intervals when high disturbance activities, such as a food delivery by barge or cage cleaning using high pressure hoses, were occurring. After these activities ended, porpoises were typically observed in the vicinity within 5 to 10 min (Haarr *et al*., 2009).

Previous behavioural avoidance studies with harbour porpoises also provide evidence for avoidance threshold levels. ACME signals in the 8-18 kHz range produced avoidance thresholds SPLs of 97 to 111 dB re 1 µPa (Kastelein *et al*., 2005a), 50 kHz tonal signals of 108 dB re 1 µPa (Kastelein *et al*., 2008a), and 70 kHz tonal signals of 124 to 130 dB re 1 µPa (Kastelein *et al*., 2008b). Estimated corresponding sensation levels (based on a tonal audiogram; Kastelein *et al*., 2010) are respectively approximately 54, 59, and 77 dB. These are in the same range as the sensation levels causing a small behavioural response in the present study; the thresholds we found for stronger deterring effects were 15-30 dB higher.

There is no direct relationship between the audibility of AMD sounds and the behavioural responses they elicit. However, this study has shown that audibility can be estimated roughly from the difference of the signal's 1/3-octave band spectrum and the tonal audiogram. Based on this, we suggest that thresholds should be presented in terms of the 'sensation level' at which behavioural effects occur. This is comparable to the use of the A-weighting to give a rough indication of 'loudness' of airborne noise to humans.

5.3. Evaluation of the harbour seal AMD audibility study

Because both seals were tested within the same sessions, any differences in AMD sound thresholds obtained for the two animals were probably due to differences in the seals' hearing sensitivity or motivation, and were not caused by differences in equipment, equipment settings, methodology, personnel, or background noise. The hearing thresholds of both seals were similar (differences 2-5 dB), so the results of the present study can be assumed to be representative for young adult harbour seals with good hearing.

The comparison of the 1/3-octave spectra of the original recordings and the Sensation Level of the reproduced sounds showed that the hearing threshold for all three AMD signals would be similar. However, for unknown reasons, the actual measured hearing threshold levels of the harbour seals were 2-7 dB higher than the predicted levels. This suggests that the assumption that the seal's hearing behaves as an energy detector is only partially valid.

The 50% hearing threshold was measured for an attentive seal listening for a familiar signal, in the direction of (assumed) maximum hearing sensitivity (sound coming from in front of the seal). The hearing distances would probably be lower for inattentive seals and for sounds coming from other directions.

5.4. Evaluation of the harbour seal AMD behavioural response study

The study was conducted with only two animals. Their hearing was probably representative for harbour seals of their age; for tonal signals and 1/3-octave noise bands it was similar between the two animals (Kastelein *et al.*, 2009a,b; 2010a).

The fact that seal 02 hauled out more frequently than seal 01 could be due to her higher hearing sensitivity for the AMD sounds (seal 02's hearing thresholds for the AMDs were on average 3 dB lower [i.e., her hearing was more sensitive] than those of seal 01; section 4.3), or due to her higher level of anxiety. Individual differences may be expected in behavioural responses to sounds, and were also observed in belugas (*Delphinapterus leucas*) in a pool subjected to playbacks of offshore oil drilling noise (Thomas *et al*., 1990), and in harbour seals in response to underwater data communications signals (Kastelein *et al*., 2006 a). This suggests that this type of study should be conducted with as many individual animals as possible, to obtain a representative range of behavioural responses.

The results from the present study can be applied to young adult male and female harbour seals, as the species has limited sexual dimorphism. It is impossible to say whether the results from the harbour seals are also applicable to grey seals (*Halichoerus grypus*). The best way to find out would be to establish the audiogram of grey seals for tones. If the hearing thresholds of the grey seal are similar to those of the harbour seal, the detection ranges of grey seals for AMDs are probably similar to those of harbour seals. The effect of the AMDs on the behaviour of grey seals probably differs from that of harbour seals, as the general behaviour of grey seals is very different from that of harbour seals. In addition, the grey seal is highly sexually dimorphic, and the sexes differ greatly in their behaviour.

The hearing thresholds of the seals for the AMD sounds were tested before and sometimes immediately (the first animal within 5 min, the second animal after 15 min) after the 30-minute exposure to the AMD sounds (all three received levels), and no hearing threshold shift was measured. A temporary hearing threshold shift (TTS) is a mechanism to reduce the perceived level of long duration, loud, sounds. Even the highest received level in the present study did not lead to noticeable TTS in the seals, unless the recovery time was less than 5 min. The seals' immediate participation in the audibility study (see section 3.4) after the sessions also showed that there were no lingering behavioural effects of the 30-minute sound exposure; the seals' behaviour was observed to return to normal very quickly.

Due to the characteristics of the pool, no SPL gradient occurred when the AMD sounds were produced, and the variation between the 33 measurement locations was very small for the AquaMark 100 and Ace Aquatec (SD 1 dB), and only slightly greater for the Lofitech (SD 4 dB). This even SPL distribution probably explains why the seals did not move away from the sound source, as the received level was the same throughout the body of water.

Previous behavioural response studies with harbour seals also provide evidence for avoidance threshold levels. ACME signals in the 8-18 kHz range produced avoidance thresholds SPLs of 107 dB re 1 µPa (Kastelein *et al*., 2006a), 8 kHz tonal signals of 130 dB re 1 µPa, 16 kHz tonal signals of 119 dB re 1 µPa, 32 kHz signal of 119 dB µPa, and 45 kHz signals of 129 dB (Kastelein *et al*., 2006b). Estimated corresponding sensation levels (based on the seals' tonal audiogram; Kastelein *et al*., 2009a,b) are respectively approximately 49, 72, 56, 56, and 61 dB. These are in the same range as the sensation levels causing small behavioural responses in the present study; the thresholds we found for stronger behavioural responses were 18-27 dB higher.

There is no direct relationship between the audibility of AMD sounds and the behavioural response they elicit. However, this study has shown that audibility can be estimated roughly from the difference of the signal's 1/3-octave band spectrum and the tonal audiogram. Based on this, we suggest that behavioural avoidance thresholds should be presented in terms of the 'sensation level' at which behavioural responses occur. This is comparable to the use of the A-weighting to give a rough indication of 'loudness' of airborne noise to humans.

5.5 Implications for environmental management

The present research allowed the calculation of deterring distance ranges of the three AMDs for harbour porpoises and harbour seals under various conditions. However, in order to determine if the AMDs tested in the present study can deter harbour porpoises and harbour seals far enough away from pile driving sites to prevent hearing damage (permanent hearing threshold shift; PTS) or temporary hearing loss (temporary hearing threshold shifts; TTS), and how they should be used, the following information is needed:

1) The received level and duration combinations of pile driving noises which cause TTS in harbour porpoises and harbour seals (SEAMARCO has been commissioned by the Dutch government to carry out research on this between September 2010 and April 2012 for harbour porpoises, and between July 2010 and April 2011 for harbour seals).
3) The received level and duration combinations of pile driving noises which cause PTS in harbour porpoises and harbour seals. This can be calculated from the TTS level/duration combination (by extrapolation from data on terrestrial mammals).
4) The required safety distance from pile driving activities at which harbour porpoises and harbour seals should be deterred, which can be calculated from the Source Level of a pile driving strike, the propagation conditions, the background noise level, and the estimated PTS level/duration combination for each of the two species.
5) A comparison of the required safety zones for pile driving noise with the deterring zones of the tested AMDs.
6) The time porpoises and seals need, after the onset of AMD signals, to swim far enough away before the higher amplitude pile driving strikes begin.
7) The time-frame within which porpoise and seal behaviour returns to normal after pile driving ceases, so that, during lulls in pile driving activities, AMDs can be used if necessary to prevent porpoises and seals from entering the danger area.

Alongside the source level, the operational characteristics and use of AMDs are important factors in their efficacy. Harbour porpoises and harbour seals are unlikely to habituate to, and thus ignore, AMD sounds if they are presented for only a few hours before the onset of pile driving. Kastelein *et al*. (2006) noted that seals did not habituate to daily 45-minute presentations of high amplitude sounds over a period of 40 days. Conversely, Jacobs and Terhune (2002) observed that wild harbour seals continuously exposed to acoustic harassment device (AHD) sounds for weeks or months in a row did not react when an AHD (at a lower amplitude than usual) was activated.

5.6 Future studies and improvements in design

5.6.1. Acoustics

Most of the energy of the AMD sounds was around 40 kHz, but each sound also contained some energy in the ultrasound range above 40 kHz, which could not be reproduced with the available sound cards. The sounds were not made available to SEAMARCO in time for alternative sound cards to be purchased. For harbour seals, which have sensitive hearing up to 40 kHz, the available sound cards were suitable. The playbacks to harbour porpoises of the AquaMark 100 sound were slightly constrained by the sound cards used. However, most of the energy in the spectra of all 3 AMD sounds was below 44 kHz, and at 44 kHz, the porpoise audiogram levels off, which means that higher parts of the spectrum would need to contain as much energy or more than the energy at 44 kHz to have an impact. This is not the case. To avoid this limitation, future studies of the effects of AMD sounds containing ultrasonic components on harbour porpoises should make use of sound cards suitable for sounds up to 140 kHz.

The AMDs were selected by Subacoustec, without input from SEAMARCO, and the selection criterion was not the sounds' potential efficiency in deterring harbour porpoises and harbour seals, but the willingness of the AMDs' manufacturers to make their products available for free for the project. Therefore, other, more effective, AMDs may be commercially available, and should also be tested.

AMDs are not acoustical devices developed for scientific research, but practical devices for use at sea. Even within AMDs from the same production series, some variation in output is likely.

Therefore, the differences between the original recordings made by Subacoustech and the reproduced sounds are probably smaller than acoustical differences between individual AMDs of one type. The margins applied by the manufacturers for the specifications of the AMDs are unknown, and wind park developers should check the output of AMDs before using them.

5.6.2. Test environment

Conducting the two behavioural response studies in pools had advantages: the background noise could be controlled and was very low, and the animals' behaviour could be filmed 100% of the time. The disadvantage was that only small SPL gradients could be achieved in the pools. The gradient depends in part on the frequency of the sounds: the higher the frequency, the steeper the gradient. Because of the small gradient, instead of offering one received level to the animals it was decided to determine three received levels during pre-tests, thus allowing a gradient in responses to be demonstrated. Future behavioural response studies could arguably better be conducted in larger water bodies where a gradient could be achieved, but a trade-off occurs: in large water bodies (e.g., lagoons and harbours), controlling the background noise and recording the animals' behaviour would become challenges.

5.6.3. Animals

Previous, recent studies had established that the young adult study animals had excellent hearing, so that the small sample sizes available (one porpoise and two seals) were considered sufficient for hearing threshold studies. For this type of study small sample sizes are deemed acceptable in part because of the time needed to train individuals to participate. However, behavioural response studies should, ideally, be conducted with as many animals as possible, as responses to acoustic stimuli vary between individuals. This was illustrated by the present research: one of the seals regularly hauled out at the highest received levels of all three AMD sounds, whereas the other did not.

Behavioural response studies on more than one animal, such as the harbour seal study, could be further improved by making each test animal recognisable even from video recordings. This could be achieved by marking the animals in some way.

5.6.4. Future studies

Based on the results of the present study, the following future actions are recommended:

1) Conduct similar studies on other commercially available AMDs (selected based on the acoustic specifications).
2) Test hearing thresholds for recordings made at longer distances from the AMDs, to allow the determination of transmission distance effects on hearing thresholds. The spectra of the reproduced AMD sounds that were offered to the porpoise and seals were based on original recordings made at a distance of 1 m from the AMDs. As the distance between an AMD and an animal increases, the energy in the high frequency components of the signals is reduced by attenuation more than that in the low frequency components. In addition, the multipath arrival pattern of sound is different, and more variable, under natural circumstances at sea than in a pool. Detecting sounds at some distance depends not only on the level, but also on the nature of the sounds. For the Ace Aquatec in particular, because of multipath arrival times, the 18 very short pulses produced over <0.5 s would merge into a single pulse with a duration >0.5 s.
3) Determine the received level and duration combinations of pile driving noises which cause TTS in harbour porpoises and harbour seals. SEAMARCO has already been commissioned by the government of the Netherlands to carry out this research.

6. Conclusions

6.1. Achievement of original and extra objectives

The four objectives listed in the original research proposal to COWRIE were met. In addition, four extra objectives were achieved, as can be seen in **Table 16**.

TABLE 16. The objectives and results of this research.

Objectives in proposal to COWRIE	**Results**
Determine hearing thresholds of a harbour porpoise for 5 AMD sounds	Achieved for one porpoise
Determine hearing thresholds of harbour seals for 5 AMD sounds	Achieved for two seals
Determine avoidance threshold levels (behavioural response) of a harbour porpoise for 3 AMD sounds	Achieved for one porpoise for three received levels
Determine avoidance threshold levels (behavioural response) of harbour seals for 3 AMD sounds	Achieved for two seals for three received levels
Extra objectives	**Extra results**
Estimate distance at which harbour porpoises can hear AMD sounds	Achieved for two conditions
Estimate distance at which harbour seals can detect AMD sounds	Achieved for two conditions
Estimate distance at which AMDs elicit behavioural responses in harbour porpoises	Achieved for two conditions for three received levels
Estimate distance at which AMDs elicit behavioural responses in harbour seals	Achieved for two conditions for three received levels

6.2. AMD audibility studies

The following conclusions can be drawn from the AMD audibility studies:

1) The distance at which porpoises and seals were able to hear the AMD sounds varied greatly between the three AMDs. The AquaMark 100, which was designed for dolphins, could not be heard from as great a distance as the other two AMDs, because it had a lower source level and operated at higher frequencies, which are more readily attenuated than lower frequencies.
2) The audibility distance for a given AMD's sound may vary by a factor of 6 or more, depending on the conditions (propagation conditions and background noise).
3) The hearing threshold of both species for AMDs is so low, that detection is mainly dependent on the background noise level (for the harbour porpoise, the hearing thresholds at frequencies above 2.5 kHz are below the background noise level of that during Sea State 0; for the harbour seal, hearing thresholds in the 200 Hz-10 kHz range are below the background noise level of Sea State 0).

6.3. AMD behavioural response studies

The following conclusions can be drawn from the AMD behavioural response studies:

1) The AquaMark 100, which was designed for dolphins, with its normal source level at least, is unlikely to be effective at deterring harbour porpoises and harbour seals. The two other AMDs (Ace Aquatec and Lofitech, which were designed for seals) have the potential to deter harbour porpoises and harbour seals at useful distances: provided that there is no attraction (such as a food source) in the immediate area of the AMD, porpoises and seals are expected to move away.

2) When AMDs are used at sea, variation in environmental variables leads to high levels of variability in response distances. For this reason, distance ranges, rather than exact distances, at which each AMD elicits a particular response, should be considered when planning mitigation measures.

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