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SEAMARCO final report 2018-01
February 2018

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Hearing thresholds, for underwater sounds, of harbor seals (*Phoca vitulina*) swimming at the water surface

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Abstract:

High amplitude impulsive sounds produced by pile driving and airguns may result in hearing damage in nearby seals. By swimming close to the surface, seals may reduce their exposure to underwater sound, as sound pressure levels (SPLs) are often lower just below the surface. There is evidence that seals can make physiological adjustments such that they can switch between having maximum sensitivity for either aerial or underwater sounds. This could mean that hearing sensitivity for underwater sound might be reduced when swimming at the water surface (when hearing might be focused on aerial sounds) compared to when swimming at depth, which - if confirmed - might reduce the perceived sound level still further. To investigate this possibility, hearing thresholds of two female harbor seals for 11 narrow-band sweeps (center frequency range: 0.125 – 63 kHz) were quantified psychophysically, while their heads were in the position normally adopted while swimming at the surface. The seals' water surface hearing thresholds were similar to each other (mean difference = 2.3 ± 2.2 dB) and to previous measurements (mean difference of 3.5 ± 2.8 dB) made at 1 m depth. When calculating the cumulative sound exposure level for hearing damage assessment, the SPL just below the water surface needs to be measured, or modeled, and the proportion of time seals normally swim at the water surface needs to be estimated, in order to estimate the sound energy that reaches the seals' ears.

I. INTRODUCTION

The harbor seal (*Phoca vitulina*) has the most extensive geographical range of all seal species. It inhabits the eastern Baltic Sea as well as both eastern and western coasts of the North Atlantic and North Pacific Oceans. It leads an amphibious life, resting and pupping on land, but migrating, foraging and performing courtship underwater (Burns, 2002).

Impulsive sounds have been produced by offshore human activities for many decades (Hersey, 1977), first by explosions and later airgun arrays (Krail, 2010) used for geophysical prospecting, and more recently by percussive pile driving used for offshore construction, especially of wind farms. During the last decade, offshore wind farms have been built in relatively shallow water in some of the areas occupied by harbor seals. So far, most wind farms have been built by using percussion pile drivers to drive large monopiles into the substrate, which can continue for up to ~4 hrs for one monopole. This piling activity produces high-amplitude sound, both in the air and under water. Harbor seals can hear both in air (Møhl, 1968, Terhune, 1991; Wolski *et al*., 2003; Reichmuth *et al*., 2013) and under water (Møhl, 1968; Terhune, 1988, 1989; Turnbull and Terhune, 1990; Kastak and Schusterman, 1998; Southall *et al*., 2005; Kastelein *et al*., 2009a, b, 2010; Reichmuth *et al*., 2013).

Underwater sounds propagate over larger distances than aerial sounds, and impulsive sounds from offshore percussion pile drivers (Source level of 226 dB re 1 µPa at 1 m; Bailey et al., 2010) can potentially be heard by seals at distances of hundreds of kilometers from the sound source, depending on the circumstances (Kastelein *et al*., 2013). However, exposure to high amplitude pile driving sounds (nearer to the sound source) can cause temporary or permanent hearing threshold shift (TTS, PTS) in harbor seals (depending on the exposure level and duration). As seals are amphibious, they may be able to keep their heads out of the water while fleeing from areas with intense underwater sound, in order to reduce the received level of the underwater sound. However, Kastelein *et al*. (2012) showed that the same amount of TTS was caused when the seals were exposed simultaneously to loud underwater sound and to aerial sound (1-octave noise band centered at 4 kHz) as when they were only exposed to the underwater sound. This suggests that when the seals swam at the water surface, underwater sound reached the middle and inner ears via tissue conduction, in the same way as when the seals were fully submerged.

Earlier studies of the hearing of phocids in air have shown that their thresholds are higher (indicating less sensitive hearing) than those of humans and terrestrial mammals (Møhl, 1968; Terhune and Ronald, 1971; Terhune, 1991; Turnbull and Terhune, 1993). In these studies, the seals were swimming in a small tank or net pen in a harbor and rising out of the water momentarily to listen to the aerial test sounds. Three researchers have reported in-air hearing thresholds for harbor seals (Reichmuth *et al*., 2013), spotted seals (*Phoca largha*; Sills *et al*., 2014) and ringed seals (*Pusa hispida*; Sills *et al*., 2015) that were similar to human hearing threshold levels. The studies were conducted in a quiet hemi-anechoic chamber and the seals were on land for long periods, suggesting that it takes some time for the hearing to switch from the aquatic mode to the aerial mode. The discrepancies between the two sets of measurements (an animal coming just from the water for an in-air hearing test, compared to an animal spending the entire hearing session out of the water inside an hemi-anechoic chamber) may be related to anatomical features of the outer and middle ears of seals, and to the related acoustic impedance differences between fully adapted underwater and in-air sound reception systems (Terhune, 1991; Sills *et al*., 2015). An air-adapted mammalian hearing system receives sound via the middle ear apparatus, which matches the acoustic impedance of sound travelling through air to the impedance of the fluid-filled cochlea (Møller, 1972). A mammalian underwater-adapted hearing system presumably has an acoustic impedance equal to that of water. The differences in the acoustic impedances of air and water are such that a

30 dB transmission loss is expected across an air-water or water-air interface (Møhl, 1968). The in-air thresholds of harbor seals were 15-32 dB higher than their underwater thresholds when sensitivity was compared in terms of their equivalent plane wave intensity level (EPWIL, in dB re 1 μW/cm^2) (Møhl, 1968; Terhune, 1991, 1993; Turnbull and Terhune, 1993). These differences were attributed to an acoustical impedance mismatch of a putative water-adapted hearing system receiving sound through air. The walls of the external auditory meatus and middle ear structures of seals contain extensive cavernous sinuses which can reduce the volume of the enclosed air spaces by becoming engorged with blood (Møhl, 1967; Ramprashad *et al*., 1973). This action may help maintain equal pressure on each side of the tympanic membrane during diving. While the blood sinuses are engorged, the impedance matching function (air to water) of the middle ear system may be interrupted and the external opening and lumen of the external auditory meatus may be closed, thus adapting the seal to receiving sound through water. To switch to a greater sensitivity while listening in air, the external auditory meatus has to be opened up and the blood sinuses drained to permit the full impedance matching transformer function of the middle ear. A cochlear microphonic study of harp seals *(Pagophilus groenlandicus)* showed that underwater sensitivity sometimes suddenly decreases as the seal's nose reaches or approaches the surface (Møhl and Ronald, 1975). This suggests that the seal may be able to switch between underwater and in-air hearing modes. The elevated in-air thresholds of harbor seals in earlier studies (Terhune and Ronald, 1971; Terhune, 1991) may have resulted from the subjects not having had time, during testing, to switch over from an underwater mode to an in-air mode. If the seals adjust the blood volumes in the outer and middle ear sinuses to facilitate receiving sound through air, the reception of sound under water may be compromised.

In environmental impact assessments of activities that produce loud impulsive underwater sounds, an estimate of the potential hearing damage to marine animals is often required. If the exposure to underwater sound at the surface would be reduced by the seals having their heads half out of the water during swimming at the surface, a smaller impact should be expected. The aim of the present study was to test the hearing sensitivity of harbor seals for underwater sounds while their heads were in the position normally adopted during swimming at the water surface (i.e., heads half out of the water, so that the auditory meatal orifices are above the water surface). The resulting "water surface audiogram" will be compared with the fully submerged underwater audiograms (at 1 m depth) of the same animals for the entire frequency hearing range.

II. MATERIALS AND METHODS

A. Study animals

The study animals were two female harbor seals (ID nos. 01 and 02), which were born at Ecomare (The Netherlands), and were moved to the SEAMARCO Research Institute (The Netherlands) soon after they had been weaned. During the study they were healthy, 8 years old, and their body weight varied between 40 kg (in summer) and 65 kg (in winter). The animals had participated in several previous psychophysical hearing studies, and had sensitive and very similar hearing thresholds, and which remained unchanged in the years they were measured (Kastelein *et al*., 2009 a, b; 2010; 2012; 2013). The seals consumed thawed fish divided into four meals per day, three of which were given during research sessions. Variation in the animals' performance was minimized by making weekly adjustments (usually in the

order of 100 g) to their daily food ration, based on their weight and performance during the previous week, and the expected change in water and air temperatures in the following week.

B. Study area and staff

The study was conducted at the SEAMARCO Research Institute, which is in a remote area that was specifically selected for acoustic research. The measurements were conducted in an outdoor pool (8 m x 7 m, 2 m deep) with an adjacent haul-out platform (**Fig. 1**). To reduce sound reverberation in the pool (for signals above 25 kHz), the inner walls were covered with 3-cm-thick mats of coconut fiber embedded in 4-mm-thick rubber. The coconut mats extended 10 cm above the water level to reduce splashing noises caused by waves. The bottom of the pool was covered with a layer of sand approximately 20 cm thick. Skimmers kept the water level constant, and seawater was pumped in directly from a nearby lagoon of the North Sea. Most of the water (80%) was re-circulated daily through a biological filter system to ensure year-round water clarity, so that the animals' behavior could be observed via underwater cameras during the test sessions.

To limit the amount of noise that the seals were exposed to on a regular basis, the water circulation system and the aeration system for the bio-filter were designed to be as quiet as possible. This was done by choosing low-noise water pumps, mounting the pumps on rubber blocks, and connecting the pumps to the circulation pipes with very flexible rubber hoses. There was no current in the pool during the experiments, as the water circulation pump and the air pump of the bio-filter were switched off at least 10 minutes before test sessions, and were off during sessions. This also reduced flow noise from the skimmers. The water temperature varied between 3 °C in January and 21 °C in July, and the salinity was around 3.4 %.

During the 15-minute hearing test sessions, the animal not being tested was trained to keep very still on the haul-out platform (not in the water, as any waves may increase the hearing threshold of the test animal). Fish were given to the non-test animal when the test animal was rewarded, to prevent distraction during the trials. The signal operator and the equipment used to produce the stimuli and listen to underwater sounds were in a research cabin next to the pool, out of sight of the animals (**Fig. 1**).

Top view
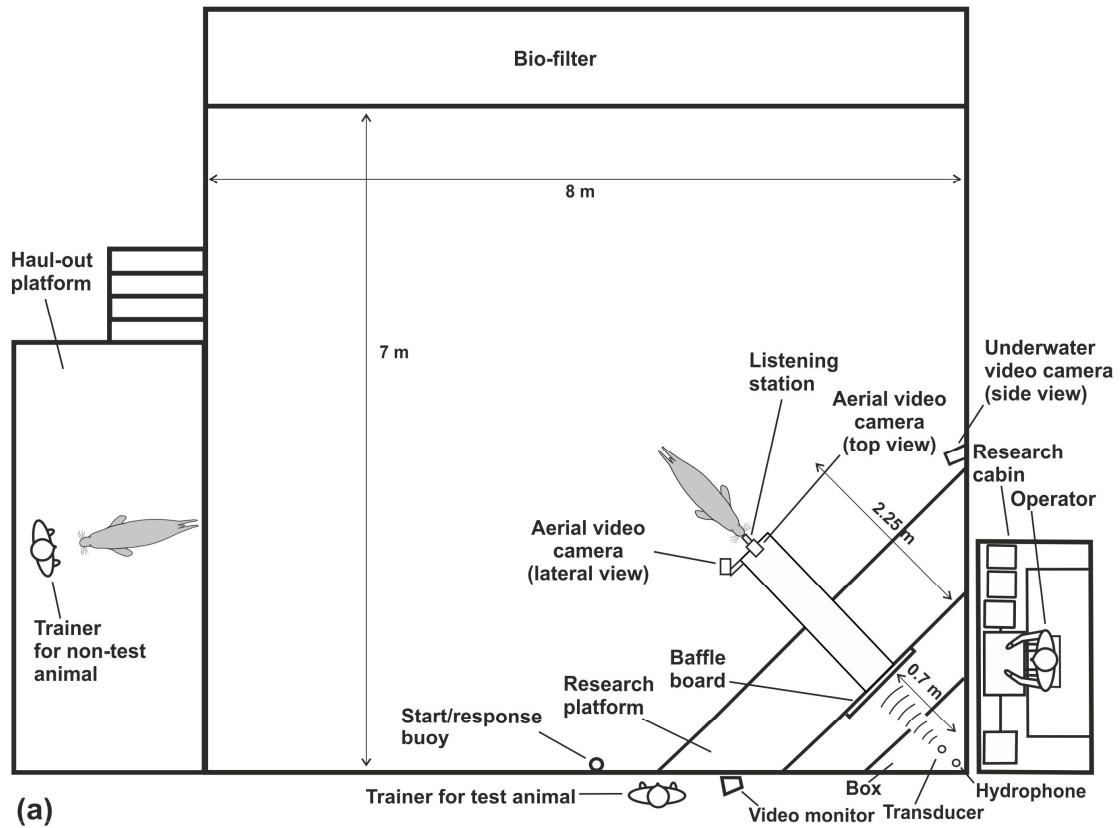
(a)

Side view
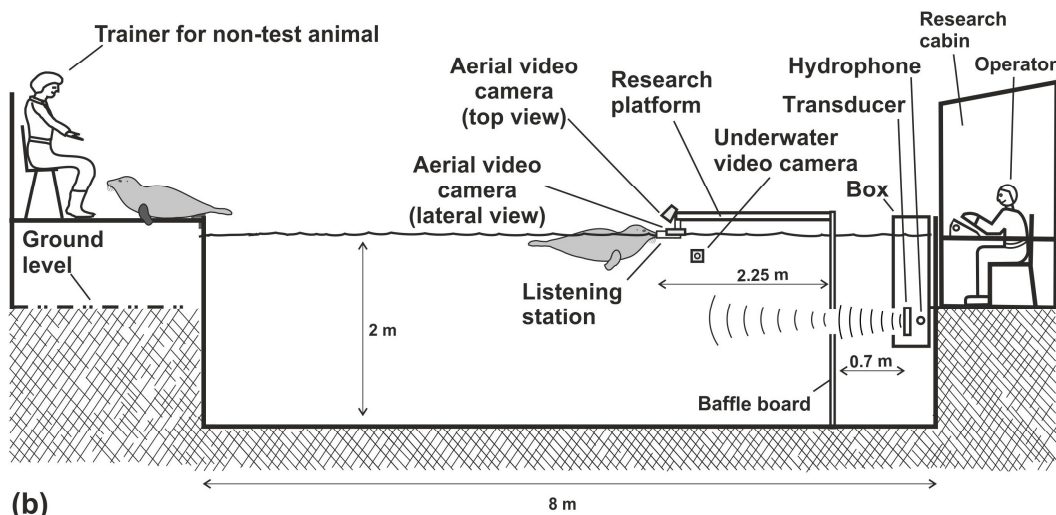
(b)

FIG. 1. The study area, showing the harbor seal being tested with her head in position at the water surface near the listening station, and the animal not being tested with the other trainer on land; (a) top view and (b) 45° angle side view, both to scale.

C. Hearing test stimuli level calibration and background noise measurement

A schematic of the equipment used to configure and emit outgoing signals is shown in **Figure 2**. Narrow band up-sweeps (linear frequency modulated tones) were used as hearing test signals instead of pure tones, because sweeps lead to very stable and precise thresholds. The hearing test signals were generated digitally (Adobe Audition, version 3.0; sample rate: 768 kHz). The linear up-sweeps started and ended at ± 2.5% of the center frequency, and had durations of 1s, including a linear rise and fall in amplitude of 50 ms. Eleven sweeps with center frequencies: 0.125, 0.25, 0.5, 1, 2, 8, 16, 32, 40, 50 and 63 kHz were used to test the seals' hearing. The WAV files used as hearing test signals were played on a laptop computer (Acer Aspire 5750 Model P5WEO) with a program written in LabVIEW, to an external data acquisition card (NI - USB6251), the output of which was controlled in 1 dB steps with the LabVIEW program. The output of the card went through a ground loop isolator, a custom-built buffer, a passive second order low-pass filter (set on 1 kHz, 12 dB/octave) and an attenuator, and drove three transducers: 1) via a digital active filter (Krohn-Hite, Model 3326) and an amplifier (HLLY, Model M-1), a low frequency moving coil transducer (Underwater Sound Reference Division**,** Model J-11; 0.125, 0.25, and 0.5 kHz), 2) through an isolation transformer (Lubell, Model AC202) to a balanced tonpilz piezoelectric acoustic transducer (Lubell, Model LL916; 1- 8 kHz), and 3) an omni-directional transducer in the horizontal plane (Edo Western, Model 337; 16-63 kHz). The sound pressure level (SPL) in water at the harbor seal's head while it was at the listening station was varied by the operator in 2 dB increments.

Multi-path arrivals and standing waves can introduce both temporal and spatial variations in the observed SPL at the listening station. Therefore, the transducers were placed in the eastern corner of the pool in a protective wooden box, lined with rubber with an irregular surface area. The transducers were hung from cords, and made no contact with the box. The transducers were 295 cm from the tip of the listening station (**Fig. 1**). To reduce reflections from the bottom of the pool and water surface reaching the listening station (so reflections could not interfere with the sounds coming to seals in the direct path), 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 center at the same level as the transducer (1 m below the water surface).

The background noise was measured and the hearing test signals were calibrated twice during the study period. The sound measurement equipment consisted of two hydrophones (Bruel & Kjear (B&K), Model 8106) with a multichannel high frequency analyzer (B&K PULSE, Model 3560 D), and a laptop computer with B&K PULSE software (Labshop, version 12.1). The system was calibrated with a pistonphone (B&K, Model 4223). The SPL (dB re 1 μPa; ANSI, 1994) of each hearing test signal was derived from the received 90% time-integrated squared sound pressure, divided by the corresponding 90%-time duration (Madsen, 2005).

The received SPL of each hearing test signal was measured at the position of the harbor seal's head during the hearing tests. The calibration measurements were conducted with two hydrophones, one at the location of each auditory meatus of the harbor seal when it was positioned at the listening station (6 cm below the water surface). The SPL at the two locations differed by 0 to 3 dB, depending on the test frequency. The average SPL of the two hydrophones was used to calculate the stimulus level during hearing threshold tests (as the presence of the seal at the listening station may alter the sound field) The received SPLs were calibrated at levels of approximately 15 dB above the threshold levels found in the present

study. The linearity of the transmitter system was checked during each calibration and was found to be consistent to 1 dB within a 20 dB range.
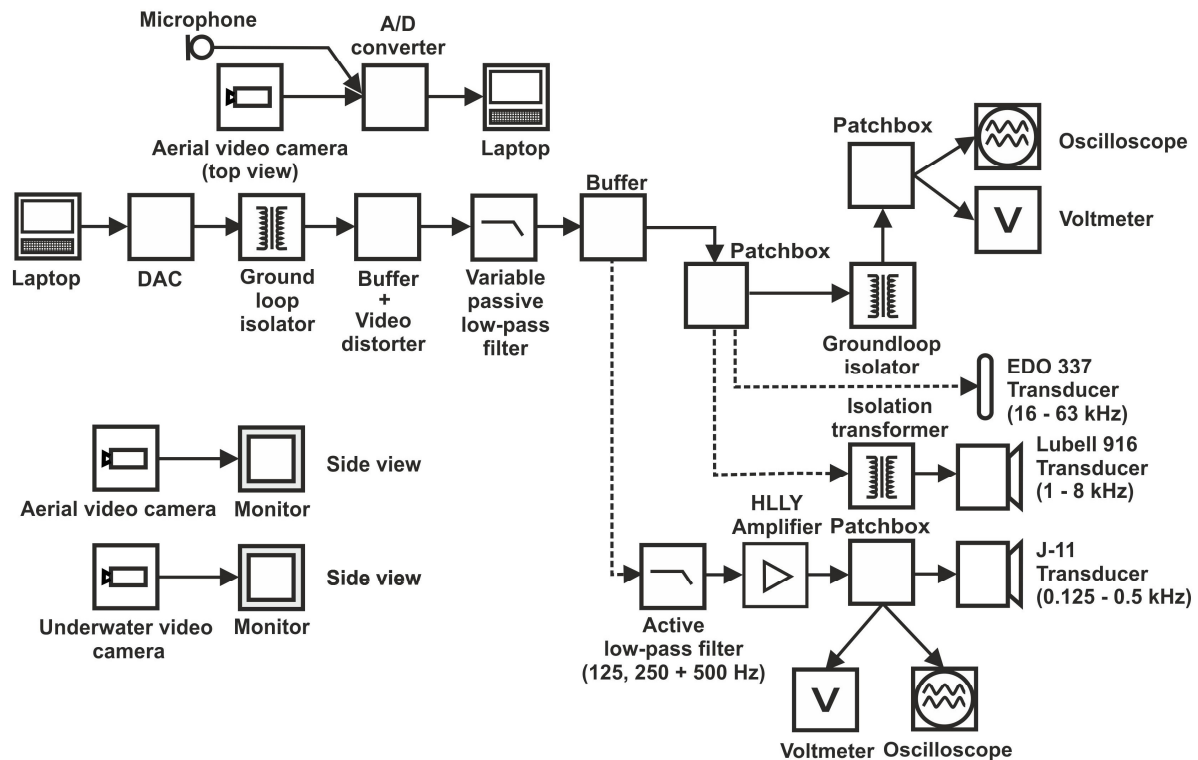
FIG. 2. Block diagram of the transmitting, listening and video systems.

Audiograms are easily influenced by background noise in the test area. Therefore, apart from noise reducing features of the seal facility during the construction phase, 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 under the same conditions as during the test sessions (i.e., in various weather conditions, but without rain, and with Beaufort wind force of ≤ 2, as the pool was outdoors).

One-third octave band background noise sound pressure levels were determined in the range 25 Hz - 160 kHz. The general background noise in the pool was very low (in the low frequency range it was below that of Sea State 0).

E. Experimental procedure and analysis

Once a week, the acoustic equipment producing the stimuli was checked with a spectrum analyzer (Velleman PCSU 1000) to ensure that it was functional and that the stimuli produced were identical to the calibrated values. Also, the background noise level was checked before each session to ensure it was not too high for testing.

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 animal not being tested was on the platform with one trainer, and the seal to be tested was positioned with its head at the start/response buoy at the edge of the pool next to the research trainer (**Fig.1 a**). When the trainer gave the animal being tested a vocal command accompanied by a gesture (pointing downwards), the animal swam to the listening station (an L-shaped, 32 mm-diameter, water-

filled polyvinylchloride tube with an end cap), so that its external auditory meata were 4 cm above the water surface and its inner ear was approximately 310 cm from the sound source (**Fig. 1 b, 3b**).

The test animal's position could be viewed by means of three video cameras. One aerial camera was attached to the listening station to view the head from above; the other aerial camera was attached to the listening station to the right of the animal's head, to view the head from the side (**Fig. 1**). One underwater camera was attached to the pool wall to view the horizontal position of the seal's entire body. The image from above was visible to the trainer near the start/response buoy (the trainer was out of the animal's view when it was at the listening station) and the images of all three cameras were visible to the operator in the research cabin. During trials, the seals' head positions (while they were at the listening station) were carefully monitored, and were kept consistent to within a few cm.

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 (1 s increments determined by a random number generator, so that the animal could not predict the moment the signal was produced) after the test 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 approach to the listening station, to dissipate. The time the meata were completely above the water and the signal presentation was thus between 4 and 10 s.

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. 1 a**). The signal operator then 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 signal operator indicated to the trainer that she had failed to detect the signal (a miss). The trainer then indicated to the animal (by tapping 3 times 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 signal operator indicated that the trainer should end the trial without rewarding the animal. After a prestimulus response, the animal was ignored for 10 s by the trainer. When a prestimulus response was clearly triggered by an external sound that was also detected by the operator, data from the trial were not used, and the trial was immediately repeated.

In signal-absent trials (catch trials), the signal operator gave a hand signal to tell the trainer to end the trial after a 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 reduce variation in the seal's exposure level between the test signals and the acoustic signals from the trainer. We believe this helped the animal to focus on very faint sounds throughout the sessions. If the animal 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 pre-stimulus response), the signal operator indicated that the trainer should end the trial without rewarding the animal (the trainer ignored the animal for 10 s). The same amount of fish was given as a reward for correct responses in signal-present trials and signal-absent trials. In both signal-present and signal-absent trials, the trainer was unaware of the trial type when he/she sent the animal to the listening station. If an animal was not in the correct position at the start of a trial, it was called back and the trial was repeated.

A session with one animal generally consisted of 30 trials and lasted for about 15 minutes. The seals were not always tested in the same order, but they were always tested

immediately after each other. Sessions consisted of ~70% signal-present and ~30% signal-absent trials presented in random order, and only one signal frequency was presented each day (this proportion of signal-present to signal-absent trials has been used for years at SEAMARCO, as it optimizes the data collection rate, and still allows us the monitor the animals performance). In each session, the signal amplitude was varied according to the simple 1 up-1 down staircase procedure, following a conventional psychometric technique (Robinson and Watson, 1973). This is a variant of the method of limits, and results in a hearing threshold for 50% correct detection (Levitt, 1971). During preliminary sessions, a rough threshold was determined for each test frequency. During subsequent experimental sessions, the starting SPL of the signal was 6 dB above the rough threshold. Following each hit, the signal amplitude in the next signal-present trial was reduced by 2 dB. Following each miss, the signal level was increased in the next signal-present trial by 2 dB. Prestimulus responses did not lead to a change in signal amplitude for the next trial. A switch in the seal's response from a detected signal (a hit) to an undetected signal (a miss), or *vice versa*, is called a reversal.

Thresholds were determined for eleven narrow-band sweeps. For each center frequency, at least 60 reversals were obtained in a minimum of 6 sessions per frequency. The frequencies were tested in the following order: 8, 63, 50, 40, 32, 16, 1, 2, 0.25, 0.125, 0.5 kHz. Hearing thresholds (especially for test sounds below 8 kHz) could be influenced by sounds caused by small waves washing against the seals' heads while they were at the water surface, so tests were only conducted when Beaufort wind force was ≤ 2. Usually, three experimental sessions were conducted daily, five days per week (at 0900, 1100, 1400 or 1600 h). Data were collected between August 2013 and June 2014 (it took so long because the hearing tests were only conducted when the ambient noise conditions were optimal).

Sessions were only used for analysis after the session threshold leveled off, which usually occurred after about 2-4 sessions with a particular center frequency. The reported 50% detection hearing thresholds for each seal were based on the mean of all reversal pair values per frequency.
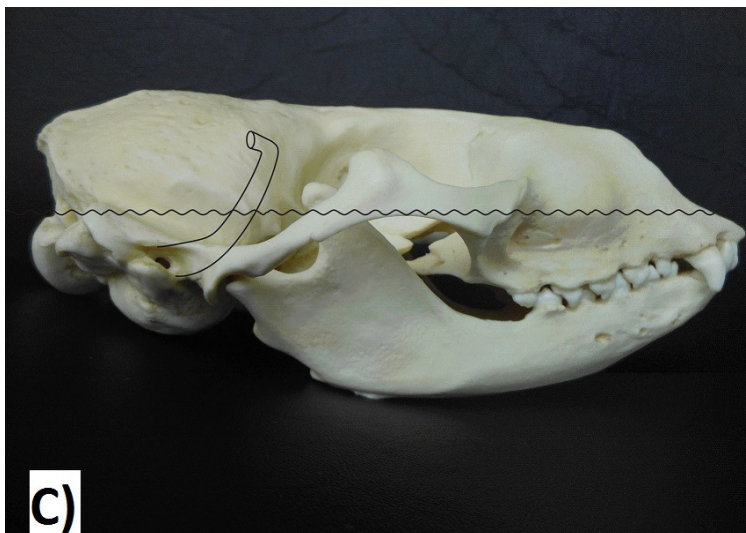
FIG. 3. Lateral view of the general level of a seal's head during natural swimming and resting at the water surface (a), a seal at the listening station during a hearing test, indicating the correct set-up (b), and a seal's skull, showing the schematic positions of the meatal orifice, the outer ear canal, the middle and inner ears, and the approximate water level (wavy line) when harbor seals swim at the water surface with its nose above the water (c).

III. RESULTS

The mean pre-stimulus response rate (for both signal-present and signal-absent trials) varied, depending on the frequency, between 0.6% and 10.5% for seal 01, and between 3.0% and 11.6% for seal 02 (**Table I**). The pre-stimulus response rate did not seem to be correlated to the test frequency, and no consistent pattern was seen between the pre-stimulus response rates of the 2 seals.

No movement of the external auditory meata was observed from the camera with the lateral view of the seal's head.

The mean 50% surface hearing thresholds of the two seals were similar, except at the lowest frequency tested (125 Hz; the difference was 8 dB). The water surface audiograms for the two seals showed the typical mammalian U-shape. However, the bottom part of the U was very flat and wide. The low-frequency sensitivity decreased gradually below 1 kHz, and the high-frequency cutoff (> 40-50 kHz) was steep (**Fig. 4** and **Table I**). The range of best hearing (here defined as 10 dB from the maximum sensitivity at 1 kHz, for which the SPL hearing threshold was 56-59 dB re 1 µPa) was very wide: from 0.5 to 40 kHz (6.3 octaves). The seals' water surface hearing thresholds were similar to each other (mean difference of 2.3 ± 2.2 dB) and to previous measurements (mean difference of 3.5 ± 2.8 dB) made at 1 m depth. Using paired-t tests, there were no significant differences between any of the combinations of threshold values given in Table I ($t \leq 1.45$, $d.f. = 10$, $p \geq 0.277$).
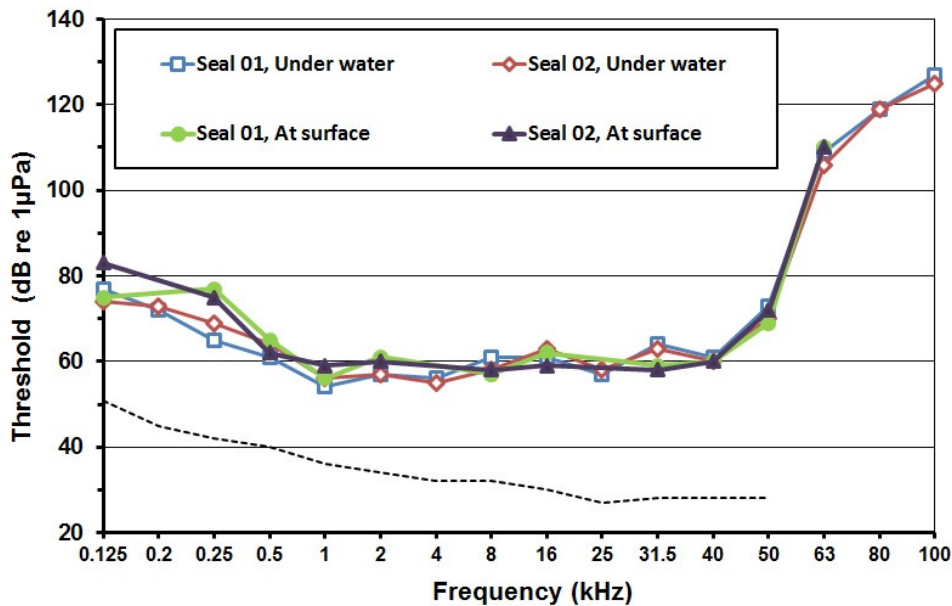

FIG. 4. The mean water surface audiograms of harbor seals 01 and 02 in the present study, showing their 50% hearing thresholds (2 dB steps) for underwater sounds while they were at the water surface (in normal swimming position), and the mean underwater audiogram (5 dB steps) of the same animals for underwater tonal signals (Kastelein *et al.*, 2009a). Also shown is the average underwater background noise level in the pool in dB re 1µPa/√Hz under test conditions (only researchers involved in the study allowed within 15 m of the pool, water circulation system off, no rain, and Beaufort wind force ≤ 2).

TABLE I. The 50% detection surface hearing thresholds, shown as means ± standard deviations, of 8-year-old female harbor seals 01 and 02 for underwater sounds while at the water surface (linear up-sweeps started and ended at ± 2.5% of the center frequency, and had durations of 1s, including a linear rise and fall in amplitude of 50 ms). Thresholds are based on at least 60 reversal pairs per frequency. Pre-stimulus response rates are also shown (based on all false responses in both signal-present and signal-absent trials). Also shown are the mean underwater hearing thresholds of the same animals 6 years previously (Kastelein *et al.*, 2009a).

Center frequency of narrow-band sweep (kHz)	Seal 01			Seal 02		
	Mean surface hearing threshold (±SD)	Pre-stimulus response rate	Mean underwater hearing threshold (±SD)	Mean surface hearing threshold (±SD)	Pre-stimulus response rate	Mean underwater hearing threshold (±SD
	dB re 1 µPa	%		dB re 1 µPa	%	
0.125	75 ± 2	2.0	77±4	83 ± 2	6.7	74±4
0.250	77 ± 2	1.1	65±4	75 ± 3	3.7	69±5
0.5	65 ± 2	0.6	61±4	62 ± 2	4.3	64±5
1	56 ± 2	5.7	54±4	59 ± 2	3.3	56±4
2	61 ± 2	2.5	57±4	60 ± 2	4.4	57±5
8	57 ± 2	3.5	61±4	58 ± 2	11.6	58±4
16	62 ± 2	8.3	61±4	59 ± 2	7.0	63±4
32	59 ± 2	10.5	64±4	58 ± 2	3.0	63±4
40	60 ± 2	5.4	61±4	60 ± 2	6.5	60±4
50	69 ± 2	8.4	73±3	72 ± 2	7.8	70±4
63	110 ± 2	2.3	109±3	110 ± 2	3.2	106±5

IV. DISCUSSION AND CONCLUSIONS

A. Evaluation

The seals were tested within the same sessions, so any differences between the thresholds obtained for the two animals must have been due to differences in their hearing sensitivity and/or individual differences in their response criteria, motivational state, or behavior. Differences could not have been caused by differences in equipment, equipment settings, methodology, personnel or background noise.

The pre-stimulus response rates of the animals in the present study are acceptable for a psychophysical hearing test (Dancer *et al.*, 1976; Dancer and Conn, 1983), and fall within the range of the animals' performance in other hearing tests. These low values indicate that the animals were well aware of the go-no procedure and did not gamble much, in the hope to respond accidently at the right moment during a signal presentation trial.

The biggest challenge in hearing studies is to maintain a low background noise level; great care was taken to do this in the present study. The main factor influencing the low-frequency part of the background noise spectrum in the pool was the wind (therefore the study

was only conducted in Beaufort wind force of ≤ 2). Any changes (increases or decreases) in wind force influencing the background noise level during the 30 minutes in which the two seals were tested are unlikely to have caused systematic bias over the six sessions (at least) on which the thresholds for each frequency were based.

It became clear during the beginning of the study that even very small waves caused by wind did mask the test signals (especially for signals below ~8 kHz). The collision of the very small waves (a few cm high) with the seal's skin in the area of the outer ears caused sound that must have masked the sounds of the test signals. Therefore, the tests could only be conducted under very low wind conditions (Beaufort wind force of ≤ 2). At sea, waves are usually higher than in the test conditions in the present study, and thus the hearing thresholds for signals < 8 kHz of harbor seals swimming at the water surface are likely to be higher than those reported in the present study, due to masking.

B. Comparison with underwater audiograms of the same harbor seals

Comparison of the water surface audiograms derived in the present study with the underwater audiograms of the same seals (Kastelein et al., 2009a) shows that, in the absence of masking sounds, there is no difference in the perception of underwater sound by seals swimming at the water surface and while fully submerged (**Fig. 4, Table I**). In seal 01, the differences between the underwater and surface hearing thresholds were generally between 2 and 4 dB and 12 dB for the 0.25 kHz signal. In seal 02, the differences between the underwater and surface hearing thresholds were generally between 0 and 4 dB and 9 dB for the 0.125 kHz signal. Considering the SDs, the only real differences between surface and underwater thresholds were for the 0.25 kHz (seal 01) and 0.125 kHz (seal 02) signals. Apart from the location of the seals relative to the water surface, the main difference in methodology between the two threshold studies shown in **Figure 4 and Table 1** is the dB step size used in the audiometric tests: 5 dB steps were used in the study reported by Kastelein et al. (2009a) and 2 dB steps in the present study. This difference in step size during testing could account for a difference in mean hearing thresholds of up to ~2 dB.

Although the animals were 6 years older in the present study than they were in the study reported by Kastelein et al. (2009a), their underwater hearing sensitivity was unchanged; between the studies, the hearing of the seals has been measured almost daily (see also Kastelein et al., 2009b; 2010) and no differences in hearing thresholds have been observed over the years. Therefore, there is no evidence of any effect of age on hearing sensitivity over the 6 years prior to the present study.

C. Seals may switch between greater hearing sensitivity in air or water

The results of the present study help to explain the findings of Kastelein *et al.* (2012): when free-swimming seals in a pool were exposed to only underwater sound (1/3-octave band noise centered at 4 kHz), this elicited the same TTS as when they were exposed to the same sound projected both under water and above the water. In the condition without aerial sound, the swimming seals did not reduce their received level of underwater noise by swimming at the water surface, because the sound energy arrived at their middle and inner ears via tissue conduction and because, due to the reverberations in the pool, the SPL near the surface was similar to that further down the water column for the frequencies tested. The SPL distribution was homogeneous throughout the pool (Kastelein *et al.,* 2012).

The area of maximum sensitivity to underwater sound extends in a 2-3 cm wide band from the opening of the external auditory meatus to 5-7 cm down the side of the head (Møhl

and Ronald, 1975). So only by raising its entire head well out of the water, is it likely that a seal could reduce the received level of underwater sound by the middle and inner ears. The results of a cochlear microphonic study suggest that seals can switch between greater sensitivity in either air or water (Møhl and Ronald, 1975). At present the physiological changes associated with achieving greater sensitivity in either air or water are not understood but the change-over takes longer than 10 seconds (Sills *et al*., 2015). Seals swimming at the surface and submerging for only very short periods are expected to maintain their greater sensitivity in the underwater mode (Terhune, 1991). However, at sea, the SPL just below the water surface is often much lower than that deeper in the water column (the reduction in SPL depends on the propagation conditions and the distance to the sound source; Weston, 1980; Sertlek and Ainslie, 2014). Thus, by swimming near or at the water surface, a seal can reduce its received level, but this is not due to a reduced coupling of its hearing with the water.

For a short while, a seal can lift its entire head out of the water; in this way it can probably temporarily reduce the sound level received by the middle and inner ears substantially. When exposed to high amplitude signals from a nearby seal acoustic harassment device, many harbor seals raised their heads above the water. When they submerged enough to have their external meatus openings below the water surface, they generally then leapt out of the water in a porpoising dive and swam rapidly away under water (Mate *et al*., 1987). The seals were probably unable simultaneously to reduce the received sound level and swim away quickly; it would be difficult for them to swim for long periods with their heads well out of the water.

The results of a cochlear microphonic study suggest that seals can switch between greater sensitivity in either air or water, and that there is a discrete location where underwater sound enters each side of the head (Møhl and Ronald, 1975). A putative underwater sound transmission pathway therefore exists: through the skin immediately below the external auditory meatus and adjacent tissues directly to each cochlea (Møhl and Ronald, 1975). Human divers also hear underwater sound via tissue conduction (Hollien and Feinstein, 1975). The in-air sound transmission pathway in seals is through the opened external auditory meatus, down the ear canal to the tympanic membrane, and across the middle ear cavity via the ossicles to the cochlea. The middle ear reflex (contraction of the tensor tympani and/or stapedius muscles in response to high amplitude sound; Møller, 1972) of a seal listening in air or under water results in a lower amplitude cochlear microphonic voltage (Møhl and Ronald, 1975). This may be a result of the interruption of the acoustic transformer function of the middle ear for a seal listening in air, or a possible loading of the oval window by the stapes for a seal listening under water, both of which would interfere with the fluid motions of the cochlea. Engorgement of the cavernous tissues while swimming may be involved in the underwater sound pathway or it may simply be a mechanism to help maintain equal pressure on each side of the tympanic membrane during diving in order to prevent rupture of the eardrum. This conjecture remains untested, but there is evidence to suggest that seals can switch between greater sensitivity to sound in either air or water.

The seal in-air thresholds reported by Reichmuth *et al*., (2013) and Sills *et al*., (2014, 2015) were obtained under lower background noise levels than the earlier studies of Terhune and Ronald (1971) and Terhune (1991). Thresholds below 2.8 kHz were possibly masked by the background noise, but at higher frequencies masking was unlikely and at all frequencies the nearby researcher could detect all of the test tones at the seal's thresholds (Terhune, 1991). The lower background noise levels in the more recent threshold determinations do not explain the previously published higher in-air thresholds of the seals.

Sills *et al*. (2015) measured the in-air thresholds of a ringed seal at 12.8 kHz, both when the seal had been on land for many minutes (during normal in-air threshold measuring procedures) and when the seal had been under water or with its head at the water surface, and

then raised its head above water for 10 s before listening to the test sound. The thresholds when the seal's head had only been fully in air for 10 s were 34 dB higher than the measured in-air thresholds when the seal had been out of the water for a longer period of time. This supports the hypothesis that seals are able to switch between greater sensitivity in air or under water, and suggests that the switch requires some time to occur. This may explain the higher thresholds previously reported in air for harbor seals (Møhl, 1968; Terhune, 1991) and harp seals (Terhune and Ronald, 1971); the seals were swimming and only raised their heads above the surface for a few seconds while listening to the test tone. There did not appear to be time for the auditory system to make the adjustments needed, presumably in the blood vascular system of the middle ear, to switch between the two modes of hearing. Thus, the hearing ability of a seal swimming at the surface is likely to be maximized for receiving sound through the water rather than in the air. Seals that occupy a haul-out site for some time are likely to experience greater sensitivity to in-air sounds. The time required to switch between the two modes is unknown, but must be greater than 10 s. The acoustic impedance matching requirements of sound transmission to the cochlea and the various in-air threshold detection measurements suggest that it is unlikely that a seal could have optimal sound reception in both media simultaneously.

D. Ecological significance

The aerial hearing of seals probably mainly plays a role in the detection of terrestrial predators (such as bears, foxes, and wolves) while seals are hauled out on land for resting and, in females, during delivery and suckling of their pups (Nordstrom, 2002). Their ability to switch to perceiving low-amplitude sounds via air while hauled out on land enhances their predator avoidance and mother-pup acoustical communication.

The results of the present study indicate that, while they are swimming at the water surface, seals' hearing is focused on perceiving underwater sounds. This makes sense, as harbor seals have no aerial predators while at sea, but need to listen for sounds from conspecifics (Schusterman et al., 1970; Van Parijs et al., 2003; Van Parijs and Kovacs, 2002), prey (fish; e.g., Wilson et al., 2004) and marine predators, such as killer whales (Orcinus orca; Deeke et al., 2002), which come from below. The present study suggests that, to reduce masking of underwater sounds while at the water surface when the sea is calm, seals may have to stop swimming to reduce noise generated by waves hitting their heads.

E. General conclusion

When swimming at the water surface, seals receive underwater sounds as if they are fully submerged. Only when they stick their entire heads fully out of the water can they probably lower the received SPL. For assessment of the total exposure, and thus the risk of hearing damage, when calculating the cumulative sound exposure level (SEL_{cum}) for harbor seals due to sounds that occur over long periods, the same hearing sensitivity below the water as at the surface can be assumed, and only the difference between the SPL at depth and the SPL just below the water surface should be taken into account. Therefore also, via transmitter research with D-tags, the proportion of time seals normally swim at the water surface needs to be estimated and should be taken into account (as the SPL near the water surface is usually lower). The SPL of aerial sounds, during for instance offshore pile driving, is of no relevance to the total sound exposure, because the received SPL of underwater sound is greater than that of aerial sound, and because aerial hearing is reduced when seals are swimming at sea.

ACKNOWLEDGMENTS

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