

Comparative assessment of amphibious hearing in pinnipeds

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Abstract Auditory sensitivity in pinnipeds is influenced by the need to balance efficient sound detection in two vastly different physical environments. Previous comparisons between aerial and underwater hearing capabilities have considered media-dependent differences relative to auditory anatomy, acoustic communication, ecology, and amphibious life history. New data for several species, including recently published audiograms and previously unreported measurements obtained in quiet conditions, necessitate a re-evaluation of amphibious hearing in pinnipeds. Several findings related to underwater hearing are consistent with earlier assessments, including an expanded frequency range of best hearing in true seals that spans at least six octaves. The most notable new results indicate markedly better aerial sensitivity in two seals (*Phoca vitulina* and *Mirounga angustirostris*) and one sea lion (*Zalophus californianus*), likely attributable to improved ambient noise control in test enclosures. An

updated comparative analysis alters conventional views and demonstrates that these amphibious pinnipeds have not necessarily sacrificed aerial hearing capabilities in favor of enhanced underwater sound reception. Despite possessing underwater hearing that is nearly as sensitive as fully aquatic cetaceans and sirenians, many seals and sea lions have retained acute aerial hearing capabilities rivaling those of terrestrial carnivores.

Keywords California sea lion · Harbor seal · Northern elephant seal · Hearing threshold · Amphibious hearing · Audiogram

Introduction

The phocids (true seals), otariids (sea lions and fur seals), and odobenids (walruses) are the most amphibious of all mammals—dividing their time to various extents between the shore and the sea. As a result, these pinnipeds are especially relevant to an examination of the selective pressures imposed by semi-aquatic living. Their sensory systems are challenged by the need to function efficiently both above and below the water's surface to support vital

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behaviors, a difficult proposition given that such systems are shaped in large part by the physical properties of the environments in which they operate. In terms of the auditory sense, hearing in air and hearing in water require different adaptations to accommodate the manner by which sound propagates through the surrounding medium, enters an animal, and eventually reaches the primary receptor system of the inner ear. Given this, it is interesting to consider the extent to which typical mammalian ears may be altered to enable sound detection in species that shift readily between terrestrial and aquatic habitats. The auditory systems of pinnipeds could be tuned for hearing either in air or in water, or perhaps for establishing a balance between the two (Møhl 1968a; Wartzok and Ketten 1999). Information about functional hearing is required to understand how these amphibious animals are adapted to use sound in both environments.

The auditory anatomy of various pinnipeds, including probable pathways for sound reception, has been previously described (Møhl 1968b; Repenning 1972; Ramprasad 1975; Nummela 2008) and considered in the contexts of hearing sensitivity, acoustic communication, foraging ecology, and degree of aquatic specialization (Kastak and Schusterman 1998; Supin et al. 2001). These mammals appear to use the conventional (terrestrial) conductive sound pathway for hearing in air, whereas under water, bone and tissue conduction are believed to contribute substantial sound energy to the inner ear from various places along the auditory pathway. The involvement of bone and tissue conduction in underwater hearing does, however, impair sound localization (Nummela 2008). As a compensatory mechanism, the ear complex in pinnipeds is partially detached from the skull. True seals, and to some extent the walrus, show additional derived morphological features which include loss of the external pinnae, muscular control of the meatal openings, cavernous tissue lining the outer and middle ear, inflated auditory bullae, hypertrophy of middle ear ossicles, and enlargement of the tympanum (Wartzok and Ketten 1999). These distinctive features have recently led to the description of a unique mammalian ear type (“phocid ear type”) that is shared by both phocids and odobenids (Nummela 2008).

The anatomical modifications present in the auditory systems of different pinnipeds may be the result of selective pressures for enhanced underwater hearing or for tolerating conditions experienced while foraging at sea. Of particular interest is the influence of these modifications on the perceptual experience of individuals, and further, how differences in the ecological niches of various species are expressed in their particular auditory capabilities. Functional hearing in pinnipeds has been studied for nearly five decades. Early auditory research on these amphibious marine mammals paralleled studies of dolphin hearing and biosonar

(see Mooney et al. 2012), with emphasis on high-frequency underwater hearing and possible specializations for echolocation (see Schusterman et al. 2000). This emphasis has since shifted further toward understanding structure–function relationships within the auditory systems of pinnipeds (see Wartzok and Ketten 1999; Nummela 2008) and evaluating the potentially harmful effects of anthropogenic noise in marine environments (see Southall et al. 2007).

Møhl (1968a, b) was the first to train captive seals for voluntary participation in hearing tests and to attempt to directly compare their aerial and underwater hearing abilities. Subsequently, there were several other studies of amphibious hearing in pinnipeds (Terhune and Ronald 1971, 1972; Turnbull and Terhune 1990; Terhune 1991; Schusterman et al. 1972, 1974a; Moore and Schusterman 1987; Babushina et al. 1991), all of which concluded that pinnipeds are primarily water-adapted with respect to hearing. Comparisons of aerial and underwater thresholds in these studies were typically made by converting pressure thresholds to common units of acoustic intensity (see e.g., Møhl 1968a; Fobes and Smock 1981; Watkins and Wartzok 1985; Schusterman et al. 2000) as this technique had previously been used to describe the amphibious hearing capabilities of human divers (Wainwright 1958). A comprehensive assessment of low-frequency hearing by Kastak and Schusterman (1998) added to this body of work by presenting amphibious hearing data for three pinniped species, including aerial hearing thresholds obtained using headphones. The authors suggested for the first time that not all pinnipeds are more sensitive to sounds received under water than in air, and further, that acoustic pressure may be more relevant than intensity as a metric of comparison for hearing thresholds measured in different physical environments.

Direct hearing measurements obtained from cooperative subjects can be applied to inform anatomical and biomechanical models of auditory function and evolution (Ketten 1992; Hemilä et al. 2006; Nummela 2008). However, the development and utility of these models is influenced by the acoustic metrics used, especially in the case of amphibious species that receive sounds in different environments. These models are also influenced by the amount and quality of available data concerning perceptual capabilities in different species. Despite substantial research efforts to date, audiometric data collected under sufficiently controlled conditions are still needed for pinnipeds, as well as for the other amphibious marine carnivores including the sea otter (*Enhydra lutris*) and polar bear (*Ursus maritimus*).

Here, we revisit the findings and conclusions of Kastak and Schusterman (1998, 1999) concerning amphibious hearing in the harbor seal (*Phoca vitulina*), the northern elephant seal (*Mirounga angustirostris*), and the California sea lion (*Zalophus californianus*). We report hearing thresholds obtained for four trained subjects using

psychophysical methods over a ten-year period. The measurements of auditory sensitivity and associated ambient noise conditions, particularly those obtained in air, help to resolve prior uncertainties in the hearing profiles of these species. Further, the updated comparisons of hearing sensitivity made possible by this study provide new perspectives on the functional relevance of derived anatomical characteristics, the potential for reasonable extrapolation of hearing capabilities among phylogenetic groups, and the trade-offs required for efficient amphibious hearing relative to other terrestrial and fully aquatic species.

Materials and methods

General methods

Experimental animals and study site

Absolute hearing measurements were obtained from four pinnipeds at Long Marine Laboratory, at the University of California Santa Cruz, in Santa Cruz, CA, USA, over a time period extending from June 2001 to December 2011. The animals were long-term residents of the research facility and lived in enclosures with saltwater pools and adjacent haul-out decks. They were trained to participate in research, husbandry, and enrichment activities using standard operant conditioning methods and positive (fish) reinforcement. All but one subject had extensive prior experience in performing psychoacoustic experiments. One adult male harbor seal identified as Sprouts (NOA0001707) was tested in Experiments 1, 2, and 3. One adult female northern elephant seal identified as Burnyce (NOA0004829) and one adult female California sea lion identified as Rio (NOA0004827) were tested in Experiment 2. A juvenile female California sea lion identified as Ronan (NOA0006602) was tested in Experiment 3. Ronan lacked previous experience in psychoacoustic testing but had been trained for voluntary participation in other behavioral paradigms; she received extensive training on the current hearing test procedure prior to beginning data collection.

The animals typically received one-third to one-half of their daily diets of freshly thawed, cut herring and capelin fish during experimental sessions. These sessions were conducted once or twice per day for up to 5 days per week. Subjects were maintained at a healthy weight and their diets were not constrained for experimental purposes.

Testing enclosures

Audiometric testing was conducted in three settings. The first two were the same testing environments used by Kastak and Schusterman (1998, 1999). The outdoor aerial

hearing tests were conducted on an elevated wooden deck adjacent to the test pool. The underwater tests were conducted in a concrete, epoxy-lined, partially in-ground pool. This circular test pool was 7.6 m in diameter and 1.8 m deep and filled with natural seawater that ranged from 10 to 14 °C. Ambient conditions were kept as quiet as possible during testing in both of these areas; experiments occurred only in calm conditions and there was no flowing water or activity within 20 m of the enclosure. The third test enclosure was a custom-built, hemi-anechoic chamber (Eckel Industries, Cambridge, MA, USA) that was constructed at Long Marine Laboratory in 2001. This free-standing acoustic chamber provided a 3.3 m × 2.3 m × 2.2 m testing room with environmental controls appropriate for conducting audiometric research with animals. It was mounted on a semi-isolated cement foundation, constructed of double-paneled, eight-gauge stainless steel walls, and lined with fiberglass-filled, aluminum-covered wedges. The cement floor was covered with several 2.6 cm thick neoprene mats. This facility was used for the first time during the experiments reported here; see Southall et al. (2003) for additional description.

Experiments in all three settings were conducted remotely from a sound-isolated control room adjacent to the acoustic chamber. This room contained the hardware used for stimulus generation and monitoring and allowed the experimenter to remain out of view of the animals during testing. Surveillance cameras located in the testing enclosures were connected to a video monitor in the control room so that experimental sessions could be observed in real time.

Psychoacoustic testing

The behavioral procedures used for threshold measurement were similar for all subjects in all testing environments. The experimental task was an auditory go/no-go procedure. Subjects were cued by a trainer to enter the experimental enclosure and to work at a simple apparatus positioned in a calibrated sound field. The apparatus included a chin station that precisely controlled the head position of the subject during each trial, a light that indicated the duration of each trial interval to the subject, and a response target at least 30 cm to the left of the station that the subject could press to indicate detection of a signal. Subjects were trained to initiate each trial by placing their chin into a fitted station and positioning their head and body on axis with the trial light. When they did so, the trial light was activated, delineating the start of the 4 s listening interval during which a signal could be presented. A response was defined as correct if the subject moved from the station and touched the response target when a test signal was triggered during the trial interval (a correct detection), or

remained in position at the station if no signal was triggered (a correct rejection). Both correct responses were marked by an acoustic conditioned reinforcer followed by an equivalent fish reward. A response was considered incorrect if the subject remained at the station when a signal was triggered (a miss) or touched the response target when no signal was triggered (a false alarm). Incorrect responses were not reinforced.

Within a single test session, frequency was held constant and signal amplitude was systematically varied across the signal-present trials. A typical session included 50–60 trials with a pre-determined ratio of signal-present and signal-absent trials. The sequence of trials was randomized for every session but constrained to include no more than four signal-present or signal-absent trials in succession (see Reichmuth et al. 2012). To maintain a stable response bias during testing (see Schusterman 1974b), the proportion of signal-present trials in each session was varied between 0.50 and 0.75. Response bias was controlled throughout testing by evaluating false alarm rates, or the proportion of signal-absent trials on which the subject reported detection of a signal, and adjusting the probability of signal-present trials to yield a false alarm rate that was higher than 0 and less than 0.3.

Hearing thresholds were determined for each frequency using two psychoacoustic methods in succession. First, an adaptive staircase method (Cornsweet 1962) was used to estimate hearing threshold over multiple sessions. At the start of a session, the test signal was set to an easily detectable level and was then varied on subsequent trials dependent on the subject's response. Following each correct detection by the subject, the signal amplitude was lowered in 4 dB steps until the subject failed to respond. The signal amplitude was then raised by 2 dB (or 4 dB in the case of the sea lion in water) following each miss and lowered by 2 dB following each correct detection until the subject had reversed from a correct detection to a miss at least five times. At the end of a session, several supra-threshold level signals were given on signal-present trials to ensure stimulus control on the task. When performance over three successive staircase sessions had stabilized to within 3 dB, the data were averaged to determine a preliminary threshold estimate and the subject moved on to testing with the method of constant stimuli (MCS) for final threshold determination.

Threshold determination using MCS (Stebbins 1970) was accomplished by first selecting at least five stimulus levels that bracketed the estimated threshold in 2 dB increments (e.g. -4 dB, -2 dB, 0 dB, +2 dB, +4 dB re preliminary threshold). Each of these stimulus levels was presented five times in a randomized order within a test session so that probabilistic detection data for each sound pressure level (SPL) could be obtained. Probit

analysis (Finney 1971) was used to fit the psychometric function to the observed proportion of correct detections at each stimulus level. An inverse prediction was then used to determine threshold, defined as the stimulus level corresponding to the 50 % correct detection probability. A minimum of two sessions (or 10 trials at each stimulus level) was used for probit analysis. If the data pooled from these sessions showed 95 % confidence limits within 3 dB of the measured threshold, and false alarm rates during testing (excluding any warm-up or cool-down trials) were above 0 and below 0.3, then the calculated threshold was accepted. If not, additional sessions were conducted until these criteria were met. Each of the experiments described below involved threshold determination for multiple frequencies. The order in which the frequencies were tested was always randomized.

Experiment 1: comparison of direct-field and headphone thresholds

Experimental design

The subject of this experiment was the adult male harbor seal (Sprouts). Kastak and Schusterman (1998) had previously reported low-frequency aerial hearing thresholds for this harbor seal at age 7. Hearing thresholds in that study had been determined at seven frequencies (100, 200, 400, 800, 1600, 3200, and 6400 Hz) using a go/no-go procedure and similar psychoacoustic methods (initial adaptive staircase testing followed by MCS testing). Testing was conducted outdoors during calm conditions, and stimuli were presented to the seal through custom-fitted headphones secured by a neoprene harness that fit snugly around his head. In the current experiment, the hearing of the seal was re-tested at age 13 at the same seven frequencies, this time in the hemi-anechoic acoustic chamber with stimuli projected from speakers to generate a calibrated sound field. Differences between the two data sets were resolved by conducting additional testing using headphones for stimulus presentation. This testing was conducted at a subset of four frequencies (200–1600 Hz) in both the outdoor environment and in the acoustic chamber.

Signal generation and calibration

The stimuli used during testing were 100, 200, 400, 800, 1600, 3200, or 6400 Hz pure tones with 500 ms duration including a 4 % linear rise/fall time. The tones were generated by a custom National Instruments (NI) LabVIEW virtual instrument (National Instruments Corporation, Austin, TX, USA) and sent out through an NI PXI-6070E data acquisition card mounted in an NI PXI-1010 chassis. The update rate for sound generation was at least 200 kHz.

The signal passed through an NI SCB-68A connector block, a Krohn-Hite 3550 filter (Krohn-Hite, Brockton, MA, USA) and a HP 350C (Hewlett Packard, Palo Alto, CA, USA) attenuator prior to reaching the transducer. The transducers (JBL Incorporated, Northridge, CA, USA) used to project stimuli into the acoustic chamber were a JBL 2245H (100–400 Hz), a JBL 2123H (800 Hz), and a JBL 2404H speaker (1600–6400 Hz). The speakers were mounted to the chamber wall approximately 0.8 m in front of the position of the seal's head during testing. The transducers used in the two headphone conditions were Telephonics TDH-39 earphones (Telephonics Corporation, Farmingdale, NY, USA) mounted in rigid rubber MX-41/AR Amplivox Audiocups (Amplivox, Eden Prairie, MN, USA). This was the same setup used by Kastak and Schusterman (1998).

The stimuli projected into the acoustic chamber simulated a free-field condition; however, the term direct field was used to characterize the enclosed space of the testing environment. Stimulus calibration was conducted with an Etymotic ER-7C (Etymotic Research Incorporated, Elk Grove Village, IL, USA) clinical probe microphone (with an internal reference calibration) placed at the position corresponding to the center of the seal's head during testing. Before and after each session, the level of a calibration tone at the test frequency was measured (in dB re 20 μ Pa) at several transmitting voltages. The calibration signal was returned from the microphone to the same NI hardware and custom software used to generate the signals. The update rate on the incoming signal was always more than twice the test frequency. The signal spectrum was inspected in the frequency domain using fast Fourier transform (FFT) analysis to confirm the absence of artifacts at other frequencies. Prior to the start of testing for each frequency, the sound field was measured at 25 positions within a 20 cm \times 20 cm \times 20 cm grid surrounding the calibration position to ensure that received SPLs at each location were within 3 dB of the calibration position.

For each of the four frequencies tested in the headphone condition, signal calibration was conducted before and after every test session with the animal in position in the test enclosure (the outdoor environment or the acoustic chamber). The animal was trained to remain still during stimulus calibration. The flexible tip of the probe microphone was placed underneath each headphone within 1 cm of the meatus. Daily calibration and stimulus monitoring were accomplished as in the direct-field condition. Ambient noise was not measured during this experiment, mainly due to the difficulty of accurately characterizing noise levels underneath the headphones, and because the purpose of the study was merely to compare hearing thresholds obtained in the different environments as a function of the type of stimulus presentation.

Psychoacoustic procedure

The testing procedure followed the general psychoacoustic method provided earlier. During direct-field testing in the acoustic chamber, the seal worked without a trainer. The conditioned reinforcer that marked each correct response was played through a speaker, and reinforcement was delivered through a PVC feeder tube that could be extended from the control room through the wall of the acoustic chamber. During headphone testing in the outdoor environment and in the acoustic chamber, a trainer that was blind to the experimental conditions checked the fit and placement of the headphones on the subject prior to each trial; the transducer was centered over the meatus and the tapered rubber edges of the ear cushions provided a smooth coupling to the skin. The trainer wore a headset that was linked to the remote experimenter, and delivered the conditioned and primary reinforcement to the seal when instructed to do so.

Analysis

Aerial auditory thresholds calculated by probit analysis (and corresponding false alarm rates) were determined for seven frequencies (100, 200, 400, 800, 1600, 3200, 6400 Hz) tested in the direct-field condition (acoustic chamber, no headphones) and compared to those obtained for the same animal (outdoor condition, with headphones) 6 years earlier (Kastak and Schusterman 1998). Auditory thresholds determined for four frequencies (200, 400, 800, 1600 Hz) tested with headphones in both environments were compared to these data and to one another.

Experiment 2: measurement of absolute aerial audiograms

Experimental design

The subjects of this experiment were the adult male harbor seal tested in Experiment 1 (Sprouts), the adult female northern elephant seal (Burnyce), and the adult female California sea lion (Rio). These animals were 17, 8, and 16 years old (respectively) at the time of testing. The aerial hearing capabilities of the harbor seal and the northern elephant seal had been tested seven to ten years earlier at frequencies from 100 to 6400 Hz using headphones in outdoor conditions (Kastak and Schusterman 1998). In order to describe absolute (unmasked) aerial audiograms for each of these individuals, hearing thresholds for pure-tone stimuli were measured for 12 frequencies between 100 and 32500 Hz. All testing was conducted in the calibrated, direct-field conditions of the acoustic chamber.

Signal generation and calibration

The stimuli used during testing were 100, 200, 400, 800, 1600, 3200, 6400, 12000, 18000, 24000, 28000, 30000, or 32500 Hz pure tones with 500 ms duration including a 4 % linear rise/fall time. The tones were generated and calibrated with the same or similar hardware and software configurations as described in Experiment 1. To accommodate testing above 6400 Hz, the JBL 2404H speaker (6400–24000 Hz) and a Fostex FT96H (Fostex Company, Tokyo, JPN) super tweeter (28000–32500 Hz) were used. In addition to the Etymotic ER-7C probe microphone, a calibrated Josephson C55H (Josephson Engineering, Santa Cruz, CA, USA) and a calibrated ACO Pacific 4012 (ACO Pacific Incorporated, Belmont, CA, USA) microphone were used for stimulus calibration and mapping, depending on the frequency of interest. Calibration procedures were conducted before and after each session as in Experiment 1, and spatial mapping of the sound field surrounding the position of each subject's head at the station was completed to ensure minimal variability in received SPLs (± 3 dB from SPL measured at the calibration position) at each test frequency.

Ambient noise was measured regularly in the acoustic chamber with a battery-powered Brüel and Kjær 2250 sound analyzer with calibrated 4189 free-field microphone (Brüel and Kjær A/S, Nærum, DK). Each 1 min sample was recorded at the calibration position and then 1 min L_{eq} levels (equivalent continuous SPLs) and percentile statistics of 1/3-octave band levels were calculated to describe temporal variance in the noise. The final ambient noise profiles comprised power spectral density levels [in dB re $(20 \mu\text{Pa})^2/\text{Hz}$] calculated from the median of 50th percentile levels for 1/3-octave bands from 40 to 20000 Hz.

Psychoacoustic procedure

The procedures used were the same as in the direct-field condition in Experiment 1. The subjects were trained to enter and work in the acoustic chamber without a trainer present, as shown in the video Online Resource 1. Remote experimenters who observed the session in real time on closed-circuit video controlled the experimental stimuli and delivery of reinforcement.

Analysis

Aerial auditory thresholds calculated by probit analysis (and corresponding false alarm rates) were determined for the three subjects at every frequency tested. Thresholds were plotted as a function of test frequency to depict audiograms. The frequency range of best hearing was

estimated from the thresholds falling within 20 dB of the lowest hearing threshold measured. Best hearing sensitivity was reported in terms of the directly measured SPL (dB re 20 μPa). The hearing data obtained for the three pinnipeds in this study were compared to previously published values for the same species.

Experiment 3: measurement of absolute underwater audiograms

Experimental design

The subjects of this experiment were the adult male harbor seal (Sprouts) tested in Experiments 1 and 2 and the juvenile female California sea lion (Ronan). At the time of testing, these subjects were 19 and 3 years old, respectively. Underwater audiograms had previously been measured under similar conditions for the northern elephant seal (Kastak and Schusterman 1999) and the adult female California sea lion (Reichmuth and Southall 2012) that were tested in air in Experiment 2. Underwater hearing thresholds were determined for the harbor seal at 12800, 18102, 25600, 36204, 51200, and 72408 Hz. These data were combined with previously reported low-frequency data for this subject (Kastak and Schusterman 1998; Southall et al. 2005) to generate a composite audiogram. Underwater hearing thresholds for the juvenile California sea lion were obtained across the frequency range of hearing at 100, 200, 400, 800, 1600, 3200, 6400, 12800, 25600, 36204, and 43054 Hz to provide a complete audiogram. All testing was conducted in the underwater enclosure described previously.

Signal generation and calibration

Signal generation for underwater testing was accomplished using NI LabVIEW-based software (HTP) described by Finneran (2003). Narrow-band linear frequency-modulated (FM) sweeps (± 5 % from center frequency), rather than pure tones, were used as test stimuli. These FM signals generate equivalent thresholds to pure-tone stimuli but reduce multipath interference in reverberant environments (Finneran and Schlundt 2007). Sweep duration was 500 ms including a 5 % linear rise/fall time. Signals were sent through an NI USB-6259 BNC M-series DAQ module with an update rate of 500 kHz, a Krohn-Hite 3350 or 3364 filter, a TDT PA5 digital attenuator (Tucker-Davis Technologies, Alachua, FL, USA) and a Hafler P1000 (Hafler Professional, Tempe, AZ, USA) power amplifier (for frequencies 6400 Hz and below) to a transducer suspended into the test pool. The transducers used were a Naval Undersea Warfare Center (Newport, RI, USA) J-11 projector

(100–200 Hz), a Lubell Labs (Columbus, OH, USA) 1424HP speaker (400–6400 Hz), and an ITC (International Transducer Corporation, Santa Barbara, CA, USA) 1042 omni-directional transducer (12800 Hz and higher).

In the underwater configuration, the chin station and response target were affixed to a submerged PVC frame suspended from the edge of the pool. This apparatus was positioned to provide a calibration depth of 0.8 m for the harbor seal and 1 m for the sea lion, and included an underwater trial light and video camera mounted in front of the animal. The position of the station in the pool was always the same for each subject. The relative position of the transducer was determined through mapping of the sound field at each frequency in the grid surrounding the calibration position. Mapping was conducted at 25 positions within a 20 cm × 20 cm × 20 cm grid (for the harbor seal) or a 14 cm × 14 cm × 14 cm grid (for the sea lion) with another calibrated ITC 1042 hydrophone (nominal sensitivity -201 dB re 1 V/ μ Pa) used as a receiver to ensure acceptable variability in the sound field (± 3 dB from SPL measured at the calibration position). If the spatial variation within the grid was too high, the transducer was moved until a suitable position was identified. In all cases, the distance between the station and the transducer exceeded the theoretical near-field boundary (Siler 1969); at the lowest frequencies tested (100–200 Hz) the transducer was 5.25 m from the station. Once an appropriate transducer configuration had been established, signal calibration was conducted prior to each session at the center position of the subject's head during testing. A calibrated Reson TC4032 low-noise hydrophone (Reson A/S, Slangerup, DK) with built-in pre-amplifier (nominal sensitivity -170 dB re 1 V/ μ Pa) was used to measure signal SPL (dB re 1 μ Pa) at several transmitting voltages. Calibration signals were returned through the same filter and NI DAQ card—with an update rate that was at least twice the test frequency—and examined using FFT analysis to confirm absence of harmonics or other artifacts at frequencies other than the test signal.

Underwater ambient noise measurements were taken daily with the Reson TC4032 hydrophone and the Brüel and Kjær 2250 sound analyzer for frequencies between 40 and 20000 Hz. For frequencies between 20000 and 78000 Hz, the noise floor was measured several times during typical testing conditions using the Reson TC4032 hydrophone and a battery-powered Fostex FR-2 Field Memory Recorder (Fostex Company, Tokyo, JPN). The 1 min noise samples were obtained in the position corresponding to the center of the animal's head during testing. As with the aerial ambient noise measurements, power spectral density levels [in dB re $(1 \mu\text{Pa})^2/\text{Hz}$] were determined from the median of 50th percentile 1/3-octave band levels for all samples.

Psychoacoustic procedure

Audiometric testing in the underwater setting was similar to that conducted in other studies (Kastak and Schusterman 1998, 1999; Southall et al. 2005; Reichmuth and Southall 2012). The subjects performed the go/no-go task at the submerged apparatus in the calibrated sound field, as shown in the video Online Resource 2. A trainer that was blind to the experimental conditions cued the subject to position at the underwater station. A delay of several seconds preceded the activation of the trial light to allow water movements to dissipate. Trials proceeded as in aerial testing with slight differences for the two subjects. The sea lion performed individual trials. Each correct response was marked by a conditioned reinforcer and followed by an equivalent fish reward. After an incorrect response, the trial light was extinguished and the subject returned to the surface without reinforcement. For the harbor seal a more efficient, multiple response procedure was used (see Kastak and Schusterman 2002). Sessions were conducted in blocks of 4–9 trials. After diving to the station, the harbor seal completed each trial and returned immediately to the station (without resurfacing) and waited for the next trial to begin. A conditioned reinforcer marked each correct response, but the delivery of accumulated fish rewards was delayed until the end of the trial block, when the subject was cued to return to the surface by the trainer. Prior testing with this subject indicated no difference in thresholds obtained using single-response versus multiple-response audiometry.

Analysis

Underwater auditory thresholds calculated by probit analysis and corresponding false alarm rates were determined for the two subjects at every frequency tested. Thresholds were plotted as a function of frequency to depict audiograms. Thresholds for frequencies from 100 to 6400 Hz for the harbor seal were averaged from previously published data (Kastak and Schusterman 1998; Southall et al. 2005) and added to the data from 12800 to 72408 Hz obtained in this study. The frequency range of best hearing for each subject was estimated from the thresholds falling within 20 dB of the lowest measured threshold. Best hearing sensitivity was reported in SPL (dB re 1 μ Pa). The thresholds obtained were compared to previously published values for the same species.

Comparisons of aerial and underwater audiograms

Hearing thresholds were compared within each of the three species in a manner that highlighted inter-subject differences within a medium and intra-subject differences

between media. The audiograms obtained in air for the three pinnipeds were compared first to one another, and then to several representative carnivores tested in similar acoustic conditions. These comparisons were made in terms of aerial sound pressure. The audiograms obtained under water for the harbor seal and California sea lion were compared to previously published values for the northern elephant seal (Kastak and Schusterman 1999), and to several representative fully aquatic mammals. These comparisons were made in terms of underwater sound pressure. To enable comparisons within individuals between the media, hearing thresholds were plotted in terms of equivalent SPL, incorporating the 26 dB difference between the reference pressures.

Results

Experiment 1 revealed large differences between hearing thresholds measured in the outdoor environment and those obtained in the acoustic chamber. Thresholds measured in the direct-field condition of the chamber were 10–39 dB lower than those reported by Kastak and Schusterman (1998) six years earlier for the same harbor seal tested outdoors with headphones (Fig. 1). Additional testing with a subset of frequencies showed no difference in thresholds with headphones in the outdoor environment for this seal at age 7 and age 13. In contrast, the thresholds obtained with headphones in the acoustic chamber at age 13 were 18–29 dB lower than those measured with headphones in the outdoor condition, but not different from those measured without headphones in the chamber condition. These comparisons show that the difference in measured sensitivity is attributable to the testing environment rather than to the method of stimulus presentation.

Experiments 2 and 3 provided complete aerial and underwater audiograms in the direct-field condition of the acoustic chamber and in the underwater testing environment. The hearing thresholds for each test frequency and associated false alarm rates for the animals tested in each condition are given in Table 1 along with corresponding background noise levels. The psychometric functions associated with the reported hearing thresholds are available in Online Resources 3–7. The ambient noise measurements obtained in the acoustic chamber and in the underwater testing environment are further described in Fig. 2. The audiograms are plotted with previously published thresholds for the same species, as well as for other terrestrial and marine mammals, in Fig. d. The aerial and underwater thresholds are shown in the left and right panels of this figure, respectively. The Y-axes corresponding to the aerial plots on the left and the underwater plots on the right are offset by 26 dB such that equal heights above the

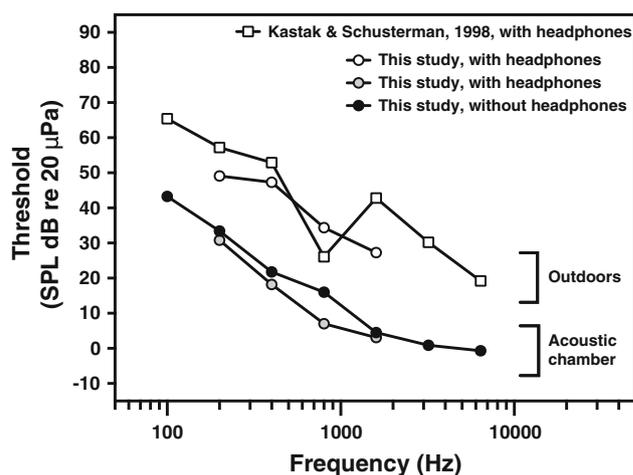


Fig. 1 Auditory thresholds for a trained harbor seal measured under different environmental (outdoors or in acoustic chamber) and stimulus presentation (with and without headphones) conditions. The *open squares* show data for this seal at age 6, when tested outdoors using headphones (Kastak and Schusterman 1998). The *circular symbols* show data for the same seal at age 13 tested under three conditions in this study: outdoors using headphones (*open circles*), in the acoustic chamber using headphones (*gray circles*), and in the acoustic chamber with stimuli presented from mounted speakers (*black circles*)

X-axes correspond to equal sound pressures in each medium (see Supin et al. 2001).

Of the three subjects tested in air, the harbor seal had the most sensitive hearing. The lowest measured threshold was -4 dB re $20 \mu\text{Pa}$ at 3200 Hz. The bandwidth of hearing within 20 dB of this value was about 500 to 14000 Hz, above which thresholds increased by roughly 30 dB/octave. The elephant seal had by far the least sensitive hearing. The lowest measured threshold was 27 dB re $20 \mu\text{Pa}$ at 400 Hz. The 20-dB bandwidth of best sensitivity was unusually broad and flat, extending from about 150 to 27000 Hz. However, the low- and high-frequency roll-offs were similar to those observed for the harbor seal, causing the base of the resultant audiogram to appear truncated. The adult California sea lion had a lowest measured threshold of 1 dB re $20 \mu\text{Pa}$, similar to that of the harbor seal but shifted upwards in frequency (12000 Hz). The 20-dB bandwidth of best sensitivity was about 1200 to 26000 Hz. The sensitivity of the sea lion in air was substantially worse than that of the two seals at frequencies below 1000 Hz, and the high-frequency roll-off was notably sharper (approximately 30 dB over a quarter octave).

The mean false alarm rates for the harbor seal, northern elephant seal, and California sea lion during aerial hearing testing were 0.09, 0.15, and 0.11, respectively. These rates are quite comparable to one another and indicate that the subjects did not have a particularly conservative response

Table 1 Hearing thresholds, false alarm (FA) rates, and corresponding ambient noise levels for the previously unpublished audiograms presented in Fig. 3

F(kHz)	Aerial audiograms							Underwater audiograms				
	Harbor seal		Northern elephant seal		California sea lion		Ambient noise dB re (20 μPa) ² /Hz	Harbor seal		California sea lion		Ambient noise dB re (1 μPa) ² /Hz
	Threshold dB re 20 μPa	FA rate	Threshold dB re 20 μPa	FA rate	Threshold dB re 20 μPa	FA rate		Threshold dB re 1 μPa	FA rate	Threshold dB re 1 μPa	FA rate	
100	43	0.09	58	0.24	76	0.21	14	96	0.05	111	0.29	74
200	32	0.10	42	0.04	56	0.14	-3	82	0.06	88	0.27	61
400	19	0.08	27	0.12	41	0.07	-14	81	0.09	76	0.11	48
800	8	0.14	32	0.10	35	0.05	-19	77	0.10	69	0.21	44
1600	-1	0.06	29	0.19	10	0.16	-20	66	0.08	67	0.07	41
3200	-4	0.10	36	0.11	20	0.14	-23	60	0.10	60	0.16	37
6400	1	0.07	39	0.17	13	0.11	-22	62	0.08	58	0.19	33
12000	10	0.16	39	0.14	1	0.12	-28					
12800								60	0.23	62	0.26	31
18000	29	0.05	45	0.18	7	0.15	-28					
18102								55	0.04			29
24000	43	0.06	42	0.23	12	0.08	-					
25600								59	0.17	60	0.22	30
28000	39	0.05	49	0.08	38	0.00	-					
30000					58	0.08	-					
32500	47	0.05	56	0.22			-					
36204								64	0.03	75	0.28	28
43054										108	0.29	28
51200								97	0.24			29
72408								119	0.19			28

Aerial hearing thresholds are provided as a function of frequency for a 16-year-old female California sea lion (Rio), a 17-year-old harbor seal (Sprouts), and an 8-year-old northern elephant seal (Burnyce). Underwater hearing thresholds are provided as a function of frequency for a 3-year-old female California sea lion (Ronan) and a 19-year-old harbor seal (Sprouts). False alarm rates for each threshold are shown as the proportion of associated control (blank) trials presented during MCS testing in which the subject reported detection of a signal. Data for all subjects are from this study, with the exception of the low frequency (100–6400 Hz) underwater hearing thresholds for the harbor seal, which were previously measured by Kastak and Schusterman (1998) and Southall et al. (2005) and are shown as a composite of these data. For all subjects, 95 % confidence limits were within 3 dB of reported thresholds. Typical ambient noise power spectral density levels were determined from one-third-octave band measurements that included each test frequency. The psychometric functions associated with each threshold are provided in Online Resources 3–7

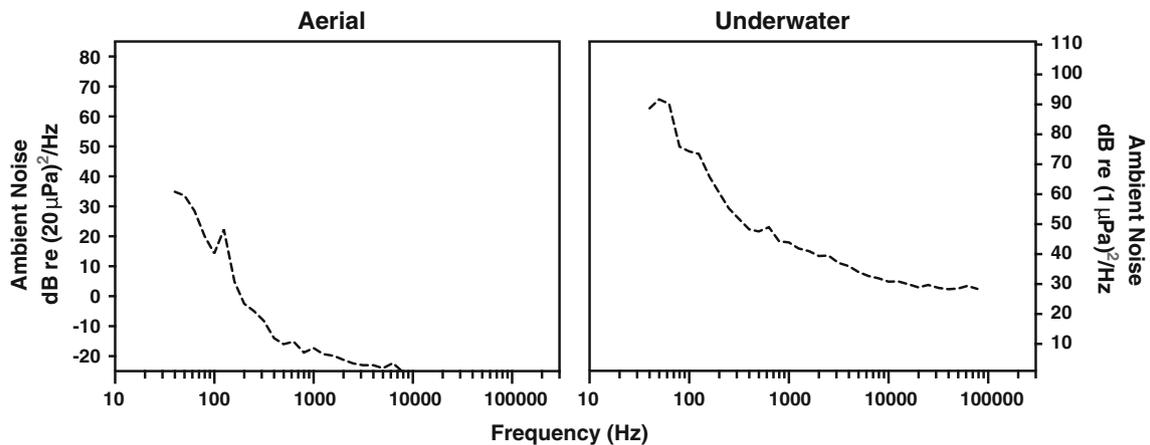


Fig. 2 Typical ambient noise levels in the acoustic chamber (*left*) and the underwater testing enclosure (*right*), shown in units of power spectral density referenced to 20 μPa in air and 1 μPa under water. The aerial noise floor is limited by self-noise of the instrument above 800 Hz

bias during testing. Threshold-to-noise offsets were at least 40 dB at all frequencies for the elephant seal. This offset is sometimes lower (<30 dB) within the range of best sensitivity for the harbor seal and the California sea lion.

The aerial audiograms determined in the current study are markedly lower than previously published thresholds. When compared to other carnivores tested in sound-isolated chambers, the hearing capabilities of the harbor seal and the sea lion appear remarkably similar to those of terrestrial species. Lowest reported thresholds average approximately -4 dB re 20 μ Pa for the four carnivores shown in the lower left panel of Fig. 3, the same as that observed for the harbor seal in this study. The bandwidth of best sensitivity is broader and extends to higher frequencies in the terrestrial carnivores but overlaps that of the pinnipeds tested. The upper frequency limits of hearing are somewhat higher in the terrestrial species.

In contrast to the aerial thresholds from the current study, underwater thresholds measured in the sea lion and the harbor seal are generally quite similar to most previously reported data for these two species. However, there are some notable differences at the lowest frequencies for the harbor seal, where thresholds reported by Kastelein et al. (2009) are roughly 15 dB lower. The harbor seal in the current study had a lowest underwater threshold of 55 dB re 1 μ Pa near 18000 Hz, and a 20-dB bandwidth of best sensitivity from about 900 to 41000 Hz. This bandwidth is even wider, extending below 200 Hz, for the two harbor seals tested by Kastelein et al. (2009). There is good agreement in high-frequency underwater sensitivity among all available data for harbor seals, showing that functional hearing under water extends to at least 72000 Hz.

The northern elephant seal tested by Kastak and Schusterman (1999) had a lowest underwater threshold of 58 dB re 1 μ Pa near 30000 Hz, and a broad 20-dB bandwidth of best sensitivity, spanning more than eight octaves from 150 to 42000 Hz. The elephant seal showed high-frequency hearing comparable to that of the harbor seal, extending to at least 60000 Hz, the highest frequency tested. This northern elephant seal was the same animal tested in air in the present study and by Kastak and Schusterman (1999), and remains the only individual of this species for which audiometric data are available.

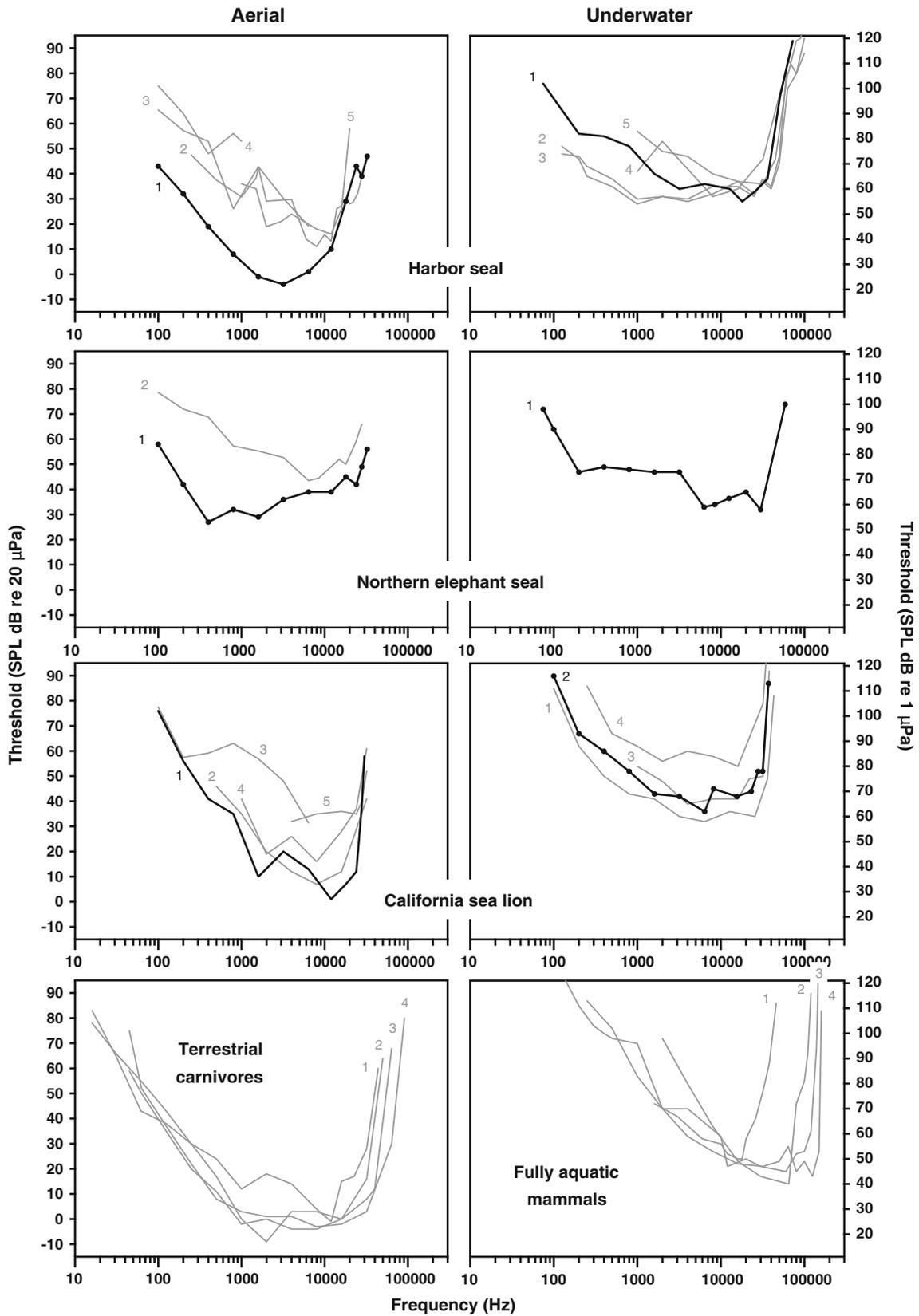
The juvenile California sea lion had the same lowest hearing threshold as the elephant seal under water (58 dB re 1 μ Pa), but her best hearing was at a lower frequency (6400 Hz). The 20-dB bandwidth of best sensitivity for this sea lion extended from 350 to 37000 Hz. High-frequency hearing rolled off sharply above this range and declined by 30 dB within a quarter octave. This underwater audiogram includes the lowest thresholds reported for this species, but is in generally good agreement with those available from prior studies (Mulsow et al. 2012a; Reichmuth and Southall

Fig. 3 Psychoacoustic hearing thresholds for three pinniped species obtained with comparable narrow-band stimuli. Harbor seals in air: 1 this study, 2 (Wolski et al. 2003), 3 (Kastak and Schusterman 1998), 4 (Terhune 1991), 5 (Møhl 1968a). Harbor seals under water: 1 this study including low frequency data from Kastak and Schusterman (1998) and Southall et al. (2005), 2, 3 (Kastelein et al. 2009), 4 (Terhune 1988), 5 (Møhl 1968a). Northern elephant seals in air: 1 this study, 2 (Kastak and Schusterman 1999). Northern elephant seals under water: 1 (Kastak and Schusterman 1999). California sea lions in air: 1 this study, 2 (Mulsow et al. 2011), 3 (Kastak and Schusterman 1998), 4 (Moore and Schusterman 1987), 5 (Schusterman 1974a). California sea lions in water: 1 this study, 2 (Reichmuth and Southall 2012), 3 (Mulsow et al. 2012a), 4 (Schusterman et al. 1972). The bold curves in each row compare aerial and underwater hearing in the same individual, obtained under the most optimal testing conditions. To facilitate these pairwise comparisons, the Y-axes are shifted by 26 dB to account for the difference in reference pressures. The lower panels show audiograms for four selected terrestrial carnivores in air: 1 domestic ferret, *Mustela putorius* (Kelly et al. 1986), 2 domestic dog, *Canis familiaris* (Heffner 1983), 3 least weasel, *Mustela nivalis* (Heffner and Heffner 1985b), 4 domestic cat, *Felis catus* (Heffner and Heffner 1985a), and four selected fully aquatic mammals in water: 1 West Indian manatee, *Trichechus manatus* (Gerstein et al. 1999), 2 false killer whale, *Pseudorca crassidens* (Thomas et al. 1988), 3 bottlenose dolphin, *Tursiops truncatus* (Johnson 1967), 4 harbor porpoise, *Phocoena phocoena* (Kastelein et al. 2002)

2012; the Schusterman et al. (1972) data have been reconciled with more recent work).

The mean false alarm rates for the harbor seal and California sea lion during underwater hearing testing were 0.16 and 0.21, respectively. These rates are similar to those obtained in Experiment 2, and again, suggest that these subjects did not have a particularly conservative response bias during testing. The relationship between the measured thresholds and the ambient noise in the testing pool can be determined by subtracting the power spectral density level of the noise from the hearing threshold at each frequency reported in Table 1. This comparison indicates a threshold-to-noise offset of at least 40 dB at frequencies above the range of best sensitivity. Within and below this range, the offset is lower, between 21 and 37 dB for both subjects.

In general, while the pinnipeds were sensitive to underwater sound over a wide range of frequencies, their lowest thresholds—55 to 58 dB re 1 μ Pa—are slightly elevated relative to the lowest thresholds reported for fully aquatic marine mammals, which occur between 40 and 50 dB re 1 μ Pa. Additionally, the high-frequency hearing limits of the pinnipeds occurred at lower frequencies than those of fully aquatic mammals (with the exception of the manatee). This difference is most pronounced in the case of the California sea lion, whose high-frequency hearing is markedly constrained in contrast to the high-frequency hearing limits of the phocid seals, which approach those of echolocating odontocetes. In terms of low-frequency hearing, the pinniped thresholds are significantly lower than those of odontocetes. This is especially apparent in



light of the harbor seal thresholds reported by Kastelein et al. (2009).

With regard to comparisons of aerial and underwater hearing capabilities, the shapes of the audiograms in each medium are most similar in the California sea lion. Both the aerial and underwater audiograms for this species have a U-shaped curve with the same, sharply declining high-frequency roll-off in the region of 25000 to 40000 Hz. This contrasts dramatically with the audiograms of the phocid seals, whose amphibious hearing capabilities are characterized by a substantial increase in the bandwidth of best sensitivity under water relative to that shown in air. The high-frequency roll-off in sensitivity occurs in the region of 40000 to 60000 Hz for these species in water, compared to a more gradual roll-off between 14000 and 30000 Hz in air. For all three species, the slope of the roll-off at low frequencies is shallower than at high frequencies. Within the range of best hearing at the base of the audiograms, thresholds for the sea lion and harbor seal occurred at lower equivalent pressures in air compared to those under water. This can be seen in Fig. 3, as the aerial audiograms for these species fall closer to the X-axis than the underwater curves. The elephant seal's aerial and underwater thresholds are generally more similar in terms of pressure, although aerial thresholds were lower at low frequencies, with the opposite being true at high frequencies.

Discussion

Aerial hearing in pinnipeds

A significant factor in past studies of marine mammal hearing has been variability in environmental controls during aerial testing. This problem seems to have been rooted to some extent in a priori assumptions that these animals would be less sensitive to sounds received in air than in water, and therefore, that the typical acoustic controls used during testing of terrestrial carnivores were not considered to be as necessary. In harbor seals, for example, successive studies of aerial hearing utilized a variety of testing environments including typical outdoor conditions (Møhl 1968a), enclosed indoor conditions (Terhune 1991), sound-attenuating headphones used outdoors (Kastak and Schusterman 1998), and sound-attenuating boxes (Wolski et al. 2003). As the ambient noise in these environments was often difficult to measure accurately with available instrumentation—especially at low frequencies—the possible masking effects of noise on hearing may have been overlooked or mischaracterized. More accurate and accessible battery-powered instrumentation and better information about critical ratios for harbor

seals (Turnbull and Terhune 1990; Southall et al. 2000, 2003) now allow for even low levels of ambient noise to be measured and considered in tests of aerial hearing.

In the present study, the availability of a specialized chamber suitable for audiometric testing allowed experimental issues related to the measurement of aerial hearing thresholds in harbor seals to be systematically addressed. The results of Experiment 1 showed that the low-frequency thresholds obtained in the acoustic chamber were markedly lower (up to 39 dB lower) than those measured outdoors with headphones. One explanation for these large differences is that the previous thresholds were limited by ambient noise, even though the noise floor under the headphones was carefully measured and the reported thresholds appeared to be well above noise power spectral density levels within relevant one-third-octave bands (Kastak and Schusterman 1998). Another possibility is that sound pathway differences between direct-field and headphone conditions contributed to threshold differences, as observed in humans (Killion 1978; Yost 2000). Because the hearing thresholds for the harbor seal were lower in the acoustic chamber than outdoors regardless of how the tones were presented, the contribution of sound pathway differences can be ruled out. Therefore, it is likely that elevated thresholds reported for this harbor seal outdoors—as well as for other harbor seals tested in air prior to this experiment—resulted from a lack of ambient noise control.

In Experiment 2, the assessment of aerial hearing thresholds was extended across the frequency range of hearing for this harbor seal as well as for the other two species tested. The quiet environment of the acoustic chamber enabled the measurement of very low thresholds at mid to low frequencies. The noise offsets determined for these thresholds can be evaluated using published critical ratio data to conservatively estimate the potential for masking by natural ambient noise. Fortunately, critical ratios have been previously measured (from 200 to 8000 Hz) for the three subjects of Experiment 2 (Southall et al. 2003). These data indicate that the aerial audiogram reported for the northern elephant seal is completely unmasked. In the case of the harbor seal, however, the thresholds measured between 1600 and 6400 Hz are a critical ratio above the measured noise floor, suggesting that even the acute sensitivity observed in this range may be constrained by noise. For the California sea lion, a similar problem likely occurs at the frequency of best sensitivity (12000 Hz), where the threshold is approximately one critical ratio above the measurable background noise. While it is possible that masking noise influenced these thresholds for the harbor seal and sea lion, this is difficult to confirm. At these frequencies, the noise levels in the environment fall at or below the detectable limits of the instrumentation used (Brüel and Kjær 2008).

Regardless, the measurements reported here represent conservative estimates of best hearing capabilities that fall well below previously measured aerial hearing thresholds for these species.

Underwater hearing in pinnipeds

The consideration of the effect of background noise in underwater testing enclosures is equally important to evaluating absolute hearing capabilities. The influence of time-varying fluctuations in low-level noise is often difficult to account for, even when critical ratio data are available to determine potential masking effects on thresholds (e.g., Branstetter and Finneran 2008). These fluctuations were greater in the test pool than the acoustic chamber. The 50th percentile statistical method used in both environments to measure ambient noise in one-third-octave bands provides some advantages over more typical L_{eq} measures in accounting for temporal variability (see Mulsow and Reichmuth 2010). However, while these measurements were obtained daily in the current study, they were based on a statistical representation of average noise during threshold testing rather than instantaneous measurements obtained coincidentally with signal presentations. Therefore, underwater threshold-to-noise offsets that are close to a critical ratio should be interpreted with caution. For the harbor seal and California sea lion, critical ratios are available up to 8000 Hz (Southall et al. 2000, 2003). Using these data and rough extrapolations to higher frequencies, the frequencies of some concern for potential masking in the current study are 3200, 18102, and 25600 Hz for the harbor seal, and 3200 through 25600 Hz for the sea lion. At lower and higher frequencies, masking effects are not likely to have limited hearing thresholds. Even with some uncertainty in underwater noise conditions, convergence in audiometric data obtained across studies under water (as shown in Fig. 3) suggests that threshold-to-noise levels are often sufficient for measurement of absolute detection thresholds.

The low-frequency underwater data for the harbor seal is the most difficult to reconcile with prior reports. Kastelein et al. (2009) measured thresholds about 15 dB lower than those reported by Kastak and Schusterman (1998) and Southall et al. (2005) for the harbor seal in the present study, suggesting that his thresholds below 1000 Hz may have been constrained by background noise. However, even lower thresholds were measured in the same environment for the northern elephant seal (Kastak and Schusterman 1999). Furthermore, there is no obvious indication from the available ambient noise and critical ratio data that the harbor seal's thresholds were masked in this frequency range. It is possible that the observed threshold differences are related to variation in hearing

between individuals, however, as low-frequency underwater thresholds were not re-measured for the harbor seal in the present study, along with corresponding environmental noise levels, this issue cannot be resolved at this time.

Amphibious comparisons

As noted earlier, the units in which hearing thresholds are reported are an important consideration when making amphibious comparisons. Due to the acoustic impedance difference between air and water, it has been argued that thresholds should be plotted in terms of acoustic intensity, which takes impedance differences into account (see e.g., Wainwright 1958; Møhl 1968a; Richardson et al. 1995; Wartzok and Ketten 1999; Hemilä et al. 2006). However, in practical terms, the experiments reported here used an acoustic pressure transducer (either a microphone or hydrophone) to measure sound levels in testing enclosures. Conversions of these measured acoustic pressures to intensities could be made easily using the data provided here and the relationship $I = p^2/\rho c$. This would assume plane wave propagation conditions, which almost certainly differ from the actual conditions in relatively small, enclosed testing environments—especially in the case of the relatively reflective pool. There are other reasons that argue toward comparing pinniped aerial and underwater hearing in units of pressure, including the suspected involvement of the middle ear in aerial and underwater sound reception (see Kastak and Schusterman 1998, for a detailed discussion). Further support for this view comes from Finneran et al. (2002), who showed experimentally that acoustic pressure is the relevant metric of sound detection for the bottlenose dolphin. The use of pressure as the appropriate comparative metric for amphibious hearing remains a working hypothesis, but is the approach taken here based on the above arguments. Of particular relevance to this study are direct pressure comparisons made within media to other mammals specialized for hearing in the same environment to provide an alternative perspective on the extent of terrestrial or aquatic adaptation.

The air–water differences observed in prior studies have supported conclusions that harbor seal hearing is adapted primarily for underwater (Møhl 1968a; Terhune 1991; Richardson et al. 1995; Wartzok and Ketten 1999) or amphibious (Kastak and Schusterman 1998) sound reception, depending on the metric of comparison used. In light of the much better hearing sensitivity shown for the harbor seal in Experiments 1 and 2—relative to four prior studies—it is important to consider how artificially elevated thresholds in air may have confounded previous comparative assessments of amphibious hearing. Contrary to earlier interpretations, the new audiogram suggests that aerial

hearing in harbor seals is not compromised in order to enhance underwater sensitivity. This is evident whether amphibious comparisons are made in terms of common units of pressure or intensity. The most straightforward representation of this finding is achieved through comparison of the hearing sensitivity of harbor seals with that of other mammals specialized for hearing in each medium. The ability of harbor seals to hear as well in air as many terrestrial carnivores—and nearly as well in water as fully aquatic cetaceans and sirenians—illustrates that the extraordinary adaptations of the seal's peripheral auditory system (Møhl 1968b; Repenning 1972; Lipatov 1992) are not in conflict with retention of acute aerial hearing. This system is well equipped to meet the challenges imposed by both acoustic environments.

This rationale, when extended to the other species in the current study, provides insight into their demonstrated hearing capabilities. The updated thresholds for the California sea lion in air, along with a recent report for another individual (Mulsow et al. 2011), have resolved prior ambiguities concerning aerial sensitivity for this species. It is now apparent that within their best frequency range, California sea lions have highly sensitive hearing that is comparable to that of harbor seals and terrestrial carnivores. The data from this study also support a converging view of underwater hearing in sea lions (Mulsow et al. 2012a; Reichmuth and Southall 2012) that is likely reflective of all otariids as a functional group (Mulsow and Reichmuth 2010; Mulsow et al. 2012b). Despite an auditory system that is less adapted for an aquatic lifestyle (Repenning 1972; Nummela 2008), sea lions still manage to hear quite well under water, albeit over a narrower frequency range than that of phocids and most fully aquatic mammals. Taken together, the robust hearing data now available for California sea lions show similar hearing curves in air and water, with auditory capabilities that remain primarily tuned for receiving airborne sounds.

The northern elephant seal remains somewhat mysterious. While the newer aerial hearing thresholds for the elephant seal are significantly lower than those previously reported for the same animal, they are still elevated across much of the frequency range of hearing with an oddly flattened contour. The audiogram has similar features to those of individuals with conductive hearing loss (Gelfand 2001). It is likely that this loss of sensitivity (relative to the harbor seal and to terrestrial carnivores) is due in part to occlusion of the peripheral auditory pathways. This modification may be more adaptive for withstanding extreme pressure effects during deep diving than for enhancing sound conduction in water (Kastak and Schusterman 1999). The flattening of the aerial audiogram may similarly be related to adaptations of the middle ear associated with pressure tolerance. Elephant seals dive to depths exceeding

1000 m (Robinson et al. 2012) where they are exposed to pressures exceeding 100 times that of the surface atmosphere. The related middle ear modifications include the dramatically inflated auditory bullae and the enlargement and thickening of the ossicles, which are nearly nine times the mass of the hypertrophied ossicles of the harbor seal (Nummela 2008). The fact that the loss of sensitivity in air does not coincide with a concomitant gain in water lends some support to the idea that the balance between aerial and underwater hearing in this species is constrained primarily by adaptations associated with their extreme foraging ecology. While only one elephant seal has been tested using behavioral methods to date, and it is possible that the results are anomalous, the application of neurophysiological techniques uphold the finding of relatively low sensitivity to airborne signals in northern elephant seals (Houser et al. 2007, 2008; Mulsow and Reichmuth 2007).

In the context of existing anatomical interpretations, an issue worthy of further discussion is the notably expanded frequency range of hearing displayed by both seals under water, but not by the sea lion. Hemilä et al. (2006) proposed an explanation for this difference on the basis of psychoacoustic data available at the time. The authors noted that the high-frequency hearing of otariids—similar to that of terrestrial carnivores—is almost certainly limited by the upper frequency-encoding capability of the inner ear in both air and in water. Their conclusion of cochlear constraints on hearing is supported by the similar, abrupt high-frequency roll-offs observed in both media for California sea lions (as discussed in the present study) as well as by audiometric data for northern fur seals, *Callorhinus ursinus* (Moore and Schusterman 1987; Babushina et al. 1991). The expanded frequency range of hearing for phocids in water indicates that their cochlear frequency-encoding capability extends much higher than in otariids. Hemilä et al. (2006) argues that the reduced high-frequency hearing limit measured for phocids in air can be explained by the dampening action of inertial constraints imposed by the hypertrophied ossicles of the middle ear. These constraints are less relevant in water where bone conduction may contribute significantly to sound reception (see Nummela 2008) and the middle ear ossicles may function in a different manner than in air. The present study strongly supports this view. A comparison of the audiograms obtained in air for the three species tested confirms more gradual high-frequency roll-offs for the seals relative to the sea lions—explainable by the effects of ossicular inertia in the seals versus the more abrupt cochlear limit in the sea lions. A comparison of the audiograms obtained in both media for the same three species demonstrates that the seals show lower and more gradual high-frequency roll-offs in air, and higher, steeper high-frequency roll-offs in

water. This confirms that differential constraints are operating in each medium for the seals, in contrast to the sea lions.

It is difficult to determine the extent to which the hearing capabilities of pinnipeds are extraordinary, as amphibious data do not exist for most other mammals. However, gross cross-media comparisons can be made for humans and dolphins. When tested under water, human divers show significant impairment relative to their sensitivity in air, regardless of the metric of comparison used (Wainwright 1958; Parvin and Nedwell 1995). Conversely, the limited available data indicate that dolphins have reduced sensitivity in air relative to land-living mammals (Babushina 1979; Kastelein et al. 1997; Liebschner et al. 2005). Therefore, the ability of at least some pinnipeds to hear well in both media appears to be a special characteristic of these species.

Rethinking amphibious hearing in pinnipeds

The observed differences in the hearing of true seals and sea lions, and comparisons of their hearing to terrestrial and fully aquatic mammals, address questions about the sensory adaptations of carnivores transitioning from terrestrial to semi-aquatic lifestyles. Of particular interest are the influences of selective pressures on hearing related to the ecology of each species. Some modifications of the auditory system seem to be a direct result of aquatic adaptation, such as the modification of outer and middle ear structures and the resultant poor aerial sensitivity in the deep-diving northern elephant seal. Others are more complex and surprising, such as the coexistence of sensitive underwater and aerial hearing sensitivity in harbor seals and California sea lions. As the availability of data increases for representative species, the structure–function relationships are starting to become clearer. However, the ultimate reasons for some adaptations, such as the notably expanded range of high-frequency hearing in phocid seals, are yet to be fully resolved.

The topic of amphibious hearing in pinnipeds has long interested investigators because of the presumed trade-offs in aerial hearing that allow adaptations for underwater sound detection. Given this premise, the assessment of auditory capabilities in these mammals is relevant to understanding hearing mechanisms in both media and elucidating the potential selective pressures acting on their auditory systems. To date, audiometric data showing that hearing sensitivity in pinnipeds is nearly as good as in fully aquatic mammals have been accepted as evidence of their reliance on underwater sound for a variety of essential life functions (see Schusterman et al. 2000). However, data showing relatively poor sensitivity in air have been more difficult to explain (see Supin et al. 2001) given the

terrestrial ancestry of pinnipeds and their persistent ties to land. The results provided in the current study allow previous, disparate conclusions drawn from experimental, anatomical, and ecological studies to be re-considered in light of updated audiometric data for representative species. A comparative assessment of these findings yields a fundamentally altered view of amphibious hearing in pinnipeds, relative to conventional perspectives.

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References

- Babushina Ye S (1979) Localization by the dolphin of the source of tonal and pulse signals in water and in air. *Vestnik Leningradskogo Universiteta Biologiya* 3:119–121
- Babushina Ye S, Zaslavskii GL, Yurkevich LI (1991) Air and underwater hearing characteristics of the northern fur seal: audiograms, frequency and differential thresholds. *Biophysics* 36:909–913
- Branstetter BK, Finneran JJ (2008) Comodulation masking release in bottlenose dolphins (*Tursiops truncatus*). *J Acoust Soc Am* 124:625–633
- Brüel and Kjær (2008) Technical documentation: hand-held analyzers types 2250 and 2270. Brüel and Kjær, Sound and Vibration Measurement A/S, Nærum
- Cornsweet TN (1962) The staircase method in psychophysics. *Am J Psychol* 75:485–491
- Finneran JJ (2003) An integrated computer-controlled system for marine mammal auditory testing. SSC, San Diego, CA, 102 p
- Finneran JJ, Schlundt CE (2007) Underwater sound pressure variation and bottlenose dolphin (*Tursiops truncatus*) hearing thresholds in a small pool. *J Acoust Soc Am* 122:606–614

- Finneran JJ, Carder DA, Ridgway SH (2002) Low-frequency acoustic pressure, velocity, and intensity thresholds in a bottlenose dolphin (*Tursiops truncatus*) and white whale (*Delphinapterus leucas*). *J Acoust Soc Am* 111:447–456
- Finney DJ (1971) Probit analysis, 3rd edn. Cambridge UP, Cambridge
- Fobes JL, Smock CC (1981) Sensory capacities of marine mammals. *Psychol Bull* 89:288–307
- Gelfand SA (2001) Essentials of audiology, 2nd edn. Thieme, New York
- Gerstein ER, Gerstein L, Forsythe SE, Blue JE (1999) The underwater audiogram of the West Indian manatee (*Trichechus manatus*). *J Acoust Soc Am* 105:3575–3583
- Heffner HE (1983) Hearing in large and small dogs: absolute thresholds and size of the tympanic membrane. *Behav Neurosci* 97:310–318
- Heffner RS, Heffner HE (1985a) Hearing range of the domestic cat. *Hear Res* 19:85–88
- Heffner RS, Heffner HE (1985b) Hearing in mammals: the least weasel. *J Mammal* 66:745–755
- Hemilä S, Nummela S, Berta A, Reuter T (2006) High-frequency hearing in phocid and otariid pinnipeds: an interpretation based on inertial and cochlear constraints. *J Acoust Soc Am* 120:3463–3466
- Houser DS, Crocker DE, Reichmuth C, Mulow J, Finneran JJ (2007) Auditory evoked potentials in northern elephant seals (*Mirounga angustirostris*). *Aquat Mamm* 33:110–121
- Houser DS, Crocker DE, Finneran JJ (2008) Click-evoked potentials in a large marine mammal, the adult male northern elephant seal (*Mirounga angustirostris*). *J Acoust Soc Am* 124:44–47
- Johnson CS (1967) Sound detection thresholds in marine mammals. In: Tavolga WN (ed) *Marine bio-acoustics*, vol 2. Pergamon Press, Oxford, pp 247–260
- Kastak D, Schusterman RJ (1998) Low-frequency amphibious hearing in pinnipeds: methods, measurements, noise, and ecology. *J Acoust Soc Am* 103:2216–2228
- Kastak D, Schusterman RJ (1999) In-air and underwater hearing sensitivity of a northern elephant seal (*Mirounga angustirostris*). *Can J Zool* 77:1751–1758
- Kastak D, Schusterman RJ (2002) Changes in auditory sensitivity with depth in a free-diving California sea lion (*Zalophus californianus*). *J Acoust Soc Am* 112:329–333
- Kastelein RA, Nieuwstraten SH, Staal C, van Ligtenberg CL, Versteegh D (1997) Low-frequency aerial hearing of a harbor porpoise (*Phocoena phocoena*). In: Read AJ, Wiepkema PR, Nachtigall PE (eds) *The biology of the harbor porpoise*. De Spil Publishers, Woerden, pp 295–312
- Kastelein RA, Bunskoek P, Hagedoorn M, Au WWL, de Haan D (2002) Audiogram of a harbor porpoise (*Phocoena phocoena*) measured with narrow-band frequency-modulated signals. *J Acoust Soc Am* 112:334–344
- Kastelein RA, Wensveen PJ, Hoek L, Verboom WC, Terhune JM (2009) Underwater detection of tonal signals between 0.125 and 100 kHz by harbor seals (*Phoca vitulina*). *J Acoust Soc Am* 125:1222–1229
- Kelly JB, Kavanagh GL, Dalton JCH (1986) Hearing in the ferret (*Mustela putorius*): thresholds for pure tone detection. *Hear Res* 24:269–275
- Ketten DR (1992) The marine mammal ear: specializations for aquatic audition and echolocation. In: Webster DB, Fay RR, Popper AN (eds) *The evolutionary biology of hearing*. Springer, New York, pp 717–750
- Killion MC (1978) Revised estimate of minimum audible pressure: where is the “missing 6 dB”? *J Acoust Soc Am* 63:1501–1508
- Liebschner A, Hanke W, Miersch L, Dehnhardt G (2005) Sensitivity of a tucuxi (*Sotalia fluviatilis guianensis*) to airborne sound. *J Acoust Soc Am* 117:436–441
- Lipatov NV (1992) Underwater hearing in seals: the role of the outer ear. In: Thomas JA, Kastelein RA, Supin AY (eds) *Marine mammal sensory systems*. Plenum Press, New York, pp 249–256
- Møhl B (1968a) Auditory sensitivity of the common seal in air and water. *J Aud Res* 8:27–38
- Møhl B (1968b) Hearing in seals. In: Harrison RJ, Hubbard RC, Peterson RS, Rice CE, Schusterman RJ (eds) *The behavior and physiology of pinnipeds*. Appleton-Century-Crofts, New York, pp 172–195
- Mooney TA, Yamamoto M, Branstetter BK (2012) Hearing in cetaceans: from natural history to experimental biology. *Adv Mar Biol* 63:197–246
- Moore PWB, Schusterman RJ (1987) Audiometric assessment of northern fur seals, *Callorhinus ursinus*. *Mar Mamm Sci* 3:31–53
- Mulow J, Reichmuth C (2007) Electrophysiological assessment of temporal resolution in pinnipeds. *Aquat Mamm* 33:122–131
- Mulow JL, Reichmuth C (2010) Psychophysical and electrophysiological aerial audiograms of a Steller sea lion (*Eumetopias jubatus*). *J Acoust Soc Am* 127:2692–2701
- Mulow J, Finneran JJ, Houser DS (2011) California sea lion (*Zalophus californianus*) aerial hearing sensitivity measured using auditory steady-state response and psychophysical methods. *J Acoust Soc Am* 129:2298–2306
- Mulow J, Houser DS, Finneran JJ (2012a) Underwater psychophysical audiogram of a young male California sea lion (*Zalophus californianus*). *J Acoust Soc Am* 131:4182–4187
- Mulow J, Reichmuth C, Houser D, Finneran JJ (2012b) Auditory evoked potential measurement of hearing sensitivity in pinnipeds. In: Popper AN, Hawkins A (eds) *The effects of noise on aquatic life*. Springer, Berlin, pp 73–76
- Nummela S (2008) Hearing in aquatic mammals. In: Thewissen JGM, Nummela S (eds) *Sensory evolution on the threshold: adaptations in secondarily aquatic vertebrates*. University of California Press, Berkeley, pp 211–231
- Nummela S, Thewissen JGM (2008) The physics of sound in air and water. In: Thewissen JGM, Nummela S (eds) *Sensory evolution on threshold: adaptations in secondarily aquatic vertebrates*. University of California Press, Berkeley, pp 175–181
- Parvin SJ, Nedwell JR (1995) Underwater sound perception and the development of an underwater noise weighting scale. *Underw Tech* 21:12–19
- Ramprasad F (1975) Aquatic adaptations in the ear of the harp seal *Pagophilus groenlandicus* (Erxleben, 1777). *Rapp P-v Reun Cons Int Explor Mer* 169:102–111
- Reichmuth C, Southall BL (2012) Underwater hearing in California sea lions (*Zalophus californianus*): expansion and interpretation of existing data. *Mar Mamm Sci* 28:358–363
- Reichmuth C, Ghaul A, Southall BL (2012) Temporal processing of low-frequency sounds by seals (L). *J Acoust Soc Am* 132:2147–2150
- Repenning CA (1972) Underwater hearing in seals: Functional morphology. In: Harrison RJ (ed) *Functional anatomy of marine mammals*, vol 1. Academic Press, London, pp 307–331
- Richardson WJ, Greene CR, Malme CI, Thomson DH (1995) *Marine mammals and noise*. Academic, San Diego
- Robinson PW, Costa DP, Crocker DE, Gallo-Reynoso JP, Champagne CD, Fowler MA, Goetsch C, Goetz KT, Hassrick JL, Huckstadt LA, Kuhn CE, Maresh JL, Maxwell SM, McDonald BI, Peterson SH, Simmons SE, Teutschel NM, Villegas-Amtmann S, Yoda K (2012) Foraging behavior and success of a mesopelagic predator in the northeast Pacific Ocean: insights from a data-rich species, the northern elephant seal. *PLoS ONE* 7:e36728
- Schusterman RJ (1974a) Auditory sensitivity of a California sea lion to airborne sound. *J Acoust Soc Am* 56:1248–1251
- Schusterman RJ (1974b) Low false-alarm rates in signal detection by marine mammals. *J Acoust Soc Am* 55:845–848

- Schusterman RJ, Balliet RF, Nixon J (1972) Underwater audiogram of the California sea lion by the conditioned vocalization technique. *J Exp Anal Behav* 17:339–350
- Schusterman RJ, Kastak D, Levenson DH, Reichmuth CJ, Southall BL (2000) Why pinnipeds don't echolocate. *J Acoust Soc Am* 107:2256–2264
- Siler W (1969) Near-and far fields in a marine environment. *J Acoust Soc Am* 46:483–484
- Southall BL, Schusterman RJ, Kastak D (2000) Masking in three pinnipeds: underwater, low-frequency critical ratios. *J Acoust Soc Am* 108:1322–1326
- Southall BL, Schusterman RJ, Kastak D (2003) Masking in three pinnipeds: aerial critical ratios and direct critical bandwidth measurements. *J Acoust Soc Am* 114(3):1660–1666
- Southall BLS, Schusterman RJ, Kastak D, Reichmuth Kastak C (2005) Reliability of underwater hearing thresholds. *Acoust Res Lett Onl* 6:243–249
- Southall BL, Bowles AE, Ellison WT, Finneran JJ, Gentry RL, Greene CR, Kastak D, Ketten DK, Miller JH, Nachtigall PE, Richardson WJ, Thomas JA, Tyack PL (2007) Marine mammal noise exposure criteria: initial scientific recommendations. *Aquat Mamm* 33:412–521
- Stebbins WC (1970) Principles of animal psychophysics. In: Stebbins WC (ed) *Animal psychophysics: the design and conduct of sensory experiments*. Appleton-Century-Crofts, New York, pp 1–19
- Supin AY, Popov VV, Mass AM (2001) The sensory physiology of aquatic mammals. Kluwer Academic Publishers, Boston
- Terhune JM (1988) Detection thresholds of a harbour seal to repeated underwater high-frequency, short-duration sinusoidal pulses. *Can J Zool* 66:1578–1582
- Terhune JM (1991) Masked and unmasked pure tone detection thresholds of a harbour seal listening in air. *Can J Zool* 69:2059–2066
- Terhune JM, Ronald K (1971) The harp seal, *Pagophilus groenlandicus* (Erleben 1777). X. The air audiogram. *Can J Zool* 49:385–390
- Terhune JM, Ronald K (1972) The harp seal, *Pagophilus groenlandicus* (Erleben 1777). III. The underwater audiogram. *Can J Zool* 50:565–569
- Thomas J, Chun N, Au W, Pugh K (1988) Underwater audiogram of a false killer whale (*Pseudorca crassidens*). *J Acoust Soc Am* 84:936–940
- Turnbull SD, Terhune JM (1990) White noise and pure tone masking of pure tone thresholds of a harbor seal listening in air and underwater. *Can J Zool* 68:2090–2097
- Wainwright WN (1958) Comparison of hearing thresholds in air and in water. *J Acoust Soc Am* 30:1025–1029
- Wartzok D, Ketten DR (1999) Marine mammal sensory systems. In: Reynolds JE III, Rommel SA (eds) *Biology of marine mammals*. Smithsonian Institution, Washington, D.C., pp 117–175
- Watkins WA, Wartzok D (1985) Sensory biophysics of marine mammals. *Mar Mamm Sci* 3:219–260
- Wolski LF, Anderson RC, Bowles AE, Yochem PK (2003) Measuring hearing in the harbor seal (*Phoca vitulina*): comparison of behavioral and auditory brainstem response techniques. *J Acoust Soc Am* 113:629–637
- Yost WA (2000) *Fundamentals of hearing: an introduction*, 4th edn. Academic Press, San Diego