Reliability of underwater hearing thresholds in pinnipeds

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Abstract: Repeated measures of low-frequency underwater hearing sensitivity in individuals of three pinniped species tested over 4–7 years are presented. Despite changes in the experience of the subjects and certain testing parameters (e.g., equipment and research personnel), measured underwater hearing thresholds within subjects over relatively long periods of time were quite similar at the frequencies tested (0.2–6.4 kHz). These data address the reliability of acoustic signal detection measurements over time in pinnipeds using psychophysical techniques. They are also relevant in considering the cumulative effects of aging, experience, and noise exposure on pinniped hearing in certain frequency bands.
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1. Introduction

Most if not all marine mammals, including the pinnipeds (seals, sea lions, and walrus), rely heavily on hearing in performing important life functions. Concern regarding the effects of human noise on these species has stimulated considerable interest and research (Richardson *et al.*, 1995; Wartzok and Ketten, 1999; National Research Council, 2003). Recent advances include quantifying simultaneous and residual effects of noise on hearing in several species (e.g., Kastak *et al.*, 1999; Schlundt *et al.*, 2000; Finneran *et al.*, 2002; Nachtigall *et al.*, 2003; Southall *et al.*, 2000). However, "absolute" (unmasked) hearing data are available for just 15% or so of the approximately 128 species of marine mammals. Fundamental questions regarding audition remain, including the reliability of independent estimates of hearing ability, variation in auditory sensitivity within and between species, and the effects of aging and repeated noise exposure on hearing. This paper presents longitudinal hearing data for individuals of three marine mammal species bearing on some of these issues.

Studies of marine mammal sensory capabilities have, without exception, involved small numbers of test subjects. This is because housing and training subjects to participate in psychophysical experiments is both costly and time-consuming. Moreover, individual marine mammals trained to perform a variety of signal detection tasks frequently show strong response bias, or the tendency to respond to test stimuli either more conservatively or more liberally (e.g., Schusterman, 1980). Advances in electrophysiological techniques in which hearing capabilities are estimated by measuring neural activity (see Supin *et al.*, 2001) may eventually minimize the effect of these factors. When electrophysiological techniques are more standardized and the resulting data systematically compared with reliable behavioral measures of hearing, these methods may improve assessment of intra- and inter-individual auditory variability for some marine mammal species. However, psychophysical data currently provide the most accurate measure of auditory sensitivity, and will remain essential at least until the precise relationship

between techniques is well demonstrated. All possible sources of variability must be considered in order to accurately interpret behavioral data.

As in most areas of comparative audiology, much more is known about longitudinal changes in human hearing than in other mammals. Both age and sex-based differences in the hearing abilities of children (e.g., Roche *et al.*, 1978) and adults (e.g., Morrell *et al.*, 1996) have been documented. These and other data indicate that there are considerable intra- and inter-individual differences in measured human auditory sensitivity based on both gender and age, in addition to various methodological factors.

Hearing studies involving small sample sizes of several other mammalian species have demonstrated inter-individual variability (see Fay, 1988; Richardson *et al.*, 1995) as well as methodological variables that limit comparisons between studies (Long, 1994). Longitudinal studies specifically addressing intra-individual auditory variability in nonhuman mammals are unavailable because most species used in behavioral hearing research (generally small primates, cats, and rodents) are typically not maintained as subjects for long periods of time. However, anecdotal data from individual subjects retested over several years suggest that absolute sensitivity may remain relatively stable in chinchillas (Trahiotis, private communication) and mice (Moody, private communication).

In contrast to most terrestrial animals, some individual marine mammals may participate in behavioral hearing tasks over many years. These situations, given appropriate controls, are particularly conducive to investigations of methodological and longitudinal variables. Hearing assessments of older marine mammal subjects, as compared either to younger individuals or to themselves when younger, have revealed patterns of age-related hearing loss similar to those observed in humans (Ridgway and Carder, 1997; Brill *et al.*, 2001; Schusterman *et al.*, 2002). However, as in terrestrial species, there are no published data addressing variability in measured hearing performance in nonsenescent marine mammals tested across multiple years.

The specific question addressed here is how reliable are underwater, behavioral threshold measurements over time in three pinniped species. The data evaluated comprise repeated measures of absolute hearing sensitivity for individuals from three species: northern elephant seal (*Mirounga angustirostris*), harbor seal (*Phoca vitulina*), and California sea lion (*Zalophus californianus*). Subjects were tested over 4–7 years, comprising roughly 25% of their life expectancy. The testing enclosure and psychophysical procedures were the same throughout testing, but the equipment and experimental personnel were not.

2. Materials and methods

Underwater behavioral hearing thresholds were obtained for a female northern elephant seal, a male harbor seal, and a female California sea lion at multiple frequencies between the years of 1994–1996 (Kastak and Schusterman, 1998) and again in 2000–2001. The elephant seal (Burnyce) was 1–3 years old during the initial tests and 7–8 during replication, the harbor seal (Sprouts) was 5–7 and 12 years old, respectively, and the sea lion (Rio) was 9–11 and 15 years old, respectively. There were no known insults to the auditory systems of these subjects other than intermittently recurrent otitis externa in the elephant seal, which was not thought to significantly affect bilateral hearing capabilities (Kastak and Schusterman, 1998).

The subjects were long-term resident research animals housed at Long Marine Laboratory at the University of California, Santa Cruz (UCSC) and maintained under National Marine Fisheries Service (NMFS) permit No. 259-1481-00. The animal care, training, and testing protocols used in these studies were approved by NMFS and the Chancellor's Animal Research Committee at UCSC and complied with current U.S. laws and standards governing marine mammal research.

The materials and methods for this study were similar to those described by Kastak and Schusterman (1998) and subjects were tested and subsequently retested in the same relatively quiet pool. Subjects received 25%–75% of their daily allotment of fish during one to three experimental sessions in each testing phase, five to seven mornings per week. Only significant differences in testing equipment and procedures between 1994–1996 and 2000–2001 are

described in detail here. Each of the subjects participated in a variety of audiometric experiments involving similar psychophysical procedures between the initial and replication studies (Kastak *et al.*, 1999, 2003; Southall *et al.*, 2000), so that no additional training was required for this study. These intervening studies investigated auditory system responses to noise exposure, including simultaneous (masking) and residual (temporary threshold shift (TTS)) impacts on hearing. Careful attention was taken throughout each study to ensure that no permanent changes in hearing resulted, despite the fact that hundreds of controlled noise exposures were conducted with each subject. The primary differences in subjects between the two phases of testing were their ages, cumulative exposure to controlled noise, and the extent of their experience in hearing tasks. Each phase of testing was conducted under the careful oversight of R.J.S. and there was some overlap in the human personnel involved in obtaining and analyzing audiometric data. However, the experimenters and trainers actually conducting experiments were generally different.

A similar-sized PVC testing apparatus was used in both the original and subsequent studies. The primary difference from the initial apparatus was the removal of a sliding door to eliminate noise associated with its movement. Pure tone signals [500 ms signal duration, 40 ms rise/fall times] were produced, triggered, and analyzed using similar hardware/software configurations in each phase of testing. However, the projecting and receiving transducers differed between experimental sessions conducted in 1994–1996 and 2000–2001. In 2000–2001, a Lubell Labs LL-1424 underwater speaker was used in projecting test stimuli rather than a Naval Undersea Warfare Center (NUWC) J-9 and an International Transducer Corporation (ITC) 8212 hydrophone was used in calibration rather than a NUWC H56. During both phases, test stimuli were carefully mapped at 26 positions within a $20 \times 20 \times 20$ cm region that encompassed all reasonable positions of the subject's head during testing to achieve maximum uniformity in signal fields (as in Kastak and Schusterman, 1998; Southall *et al.*, 2000). Calibrated low frequency ambient noise measurements in the testing enclosure were also obtained.

The behavioral techniques used in determining frequency-specific hearing thresholds were identical between studies. The sequence of testing involved a staircase procedure (Cornsweet, 1962) for initial threshold estimates, each test involving ten reversals between sequences of hits and misses (as in Kastak and Schusterman, 1998). Generally between three and eight staircase sessions were conducted for each test frequency. Based on threshold estimates from the staircase procedures, a modified method of constant stimuli technique (Stebbins, 1970) was used for the final threshold determination. Final hearing thresholds were calculated as the 50%-correct detection point of pooled results over a range of 16–24 dB from all method of constant stimuli sessions using probit analysis (Finney, 1971). Generally between three and five methods of constant stimuli sessions (or approximately 12–20 trials per stimulus level) were required to ensure that 95% confidence intervals for detection thresholds fell within 3 dB of the calculated value.

In 2000–2001, estimates of hearing sensitivity were obtained at six test frequencies: 200, 400, 800, 1600, 3200, and 6400 Hz. In 1994–1996, Kastak and Schusterman (1998) tested each of these frequencies, but used 6300 Hz rather than 6400 Hz. The lowest two frequencies tested in 1994–1996 (75 and 100 Hz) were not retested in 2000–2001 due to equipment limitations. A *t*-test for paired comparisons was used to determine statistical differences between initial and replicated hearing thresholds for each subject. Thresholds were compared within subjects at different ages for mutual paired test frequencies. The percentage of "false alarms" (positive responses on signal-absent trials) was calculated for all experimental sessions at each test frequency. Signal-present to signal-absent trial ratios were 1:1 unless false alarm rates deviated repeatedly outside a target range. If a subject had three consecutive sessions with false alarm rates below 5%, the signal-present to signal-absent ratio was changed to 2:1 until false alarm rates increased. If a subject had three consecutive sessions with a false alarm rate above 20%, the ratio was changed to 1:2 until false alarm rates decreased. Only sessions with a 1:1 signal-present to signal-absent ratio were included in data analysis.

3. Results

Underwater, low frequency, behavioral hearing thresholds in the elephant seal and California sea lion were not significantly different compared to those obtained 4–7 years earlier; thresholds for the harbor seal were slightly but significantly lower than those obtained previously (Fig. 1). Neither the initial nor subsequent behavioral hearing thresholds appear to be masked by low frequency ambient noise in the testing enclosure (see Kastak and Schusterman, 1998; Fig. 1). Additionally, subjects maintained comparable response biases in both the 1994–1996 and 2000–2001 studies. If false alarm rates deviated from the defined acceptable range (5%–20%), a change in signal probability usually resulted in a swift modification in response bias.

For the elephant seal, thresholds obtained in 2000–2001 were very similar to those obtained in 1994–1996 [range: −2.5 to +2.5 dB]; thresholds paired by test frequency were not significantly different ($t_{[1,5]}=0.641$; $p=0.57$). Standard deviations of hearing thresholds were similar in 2000–2001 [0.4–0.6 dB] and 1994–1996 [0.6–1.3 dB]. False alarm rates ranged from 2.2% to 11.5% in 2000–2001 as compared to 1.1% to 4.1% in 1994–1996.

Replicated hearing thresholds for the harbor seal were lower for each frequency retested [range: −2.1 to −6.2 dB]. While these differences were relatively small, they were statistically significant ($t_{[1,4]}=5.262$; $p=0.01$). Standard deviations of hearing thresholds were virtually identical in 2000–2001 [0.3–1.0 dB] and 1994–1996 [0.3–1.0 dB]. False alarm rates for the harbor seal ranged from 4.8% to 12.2% in 2000–2001 compared with 3.3%–10.1% in 1994–1996.

For the sea lion, 2000–2001 thresholds for the three lowest test frequencies were considerably lower than in 1994–1996, while the remaining values were higher than those obtained initially [range: −15.0 to +8.6 dB]. Differences between the initial and replicated hearing thresholds across all paired test frequencies were not statistically different ($t_{[1,4]}=0.6864$; $p=0.53$). Standard deviations of hearing thresholds were similar in 2000–2001 [0.4–1.6 dB] and 1994–1996 [0.3–0.8 dB]. False alarm rates for the sea lion ranged from 4.4% to 19.1% in 2000–2001 as opposed to 2.9% to 12.0% in 1994–1996.

4. Discussion

Despite changes in subject age and experience in signal detection tasks, testing equipment, and experimental personnel, underwater hearing thresholds in three pinnipeds over a 4–7 year time period were remarkably consistent. There were no measurable differences in hearing sensitivity in two of the subjects and a slight but statistically significant increase in sensitivity (i.e., lower hearing thresholds) in another. There were no measurable reductions in hearing sensitivity for the frequencies tested despite the fact that research conducted between 1996 and 2000 involved several hundred controlled noise exposures at similar frequencies resulting in auditory masking (Southall *et al.*, 2000) and a lesser number of exposures known to induce temporary hearing losses of 6 dB or greater [elephant seal: 12 occurrences; California sea lion: 18; harbor seal: 20] (Kastak *et al.*, 1999, 2003). The combined results of the 1994–1996 and 2000–2001 measurements suggest that hearing abilities in some mammals, including those regularly exposed to moderate levels of noise, may remain relatively unchanged over multiple years prior to senescence. Furthermore, audiometric data can be reliably replicated with psychophysical techniques over long periods in marine mammals. It is likely that the careful attention given to minimizing variability in critical factors, including testing methodology, signal calibration and control, ambient noise in the testing enclosure, and subject response tendencies was very important in replicating earlier measurements.

Methodological variables that may introduce apparent differences in hearing sensitivity (Long, 1994) were controlled in both the 1994–1996 and 2000–2001 measurements. Test stimuli and background noise conditions were also controlled to the greatest practical extent within the testing enclosure. These controls were essential in replicating the initial results, despite some differences in equipment and experimental personnel. Further, the ability to maintain relatively consistent subject response biases throughout the investigations was likely important in replicating the earlier results.
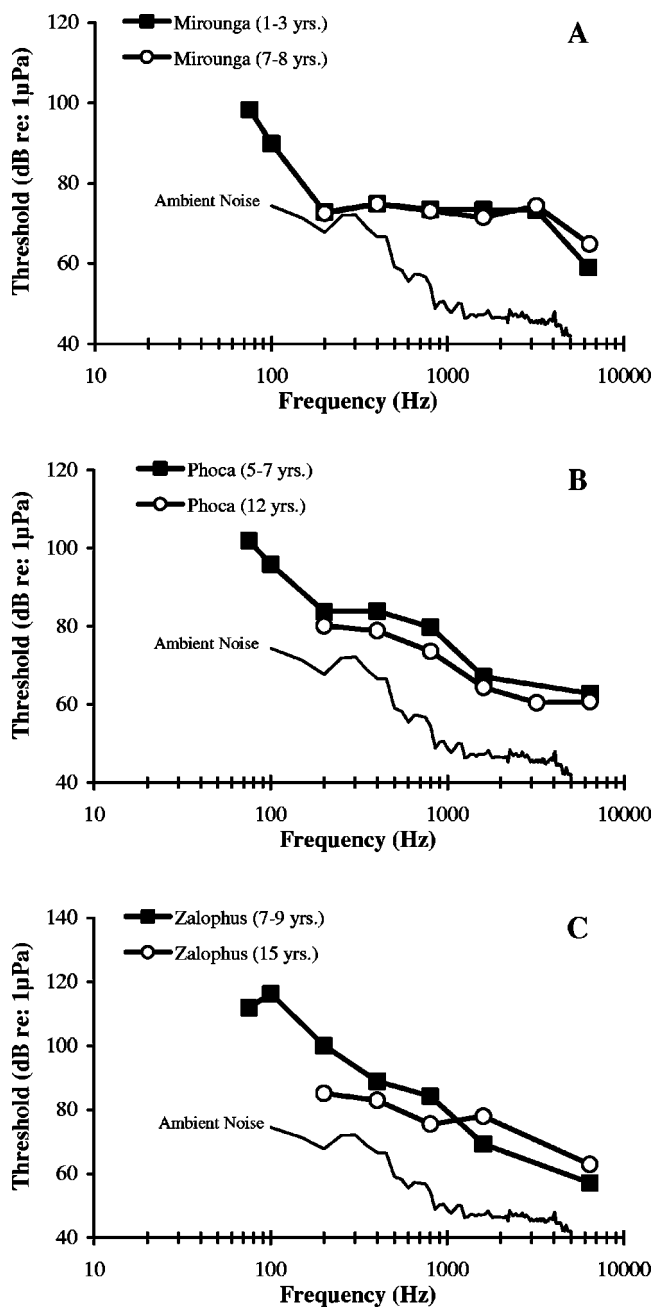
Fig. 1. Underwater hearing thresholds obtained at low test frequencies for three pinniped subjects [(a) northern elephant seal (*Mirounga angustirostris*), (b) harbor seal (*Phoca vitulina*), and (c) California sea lion (*Zalophus californianus*). Note difference in vertical scale for (c). Closed squares indicate hearing thresholds measured in 1994–1996 and open circles denote those measured in 2000–2001. Ambient noise measurements (in dB re: 1 μPa^2/Hz) given here were collected in conjunction with 2000–2001 thresholds; ambient noise measurements accompanying 1994–1996 thresholds are presented in Kastak and Schusterman (1998).

Subject variables that are less easily controlled include age and testing experience. The present study was undertaken in part to evaluate age-related changes in the hearing sensitivity of individual subjects. As described, these systematic longitudinal factors appear not to have affected measured hearing sensitivity at the frequencies tested. Subject experience in signal detection tasks also changed (increased) over the period of testing, but this also did not appear to affect measurements of hearing sensitivity. It is important to note, however, that subjects were already fairly experienced in performing signal detection tasks prior to 1994–1996. One might imagine an interaction between subject experience and age-related hearing loss that might produce the observed results. However, it is unlikely that age-related reductions and experience-related improvements in measured sensitivity would be so uniform across the range of frequencies tested for each of the three test subjects, because of the frequency dependence of age-related hearing loss. Mammals investigated thus far, including pinnipeds (Schusterman *et al.*, 2002), tend to lose sensitivity first and most dramatically at relatively high frequencies. All of the measurements reported here were conducted at low frequencies relative to the overall range of hearing sensitivity of the subjects. Kastak and Schusterman (1998) selected these frequencies for the 1994–1996 measurements because of the limited data on pinniped hearing at low frequencies and the fact that many natural and human noise sources have considerable energy at low frequencies. It is possible that age-related hearing losses were not detected between studies because of the frequencies tested. It is also possible that the elevated hearing thresholds for the California sea lion at the highest frequencies used in this study represent real hearing losses that are expected to increase with both test frequency and age. Results obtained from this subject and an older California sea lion at higher frequencies support this idea (Schusterman *et al.*, 2002).

Because so little is known about basic hearing capabilities in marine mammals and the effects of noise, sensory research laboratories holding subjects for relatively long periods should periodically repeat absolute hearing measurements under controlled conditions. Ideally, these experiments would involve comparative analyses using various behavioral and electrophysiological procedures. Such data should provide needed insight into the effects of subject age/experience as well as methodological variables on measured sensory capabilities. Differences in environmental variables, such as ambient noise in testing enclosures, should be particularly considered in future longitudinal audiometric analyses (Holt *et al.*, 2001).

Acknowledgments

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