

Changes in auditory sensitivity with depth in a free-diving California sea lion (*Zalophus californianus*)

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All current data on underwater hearing in pinnipeds are based on tests conducted in small tanks, and may not accurately represent the auditory functioning of free-ranging animals, especially if hearing sensitivity changes with water depth. Underwater auditory thresholds were determined for a California sea lion at depths ranging from 10 to 100 meters. The following results were obtained: (1) False alarm probabilities (responding in the absence of a signal) decreased significantly with depth, indicating that the sea lion adopted a more conservative response criterion in deeper water. (2) Hearing sensitivity generally worsened with depth. (3) There was a significant interaction between depth and frequency, the depth effect being most pronounced at 10 kHz and reversing at 35 kHz. Increasing pressure related to diving probably alters the impedance characteristics of the pinniped ear, in particular affecting the size of the middle-ear air space via expansion of cavernous tissue in the middle-ear cavity. These results show that the middle ear plays a functional role in underwater sound detection in sea lions. However, contrary to previous speculation, the presence of cavernous tissue in the sea lion middle ear does not appear to enhance sensitivity at depth. © 2002 Acoustical Society of America. [DOI: 10.1121/1.1489438]

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I. INTRODUCTION

One of the distinctive features of the middle ear of seals and sea lions is the presence of a thickened mucosa with three distinct layers, thought to engorge with blood when submerged. The cavernous tissue of the pinniped middle ear has been thoroughly described by Tandler (1899; harbor seal), Odend'hal and Poulter (1996; California sea lion), Møhl (1968; harbor seal), and Welsch and Riedelsheimer (1997; Weddell seal). The function of this middle-ear mucosal layer has been suggested by these authors as (1) regulating middle-ear volume at depth by expanding under increased static pressure, through either passive or active means, and (2) allowing for movement of the middle-ear ossicles at depth, by ensuring that they were maintained in an air-filled space upon submergence (i.e., the epitympanic recess would be the last reservoir of air in the middle ear as the cavernous tissue expanded during a dive). Møhl (1968) first hypothesized that the cavernous tissue played a potential role in acoustic impedance switching, allowing the pinniped ear to function amphibiously. In air, for example, the input impedance of the middle ear would approximate that of air; in water, the inflation of cavernous tissue would alter the middle-ear impedance to equal that of the aquatic environment. Although the sound conduction mechanisms between the environment and the pinniped inner ear have not been elucidated in water or in air, Repenning (1972) believed that expansion of the cavernous tissue on both sides of the tympanic membrane would allow sound transmission along a pathway identical to that of terrestrial mammals (i.e., tympanic-ossicular transmission), based on a similar concept of impedance matching due to cavernous tissue expansion.

The role of the middle ear in underwater hearing is unknown for terrestrial, marine, or amphibious mammals. In

air, the middle ear is commonly thought to serve as an impedance-matching device, converting airborne sound waves (low-pressure, high-particle velocity) into fluid waves (high-pressure, low-particle velocity) in the cochlea. In water, the air-filled middle ear is generally described as an impediment to the transfer of sound energy, because of the air-water barrier at the tympanic membrane, where theoretically, almost all incident sound energy is reflected rather than transmitted (Repenning, 1972). As a result, mammals are thought to hear primarily through bone conduction when under water, "bone conduction" referring to any and all sound conduction pathways not utilized in the normal (i.e., outer to middle to inner ear) sense. Vibration of the head, transmission of pressure or flexural waves through the bones of the skull, conduction of particle motion from the environment through the skull to the inner ear, and compression of the cochlear capsule have all been proposed as bone conduction mechanisms (Tonndorf, 1972). The retention of air spaces in the external meatus and middle ear has been viewed solely as a barrier to sound conduction and thus, an impediment to underwater hearing (but see Lipatov, 1992 for a discussion of the role of intrameatal air on hearing sensitivity in pinnipeds).

In contrast to humans, pinnipeds are generally more sensitive to underwater sound than to airborne sound across the audible frequency range (Hamilton, 1957; Hollien and Feinstein, 1975; Schusterman, 1981—for a review of underwater hearing in humans, see Kirkland *et al.*, 1989). Additionally, when tested in water, pinnipeds also have shown an extended upper-frequency hearing limit compared to the upper limit shown in air. One reason for the increase in upper-frequency limit may be related to changes in acoustic impedance—increasing the stiffness of the ossicular suspension upon submergence ought to facilitate high-frequency sound transmis-

sion through the middle ear. As Repenning (1972) notes, there is no evidence that the pinniped middle-ear bones do not function in a relatively fluid environment (i.e., partially or wholly surrounded by cavernous tissue); therefore, if cavernous tissue surrounds the ossicles and contacts both sides of the tympanic membrane at depth, then acoustic energy should be transmitted across the tympanic membrane with the same efficiency as when transmitted from air to the ear. Thus, hearing sensitivity would be better than that expected for a terrestrial ear (Repenning, 1972). These changes in underwater sensitivity should directly reflect the degree to which the cavernous tissue is inflated, and therefore, hearing sensitivity in pinnipeds should change with depth.

To date, all pinniped auditory thresholds have been obtained in shallow tanks, as has all work on masking and the effects of noise. However, conclusions drawn from work conducted in shallow tanks are suspect because of the presence of reflective barriers, pressure release surfaces, and near-field effects. Free-field relationships between sound pressure and particle velocity do not apply in such confined situations, so estimates of acoustic impedance and sound intensity are unlikely to be close approximations of the experimental conditions. Testing under conditions more closely resembling a free field (i.e., open ocean) would provide a means to ecologically validate results obtained in the laboratory. Another advantage of open ocean work relates to the effects of noise on marine mammals: In order to make rational policy decisions regarding anthropogenic noise limits, it is necessary to determine how realistic our estimates of noise exposure for these animals are, given that the work in shallow tanks might over- or underestimate sensitivity of free-ranging (diving) animals to noise.

To resolve the role of the middle ear in hearing at depth, and to provide a comparison open-water study of hearing in pinnipeds, we trained a free-diving male California sea lion to report detection of acoustic signals at depths of 10, 50, and 100 m. We obtained auditory thresholds at each of these depths over a range of frequencies to determine whether pressure-related changes in auditory sensitivity occur.

II. METHODS

A. Subject

The subject of this experiment was "Newman" (ZC701), a 12-year-old male California sea lion (*Zalophus californianus*), housed in an open ocean pen at Space and Naval Warfare Systems Center San Diego in San Diego Bay, California. He was fed a daily diet of freshly thawed herring (*Clupea spp.*), capelin (*Mallotus villosus*), smelt, (*Osmarus spp.*) and squid (*Ilex spp.*) totaling about 4 to 5 kg. He consumed about 25%–50% of his daily ration during experimental sessions.

B. Apparatus

The response apparatus consisted of a platform comprising a 31-cm-diameter PVC tube on which were mounted a bite plate, response paddle, and video camera, as shown in Fig. 1. The bite plate provided a stationing area for the subject, who could maintain an invariant head position at a par-

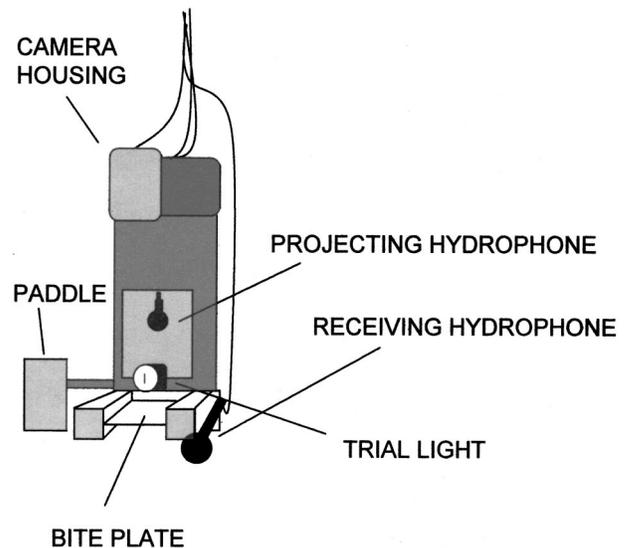


FIG. 1. Diagram of the apparatus for obtaining thresholds at depth from a California sea lion.

ticular depth in the water column by biting on to the neoprene-covered bite-plate surface. The response paddle was an aluminum target plate positioned to the left side of the bite plate. The video camera provided a view of the bite plate and response paddle from above. A dive light, used to delineate trial intervals, was positioned in front of the bite plate and in view of the subject while stationed. A projecting hydrophone (ITC 1032) was suspended in the center of the tube, and an identical receiving hydrophone was mounted on the bite plate. The receiving hydrophone was positioned for calibration purposes in the space that would be occupied by the subject's head during test sessions, and could be pivoted away for testing. The projecting hydrophone was positioned in front of and above the subject, at a distance of 80 cm from the center of the subject's head.

The apparatus was suspended via cable from the side of a 26-ft. research vessel, modified to house the equipment for signal production and audio and video monitoring. Test signals (500-ms duration, sampled at 500 kHz, 5-ms rise/fall time, manually triggered) were generated by an experimenter on a personal computer, attenuated, amplified, and projected from the transmitting hydrophone. The incoming signal and ambient noise from the receiving hydrophone were bandpass filtered, amplified, and analyzed using an oscilloscope and a PC-based real-time spectrum analyzer. The dive light was operated manually from the control booth. The trainer monitored live video feed on a constant basis during training and testing. The procedure was blind; that is, the experimenter could not see when a response was made, and the trainer did not know when a signal was triggered. The subject's responses were relayed by the trainer to the experimenter. The experimenter subsequently informed the trainer whether the response was correct or incorrect.

C. Training

The sea lion was trained via classical and instrumental conditioning procedures to dive to the bite plate in shallow water and hold on for a period of time ranging from several

seconds to just over a minute. Subsequently, the subject was trained to respond to a 2-s duration 5-kHz pure tone by pressing the paddle with its nose. In the training phase, we used a discrete trial procedure; after every presentation of a tone, the subject was recalled to the surface using a pinger that produced a series of brief 10-kHz pulses. After acquisition of the stationing and paddle press response, a dive light was introduced to delineate 4-to 6-s trial intervals. At this point in training, a signal could only occur during trial intervals, when the light was turned on. To control for false reporting during the psychophysical phase of the experiment, catch trials, during which the dive light was switched on for 4 to 6 s, but no signal was delivered, were introduced at this time. The behavior of maintaining position (rather than pressing the paddle) was reinforced on catch trials. Following each correct response, the subject was provided feedback by being presented an acoustic conditioned reinforcer (a buzz delivered under water from a small, sealed PVC enclosure).

Following acquisition of single-trial responses, multiple trials were grouped together within a single dive, the subject being trained to restation rather than to surface between trials. Training continued in shallow water until the subject reliably performed ten trials without surfacing until recalled. Fish reinforcement was delivered following completion of a pseudorandomly predetermined number of trials. The amount of reinforcement was determined by the overall amount of correct responding during a trial sequence.

Subsequent to completion of the auditory detection task, the sea lion was trained to enter a cage for transport via the research vessel to the experimental site in 250 m of water, and approximately 10 km off the San Diego coast. Acclimation to open ocean testing took place in stages starting in shallow water near the subject's home pen. Gradually, training moved into San Diego Bay up to depths of about 10 m. The training phase ended following demonstration of normal testing behavior (a series of four dives without spontaneous surfacing) at a depth of 100 m.

D. Psychophysics

Audiometric data were obtained using the go/no-go procedure described above, and a staircase psychophysical method (Stebbins, 1970). The sequence of signal and catch trials was predetermined. For each session, the initial signal level was well above threshold. This signal was attenuated by 4 dB following each HIT (correct detection) until the first MISS (failure to detect), which defined the first reversal point. Subsequently, the signal level was increased by 2 dB following each MISS and decreased by 2 dB following each HIT. Each change of direction in a sequence of signal amplitudes defined a reversal, and sessions comprised a minimum of seven and a maximum of ten reversals. False alarm rates were calculated as the number of FALSE ALARMS (responding in the absence of a signal) divided by the total number of catch trials. Thresholds defined as signal levels corresponding to 50% correct detections were calculated as the mean of the reversal points (Dixon and Mood, 1948).

The thresholds determined by the staircase procedure were ultimately transformed to constant d' thresholds. This calculation adjusted the initial threshold determination based

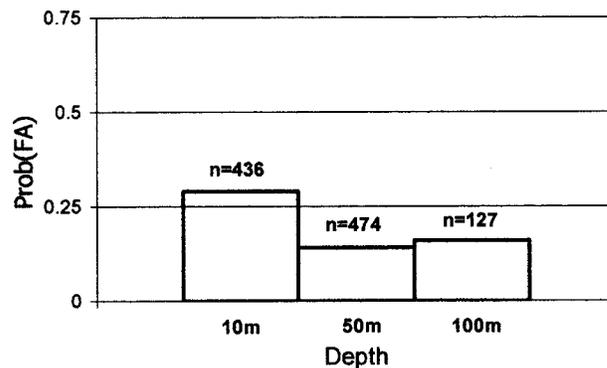


FIG. 2. Proportion of false alarm responding $P(FA)$ vs depth. $P(FA)$ was calculated by pooling training phase data and dividing the number of false alarms by the number of catch trials for a given depth. False alarm responding was significantly greater at 10-m depth than at 50 and 100 m.

on the number of false alarms per session. Thus, all sensitivity data reported herein are thresholds corresponding to a d' value of 1.

Auditory sensitivity at 10 and 50 m was measured at frequencies of 2.5, 6, 10, and 35 kHz. Additionally, thresholds at 100 m were obtained at frequencies of 2.5 and 6 kHz. However, because of small numbers of reversals and high variability, the threshold data at 100 m were excluded from comparison with shallow-water thresholds. The testing order was randomized with respect to depth and frequency in order to eliminate potentially confounding practice effects. The only constraints on testing at particular depths and frequencies on a given day were ambient noise levels and weather conditions. If noise levels were high enough to mask the test signal, dive sequences were run, but the data were not included in threshold calculations. A minimum of two and a maximum of six sessions were used to calculate thresholds at each depth/frequency combination.

III. RESULTS

During training and testing, the subject's response bias changed with depth. Results of pooled training sessions at depths of 10, 50, and 100 m are shown in Fig. 2. False alarm rates were double for sessions conducted at 10-m depth relative to those conducted at 50- and 100-m depth. There were no significant differences in false alarm responding between 50 and 100 m.

Figure 3 shows auditory thresholds (± 1 standard deviation) obtained at depths of 10 and 50 m. Included in this figure are data from Schusterman *et al.* (1972) obtained from a 5–6-year-old male California sea lion in a shallow tank at a depth of 1 m. These data fairly closely match the 10-m data obtained in the present study. As expected, thresholds varied significantly with frequency, resulting in the typical “u-shaped” audiogram. There was also a significant effect of depth on thresholds ($F_{1,26} = 12.25$; $p < 0.01$) and a significant interaction between depth and frequency ($F_{3,26} = 11.05$; $p < 0.01$); thresholds were lower in shallow water, except at the highest frequency tested (35 kHz), where this trend was reversed.

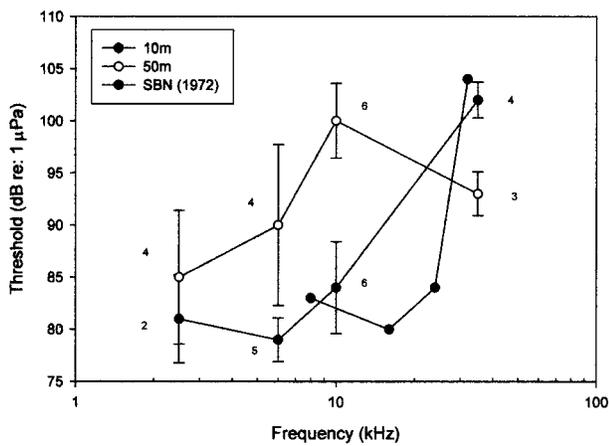


FIG. 3. California sea lion audiograms at 10 and 50 m. Error bars represent standard deviations. Numbers alongside data points represent numbers of sessions conducted at each frequency-depth combination. The plotted points without error bars are reported values for a California sea lion in a small pool (SBN=Schusterman *et al.*, 1972).

IV. DISCUSSION

The California sea lion tested in this study showed a clear tendency to withhold responding at depth, the reasons for which are unclear. It is possible that the subject was more comfortable near the surface, while within view of the boat, and was simply more likely to release the bite plate in order to respond when it was closer to the surface. At depth, if the sea lion were fearful or uncomfortable, it may have been less likely to let go of the bite plate in general, thus less likely to respond in the presence of a signal. The subject's more conservative behavior at depth may also have been a response to the generally increasing task difficulty associated with deeper diving and longer transit time to and from the apparatus at depths of 50 and 100 m. Whatever the reason for the subject's conservative behavior, the difference in response bias among the three depths tested would have had a small but significant effect on estimates of sensitivity had the traditional measure of the 50%-correct detection level been used to define a threshold. Nearly all threshold estimates at 50-m depth would have been high, while estimates obtained at 10 m would have been low, exaggerating the differences evident in the data shown in Fig. 3. It is also possible that small sample sizes for estimating false alarm rates (20 to 30 catch trials per session) led to poor estimates of detectability. However, such errors would have had to be extremely large in magnitude to account for differences of 10 dB or more. Because the 10-m thresholds obtained in this study were similar to those obtained by Schusterman *et al.* (1972) for the same species in a shallow tank, we conclude that audiometric data collected from captive subjects are probably fairly representative of hearing capabilities of free-ranging animals. However, based on the results at depth, this comparison may be valid *only* for shallow water.

Physiological mechanisms must be taken into consideration as the primary cause of the sensitivity changes with depth. It may make little sense to think of the air-water interface at the tympanic membrane as a reflective barrier to the transmission of sound, especially at low frequencies, where the sound wavelengths are many times larger than the

structures of the ear. If, at these frequencies, the middle-ear structures act as a damped resonant air space, transmitting acoustic particle motion directly to the inner ear, then changes in pressure, density, and volume would be expected to have frequency-dependent effects on hearing sensitivity. The interface between the environment and the middle-ear space might only become important as a barrier to sound transmission at high frequencies where the wavelengths are similar in size to the middle ear, external meatus, and other tissue and bony structures surrounding the ear. Thus, in shallow water, high-frequency sensitivity would be relatively poor, because of the significant reflection of sound energy at the middle ear. In deeper water, sensitivity might improve because of better impedance matching due to the expansion of cavernous tissue on both sides of the tympanic membrane.

Two separate types of change in the middle ear might account for the patterns of sensitivity change that occurred with depth. At low frequencies, changes in the resonance properties of the middle-ear space should affect sound transmission in a frequency-dependent manner. At high frequencies, expansion of cavernous tissue increases the efficiency of sound transmission through the ossicular pathway, also leading to frequency-dependent alterations in sound transmission with depth, but in the opposite direction. These proposed mechanisms doubtless oversimplify the problem of underwater hearing in pinnipeds, and do not take into consideration sound channels through tissue and bone or the possible role of the middle-ear ossicles acting as a load on the inner ear (which might be stimulated through some other mechanism altogether). However, these ideas are consistent with the results of this study and imply not only that high-frequency sensitivity improves with depth in the California sea lion, but that the upper-frequency hearing limit might also increase with depth. It is interesting to note that in a recent study by Ridgway *et al.* (2001), two belugas tested at depths of up to 300 m showed no evidence of changes in hearing sensitivity at frequencies ranging from 500 Hz to 100 kHz. These authors concluded that in the beluga, underwater hearing does not require the usual ossicular sound conduction/amplification found in terrestrial mammals. The odontocete middle ear, however, is highly specialized compared to that of the pinniped, and differences in structural anatomy and sound conduction pathways are likely to account for the different effects of depth on hearing in these two taxa.

It is evident from this study that the sea lion middle ear is functional under water, in depth- and frequency-dependent ways. Future investigations of the effects of pressure on hearing should focus on several problems. First, experimental variables such as temporal patterns of pressure change, trial and session length, and response bias should be better controlled. These problems might be addressed by testing in a hyperbaric chamber, where the time taken to simulate descent to various depths could be controlled. Second, the physiological and anatomical correlates of diving should be monitored. This might take place through ultrasound or magnetic resonance imaging when a dive response can be induced in sedated or trained subjects, answering the question of whether (a) middle-ear cavernous tissue does engorge, and

(b) whether blood flow to the middle ear is actively regulated (as part of the dive response) or passively controlled, in response to pressure changes. Finally, similar experiments should be conducted with the true seals, or phocids, which generally have larger middle-ear spaces, more extensive cavernous tissue, and are deeper divers. Only after data addressing these issues are obtained will the mechanisms of underwater auditory functioning in pinnipeds be fully understood.

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