Hearing and whistling in the deep sea: depth influences whistle spectra but does not attenuate hearing by white whales (*Delphinapterus leucas*) (Odontoceti, Cetacea)

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Summary

Hearing is attenuated in the aerial ear of humans and other land mammals tested in pressure chambers as a result of middle ear impedance changes that result from increased air density. We tested the hypothesis, based on recent middle ear models, that increasing the density of middle ear air at depth might attenuate whale hearing. Two white whales *Delphinapterus leucas* made dives to a platform at a depth of 5, 100, 200 or 300 m in the Pacific Ocean. During dives to station on the platform for up to 12 min, the whales whistled in response to 500 ms tones projected at random intervals to assess their hearing threshold at each depth. Analysis of response whistle spectra, whistle latency in response to tones and hearing thresholds showed that the increased hydrostatic pressure at depth changed each whale's whistle response at depth, but did not attenuate hearing overall. The finding that whale hearing is not attenuated at depth suggests that sound is conducted through the head tissues of the whale to the ear without requiring the usual ear drum/ossicular chain amplification of the aerial middle ear. These first ever hearing tests in the open ocean demonstrate that zones of audibility for human-made sounds are just as great throughout the depths to which these whales dive, or at least down to 300 m.

Key words: white whale, *Delphinapterus leucas*, hearing, whistling, deep sea, middle ear, eardrum, response, time, threshold, Eustachian tube, nasal cavity, phonic lip, auditory reaction time.

Introduction

Recently, scientists have debated the possible harmful effects of intense human-generated sound on the sensitive ears of sea mammals (Mulroy, 1991; Revelle, 1991; Green et al., 1994; Richardson et al., 1995). Sounds that whales hear (i.e. suprathreshold sounds) may influence their behavior in various ways. For example, a whale might approach to investigate or swim away to avoid the sound. Whales might emit a sonic response or fall silent. Intense anthropogenic sounds might mask echolocation, communication or other auditory cues important to the individual and its group. Harmful effects of the most intense sound exposure could include deafness. Deafness can occur when the level of exposure exceeds the dynamic range of the ear for a sufficient period to cause irreversible damage to hair cells. The dynamic range of the ear may vary depending on the hearing threshold.

The zone of sonic influence for the auditory system of the whale is the region around a sound source within which the projected sound intensity exceeds the hearing threshold. The extent of this region depends not only on the hearing threshold but also on the efficiency of the path from the source to the whale, on the level of background noise around the whale and on the frequency spectrum of the source. The efficiency of the sound path can be affected by factors such as water temperature profiles, the presence of organisms, suspended air bubbles or particulate matter in the water (Urick, 1982) and water depth, bottom and surface conditions. For example, background noise can be generated by wind and wave motion, by sonically active animals, by pounding surf, by grinding sea ice or by ships and aircraft (Green et al., 1994; Richardson et al., 1995). Higher frequencies tend to propagate for shorter distances because of their more rapid absorption in sea water (Urick, 1982). Although the efficiency of the sound path in the ocean can vary considerably (Urick, 1982), an efficient path over great distances, a path of minimum sonic attenuation, is the deep sound channel (DSC). For example, a small (0.5 kg) explosion in the DSC can be heard several thousand kilometers away (Urick, 1982). It has been suggested that whales might use the DSC for long-distance communication (Payne and Webb, 1971). In the tropics, the DSC may be 1000 m deep, while in the Arctic it may be only 100 m or less (Urick, 1982).

Arctic white whales (*Delphinapterus leucas*), members of the largest cetacean superfamily Delphinoidea, are known to dive to depths below 600 m (Ridgway et al., 1984; Martin and Smith, 1992; Heide-Jørgensen et al., 1998). Termed 'sea canaries' because they are among the most sonically active of all whales, white whales whistle, squeal, roar, growl and click (Slijper, 1979). White whales also have a keen sense of hearing. Hearing thresholds approach 10^{-14} W m^{-2} (42 dB re: 1 µPa) at the most sensitive frequencies in water, with an overall bandwidth of 40 Hz to approximately 150 kHz (Awbrey et al., 1988; Johnson et al., 1989; Au, 1993). Humans with thresholds at the most sensitive frequency in air of 10^{-12} W m^{-12} (0 dB re: 20 µPa) have approximately one-eighth the bandwidth of *D. leucas* (Au, 1993).

A key issue bearing on the debate about the effects of human-generated sound on whales in the ocean is the function of the ear drum and middle ear under water, especially at depth. Hydrostatic pressure could raise auditory threshold (Fluur and Adolfson, 1966; Thomas et al., 1974; Pantev and Pantev, 1979; Levendag et al., 1981) and, therefore, reduce the potential zone of sonic audibility for these deep-diving mammals through the effect of hydrostatic pressure on the ear.

The ear can be divided into three parts: inner, middle and outer. When predecessors of mammals came from an aquatic environment onto the land, a primitive inner ear had already developed. Having evolved in the water, the fluid-filled inner ear was insensitive to pressure fluctuations as the animal changed depth; however, to be an effective receiver for sound in the aquatic environment, the primitive inner ear must have been sensitive to the rapid pressure changes of a sound wave in water. For sensitive hearing in air, mammals had to evolve a middle ear providing sufficient amplification of sounds received at the outer ear to overcome the enormous impedance mismatch (a ratio of almost 3600-fold) between the air and the inner ear fluid, which is more like sea water. Three suspended bones or ossicles (malleus, incus and stapes) within the middle ear connect the ear drum to the oval window of the inner ear and convert low-pressure, high-amplitude velocity excursions of sound waves in air to high-pressure, low-amplitude velocity waves in the perilymphatic fluid of the inner ear. Hair cells along the basilar membrane of the inner ear convert these high-pressure, low-amplitude velocity waves into neural signals that the brain perceives as sound.

The aquatic ear of whales evolved from land mammal ears adapted for hearing in air (Gingerich et al., 1983; Thewissen and Hussain, 1993). In evolving back to a totally aquatic life, white whales, like all cetaceans, have lost the most obvious physical feature of the outer ear of their land mammal ancestors, the pinnae. The ear canal, as in other delphinoid cetaceans, has an incomplete or microscopic lumen (McCormick et al., 1970; Popper, 1980). White whales, however, retain the ear drum, ossicles, Eustachian tube and middle ear structures that evolved on land in their distant quadruped ancestors during the initial period of mammalian ear evolution in air (Gingerich et al., 1983; Thewissen and Hussain, 1993). The middle ears in both whales and land mammals are air-filled cavities within the temporal bone or bulla, connected by the Eustachian tube (Fig. 1) to the nasal cavity or nasopharynx, allowing for equalization of pressure between the air cavities and the external environment.

Despite the loss of the external ears or pinnae and the atrophy and stricture of the ear canal, the auditory system of white whales and other delphinoids reached an advanced state of development considered to be crucial to their success as ocean predators (Norris, 1968).

There is little understanding of the mechanism of hearing by which these descendants of aerial-hearing mammals function at great depth. While middle ear function in land mammals in air is documented (Rosowski, 1994; Ravicz and Rosowski, 1997), middle ear function for aquatic cetaceans is still debated (Popper, 1980; Ketten, 1992). One of the most frequently cited theorists, Gerald Fleischer (1978) suggests that, 'During deep
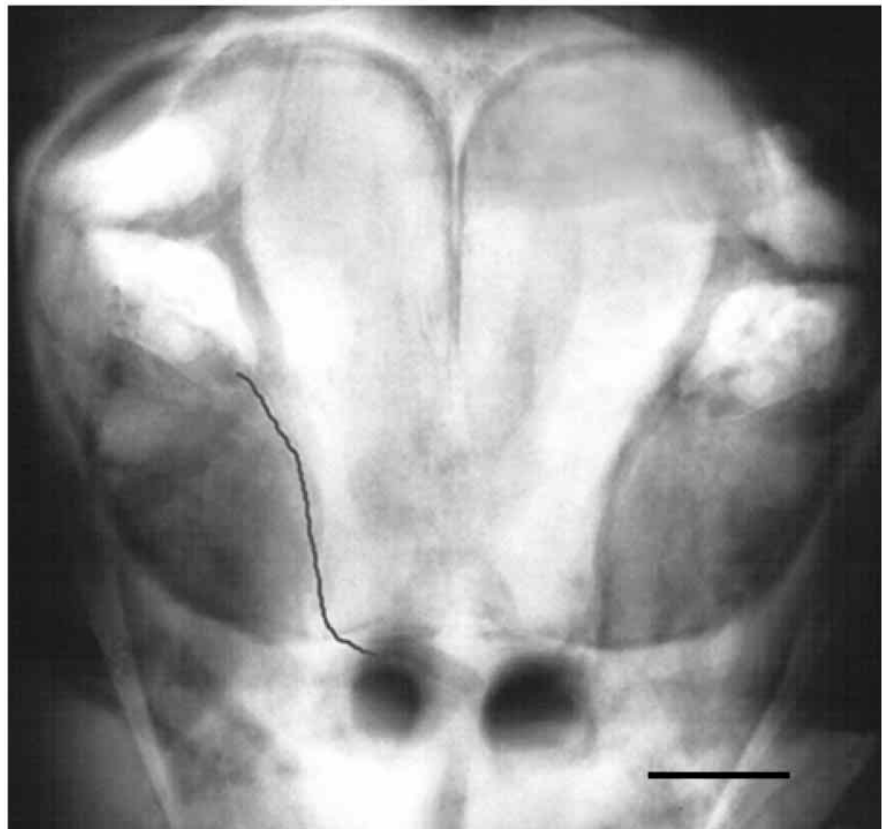

Fig. 1. Radiograph of the nasal and auditory areas of the head of a white whale adult (*Delphinapterus leucas*) that died of natural causes (not one of the subjects of this report). The path of the Eustachian tube to the nasal cavity is traced on one side. Scale bar, 5 cm.

diving the frequency response of the ear changes, predominantly because of the increased stiffness of the air volume in the middle ear cavity'.

Ample evidence reveals that the middle ear is influenced by hydrostatic pressure changes in air. When subjects occupied an air-filled chamber at a barometric pressure of several atmospheres or more, both human hearing and non-human laboratory mammal hearing was attenuated by up to 40 dB as a result of the increased gas density in the middle ear (Fluur and Adolfson, 1966; Thomas et al., 1974; Pantev and Pantev, 1979; Levendag et al., 1981).

The primary goal of this study was to test the hypothesis that depth would produce a progressive attenuation in whale hearing to the extent that middle ear models derived from land mammals would predict (Ravicz and Rosowski, 1997). The effect of depth can be tested not only by determining threshold but also by measuring response latency or reaction time (Stebbins, 1966). Since white whales are very active producers of sound, a secondary goal was to determine whether the animal's response whistles changed in amplitude, frequency or response latency at depth compared with whistles near the surface.
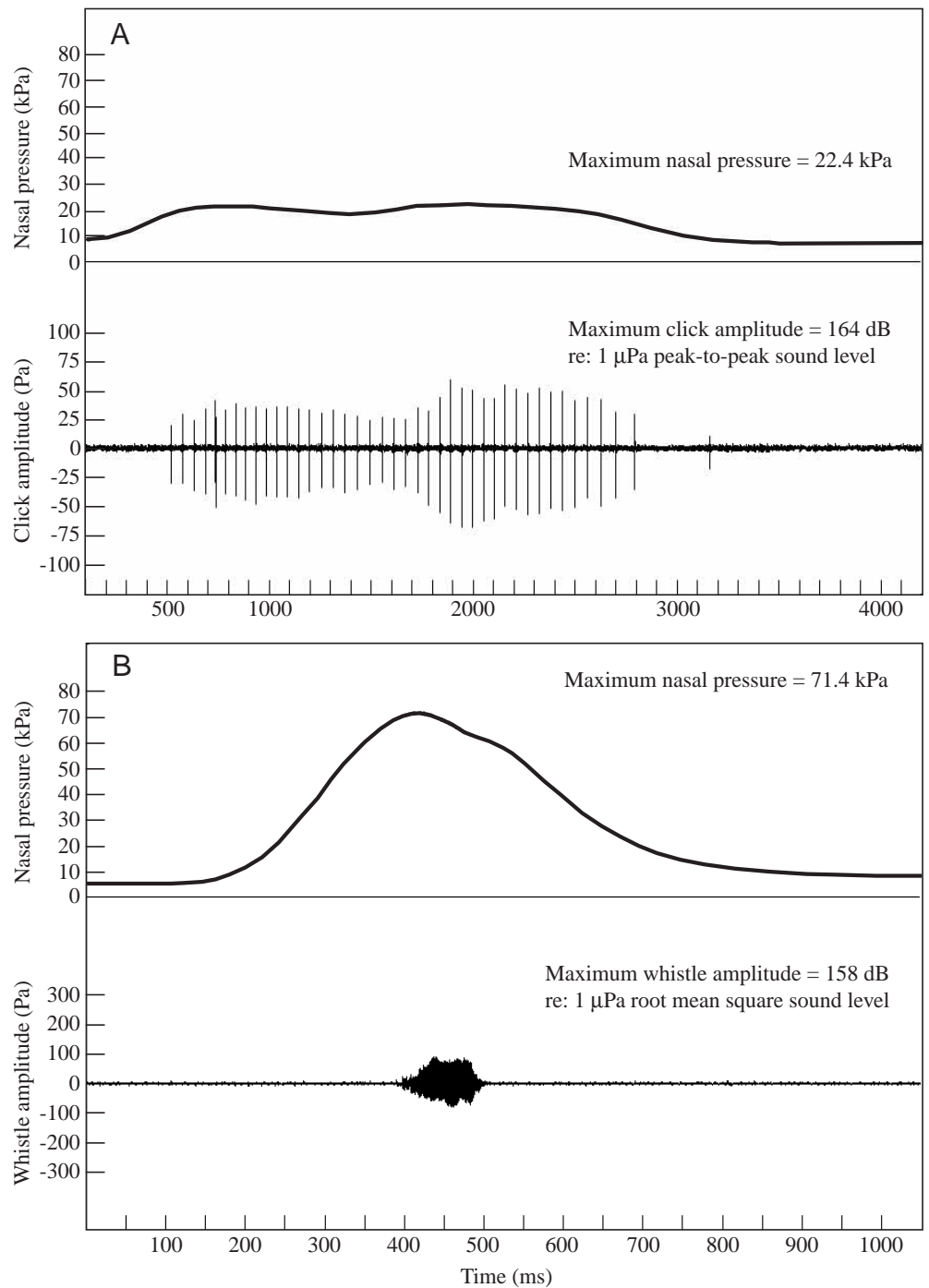

Fig. 2. A comparison of nasal cavity pressure and hydrophone recording during clicking (A) and whistling (B) for white whale MUK. For this recording, the animal was stationed at a depth of 1 m in San Diego Bay. The upper trace in each part shows pressure rising in the nasal cavity before the animal produces sound. The trace in B illustrates nasal pressure in response to a 500 ms tone. In response to the tone, the whale whistles approximately 400 ms after the suprathreshold tone is presented. The rise in nasal cavity pressure begins approximately 200 ms after the onset of the tone (not shown). Soon (220 ms) after the pressure rise begins, the whistle is recorded. Whistling requires considerably more pressure than clicks of similar amplitude (see also Ridgway and Carder, 1988). Because the click train is longer in duration, the axes are different for the two recordings (A and B).

Materials and methods

Test animals

To test the influence of greater static pressure at depth, we employed two white whales *Delphinapterus leucas* (Pallas, 1776), a male and a female, trained both for diving (Ridgway et al., 1984; Shaffer et al., 1997) and for hearing tests at depths of 5, 100, 200 and 300 m in the open ocean.

The whales were collected in 1977 at Churchill, Manitoba, Canada, under Canadian permit and under supervision by the Canadian authorities. On the basis of body size and skin coloration, their ages at capture were estimated to be 1 year (smaller and gray) and 10 years (larger and white) for the male and female, respectively. Since 1977 and for the duration of this study, the two whales have been housed in open ocean or bay enclosures and kept in conformance with US regulations promulgated under the Animal Welfare Act and Marine Mammal Protection Act. When these studies commenced, both whales had been with our program for 18 years. At that time, the male, NOC, was estimated to be 19 years old. He was 3.96 m in length and weighed 660 kg. The female, MUK, was estimated to be 28 years old. She was 3.50 m in length and weighed 550 kg. Audiograms carried out in San Diego Bay prior to the experiments reported here showed that NOC had normal hearing compared with whales tested previously (Awbrey et al., 1988; Johnson et al., 1989). Although MUK had a notch, a reduction in sensitivity, in her audiogram centered around 40 kHz, she had thresholds comparable with those of NOC and of other whales tested (Johnson et al., 1989) at all other frequencies tested for the present study. As result of MUK's 30–40 kHz notch, we tested only NOC at 32 kHz.

Training whales to listen and report tones

We chose to employ a whistle by the whale to report tone detection because this type of response could be readily recorded at depth. The use of the whistle also allowed us to obtain an accurate record of response time or reaction time for each tone stimulus heard by the whale. Reaction time has been shown to vary as a function of stimulus magnitude perceived by the animal (Stebbins, 1966; Ridgway et al., 1991). In addition, our previous work had demonstrated that the whales pressurize their nasal cavity (Ridgway and Carder, 1988) by up to 101 kPa (1 atmosphere) over surface ambient pressure before whistling (Fig. 2). We were interested to see how changing air density with depth might influence the whales' whistles and their ability to produce them.

The first task was to get the whales to whistle on cue. Trainers noticed that the area around the whales' closed blowhole moved when they produced spontaneous whistles. Therefore, in the initial training stages, the trainers induced whales to repeat whistles by touching the area around the blowhole where movement had been detected. When whistles were reliably elicited by touching the closed blowhole, the signal was transferred to a touch to the forehead of the whale. Then, with the whale under water in front of the trainer, the forehead touch was paired with a suprathreshold tone until the animal reliably gave the whistle each time the tone was projected.

The animals were trained to station on a hearing test platform (HTP) constructed of polyvinylchloride. Previously, Johnson et al. (1989) had shown that this material was a good acoustic match to sea water. To signal correct behavior when
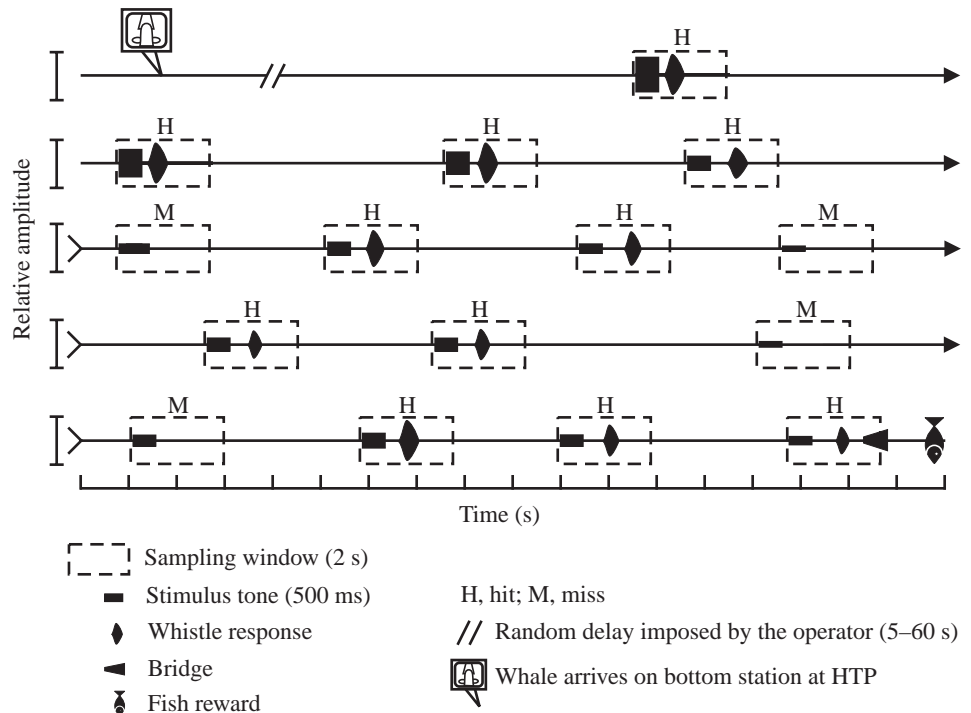

Fig. 3. Up to 40 tones were presented to the whale during a dive. This example timing scheme shows 15 tones presented together with 12 responses (hits, H) and three misses (M) during the last 86 s of one dive. Together with each test tone, the computer digitized a 2 s sampling window. After the last correct response, the trainer sounded a 500 ms bridge sweeping up in frequency and amplitude, signaling the whale to return to the surface for a reward of several fish. HTP, hearing test platform.

Sampling window (2 s)

Stimulus tone (500 ms)

Whistle response

Bridge

Fish reward

H, hit; M, miss

// Random delay imposed by the operator (5–60 s)

Whale arrives on bottom station at HTP

a whale whistled in response to a suprathreshold tone, a secondary reinforcer or bridging stimulus (BSt) was used. This was a 500 ms frequency sweep from 8 to 16 kHz delivered under water through a projector. Initially, the whale was given the BSt and rewarded each time it whistled after a suprathreshold tone. Gradually, the time on the HTP was increased until the animal made 10–40 whistle responses in a row (Fig. 3) after an equal series of suprathreshold tones. The BSt was given immediately after the last correct tone/whistle combination in the series (Fig. 3) and was followed by a reward of several fish when the whale returned to the surface

to breathe. Both time on the HTP (Figs 4, 5) and the number of tones presented during this period were varied in a random fashion.

Practice is known to improve auditory threshold testing in humans (Zwislocki et al., 1958). Prior to the diving tests to determine threshold at depth, MUK made approximately 15 870 responses to tones and NOC made approximately 10 860. This as yet unpublished work was a follow-on project to a previous dolphin auditory reaction time study (Ridgway et al., 1991). After the initial 1000 responses during this practice, both whales maintained a very low false alarm rate
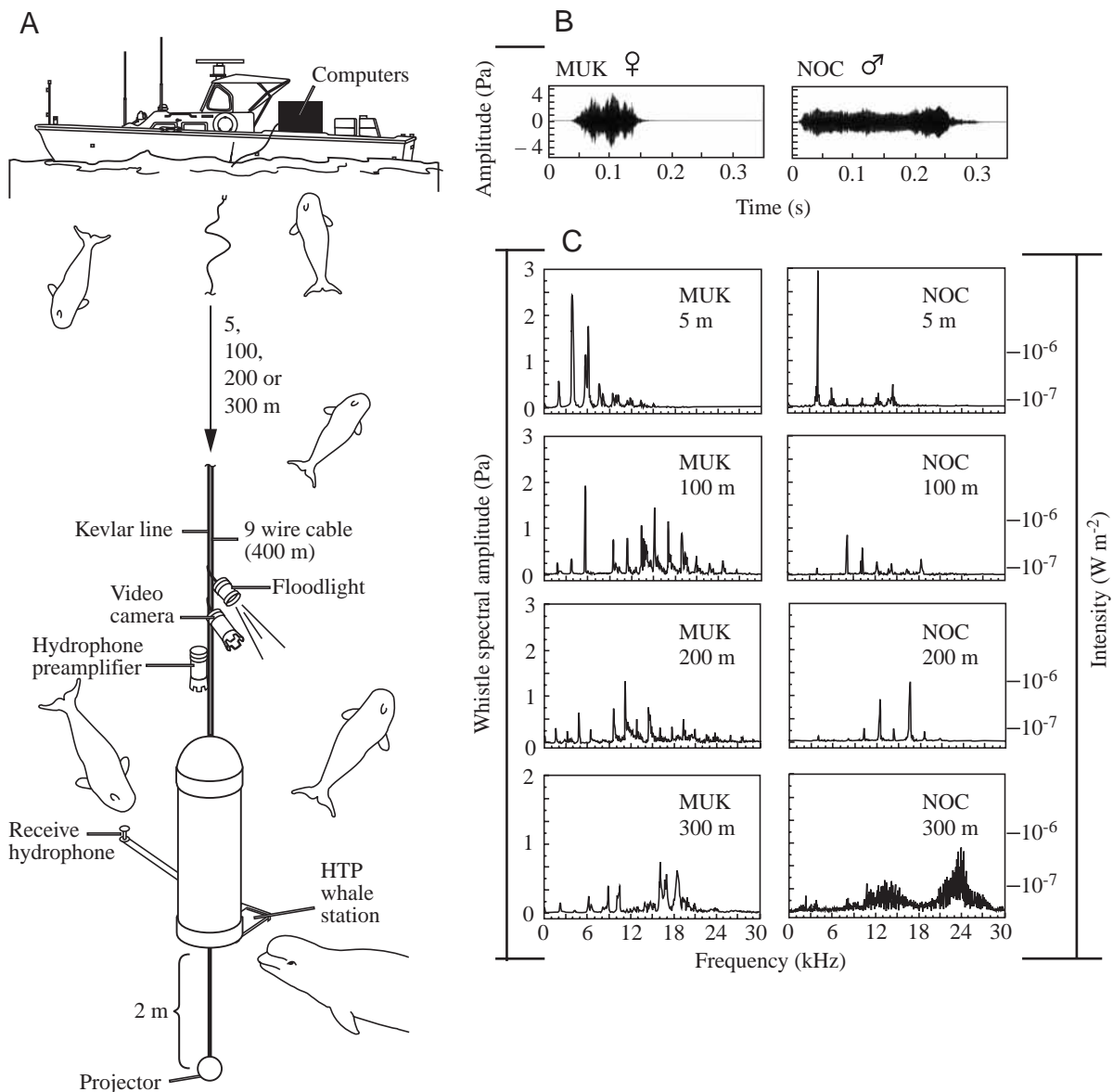

Fig. 4. (A) The boat contained the computer and other equipment together with necessary battery power and cable storage. The hearing test platform (HTP) was suspended by a Kevlar line and by an electrical cable. The cable carried power for lights, video, preamplifier, projector and hydrophone lines. A projector below the HTP delivered tones. A hydrophone in front of the whale station received the projected tones, the whale whistles and background noise. A video camera above the HTP allowed us to observe the whale to ensure correct positioning. (B) An amplitude/time series of a typical whistle employed by each whale as a response to tones it heard. MUK's whistle lasted approximately 100 ms, while NOC's whistle lasted 300–400 ms. (C) Typical spectra of whistles (all levels are received levels). The only response from NOC at 300 m was a pulse train, and this was not included in the whistle spectral analysis.

(under 6% of trials), which is consistent with results from other marine mammal signal-detection studies (Schusterman, 1974).

Training whales to dive in the open sea

On each test day, we called the whales out of an enclosure to swim, without any restraint or tether, beside a boat, to deep water 2–4 km off shore of San Clemente Island, California, USA, where the tests were conducted (Fig. 4A). Both whales had previously participated in diving experiments off San Clemente Island, where the female had made dives as deep as 647 m and the male somewhat shallower but well past the 300 m required for the current project. The training methods employed in the previous diving tests have been described elsewhere (Ridgway et al., 1969, 1984). For the present study, the whales were required to make dives to the HTP, which could be positioned at any of four different depths: 5 m (surface), 100 m, 200 m and 300 m. A non-related investigation into the diving and swimming performance of the same whales was conducted concurrently with the initial portion of the present study (Shaffer et al., 1997).

Hearing test series during dives

A hearing-test series (see Fig. 3) began only after the whale swam from the surface, reached the platform (Fig. 4A) and was
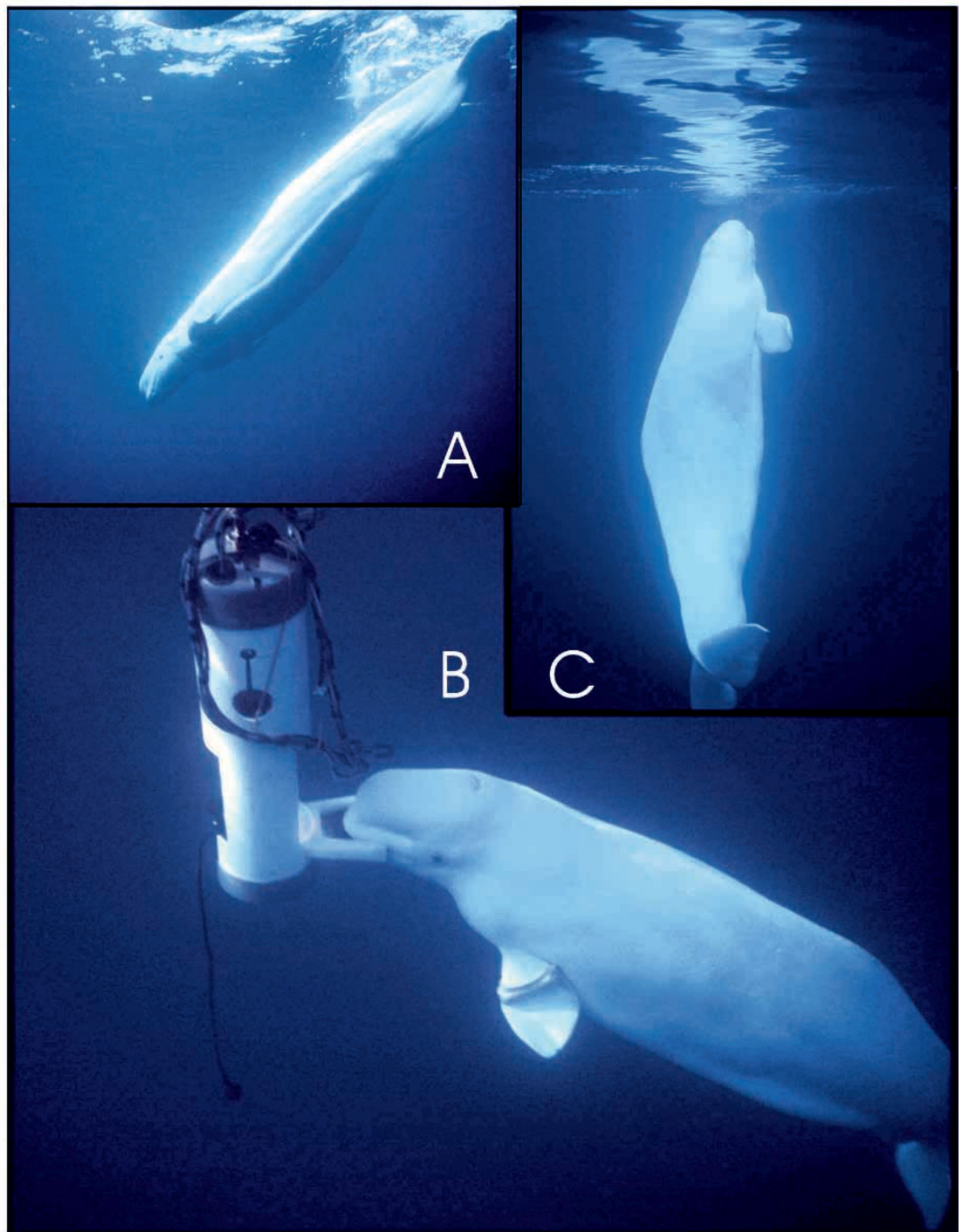

Fig. 5. On a cue, the whale dived from the surface (A) to the hearing test platform (HTP) and stationed (B) to listen for tones from the projector 2 m below the HTP. The whale responded to a series of tones projected at random intervals (see Fig. 3) until the trainer signaled the end of the test dive by sounding a frequency-swept bridge tone (BSt). On hearing the BSt, the whale returned to the surface (C) for a fish reward.

observed positioned properly on the HTP through the video system. The actual video perspective during hearing tests provided an overhead view of the whale on the HTP only. The images in Fig. 5 were taken by a diver to illustrate the HTP with the whale in place. Fig. 5A shows the whale descending to the HTP. Fig. 5B shows a wide-angle view of the whale on the HTP. This figure also shows the relative positions of the test tone projector, monitoring/receive hydrophone and video system. Fig. 5C shows the whale ascending from the HTP after completion of a series of test tones.

Hearing tests were conducted in deep water during sea states 1 or less to ensure low background noise levels at all depths. With the whale properly stationed, a 500 ms tone was presented through the projector. The receive hydrophone (Figs 4A, 5) output was digitized for 2 s. If the whale responded with a whistle during this 2 s sampling window, a 'hit' was recorded; if the whale did not respond, a 'miss' was recorded. Whistles outside the 2 s sampling window were regarded as false alarms. While the whale was on the station, we projected up to 40 tones at random interstimulus intervals of 3–60 s using a modified staircase procedure for stimulus presentation. The whale remained on the HTP station for 3–12 min responding with whistles to tones it heard until it received a BSt through the projector, signaling the animal to return to the surface for a fish reward. A reversal occurred when tone intensity was decreased until the whale failed to respond, was increased until it responded again and was then decreased until it again did not respond (Au, 1993). We used an average of the five lowest reversals at each frequency and at each depth to indicate threshold.

Our response paradigm for testing hearing, a modification of the method of free response described by Egan et al. (1964), was similar to methods employed previously by others (Au, 1993). The earlier methods required a subject to leave the test station and swim to an alternative area and/or press a response paddle to indicate when it heard a tone. Our method required the whales to remain at the test station and whistle to indicate when they heard the tone (Ridgway and Carder, 1997). This is not the first time a sonic response had been used to measure auditory thresholds in a marine mammal. Schusterman et al. (1972) used a vocal response, a bark, to measure auditory thresholds for another marine mammal, a sea lion, *Zalophus californianus*. The sea lion was near the surface and was rewarded with a small piece of herring for each correct response. Our whales made up to 40 responses during a dive and were rewarded only after completion of the dive (Fig. 3), thereby allowing a considerable quantity of data to be collected in a relatively short time. This advantage was especially important when testing at the deepest depths.

Hearing test equipment and analysis method

The tests were controlled from a computer linked by fiber-optic cables to Tucker-Davis Technologies (TDT; Gainesville, FL, USA) analog/digital acoustic instrumentation. The user at the computer on the boat (Fig. 4A) selected the desired
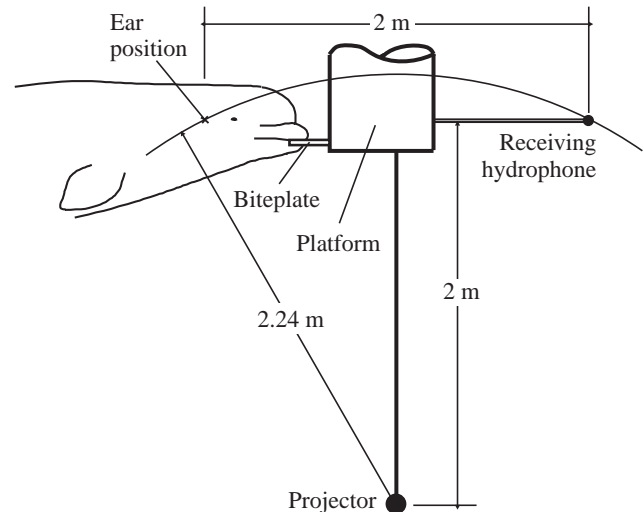

Fig. 6. Drawing illustrating the geometry of the location of the sound projector relative to the whale's ears and the receiving hydrophone. Note that the projector was 2 m below the platform (HTP), 2.24 m from the whale's ears and 2.24 m from the receiving hydrophone. The receiving hydrophone was also 2 m from the whale's blowhole. All values reported here are received levels at the location of the receiving hydrophone or the whale's ears.

frequency and amplitude for the next test tone then struck a key on the keyboard to initiate tone presentation. After a random delay of 2–5 s implemented by the computer program, the 500 ms tones were shaped by a function generator (Wavetek 275; San Diego, CA, USA), controlled through a GPIB interface with the computer, and gated using a cosine squared function with a rise/fall time of 5 ms. A tone was then projected with an ITC 1032 projector (International Transducer Corp., Santa Barbara, CA, USA). For projector calibration, a calibrated hydrophone was placed in the same position where we estimated the whale's ears to be inside the whale's head while stationed (Fig. 5, Fig. 6).

Another calibrated hydrophone (Brüel & Kjær 8103, Denmark) was placed on the HTP for recording background noise, the projected 0.5 s tones and the whale's whistles during the 2 s sampling window. The hydrophone was positioned on the HTP in front of the whale (Fig. 4A). The geometry of this arrangement was such that the hydrophone and the whale's ears were along the base of a downward-pointing equilateral triangle and were the same distance (2.24 m) from the projector 2 m below the HTP (Fig. 6). A preamplifier (Brüel & Kjær 2635) on the HTP was used to improve the signal-to-noise ratio along the 400 m of cable connecting the HTP to the computer (Fig. 4A). On the surface, a TDT module converted the analog signal to a digital signal. The sampling rate was 500 kHz and the sampling window was 2 s. The digital signals were transferred to the computer through the fiber-optic interface and then saved on computer disk together with files containing system settings and all other information pertinent to the current session. Each digitized 2 s sampling window

was analyzed using a software package (DADiSP; DSP Development Corp., Cambridge, MA, USA).

Sound measurement units

Hearing thresholds were based on received levels at the whales' ears, while whistle amplitudes were based on received levels at the hydrophone (Fig. 6). Different units have been used in hearing tests in air and in water. Air and water differ in impedance; therefore, it has been suggested that land mammal and sea mammal hearing are best compared by converting units of pressure in dB or Pa to intensity as $W\,m^{-2}$ (see Moore, 1997). Where appropriate, we have given units in intensity as well as in pressure in pascals (Pa) or dB re: 1 µPa for our measures. Human hearing and laboratory animal measures made in air are usually stated in dB re: 20 µPa. We have maintained the original units when citing such publications.

Results

Changes in whistles with depth

Video recordings from a camera on the HTP (Fig. 4A) enabled us to see the top of the whale's head stationed properly for hearing tests (Fig. 3; see Fig. 5 for a wide-angle view of proper stationing). While stationed, each of the two whales chose to use different whistles to respond to the test tones from a projector positioned 2 m below the HTP (Figs 4–6). MUK employed a whistle of approximately 100 ms in duration, while NOC responded with a whistle of 300–400 ms (Fig. 4B).

Time series for typical whale responses to the 500 ms test tones are shown for each whale in Fig. 4B. Response intensity for both whales usually ranged from approximately 10^{-7} to $10^{-5}\,W\,m^{-2}$ (approximately 112–132 dB re: 1 µPa). On occasion, however, much more intense responses were recorded (Fig. 7). The fundamental frequency of the response remained at 1–2 kHz for MUK and at 3–4 kHz for NOC. Harmonics were evident in whistles at all depths. Typical whistle spectra from each whale at the various depths are illustrated in Fig. 4C. The spectra of 20 whistle responses from each animal at each depth [MUK, 5 m (surface), 100 m, 200 m, 300 m (N=80); NOC, 5 m (surface), 100 m, 200 m (N=60)] were analyzed to determine whether depth had an effect on the amplitude (Pa) of the highest peak of the whistle spectrum. The mean values of the 20 peak whistle spectral amplitudes for each whale by depth are reported in Table 1. Results of within-subject, one-way analyses of variance (ANOVAs) indicated that the maximum intensity of the highest peak of the spectrum decreased with depth for both MUK ($F_{3,76}$=17.98, P<0.001) and NOC ($F_{2,57}$=35.06, P<0.001).

The spectra of the same whistle responses were also analyzed to determine whether depth had an effect on the frequency (kHz) of the highest-intensity peak. The mean values of the 20 peak whistle spectral frequencies for each whale by depth are reported in Table 1. Although there was some variation in spectral content from response to response,
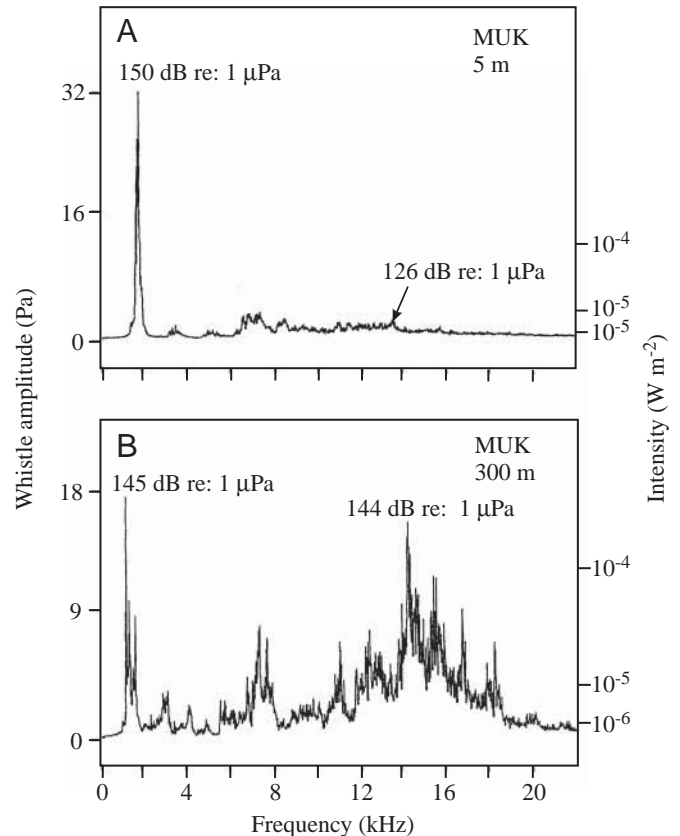

Fig. 7. Spectra of two response whistles that were higher in amplitude than the usual responses seen in Fig. 4C. Note that the 5 m whistle spectra show a higher low-frequency amplitude and much lower high-frequency amplitude than the 300 m whistle.

the results of within-subject, one-way ANOVAs indicated that the frequency of the highest-intensity peak of the whistle spectra increased with depth for both MUK ($F_{3,76}$=8.32, P<0.001) and NOC ($F_{2,57}$=54.57, P<0.001). The effect of depth on the peak frequency of the whistle responses of each whale can be seen in Fig. 4C.

Table 1 presents the mean values for the duration of the same set of whistle responses from each whale at each depth used in the spectral analysis above. Whistle duration for MUK, on average, was shortest at 300 m; however, the results of a one-way ANOVA indicated that her overall whistle duration was not significantly different between depth and the surface ($F_{3,76}$=1.80, P>0.15). Conversely, the results of a one-way ANOVA indicated that the whistles of NOC were significantly shorter at 100 and 200 m than at the surface ($F_{2,57}$=49.16, P<0.001).

On occasion, MUK did produce whistles that were much more intense than those shown in Fig. 4C. Near the surface, these more intense whistles had fundamentals of approximately 10^{-4} to $10^{-1}\,W\,m^{-2}$ (approximately 142–172 dB re: 1 µPa) and no higher-frequency peaks above approximately $10^{-6}\,W\,m^{-2}$ (in the 120–126 dB re: 1 µPa range). At 300 m, these rarely produced, intense whistles had amplitudes of approximately 10^{-4} to $10^{-3}\,W\,m^{-2}$ (approximately 140–152 dB re: 1 µPa) at the

Table 1. *Mean values for whistle responses at each depth for each white whale*

White whale	Depth (m)	Peak frequency (kHz)	Amplitude (Pa)	Duration (ms)	Response time at threshold (ms)
MUK	5	3.38	2.23	113	953
	100	8.61	3.20	125	952
	200	8.42	1.33	113	861
	300	10.43	0.54	112	870
NOC	5	5.00	2.03	381	808
	100	10.64	0.57	313	845
	200	13.20	0.59	314	870

Table 2. *Hearing threshold values (dB re: 1 µPa) at depths of 5, 100, 200 and 300 m for the two whales at the test frequencies*

White whale	Depth (m)	Frequency (kHz)								
		0.5	2	4	8	16	24	32	64	100
MUK	5	78	69	61	52	50	54	–	55	61
	100	77	60	55	47	51	53	–	63	72
	200	67	65	60	54	49	55	–	60	74
	300	72	60	65	54	–	56	–	59	71
NOC	5	82	67	62	56	51	60	71	63	72
	100	82	65	61	53	43	55	66	64	73
	200	82	–	66	64	–	61	66	58	74

fundamental frequency and, also, equally high peaks in the 10–18 kHz range (Fig. 7A,B).

Depth and auditory reaction time at threshold

Although overall whistle amplitude, frequency and, for NOC, duration characteristics changed with depth as shown in Fig. 4C and Table 1, auditory reaction time from the onset of hearing the test tone to the onset of the whale's whistle response remained essentially the same across depths. The mean reaction times for the same 20 whistle responses used in the previous analyses from both subjects at the various depths are presented in Table 1. The results of within-subject one-way ANOVAs indicated that the whales' general reaction times were not significantly different between the surface and depth for either MUK ($F_{3,76}$=0.39, P=0.76) or NOC ($F_{2,57}$, P=0.07)].

Response times to tones projected specifically around threshold were also analyzed to determine any effect of depth. The mean reaction times of both subjects to tones projected at near-threshold levels at the various depths are presented in Table 1. Although, as expected, the reaction times at threshold increased over the reaction times in general, which included suprathreshold responses, for both animals, the results of within-subject, one-way ANOVAs indicated that the whales' reaction times at threshold were also not significantly different between the surface and depth (MUK, $F_{3,87}$=1.73, P=0.17; NOC, $F_{2,69}$=1.32, P=0.27).

Hearing threshold and depth

In contrast to the changes in the whistle responses of the whales (Fig. 4C), the hearing thresholds of the whales at depth were mostly unchanged (Table 2). Thresholds (dB re: 1 µPa) for each whale at each test frequency were analyzed to determine whether there was an effect of depth. The results of within-subject two-way ANOVAs (frequency×depth) revealed no significant effect of depth on hearing thresholds for either MUK or NOC. That is, there was no significant difference between either whale's thresholds estimated at the surface and those estimated at depth when all frequencies were considered together. Depth did not attenuate whale hearing.

Discussion

Modification of whale response whistles at depth

It was apparent that the whistles of both whales were modified at depth. On average, their whistle amplitude was lower at depth (Table 1), probably because of the increasing density of the air in the nasal system where the whistles are produced (Dormer, 1979; Ridgway et al., 1980; Mackay and Liaw, 1981; Ridgway and Carder, 1988; Cranford et al., 1996). The high-frequency components of the response whistle increased with depth. This is the first instance of white whale whistles being recorded at known depths. Investigators studying such acoustic behavior should be aware of the possibility that whistle repertoire might be influenced by depth.

Air pressure in deep-sea test chambers modifies human speech. When humans in an air pressure chamber speak, the increased air density reduces the impedance mismatch between the vocal tract air and the vocal cavity walls, resulting in speech formant frequency increases (Fant and Sonesson, 1967). Mackay and Liaw (1981) observed that, at 30 m pressure, Mackay could whistle only with difficulty but could easily use a rigid whistle or 'bird call'. They suggested that dolphin whistles might involve the excitation of resonances in the nasal system. Barham (1973) has suggested the whale's lung volume constitutes a low-frequency resonant receiver that would change with depth.

In general, NOC was less cooperative than MUK and was more reluctant to dive to 300 m. The only 300 m response from NOC, seen on the lower right in Fig. 4C, was a rapid pulse train rather than the usual whistle used at the 5, 100 and 200 m levels. We suspect that the whistles of NOC, which were 3–4 times as long as those of MUK, became more difficult to produce in many repetitions at the deeper depths. Since pulse trains require less nasal pressure to produce than whistles (Ridgway and Carder, 1988) (see also Fig. 2), and therefore use less muscle energy, we suspect that NOC was reverting to an easier means of responding to the test tones at the 300 m depth.

The present data suggest that white whales produce their whistles by muscle action pressurizing the paired nasal cavities (Ridgway and Carder, 1988), squeezing air past the internal lips of the blowhole (Fig. 8). Termed 'phonic lips' (or 'monkey

lips'), these structures have recently been well described by Cranford et al. (1996) and Cranford (1999). Air in the pressurized bony nasal cavities (Fig. 8) moves between the lips into the upper nasal vestibular sacs (Fig. 9), where it may be held for recycling (Dormer, 1979; Ridgway et al., 1980). Such air recycling appears to be essential for a whale making repetitive whistles at depth. Along the lips, several edges and areas of corrugation could cause air-flow instability that produces whistles (Crawford, 1974; Nakamura and Fukamachi, 1991; Petrie and Huntley, 1980). The observed increase in the relative amplitude of the higher frequencies of whistle spectra at depth (Fig. 4C) could be the result of air-flow changes caused by increasing air density, and a concurrent reduction in total air volume, with depth. In addition, changes in air-cavity resonance (Barham, 1973; Mackay and Liaw, 1981) and a reduction in impedance mismatch (Fant and Sonesson, 1967) could be responsible for the relative increase in amplitude of the higher frequencies that was observed at depth.

Auditory reaction time and depth

Stebbins (1966) showed that the auditory reaction time of monkeys varied with sound intensity, and he was able to construct differential loudness curves for the animals on the basis of their reaction time. Ridgway et al. (1991) reported that the auditory reaction time of the bottlenose dolphin *Tursiops truncatus* also varied with sound stimulus intensity. Auditory reaction time at threshold in the present study did not change

significantly with depth (Table 1). Therefore, it appears that the whale's perceived loudness of the hearing test tones at threshold was not changed by depth. This is further evidence that hearing is not attenuated at depth.

Hearing test geometry and comparison of results with earlier work

Our deep-water thresholds for 500 Hz were 16–21 dB lower than thresholds we obtained for the same animals in San Diego Bay with the projector positioned in front of the whale. In San Diego Bay with front-projected sound at a depth of 2 m, MUK had a 500 Hz threshold of 96 dB, while the threshold for NOC was 106 dB. In contrast to the low-frequency thresholds, the thresholds above 30 kHz in the present study were higher than when the projector was positioned in front of the whale, in agreement with Au and Moore (1984) (see also Schlundt et al., 2001). The geometry of the test was kept the same for high and low frequencies in this series because the position of the much smaller receive hydrophone in front (Fig. 4, Fig. 5) was critical for response whistle, projected tone and background noise recording. In addition, placement of the larger projector and a stabilization weight below the HTP added to the stability of the apparatus in ocean currents.

Differences in psychophysical methods compared with published whale audiograms

In addition to projector position relative to the whale and to the great environmental differences between the deep open
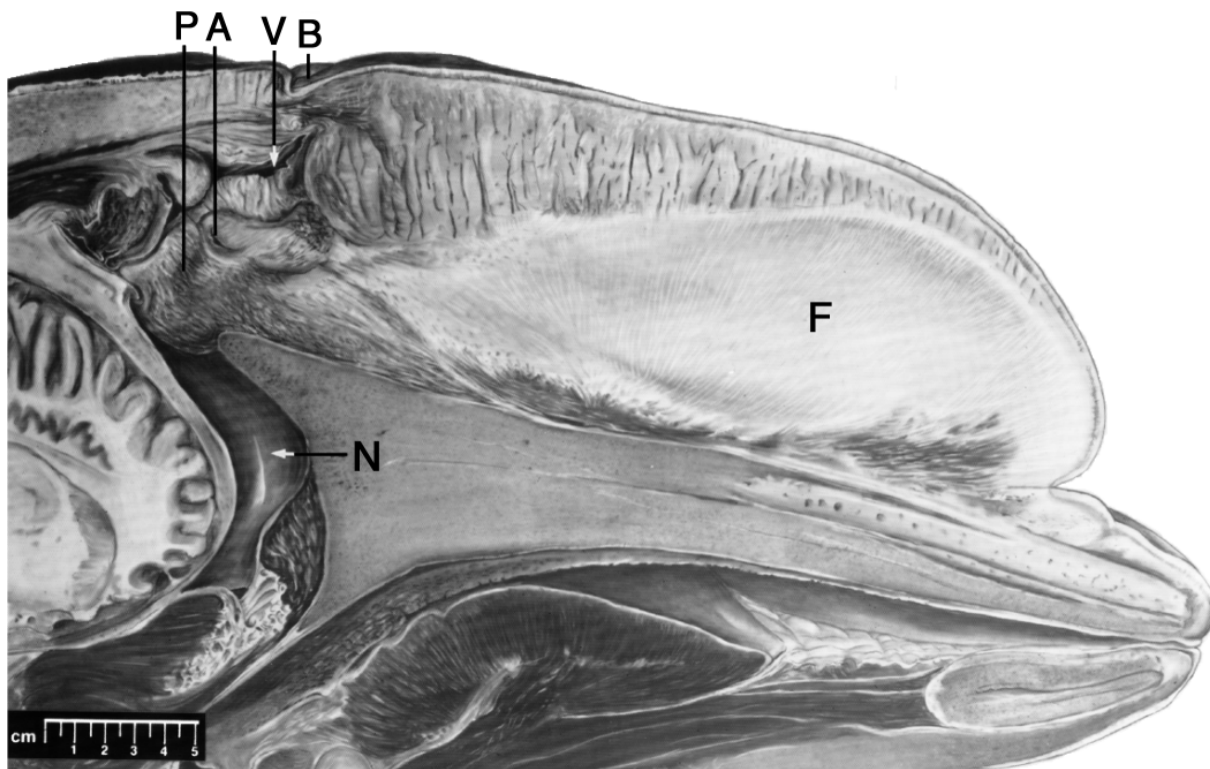

Fig. 8. Illustration of a sagittal section through the right nasal cavity of a white whale. P, phonic lips; V, vestibular sac; B, blowhole position; F, fatty melon tissue; A, right anterior nasofrontal sac; N, nasal cavity where air is pressurized by muscle action for sound production.

ocean and tanks or bays, the difference in the psychophysical methods in these hearing tests reduce the comparability with previous thresholds. Hearing threshold may vary according to the test methods used. Schusterman (1974) has pointed out how animal detection thresholds can be biased by the behavioral approach used. Differences in hearing test methods may account for differences between the thresholds presented here and those published previously (Awbrey et al., 1988; Johnson et al., 1989) for the white whale. It is important to note that the purpose of these experiments was to investigate the effect of depth on hearing threshold. Thus, it is the consistency of results within the method that is important to these findings, not that the results should closely match previously obtained thresholds at all frequencies.

At depths of 5–300 m, the whales in this study made multiple responses to multiple tone presentations for each BSt and for each fish reward. Schusterman (1974) has shown that marine mammals are in general very conservative in their response criteria in psychophysical experiments. The very low false alarm rate (under 6 % of trials) from our whales might suggest that the animals were excessively conservative in their criteria for responding. This might have resulted in thresholds that were higher than if a less conservative criteria were used; however, we suspect that the whales' extensive practice responding to tones of all frequencies in prior diving tests resulted in the reduced rate of false alarms observed (MUK made approximately 15 870 responses to tones and NOC approximately 10 860). Practice resulted in lower auditory thresholds in humans (Zwislocki et al., 1958). For testing hearing down to 300 m, the alternative methods in which responses are collected one at a time were not adequate. Therefore, a method was devised with similarities to the free response method of Egan et al. (1964) and with threshold determination with reversals similar to the previous studies of Awbrey et al. (1988) and Johnson et al. (1989).
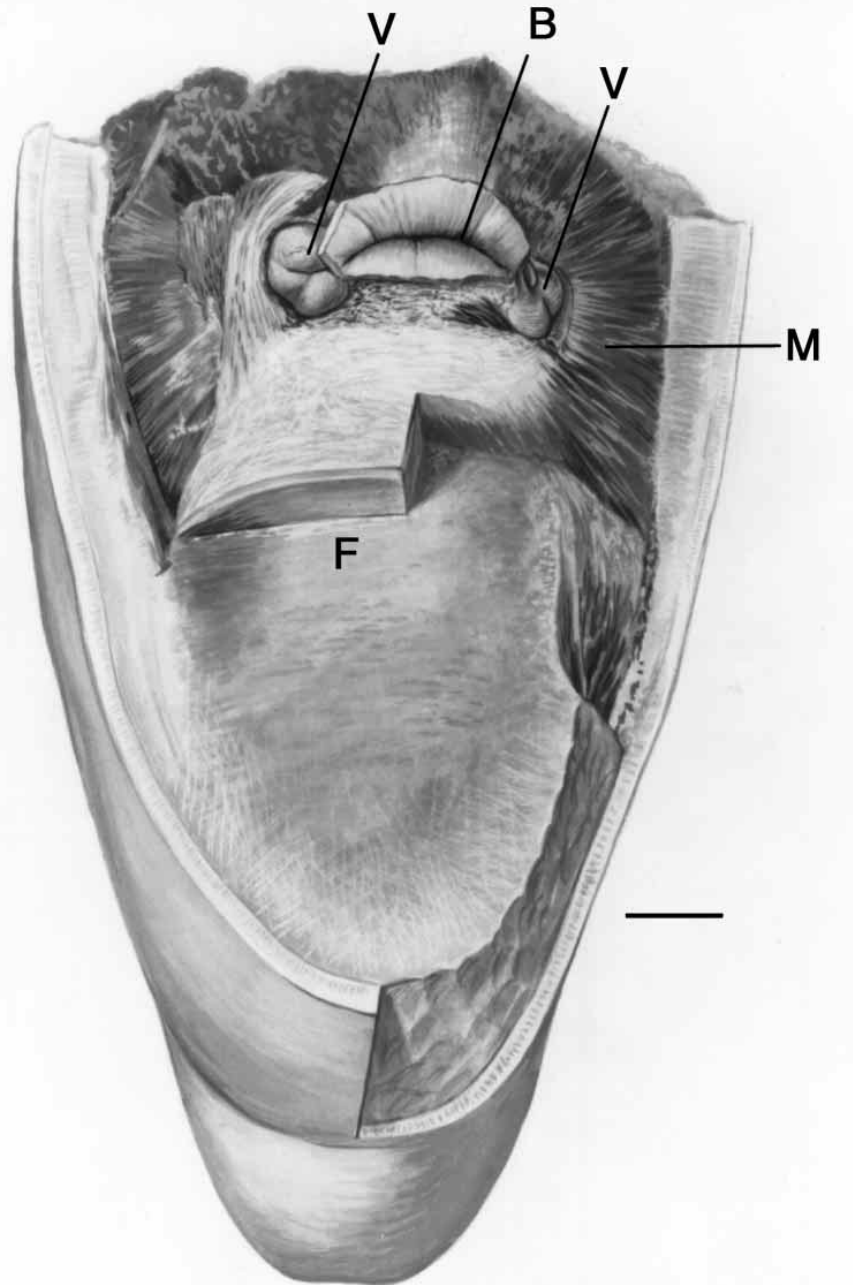

Fig. 9. A dorsal view showing the large vestibular sacs (V) on either side of the blowhole (B). F, fatty melon tissue; M, muscle. The vestibular sacs are important reservoirs holding air for recirculation for sound production, especially during dives. Scale bar, 5 cm.

Whale ear function is not diminished at depth

The increased density of air in the middle ear causes an approximately 20–40 dB attenuation of hearing of humans and laboratory animals (Fluur and Adolfson, 1966; Thomas et al., 1974; Pantev and Pantev, 1979; Levendag et al., 1981). With the exception of the 100 kHz responses, the very lowest thresholds of the whales were found when they were at depth (100, 200 or 300 m). However, overall, thresholds at depth did not differ significantly from those at the near-surface depth of 5 m. As a separate measure of tone loudness (Stebbins, 1966) near threshold, we measured the auditory reaction time of each whale (the time from the onset of the test tone to the onset of the response whistle) and found no difference between surface and depth auditory reaction times.

When under water, humans are said to hear by bone

conduction and sustain considerable hearing attenuation; however, at shallow depths down to 32 m (105 feet), their sensitivity is not further attenuated (Brandt and Hollien, 1969). Under water, delphinoids are, on an intensity basis, more sensitive (10^{-14} *versus* 10^{-9} W m^{-2}; 42 dB *versus* 92 dB re: 1 µPa), and bone conduction theories for whales are under debate (McCormick et al., 1970, 1980; Fleischer, 1978; Ketten, 1992; Popper, 1980). One delphinoid middle ear model (Fleischer, 1978) predicts a frequency shift in threshold attributed to the 'increased stiffness' of the middle ear air. At 200 and 300 m, MUK was slightly more sensitive to 500 Hz and less sensitive to 100 kHz; however, there were neither consistent differences at other frequencies nor at the same frequencies with NOC (Table 2). Incorporating a 3000 kPa (30 atmosphere) air density, equivalent to a depth of 300 m, in the most recent mammalian aerial ear model (Ravicz and Rosowski, 1997) results in a 15 dB attenuation across frequencies to at least 16 kHz. Whales did not show such attenuation (Table 2).

These findings are of interest to those considering how animals hear under water and at the increasing pressure at depth. Moreover, the findings have practical significance in making decisions concerning the potential effects of intense sound on sea mammals. For example, it has been suggested that working human divers could tolerate a higher level of environmental noise at depth because middle ear impedance mismatching at depth would make them less sensitive to sound (Mittleman, 1976). Our findings show unchanged sensitivity at depth, suggesting that middle ear impedance mismatching does not make whales less sensitive to sound at depth.

These first controlled tests of hearing of any marine mammal species in the open sea suggest that high-pressure, low-amplitude velocity sound waves in the ocean reach the ear without requiring the usual ear drum/ossicular chain amplification of the aerial mammalian middle ear. This is consistent with the theory first developed by Norris (1968) suggesting that sound arrives at the ear through the lower jaw of dolphins and other toothed whales. Bullock et al. (1968), McCormick et al. (1970) and Brill et al. (1988) have provided supporting evidence for the lower jaw path. Our results are not inconsistent with the idea that the tympanic bulla and the ossicles play a role in whale hearing as an inertial mass. This has been mentioned by several authors (McCormick et al., 1970, 1980; Ketten, 1992; Thewissen and Hussain, 1993; Hamilä et al., 1999).

However, an erroneous view of whale hearing could be derived from experimental and modeling results from studies of middle ear function in terrestrial animals, such that reduced hearing sensitivity with increasing depth would be expected. Even in the deep sound channel, white whales, at least, hear as well at depth as near the surface, and zones of influence for human-made sound are therefore just as great at depth.

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