Title

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Journal

The Journal of The Acoustical Society of America, 117(6)

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Publication Date

2005-05-31

Peer reviewed

Pure tone audiograms and possible aminoglycoside-induced hearing loss in belugas (*Delphinapterus leucas*)

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(Received 7 October 2005; revised 10 February 2005; accepted 22 February 2005)

A behavioral response paradigm was used to measure pure-tone hearing sensitivities in two belugas (*Delphinapterus leucas*). Tests were conducted over a 20-month period at the Point Defiance Zoo and Aquarium, in Tacoma, WA. Subjects were two males, aged 8–10 and 9–11 during the course of the study. Subjects were born in an oceanarium and had been housed together for all of their lives. Hearing thresholds were measured using a modified up/down staircase procedure and acoustic response paradigm where subjects were trained to produce audible responses to test tones and to remain quiet otherwise. Test frequencies ranged from approximately 2 to 130 kHz. Best sensitivities ranged from approximately 40 to 50 dB *re* 1 μPa at 50–80 kHz and 30–35 kHz for the two subjects. Although both subjects possessed traditional ''U-shaped'' mammalian audiograms, one subject exhibited significant high-frequency hearing loss above 37 kHz compared to previously published data for belugas. Hearing loss in this subject was estimated to approach 90 dB for frequencies above 50 kHz. Similar ages, ancestry, and environmental conditions between subjects, but a history of ototoxic drug administration in only one subject, suggest that the observed hearing loss was a result of the aminoglycoside antibiotic amikacin.
[DOI: 10.1121/1.1893354]

PACS numbers: 43.80.Lb [WA] Pages: 3936–3943

I. INTRODUCTION

The first cetacean audiogram was obtained by Johnson (1966, 1967), who measured pure-tone thresholds in a trained bottlenose dolphin. Since that time, researchers have investigated hearing sensitivity, frequency selectivity, masking, auditory fatigue, temporal integration, and localization in dolphins and other marine mammal species (review Nachtigall, 1986; Johnson, 1986; Au, 1993; Nachtigall *et al.*, 2000). The majority of studies on the auditory capabilities of marine mammals have used psychophysical or behavioral response paradigms similar to that employed by Johnson (1966, 1967). In the behavioral method, the subject is trained to give a specific response to a particular acoustic stimulus and to withhold the response (or provide an alternate response) in the absence of the stimulus. Behavioral techniques allow direct measurements of hearing sensitivity and are generally considered the ''standard'' to which other sensitivity measures (e.g., electrophysiological measures) are compared (e.g., Szymanski *et al.*, 1999).

Behavioral methods are limited, however, by the difficulty and costs involved with training marine mammals to participate in hearing tests. Most marine mammal psychoacoustic studies have used one or two experimental subjects (Green *et al.*, 1994). Little attention has been given to replicating earlier work with additional subjects. The small number of individuals for whom data are available has resulted in lingering questions regarding ''normal'' hearing for marine mammal species, typical intraspecific variability, and typical hearing loss for different ages and genders.

In this paper we report behavioral audiograms for two belugas (*Delphinapterus leucas*). These data augment the beluga hearing threshold data presented by White *et al.* (1978), Awbrey *et al.* (1988), Klishin *et al.* (2000), and Ridgway *et al.* (2001). Taken together, these data allow estimates to be made of ''normal'' hearing sensitivity in belugas. The data from the present study were also unique because they

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revealed significant hearing loss in a subject previously treated with aminoglycoside antibiotics.

II. METHODS

A. Subjects

Test subjects were two male belugas: Beethoven (9–11 years old, mass approximately 640 kg) and Turner (8–10 years, approximately 590 kg). Both subjects were born in an oceanarium, had the same father, and had been housed together since shortly after Turner was born. Neither subject had any previous experience with hearing tests or other psychophysical test procedures.

The health of the subjects was ascertained through periodic medical examinations by veterinarians. Subjects were healthy during the course of the study, with the exception that from June 2002 until the end of the study Turner was treated off and on for glomerulonephritis as evidenced by periodic hemituria. No ototoxic drugs were used during these periods of treatment. Beethoven required no medical treatment during the study period.

Tests were conducted according to a protocol approved by the Institutional Animal Care and Use Committees at the Space and Naval Warfare Systems Center, San Diego and the Point Defiance Zoo and Aquarium. The described experiments were conducted in accordance with the Acoustical Society of America's *Guiding Principles in the Care and Use of Animals* and followed all applicable U.S. Department of Defense guidelines.

B. Experimental apparatus

Figure 1(a) shows the test site located in the ''Rocky Shores'' beluga habitat at the Point Defiance Zoo and Aquarium, in Tacoma, WA. The exhibit contained approximately 1150 m^3 (304 000 gal) of filtered, ozonated seawater within a large main pool, a shallow connection channel, and an off-exhibit holding pool. The holding pool, which was 9 m in diameter with a depth of 2.7 m, was used to separate the subjects so each could be tested independently. The hearing tests were conducted in the main pool. The main pool was irregularly shaped, roughly 15×20 m, with a surface of sprayed gunite and varying bottom depth and topography. The maximum depth was 4.4 m, sloping up to 1.5 m at the entrance to the channel. The average depth was about 3 m. The main pool volume was 920 m^3 (243 000 gal) and the surface area was 30 m^2.

The trainer was positioned near a shallow beaching area at the northwest edge of the pool. The test apparatus was located along the south side of the pool, near an approximately 10-m-long underwater viewing window built into the southwest wall of the pool. A personal computer (PC), video monitor, and other electronics were housed in a small enclosure located in the underwater viewing area.

The test apparatus [Fig. 1(b)] consisted of a submerged polyvinyl chloride (PVC) frame containing an underwater sound projector (ITC 10001, ITC 1032, or ITC 1042, depending on the test frequency), receiving hydrophone (B&K 8105), video camera, and a neoprene-covered plastic ''biteplate.'' Subjects were trained to dive underwater and position

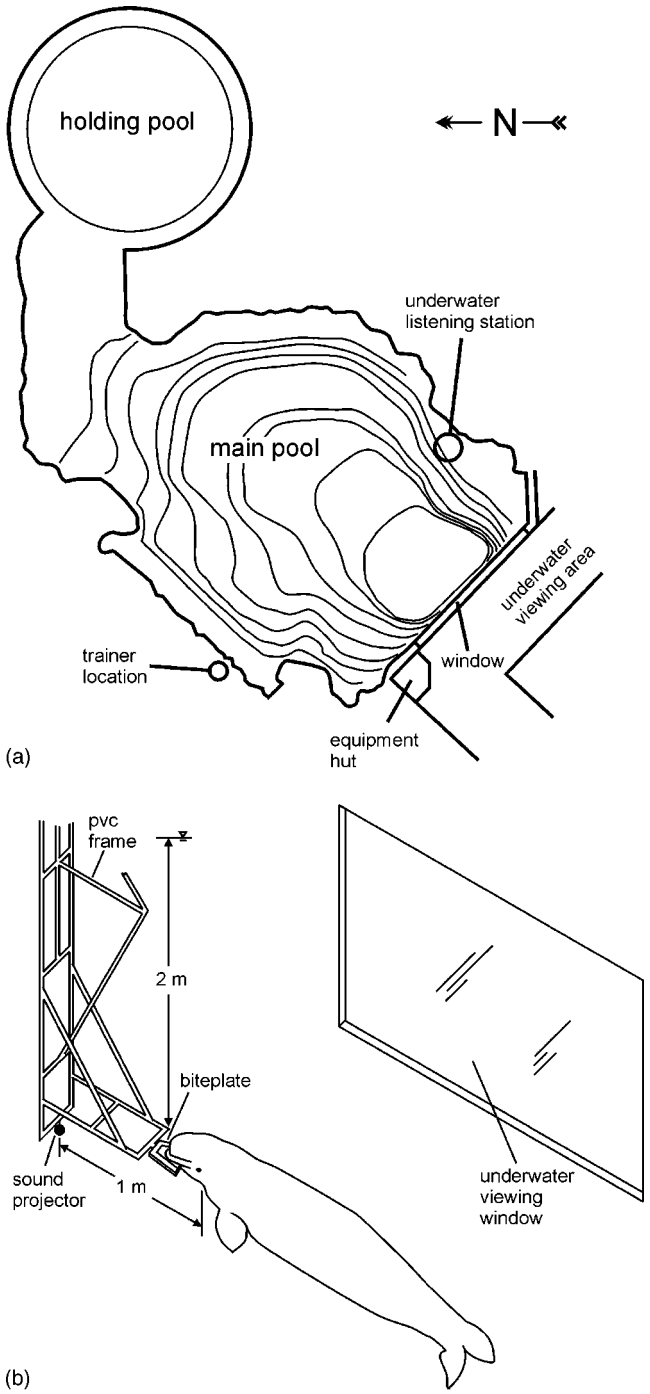

FIG. 1. (a) Top-view schematic of the beluga habitat at the Point Defiance Zoo and Aquarium showing the main pool and holding pool. Locations of the test apparatus and trainer positions are marked. Depth contours are approximate. (b) Schematic of the test apparatus with subject positioned on the biteplate.

themselves on the biteplate, which put their head in a fixed, repeatable position with respect to the sound projector and receiving hydrophone. Water depth at the apparatus was approximately 4 m. The depth of the sound projector and subject was approximately 2 m. The sound projector was positioned so that the distance between the projector and the subject's ears was approximately 1 m.

Hearing test tones were generated by a multifunction data acquisition board (National Instruments PCI-MIO-16E-1) residing within the PC. The generated tones were

attenuated (HP 355D or TDT PA-5), filtered (Ithaco 4302), and amplified (BGW PS2), before being input to the sound projector: ITC 1001 (2–20 kHz), ITC 1032 (12–50 kHz), or ITC 1042 (18–130 kHz). Tones were 500 ms in duration, including a 50-ms linear rise and fall time. Sound levels presented to each subject were calibrated before and after each session, without the subject present, with the receiving hydrophone located at a position estimated to lie on the subject's midline at the location of the ears. The hydrophone output was amplified (B&K 2635) and filtered (Ithaco 4302) before being digitized by the PCI-MIO-16E-1. During the hearing tests the hydrophone was positioned above and in front of the subject and used to monitor the sound in the water, including the hearing test tones and any sounds produced by the subject. Custom software (Finneran, 2003) was used to calibrate the sound system, control the hearing test, and analyze the resulting data.

C. Procedure

1. Hearing test

Hearing test sessions were conducted once or twice a day with each subject. Each session produced a single threshold estimate at a particular frequency. Session durations were approximately 15 to 20 min. The hearing test procedure was based on the method of free response (MFR) (Egan *et al.*, 1961). Similar test methods were used by Finneran *et al.* (2000, 2002a, b, c, 2003) and Schlundt *et al.* (2000) to measure marine mammal hearing thresholds.

Each hearing test session was divided into a number of observation periods, referred to here as ''dives.'' During each dive, the subject was instructed to swim to the test apparatus, submerge, and position at the underwater biteplate. A variable number of trials was then presented. To conclude the dive, the trainer sounded an underwater buzzer and signaled the subject to return for fish reward.

Each trial was 2 s in duration. The intertrial interval (defined from the start of one trial to the start of the next trial) was randomized between 4 and 7 s. Fifty percent of the trials (determined from a Gellerman series) contained a hearing test tone, 50% were signal-absent or ''catch'' trials. Tone trials contained a 500-ms duration pure tone at the test frequency. The tone onset coincided with the trial start. Subjects were trained to produce a specific audible response to each hearing test tone and to stay quiet otherwise. The time period between 0.1 and 2.0 s immediately following each tone start was designated as the ''hit interval.'' Responses occurring within a hit interval (following a tone) were recorded as ''hits.'' No response to a tone trial was classified as a ''miss.'' Catch trials were identical to tone trials except that the PC-generated tone amplitude was set to 0 V. Subjects were not aware of the trial start times, thus the catch trials were essentially extensions of the randomized intertrial interval from the proceeding tone and functioned as equipment catch trials (to ensure that the generation/recording process was not producing acoustic artifacts audible to the subjects). Whistle responses to catch trials were recorded as ''false alarms'' (whistle responses by a subject outside of any trial period were also recorded—see below). No response to a catch trial was recorded as a ''correct rejection.'' Tone amplitudes were adjusted using a modified up/down staircase procedure (e.g., Cornsweet, 1962): the amplitude was decreased 2 dB after each hit and increased 2 dB after each miss. Hearing thresholds for a single session were estimated from the mean sound pressure of 10 hit-miss/miss-hit reversal points collected within that session.

The trainer and computer operator monitored the sound in the water for any responses by the subject. A small LCD was used to display trial parameters (e.g., stimulus level, tone or catch trial, dive time) for the trainer. The display was updated just before the start of a trial. The number of trials per dive was randomized within the following guidelines: Dives were ended only after correct responses. An attempt was made to reinforce responses to low-level tones (i.e., at a lower level than any previously responded to). The first hit following several misses was generally not reinforced. The dive times were normally kept under 2 min. The amount of fish reward was scaled to the performance of the subject during the dive (e.g., more reinforcement was given for longer dives and/or responding to low-level tones).

2. False alarms

Previous studies of marine mammal audition have demonstrated the importance of the subject's motivational state and response bias (e.g., Schusterman *et al.*, 1975). In the present study, the response bias was assessed using two techniques. In the first, more traditional method, the false alarm rate R_{FA} was defined as

$$R_{\mathrm{FA}}=\frac{N_{\mathrm{FA}}}{N}, \tag{1}$$

where N_{FA} is the number of false alarms and N is the total number of trials. Miller (1969) presented an alternate method to assess response bias in the MFR:

$$r_{\mathrm{FA}}=\left(\frac{n_{\mathrm{FA}}}{T-N_T T_1}\right)T_1, \tag{2}$$

where r_{FA} is the false alarm rate, n_{FA} is the total number of whistle responses occurring outside of a hit interval, T is the total amount of time the subject spent on the biteplate, N_T is the number of tones presented, and T_1 is the hit interval duration. The term in parentheses is the number of ''false positive'' responses divided by the total amount of time during which the subject was on the station with no hit interval present. This term is multiplied by T_1 to obtain a dimensionless quantity. For the MFR, r_{FA} is analogous to R_{FA}; however, this study employed a modified version of the MFR where the intertrial interval was constrained between 4 and 7 s—it was not a Poisson distribution. For this reason, we report both false alarm measures. In most sessions, there were few responses outside of any response interval, so the two measures are nearly proportional.

3. Threshold estimates

Hearing thresholds were measured over a 20-month period. Thresholds were measured for Beethoven at 29 frequencies between 2 and 130 kHz. For Turner, thresholds

were measured at 28 frequencies between 2 and 100 kHz. Above 100 kHz, Turner did not respond to tones with an SPL of 160 dB *re* 1 μPa and was therefore not tested at these frequencies.

Test frequencies were separated into three overlapping groups, dictated by the usable ranges of the three available sound projectors: low (2–20 kHz), mid (12–50 kHz), or high (18–130 kHz). Frequencies belonging to more than one group were tested with multiple projectors to ensure consistency in the thresholds despite changes in the sound source. Testing began at the low frequencies, then progressed to the mid and high frequencies. Within each group, the frequency was varied from day to day. After several months, testing shifted back to the low frequency group and the process was repeated for most frequencies, so thresholds were obtained several months apart.

Each frequency was tested at least three times; most frequencies were tested five or more times. Each test yielded an independent threshold based on ten reversals. The number of times a particular frequency was tested depended, in part, on the variability of the threshold measurements at a single frequency and between nearby frequencies. Additional tests were conducted at frequencies where measurements were highly variable and at frequencies where thresholds showed large differences compared to neighboring frequencies. The threshold and false alarm data at each frequency were used to calculate the mean and standard deviations for the threshold and false alarm rates as functions of the sound frequency.

III. RESULTS

Table I and Fig. 2 present the hearing thresholds, R_{FA}, and r_{FA} values for Beethoven. The symbols in Fig. 2 indicate the mean values; the error bars represent the 95% confidence intervals. Figure 2(a) includes a representative sample of the mean ambient noise spectral density level (in dB *re* 1 μPa^2/Hz) measured in the test pool. Above approximately 20 kHz ambient noise levels were below the self-noise of the measuring hydrophone and amplifier (B&K 8105 and B&K 2635). Table I includes the number of measurements conducted at each frequency (n). Table II and Fig. 3 present analogous data for Turner.

Audiograms for both subjects have the ''U-shape'' typically seen in mammals. Beethoven had best sensitivity from approximately 50 to 80 kHz and functional hearing (defined here as thresholds $<$120 dB *re* 1 μPa) above 100 kHz. Notches and peaks in sensitivity were observed at 20 and 50 kHz, respectively. Turner had best sensitivity from 30 to 35 kHz and functional hearing up to only about 50 kHz. False alarm rates in both subjects averaged near 5% and 1% for R_{FA} and r_{FA}, respectively. False alarm rates were variable from session to session, leading to relatively high standard deviations. There were no substantial differences in false alarm rates with frequency in either subject.

Figure 4 compares the data from the present study to beluga hearing thresholds previously measured by White *et al.* (1978), Awbrey *et al.* (1988), Klishin *et al.* (2000), and Ridgway *et al.* (2001). The adult male tested by Awbrey *et al.* was one of the subjects tested by White *et al.* White *et al.*, Awbrey *et al.*, and Ridgway *et al.* used behavioral methods; Klishin *et al.* used an electrophysiological technique. Ridgway *et al.* conducted measurements in the open ocean at 5-m depth; the other data were obtained in pools with depths from about 1.5 to 4 m. Klishin *et al.* lowered water depth to 40 cm during measurements, so that the electrodes remained above the waterline.

TABLE I. Hearing thresholds and false alarm rates for Beethoven. SD =standard deviation. n=number of independent threshold estimates (each based on ten reversals).

	Threshold		R_{FA}		r_{FA}		
Frequency (kHz)	Mean (dB *re* 1 μPa)	SD (dB)	Mean (%)	SD (%)	Mean (%)	SD (%)	n
2	89	5.3	1.6	3.7	0.3	0.6	12
4	82	5.4	5.7	6.5	1.4	1.6	13
5	77	2.1	5.4	7.3	1.7	1.3	6
7	67	3.6	3.8	5.0	1.6	2.0	6
8	67	1.8	2.3	3.6	1.6	1.8	6
10	68	3.3	3.1	5.3	1.1	2.0	10
12	67	4.4	3.5	3.3	0.7	0.7	7
14	62	4.1	4.6	7.4	0.7	1.2	7
15	65	1.7	13.0	2.0	2.2	0.4	4
18	61	3.3	10.2	7.6	1.7	1.3	4
20	77	4.3	3.8	6.9	0.6	1.1	16
25	61	6.8	4.3	4.2	0.8	0.8	7
30	60	4.5	7.6	8.8	1.0	1.1	5
40	57	2.1	3.0	5.9	0.5	1.0	7
45	59	1.0	5.8	6.3	1.0	1.1	3
50	43	2.4	2.6	3.6	0.5	0.6	5
55	55	4.8	1.8	3.6	0.3	0.6	4
60	53	1.2	7.2	7.7	1.2	1.2	3
70	53	4.6	9.8	6.1	1.7	1.2	4
80	56	1.9	7.1	8.2	1.3	1.5	4
90	59	7.7	5.6	6.2	0.9	1.1	13
91	72	8.9	1.1	2.5	0.2	0.5	5
95	75	2.9	2.0	3.4	0.4	0.8	3
100	74	4.0	6.5	5.9	1.1	1.0	14
110	79	3.9	13.4	8.9	2.5	2.0	7
115	86	3.1	2.6	4.4	0.5	0.9	3
117	92	1.0	2.1	3.6	0.4	0.7	3
120	101	2.3	2.3	3.6	0.4	0.6	6
130	103	2.7	5.1	8.7	0.8	1.4	5

At the lower frequencies (below 10 kHz), the data from the present study are consistent with the White *et al.* and Awbrey *et al.* data, which were also obtained in pools. Differences in ambient noise levels may explain the relatively low thresholds obtained by Ridgway *et al.* at these frequencies. At the higher frequencies, Beethoven's thresholds are close to those of the subjects tested by Ridgway *et al.* and the male tested by White *et al.* The relatively high thresholds reported by Klishin *et al.* may be the result of the evoked potential methodology, the very shallow water, which could have created problems in accurately assessing the received SPL, or the short duration stimuli (about 20 ms), which may have resulted in higher thresholds due to temporal integration (Johnson, 1968). Both subjects tested by White *et al.* exhibited peaks and notches in sensitivity similar to those seen in Beethoven. The upper cutoff frequency in Beethoven is close to that observed in the other subjects. Turner had lower sensitivity at the higher frequencies compared to other belugas. The dramatic increase in Turner's thresholds above

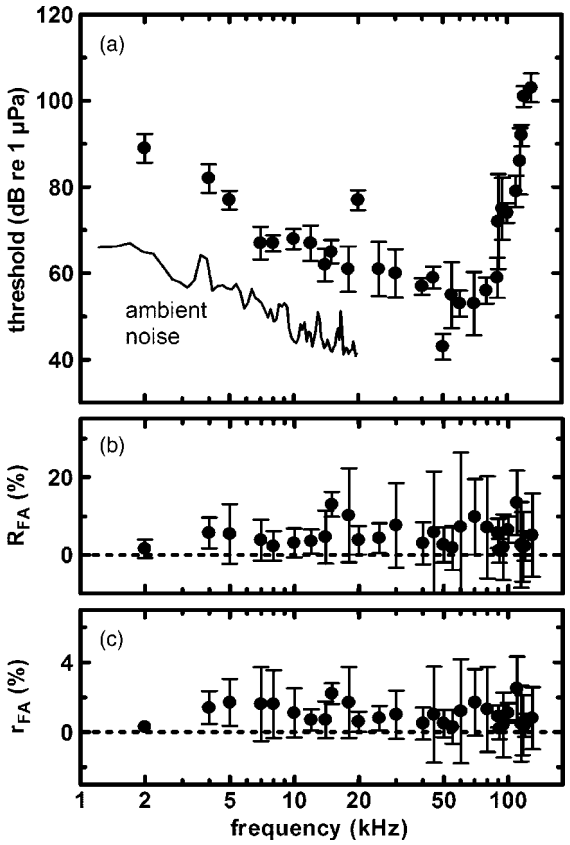

FIG. 2. (a) Hearing thresholds, (b) R_{FA}, and (c) r_{FA} values as functions of frequency for Beethoven. The symbols indicate mean values, the error bars represent the 95% confidence intervals.

TABLE II. Hearing thresholds and false alarm rates for Turner. SD =standard deviation. n=number of independent threshold estimates (each based on ten reversals).

	Threshold		R_{FA}		r_{FA}		
Frequency (kHz)	Mean (dB *re* 1 μPa)	SD (dB)	Mean (%)	SD (%)	Mean (%)	SD (%)	n
2	93	3.5	1.3	2.8	1.3	0.6	5
4	81	4.8	4.5	4.5	5.7	2.1	11
5	76	1.3	0.9	2.4	2.3	0.9	7
7	72	2.4	2.6	4.3	5.2	1.9	6
8	68	3.2	1.7	2.9	1.1	0.5	7
10	64	2.2	2.9	3.4	1.7	0.7	8
12	72	5.1	4.9	6.9	4.6	2.0	7
14	67	1.5	0.0	0.0	0.0	0.0	4
15	68	2.4	6.3	7.7	2.9	1.4	4
20	60	2.1	1.7	3.7	1.8	0.7	10
25	54	1.3	8.8	10.4	3.6	1.9	4
30	63	5.3	4.5	6.6	3.7	1.2	11
32	52	4.5	4.1	4.9	1.6	0.8	4
34	52	1.7	4.2	8.3	2.9	1.5	4
35	55	4.0	8.5	9.1	3.9	1.7	4
37	65	5.5	5.9	6.1	2.8	1.2	5
38	67	3.5	3.2	5.5	1.7	1.0	3
40	79	3.3	3.6	5.6	1.9	1.0	6
42	84	3.6	10.9	3.9	3.0	0.7	5
44	102	3.5	4.1	3.7	1.3	0.7	3
46	114	3.6	9.7	0.5	1.9	0.3	3
48	133	5.1	8.2	4.3	2.4	0.8	6
50	142	2.7	8.6	6.7	3.7	1.3	6
60	140	3.3	10.8	7.9	3.2	1.4	4
70	146	5.6	8.6	8.3	3.1	1.6	3
80	143	3.0	6.7	6.7	2.4	1.2	3
90	151	3.5	7.6	2.3	1.9	0.5	3
100	161	2.6	2.1	3.6	1.2	0.7	3

35 kHz is unique among the individual belugas for which data exist.

Although each of the previous studies of beluga hearing utilized only one to three individual subjects, pooling these data allows a larger sample size to be obtained. From this, estimates may be made of what typical means and variations may be in beluga hearing thresholds. To accomplish this, the data from Fig. 4 were collapsed at each frequency to create a single composite audiogram. The following restrictions were used when pooling the data: Only behavioral psychoacoustic data was used, so the data from Klishin *et al.* (2000) were excluded. Turner's thresholds were also excluded. The individual subject tested by both Awbrey *et al.* (1988) and White *et al.* (1978) was only included once (the White *et al.* data were used). Linear interpolation was used to estimate thresholds at intermediate frequencies. Figure 5 shows the resulting composite beluga audiogram. The solid and dashed lines indicate the mean and the mean±one standard deviation, respectively.

Figure 5 allows comparisons to be made between Turner's hearing thresholds and ''normal'' thresholds for belugas. Figure 6 shows the differences between Turner's hearing thresholds and the mean values from Fig. 5 and represents the estimated hearing loss for Turner at each frequency.

IV. DISCUSSION

The hearing thresholds presented in Figs. 2 and 3 exhibit the typical mammalian ''U-shape;'' however, the large differences between Turner and Beethoven's thresholds raise numerous questions. The large differences between thresholds are especially interesting considering the identical environments and test conditions to which the animals were subjected. Hearing loss up to 15 to 25 dB is often considered ''normal'' for humans and to represent no impairment or handicap (Davis and Silverman, 1978; Kinsler *et al.*, 1982; Glorig, 1988; ASLHA, 2004). According to this definition, Turner's thresholds would be considered normal below about 37 kHz. At 50 kHz and above Turner's hearing loss is approximately 90 dB, which would be considered ''severe'' hearing loss in humans (Davis and Silverman, 1978; Kinsler *et al.*, 1982; ASLHA, 2004).

Hearing loss in mammals can be caused by a variety of factors, including aging, exposure to high intensity sound, exposure to ototoxic drugs, or congenital factors (review Pickles, 1988; Yost, 1994). Ridgway and Carder (1993, 1997) reported hearing deficits in three male dolphins (23, 26, and 34 years) and one female dolphin (33 years). Brill *et al.* (2001) also reported hearing loss above 55 kHz in a 33-year-old male dolphin. However, Turner's young age (8–10 years) and age relative to Beethoven (13 months younger) suggest that typical mammalian age-related hearing is not a plausible explanation. Environmental noise exposure may also be ruled out, since Beethoven and Turner were housed together for nearly all of Turner's life and Beethoven's hearing appears normal. This leaves ototoxic

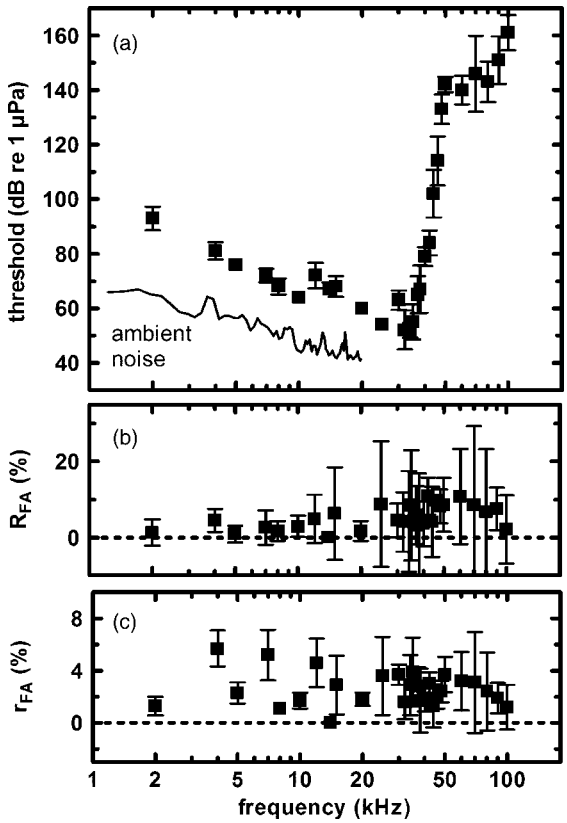

FIG. 3. Hearing thresholds, (b) R_{FA}, and (c) r_{FA} values as functions of frequency for Turner. The symbols indicate mean values, the error bars represent the 95% confidence intervals.

drug exposure as a leading candidate for the observed hearing loss.

Many drugs are toxic to the hair cells of the cochlea or vestibular system. Known ototoxic drugs include the salicylates (e.g., aspirin and aspirin-containing products), quinines, loop diuretics, aminoglycoside antibiotics (e.g., streptomycin, gentamicin, kanamycin, amikacin) and some antineoplastics (Griffin, 1988; Rybak, 1986; Tange, 1998). The extent of ototoxicity and site of damage (vestibular or cochlear) vary. For example, for the salicylates, ototoxicity occurs in approximately 1% of people receiving the drug, occurs in the cochlea, and is temporary (Jung *et al.*, 1993; Tange, 1998). Initial damage from ototoxic drugs is normally in the basal end of the cochlea, leading to high frequency hearing loss (Prosen *et al.*, 1978; Sande and Mandell, 1985; Aran *et al.*, 1995; Tange, 1998; Tan *et al.*, 2001).

To assess the possibility that exposure to ototoxic drugs was responsible for Turner's hearing loss, the health records of Beethoven and Turner were examined to determine the extent to which either had been administered ototoxic drugs. Beethoven had no history of ototoxic drug treatment; however, Turner had received aminoglycoside antibiotics. In April 1994, at six months age, Turner was diagnosed with *Nocardia* spp. infection. *Nocardia* is an infection caused by a funguslike bacterium that begins in the lungs and can spread to the brain (Turkington, 1999). Nocardial infections have a very high mortality rate (Turkington, 1999) and have been

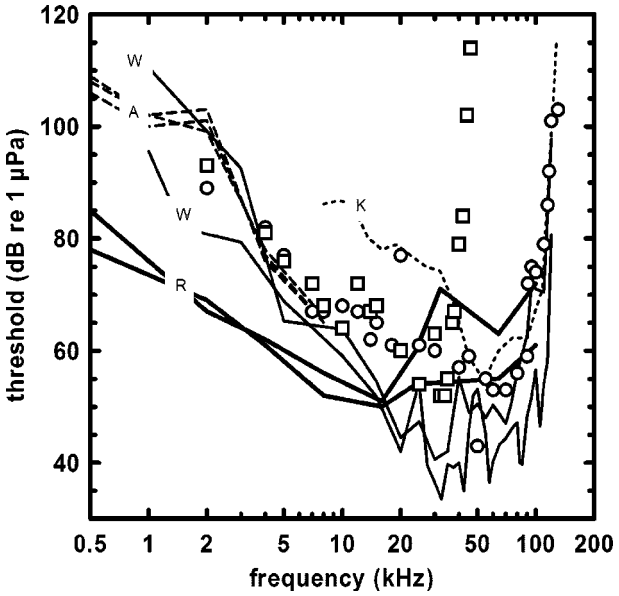

FIG. 4. Comparison between data from the present study and previously published beluga hearing thresholds. Circles—Beethoven; squares—Turner; w—White *et al.* (1978), adult male and female; A—Awbrey *et al.* (1988), adult and juvenile males and adult female; K—Klishin *et al.* (2000), adult male; and R—Ridgway *et al.* (2001), adult male and female.

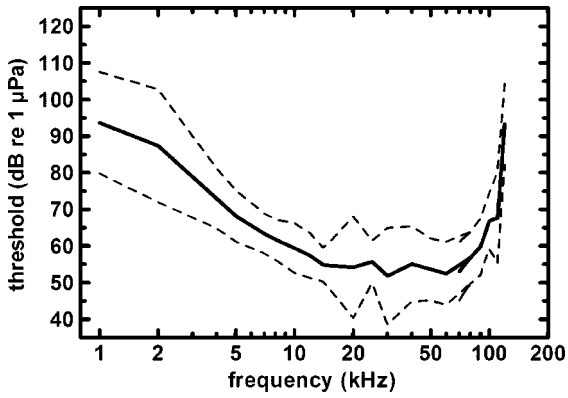

FIG. 5. Composite beluga audiogram created by collapsing the data from Fig. 4 at each frequency. Data from Klishin *et al.* (2000) and subject Turner from the present study were not included. Dotted lines represent the mean ±one standard deviation. The number of data points at each frequency were as follows: 1 kHz, six; 2–8 kHz, seven; 10–100 kHz, five; and 110–120 kHz, three.

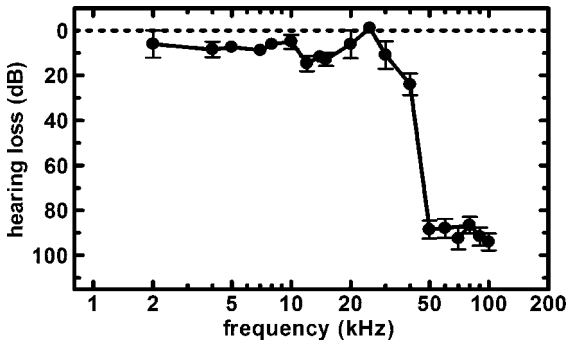

FIG. 6. Estimated hearing loss for Turner as a function of frequency. Error bars represent the 95% confidence intervals and are only shown at frequencies where data existed for both Turner and the composite audiogram shown in Fig. 5. Linear interpolation was used to estimate the ''normal'' beluga hearing thresholds at other frequencies.

reported in several cetacean species, including *Delphinapterus* (Dunn *et al.*, 2001).

Recommended treatment for *Nocardia* includes the aminoglycoside antibiotic amikacin (Dunn *et al.*, 2001), which is known to possess cochlear toxicity (Sande and Mandell, 1985; Matz, 1986). In April 1994 Turner received 8.27 mg/kg twice daily (BID) for 23 days. In October 1995, Turner received 15 mg/kg amikacin once daily (SID) for 34 days. During the course of Turner's amikacin therapy, periodic serum trough levels were documented. His 12- and 24-hour trough levels ranged between a high of 2.6 mcg/ml of serum to a low of $<$1.0 mcg/ml of serum. Peak levels of amikacin were measured during the time he received 15 mg/kg SID. The measured level was 52 mcg/ml, within the 50–60-mcg/ml range targeted for peak amikacin serum levels when utilizing SID therapy.

Amikacin, like the aminoglycoside antibiotics kanamycin and neomycin, is toxic to cochlear outer hair cells and affects those cells in the basal end of the cochlea (higher frequencies) first (Prosen *et al.*, 1978; Hawkins, 1959; Aran *et al.*, 1995; Tange, 1998). The toxicity of amikacin is similar to that of kanamycin and neomycin, with incidences of ototoxicity as high as 13% of those receiving treatment (Matz, 1986; Griffin, 1988). The relatively high amikacin dosages given to Turner and long treatment period, coupled with the observed high-frequency hearing loss, suggest that this hearing loss was a result of the amikacin treatment.

It should be pointed out that aminoglycoside antibiotics may be used to treat life-threatening infections that are resistant to other types of drugs—there may be no other choice but to use them. In the present case, Turner's severe high-frequency hearing loss must be weighed relative to the high mortality rate associated with *Nocardia*, especially in cetaceans. Recently, Bates (2003) has published data suggesting that a decrease in hearing loss induced by aminoglycosides is observed when antioxidants or iron chelator therapy is given concomitantly with aminoglycoside antibiotics. This has yet to be tested in cetaceans but might be useful to consider when infections must be treated with amikacin or other aminoglycoside antibiotics. It is interesting to note that Turner received 750 IU Vitamin E and 750 mg Vitamin C orally as a part of his routine daily dietary vitamin supplementation. It may also be noted that Turner's serum iron levels fluctuated, during the amikacin treatment periods, from his normal levels to very low levels which are common during illness in belugas.

V. CONCLUSIONS

Despite similar ages, ancestry, and environmental conditions, large differences were observed in high-frequency hearing thresholds between the two subjects. While Beethoven's thresholds were consistent with previously published data for belugas, Turner exhibited significant high-frequency hearing loss above 37 kHz, with hearing loss approaching 90 dB for frequencies above 50 kHz. An analysis of environmental factors and previous drug treatments suggests that the observed hearing loss was a result of treatment with the aminoglycoside antibiotic amikacin, which is known to be toxic to outer hair cells of the cochlea in terrestrial mammals. Amikacin and other aminoglycoside antibiotics may be used to treat life-threatening infections that are resistant to other types of drugs, so there may be no choice but to use them in certain situations. In these cases, careful dosage and/or monitoring of serum levels and possibly concomitant protective therapy may help to lower the risk of substantial hearing loss.

ACKNOWLEDGMENTS

This work supported by the U.S. Office of Naval Research Marine Mammal S&T Program and the U.S. Navy CNO(N45). We thank the staff at the Point Defiance Zoo and Aquarium for animal training and other valuable assistance.

American Speech-Language-Hearing Association (ASLHA) (**2004**). ''Types of Hearing Loss,'' http://www.asha.org/public/hearing/disorders/types.htm

Aran, J.-M., Chappert, C., Dulon, D., Erre, J.-P., and Aurousseau, C. (**1995**). ''Uptake of amikacin by hair cells of the guinea pig cochlea and vestibule and ototoxicity: Comparison with gentamicin,'' Hear. Res. **82**, 179–183.

Au, W. W. L. (**1993**). *The Sonar of Dolphins* (Springer, New York).

Awbrey, F. A., Thomas, J. A., and Kastelein, R. A. (**1988**). ''Low-frequency underwater hearing sensitivity in belugas, *Delphinapterus leucas*,'' J. Acoust. Soc. Am. **84**, 2273–2275.

Bates, D. E. (**2003**). ''Aminoglycoside ototoxicity,'' Drugs Today **39**, 277–285.

Brill, R. B., Moore, P. W. B., and Dankiewicz, L. A. (**2001**). ''Assessment of dolphin (*Tursiops truncatus*) auditory sensitivity and hearing loss using jawphones,'' J. Acoust. Soc. Am. **109**, 1717–1722.

Cornsweet, T. N. (**1962**). ''The staircase method in psychophysics,'' Am. J. Psychol. **75**, 485–491.

Davis, H., and Silverman, S. R. (**1978**). *Hearing and Deafness*, 4th ed. (Rinehart and Winston, New York).

Dunn, J. L., Buck, J. D., and Robeck, T. R. (**2001**). ''Bacterial diseases of cetaceans and pinnipeds,'' in *Marine Mammal Medicine*, edited by L. A. Dierauf and F. M. D. Gulland (CRC, Boca Raton, FL), pp. 309–335.

Egan, J. P., Greenberg, G. Z., and Schulman, A. I. (**1961**). ''Operating characteristics, signal detectability, and the method of free response,'' J. Acoust. Soc. Am. **33**, 993–1007.

Finneran, J. J., Schlundt, C. E., Carder, D. A., Clark, J. A., Young, J. A., Gaspin, J. B., and Ridgway, S. H. (**2000**). ''Auditory and behavioral responses of bottlenose dolphins (*Tursiops truncatus*) and white whales (*Delphinapterus leucas*) to impulsive sounds resembling distant signatures of underwater explosions,'' J. Acoust. Soc. Am. **108**, 417–431.

Finneran, J. J., Schlundt, C. E., Dear, R., Carder, D. A., and Ridgway, S. H. (**2002a**). ''Temporary shift in masked hearing thresholds (MTTS) in odontocetes after exposure to single underwater impulses from a seismic watergun,'' J. Acoust. Soc. Am. **111**, 2929–2940.

Finneran, J. J., Schlundt, C. E., Carder, D. A., and Ridgway, S. H. (**2002b**). ''Auditory filter shapes for the bottlenose dolphin (*Tursiops truncatus*) and the white whale (*Delphinapterus leucas*) derived with notched-noise,'' J. Acoust. Soc. Am. **112**, 322–328.

Finneran, J. J., Carder, D. A., and Ridgway, S. H. (**2002c**). ''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.

Finneran, J. J. (**2003**). ''An integrated computer-controlled system for marine mammal auditory testing,'' SSC San Diego Technical Document 3159, June.

Finneran, J. J., Dear, R., Carder, D. A., and Ridgway, S. H. (**2003**). ''Auditory and behavioral responses of California sea lions (*Zalophus californianus*) to underwater impulses from an arc-gap transducer,'' J. Acoust. Soc. Am. **114**, 1667–1677.

Glorig, A. (**1988**). ''Damage-risk criteria for hearing,'' in *Noise and Vibration Control*, 1988 edition, edited by L. Beranek (Institute of Noise Control Engineering, Washington, DC).

Green, D. M., DeFerrari, H. A., McFadden, D., Pearse, J. S., Popper, A. N., Richardson, W. J., Ridgway, S. H., and Tyack, P. L. (**1994**). *Low-Frequency Sound and Marine Mammals: Current Knowledge and Research Needs* (National Academy, Washington, D.C.).

Griffin, J. P. (**1988**). ''Drug-induced ototoxicity,'' Br. J. Audiol. **22**, 195–210.
Hawkins, Jr., J. E. (**1959**). ''The ototoxicity of kanamycin,'' Ann. Otol. Rhinol. Laryngol. **68**, 698–714.
Johnson, C. S. (**1966**). ''Auditory thresholds of the bottlenosed porpoise (*Turisops truncatus*, Montagu),'' U.S. Naval Ordnance Test Station NOTS TP 4178.
Johnson, C. S. (**1967**). ''Sound detection thresholds in marine mammals,'' in *Marine Bio-Acoustics, Vol. 2*, edited by W. N. Tavolga (Pergamon, New York), pp. 247–260.
Johnson, C. S. (**1968**). ''Relation between absolute threshold and duration of tone pulses in the bottlenosed dolphin,'' J. Acoust. Soc. Am. **43**, 757–763.
Johnson, C. S. (**1986**). ''Dolphin audition and echolocation capacities,'' in *Dolphin Cognition and Behavior: A Comparative Approach*, edited by R. J. Schusterman, J. A. Thomas, and F. G. Wood (Erlbaum, Hillsdale, NJ).
Jung, T. T., Rhee, C. K., Lee, C. S., Park, Y. S., and Choi, D. C. (**1993**). ''Ototoxicity of salicylate, nonsteroidal anti-inflammatory drugs, and quinine,'' Otolaryngol. Clin. North Am. **26**, 791–810.
Kinsler, L. E., Frey, A. R., Coppens, A. B., and Sanders, J. V. (**1982**). *Fundamentals of Acoustics*, 3rd ed. (Wiley, New York).
Klishin, V. O., Popov, V. V., and Supin, A. Ya. (**2000**). ''Hearing capabilities of a beluga whale, *Delphinapterus leucas*,'' Aquat. Mammals **26**, 212–228.
Matz, G. J. (**1986**). ''Aminoglycoside ototoxicity,'' Am. J. Otolaryngol. **7**(2), 117–119.
Miller, H. (**1969**). ''The FROC curve: a representation of the observer's performance for the method of free response,'' J. Acoust. Soc. Am. **46**, 1473–1476.
Nachtigall, P. E. (**1986**). ''Vision, audition, and chemoreception in dolphins and other marine mammals,'' in *Dolphin Cognition and Behavior: A Comparative Approach*, edited by R. J. Schusterman, J. A. Thomas, and F. G. Wood (Erlbaum, Hillsdale, NJ).
Nachtigall, P. E., Lemonds, D. W., and Roitblat, H. L. (**2000**). ''Psychoacoustic studies of dolphin and whale hearing,'' in *Hearing by Whales and Dolphins*, edited by W. W. L. Au, A. N. Popper, and R. R. Fay (Springer, New York), pp. 330–363.
Pickles, J. O. (**1988**). *An Introduction to the Physiology of Hearing*, 2nd ed. (Academic, San Diego).
Prosen, C. A., Petersen, M. R., Moody, D. B., and Stebbins, W. C. (**1978**). ''Auditory thresholds and kanamycin-induced hearing loss in the guinea pig assessed by a positive reinforcement procedure,'' J. Acoust. Soc. Am. **63**, 559–566.
Ridgway, S. H., and Carder, D. A. (**1993**). ''High-frequency hearing loss in old (25+ years-old) male dolphins,'' J. Acoust. Soc. Am. **94**, 1830(A).
Ridgway, S. H., and Carder, D. A. (**1997**). ''Hearing deficits measured in some *Tursiops truncatus*, and discovery of a deaf/mute dolphin,'' J. Acoust. Soc. Am. **101**, 590–594.
Ridgway, S. H., Carder, D. A., Kamolnick, T., Smith, R. R., Schlundt, C. E., and Elsberry, W. R. (**2001**). ''Hearing and Whistling in the Deep Sea: Depth Influences Whistle Spectra But Does Not Attenuate Hearing by White Whales (*Delphinapterus leucas*, Odontoceti, Cetacea),'' J. Exp. Biol. **204**, 3829–3841.
Rybak, L. P. (**1986**). ''Drug ototoxicity,'' Annu. Rev. Pharmacol. Toxicol. **26**, 79–99.
Sande, M. A., and Mandell, G. L. (**1985**). ''Antimicrobial agents: the aminoglycosides,'' in *Goodman and Gilman's: The Pharmacological Basis of Therapeutics*, edited by A. Goodman-Gilman, L. S. Goodman, T. W. Rall, and F. Murad (Macmillan, New York), p. 1150.
Schlundt, C. E., Finneran, J. J., Carder, D. A., and Ridgway, S. H. (**2000**). ''Temporary shift in masked hearing thresholds (MTTS) of bottlenose dolphins, *Tursiops truncatus*, and white whales, *Delphinapterus leucas*, after exposure to intense tones,'' J. Acoust. Soc. Am. **107**, 3496–3508.
Schusterman, R. J., Barrett, R., and Moore, P. W. B. (**1975**). ''Detection of underwater signals by a California sea lion and a bottlenose porpoise: variation in the payoff matrix,'' J. Acoust. Soc. Am. **57**, 1526–1532.
Szymanski, M. D., Bain, D. E., and Kiehl, K. (**1999**). ''Killer Whale (*Orcinus orca*) hearing: Auditory brainstem response and behavioral audiograms,'' J. Acoust. Soc. Am. **106**, 1134–1141.
Tan, C.-T., Hsu, C.-J., Lee, S.-Y., Liu, S.-H., and Lin-Shiau, S.-Y. (**2001**). ''Potentiation of noise-induced hearing loss by amikacin in guinea pigs,'' Hear. Res. **161**, 72–80.
Tange, R. A. (**1998**). ''Ototoxicity,'' Adverse Drug React Toxicol. Rev. **17**, 75–89.
Turkington, C. A. (**1999**). ''Norcardiosis,'' in Gale Encyclopedia of Medicine, edited by K. Boyden and D. Olendorf (Gale Research Group, Farmington Hills, MI).
White, M. J., Jr., Norris, J. C., Ljungblad, D. K., Barton, K., and di Sciara, G. N. (**1978**). ''Auditory thresholds in two beluga whales (*Delphinapterus leucas*),'' in Hubbs/Sea World Research Institute Tech. Rept., pp. 78–109 (Hubbs Research Institute, San Diego, CA).
Yost, W. A. (**1994**). *Fundamentals of Hearing* (Academic, San Diego).