Title: Direct hearing measurements in a baleen whale suggest ultrasonic sensitivity

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Abstract: Predicting and mitigating anthropogenic ocean noise impacts on marine animals is hindered by a lack of information on hearing in these species. We established a catch-and-release program to temporarily hold adolescent minke whales (*Balaenoptera acutorostrata*) for hearing tests during their summer migration. In 2023, two minke whales provided measures of the auditory brainstem response and data on the frequency range of their hearing. Results show that minke whales are sensitive to sound frequencies as high as 45 – 90 kHz. These tests provide information on the types of anthropogenic noise that could affect minke whales and, at least, other related baleen whale species.

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Main Text: Concerns about ocean noise impacts on marine mammals have existed since the 1980s (e.g., *1, 2*). This issue gained broader attention in the 1990s and early 2000s with the Heard Island Experiment, the Acoustic Thermometry of Ocean Climate research program, and several high-profile whale strandings coincident with naval sonar activity (*3-7*). The development of criteria and thresholds for estimating impacts on noise-exposed marine mammals has advanced significantly since that time (*8-10*). Behavioral responses to noise exposure, impacts to the auditory system (mainly noise-induced hearing loss), and other physiological responses have been proposed as impact criteria, and marine mammal research funding has shifted toward experimentally determining thresholds of noise exposure at which criteria will be met. Determining impact thresholds is, however, complicated because they can vary significantly across diverse marine mammal taxa.

The ear is the most sensitive organ to sound and the audiogram, a graphical depiction of an animal's frequency-specific sensitivity to sound, is the most fundamental piece of information necessary to evaluate whether a marine mammal is likely to be directly affected by exposure to anthropogenic noise. Of the roughly 130 species of extant marine mammals, audiograms exist for at least one representative species within the pinnipeds (seals, sea lions, and walrus), odontocetes (toothed whales, such as dolphins and porpoises), mustelids (sea otters), sirenians (dugongs and manatees), and polar bear. The one major phylogenetic group for which no audiogram exists is the parvorder Mysticeti, which are the mysticete, or baleen, whales. These whales are too large to keep under human care or train for behavioral hearing tests, and hearing range estimates have largely relied upon vocalization frequencies, anatomical modeling, and some behavioral response studies (*11-15*). The only feasible direct hearing measurements will likely be through auditory evoked potential (AEP) tests, which estimate hearing sensitivity by measuring the electrical signals produced by the brain in response to sound. For less common marine mammal species, this approach is hindered by animal access (often relegated to stranding situations) and is limited by subject animal size (*16*), i.e. it becomes increasingly difficult to record AEPs as the distance between the skin-surface recording electrodes and neurons generating the potentials increases with increasing head size. To date, the only attempt to measure hearing in a baleen whale using AEP methods was made in a gray whale (*Eschrichtius robustus*) calf during post-stranding rehabilitation (*17*). The effort was largely unsuccessful.

We designed an oceanic catch-and-release site to temporarily hold whales for AEP hearing tests; a detailed description of the site and catch methodology can be found elsewhere (*18*). Adolescent minke whales (*Balaenoptera acutorostrata*) were targeted because their small size (3-5 m; ~240-1050 kg), compared to other baleen whales, make them amenable to testing (*16*). Additionally, adolescent minke whales have a predictable northward migration along coastal Norway during the late spring and early summer. The site was therefore selected off Stamsund in Lofoten (Vestfjorden, Norway) because the coastal migration brings the whales through numerous small islands that litter the coastline of this region (Fig. 1A).

The experimental setup consisted of ~1.8 km of weighted purse seine net up to 50-m depth arranged such that whales moving westward along the north side of the fjord were guided into a channel between two islands (Fig. 1 in *18*). The west end of the channel was blocked with a barrier net. After a whale entered the channel, another barrier net was maneuvered to block the east entrance to the channel. Once contained, the whale was observed for 2 hours before it was corralled into an adjacent circular (90-m circumference) fish farm enclosure that had been modified with a drop-down net door. A roller system, used to pull the net up when harvesting

fish, was used to pull the net up until the whale was held partially submerged at the water surface for testing (Fig. 1B).

Two adolescent minke whales were caught for tests in June 2023. The first whale (Ba23_2606a) had a preexisting, potentially fatal net entanglement around its rostrum that was removed prior to testing. The whale was otherwise healthy. Test durations were dependent upon how each whale tolerated handling; the first whale was held and tested for ~90 minutes, the second for ~30 minutes. Auditory evoked potentials were noninvasively recorded utilizing cabled, gold-plated EEG electrodes attached to the skin with silicone suction cups. Acoustic stimuli were projected to the whales from an underwater sound transducer placed in front of them ~1.75 m from the ears (Fig. 2A). A satellite tag was attached to the dorsal fin of each whale so that behavior could be monitored following release (Fig. 2B). Post-release monitoring indicated that both whales returned to normal diving behavior after release. No lasting negative effects due to handling were observed (*19*).

Measuring the auditory brainstem response

Optimal acoustic and evoked response recording parameters for performing AEP hearing tests are species-specific. Since AEP recordings had never been attempted in a minke whale, acoustic stimulus and recording parameters were explored in the first subject (Ba23_2606a) to determine suitable settings for recording the auditory brainstem response (ABR), an AEP consisting of a series of neural potentials ("waves") generated by the ascending auditory pathway in response to an acoustic stimulus. A series of "chirps," which are short-duration, increasing-frequency tones that enhance the ABR by accounting for temporal dispersion along the cochlear partition (*20, 21*), were used as test stimuli. Chirps swept from 2.8-32 kHz and ranged from 125 to 1000 μs in duration. All chirps produced an ABR characterized by a dominant wave at a latency of ~9 ms (after accounting for the acoustic delay and duration of the chirp). The highest amplitude wave (450-575 nV) observed in Ba23_2606a was produced with a chirp duration of 710 μs (Fig. 3A).

The dominant ABR wave observed in the minke whale is likely analogous to the P4-N5 wave observed in odontocetes and pinnipeds (Fig. 3B). It is typically the highest amplitude wave in odontocetes, exceeding 10 μV under optimal conditions (*22*), and presumably originates from the inferior colliculus (*16, 23, 24*). Due largely to specializations supporting echolocation, e.g., enlargement of the auditory nerve and auditory centers of the brain (*25*), the odontocete ABR amplitude is an order of magnitude larger than that observed in the minke whales. The large body size of the minke whale also diminishes the amplitude of the signal given the increased distance between the source of the ABR and the epidermal recording site (*16*). Nevertheless, the dominant ABR wave was observable with chirps of all durations and waves of presumably more distal origin in the ascending auditory pathway were observed between 6 and 9 ms with most chirps (Fig. 3B).

Upper-frequency limit of hearing

Broadband chirps have limited utility in determining frequency-specific sensitivity to sound. Tone burst stimuli are more narrowband and allow more frequency-specific testing to be performed. Tone bursts projected in series produce an auditory steady-state response (ASSR) where ABR waves to individual tone bursts become superimposed (*26*). Fourier analysis of the ASSR waveform produces a spectral peak that corresponds to the stimulus repetition rate. Given a consistent received sound pressure level (SPL, in dB re 1 μPa), the spectral peak amplitude will vary as a function of the stimulus repetition rate. The maximum spectral peak across all tested repetition rates corresponds to the optimal stimulus presentation rate. The relationship between

stimulus repetition rate and spectral amplitude, and the optimal stimulus presentation rate are species-specific. A transfer function relating ASSR spectral amplitude to tone burst repetition rate can be measured to determine the optimal rate, but the process is time consuming and was not used here due to limited animal handling times. Instead, peaks in the ABR spectrum were used to estimate the optimal repetition rate as the ABR spectrum largely mirrors the transfer function (*16, 27*). Peaks in the ABR spectrum obtained from the first whale (Ba23_2606a) suggested that optimal stimulus repetition rates were either 200 or 600 Hz, which is lower than optimal rates for odontocetes but similar to those for sea lions (*28, 29*). Subsequent tests demonstrated that 600 Hz provided an ASSR with a statistically detectable spectral peak (Fig. 3C).

The ABR was recorded in the second whale (Ba23_2706c) using a 710-µs chirp to ensure comparable results to those from the first whale. The dominant wave had a similar latency (~8 ms) and slightly larger amplitude (700 nV; Fig. 3B). A series of repetitive five-cycle tone bursts, without an amplitude envelope to mitigate spectral spread, was then presented to the whale at a 600 Hz repetition rate. The tone bursts were presented with center frequencies (F_c) ranging from 4 to 64 kHz and at peak-to-peak SPLs sufficiently high to evoke a detectable ABR (see Table S2 for stimulus levels and bandwidths). Spectral peaks were observed at 600 Hz for all tested frequencies, except 5.7 kHz (Fig. 3C). Given insufficient time was available to measure a full AEP audiogram, it was determined that the upper-frequency limit (UFL) of hearing should be probed and the whale released. Trains of cosine-enveloped tone bursts that narrowed the stimulus bandwidth were therefore transmitted to the whale. The tone bursts had a two-cycle rise time, one cycle at maximum amplitude, and a two-cycle fall time, and were transmitted with F_c of 45, 53, 64, 90 and 128 kHz (Table S2). All except the 128-kHz tone burst elicited peaks in the ASSR spectrum indicating sensitivity to stimuli with F_c<128 kHz (Fig. 3D).

Significant bandwidth in the suprathreshold test signals (Fig. 3E) precluded an accurate UFL assessment; audible acoustic energy spread into frequencies below the stimulus F_c due to steeply declining hearing sensitivity at frequencies near the UFL (*30*). To refine the UFL estimate from the available minke whale data, a trained bottlenose dolphin with a known UFL participated in the same AEP tests conducted with Ba23_2706c. Identical acoustic stimuli were used but in the presence of high-pass masking noise, which simulated the UFL by masking sounds above the high-pass frequency. By constraining the frequency contribution of the tone pips to the evoked response with masking noise, we were able to simulate subjects with UFLs ranging from 45 – 128 kHz (*19, 31, 32*). Similar results were obtained to those from Ba23_2706c at a subset of the test conditions (Table 1); however, the dolphin detected the 128-kHz F_c stimulus even when high-pass masking above 90 kHz was provided. The minke whale did not detect the 128-kHz F_c stimulus when unmasked, even though the 10-dB bandwidth was 41.8 kHz (Table S2). This suggests that the minke's UFL does not exceed 90 kHz, and likely occurs between 45 and 90 kHz.

Discussion

Mysticete whales have long been assumed to be low-frequency hearing specialists. This assumption should be reconsidered in a broader ecological context given the current findings. For example, the high-frequency hearing we detected in the minke whale might support the detection and localization of echolocation clicks produced by its predator, the killer whale (*Orcinus orca*) (*33-36*). Killer whale echolocation clicks have F_c ranging from 45 to 80 kHz and

bandwidths between 35 and 50 kHz (*37*), suggesting that minke whales are well-suited to their detection. The evolution of counter-predator sensory capabilities is likely common. For example, predation risk has been hypothesized as an evolutionary driver in porpoises, pygmy and dwarf sperm whales, and certain dolphin species that generate narrow-band echolocation signals at frequencies above the killer whale hearing range to avoid detection by these predators (*38-40*).

Table 1. Simulating the UFL. Detection of the ASSR to tone bursts in the presence of high-pass masking noise in a trained bottlenose dolphin and a minke whale. Check marks indicate that the ASSR recorded with skin-surface electrodes was detected. Xs indicate that no ASSR was detected. The simulated UFL corresponds to the high-pass frequency of the masking noise.

tone burst F_c (kHz)	minke whale	simulated bottlenose dolphin UFL (kHz)				
		45	53	64	75	90
45	☑	☑	☑	☑	☑	☑
64	☑	☑	☑	☑	☑	☑
90	☑	X	☑	☑	☑	☑
128	X	X	X	X	X	☑

Although there remains ambiguity regarding the exact frequency of the UFL, this study's findings have consequences for understanding potential anthropogenic noise effects on minke whales. Currently, ocean noise regulators cautiously evaluate potential impacts of mysticete whale noise exposure because of a lack of quantitative information on mysticete hearing. Assumptions regarding mysticete hearing are driven by vocalization frequencies, anatomical models, behavioral reactions to sound, and extrapolations from other marine mammals (*8, 12-14, 41-45*). However, no model predicted minke whales might hear frequencies >45 kHz. Some anthropogenic sound sources audible to minke whales might therefore have been eliminated from regulatory consideration because of what seemed an unlikely hearing range. Is it possible that similar assumptions about hearing in other mysticetes are also incorrect?

The minke whale is the first baleen whale to undergo a successful electrophysiological hearing test. Electrophysiological methods remain a promising approach for directly measuring hearing in other mysticete species, but application of the methods is challenged by the large size of mysticete whales and the logistical complexities of gaining subject access. In addition, AEP methods will be unusable below some frequency since inner hair cells lose phase locking and firing synchrony toward the cochlear apex, impeding production of a measurable evoked response. The frequency below which this happens is species-specific and dependent upon cochlear structure; for example, loss of firing synchrony occurs in bottlenose dolphins, a high-frequency specialist, at stimulus frequencies <10 kHz (*31*). The frequency below which phase locking and firing synchrony are lost is unknown for all mysticetes. Indirect and inferential methods of estimating hearing will therefore continue to be of value. However, vocalization frequencies may underestimate hearing ranges (*46*), anatomical models require validation before predictions are confidently accepted, and behavioral response studies in the wild will leave uncertainty regarding whether an animal remains unresponsive despite hearing a sound. For

example, sound playbacks to estimate hearing sensitivity in gray whales provided important information about their hearing, including at frequencies <1 kHz (*15, 47*). However, these approaches recorded spontaneous rather than conditioned responses to acoustic stimuli and the response motivation (or lack thereof) was unknown, complicating determining whether some stimuli were heard.

Further AEP method optimization for minke whales can be performed. Although the optimal chirp duration was assessed by stepping through multiple chirps of different durations, the chirp maximum frequency was below the minke whale UFL. Adjusting the chirp frequency range to match the minke whale UFL should increase chirp-evoked potential amplitudes. Optimal placement of the non-inverting electrode on whales is assessed by systematically varying the electrode placement distance from the whale's blowhole and measuring the ABR amplitude to a standard stimulus at each site. Because of welfare-determined limitations on animal hold times, only three locations were tested in this study - 5, 8 and 11 cm. The findings were suitable for an initial hearing range assessment, but further electrode placement optimization could shorten test times and increase result quality.

The potential for success with mysticete AEP hearing tests would substantially improve if applied to calves of certain species, as this would mitigate signal attenuation due to animal size (i.e., due to the distance from the brain to the skin surface). The results would also present the most liberal estimate of the frequency range of hearing and sensitivity to sound (i.e., their hearing is unlikely to be affected by presbycusis (age-related hearing loss), injury, or disease). For some species, e.g., humpback whales (*Megaptera novaeangliae*) and gray whales, calves demonstrate a relatively high rate of entanglement or stranding in certain parts of the world. Conducting AEP hearing tests in these individuals may be controversial, but might provide the only means to reliably assess the frequency range of hearing in the world's largest mammals.

The study conducted here presented risks to both whales and researchers. Though every effort was made to mitigate potential problems with the catch-and-release procedures (*18*), risk reduction can be improved as experience is gained with the procedures. Capturing wild odontocetes for research purposes is not new, e.g., wild belugas and bottlenose dolphins are regularly caught for health assessments (*48, 49*), and capture and handling techniques for these species have evolved over time. It should be reasonably expected that the same evolution will occur with mysticetes, although the benefit of performing such procedures must continue to be weighed against potential animal risk.

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Supplementary Materials

Materials and Methods

Supplementary Text

Figure S1

Tables S1 to S2

References (*50*-*56*)

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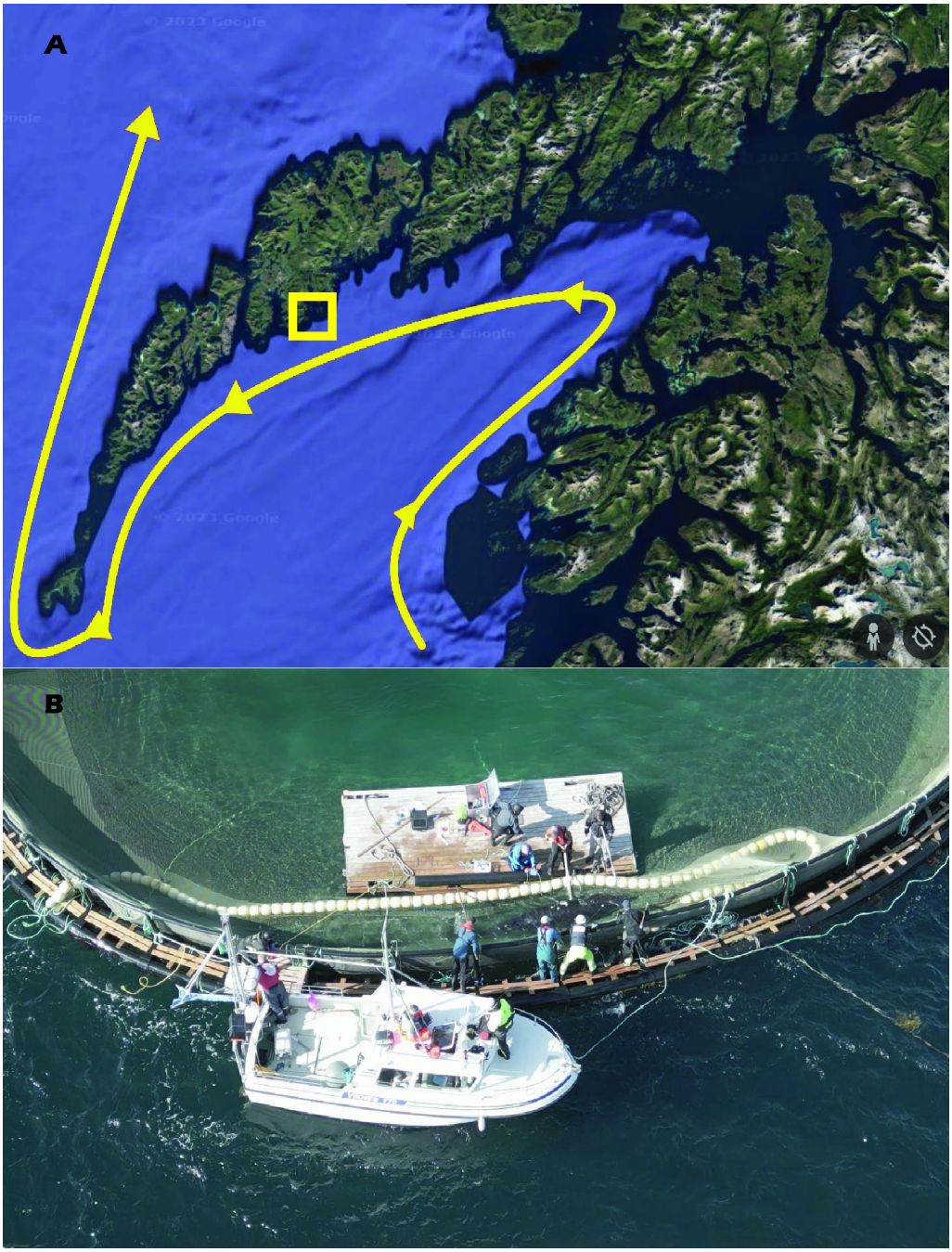

Figure 1. Experimental setup and holding procedures conducted with minke whales. (**A**) Migratory path of adolescent minke whales traveling to the Arctic. The yellow square designates the location of the experimental site. (Map Data: Google, IBCAO, Landsat / Copernicus) (**B**) Overhead view of the minke whale secured in the fish farm net hammock. The net is supported by the rollers and fish farm frame to either side. The whale receives added support from a padded strap passed underneath its body at the axillae. Details of the experimental site and catch methodology are given in (*16*).

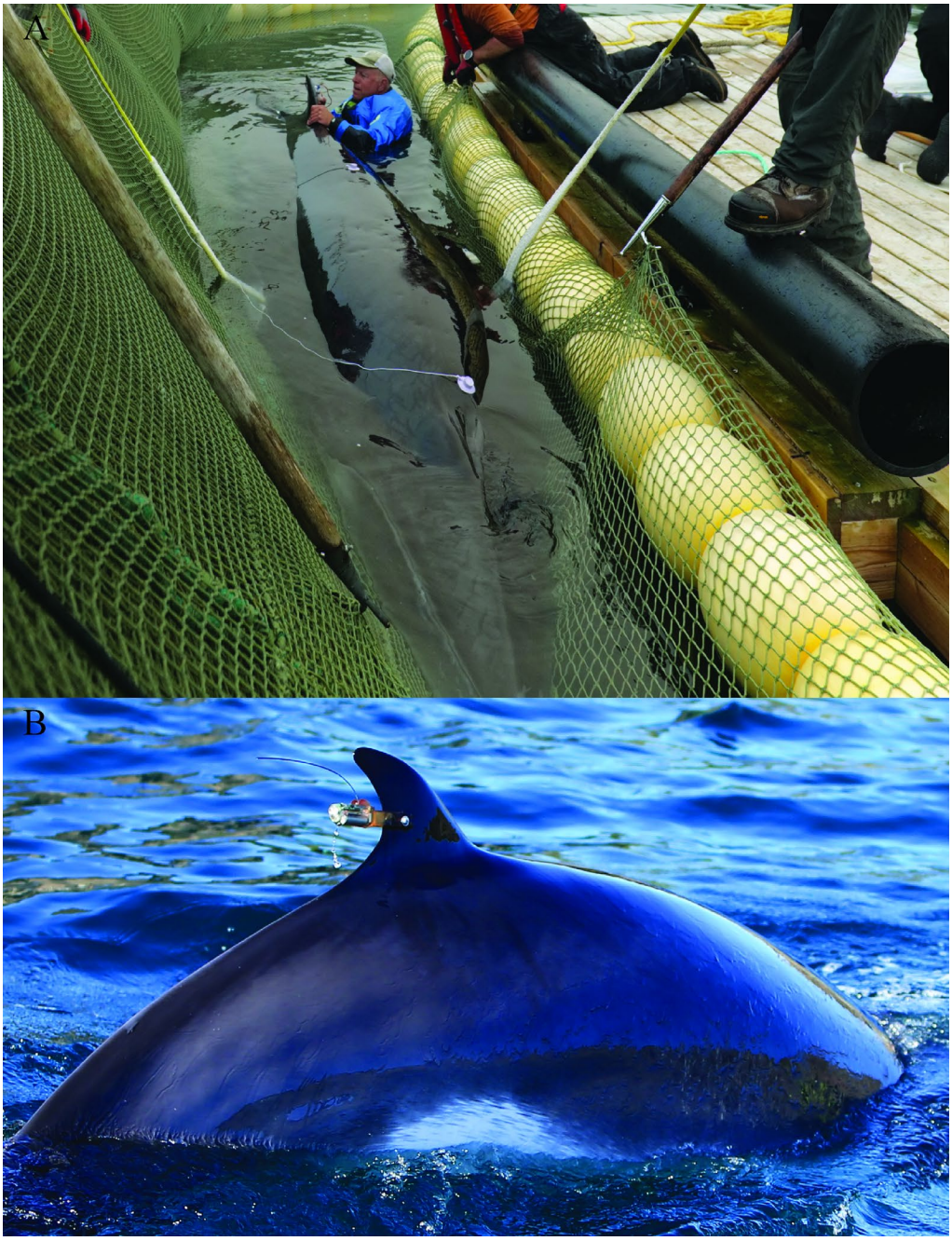

Figure. 2. Animal handling procedures. (**A**) Minke whale undergoing an AEP hearing test. The non-inverting electrode and reference electrode are caudal of the blowhole and rostral of the dorsal fin, respectively. The attending veterinarian (C.A. Harms) is pictured collecting a blood sample from the dorsal fin in this image. (**B**) Post-release view of the dorsal fin-mounted satellite tag.

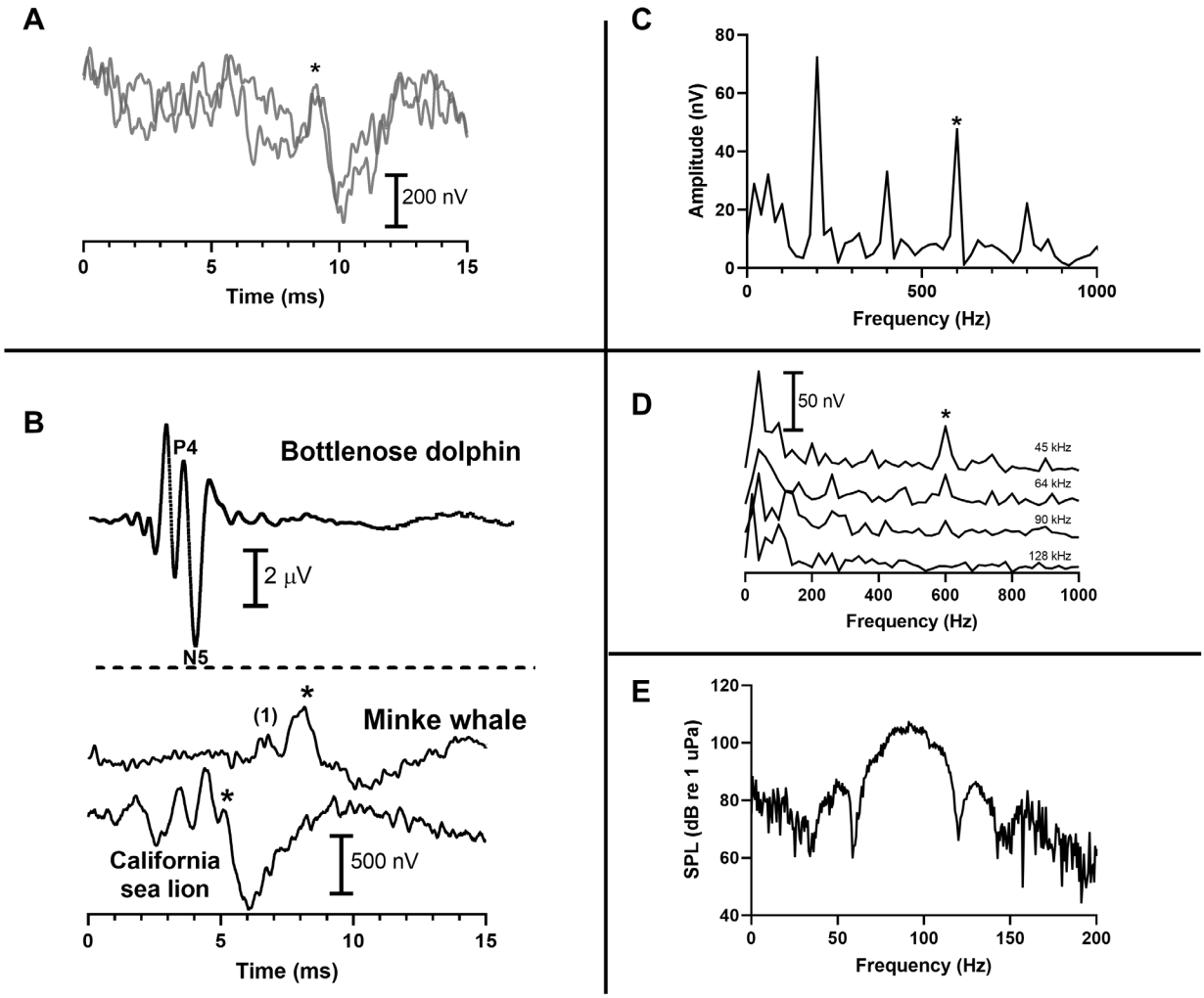

Figure. 3. Auditory evoked potential results. (**A**) Two ABR traces from the 710-µs chirp in Ba23_2606a. The * marks the beginning of the dominant ABR wave. The waveforms are offset in the vertical to facilitate viewing. (**B**) Comparison of a chirp-evoked ABR from Ba23_2706c, a California sea lion (*Zalophus californianus*), and a bottlenose dolphin (*Tursiops truncatus*). The dolphin ABR is separated along the y-axis because of the larger ABR amplitude (note scale differences), but is aligned with the time scale of the x-axis. Note the designation of the P4-N5 complex. Asterisks mark the start of a presumably analogous wave in the sea lion and minke whale, whereas (1) denotes earlier waves of the ABR in the minke whale. (**C**) Frequency-domain analysis of the ASSR evoked by repetitive tone bursts with F_c=45 kHz. The statistically determined presence of the ASSR is denoted by an * and corresponds to the 600-Hz rate at which stimuli were projected. (Note the spectral peaks at the harmonics and sub-harmonics of the 600-Hz rate.) (**D**) Spectra of the ASSR generated with enveloped tone burst stimuli at F_c of 45, 64, 90 and 128 kHz. Note the presence of a statistically detectable signal at 600 Hz (indicated by *), which was detected in all the ASSRs except that produced by repetitive tone bursts with F_c=128 kHz. (**E**) Spectrum of an enveloped tone burst with F_c=90 kHz. Note the broad bandwidth of the signal due to its short duration.

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Supplementary Materials for

Direct hearing measurements in a baleen whale suggest ultrasonic sensitivity

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The PDF file includes:

Materials and Methods

Ethics

All procedures were reviewed and approved for ethics and animal welfare by the Institutional Animal Care and Use Committee of the National Marine Mammal Foundation (protocols #15-2019 and #17-2021). Procedures were subsequently reviewed and approved by the US Navy Bureau of Medicine and Surgery (BUMED). Permits for the capture of minke whales in Norwegian territorial waters were approved by the Norwegian Animal Research Authority (permit nos. 19/84343 and 22/241930) and the Norwegian Fisheries Directorate (permit nos. 22/672 and 23/2507). Additional permits for coastal waterway blockage due to the presence of the barrier nets were obtained from the Norwegian Coastal Agency (permit no 2021/6-11/20/40). Subsequent procedures to estimate the upper-frequency limit of hearing (UFL) were also approved by the Institutional Animal Care and Use Committee of the Navy Information Warfare Center Pacific, and BUMED.

Experimental site setup

The setup and logistics of the catch-and-release site with lead nets, barrier nets and an aquaculture pen, as well as the corralling procedure for getting the whale into the aquaculture farm ahead of AEP measurements, are described in detail elsewhere (16). Briefly, a system of barrier and guide nets were placed offshore of the town of Stamsund, Norway. The purse seine vessel, "*Roger*," which had a triplex net handling system for seine nets, was contracted from Rowenta AS (Kjeller, Norway) to place the nets. Over an 18-hour period, the *Roger* assisted with placing and connecting barrier and guide nets to previously moored, 1200-kg sea anchors or to land anchors placed on the small islands making up the site.

The catch basin for initially catching whales was created utilizing the channel between the islands, Æsøya (68° 6' 2.4372", 13° 48' 17.0208") and Kvannholmen (68° 5' 49.941", 13° 48' 37.9656"). Once contained within the catch basin and determined to be suitable for testing by veterinary staff, the whale was corralled into an aquaculture pen (10,000 m^3 volume) using two small boats with outboard engines and a corralling net (150 m x 40 m). After additional observation by the veterinary staff and obtaining their approval to continue, a system of rollers used in the harvesting of fish was used to slowly reduce the water volume in the aquaculture pen until the whale was held in a net hammock supported on one side by the ring of the aquaculture farm and by the roller system on the other side (Fig. 1B).

The hearing test and satellite tag attachment were performed with the whale in the hammock (*16*). When the hearing test and satellite tag attachment were completed, the whale was released back into the aquaculture pen by slowly lowering the nets of the aquaculture pen. The rate at which the nets were released was based upon the respirations of the whale and its activity (e.g. trying to swim). Once the whale was fully released back into the aquaculture pen with the full volume of water, it was observed for 2 additional hours to ensure that there were no apparent issues from the testing and satellite tag deployment (*16*).

Auditory evoked potential (AEP) hearing tests

Sound presentation to the whales was performed with an underwater transducer (M18c-4.0; Geospectrum Technologies, Inc., Nova Scotia, Canada) placed at approximately 0.5 m depth and either 1 m lateral of the whale's right ear or directly in front of the animal at 1.75 m from the acoustic meatus (see Supplementary Text, below). Acoustic stimuli were digitally synthesized

and converted to analog (1 MHz update rate and 16-bit resolution), low-pass filtered at 200 kHz (8-pole Butterworth, Krohn-Hite 3C series), and attenuated (custom, 0–70 dB range) before being transmitted to the underwater transducer.

Initial AEP measurements utilized clicks and swept-frequency tones with increasing frequency ("chirps") to elicit the transient auditory brainstem response (ABR). Click and chirp spectra were broadband and colored by the transmitting voltage response of the transducer. Clicks were created by transmitting a 5-µs DC electrical pulse to the sound transducer. The instantaneous frequency of the chirp waveforms varied according to:

$$f(\tau) = k(\tau)^{-1}$$

where f is frequency (in kHz), τ is instantaneous time during the chirp (in ms), and k is a parameter governing the chirp sweep rate (larger k leads to slower sweep rates and longer chirps). Chirps were frequency swept from 2.8 to 32 kHz and had durations of 125, 250, 375, 500, 710 or 1000 µs. The presentation rate for clicks and chirps was ~13 Hz.

Later AEP testing utilized more frequency-specific stimuli consisting of repetitive tone bursts, which results in a steady-state AEP (the auditory steady-state response, ASSR) formed when successive ABRs overlap. Tone bursts consisted of either five cycles of a tone without an envelope, or a "2-1-2" tone burst in which there was a two-cycle rise, one cycle amplitude plateau, and a two-cycle fall under a cosine envelope. Based on estimates of optimal stimulus presentation rates (see Supplementary Text, below), tone bursts were presented at a rate of either 200 Hz or 600 Hz. Tone bursts were presented in 50-ms sequences consisting of either 10 (200 Hz rate) or 30 (600 Hz rate) individual tone bursts. Each tone burst sequence was followed by ~25 ms of silence before the next sequence began.

Given the exploratory nature of the initial hearing test procedures and the short procedure durations, the peak-to-peak equivalent sound pressure level (SPL_{ppe}; dB re 1 µPa) of the chirp and tone burst stimuli were varied within and across the two tests; stimulus received levels are provided in Tables S1 and S2, and discussed in the Supplementary Text for each whale.

All extraneous noise sources (e.g. ship engines, compressors, generators) were stopped during the measurements and the electronic equipment necessary for AEP testing was battery powered to minimize the presence of electrical noise. Ambient noise recordings were collected using a SoundTrap 300HF prior to, during and following AEP measurements. The SoundTrap was placed at a depth of 1 m just outside of the door of the outer net. The SoundTrap digitized signals with 16-bit resolution at a continuous sampling rate of 576 kHz and had a sensitivity of -179 dB re 1 µPa. For most of the frequency range of interest, the noise floor was near the self-noise of the hydrophone system (Figure S1); average noise pressure spectral density was ~30 dB re 1 $\mu Pa^2/Hz$ above 1 kHz, but increased at lower frequencies. The noise floor was sufficiently low that all the acoustic stimuli used in the hearing test were free from masking.

Evoked potentials were measured using three 10-mm gold cup surface electrodes embedded in silicon suction cups; a noninverting electrode was located on the dorsal midline posterior to the blowhole, an inverting electrode was placed approximately at the midpoint between the blowhole and the dorsal fin, and a ground electrode was positioned in the ocean near the whale (the initial

placement of the noninverting electrode was ~5-8 cm posterior of the blowhole lip, but this was changed after testing indicated a higher amplitude evoked response was received 11 cm posterior of the blowhole; see Supplementary Text.) Conductive paste was used to couple the electrodes to the whale's skin surface. Electrodes were cabled to a biopotential amplifier (Grass ICP-511) where the voltage between the noninverting and inverting electrodes was amplified (94 dB) and filtered (either 0.3-3 kHz or 0.03-3 kHz, see Supplementary Text, below). The amplifier output was digitized (16-bit resolution) and saved to hard disk at a rate of 250 kHz.

Amplified and filtered EEG signals were divided into 75.3-ms segments ("sweeps") temporally aligned with the stimulus onset and were then synchronously averaged. Sweeps with peak instantaneous voltage above 98 μV were excluded from averaging. To analyze the ASSR in real time, a statistical test was performed after each integral multiple of 256 sweeps to see if an evoked response was detected. Sweeps were averaged using a weighted averaging technique and the presence of the ASSR determined by applying the magnitude squared coherence (MSC) statistic using eight "sub-averages" and a critical value of α=0.01 (*50-52*). If the ASSR was detected, the measurement was completed. If the ASSR was not detected, an additional 256 sweeps were collected and the statistical test for the response was again performed. A maximum of 512 or 1024 sweeps was collected for any stimulus presentation (see Supplementary Text, below). All stimulus presentations, EEG recordings, and statistical analyses were performed using the Evoked Response Study Tool (*53*)

Simulating the upper frequency limit (UFL) of hearing with a bottlenose dolphin

Results suggested that minke whales hear at frequencies above 45 kHz (see main text). However, the bandwidth of the tone-bursts (see Table S2) used in the testing was too broad to define an exact UFL (Fig. 3E). To address this ambiguity, a series of tests were conducted with a trained bottlenose dolphin (*Tursiops truncatus*) at the US Navy Marine Mammal Program to estimate the possible UFL of hearing in the minke. The dolphin was tested for the presence of the ASSR utilizing the same equipment, stimulus and recording settings, and tone-burst center frequencies as those used for the minke whales. The dolphin was tested at a distance of 1 m from the sound source and placement of the non-inverting electrode and inverting electrode were adjusted for optimal placement in the species. The hearing tests were performed in the presence of high-pass masking noise to simuate different UFLs. The masking noise consisted of "pink noise," which has equal sound pressure levels in each one-third octave (OTO) band. The noise high-pass frequency varied in ¼ octaves, from 45 to 128 kHz, and the low-pass frequency was 150 kHz. The masking noise was broadcast at an OTO SPL of 125 dB re 1μPa. Changes in the high-pass frequency emulated different UFLs of hearing by eliminating any synchronized auditory system response to frequencies above the high-pass frequency. Comparisons of the detection of ASSRs in the dolphin with various simulated UFLs with the results from the minke suggested that the upper-frequency limit of hearing likely occured between 45-90 kHz (see Table 1).

Satellite tag placement

The dorsal fin was prepared for satellite tag attachment in parallel with the AEP measurements, following the method described in (*54*). The dorsal fin was aseptically prepared with alternating scrubs of chlorhexidine and isopropyl alcohol. The satellite tag anchor point was identified, and the skin was scored with a needle for visibility. The width of the dorsal fin at that point was measured with calipers. Prilocaine 2.5% topical anesthetic cream was applied to both sides of the fin at the anchor location, and approximately 2 ml of lidocaine (20 mg/ml) with epinephrine

1:200,000 was infiltrated. Meanwhile a polypropylene pin was cut to an appropriate length based on the caliper measurement, allowing approximately 5 - 10 min for local anesthetics to take effect. Aseptic preparation of the dorsal fin was repeated, and a tissue core was drilled at the anchor location. The anchor pin was coated with triple antibiotic ointment (neomycin, polymixin, bacitracin) prior to insertion into the dorsal fin hole. Holes in the wings of the satellite tag harness were aligned on either side of the anchor pin, and nuts were screwed onto either side of the pin to hold the tag in place.

Supplementary Text

Hearing tests on the minke whales were exploratory because the species had not been tested prior to this study. Tests conducted on the first whale were performed to assess the stimulus and recording parameters necessary for eliciting and capturing the ABR. Based upon the parameter settings determined from the first whale, efforts to generate and record the ASSR were focused on the second whale. Details of each test session follow.

Results – Ba23_2606a

The whale had a net entanglement around the maxilla at the anterior portion of the rostrum. The net had been overgrown by tissue and was deemed by the veterinary team as likely to result in mortality; as the whale grew, the net would become more deeply embedded and affect bone formation. The net was therefore cut and pulled free of the tissue prior to the start of the hearing test. The whale suffered no observable ill effects of the entanglement removal.

The whale was initially tested with chirps with sweep durations from 125 to 1000 μs, as well as 32-kHz repetitive tone bursts. The amplitude of the dominant ABR wave for each condition is presented in Table S1. The transducer placement relative to the whale's ears was changed during the series of tests to obtain a higher amplitude evoked response. The transducer was initially placed ~1 m to its right and in line with the right ear. This position would produce a response that was dominated by sound reception at the right ear. The position of the transducer was changed to directly in front of the whale 1.75 m from its ears to produce a binaural response (see Table S1 for the time that the transducer was moved), which modestly increased the ABR amplitude. The number of sweeps included in the grand average for the ABR was 512 for all stimuli except the tone burst sequence, which used 1024 sweeps. The biopotential amplifier filter settings were 0.3-3 kHz and the active electrode was placed 5-8 cm caudal of the blowhole lip.

The whale was female, as determined by palpation of the anogenital slit, measuring 4.35 m in total length and with an estimated mass of 680 kg (*55*). The whale was held for ~90 minutes, during which time the satellite tag was attached and the hearing tests were performed. Once procedures were completed, the whale was released back into the aquaculture pen and observed for an additional 2 hours. The animal immediately returned to pre-handling dive behavior upon release from the net hammock. After the observation period, the outer net door of the aquaculture pen and the door to the catch basin were opened. The whale remained in the fish farm for approximately 2 more hours before departing into the basin and out of the experimental site. The following 2 weeks, the whale migrated westwards; it first swam offshore, then it returned to the Norwegian coastline and followed it north and east around North Cape (71° 10' 11", 25° 46' 58"). The whale traveled a total distance of more than 950 nautical miles during the observation period.

Results – Ba23_2706c

Based on results obtained with Ba23_2606a, the biopotential amplifier filter passband was widened (0.03-3 kHz) to ensure that no portion of the ABR was unintentionally attenuated. Additionally, movement of the noninverting electrode suggested that a more optimal location for placement of the electrode was 11 cm caudal of the blowhole lip. The transducer was positioned for binaural stimulation throughout testing.

Testing with Ba23_2706c began with a 710-µs duration chirp, which produced an ABR with a dominant wave peak-to-peak amplitude of 712 nV. Subsequently, a series of tone burst trains without an amplitude envelope were presented to the whale at a rate of 200 Hz. This was followed by the presentation of enveloped tone burst trains at a rate of 600 Hz; the envelope was used to narrow the stimulus bandwidth, but this also reduced the amplitude of the spectral peak due to fewer neurons synchronously firing (i.e. the narrower bandwidth meant that fewer hair cells were involved in the ASSR). The center frequency of the tone burst trains and the amplitude of the spectral peak corresponding to the stimulus presentation rate for each tone burst train are provided in Table S2. A total of 512 sweeps was averaged for each tone burst train.

The whale was another female, measuring 4.9 m in total length and with an estimated mass of 991 kg. The whale was held in the hammock for ~30 minutes, during which time the satellite tag was attached and AEP testing occurred. The whale was released earlier than the first whale because its behavior indicated a more rapid decompensation due to handling. Post-testing procedures mirrored those reported for Ba23_2606a, and the whale exited the fish farm and basin approximately 2 hours after the fish farm door was opened. The next day, Ba23_2706c moved 30 nm southeast, crossing the Vestfjorden. The whale stayed in that region until the tag stopped transmitting 2 weeks later.

Point-of-care blood analyzer results

Point-of-care blood analysis was performed as part of monitoring animal health and welfare. Low lactate values (1.2 and 2.0 mmol/L in Ba23_2606a and Ba23_2706c, respectively) indicated negligible anaerobic metabolism that could occur with capture and restraint. Blood glucose values were elevated (8.2 and 11.9 mmol/), consistent with an expected hypothalmamic-pituitary-adrenal axis stress response. Sodium (145 and 151 mmol/L), potassium (3.6 and 3.6 mmol/L), ionized calcium (0.99 and 1.31 mmol/L), and hematocrit (41 and 44%) values were all considered within reasonable limits, based on values published for other cetaceans (*56*). Blood gas (pH, pO_2, pCO_2) results were discounted due to air exposure prior to analysis artificially elevating (pH, pO_2) and depressing (pCO_2) values.

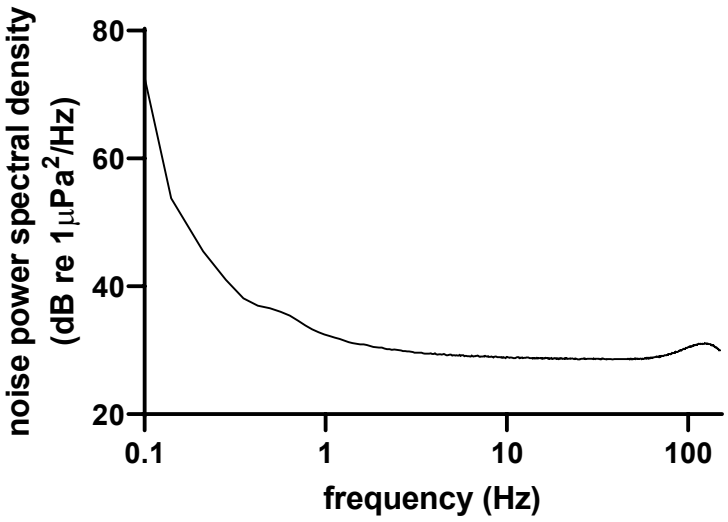

Fig. S1.

Ambient noise pressure spectral density collected during the hearing tests.
The noise pressure spectral density was low, potentially within the self-noise of the recording system. Ambient noise was sufficiently low that hearing test signals were unmasked.

Stimulus (duration)	Received SPL_{ppe} (dB re 1 µPa)	Number of sweeps	Max ABR amplitude (nV)
chirp (125 µs)	142	512	376
chirp (250 µs)	148	512	282
chirp (375 µs)	149	512	435
chirp (500 µs)	150	512	519
chirp (500 µs)*	146	512	543
chirp (710 µs)	147	512	671
chirp (1000 µs)	147	512	413
32-kHz tone burst (15.6 µs)	142	1024	440

Table S1.

Type of acoustic stimulus, received SPL_{ppe} of acoustic stimuli used in the testing of Ba23_2606a, the number of sweeps averaged to calculate the averaged ABR, and the maximum peak-peak amplitude of the dominant wave in the ABR. * indicates the point at which the transducer was moved from a monaural-dominant position (1 m to the right of the whale's right ear) to a truly binaural position directly in front of the whale (1.75 m from the whale's ears). Stimuli are presented in the order of presentation during data collection.

Tone burst center frequency (kHz)	Repetition rate (Hz)	Received SPL_{ppe} (dB re 1 µPa)	10-dB bandwidth (kHz)	Spectral peak amplitude (nV)
4*	200	118	1.0	56
5.7*	200	117	1.0	42
8*	200	116	2.3	59
16*	200	116	4.4	43
23*	200	118	6.6	66
32*	200	120	9.5	85
45*	200	120	12.2	72
64*	200	118	16.8	61
45	600	124	12.4	42
64	600	129	18.2	28
90	600	132	32.4	17
128	600	120	41.8	6†

Table S2.

Center frequency of tone bursts transmitted at a repetition rate of 600 Hz, received SPL_{ppe} of the tone burst train, and amplitude at the spectral peak corresponding to the stimulus repetition rate in Ba23_2706c. All tone burst stimuli with * were transmitted without an amplitude envelope. All other stimuli were transmitted with a cosine envelope with two cycles on the rise and fall and one cycle at peak amplitude. († indicates that the spectral peak was not significantly different than the background noise.)