

Collection of auditory evoked potential hearing thresholds in minke whales (*Balaenoptera acutorostrata*)

FINAL REPORT FOR CONTRACT MMC19167

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SUMMARY

A four-year field effort to temporarily catch and release minke whales (*Balaenoptera acutorostrata*) for the purpose of obtaining auditory evoked potential (AEP) hearing thresholds was completed in Lofoten, Norway from 2020-2024. A net-based barrier and guide system was created to temporarily catch adolescent minke whales during their summer migration to the Arctic. Whales were subsequently held in a fish farm and constrained in a net hammock for the hearing test prior to release back to the wild. Four whales were caught and tested over the four-year period. Stimuli were evaluated to determine the optimal stimulus for obtaining the auditory brainstem response (ABR). Subsequently, the ABR was obtained from all four whales, the upper-frequency limit of hearing was determined and validated in three whales, and frequency-specific thresholds of hearing were obtained from two whales. The hearing data obtained from this effort will contribute to the development of a mysticete audiogram that will support environmental assessments of the potential impacts that US Navy sound sources may have on mysticete whales.

INTRODUCTION

Currently, the Navy utilizes an audiogram-based approach to estimate the impacts that ocean noise might have on marine mammals. The audiogram is a graphical representation of an animal's frequency-specific sensitivity to sound. Audiograms are used to determine whether Navy sound sources could potentially impact marine mammals (e.g. *de minimis* source selection), and are also used in the creation of auditory weighting functions. These bandpass filter functions provide a means of emphasizing frequencies of sound to which animals are sensitive while de-emphasizing sounds to which they are not sensitive, and are used to assess potential temporary and permanent losses of hearing. Audiograms exist for one or more representative species and/or individuals within the pinnipeds, odontocetes, mustelids, sirenians, and polar bears. However, no audiogram exists for any baleen whale (parvorder Mysticeti) making it necessary to infer hearing abilities from vocalization patterns, extrapolate from other marine mammal species, or use anatomical models to predict baleen whale hearing abilities.

Direct hearing measurements in baleen whales cannot be conducted using behavioral methods because of the inability to hold baleen whales for sufficient time to train them for a hearing test. Direct measurements are most likely to be made through AEP tests, which measure electrical signals produced by the brain in response to sound. Recording AEPs becomes increasingly difficult as the distance between the recording electrodes on the skin surface and the neurons generating the potentials increase with increasing head size. For this reason, AEP hearing tests are most likely to be successful in the smallest of the baleen whales.

The objective of this effort was to catch and release minke whales for the purpose of obtaining AEP hearing thresholds, thus providing data that can contribute to the development of a mysticete audiogram and/or weighting function. Utilizing regional knowledge about minke whale migration routes, small, adolescent minke whales (3-5m) migrating along the coast of Norway were temporarily caught in a basin between two small islands and then corralled into a modified fish farm. Once contained in shallower water, the whales were constrained by nets and their AEPs measured. Adolescent minke whales were targeted due to their small size, which increased the likelihood that the AEP method would work.

The minke AEP hearing data obtained from this study provide the first direct measurement of hearing in a mysticete whale. The data will be invaluable to regulators, scientists, and action proponents concerned with the potential impact of sound on mysticetes; data can be used to determine which sound sources have the potential to impact mysticetes and can be used to guide mysticete auditory weighting function development. The data can also be used to validate anatomic models of mysticete whale hearing. Techniques developed for mysticete hearing tests during this study will facilitate future mysticete hearing tests, should follow-on work be planned.

METHODS, ASSUMPTIONS, AND PROCEDURES

This project was a research collaboration between the National Marine Mammal Foundation (NMMF; Dorian Houser and Jason Mulsow), Forsvarets forskningsinstitutt (FFI; Petter Kvasdheim), LKARTS Norway (Lars Kleivane), Kristiansand Dyrepark (Rolf Arne Ølberg), Aarhus University (Jonas Teilmann) and the US Navy Marine Mammal Program (MMP; James Finneran). Dr. Craig Harms (North Carolina State) was added as a collaborator during the first year of captures in order to bolster the experience base with mysticete sedation and large whale veterinary care.

Small, adolescent minke whales (3-5 m) migrating through coastal waters were guided into an enclosed area with barrier nets. Within the enclosed area, called the capture basin, the whales were corralled into an adjacent circular fish farm. The fish farm was modified with a net door in the outer net, but had a second inner net that rested on the sea floor. Once inside the fish farm, the door was pulled shut and the second net pulled from the bottom and secured to the fish farm so the whale was contained safely within. This net was subsequently pulled up such that the whale was contained in a shallower body of water. A roller system was then passed underneath the net and pulled across the fish farm to constrain the whale in a hammock suspended between the edge of the fish farm and the rollers. Its body was positioned such that it was partially submerged while the blowhole and dorsal surface were maintained above water. Personnel accessed the animal either from the edge of the fish farm or from a floating platform placed on the opposite side of the rollers. Once constrained, AEP hearing tests were performed utilizing surface electrodes and chirp, click, tone pip, and sinusoidal amplitude modulated (SAM) tones as acoustic stimuli. Stimuli varied in level and frequency to cover the whale's presumed frequency range of hearing and range of sensitivity. Steady-state evoked responses were measured using standardized methods and the input-out (IO) function, which describes the AEP

amplitude as a function of stimulus level, was used to determine the threshold of audibility. Whales were fitted with a satellite tag during the hearing test procedure so that their behavior could be monitored after handling.

RESULTS AND DISCUSSION

Permitting

Institutional Animal Care and Use Committee (IACUC) approval of animal test procedures and appropriate permits from the Norwegian Coastal Administration (NCA) and Norwegian Animal Research Authority (NARA) were obtained prior to conducting the first field effort. These permits were renewed either annually (NCA) or after three years (NARA, IACUC). Navigational warnings were issued each year through the Norwegian Hydrographic Office for the region and timeframe of the field effort. After the first year of the project, the Norwegian Directorate of Fisheries requested the team file for permits to capture the whales, even though the whales were to be released back to the wild. These permit requests were submitted annually from the second through the final year of the project.

Staffing

Each field season, between ten to thirteen individuals participated in the field effort on a full-time basis. An additional two to nine people participated on a part-time basis. Personnel were split into two teams (Day and Night) to enable 20 hours of manning the capture basin. Personnel operated as a single team during setup and breakdown of the capture basin, but worked a split shift when in “catch” mode (Day Team – 0800-1800, Night Team – 2000-0600). Once a whale was in the capture basin, personnel monitored it continuously.

Catch and Release System (CARS)

The catch and release system was established around a basin between two small islands, Æsoya and Kvannholmen (Figure 1). The basin was sealed to the west with a barrier net anchored into the island rock to the north and south (net A). A salmon farm was anchored just to the east of the northern island and attached to Æsoya with an additional barrier net (net B3). Barrier nets were also extended across the east entrance of the basin (nets B2 and B3), but were positioned so that a gap of approximately 50 m existed between barrier nets. The gap served as the entry to the capture basin from the east. From each side of the entry, barrier nets were extended further eastward; the northern barrier net (net C) extended to a small island, Ausa, and the southern barrier net (nets D1 and D2) extended ~1.1 km eastward into the ocean. The east-extending barrier nets (C, D1 and D2) formed an “alley” that guided whales toward the capture basin as they migrated westward. Approximately 1.7 km of barrier netting with depths of up to 50 m were deployed, equating to more than 20+ tons of net.

Barrier nets were deployed over multiple days during mid to late May in coordination with a purse seine ship. Nets were subsequently rearranged using smaller vessels, and entanglements that occurred following deployment from the purse seiner (or during basin door operation) were resolved with the assistance of an aquaculture support vessel.

The fish farm’s location was adjusted each year to best shelter it from wind and wave action. During the first field season, the fish farm was modified by team members with boarded walkways to enable safer movement around the fish farm edge. In addition, the door to the fish farm was modified with a pulley system to enable the door to be shut rapidly once a whale entered.

The original CARS design was used during the first two years of field effort. In the third year, because numerous catch opportunities were missed after whales swam around the east end of the D1/D2 barrier nets, the CARS system was modified (Figure 2). Changes to the barrier net setup included elimination of the D2 net, which previously extended eastward from the catch site, and connecting the eastward end of the D1 net to the south side of Flat Island (Figure 3). In the fourth year, approximately 150 m of the D1 net was removed to shore up the net and its connections between sea and land anchors. Modifications made over the last two field seasons substantially improved catch rates of animals within the basin.

Significant investment in barrier/guide net repairs was required prior to the last field season. Over the course of the first three seasons, the net system suffered more damage than was anticipated at the start of the project. The A net catastrophically failed near the beginning of the third season and was replaced by the C net to salvage remaining catch opportunities. Large holes in the B nets resulted in the escape of multiple whales that were initially caught within the basin during the third season. Fixing these holes resulted in a much higher retention of basin-caught animals in the fourth year; no whale caught in the basin during the fourth field season escaped.

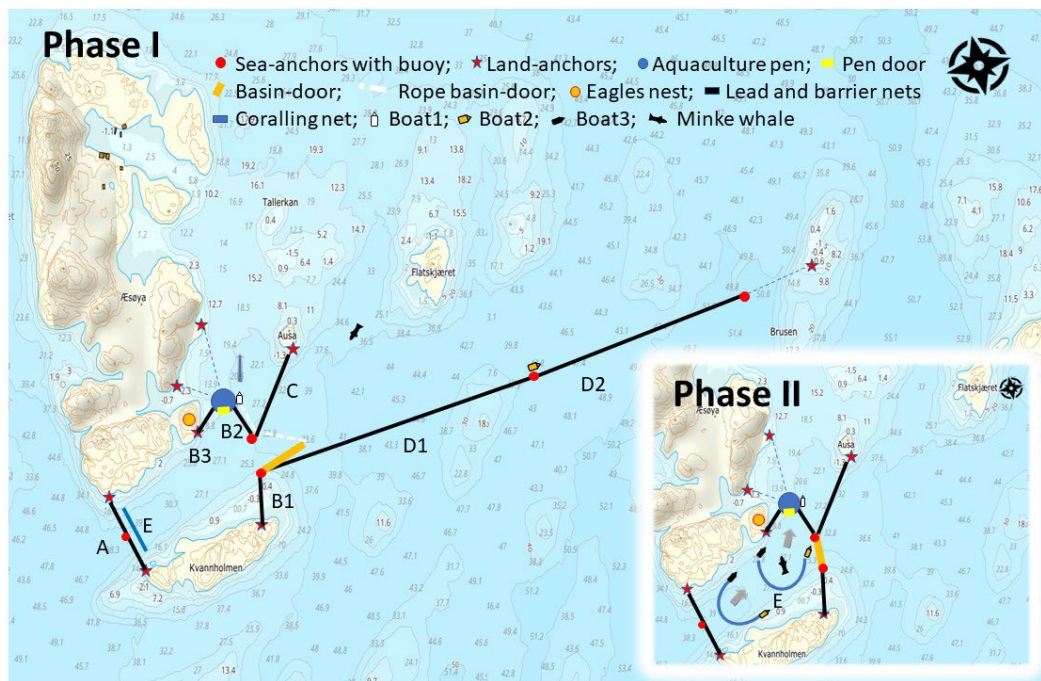


Figure 1. Design of the Catch-and-Release Site (CARS) in Lofoten, Norway during the first two field seasons. The map shows the placement of nets (solid lines, A:160 m, B1:120 m including 40-m door, B2:100 m, B3:50 m, C:160 m, D1:600 m, D2:500 m; E:100 m), anchors, fish farm (aquaculture pen), and observer platforms on the Eagles Nest, and boats (boat #1 used to close the door and boat #2 to patrol the area). Insert: Map showing the corraling of a minke whale from the catch basin into the aquaculture pen during Phase II of the catch process. The 100-m long E net is pulled between two boats from the A net eastwards towards the door of the aquaculture pen.

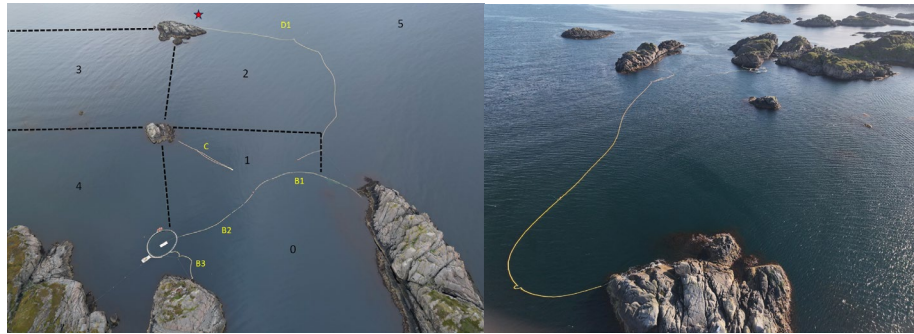


Figure 2. (left) Change in barrier net design for the CARS in 2023. Net designation is as described for Figure 1. Black numbers correspond to the zones called out when whales were first sighted. The red star designates the attachment to Flat Island. (right) Change in barrier net design for the CARS in 2024. The D1 line (yellow floats on left side of image) was shortened to prevent billowing of the net into the fjord and provide a straighter line into the catch basin. (Note that the right picture is taken from the reverse orientation of the picture on the left.)

Whale sightings

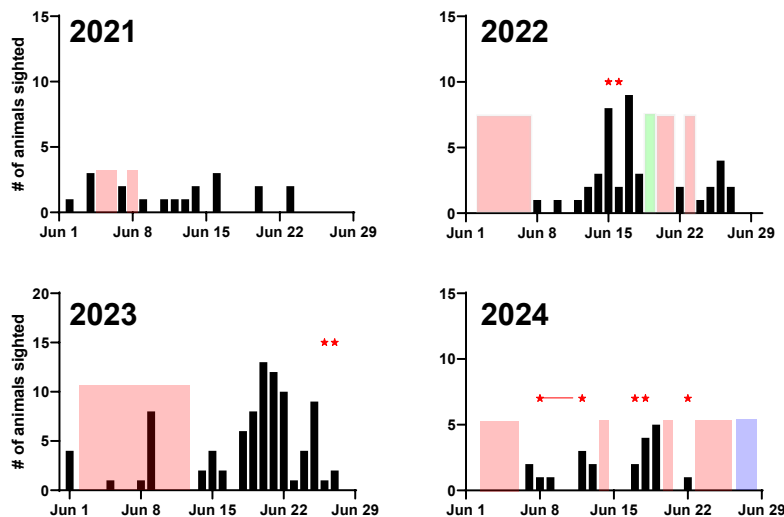


Figure 3. Number of minke whale sightings as a function of date and year. The red-shaded regions correspond to bad weather days that prevented personnel from being on the water. The purple-shaded region corresponds to the demobilization phase, which typically took the final two days of each season (only shown for 2024). The green-shaded region corresponds to a day where the full team was allowed to recover after a period of 48-72 hours where captures and attempted testing occurred; most personnel had disrupted sleep cycles with minimal sleep during this time. The red stars indicate capture of a whale in the basin with a subsequent effort to corral the whale into the aquaculture pen (n=9).

Sightings of minke whales showed substantial interannual variability (Figure 3). Nineteen whales were sighted in 2021, 44 in 2022, 88 in 2023, and 21 in 2024. Sighting effort was variable from year to year, primarily due to unpredictable weather events. Of the whales sighted, nine were caught in the basin and progressed to the corralling phase. The pulsatile nature of the sighting data suggests some form of synchrony in the migration of the adolescent whales, although the exact timing likely varies from year to year. The whales were observed to be very aware of the barrier nets and navigated the nets without difficulty.

Whale hearing tests

Five whales were corralled into the aquaculture pen and placed into the hammock. The first whale, Ba22_1606a (3.8 m, 448 kg, sex unidentified), was released 26 minutes after capture because it exhibited tonic immobility when it came into physical contact with the aquaculture pen net during the final stranding phase. The remaining four animals, which also experienced tonic immobility during the stranding phase, were subject to hearing tests.

Ba23_2606a: The whale was determined to be female (4.35 m length, 680 kg) and was held for ~90 minutes. The research team removed a net entanglement from around its maxilla; tissue had grown over the netting and the netting would have eventually cut into the maxillary bone as the animal grew, ultimately resulting in infection and likely death.

Based on prior research funded by LMR to develop acoustic stimuli that enhance the ABR, a series of frequency-uncompensated “chirps” were presented to the minke whale. The chirps ranged in duration from 125-1,000 μ s in duration and swept from 2.8-32 kHz. Additionally, some SAM tones were tested, as well as a single frequency-compensated (accounting for the response of the signal projector) chirp of 125- μ s duration. The optimal stimulus waveform for eliciting the ABR was found to be a 710- μ s uncompensated chirp. Chirps were able to elicit a measureable ABR (peak-peak amplitude of ~500 nV) with a dominant wave notable ~10 ms into the averaged EEG epoch (Figure 4A; the actual latency of the response is ~8 ms due to the acoustic travel time of the chirp.) The dominant ABR wave is likely analogous to the P4-N5 complex observed in dolphins and other small cetaceans, but the amplitude is much reduced reflecting a lack of neural specializations for echolocation and a smaller brain-to-body mass ratio (Figure 4B). The ability to reliably record the waveform set the stage for frequency-specific testing using auditory steady-state response (ASSR) methods.

Ba23_2706c: The whale was determined to be female (4.9 m length, 991 kg) and was held for approximately 30 minutes. The ABR was measured using the 710- μ s uncompensated chirp to ensure that the all equipment was working properly and confirm the ABR results from the first whale. Peaks in the frequency spectrum of the ABR waveform were used to estimate optimal rates for repetitive stimulus presentation used to produce an ASSR. Optimal rates were determined to be 200 and 600 Hz. Various waveforms with center frequencies ranging from 4-128 kHz were used to test for the presence of the ASSR. Insufficient time was available to take each frequency down to threshold (i.e. the animal was only held for 30 minutes due to decompensation), so it was determined that the frequency range of hearing should be assessed. To this end, trains of cosine-enveloped tone pips consisting of a two-cycle rise, single-cycle plateau, and two-cycle fall (2-1-2), transmitted at a repetition rate of 600 Hz were used to test for the presence of the ASSR (Figure 4C and D). All tone pips were presented at supra-threshold levels and at center frequencies of 45, 64, 90 and 128 kHz.

The results suggested that minke whales can hear at frequencies >45 kHz. However, the bandwidth of the tone pips used in the testing were too broad to define an exact upper cut-off frequency (Figure 4E). To address this ambiguity, a series of tests were conducted with bottlenose dolphins (*Tursiops truncatus*) at the US Navy Marine Mammal Program to estimate the upper-frequency limit of hearing in the minke. Dolphins were tested for the presence of the ASSR utilizing similar stimulus and recording settings and the same tone-pip center frequencies used for the minke whales. However, tests were performed in the presence of high-pass masking noise to emulate different upper-frequency limits to hearing. The masking noise consisted of pink noise with the high-pass varied in $\frac{1}{4}$ octaves. Changes in the high-pass frequency therefore emulated the possible upper-frequency limit of hearing. Comparisons of the detection of ASSRs in dolphins with conditions under which ASSRs were detected

in the minke suggested that the minke upper-frequency limit of hearing likely occurs between 45-90 kHz (Table 1).

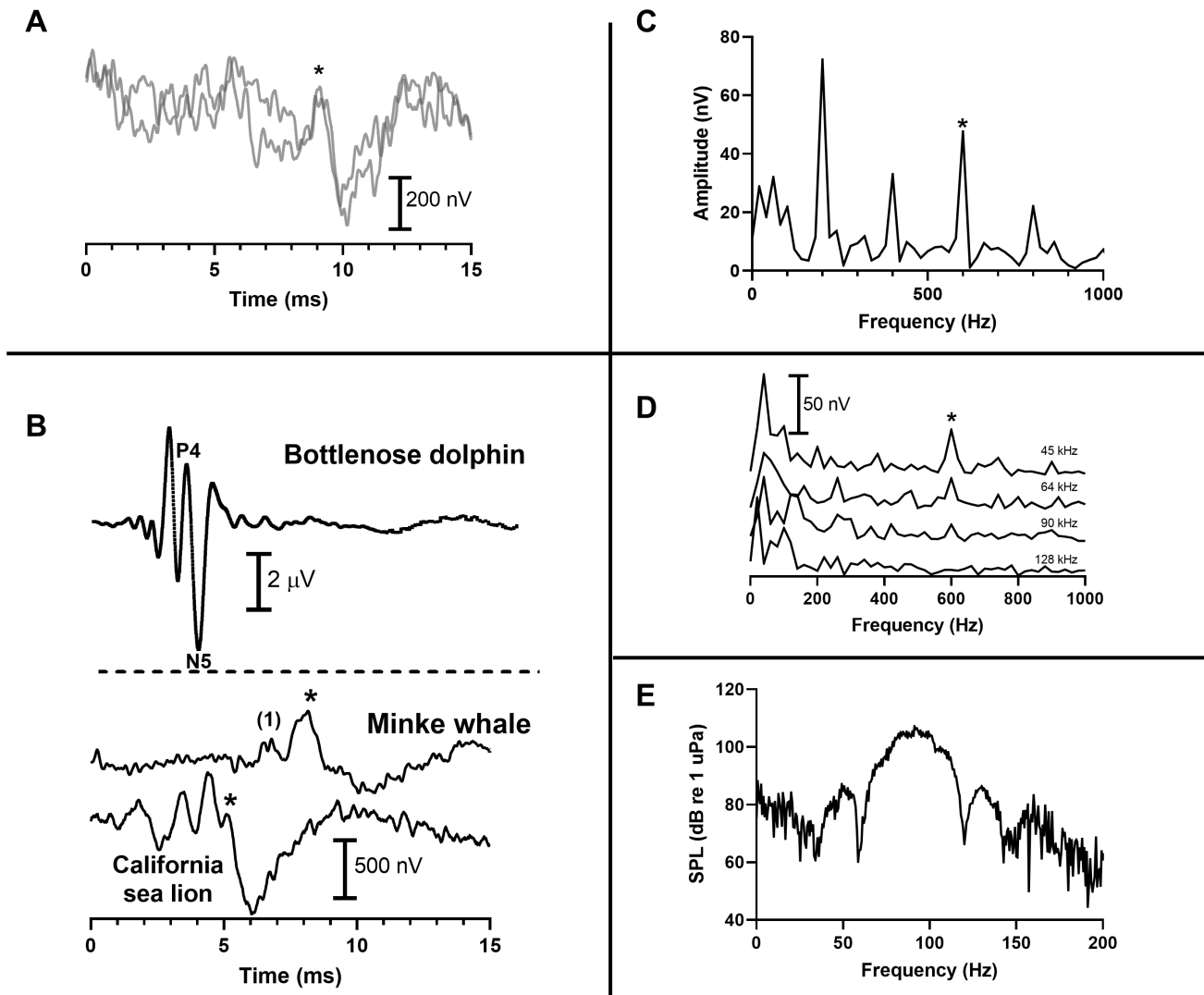


Figure 4. (Modified from Figure 3 of Houser et al. 2024) (A) Two ABR traces from the 710- μ s chirp from Ba23_2606a. The asterisk marks the beginning of the dominant ABR wave. The waveforms are offset in the vertical direction to facilitate viewing. (B) Comparison of a chirp-evoked ABR from Ba23_2706c, a California sea lion (*Zalophus californianus*), and a bottlenose dolphin. 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 pips with a center frequency (Fc) of 45 kHz. The statistically determined presence of the ASSR is denoted by an asterisk and corresponds to the 600-Hz rate at which stimuli were projected (note the spectral peaks at the harmonics and subharmonics of the 600-Hz rate.) (D) Spectra of the ASSR generated with enveloped tone pip stimuli at Fc of 45, 64, 90, and 128 kHz. Note the presence of a statistically detectable signal at 600 Hz (indicated by an asterisk), which was detected in all the ASSRs except that produced by repetitive tone pips with Fc = 128 kHz. (E) Spectrum of an enveloped tone pip with Fc = 90 kHz. Note the broad bandwidth of the signal due to its short duration.

Table 1. Detection of the ASSR to repetitive tone pips in the presence of high-pass masking noise in a trained bottlenose dolphin. Results are compared to those obtained using the same stimuli in a minke whale. Check marks indicate that the ASSR was detected, while red Xs indicate that no ASSR was detected. The high-pass frequency of the masking noise indicates the simulated upper-frequency limit of hearing.

<i>tone-pip center frequency (kHz)</i>	<i>minke whale</i>	<i>simulated bottlenose dolphin UFL (kHz)</i>				
		45	53	64	75	90
45	☑	☑	☑	☑	☑	☑
64	☑	☑	☑	☑	☑	☑
90	☑	✗	☑	☑	☑	☑
128	✗	✗	✗	✗	✗	☑

Ba24_1706a: The whale was female (4.8 m, 980 kg); however, the whale had much larger girth than prior whales so the mass is likely underestimated. The whale was tested in just under an hour. Based on results from hearing tests performed in 2023 that suggested an upper-frequency limit to hearing potentially as high as 90 kHz, the chirp used for obtaining the ABR was extended in frequency range (8-90 kHz), but the duration kept constant (710 μ s). The ABR recorded with this chirp was of similar amplitude (~700 nV) to those recorded in 2023. A SAM tone was attempted as a narrowband stimulus, but no response was observed at the highest stimulation level. Stimuli were therefore reverted back to the 2-1-2 tone pips used in 2023. Stimulus repetition rates of both 200 and 600 Hz were used to determine which might be best suited for threshold audiometry. For this whale, it was determined that the 200-Hz stimulus repetition rate was better suited and the whale was subsequently tested at frequencies from 5.6-64 kHz in half-octave steps (open circles in Figure 5). A statistical test (magnitude squared coherence) was used to objectively detect whether a response was obtained from the whale. Unfortunately, the test failed to capture marginal responses (possibly due to relative motion between whale and sound source), but this was found after the termination of the experiment.

No statistically detected response was recorded at the highest frequency tested (64 kHz), even with the stimulus at maximum amplitude. This conflicts with findings in 2023 which indicated a response at 64 kHz. However, the animal tested in 2023 (Ba23_2706c) was the smallest animal tested, whereas Ba24-1706a was the largest. Since size affects the amplitude of the AEP recorded at the surface of the animal, it is possible that the larger size of this animal impeded observation of the response. Nevertheless, the observation is likely indicative of a roll-off occurring around 64 kHz. The lowest threshold was at $F_c=32$ kHz, which could suggest that minke whales are most sensitive to this frequency.

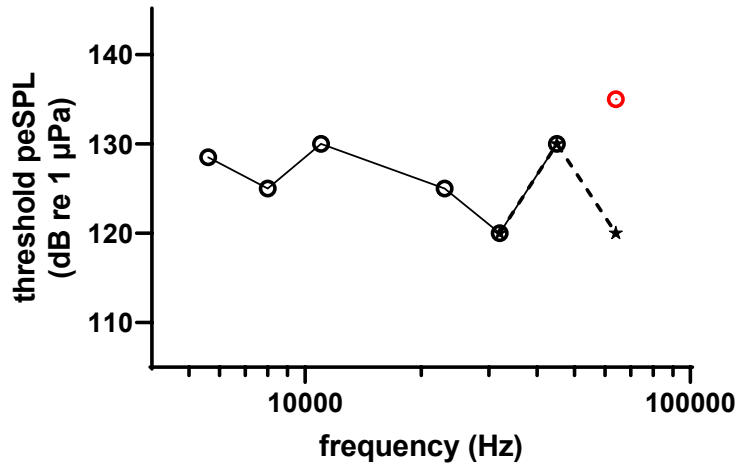


Figure 5. AEP thresholds for whales Ba24_1706a (open circles) and Ba24-2206a (stars). The red, open circle indicates that no response was obtained at the highest stimulus levels that could be generated.

Ba24-2206a: The whale was male (4.4 m, 734 kg) and was held just under an hour. Based on the findings with Ba24-1706a, the chirp stimulus was changed to a frequency range of 5.6-64 kHz (duration was kept constant). Although the whale was smaller than Ba24-1706a, the amplitude of the chirp-evoked ABR was smaller (~470 nV). The initial attempt at making threshold measurements used a 200-Hz stimulus repetition rate and tested at 45 and 64 kHz. Responses were measured at 45 kHz, but not at 64 kHz, even at the maximum stimulus level. The stimulus repetition rate was then changed to 600 Hz to see if a better response could be gotten from the whale and frequencies of 32, 45 and 64 kHz tested. All frequencies tested had an observable response; unfortunately, the whale had to be released before additional testing could occur and no other series were completed. The 32- and 45-kHz results were consistent with the higher-frequency results obtained, although the 64-kHz response was observable in this smaller animal, possibly due to its smaller body size.

The thresholds in both whales were higher than those typically reported for marine mammals, likely as a result of the poor brain-to-body mass ratio, but also due to the fact that AEP thresholds are typically higher than behavioral thresholds (by as much as 25 dB in humans and dolphins). The relatively small amount of threshold variability between 5.6 and 45 kHz suggests this region might constitute at least a portion of the flat region of sensitivity within the audiogram that correlates with the region of best hearing sensitivity (i.e., the thresholds are within 20 dB of the lowest threshold).

Animal welfare

Animal welfare was a high priority of this research project. All whales that were corralled and tested were monitored for respiration rate and heart rate. In addition, blood samples were taken opportunistically and the blood processed on site with an IStat blood analyzer to monitor for changes in blood chemistry that might reflect animal decompensation due to handling. Finally, a satellite tag was attached to each whale to monitor its swimming and diving behavior following release (see **Satellite tagging**).

The first animal corralled into the hammock exhibited tonic immobility once it physically contacted the aquaculture pen net. All subsequent animals exhibited the same behavior. In each case, the whale sank into the net, which was then pulled to the surface by the research team. All whales began

spontaneous breathing once brought to the surface. The whales differed in their tolerance to handling with handling times ranging from roughly 30 to 90 minutes. Termination of the hearing test and release of the animal was determined during the session by monitoring health metrics (respiration rate, heart rate, blood values) and behaviors associated with decompression (e.g., arching, mouth gaping).

Table 2. Health parameters monitored in minke whales during the AEP hearing tests. (ND=not detected)

	Ba22_1706g	Ba23_2606a	Ba23_2606c	Ba24_1706a	Ba24_2206A
HR (/min)	16 to 38	39 to 61	ND	low 20s to high 50s	low teens to high 30s
Lactate (mmol/L)	1.8	1.2 to 2.1	2.0	0.7 to 1.1	<0.3 to 1.8
Na (mmol/L)	ND	145	151	145	143
K (mmol/L)	ND	3.6	3.6	3.4	4.3
iCa (mmol/L)	ND	0.99	1.32	1.18	1.15
Glucose (mg/dL)	ND	148	214	155	142
HCT (%)	ND	44	41	33	48
Hb (g/dL)	ND	15.0	13.9	11.2	16.3
pH	ND	7.741	7.353	7.528	7.556
pCO2 (mmHg)	ND	22.2	70.7	48.5	39.3
pO2 (mmHg)	ND	185	70	122	129
sO2 (%)	ND	100	92	99	99
BE (mmol/L)	ND	11	14	18	13
TCO2 (mmol/L)	ND	31	41	42	36
HCO3- (mmol/L)	ND	30.2	39.3	40.3	34.8

Blood samples for the four animals suggested that the whales remained stable throughout testing, even in the presence of outward signs of decompression (Table 2). The only parameter that was deemed high was blood glucose, but the moderate elevation was consistent with an increase in circulating cortisol due to handling stress. Whale heart rates were consistent with what has been seen in stranded minke whales and showed good splits between breath-hold bradycardia and inhalation tachycardia.

Satellite tagging

Each of the four animals for which hearing tests were performed had a satellite tag (Splash10-397A) attached its dorsal fin prior to release using either a single or three-pin attachment. Whales B24_1706a and B24_2206a additionally had a CATS Cam suction cup-mounted tag placed on their dorsal surface prior to release. The CATS tag fell off both animals prior to their departure from the fish farm, but provided some interesting “animal” perspectives and observations of sensory hair orientation during free swimming. Data from the Splash tags indicated that all whales returned to normal activity following release from the aquaculture pen (Figure 6).

The first animal tagged (Ba23_2606a) traveled into the Atlantic following release, and then over the north of Norway and into the south Barents Sea where it foraged near the coast of Yuzhny Island in Russian territorial waters. The satellite tag operated for two months (26 June – 29 August).

The second animal tagged (Ba23_2706c) traveled across the fjord following release and appeared to forage in the region near Bodø. The tag failed after only two weeks of deployment (28 June – 12 July).

The third animal tagged (Ba24_1706a) headed north where it apparently foraged for several weeks south of Svalbard, and then headed east into the north Barents Sea where it continued to forage for another several weeks. In mid-November, the whale began its southward migration. The last satellite location obtained from the animal was on 14 December and showed the whale in the region of the Azores. The tag transmitted for nearly six months (17 June – 14 December).

The fourth animal tagged (Ba24_2206a) traveled north of Norway where it apparently foraged in deep waters of the Atlantic with an extended excursion into shallower waters of the south Barents Sea. The whale appeared to start heading south at the time that its satellite tag ceased transmitting. The tag transmitted for four months (22 June – 23 October).

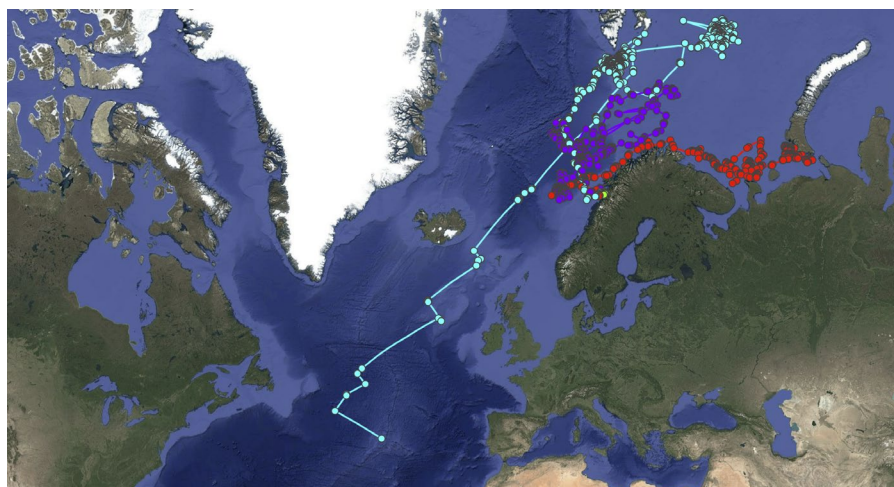


Figure 6. Post-release satellite tracks of minke whales subject to AEP hearing tests (red=Ba23_2606a, green=Ba23_2706c, light blue=Ba24_1706a, purple=Ba24_2206a).

Publications/Presentations

Kleivane L, Kvadsheim PH, Pyne Vinje AV, Mulsow J, Ølberg RA, Teilmann J, Harms C, Houser D (2024) Capture and release of minke whales offers new research opportunities. *Aquatic Mammals* 50 (4):352-368. <https://doi.org/10.1578/AM.50.4.2024.352>

Houser DS, Kvadsheim PH, Kleivane L, Mulsow J, Ølberg RA, Harms CA, Teilmann J, Finneran JJ (2024) Direct hearing measurements in a baleen whale suggest ultrasonic sensitivity. *Science* 386 (6724):902-906. <https://doi.org/doi:10.1126/science.ado7580>

Additional papers planned for 2025 and beyond:

1. Thresholds of hearing sensitivity in the common minke whale
2. Tonic immobility associated with capture stress in the common minke whale
3. Health and welfare indices of minke whales temporarily caught for hearing assessments
4. Migratory behavior of minke whales as determined through satellite telemetry

The results of this study were presented at the Effects of Sound in the Ocean on Marine Mammals (ESOMM) meeting in Den Haag, the Netherlands (September 2024), and at the 25th Biennial Conference on the Biology of Marine Mammals held in Perth, Australia (November 2024). Additional presentations are planned for the 7th International Conference on the Effects of Noise on Aquatic Life,

the Acoustical Society of America semi-annual meeting, and the 29th International Evoked Response Audiometry Study Group Symposium. All conferences are planned for 2025.

Discussion

The minke AEP hearing data obtained in this study provide the first direct threshold measurements in any mysticete whale. The data suggest that minke whales can hear frequencies higher than 45 kHz, and that the upper-frequency limit of hearing is probably close to 64 kHz. This is higher than anatomical predictions based on cochlear frequency maps, which suggested that the upper-frequency limit of hearing was likely an octave lower (i.e., ~32 kHz). This information should be useful in understanding where anatomical predictions are errant, hopefully contributing to improvement in model design and prediction accuracy. The AEP hearing curve covers the frequency range from 5.6 to 64 kHz, and the frequency of greatest sensitivity appears to be at 32 kHz. However, the limited variability in thresholds across the measured frequency range suggests that the frequency range likely represents at least a portion of the best region of hearing sensitivity for this species (i.e., a range of frequencies with thresholds within 20 dB of the lowest threshold in the audiogram).

The thresholds are high in comparison to most reported for marine mammals due to the method of testing and the distance from the brain to the surface of the animal. Given known differences between AEP thresholds and behavioral hearing thresholds in humans and delphinids, a correction for the AEP estimate could potentially be applied to the hearing thresholds. Provided the loss of signal as a function of distance between evoked response source and the surface location recording site can be estimated, an additional correction should be calculable. Collectively, these could provide an approximation of the behavioral audiogram, which would provide regulators, scientists, and action proponents concerned with the potential impact of sound on mysticetes information necessary to improve mitigation measures and environmental assessments.

The finding of a greater frequency range of hearing should be useful in adjusting the auditory weighting function for the low-frequency (LF) hearing group established in Navy environmental impact analyses. The LF weighting function was created through consideration of anatomical modeling information and extrapolations from other non-mysticete, marine mammal species. The information gathered as part of this study provides empirical evidence for increasing the upper-frequency limit of the function. Furthermore, by knowing the upper-frequency limit of hearing, action proponents and regulators can make a more accurate *de minimis* source selection, i.e., they can determine the sources of most concern based on the frequencies at which they operate when environmental assessments are initiated.

The establishment of the CARS and the method of catching minke whales is likely directly relevant only to this species because of the unique overlap of the minke whale's migratory behavior and the large number of small coastal islands scattered along the migratory path near the coast of Norway. However, techniques developed for the minke hearing tests will facilitate future mysticete hearing tests (e.g., at stranding events/planned captures). For example, it is now known that chirps are likely to be the best option for obtaining the ABR, which is a critical first step in moving toward frequency-specific hearing tests. Ideally, other mysticete species will be tested in the future. As there is substantial variation in size and vocal frequency range of the mysticetes, it cannot be assumed that minke whales are representative of all mysticetes, particularly those that communicate at frequencies in the tens of Hz (e.g., blue and fin whales). More detailed hearing information coupled with what is known regarding the acoustic ecology and auditory anatomy of the mysticete species could go a long

way toward determining whether the LF hearing group established by the Navy should be further broken down into LF and very low-frequency (VLF) hearing groups.