

Baseline hearing measurements in Alaskan belugas

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LONG-TERM GOALS

While hearing is the primary sensory modality for odontocetes, there are few data addressing baseline hearing and subsequent variation within a natural population. To determine the effects of noise on marine mammals we need to understand what they hear. This is imperative in the Arctic where there is both an increase in human activity and a concurrent increase in human-produced noise. This work examines the hearing sensitivity and variability of wild beluga whales in an effort to understand how belugas may be impacted by noise. A standard audiogram was determined from the wild animals, noting the variation between animals and the audiogram of maximal and minimal sensitivity. These novel data were compared to available hearing results from captive belugas, evaluating differences and variation within the two data sets. The hearing curves were appraised relative to basic demographic meta-data from the animals from which the measurements were made. Through these data analyses we sought to: 1) define the natural and baseline hearing abilities and variability in belugas, 2) place the results in the context of potential ecological influences and that of anthropogenic noise, and 3) evaluate the validity of captive-based hearing data in relation to wild animals. This is part of a larger effort to understand variation in the sensory biology and noise susceptibility of diverse odontocete species.

OBJECTIVES

Evaluate the audiograms of temporarily captured wild belugas from Bristol Bay, substantially increasing the sample size and consequent knowledge of how this protected species naturally detects and utilizes sound.

- 1a) Identify a standard beluga audiogram and evoked potential waveform, and their variances, from temporarily captured wild belugas from Bristol Bay and examine the individual audiograms relative to demographic and health-related meta-data.

1b) Compare these audiograms with published audiograms and new data from captive experiments to evaluate the fidelity of summing captive and wild beluga hearing data and establish a baseline comparison for captive auditory research. Also place the beluga audiogram variability in the context of other measured odontocetes populations.

APPROACH

This work sought to determine hearing sensitivity in wild and presumably healthy beluga whales, using consistent AEP methods. A primary emphasis here was placed on assessing the frequency and sensitivity ranges, as well as the audiogram variability of the measured animals.

Auditory evoked potential data were collected during a planned capture-release field project in Bristol Bay, AK. The project was run by the National Marine Mammal Laboratory of the Alaska Fisheries Science Center (NMFS Marine mammal research permit #14245), Alaska Department of Fish and Game, the Georgia Aquarium and the Alaska Sealife Center. The work involved temporarily capturing 9 beluga whales during September 1-13, 2012. Hearing abilities were measured for 7 (2 were caught prior to our arrival) restrained animals using auditory evoked potential (AEP) methods (Fig 1). Representatives from NMML, ADF&G, Georgia Aquarium and others were simultaneously acquiring additional physical health measurements (e.g., blood chemistry, stress measures), genetic samples, ultrasound images and fitting the animals with satellite tags.



Figure 1. (A) Beluga #1 during an auditory evoked potential (AEP) hearing experimental session. The whale is facing right. The three suction-cup attached sensors (right to left are active sensor, invert sensor and ground) are visible. (B) The AEP equipment being operated in its ruggedized case in the small inflatable boat while the whale is positioned adjacent to left (not visible).

The audiograms were collected using a custom-built AEP system and software which is well established for physiological hearing tests (Mooney et al. 2008) including field measures (Mooney et al. 2009; Mooney et al. 2011a). Auditory sensitivity was measured in octaves and half-octaves from 1-180 kHz (1, 2, 3, 4, 5.6, 8, 11.2, 16, 22.5, 32, 45, 54, 80, 100, 128, 150, 180 kHz) using SAM tone stimuli (except for 1-3 kHz which used 6 cycle tone pips). Sounds were presented using a suction-cup jawphone placed on the rostrum tip of the lower jaw (Mooney et al. 2008; Mooney et al. 2011b). Hearing thresholds were determined statistically using linear regression methods.

WORK COMPLETED

Audiograms were collected on all 7 belugas using AEP methods (Fig 1). “Full audiograms,” consisting of at least 7 frequencies ranging from 4-128 kHz, were tested on all animals. For one animal, lower frequencies of 1-4 kHz were also examined using tone pips.

The data collection was very successful. The goal of this project was to obtain measurements and analyses from 6 animals; thus, with 7 animals we exceeded this target. We are extremely pleased that full audiograms were recorded from all animals. These are the first audiograms of wild belugas and the first population evaluation of hearing in an odontocete species other than bottlenose dolphin (*Tursiops truncatus*). Seven full audiograms increases the current beluga published audiogram sample size by 233% and provides the only hearing data for wild belugas. We expect these data will greatly enhance our knowledge of beluga hearing and wild odontocetes in general. Background noise levels were also collected in Bristol Bay, the beluga habitat.

Physiological noise levels were quantified for each animal by calculating the rms value for a 16 ms window for five AEP records for each animal. Background acoustic noise was recorded using a DSG-Ocean acoustic data-logger with a HTI-96-Min hydrophone with a -185.8 dB re 1V/ μ PA receiving sensitivity and frequency response of ± 1 dB from 2 Hz to 40 kHz.

We analyzed and presented variance and sensitivity curves in multiple ways, including presenting means, SD's, max and min sensitivities, overall curves for all animals (grouped and ungrouped), variance relative to frequency, a best-fit polynomial-based threshold and comparisons to captive animal data. The results have been presented at the 3rd International Meeting of the The Effects of Noise on Aquatic Life and are scheduled for oral presentation at the 20th Biennial Conference on the Biology of Marine Mammals in December 2013. A book chapter and an initial paper have both been submitted for publication.

RESULTS

Our system to measure AEP responses was quite robust for establishing the audiograms of wild belugas. Envelope following responses were typically quite distinct from the background electrophysiological noise even though we used a limited number of sweeps per record. Thresholds were collected in approximately 3-5 min in order to minimize overall handling time of the animals. A mean of 9 (± 2.4 s.d., range 5-12) and a median of 10 thresholds were obtained per animal. It took an average of 45 min (range 31-55) to record the audiograms shown in Figure 2. The number of thresholds obtained were not correlated with the duration of the effort ($r^2 = 0.17$; $p > 0.5$) because recordings were often paused as the animal was repositioned, relocated to adjust for the tide, to move electrodes or while another sample type was obtained. Thus, 36-38 min was a good assessment of how quickly the procedure could be accomplished in these particular environmental and contextual conditions.

All audiograms had a general U-shape typical of mammals and odontocetes with a steeper slope at the high frequency cut-offs, and a more gradual increase in thresholds at the lower range of hearing (Fig. 2). Audiograms were grouped based on similar curves, curves of greater and the animal with the lowest average threshold. An overall mean audiogram (\pm s.d.) was calculated (Fig. 3A). Two composite audiograms were created using the highest and lowest thresholds for each frequency (Fig. 3B). The standard deviation (sd) difference of thresholds at measured hearing frequencies and fitted power trend

line showed an increase with frequency. A fitted power function showed that half of the variation ($r_2 = 0.52$) was explained by the increase (Fig. 3C). A best-fit fourth order polynomial was fit to the threshold data (Fig. 3D) to characterize a general audiometric curve and provide a view of the associated variability. This metric provided a composite audiogram that was less influenced by variability at certain frequencies (as found in the mean of seven animals) and may provide a valuable way to identify the general hearing abilities of a population.

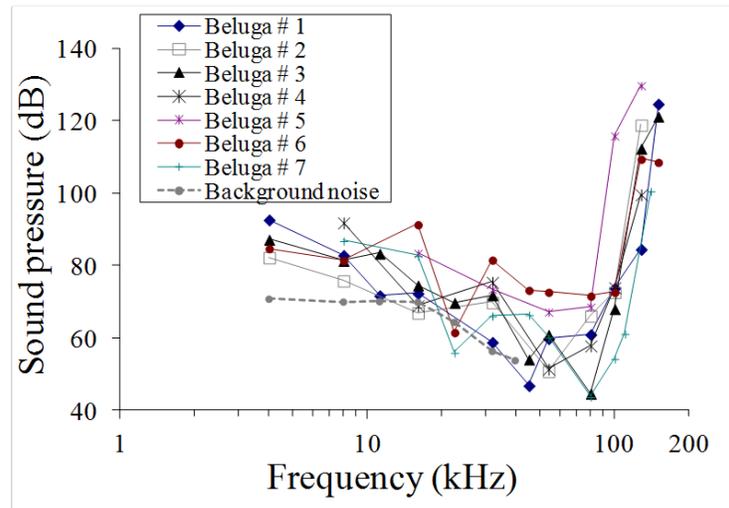


Figure 2. AEP audiograms of all seven wild belugas and Bristol Bay background noise spectrum. Sound pressure levels are in dB re 1 μ Pa.

The mean audiogram of the wild belugas from this study was compared to those of laboratory animals from other studies (Fig. 4). In general the mean audiogram of the wild animals fell within the spread of those from laboratory animals, although those belugas often had more sensitive hearing at many frequencies. The wild animals tested here heard comparatively well at higher frequencies, including demonstrated responses at 140 and 150 kHz, which is the highest recorded frequency range for beluga whales.

The four males (belugas #2, #4, #5 and #7) had upper hearing limits of 128 kHz and 140 kHz, compared to the three females which all heard up to 150 kHz. Females also had lower thresholds at 128 kHz. Otherwise, there were no substantial male-female differences.

The mean audiogram of wild belugas showed a wide range of sensitive hearing, from 22 to 110 kHz and minimum detection levels near 60 dB. The mean thresholds showed an audiogram shape similar to other odontocetes and beluga (Fig. 3A, 4). Best thresholds were from 22.5-80 kHz with the absolute lowest between 45 and 80 kHz, and 59-60 dB. There were differences in hearing among animals that was often > 20 dB (Fig. 3B). The greatest differences in hearing abilities occurred at the high end of the auditory range with 45 dB differences between two individuals at 128 kHz. The mean difference between maximum and minimum thresholds across all frequencies was 21.8 dB (19.5 dB when not including 128 kHz). Lowest mean thresholds were between 45 and 80 kHz with average thresholds of 60.3, 60.6 and 59.2 dB at 45, 54 and 80 kHz, respectively. Greatest s.d. values were at the highest frequency (128 kHz) and frequencies of maximum sensitivity (54 and 80 kHz).

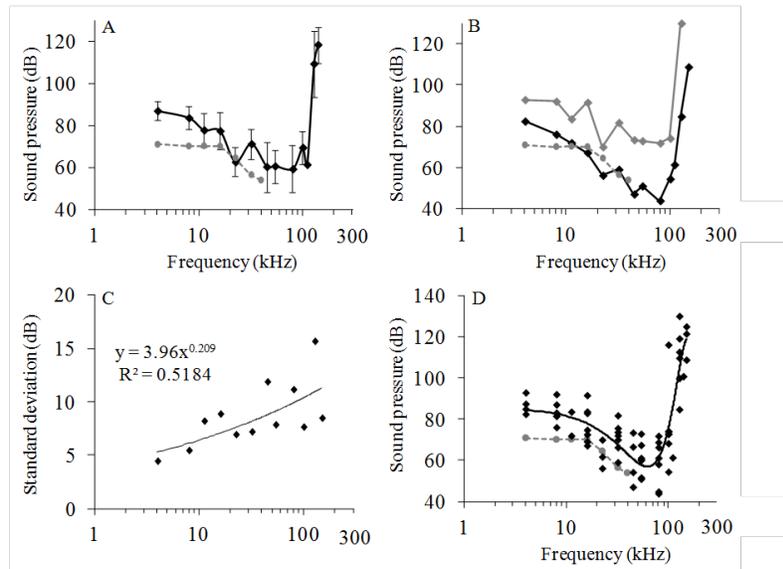


Figure 3. (A) The mean audiogram \pm s.d and Bristol Bay background noise spectrum (grey dashed line). (B) Composite audiograms constructed by plotting the thresholds of maximum (black, diamonds) and minimum sensitivity (grey triangles) and Bristol Bay background noise spectrum (grey dashed line). (C) The standard deviation (sd) difference of thresholds at measured hearing frequencies and fitted power trend line. Sd values increased with frequency. Sound pressure levels are in dB re 1 μ Pa. (D) Fourth order polynomial trend curve ($y = -1E-06x^4 + 0.0003x^3 - 0.0168x^2 - 0.2966x + 85.832$; $R^2 = 0.6919$) for all collected thresholds and frequencies and Bristol Bay background noise spectrum (grey dashed line).

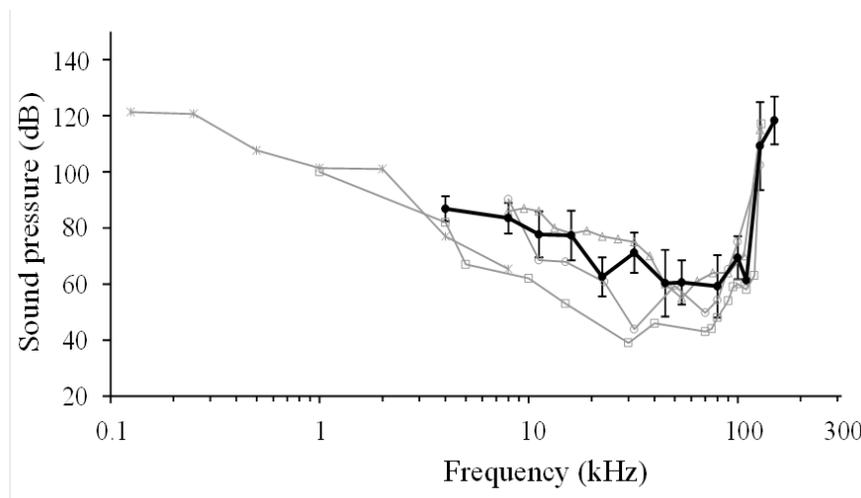


Figure 4. Mean wild beluga audiogram \pm s.d. (black, circles) compared to the audiograms (grey and/or open symbols) from belugas held in laboratory conditions or in aquaria. Other audiograms include: (White et al., 1978)-squares, (Awbrey et al., 1988)-stars, -circles, (Klishin et al., 2000)-triangles, (Finneran et al., 2005)-x's, (Ridgway et al. 2001)-dashes. The audiogram (x shapes) which cuts off near 50 kHz was considered a result of aminoglycoside antibiotic treatment. All other audiograms are similar to the wild belugas. Sound pressure levels are in dB re 1 μ Pa.

IMPACT/APPLICATIONS

Overall, these animals heard well in the upper frequencies. Based on the size of some animals, we assume that not all animals were very young. Thus, there appeared to be little sensorineural high-frequency hearing loss associated with age (i.e., presbycusis). Presbycusis in cetaceans has been documented in older bottlenose dolphins and suggested in a false killer whale; hearing loss has also been related to antibiotic treatment in belugas (Finneran et al. 2005). Why these belugas demonstrated generally good high-frequency hearing, and whether this trend would continue in other beluga or other wild populations, is uncertain. This result further supports the need for larger sample sizes.

Except for the relatively small differences in the upper auditory limit, there was little difference between female (n =3) and male (n=4) audiograms. Overall Bristol Bay belugas are a good subject population for approximating the baseline hearing for comparison with other populations inhabiting other regions impacted by anthropogenic noise. Increasing our sample size of wild belugas will be necessary to determine which to conclude.

One way to examine beluga hearing variability is to compare these audiograms with hearing measured in other belugas (Fig. 4). The hearing sensitivities reported here fall within those previously described for laboratory belugas. Results from White et al. (1978) and Aubrey et al. (1988), obtained through psychophysical methods, show slightly lower thresholds across all tested frequencies. This difference between White et al., and this study may be methodological, as psychophysical-based methods used by White et al., (1978) generally yield lower thresholds (in the order of 8-12 dB) than AEP based results in other odontocete species (Szymanski et al. 1999; Yuen et al. 2005) as well as in pinnipeds (Mulsow and Reichmuth 2010). On average, hearing thresholds from the beluga studied by Klishin et al., (2000) and the one studied by Mooney et al., (2008) fall within our observed variability in wild belugas. Only two frequencies show considerable differences. The threshold reported by Mooney et al., (2008) for 32 kHz is 27.3 dB lower than the average value from our 7 wild belugas (71.1 dB re 1 μ Pa). The threshold reported by Klishin et al., (2000) for 22.5 kHz is 14.5 dB higher than the average value from our 7 wild belugas (62.5 dB re 1 μ Pa). These small audiogram differences likely reflect individual variability in hearing. Only one animal differed substantially across these comparisons and it is suspected that this beluga's hearing loss was a result of aminoglycoside antibiotic treatment (Finneran et al. 2005).

This method to measure odontocete hearing during a capture-and-release health assessment project was successful. The success of this challenging methodology supports further efforts to gather hearing and related data during capture-release events at Bristol Bay and elsewhere. Similar hearing data were also collected from wild bottlenose dolphins during capture events (Cook and Mann 2004), however these tests did not measure the full range of odontocete hearing. Thus, these full audiograms for seven wild, healthy beluga whales provide a unique data set for odontocetes. This study has contributed to odontocete hearing in several respects. First, a wild population was sampled in a relatively non-invasive manner in that belugas were held for short periods and released. Second, the results provide full audiograms documenting the hearing of wild individuals. Previously, beluga hearing limits came from six animals held in enclosed facilities for extended periods of time, where they had received medical treatment and had been exposed to different noise environments Third, these wild-caught individuals were healthy based on preliminary results from the concurrent health assessment project. Hearing measured in stranded cetaceans provides a rare opportunity to obtain hearing information, however, it is uncertain how it compares to wild healthy animals. Lastly, this study provided data for multiple belugas of different sexes and ages from the same population.

In view of the expected increases in sound levels as human activities increase in the Arctic, expanding our knowledge of beluga hearing is key to an appropriate conservation management effort. One of the five distinct stocks of beluga whales that are currently recognized in U.S. waters, the Cook Inlet beluga population, is endangered and recovery efforts are being identified. The impact of anthropogenic noise has been identified as a serious potential threat and possible contributor to the lack of its recover (NMFS 2008). Similarly, there has been no noticeable recovery for the threatened St. Lawrence beluga population and anthropogenic noise has been identified as one of the main threats (DFO 2012). In contrast, the Bristol Bay beluga population is increasing and is considered healthy (NMFS 2008). While the Bristol Bay acoustic environment is not pristine, anthropogenic noise is more seasonal and less intense than that of Cook Inlet. Therefore, Bristol Bay belugas are a good subject population for approximating the baseline hearing for comparison with other populations inhabiting other regions impacted by anthropogenic noise. We hope that the results presented here will encourage sampling of wild cetaceans and further the understanding of the potential effects of anthropogenic noise on belugas and other odontocetes.

RELATED PROJECTS

- 1) Beluga tagging Health Assessment- NMML/Georgia Aquarium. Collaborators: Alaska Department of Fish and Game, Alaska Sealife Center, Bristol Bay Native Association, Alaska Pacific Univeristy.

Beluga captures were made for this health assessment project. The hearing study presented here was incorporated as part of the health status for the first time this season.

- 2) Satellite-linked depth-recording LIMPET tag testing in Bristol Bay Belugas – Alaska Sealife Center.

Five temporarily restrained belugas for the health assessment project were instrumented with LIMPET tags with a pneumatic gun to test the viability of this tagging method and the duration of the tag transmission.

- 3) Quantifying masked hearing in belugas with anthropogenic noise from Cook Inlet. NOAA Ocean Acoustics Program.

This proposal aims to collect AEPs to document baseline hearing and masked hearing thresholds of captive belugas when exposed to anthropogenic noise, in different angles, recorded in Cook Inlet beluga critical habitat.

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1. Castellote, M, Mooney, TA, Hobbs, R, Quackenbush, L, Goetz, C, Gaglione, E. 201X. Baseline hearing abilities and variability in wild beluga whales (*Delphinapterus leucas*). *Journal of Experimental Biology* [in revision].
2. Mooney, TA, Castellote, M, Hobbs, R, Quackenbush, L, Goetz, C, Gaglione, E. 201X. Hearing in wild belugas relative to levels of background noise. *The Effects of Noise on Aquatic Life*. Popper, A, Hawkins A (eds). Springer Science+Business Media, LLC, New York. [in press]

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