Baseline hearing abilities and variability in wild beluga whales (*Delphinapterus leucas*)

Manuel Castellote[1,2*], T. Aran Mooney[3*$], Lori Quakenbush[4], Roderick Hobbs[1], Caroline Goertz[5], Eric Gaglione[6]

[1]National Marine Mammal Laboratory, Alaska Fisheries Science Center, National Marine Fisheries Service, Seattle, WA 98115
[2]North Gulf Oceanic Society, Homer, AK 99603, USA.
[3]Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA
[4]Alaska Department of Fish and Game, 1300 College Road, Fairbanks, AK. 99701, USA
[5]Alaska SeaLife Center, Seward, AK 99664, USA
[6]Georgia Aquarium, 225 Baker St NW, Atlanta, GA 30313, USA

[*]These authors contributed equally to this work

Running title: Hearing variability in wild belugas

Key words: noise, marine mammal, cetacean, odontocete, arctic

[$]Corresponding author: amooney@whoi.edu; 508-289-3714

SUMMARY

While hearing is the primary sensory modality for odontocetes, there are few data addressing variation within a natural population. This work describes the hearing ranges (4-150 kHz) and sensitivities of seven apparently healthy, wild beluga whales (*Delphinapterus leucas*) during a population health assessment project that captured and released belugas in Bristol Bay, Alaska. The baseline hearing abilities and subsequent variations are addressed. Hearing was measured using auditory evoked potentials (AEPs). All audiograms showed a typical cetacean U-shape; substantial variation (>30 dB) was found between most and least sensitive thresholds. All animals heard well, up to at least 128 kHz. Two heard up to 150 kHz. Lowest auditory thresholds, 35-45 dB, were identified in the range 45-80 kHz. Greatest differences in hearing abilities occurred at both the high end of the auditory range and at frequencies of maximum sensitivity. In general, wild beluga hearing was quite sensitive. Hearing abilities were similar to belugas measured in zoological settings, reinforcing the comparative importance of both settings. The relative degree of variability across the wild belugas suggests that audiograms from multiple individuals are needed to properly describe the maximum sensitivity and population variance for odontocetes. Hearing measures were easily incorporated into field-based settings. This detailed examination of hearing abilities in wild Bristol Bay belugas provides a basis for a better understanding of the potential impact of anthropogenic noise on a noise-sensitive species. Such information may help design noise limiting mitigation measures that could be applied to areas heavily influenced and inhabited by endangered belugas.

INTRODUCTION

Beluga whales (*Delphinapterus leucas*) are often found in turbid, coastal waters in northern latitudes where darkness can extend for many months. They depend upon sound for many important biological functions such as foraging, navigation and communication, and they are considered to have sophisticated hearing and echolocation abilities (e.g., Ridgway et al., 2001; Turl et al., 1987). Their diverse vocal repertoire has often led them to be referred to as "canaries of the sea." Hearing studies of belugas held in laboratory settings have generally shown sensitive and broadband hearing abilities, similar to other odontocetes (Awbrey et al., 1988; Finneran et al., 2005a; Finneran et al., 2002a; Finneran et al., 2002b; Klishin et al., 2000; Mooney et al., 2008; Ridgway et al., 2001). Yet, it is unclear how these hearing abilities compare to those of wild belugas (or any odontocete). Measurements from multiple wild individuals are needed to truly evaluate what a species may hear and variations found between individuals.

With a wide distribution in the Arctic and subarctic, and as near apex predators with a complex social structure and acoustic ecology, belugas can serve as an effective sentinel of the ecosystems in which they live (Moore and Huntington, 2008). Changes in sea ice due to climate warming may affect beluga whales directly, with reductions in sea ice and related effects on prey, and by indirect increased industrial activity (e.g., shipping, oil and gas exploration) with less ice to restrict that activity; with the increase in human activity comes an increase in ocean noise.

Because both hearing and sound production are important to belugas, changes in background noise levels due to human activities may have a large impact on their ability to carry out vital activities. Anthropogenic ocean noise is believed to be a chronic, habitat-level stressor (Ellison et al., 2012) and there is special concern for Arctic ecosystems (Moore et al., 2012; Southall et al., 2007). The increase in human activities now allowed by less sea ice is increasing ocean noise in the Arctic, including areas that have been acoustically pristine (Moore et al., 2012). Although the biological consequences of elevated ambient noise are not well understood, there is sufficient evidence to suggest that at some threshold noise could negatively affect sound-dependent marine mammals (National Academy of Sciences, 2005; Richardson et al., 1995; Tyack and Clark, 2000). Therefore, understanding how noise might affect beluga sensory ecology is important to address the potential impacts of increased noise within the Arctic.

To determine the effects of noise on marine mammals it is vital to understand what they hear. There are few studies evaluating the auditory frequencies and sensitivities of most species of marine mammals, and even fewer that address variability within a population (Gerstein et al., 1999; Houser and Finneran, 2006b; Mooney et al., 2012a; Nachtigall et al., 2007b; Nachtigall et al., 2005). Approximately 20 species of cetaceans and pinnipeds have been tested, representing about 10% of all marine mammals. Most of what is known about odontocete hearing has come from individuals born or maintained in aquaria or laboratories for many years (Nachtigall et al., 2000). Few wild odontocetes have been studied and the ones that have are typically stranded due to health-related issues that could affect hearing (Andre et al., 2003; Finneran et al., 2009; Mann et al., 2010; Nachtigall et al., 2008; Nachtigall et al., 2005; Pacini et al., 2010; Pacini et al., 2011). The auditory abilities of captive or stranded odontocetes may be robust as examples of species-specific hearing but the only way to test this assumption is to compare captive to wild, healthy animals. Capture and release of wild odontocetes to study hearing has rarely been undertaken primarily because the equipment used to measure hearing has not been portable or rugged enough for reliable use under field conditions and because animals are seldom captured for short time periods. Recent advances in portable auditory evoked potential (AEP) equipment and techniques have allowed this method to be used with dolphins that were captured and temporarily restrained (Mooney et al., 2009b; Nachtigall et al., 2008).

The AEP method tests hearing using rapid neurophysiological responses to stimuli and has been used for a variety of taxa including terrestrial mammals (Dolphin and Mountain, 1992), birds (Brittan-Powell et al., 2002), fishes (Kenyon et al., 1998), reptiles (Bartol et al., 1999) and invertebrates (Mooney et al., 2010). It is well established and now used extensively in odontocetes (see reviews Mooney et al., 2012b; Nachtigall et al., 2007a). In odontocetes, neurophysiological responses to acoustic stimuli can be measured non-invasively from the surface of the skin. The ability to capture and release wild whales and test their hearing using the non-invasive AEP technique provides a method for sampling enough individuals to begin to describe hearing abilities at the population level. This addresses a recommendation of the U.S. National Research Council (National Academy of Sciences, 2003; National Academy of Sciences, 2005) that population level audiograms be obtained in order to discover population audiometrics and to determine normal variability in the hearing sensitivity for marine mammals.

Beluga whale hearing is among the best of all odontocetes (Erbe, 2000; Erbe and Farmer, 1998; Finneran et al., 2000; Johnson, 1991; Schlundt et al., 2000). Hearing sensitivity has been assessed in numerous published works (Awbrey et al., 1988; Finneran et al., 2005a; Finneran et al., 2002a; Finneran et al., 2002b; Klishin et al., 2000; Mooney et al., 2008; Ridgway et al., 2001) and one non-peer-reviewed study (White et al., 1978). However, these investigations are difficult to compare because methods or study designs have varied and samples sizes are limited. For example, one study focused only on lower frequencies (Awbrey et al., 1988), while in another hearing thresholds were elevated (n=1; Klishin et al., 2000). A third study found hearing loss was attributed to a side effect of antibiotic treatment (Finneran et al., 2005a). Most studies were limited to one beluga. Some tests involved behavioral conditioning responses (Awbrey et al., 1988; Finneran et al., 2005a; Ridgway et al., 2001; White et al., 1978) whereas others used AEP methods (Klishin et al., 2000; Mooney et al., 2008). It is clear that audiograms may vary due to a number of factors including sex, age, genetics, prior history of chemical or noise exposures, physiological or behavioral metrics, threshold evaluation methods, subject stress level, environmental test conditions and others (Burkhard et al., 2007; Webster et al., 1992; Yost, 1994). For belugas many of these factors have varied. Thus, it is often unclear whether differences in individual hearing abilities discrepancies are a result of methodological discrepancies or actual auditory differences (or both). Further, none of these studies examined belugas in natural environments; thus how these results compare to those of wild subjects was unknown. What are needed are audiograms collected on multiple wild individuals using consistent methodologies allowing us to place both individual variation and prior measurements in a relative context.

The goal of this study was to determine hearing sensitivity in wild and presumably healthy beluga whales, using consistent AEP methods, to establish a baseline audiogram and the natural variability for this species, and to compare these results to previous work in laboratory conditions.

RESULTS

Our system to measure AEP responses was quite robust for establishing the audiograms of wild belugas. EFR were typically quite distinct from the background electrophysiological noise at the higher stimulus levels (Fig. 1A) even though a limited number of sweeps were collected per record. Thresholds at each frequency were collected in approximately 3-5 min in order to minimize overall handling time of the animals. Physiological noise conditions were typically quite low; the mean of all animals was 0.979 ± 0.277 μV rms (root mean squared). Only five thresholds were measured for beluga #5 because it was more active during the health exam; its movement likely introduced neuromuscular physiological noise into the AEP records. Therefore it may not be appropriate to include #5 in the mean. Without #5, the mean was 0.710 ± 0.174 μV (Table 1). Overall, peak AEP response amplitudes were relatively high and easily identifiable, even for some relatively low, near-threshold, sound levels. The FFT (fast Fourier transform) method was robust for extracting the EFR (envelop following response) at the respective modulation rate.

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 complete data collection for each audiogram shown in Figure 3. 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 reattach 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.

The AEP responses were typical of odontocetes in general and belugas in particular. There was a physiological delay of 4-5 ms at the start of the EFRs. Peak-to-peak amplitudes were often greater than 2 μV and physiological noise levels were less than 0.1 μV. Occasionally at lower sound levels, the early AEP onset waves were not easily distinguished from noise. This was, in part, because measurements were often made very close to the lower hearing threshold where responses are not very strong and electrophysiological noise signal could change when the animal respires or moves making the results harder to interpret. At about 20 dB above threshold, however, both early-wave AEPs and individual EFR waves were distinct and similar to laboratory conditions. Therefore, the following response FFT spectra reflected clear peaks at the stimulus amplitude modulation rate (Fig. 1B) resulting in good quality audiograms.

Using the FFT method to determine thresholds, audiograms were established for each animal (Fig 2). The secondary goal of the work was to understand the variation among individuals. To address this variation, audiogram differences were shown in several ways. All animals were assessed together (Fig. 2). All audiograms had a general U-shape typical of mammals with a steeper slope at the high frequency cut-offs, and a more gradual increase in thresholds a the lower range of hearing (Fig. 2). Audiograms could be grouped based on similar shapes. The first three animals showed similarity in shape, thresholds and frequency ranges. Greater variation was found in the animals 4, 5, and 6. Animal #7 showed the lowest overall thresholds based upon individual means of the thresholds at each frequency; Table 1).

Variation was calculated in several ways. An overall mean audiogram (± 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 (s.d.) 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.

Recordings selected to measure the background noise sound pressure level spectrum did not include any foreign noise source other than water splashing against the pile where the acoustic data-logger was installed during the flooding tidal cycle; however this type of noise did not affect frequencies above 4 kHz and therefore it is assumed that the background noise curve presented here is not affected by splashing wave noise. The background noise spectrum obtained in Dillingham showed a typical curve with higher noise levels in lower frequencies, and a gradual decrease in intensity with frequency (Fig. 2,3). Both the mean audiogram and the fourth order polynomial trend curve (Fig. 3D) closely followed the shape of the background noise curve. This noise curve was often between the values of the maximum and minimum curves, but overlapped the more sensitive values at low frequencies and less sensitive values at higher frequencies. Most hearing thresholds for frequencies between 4 and 40 kHz centered around the sound pressure level of background noise suggesting the noise levels at the recorder site may have been slightly higher than several of the capture locations sites. It is uncertain whether elevated audiograms were constrained by higher noise levels, showed hearing loss, or was some reflection of methodical and individual variation.

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 upper limit of hearing was 128 kHz (*n* =3), 140 kHz (*n* = 1), and 150 kHz (*n* =3) with a mean of 139 kHz. This was defined as the last detectable response (Finneran et al., 2009; Yost, 1994). 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. Beyond the similar upper frequency limits in hearing, the audiograms of the males had little resemblance to each other. There were similarities and differences among animals. The audiograms of belugas #1-3 were very similar in shape, with little variability among thresholds. Belugas #4-6, however, showed substantial differences. For example, Beluga #6 had an area of sensitivity at 22.5 kHz which was 20-30 dB lower than surrounding frequencies 16 and 32 kHz. Belugas #4 and #6 showed differences of > 20 dB at 16 and 54 kHz. And overall, Beluga #5, while elevated and limited in its audiogram, showed relatively stable hearing thresholds with few large deviations between points. Beluga #7 had the “best” overall hearing with lowest mean thresholds (Table 1). This is because thresholds were particularly low in the audiogram center (with thresholds of 47 and 35 dB at 22.5 and 80 kHz, respectively; after 80 kHz, thresholds steeply increased until 140 kHz) and no clear responses were detected at 150 kHz. At the lower end for this animal, the 16 kHz threshold increased relatively steeply and thresholds were slightly (4 dB) above the mean at 8 kHz. No responses were detectable at 4 kHz and 120 dB maximum SPL (sound pressure level).

The mean thresholds showed an audiogram shape similar to other odontocetes and beluga (Fig. 3A, 5). “Best” or lowest thresholds were typically from 22.5-80 kHz with the absolute lowest between 45 and 80 kHz. 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 51, 52 and 50 dB at 45, 54 and 80 kHz, respectively. The mean threshold s.d. for all the frequencies was 8.7 dB, but, the greatest s.d. value was 15.7 dB at 128 kHz. Not including the upper limit of 128 kHz, 45 and 80 kHz had the greatest s.d. in mean thresholds at 11.9 and 11.2 dB. Therefore, greatest s.d. values were at the highest frequency (128 kHz) and frequencies of maximum sensitivity (54 and 80 kHz).

Health assessment data collected included blood samples to study hormones, genetics and blood chemistry (Norman et al., 2012) and fecal samples, morphometric measurements, blubber thickness by ultrasound techniques, full core biopsies in two locations and satellite transmitters were attached to the individuals. Full assessment results will be presented elsewhere but in general, no abnormal findings were found as part of field exams or in the review of results from analyses to date. After sampling and testing for hearing, belugas were released and tracked via satellite-linked transmitters to monitor behavior for the next several months. No adverse responses to the multiple sampling procedures and hearing tests were indicated by subsequent movements or dive behavior.

DISCUSSION

In order to better understand odontocete hearing it is necessary to determine what the average individual of a population hears and examine the associated variability among individuals within that population. The mean audiogram of wild belugas showed a wide range of sensitive hearing, from 22 to 110 kHz and minimum detection levels near 50 dB. Overall detection ranges were found to be from 4 to as high as 150 kHz, although the adult males only heard to 128 or 140 kHz. The low frequency limit is largely a function of the AEP methods; short-latency, rapidly-rising AEP waves are not easily detectable with longer wave-length, low frequency stimuli (Burkhard et al., 2007). Four kilohertz is often the lower limit for cetacean AEP studies (Mooney et al., 2012a). The high frequency cut-off is likely the hearing limit for each animal. These levels and the frequency range demonstrate good hearing compared to other belugas and odontocetes in general (Mooney et al., 2012b; Nachtigall et al., 2000). For example, previously tested belugas only heard up to 128 kHz. Population AEP audiograms of captive bottlenose dolphins (*Tursiops truncatus*) (Houser and Finneran, 2006b; Houser et al., 2008) show most animals have somewhat less sensitive hearing, compared to these wild belugas. Audiograms with some wild, stranded animals are closer in threshold values (Nachtigall et al., 2008; Nachtigall et al., 2005). In general, variation among individuals seems relatively large (±11 dB s.d.) at some frequencies. But most standard deviations were not greater than 7-8 dB. In dolphins, standard deviations of repeated AEP measurements in an individual are as low as 2-3 dB (Mooney et al., 2009a). But values are also often higher. The overall inter-individual variation of 7-8 dB is very similar to results from bottlenose dolphins in laboratory conditions (Houser and Finneran, 2006b; Houser et al., 2008). With a lower sample size (n = 7, vs. 13 and 42), greater variation might be expected here. Repeated measures within certain individuals would help groundtruth the level of this variation. Yet, the comparable values suggest relatively consistent hearing among the animals tested despite the differences in individual audiograms and a field-based method.

The audiogram variability between animals and within an individual audiogram is not unusual for odontocetes (Houser and Finneran, 2006b; Houser et al., 2008). For example, individual dolphin hearing measurements at a particular frequency may vary nearly 10 dB between days (Mooney et al., 2009a). Differences in hearing sensitivity of 20 dB have been reported across a relatively small range of frequencies (Houser and Finneran, 2006a; Houser and Finneran, 2006b; Pacini et al., 2011). Here, the results show the greatest variability at maximum sensitivity and highest frequencies. Both are regions where natural hearing loss likely to occur and thus great variation might be expected. It also suggests that frequencies of interested should be noted when discussing audiogram threshold variations.

Age and other factors may influence differences among individuals (Houser and Finneran, 2006b; Houser et al., 2008). Audiometric variation might also be methodological. When using AEP, such differences may be a result of several factors including background noise, physiological variability, transducer placement, electrode placement, and natural response variability. Some background bioelectrical variability was found among individuals. While this variability was highest for beluga #5, its responses were clear and the audiogram was relatively smooth suggesting that the background bioelectrical variability was not a major factor in these audiograms. Background noise levels were not measured in each test location because of limited time, the tide often changed the exact measurement site (so we would move the animal to keep a consistent depth), and environmental conditions appeared similar between locations (i.e., all muddy, estuarine environments, calm water and without external vessel traffic); thus the acoustic conditions were not expected to vary substantially among capture sites. The transducer and electrode placement may have introduced some variability even though they were placed in the same general locations for each animal. The jawphone, however, was able to produce a relatively constant stimulus condition. Thus, most of the variation shown here likely reflects the variation between the individual animals, although it was recognized that the field conditions were somewhat more variable than some (but not all) laboratory settings.

The general similarity of beluga audiograms among studies supports our field measurement equipment and methods. The background bioelectrical variability was relatively low (Fig. 1) and similar to controlled laboratory settings (Nachtigall et al., 2004), especially considering that several other sampling processes such as blood draws, satellite tagging and ultrasounds, occurred concurrently with the AEPs collection.

Overall, these animals heard well in the upper frequencies. Based on the size of some animals, it was assumed 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 (Houser and Finneran, 2006b), suggested in a false killer whale (Kloepper et al., 2010); hearing loss has also been related to antibiotic treatment in belugas (Finneran et al., 2005b). 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.

The background noise spectrum was below hearing thresholds in most cases, except for a few instances in the 16 kHz band for beluga #2 and #4 and the 22.5 kHz band for beluga #6 and #7. This indicates that the background noise levels measured in Dillingham were above the noise conditions in some of the capture locations, but also suggests that the hearing abilities of these sampled belugas was close to the natural limit imposed by the background noise of their habitat. The fact that the shape of the composite audiogram of minimum sensitivity follows very closely and even partially overlaps the background noise curve in the range 4-40 kHz supports this observation. Potential increases in background noise due to anthropogenic activities, even if moderate, could cause considerable masked hearing.

In order to evaluate beluga hearing abilities from audiograms the mean values are often used, however using a mean audiogram alone may limit our understanding of the differences among individuals. Therefore the mean population audiogram should include a measure of variation. An additional measure of hearing variation is shown in the composite audiograms of maximum and minimum sensitivity (Fig. 3B) and the respective differences between these values. At many frequencies, there was a 20-25 dB difference between the lowest and highest thresholds. While these differences are substantial, they are not as large as those found in some bottlenose dolphins, which often exceeded 40-60 dB (Houser and Finneran, 2006b). Except for the upper auditory limit, there was little difference between female (n =3) and male (n=4) audiograms. Overall, the relatively low variation among all individual belugas tested in this study suggests that either our sample size was too low to determine population level differences, wild animals may have less variation, or belugas from this population have less variation in hearing ability. Additionally, variation may be dependent upon the population and its exposure to various auditory stressors. 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), obtained through behavioral methods, show slightly lower thresholds across many frequencies. This difference between White et al., and this study may be methodological, as psychophysical-based methods used by White et al., (1978) may yield lower thresholds (in the order of 8-12 dB) than AEP based results in other odontocete species (Finneran and Houser, 2006; 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 Mooney et al. (2008) (using AEPs) fell within the observed variability in wild belugas. At the lower frequencies, the beluga studied by Finneran et al., (2005a) was also similar to the belugas examined here. The threshold reported by Klishin et al., (AEPs; 2000) were generally higher than the animals observed in this study. Alternately, the animals from Ridgway et al., (behavioral methods; 2001) demonstrated lower thresholds. Thus, there may be some difference between behavioral and physiological audiograms. Yet, the various beluga hearing measures from other studies overlap the s.d.'s of the mean audiogram in this study. This suggests these animals often heard similarly, indicating that rather than revising the beluga audiogram, these results reinforce the validity of those from laboratory studies. 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., 2005a).

Successfully measuring the hearing of multiple wild odontocetes expanded on upon previous work which collected a single full audiogram from a white-beaked dolphin during a capture-and-release procedure (Nachtigall et al., 2008). Similar hearing data were also collected from wild bottlenose dolphins during capture events (Cook et al., 2006), however these unpublished tests did not measure the full range of odontocete hearing. These audiograms for seven wild, healthy beluga whales provide a unique data set for odontocetes. This study has contributed to knowledge of 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. The method could be applicable on a broader scale. Second, the results provide nearly complete audiograms documenting the hearing of wild individuals (only the low frequencies were not measured). Not only are the data directly applicable to other wild animals, similarities to the laboratory animals supports use of their data as well. 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 recovery (National Marine Fisheries Service, 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 (National Marine Fisheries Service, 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. It is hoped 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.

METHODS

Field conditions and setup

Baseline audiograms in wild belugas were measured as a component of a health assessment project in Bristol Bay, Alaska, USA (Norman et al., 2012). Belugas were captured in a net, held briefly (<2 hrs) and released. In general, the bay consists of relatively shallow, tidally influenced, murky water with a soft mud bottom. Seven of nine beluga whales that were captured in September, 2012 were given hearing tests. Hearing was tested using AEPs (methods described below). The AEP data collection was conducted while the whales were temporarily restrained for physical health and condition measurements, some of which were collected simultaneous with the AEP. Health assessments included measurements (length and girth), ultrasound (blubber thickness) at eight locations and samples of feces, exhalation, skin and blubber (Norman et al., 2012). A satellite-linked transmitter was also attached for tracking movements after release. Sampling procedures were coordinated to minimize holding time and on-site veterinarians monitored the status of each beluga during capture and holding. The mean total capture time was 91 minutes and belugas were not held for more than 2 hrs. Collection of data for audiograms was typically completed in 45 min, including breaks to adjust the animal or focus on other measures.

Temporary capture events followed procedures similar to those established in the 1990s (Ferrero et al., 2000) and were conducted under National Marine Fisheries Service marine mammal research permit #14245 and approved by the Institutional Animal Care and Use Committee. Animals were spotted from one of three 3.5 m open-aluminum skiffs. The skiffs gradually approached the whales and guided them into shallow water (i.e., <2 m). A 125 m long × 4 m deep, 0.3 m braided square mesh net was deployed from the net boat around a single target animal. Once the whale became entangled in the net, an inflatable rubber boat with three handlers approached the whale and placed a tail rope around the peduncle and secured the whale to the boat. As the whale was removed from the net a "belly band" stretcher with hand holes was placed under the whale for ease of handling and moving the whale as water depth changed with the tide. The capture net was pulled in as soon as the captured whale was removed.

During the hearing tests the whales were positioned adjacent to the small inflatable boat in the belly band. The beluga's head typically rested on or just above the soft mud bottom. This was successful for all animals, except one (#7) for which the water level was too low and this test was conducted partly out of the water. These conditions were similar to many previous cetacean AEP hearing tests. The AEP equipment was outfitted in a ruggedized case and the operator sat in the small inflatable boat beside the beluga (Fig. 5).

Stimulus presentation

The acoustic stimuli were sinusoidally amplitude modulated (SAM) tone-bursts (Nachtigall et al., 2007a), digitally synthesized with a customized LabVIEW (National Instruments, Austin, TX, USA) data acquisition program. The sound's digital-analog conversion was made using a National Instruments PCMCIA-6062E data acquisition card. The card was implemented in a semi-ruggedized Panasonic Toughbook laptop computer. Each SAM tone-burst was 20 ms long, with an update rate of 512 kHz. The carrier frequencies were modulated at a rate of 1000 Hz, with a 100% modulation depth. Thus a neurological response by the animal to the stimulus would occur at a rate of 1000 Hz. This modulation rate was chosen based on pre-established modulation rates for belugas shown elsewhere (Klishin et al., 2000; Mooney et al., 2008). Amplitude modulated signals do show some frequency spreading but this modulation rate minimizes the leakage to 1-2 kHz (Supin and Popov, 2007). Effects to AEP thresholds would only likely be seen at the very lowest frequencies. A 30 ms break of no sound was alternated between the 20 ms stimulus presentations, thus the rate of tone-burst presentations was 20/s.

The sounds were then sent to a HP 350D attenuator (Palo Alto, CA, USA) which could control sound levels in 1 dB (re 1 µPa) increments. From the attenuator the signal was played to the beluga using a "jawphone" transducer. This method was chosen because belugas freely moved their heads during the experiments; this would have provided varying sound received levels for a free-field transducer. By always placing the jawphone at a consistent location, it was possible to easily provide comparable stimuli within a session and between animals despite movement of their heads. This suction cup was attached medially to the lower jaw, about 4 cm from the tip and sounds were presented directly to the whale through this suction cup. This location on the jaw has been identified as a region of primary acoustic sensitivity for belugas (Mooney et al., 2008). Prior studies have also shown comparable audiograms between jawphone and free-field measurements (Finneran and Houser, 2006; Houser and Finneran, 2006a). The jawphone consisted of a Reson 4013 transducer (Slangerup, Denmark) implanted in a custom silicone suction-cup (KE1300T, Shin-Etsu, Tokyo, Japan). It was attached to the animal using conductive gel (Signagel, Parker Laboratories, Fairfield, NJ, USA) which eliminated reflective air gaps between the suction cup and the skin. Frequencies (kHz) tested included: 4, 8, 11.2, 16, 22.5, 32, 45, 54, 80, 100, 110, 128, 140, 150, and 180 although not all frequencies were tested on all belugas because of the time limitations associated with each capture situation. A sequence was developed to prioritize certain frequencies when time did not allow all frequencies to be completed. First the frequency range was abbreviated in a way that would still show the animal's hearing abilities. Instead of 15 frequencies, nine were tested in the following order: 54, 16, 8, 4, 32, 80, 100, 128 and 150. The first frequency, 54 kHz, was chosen because it is a mid-frequency tone likely to be in the beluga's hearing range and generate a positive response. Once these frequencies were completed, a second series was tested to expand the frequency range and fill in between the original frequencies (i.e., 45, 11.2, 22.5, 110, 140 and 180 kHz). Sometimes the order varied slightly depending upon the initial results (e.g., the highest frequencies might not be tested if it were clear that the high-frequency cut-off had already been reached).

Jawphone stimuli were calibrated prior to the experiment using the same sound stimuli as in the hearing tests. While calibration measurements were in the free- and far-fields, it is acknowledged that jawphone presented stimuli were not received by the animal in this manner. This calibration allows for some comparisons with how sounds may be received in the far-field while recognizing the differences between free-field and contact transducer measurements (Cook et al., 2006; Finneran and Houser, 2006). Received measurements were made using a Reson 4013 transducer. During calibration, the jawphone projector and receiver were placed in salt water 50 cm apart at 1 m depth. Fifty cm is the approximate distance from jaw tip to auditory bulla in an adult beluga. The received signals were viewed on an oscilloscope (Tektronix TPS 2014, Beaverton, OR, USA) and the peak-to-peak voltages (Vp-p) were measured. These values were then calculated into sound pressure levels (dBp-p re 1 μPa) (Au, 1993). This Vp-p was converted to estimate RMS by subtracting 15 dB. This was taken as the RMS voltage and used to calculate the SPL for that frequency (Au, 1993; Nachtigall et al., 2005). Calibrations were tested with the suction cup, and neither the suction cup nor the gel impacted the received sound levels of the stimuli.

AEP measurements

AEP responses were collected from three gold, passive electrode sensors embedded in silicone suction-cups. The electrodes were standard 10 mm electroencephalogram (EEG) electrodes, the same type used for human EEGs. The suction cups were easily stuck on the dorsal surface of the beluga at the beginning of each session with the aid of conductive gel. The active electrode was attached about 3–4 cm behind the blowhole slightly off to the right approximately over the brainstem. Placement of this electrode was somewhat challenging as the beluga can move its head from side to side and the skin surface was typically wrinkled in this area, thus the cup could be easily dislodged and was frequently replaced interrupting the AEPs. The reference (inverting) electrode was attached distal to the active electrode, on the animal's back typically near the anterior terminus of the dorsal ridge. A third suction-cup sensor was also placed dorsally, typically posterior to the dorsal ridge. These general placements away from major neuromuscular activity support decreased noise measures (Supin et al., 2001).

The animal rested with its ventrum on the bottom partially supported by buoyancy during each experimental session, with its back, blowhole, head and the electrodes out of the water. This positioning allowed the animal to easily control its own respiration rates and improved evoked potential signal strength. It also kept most of the head, including the lower jaw primary sound reception pathways, under water during the hearing tests. On most animals, other measures, sampling or tag attachment could be conducted concurrent with the hearing tests and with no apparent impact to the AEP responses.

The incoming electrophysiological signals received by the active electrode were amplified 10,000x and bandpass filtered from 300-3000 Hz using a biological amplifier (Grass Technologies CP511, Warwick, RI, USA). A second Krohn-Hite filter (Warwick, RK, USA) conditioned the responses again using the same bandpass filter range. They were then conducted to the data acquisition card where a custom program sampled the signal amplitude at 16 kHz to ensure resolution of the 1 kHz signal, and then recorded and stored on the laptop computer. The responses were collected in 30 ms records that began coincident with the stimulus presentation.

There was a 20 ms break before the stimulus/AEP recording began again; 500 responses were collected for each trial stimulus amplitude at each frequency. The 500 response records were averaged into a single time series to reduce unwanted electrophysiological noise by approximately a factor of 20 and then stored as the mean response or envelope following response (also referred to as auditory steady state response-ASSR). These incoming EFRs and their FFTs were also monitored in real-time on the custom program to ensure the correct background noise conditions and generally good response levels.

The amplitudes of the transmitted SAM tone-bursts for the various carrier frequencies were reduced in 5–10 dB steps, until responses could no longer be distinguished from the background noise. Then 1-2 more responses were typically recorded near this apparent threshold to ensure responses were not "missed." Decibel step size was based on the amplitude of the signal and the animal's neurological response. An average of seven stimuli with different SPLs were presented for each tested frequency.

Data analysis

Recorded EFR waveforms were first viewed relative to time. Response amplitude was also examined in the frequency domain by calculating a 256-point FFT of the response waveforms (FFT of the EFR). Only, a 16-ms portion of the EFR, from 5 to 21 ms, was used for the FFT. This window contained 256 response samples and the majority of the stimulus period while allowing for the delay of the EFR relative to stimulus onset. The FFT-EFR provided a measure of the animal's physiological response to the frequency being tested when a peak was detected at the1000 Hz modulation rate of the signal. Thus a larger EFR response was reflected as a higher peak value. The peak value was used to estimate the magnitude of the response evoked by the SAM stimulus. These values were then plotted as response intensity against SPL of the stimulus at a given frequency. A regression line addressing the data points was hypothetically extended to zero (horizontal axis intercept of the regression), the theoretical point where there would be no response to the stimulus and the arbitrary definition of hearing threshold. In a near-threshold range, these points can be reasonable approximated by straight regression lines (r^2 = 0.97 in Fig. 1) with the five points with the highest r^2 value used to calculate the regression (Mooney et al., 2009a; Nachtigall et al., 2007a; Nachtigall et al., 2007b; Supin et al., 2001). The stimulus SPL value corresponding to the estimated zero FFT-EFR, was the estimated hearing threshold for each of the frequencies presented to the animal as described in Supin et al. (2001). From these thresholds, audiograms could then be established for each animal.

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. This window length was chosen because it equaled the FFT window for threshold determinations. Records used were the minimum sound level for five separate frequencies and no responses (waveforms or FFT peaks) were evident at these levels (or 10 dB above). Five records were averaged because animals were presented with at least five frequencies, facilitating comparisons of the mean rms value for each animal's neurophysiological responses (Table 1). These values can generally be taken as the noise level at the modulation rate FFT. But because noise values often decreased across the FFT spectrum, noise value at this frequency were more often less than 0.01 µV peak value. Analyses were conducted using EXCEL, Matlab and MINITAB software.

Background noise measurements

In order to describe the background noise levels of the acoustic environment where the sampled belugas inhabit, background acoustic noise in the bay was recorded using a DSG-Ocean acoustic data-logger (Loggerhead Instruments, Sarasota, FL, USA) with a HTI- 96-Min hydrophone (High Tech Inc. Gulfport, MS, USA) with -185.8 dB re 1V/µPA receiving sensitivity and frequency response of ± 1 dB from 2 Hz to 40 kHz. The system has a frequency response of ±0.7 dB from 20 Hz to 40 kHz. The acoustic data-logger was set to record continuously at 80 kHz sample rate and was deployed for 4 days while the beluga captures took place. The data-logger was deployed 1 m from the seafloor attached to a pile during low tide in an unused cannery pier in Dillingham, AK, facing open water. This site was 3 km (mean) from the the capture sites (stdv 0.9, max 5 km, min 2 km). This location was expected to be similar but perhaps slightly higher in ambient noise levels than most capture site because of proximity to the town. Recordings for analysis were selected based on the sea state and the tide cycle. During the selection, recordings were manually scanned to check quality, confirm that the instrument was below the surface and check whether anthropogenic noise sources were absent. A total of 45 min of recordings were selected from September 8th and 9th 2012, corresponding to periods of sea state 0-1 in ebbing (15 min), high (15 min) and flooding (15 min) tidal cycles. Recordings were analyzed in SpectraPRO 732 (Sound Technology Corporation). The selected 45 min of raw data were transformed to instantaneous pressure in µPa using the analog-to-digital conversion factor, amplification gain and hydrophone receiving sensitivity. Sound pressure level spectrum (in dB re 1µPa) from 4 kHz to 40 kHz was estimated using the Fast Fourier transform algorithm with a Hanning window of 65536 samples with 50% overlap, providing a frequency resolution of 1.2 Hz and a time resolution of 0.4 sec.

Symbols and abbreviations:

AEP – Auditory evoked potential

ASSR – Auditory steady state response

FFT – fast Fourier transform

EFR – envelope following response

SAM – Sinusoidally amplitude modulated

Acknowledgements

Project funding and field support provided by Georgia Aquarium and the National Marine Mammal Laboratory of the Alaska Fisheries Science Center (NMML/AFSC). Field work also supported by National Marine Fisheries Service Alaska Regional Office (NMFS AKR), WHOI Arctic Research Initiative, WHOI Ocean Life Institute, U.S. Fish and Wildlife Service, Bristol Bay Native Association, Alaska SeaLife Center, Shedd Aquarium and Mystic Aquarium. We greatly appreciate the support from all their directors. Audiogram analyses were funded by the Office of Naval Research award number N000141210203 (from Michael Weise). Paul Nachtigall and Alexander Supin assisted with the AEP program. We also acknowledge substantial assistance in data collection from Russ Andrews, George Biedenbach, Brett Long, Stephanie Norman, Mandy Keogh, Amanda Moors, Laura Thompson, Tim Binder, Lisa Naples, Leslie Cornick, Katie Royer, and Kathy Burek-Huntington and of course boat drivers Richard Hiratsuka, Albie Roehl, Ben Tinker and Danny Togiak and their respective first mates. All work conducted under NMFS permit no. 14245 and in accordance with approval from the

NMML/AFSC IACUC protocols (ID number: AFSC-NWFSC2012-1) and WHOI IACUC protocols (ID number: BI166330).

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Table 1. Morphometric measurements, sex, hearing thresholds. sampling duration and physiological noise levels for all belugas.

	Beluga #1 female subadult	Beluga #2 male adult	Beluga #3 female adult	Beluga #4 male adult	Beluga #5 male adult	Beluga #6 female adult	Beluga #7 male adult	
Length (cm)	272.5	350	300	375	390	310	390	341.1
Girth (cm)	68	84	190	260	245	192.5	276.5	188.0
Fluke width (cm)	26.5	37	62.5	90	95	82.5	92.5	69.4
Frequency (kHz)				Thresholds (dB re 1 μPa)				Mean
4	84	73	78			76	NR	78 (4.5)
8	74	67	72	83		73	78	74 (5.5)
11.2	63		74					69 (8.2)
16	63	58	66	60	75	82	74	68 (8.9)
22.5			61			53	47	54 (6.9)
32	50	61	63	67	65	73	57	62 (7.2)
45	38		45			64	58	51 (11.9)
54	51	42	52	43	58	64	51	52 (7.7)
80	52	57	36	49	60	63	35	50 (11.2)
100	65	64	59	65		64	45	60 (7.7)
110							52	52
128	76	110	104	91	121	101		100 (15.7)
140							92	
150	116		112			100	NR	109 (8.5)
Mean	76	76	78	74	85	83	68	
AEP sampling duration (min)	48	52	40	38	36	49	55	45
Mean noise (μV, rms)	0.44	0.4	0.561	1.068	2.592	0.888	0.9	0.979
s.d.	0.134	0.161	0.081	0.226	0.893	0.195	0.249	0.277

Figure 1. (A) Evoked potential envelope following responses to SAM tones at 54 kHz (Beluga #1). The tones decreased in amplitude from 97 to 57 dB re 1 µPa, and the EFR waveforms and (B) corresponding FFT-EFR peak values at 1 kHz decrease. The peak values (diamonds) at 1 kHz are shown with a best-fit linear regression (bold line) which, when extrapolated to zero, provides the threshold. The regression addressed the lowest 5 points and reflected an $r^2 = 0.97$. In this case the threshold is 51 dB. Sound pressure levels are in dB re 1 µPa.

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

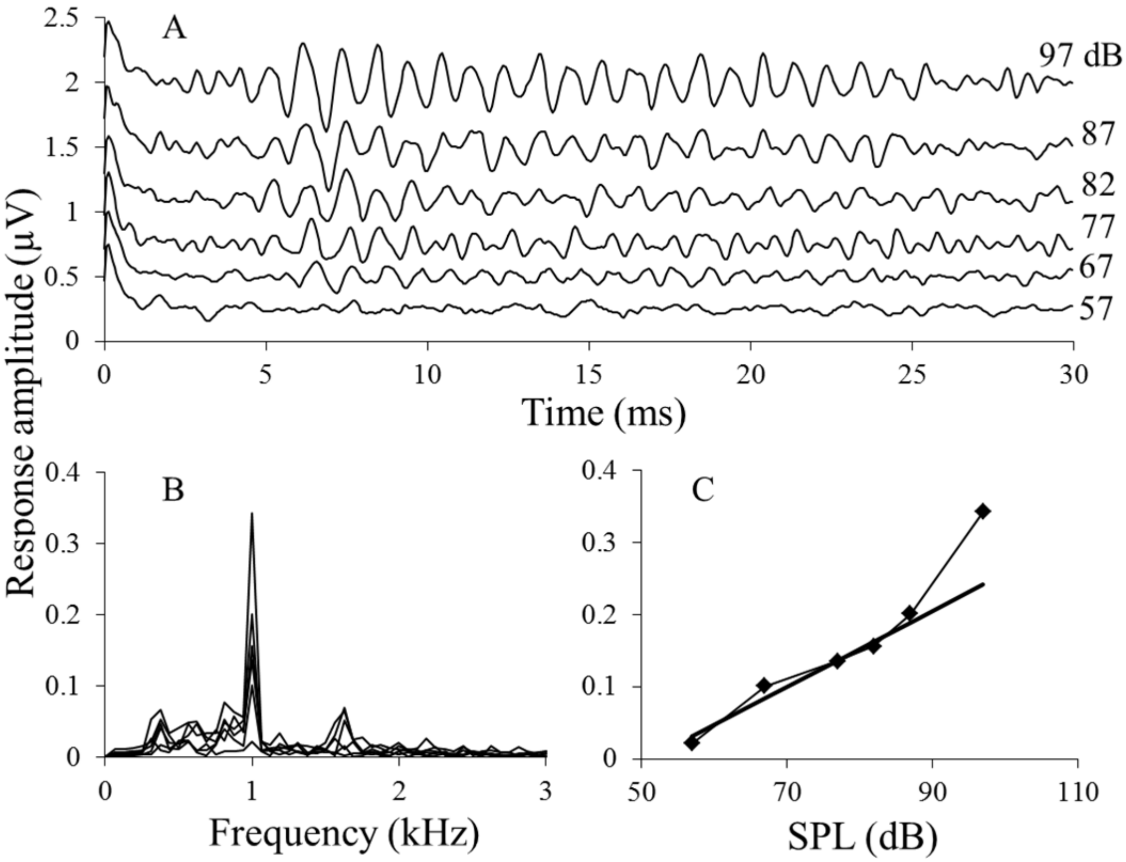
Figure 3. (A) The mean audiogram ± s.d and Bristol Bay background noise spectrum (grey dashed line). (B) Composite audiograms constructed by plotting the thresholds of maximum (black, diamonds) and minimum sensitivity (grey triangles) and Bristol Bay background noise spectrum (grey dashed line). (C) The standard deviation (s.d.) difference of thresholds at measured hearing frequencies and fitted power trend line. S.d. values increased with frequency. Sound pressure levels are in dB re 1 µPa. (D) Fourth order polynomial trend curve (y = -1E-06x4 + 0.0003x3 - 0.0168x2 - 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 ± 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, (Mooney et al., 2008)-circles, (Klishin et al., 2000)-triangles, (Finneran et al., 2005a)-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 μPa.

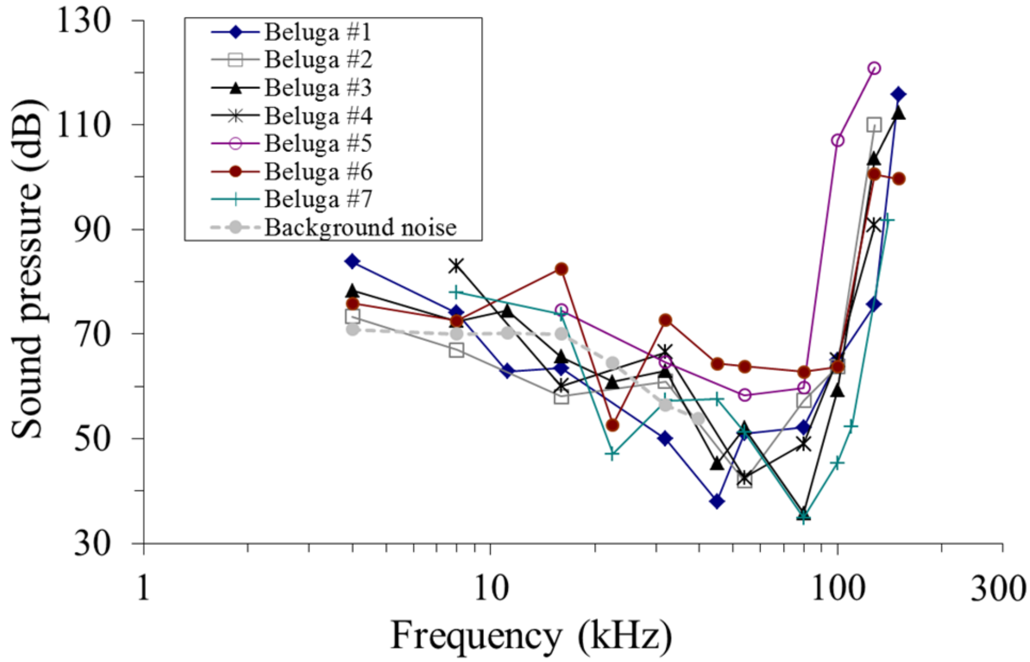
Figure 5. (A) Beluga #1 during an auditory evoked potential (AEP) hearing experimental session. The whale is facing right. The three suction-cup attached sensors (right to left are active sensor, invert sensor and ground) are visible and attached to the typical locations on the animal. (B) The AEP equipment being operated in its ruggedized case in the small inflatable boat while the whale is positioned adjacent to left (not visible).

Figure 1.


A
97 dB
87
82
77
67
57
2.5
2
1.5
1
0.5
0
0
5
10
15
20
25
30
Time (ms)
Response amplitude (µV)
B
0.4
0.3
0.2
0.1
0
0
1
2
3
Frequency (kHz)
C
0.4
0.3
0.2
0.1
0
50
70
90
110
SPL (dB)

1 Figure 2.

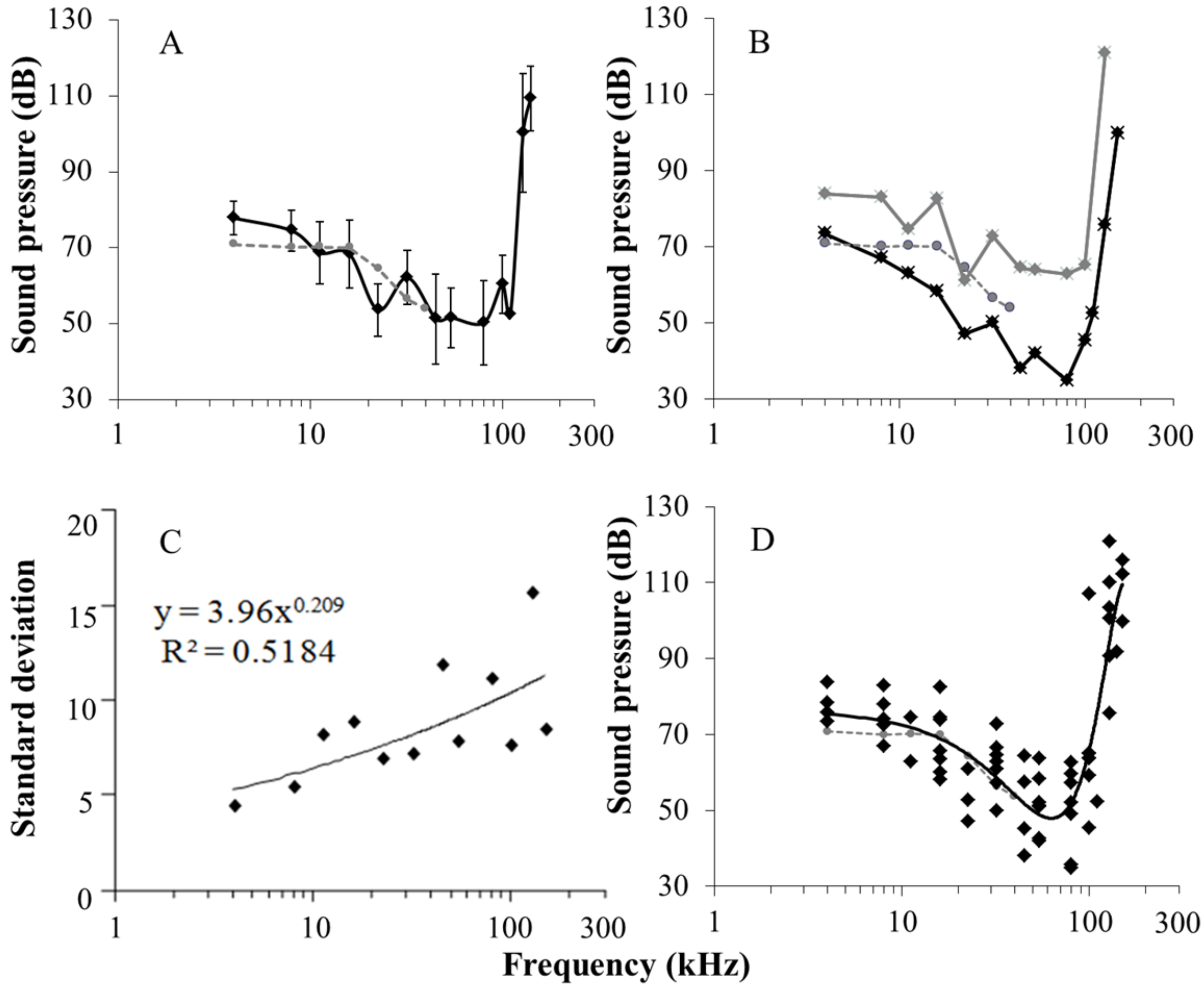
2



3

4

Figure 3.



A
B
C
D
Sound pressure (dB)
Standard deviation
y = 3.96x^0.209
R² = 0.5184
Frequency (kHz)
130
110
90
70
50
30
20
15
10
5
0
1
10
100
300

Figure 4.

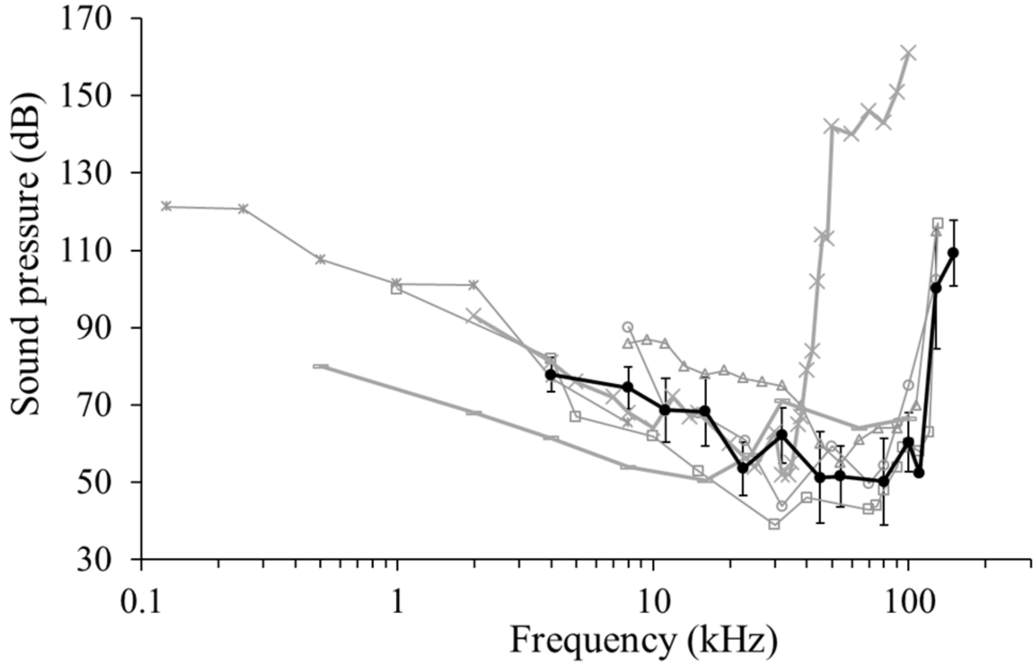

Sound pressure (dB)
170
150
130
110
90
70
50
30
0.1
1
10
100
Frequency (kHz)

Figure 1.