

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

2
3 Manuel Castellote^{1,2*}, T. Aran Mooney^{3*\$}, Lori Quakenbush⁴, Roderick Hobbs¹, Caroline
4 Goertz⁵, Eric Gaglione⁶

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6
7 ¹National Marine Mammal Laboratory, Alaska Fisheries Science Center, National Marine
8 Fisheries Service, Seattle, WA 98115

9 ²North Gulf Oceanic Society, Homer, AK 99603, USA.

10 ³Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA

11 ⁴Alaska Department of Fish and Game, 1300 College Road, Fairbanks, AK. 99701, USA

12 ⁵Alaska SeaLife Center, Seward, AK 99664, USA

13 ⁶Georgia Aquarium, 225 Baker St NW, Atlanta, GA 30313, USA

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16 *These authors contributed equally to this work

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30 ^{\$}Corresponding author: amooney@whoi.edu; 508-289-3714

1 **SUMMARY**

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

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1 **INTRODUCTION**

2 Beluga whales (*Delphinapterus leucas*) are often found in turbid, coastal waters in
3 northern latitudes where darkness can extend for many months. They depend upon sound for
4 many important biological functions such as foraging, navigation and communication, and they
5 are considered to have sophisticated hearing and echolocation abilities (e.g., Ridgway et al.,
6 2001; Turl et al., 1987). Their diverse vocal repertoire has often led them to be referred to as
7 “canaries of the sea.” Hearing studies of belugas held in laboratory settings have generally
8 shown sensitive and broadband hearing abilities, similar to other odontocetes (Awbrey et al.,
9 1988; Finneran et al., 2005a; Finneran et al., 2002a; Finneran et al., 2002b; Klishin et al., 2000;
10 Mooney et al., 2008; Ridgway et al., 2001). Yet, it is unclear how these hearing abilities compare
11 to those of wild belugas (or any odontocete). Measurements from multiple wild individuals are
12 needed to truly evaluate what a species may hear and variations found between individuals.

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

20 Because both hearing and sound production are important to belugas, changes in
21 background noise levels due to human activities may have a large impact on their ability to carry
22 out vital activities. Anthropogenic ocean noise is believed to be a chronic, habitat-level stressor
23 (Ellison et al., 2012) and there is special concern for Arctic ecosystems (Moore et al., 2012;
24 Southall et al., 2007). The increase in human activities now allowed by less sea ice is increasing
25 ocean noise in the Arctic, including areas that have been acoustically pristine (Moore et al.,
26 2012). Although the biological consequences of elevated ambient noise are not well understood,
27 there is sufficient evidence to suggest that at some threshold noise could negatively affect sound-
28 dependent marine mammals (National Academy of Sciences, 2005; Richardson et al., 1995;
29 Tyack and Clark, 2000). Therefore, understanding how noise might affect beluga sensory
30 ecology is important to address the potential impacts of increased noise within the Arctic.

1 To determine the effects of noise on marine mammals it is vital to understand what they
2 hear. There are few studies evaluating the auditory frequencies and sensitivities of most species
3 of marine mammals, and even fewer that address variability within a population (Gerstein et al.,
4 1999; Houser and Finneran, 2006b; Mooney et al., 2012a; Nachtigall et al., 2007b; Nachtigall et
5 al., 2005). Approximately 20 species of cetaceans and pinnipeds have been tested, representing
6 about 10% of all marine mammals. Most of what is known about odontocete hearing has come
7 from individuals born or maintained in aquaria or laboratories for many years (Nachtigall et al.,
8 2000). Few wild odontocetes have been studied and the ones that have are typically stranded due
9 to health-related issues that could affect hearing (Andre et al., 2003; Finneran et al., 2009; Mann
10 et al., 2010; Nachtigall et al., 2008; Nachtigall et al., 2005; Pacini et al., 2010; Pacini et al.,
11 2011). The auditory abilities of captive or stranded odontocetes may be robust as examples of
12 species-specific hearing but the only way to test this assumption is to compare captive to wild,
13 healthy animals. Capture and release of wild odontocetes to study hearing has rarely been
14 undertaken primarily because the equipment used to measure hearing has not been portable or
15 rugged enough for reliable use under field conditions and because animals are seldom captured
16 for short time periods. Recent advances in portable auditory evoked potential (AEP) equipment
17 and techniques have allowed this method to be used with dolphins that were captured and
18 temporarily restrained (Mooney et al., 2009b; Nachtigall et al., 2008).

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

1 Beluga whale hearing is among the best of all odontocetes (Erbe, 2000; Erbe and Farmer,
2 1998; Finneran et al., 2000; Johnson, 1991; Schlundt et al., 2000). Hearing sensitivity has been
3 assessed in numerous published works (Awbrey et al., 1988; Finneran et al., 2005a; Finneran et
4 al., 2002a; Finneran et al., 2002b; Klishin et al., 2000; Mooney et al., 2008; Ridgway et al.,
5 2001) and one non-peer-reviewed study (White et al., 1978). However, these investigations are
6 difficult to compare because methods or study designs have varied and samples sizes are limited.
7 For example, one study focused only on lower frequencies (Awbrey et al., 1988), while in
8 another hearing thresholds were elevated (n=1; Klishin et al., 2000). A third study found hearing
9 loss was attributed to a side effect of antibiotic treatment (Finneran et al., 2005a). Most studies
10 were limited to one beluga. Some tests involved behavioral conditioning responses (Awbrey et
11 al., 1988; Finneran et al., 2005a; Ridgway et al., 2001; White et al., 1978) whereas others used
12 AEP methods (Klishin et al., 2000; Mooney et al., 2008). It is clear that audiograms may vary
13 due to a number of factors including sex, age, genetics, prior history of chemical or noise
14 exposures, physiological or behavioral metrics, threshold evaluation methods, subject stress
15 level, environmental test conditions and others (Burkhard et al., 2007; Webster et al., 1992; Yost,
16 1994). For belugas many of these factors have varied. Thus, it is often unclear whether
17 differences in individual hearing abilities discrepancies are a result of methodological
18 discrepancies or actual auditory differences (or both). Further, none of these studies examined
19 belugas in natural environments; thus how these results compare to those of wild subjects was
20 unknown. What are needed are audiograms collected on multiple wild individuals using
21 consistent methodologies allowing us to place both individual variation and prior measurements
22 in a relative context.

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

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29 **RESULTS**

30 Our system to measure AEP responses was quite robust for establishing the audiograms
31 of wild belugas. EFR were typically quite distinct from the background electrophysiological

1 noise at the higher stimulus levels (Fig. 1A) even though a limited number of sweeps were
2 collected per record. Thresholds at each frequency were collected in approximately 3-5 min in
3 order to minimize overall handling time of the animals. Physiological noise conditions were
4 typically quite low; the mean of all animals was $0.979 \pm 0.277 \mu\text{V}$ rms (root mean squared).
5 Only five thresholds were measured for beluga #5 because it was more active during the health
6 exam; its movement likely introduced neuromuscular physiological noise into the AEP records.
7 Therefore it may not be appropriate to include #5 in the mean. Without #5, the mean was 0.710
8 $\pm 0.174 \mu\text{V}$ (Table 1). Overall, peak AEP response amplitudes were relatively high and easily
9 identifiable, even for some relatively low, near-threshold, sound levels. The FFT (fast Fourier
10 transform) method was robust for extracting the EFR (envelop following response) at the
11 respective modulation rate.

12 A mean of 9 (± 2.4 s.d., range 5-12) and a median of 10 thresholds were obtained per
13 animal. It took an average of 45 min (range 31-55) to complete data collection for each
14 audiogram shown in Figure 3. The number of thresholds obtained were not correlated with the
15 duration of the effort ($r^2 = 0.17$; $p > 0.5$) because recordings were often paused as the animal was
16 repositioned, relocated to adjust for the tide, to reattach electrodes or while another sample type
17 was obtained. Thus, 36-38 min was a good assessment of how quickly the procedure could be
18 accomplished in these particular environmental and contextual conditions.

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

29 Using the FFT method to determine thresholds, audiograms were established for each
30 animal (Fig 2). The secondary goal of the work was to understand the variation among
31 individuals. To address this variation, audiogram differences were shown in several ways. All

1 animals were assessed together (Fig. 2). All audiograms had a general U-shape typical of
2 mammals with a steeper slope at the high frequency cut-offs, and a more gradual increase in
3 thresholds at the lower range of hearing (Fig. 2). Audiograms could be grouped based on similar
4 shapes. The first three animals showed similarity in shape, thresholds and frequency ranges.
5 Greater variation was found in the animals 4, 5, and 6. Animal #7 showed the lowest overall
6 thresholds based upon individual means of the thresholds at each frequency; Table 1).

7 Variation was calculated in several ways. An overall mean audiogram (\pm s.d.) was
8 calculated (Fig. 3A). Two composite audiograms were created using the highest and lowest
9 thresholds for each frequency (Fig. 3B). The standard deviation (s.d.) difference of thresholds at
10 measured hearing frequencies and fitted power trend line showed an increase with frequency. A
11 fitted power function showed that half of the variation ($R^2 = 0.52$) was explained by the increase
12 (Fig. 3C). A best-fit fourth order polynomial was fit to the threshold data (Fig. 3D) to
13 characterize a general audiometric curve and provide a view of the associated variability. This
14 metric provided a composite audiogram that was less influenced by variability at certain
15 frequencies (as found in the mean of seven animals) and may provide a valuable way to identify
16 the general hearing abilities of a population.

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

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

7 The upper limit of hearing was 128 kHz ($n = 3$), 140 kHz ($n = 1$), and 150 kHz ($n = 3$)
8 with a mean of 139 kHz. This was defined as the last detectable response (Finneran et al., 2009;
9 Yost, 1994). The four males (belugas #2, #4, #5 and #7) had upper hearing limits of 128 kHz
10 and 140 kHz, compared to the three females which all heard up to 150 kHz. Females also had
11 lower thresholds at 128 kHz. Otherwise, there were no substantial male-female differences.
12 Beyond the similar upper frequency limits in hearing, the audiograms of the males had little
13 resemblance to each other. There were similarities and differences among animals. The
14 audiograms of belugas #1-3 were very similar in shape, with little variability among thresholds.
15 Belugas #4-6, however, showed substantial differences. For example, Beluga #6 had an area of
16 sensitivity at 22.5 kHz which was 20-30 dB lower than surrounding frequencies 16 and 32 kHz.
17 Belugas #4 and #6 showed differences of > 20 dB at 16 and 54 kHz. And overall, Beluga #5,
18 while elevated and limited in its audiogram, showed relatively stable hearing thresholds with few
19 large deviations between points. Beluga #7 had the “best” overall hearing with lowest mean
20 thresholds (Table 1). This is because thresholds were particularly low in the audiogram center
21 (with thresholds of 47 and 35 dB at 22.5 and 80 kHz, respectively; after 80 kHz, thresholds
22 steeply increased until 140 kHz) and no clear responses were detected at 150 kHz. At the lower
23 end for this animal, the 16 kHz threshold increased relatively steeply and thresholds were
24 slightly (4 dB) above the mean at 8 kHz. No responses were detectable at 4 kHz and 120 dB
25 maximum SPL (sound pressure level).

26 The mean thresholds showed an audiogram shape similar to other odontocetes and beluga
27 (Fig. 3A, 5). “Best” or lowest thresholds were typically from 22.5-80 kHz with the absolute
28 lowest between 45 and 80 kHz. There were differences in hearing among animals that was often
29 > 20 dB (Fig. 3B). The greatest differences in hearing abilities occurred at the high end of the
30 auditory range with 45 dB differences between two individuals at 128 kHz. The mean difference
31 between maximum and minimum thresholds across all frequencies was 21.8 dB (19.5 dB when

1 not including 128 kHz). Lowest mean thresholds were between 45 and 80 kHz with average
2 thresholds of 51, 52 and 50 dB at 45, 54 and 80 kHz, respectively. The mean threshold s.d. for all
3 the frequencies was 8.7 dB, but, the greatest s.d. value was 15.7 dB at 128 kHz. Not including
4 the upper limit of 128 kHz, 45 and 80 kHz had the greatest s.d. in mean thresholds at 11.9 and
5 11.2 dB. Therefore, greatest s.d. values were at the highest frequency (128 kHz) and
6 frequencies of maximum sensitivity (54 and 80 kHz).

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

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18 **DISCUSSION**

19 In order to better understand odontocete hearing it is necessary to determine what the
20 average individual of a population hears and examine the associated variability among
21 individuals within that population. The mean audiogram of wild belugas showed a wide range of
22 sensitive hearing, from 22 to 110 kHz and minimum detection levels near 50 dB. Overall
23 detection ranges were found to be from 4 to as high as 150 kHz, although the adult males only
24 heard to 128 or 140 kHz. The low frequency limit is largely a function of the AEP methods;
25 short-latency, rapidly-rising AEP waves are not easily detectable with longer wave-length, low
26 frequency stimuli (Burkhard et al., 2007). Four kilohertz is often the lower limit for cetacean
27 AEP studies (Mooney et al., 2012a). The high frequency cut-off is likely the hearing limit for
28 each animal. These levels and the frequency range demonstrate good hearing compared to other
29 belugas and odontocetes in general (Mooney et al., 2012b; Nachtigall et al., 2000). For example,
30 previously tested belugas only heard up to 128 kHz. Population AEP audiograms of captive
31 bottlenose dolphins (*Tursiops truncatus*) (Houser and Finneran, 2006b; Houser et al., 2008)

1 show most animals have somewhat less sensitive hearing, compared to these wild belugas.
2 Audiograms with some wild, stranded animals are closer in threshold values (Nachtigall et al.,
3 2008; Nachtigall et al., 2005). In general, variation among individuals seems relatively large
4 (± 11 dB s.d.) at some frequencies. But most standard deviations were not greater than 7-8 dB. In
5 dolphins, standard deviations of repeated AEP measurements in an individual are as low as 2-3
6 dB (Mooney et al., 2009a). But values are also often higher. The overall inter-individual
7 variation of 7-8 dB is very similar to results from bottlenose dolphins in laboratory conditions
8 (Houser and Finneran, 2006b; Houser et al., 2008). With a lower sample size ($n = 7$, vs. 13 and
9 42), greater variation might be expected here. Repeated measures within certain individuals
10 would help groundtruth the level of this variation. Yet, the comparable values suggest relatively
11 consistent hearing among the animals tested despite the differences in individual audiograms and
12 a field-based method.

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

22 Age and other factors may influence differences among individuals (Houser and
23 Finneran, 2006b; Houser et al., 2008). Audiometric variation might also be methodological.
24 When using AEP, such differences may be a result of several factors including background
25 noise, physiological variability, transducer placement, electrode placement, and natural response
26 variability. Some background bioelectrical variability was found among individuals. While this
27 variability was highest for beluga #5, its responses were clear and the audiogram was relatively
28 smooth suggesting that the background bioelectrical variability was not a major factor in these
29 audiograms. Background noise levels were not measured in each test location because of limited
30 time, the tide often changed the exact measurement site (so we would move the animal to keep a
31 consistent depth), and environmental conditions appeared similar between locations (i.e., all

1 muddy, estuarine environments, calm water and without external vessel traffic); thus the acoustic
2 conditions were not expected to vary substantially among capture sites. The transducer and
3 electrode placement may have introduced some variability even though they were placed in the
4 same general locations for each animal. The jawphone, however, was able to produce a relatively
5 constant stimulus condition. Thus, most of the variation shown here likely reflects the variation
6 between the individual animals, although it was recognized that the field conditions were
7 somewhat more variable than some (but not all) laboratory settings.

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

13 Overall, these animals heard well in the upper frequencies. Based on the size of some
14 animals, it was assumed that not all animals were very young. Thus, there appeared to be little
15 sensorineural high-frequency hearing loss associated with age (i.e., presbycusis). Presbycusis in
16 cetaceans has been documented in older bottlenose dolphins (Houser and Finneran, 2006b),
17 suggested in a false killer whale (Kloepper et al., 2010); hearing loss has also been related to
18 antibiotic treatment in belugas (Finneran et al., 2005b). Why these belugas demonstrated
19 generally good high-frequency hearing, and whether this trend would continue in other beluga or
20 other wild populations, is uncertain. This result further supports the need for larger sample sizes.

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

30 In order to evaluate beluga hearing abilities from audiograms the mean values are often
31 used, however using a mean audiogram alone may limit our understanding of the differences

1 among individuals. Therefore the mean population audiogram should include a measure of
2 variation. An additional measure of hearing variation is shown in the composite audiograms of
3 maximum and minimum sensitivity (Fig. 3B) and the respective differences between these
4 values. At many frequencies, there was a 20-25 dB difference between the lowest and highest
5 thresholds. While these differences are substantial, they are not as large as those found in some
6 bottlenose dolphins, which often exceeded 40-60 dB (Houser and Finneran, 2006b). Except for
7 the upper auditory limit, there was little difference between female (n =3) and male (n=4)
8 audiograms. Overall, the relatively low variation among all individual belugas tested in this
9 study suggests that either our sample size was too low to determine population level differences,
10 wild animals may have less variation, or belugas from this population have less variation in
11 hearing ability. Additionally, variation may be dependent upon the population and its exposure
12 to various auditory stressors. Increasing our sample size of wild belugas will be necessary to
13 determine which to conclude.

14 One way to examine beluga hearing variability is to compare these audiograms with
15 hearing measured in other belugas (Fig. 4). The hearing sensitivities reported here fall within
16 those previously described for laboratory belugas. Results from White et al. (1978), obtained
17 through behavioral methods, show slightly lower thresholds across many frequencies. This
18 difference between White et al., and this study may be methodological, as psychophysical-based
19 methods used by White et al., (1978) may yield lower thresholds (in the order of 8-12 dB) than
20 AEP based results in other odontocete species (Finneran and Houser, 2006; Szymanski et al.,
21 1999; Yuen et al., 2005) as well as in pinnipeds (Mulsow and Reichmuth, 2010). On average,
22 hearing thresholds from the beluga studied by Mooney et al. (2008) (using AEPs) fell within the
23 observed variability in wild belugas. At the lower frequencies, the beluga studied by Finneran et
24 al., (2005a) was also similar to the belugas examined here. The threshold reported by Klishin et
25 al., (AEPs; 2000) were generally higher than the animals observed in this study. Alternately, the
26 animals from Ridgway et al., (behavioral methods; 2001) demonstrated lower thresholds. Thus,
27 there may be some difference between behavioral and physiological audiograms. Yet, the various
28 beluga hearing measures from other studies overlap the s.d.'s of the mean audiogram in this
29 study. This suggests these animals often heard similarly, indicating that rather than revising the
30 beluga audiogram, these results reinforce the validity of those from laboratory studies. Only one

1 animal differed substantially across these comparisons and it is suspected that this beluga's
2 hearing loss was a result of aminoglycoside antibiotic treatment (Finneran et al., 2005a).

3 Successfully measuring the hearing of multiple wild odontocetes expanded on upon
4 previous work which collected a single full audiogram from a white-beaked dolphin during a
5 capture-and-release procedure (Nachtigall et al., 2008). Similar hearing data were also collected
6 from wild bottlenose dolphins during capture events (Cook et al., 2006), however these
7 unpublished tests did not measure the full range of odontocete hearing. These audiograms for
8 seven wild, healthy beluga whales provide a unique data set for odontocetes. This study has
9 contributed to knowledge of odontocete hearing in several respects. First, a wild population was
10 sampled in a relatively non-invasive manner in that belugas were held for short periods and
11 released. The method could be applicable on a broader scale. Second, the results provide nearly
12 complete audiograms documenting the hearing of wild individuals (only the low frequencies
13 were not measured). Not only are the data directly applicable to other wild animals, similarities
14 to the laboratory animals supports use of their data as well. Previously, beluga hearing limits
15 came from six animals held in enclosed facilities for extended periods of time, where they had
16 received medical treatment and had been exposed to different noise environments. Third, these
17 wild-caught individuals were healthy based on preliminary results from the concurrent health
18 assessment project. Hearing measured in stranded cetaceans provides a rare opportunity to
19 obtain hearing information, however, it is uncertain how it compares to wild healthy animals.
20 Lastly, this study provided data for multiple belugas of different sexes and ages from the same
21 population.

22 In view of the expected increases in sound levels as human activities increase in the
23 Arctic, expanding our knowledge of beluga hearing is key to an appropriate conservation
24 management effort. One of the five distinct stocks of beluga whales that are currently recognized
25 in U.S. waters, the Cook Inlet beluga population, is endangered and recovery efforts are being
26 identified. The impact of anthropogenic noise has been identified as a serious potential threat and
27 possible contributor to the lack of its recovery (National Marine Fisheries Service, 2008).
28 Similarly, there has been no noticeable recovery for the threatened St. Lawrence beluga
29 population and anthropogenic noise has been identified as one of the main threats (DFO, 2012).
30 In contrast, the Bristol Bay beluga population is increasing and is considered healthy (National
31 Marine Fisheries Service, 2008). While the Bristol Bay acoustic environment is not pristine,

1 anthropogenic noise is more seasonal and less intense than that of Cook Inlet. Therefore, Bristol
2 Bay belugas are a good subject population for approximating the baseline hearing for
3 comparison with other populations inhabiting other regions impacted by anthropogenic noise. It
4 is hoped that the results presented here will encourage sampling of wild cetaceans and further the
5 understanding of the potential effects of anthropogenic noise on belugas and other odontocetes.

6

7 **METHODS**

8 *Field conditions and setup*

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

24 Temporary capture events followed procedures similar to those established in the 1990s
25 (Ferrero et al., 2000) and were conducted under National Marine Fisheries Service marine
26 mammal research permit #14245 and approved by the Institutional Animal Care and Use
27 Committee. Animals were spotted from one of three 3.5 m open-aluminum skiffs. The skiffs
28 gradually approached the whales and guided them into shallow water (i.e., <2 m). A 125 m long
29 × 4 m deep, 0.3 m braided square mesh net was deployed from the net boat around a single target
30 animal. Once the whale became entangled in the net, an inflatable rubber boat with three
31 handlers approached the whale and placed a tail rope around the peduncle and secured the whale

1 to the boat. As the whale was removed from the net a “belly band” stretcher with hand holes was
2 placed under the whale for ease of handling and moving the whale as water depth changed with
3 the tide. The capture net was pulled in as soon as the captured whale was removed.

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

11 *Stimulus presentation*

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

25 The sounds were then sent to a HP 350D attenuator (Palo Alto, CA, USA) which could
26 control sound levels in 1 dB (re 1 μ Pa) increments. From the attenuator the signal was played to
27 the beluga using a “jawphone” transducer. This method was chosen because belugas freely
28 moved their heads during the experiments; this would have provided varying sound received
29 levels for a free-field transducer. By always placing the jawphone at a consistent location, it was
30 possible to easily provide comparable stimuli within a session and between animals despite
31 movement of their heads. This suction cup was attached medially to the lower jaw, about 4 cm

1 from the tip and sounds were presented directly to the whale through this suction cup. This
2 location on the jaw has been identified as a region of primary acoustic sensitivity for belugas
3 (Mooney et al., 2008). Prior studies have also shown comparable audiograms between jawphone
4 and free-field measurements (Finneran and Houser, 2006; Houser and Finneran, 2006a). The
5 jawphone consisted of a Reson 4013 transducer (Slangerup, Denmark) implanted in a custom
6 silicone suction-cup (KE1300T, Shin-Etsu, Tokyo, Japan). It was attached to the animal using
7 conductive gel (Signagel, Parker Laboratories, Fairfield, NJ, USA) which eliminated reflective
8 air gaps between the suction cup and the skin. Frequencies (kHz) tested included: 4, 8, 11.2, 16,
9 22.5, 32, 45, 54, 80, 100, 110, 128, 140, 150, and 180 although not all frequencies were tested on
10 all belugas because of the time limitations associated with each capture situation. A sequence
11 was developed to prioritize certain frequencies when time did not allow all frequencies to be
12 completed. First the frequency range was abbreviated in a way that would still show the
13 animal's hearing abilities. Instead of 15 frequencies, nine were tested in the following order: 54,
14 16, 8, 4, 32, 80, 100, 128 and 150. The first frequency, 54 kHz, was chosen because it is a mid-
15 frequency tone likely to be in the beluga's hearing range and generate a positive response. Once
16 these frequencies were completed, a second series was tested to expand the frequency range and
17 fill in between the original frequencies (i.e., 45, 11.2, 22.5, 110, 140 and 180 kHz). Sometimes
18 the order varied slightly depending upon the initial results (e.g., the highest frequencies might
19 not be tested if it were clear that the high-frequency cut-off had already been reached).

20 Jawphone stimuli were calibrated prior to the experiment using the same sound stimuli as
21 in the hearing tests. While calibration measurements were in the free- and far-fields, it is
22 acknowledged that jawphone presented stimuli were not received by the animal in this manner.
23 This calibration allows for some comparisons with how sounds may be received in the far-field
24 while recognizing the differences between free-field and contact transducer measurements (Cook
25 et al., 2006; Finneran and Houser, 2006). Received measurements were made using a Reson
26 4013 transducer. During calibration, the jawphone projector and receiver were placed in salt
27 water 50 cm apart at 1 m depth. Fifty cm is the approximate distance from jaw tip to auditory
28 bulla in an adult beluga. The received signals were viewed on an oscilloscope (Tektronix TPS
29 2014, Beaverton, OR, USA) and the peak-to-peak voltages (V_{p-p}) were measured. These values
30 were then calculated into sound pressure levels (dB $_{p-p}$ re 1 μ Pa) (Au, 1993). This V_{p-p} was
31 converted to estimate RMS by subtracting 15 dB. This was taken as the RMS voltage and used to

1 calculate the SPL for that frequency (Au, 1993; Nachtigall et al., 2005). Calibrations were tested
2 with the suction cup, and neither the suction cup nor the gel impacted the received sound levels
3 of the stimuli.

4 5 *AEP measurements*

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

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

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

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

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

14

15 *Data analysis*

16 Recorded EFR waveforms were first viewed relative to time. Response amplitude was
17 also examined in the frequency domain by calculating a 256-point FFT of the response
18 waveforms (FFT of the EFR). Only, a 16-ms portion of the EFR, from 5 to 21 ms, was used for
19 the FFT. This window contained 256 response samples and the majority of the stimulus period
20 while allowing for the delay of the EFR relative to stimulus onset. The FFT-EFR provided a
21 measure of the animal’s physiological response to the frequency being tested when a peak was
22 detected at the 1000 Hz modulation rate of the signal. Thus a larger EFR response was reflected
23 as a higher peak value. The peak value was used to estimate the magnitude of the response
24 evoked by the SAM stimulus. These values were then plotted as response intensity against SPL
25 of the stimulus at a given frequency. A regression line addressing the data points was
26 hypothetically extended to zero (horizontal axis intercept of the regression), the theoretical point
27 where there would be no response to the stimulus and the arbitrary definition of hearing
28 threshold. In a near-threshold range, these points can be reasonable approximated by straight
29 regression lines ($r^2 = 0.97$ in Fig. 1) with the five points with the highest r^2 value used to
30 calculate the regression (Mooney et al., 2009a; Nachtigall et al., 2007a; Nachtigall et al., 2007b;
31 Supin et al., 2001). The stimulus SPL value corresponding to the estimated zero FFT-EFR, was

1 the estimated hearing threshold for each of the frequencies presented to the animal as described
2 in Supin et al. (2001). From these thresholds, audiograms could then be established for each
3 animal.

4 Physiological noise levels were quantified for each animal by calculating the rms value
5 for a 16 ms window for five AEP records for each animal. This window length was chosen
6 because it equaled the FFT window for threshold determinations. Records used were the
7 minimum sound level for five separate frequencies and no responses (waveforms or FFT peaks)
8 were evident at these levels (or 10 dB above). Five records were averaged because animals were
9 presented with at least five frequencies, facilitating comparisons of the mean rms value for each
10 animal's neurophysiological responses (Table 1). These values can generally be taken as the
11 noise level at the modulation rate FFT. But because noise values often decreased across the FFT
12 spectrum, noise value at this frequency were more often less than 0.01 μV peak value. Analyses
13 were conducted using EXCEL, Matlab and MINITAB software.

14

15 *Background noise measurements*

16 In order to describe the background noise levels of the acoustic environment where the
17 sampled belugas inhabit, background acoustic noise in the bay was recorded using a DSG-Ocean
18 acoustic data-logger (Loggerhead Instruments, Sarasota, FL, USA) with a HTI- 96-Min
19 hydrophone (High Tech Inc. Gulfport, MS, USA) with -185.8 dB re 1V/ μPA receiving
20 sensitivity and frequency response of ± 1 dB from 2 Hz to 40 kHz. The system has a frequency
21 response of ± 0.7 dB from 20 Hz to 40 kHz. The acoustic data-logger was set to record
22 continuously at 80 kHz sample rate and was deployed for 4 days while the beluga captures took
23 place. The data-logger was deployed 1 m from the seafloor attached to a pile during low tide in
24 an unused cannery pier in Dillingham, AK, facing open water. This site was 3 km (mean) from
25 the the capture sites (stdv 0.9, max 5 km, min 2 km). This location was expected to be similar
26 but perhaps slightly higher in ambient noise levels than most capture site because of proximity to
27 the town. Recordings for analysis were selected based on the sea state and the tide cycle. During
28 the selection, recordings were manually scanned to check quality, confirm that the instrument
29 was below the surface and check whether anthropogenic noise sources were absent. A total of 45
30 min of recordings were selected from September 8th and 9th 2012, corresponding to periods of
31 sea state 0-1 in ebbing (15 min), high (15 min) and flooding (15 min) tidal cycles. Recordings

1 were analyzed in SpectraPRO 732 (Sound Technology Corporation). The selected 45 min of raw
2 data were transformed to instantaneous pressure in μPa using the analog-to-digital conversion
3 factor, amplification gain and hydrophone receiving sensitivity. Sound pressure level spectrum
4 (in dB re $1\mu\text{Pa}$) from 4 kHz to 40 kHz was estimated using the Fast Fourier transform algorithm
5 with a Hanning window of 65536 samples with 50% overlap, providing a frequency resolution of
6 1.2 Hz and a time resolution of 0.4 sec.

7
8

9 Symbols and abbreviations:

- 10 AEP – Auditory evoked potential
- 11 ASSR – Auditory steady state response
- 12 FFT – fast Fourier transform
- 13 EFR – envelope following response
- 14 SAM – Sinusoidally amplitude modulated

15
16

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3
4

5 **References**

- 6 **Andre, M., Supin, A. Y., Delory, E., Kamminga, C. and Degollada, E.** (2003). Evidence of
7 deafness in a striped dolphin, *Stenella coeruleoalba*. *Aquatic Mammals* **29**, 3-8.
- 8 **Au, W. W. L.** (1993). The sonar of dolphins. New York: Springer.
- 9 **Awbrey, F. T., Thomas, J. A. and Kastelein, R. A.** (1988). Low-frequency underwater hearing
10 sensitivity in belugas, *Delphinapterus leucas*. *Journal of the Acoustical Society of*
11 *America* **84**, 2273-2275.
- 12 **Bartol, S. M., Musick, J. A. and Lenhardt, M. L.** (1999). Auditory evoked potentials of the
13 loggerhead sea turtle (*Caretta caretta*). *Copeia* **3**, 836-840.
- 14 **Brittan-Powell, E. F., Dooling, R. J. and Gleich, O.** (2002). Auditory brainstem responses
15 (ABR) in adult budgerigars (*Melopsitacus undulatus*). *J. Acoust. Soc. Am.* **112**, 999-1008.
- 16 **Burkhard, R. F., Eggermont, J. J. and Don, M.** (2007). Auditory evoked potentials: Basic
17 principles and clinical applications. Philadelphia, PA: Lippincott, Williams and Wilkins.
- 18 **Cook, M. L. H., Verela, R. A., Goldstein, J. D., McCulloch, S. D., Bossart, G. D., Finneran,**
19 **J. J., Houser, D. S. and Mann, D. A.** (2006). Beaked whale auditory evoked potential
20 hearing measurements. *J. Comp. Physiol. A.* **192**, 489-495.
- 21 **DFO.** (2012). Recovery strategy for the beluga whale (*Delphinapterus leucas*) St. Lawrence
22 estuary population in Canada. Species at Risk Act Recovery Strategy Series. Ottawa:
23 DFO.
- 24 **Dolphin, W. F. and Mountain, D. C.** (1992). The envelope following response: Scalp potentials
25 elicited in the mongolian gerbil using sinusoidally AM acoustic signals. *Hear. Res.* **58**,
26 70-78.
- 27 **Ellison, W., Southall, B., Clark, C. and Frankel, A.** (2012). A new context-based approach to
28 assess marine mammal behavioral responses to anthropogenic sounds. *Conserv. Biol.* **26**,
29 21-28.

- 1 **Erbe, C.** (2000). Detection of whale calls in noise: Performance comparison between a beluga
2 whale, human listeners, and a neural network *Journal of the Acoustical Society of*
3 *America* **108**, 297-303
- 4 **Erbe, C. and Farmer, D. M.** (1998). Masked hearing thresholds of a beluga whale
5 (*Delphinapterus leucas*) in icebreaker noise. *Deep-Sea Research II* **45**, 1373-1388.
- 6 **Ferrero, R. C., Moore, S. E. and Hobbs, R.** (2000). Development of beluga, *Delphinapterus*
7 *leucas*, capture and satellite tagging protocol in Cook Inlet, Alaska. *Marine Fisheries*
8 *Review* **62**, 112-123.
- 9 **Finneran, J. J., Carder, D. A., Dear, R., Belting, T., McBain, J., Dalton, L. and Ridgway, S.**
10 **H.** (2005a). Pure tone audiograms and possible aminoglycoside-induced hearing loss in
11 belugas (*Delphinapterus leucas*) *Journal of the Acoustical Society of America* **117**, 3936-
12 3943.
- 13 **Finneran, J. J., Dear, R., Belting, T., McBain, J., Dalton, L. and Ridgway, S. H.** (2005b).
14 Pure tone audiograms and possible aminoglycoside-induced hearing loss in belugas
15 (*Delphinapterus leucas*). *J. Acoust. Soc. Am.* **117**, 3936–3943.
- 16 **Finneran, J. J. and Houser, D. S.** (2006). Comparison of in-air evoked potential and
17 underwater behavioral hearing thresholds in four bottlenose dolphins (*Tursiops*
18 *truncatus*). *J. Acoust. Soc. Am.* **119**, 3181-3192.
- 19 **Finneran, J. J., Houser, D. S., Mase-Guthrie, B., Ewing, R. Y. and Lingenfelser, R. G.**
20 (2009). Auditory evoked potentials in a stranded Gervais' beaked whale (*Mesoplodon*
21 *europaeus*). *Journal of the Acoustical Society of America* **126**, 484–490.
- 22 **Finneran, J. J., Schlundt, C. E., Carder, D. A., Clark, J. A., Young, J. A., Gaspin, J. B. and**
23 **Ridgway, S. H.** (2000). Auditory and behavioral responses of bottlenose dolphins
24 (*Tursiops truncatus*) and white whales (*Delphinapterus leucas*) to impulsive sounds
25 resembling distant signatures nderwater explosions. *Journal of the Acoustical Society of*
26 *America* **108**, 417-431.
- 27 **Finneran, J. J., Schlundt, C. E., Carder, D. A. and Ridgway, S. H.** (2002a). Auditory filter
28 shapes for the bottlenose dolphin (*Tursiops truncatus*) and the white whale
29 (*Delphinapterus leucas*) derved with notched noise. *J. Acoust. Soc. Am.* **112**, 322-328.

- 1 **Finneran, J. J., Schlundt, C. E., Dear, R., Carder, D. A. and Ridgway, S. H.** (2002b).
2 Temporary shift in masked hearing thresholds in odontocetes after exposure to single
3 underwater impulses from a seismic watergun. *J. Acoust. Soc. Am.* **111**, 2929-2940.
- 4 **Gerstein, E. R., Gerstein, L., Forsythe, S. E. and Blue, J. E.** (1999). The underwater
5 audiogram of the West Indian manatee (*Trichechus manatus*). *J. Acoust. Soc. Am.* **105**,
6 3575-3583.
- 7 **Houser, D. S. and Finneran, J. J.** (2006a). A comparison of underwater hearing sensitivity in
8 bottlenosed dolphins (*Tursiops truncatus*) determined by electrophysiological and
9 behavioral methods. *J. Acoust. Soc. Am.* **120**, 1713-1722.
- 10 **Houser, D. S. and Finneran, J. J.** (2006b). Variation in the hearing sensitivity of a dolphin
11 population determined through the use of evoked potential audiometry. *J. Acoust. Soc.*
12 *Am.* **120**, 4090-4099.
- 13 **Houser, D. S., Gomez-Rubio, A. and Finneran, J. J.** (2008). Evoked potential audiometry of
14 13 Pacific bottlenose dolphins (*Tursiops truncatus gilli*). *Mar. Mamm. Sci.* **24**, 28-41.
- 15 **Johnson, C. S.** (1991). Hearing thresholds for periodic 60-kHz tone pulses in the beluga whale
16 *Journal of the Acoustical Society of America* **89**, 2996-3001.
- 17 **Kenyon, T. N., Ladich, F. and Yan, H. Y.** (1998). A comparative study of hearing in fishes: the
18 auditory brainstem response approach. *J. Comp. Physiol. A* **182**, 307-318.
- 19 **Klishin, V. O., Popov, V. V. and Supin, A. Y.** (2000). Hearing capabilities of a beluga whale,
20 *Delphinapterus leucas*. *Aquat. Mamm.* **26**, 212-228.
- 21 **Kloepper, L. N., Nachtigall, P. E., Gisiner, R. and Breese, M.** (2010). Decreased echolocation
22 performance following high-frequency hearing loss in the false killer whale (*Pseudorca*
23 *crassidens*). *J. Exp. Biol.* **213**, 3717-3722.
- 24 **Mann, D., Hill-Cook, M., Manire, C., Greenhow, D., Montie, E., Powell, J., Wells, R.,**
25 **Bauer, G., Cunningham-Smith, P., Lingenfeller, R. et al.** (2010). Hearing loss in
26 stranded odontocete dolphins and whales. *PLoS ONE* **5**, e13824.
- 27 **Mooney, T. A., Hanlon, R. T., Christensen-Dalsgaard, J., Madsen, P. T., Ketten, D. R. and**
28 **Nachtigall, P. E.** (2010). Hearing by the longfin squid (*Loligo pealeii*) studied with
29 auditory evoked potentials: Sensitivity to low-frequency particle motion and not pressure.
30 *J. Exp. Biol.* **213**, 3748-3759.

- 1 **Mooney, T. A., Nachtigall, P. E., Breese, M., Vlachos, S. and Au, W. W. L.** (2009a).
2 Predicting temporary threshold shifts in a bottlenose dolphin (*Tursiops truncatus*): the
3 effects of noise level and duration. *J. Acoust. Soc. Am.* **125**, 1816–1826.
- 4 **Mooney, T. A., Nachtigall, P. E., Castellote, M., Taylor, K. A., Pacini, A. F. and Esteban, J.-**
5 **A.** (2008). Hearing pathways and directional sensitivity of the beluga whale,
6 *Delphinapterus leucas*. *J. Exp. Mar. Biol. Ecol.* **362**, 108–116.
- 7 **Mooney, T. A., Nachtigall, P. E., Taylor, K. A., Miller, L. A. and Rasmussen, M.** (2009b).
8 Comparative auditory temporal resolution of the white-beaked dolphin (*Lagenorhynchus*
9 *albirostris*). *J. Comp. Physiol. A.* **195**, 375–384.
- 10 **Mooney, T. A., Yamato, M. and Branstetter, B. K.** (2012a). Hearing in cetaceans: From
11 natural history to experimental biology. *Advances in Marine Biology* **63**, 197–246.
- 12 **Mooney, T. A., Yamato, M. and Branstetter, B. K.** (2012b). Hearing in cetaceans: From
13 natural history to experimental biology. *Advances in Marine Biology* **63**, 198–246.
- 14 **Moore, S., Reeves, R., Southall, B., Ragen, T., Suydam, R. and Clark, C. W.** (2012). A New
15 Framework for Assessing the Effects of Anthropogenic Sound on Marine Mammals in a
16 Rapidly Changing Arctic. *BioScience* **62**, 289–295.
- 17 **Moore, S. E. and Huntington, H. P.** (2008). Arctic marine mammals and climate change:
18 impacts and resilience. *Ecological Applications* **18**, S157–S165.
- 19 **Mulsow, J. and Reichmuth, C. J.** (2010). Psychophysical and electrophysiological aerial
20 audiograms of a Steller sea lion (*Eumetopias jubatus*). *J. Acoust. Soc. Am.* **127**, 2692–
21 2701.
- 22 **Nachtigall, P. E., Lemonds, D. W. and Roitblat, H. L.** (2000). Psychoacoustic studies of
23 dolphin and whale hearing. In *Hearing by whales and dolphins*. (ed. W. W. L. Au, A. N.
24 Popper and R. J. Fay), pp. 330–363. New York: Springer-Verlag.
- 25 **Nachtigall, P. E., Mooney, T. A., Taylor, K. A., Miller, L. A., Rasmussen, M., Akamatsu, T.,**
26 **Teilmann, J., Linnenschidt, M. and Vikingsson, G. A.** (2008). Shipboard
27 measurements of the hearing of the white-beaked dolphin, *Lagenorhynchus albirostris*. *J.*
28 *Exp. Biol.* **211**, 642–647.
- 29 **Nachtigall, P. E., Mooney, T. A., Taylor, K. A. and Yuen, M. M. L.** (2007a). Hearing and
30 auditory evoked potential methods applied to odontocete cetaceans. *Aquat. Mamm.* **33**, 6–
31 13.

- 1 **Nachtigall, P. E., Supin, A. Y., Amundin, M., Roken, B., Moller, T., Mooney, T. A., Taylor,**
2 **K. A. and Yuen, M. M. L.** (2007b). Polar bear *Ursus maritimus* hearing measured with
3 auditory evoked potentials. *J. Exp. Biol.* **210**, 1116-1122.
- 4 **Nachtigall, P. E., Supin, A. Y., Pawloski, J. L. and Au, W. W. L.** (2004). Temporary
5 threshold shifts after noise exposure in the bottlenose dolphin (*Tursiops truncatus*)
6 measured using evoked auditory potentials. *Mar. Mamm. Sci.* **20**, 673-687.
- 7 **Nachtigall, P. E., Yuen, M. M. L., Mooney, T. A. and Taylor, K. A.** (2005). Hearing
8 measurements from a stranded infant Risso's dolphin, *Grampus griseus*. *J. Exp. Biol.* **208**,
9 4181-4188.
- 10 **National Academy of Sciences.** (2003). Ocean noise and marine mammals. Washington, DC:
11 National Academies Press.
- 12 **National Academy of Sciences.** (2005). Marine mammal populations and ocean noise:
13 Determining when noise causes biologically significant effects. Washington, DC:
14 National Academies Press.
- 15 **National Marine Fisheries Service.** (2008). Conservation Plan for the Cook Inlet beluga whale
16 (*Delphinapterus leucas*). Juneau, Alaska: National Marine Fisheries Service.
- 17 **Norman, S., A., Goertz, C. E. C., Burek, K. A., Quakenbush, L. T., Cornick, L. A.,**
18 **Romano, T. A., Spoon, T., Miller, W., Beckett, L. A. and Hobbs, R. C.** (2012).
19 Seasonal hematology and serum chemistry of wild beluga whales (*Delphinapterus*
20 *leucas*) in Bristol Bay, Alaska, USA. *J. Wildl. Dis.* **48**, 21-32.
- 21 **Pacini, A. F., Nachtigall, P. E., Kloepper, L. N., Linnenschmidt, M., Sogorb, A. and Matias,**
22 **S.** (2010). Audiogram of a formerly stranded long-finned pilot whale (*Globicephala*
23 *melas*) measured using auditory evoked potentials. *Journal of Experimental Biology* **213**,
24 3138-3143.
- 25 **Pacini, A. F., Nachtigall, P. E., Quintos, C. T., Schofield, T. D., Look, D. A., Levine, G. A.**
26 **and Turner, J. P.** (2011). Audiogram of a stranded Blainville's beaked whale
27 (*Mesoplodon densirostris*) measured using auditory evoked potentials. *J. Exp. Biol.* **214**,
28 2409-2415.
- 29 **Richardson, W. J., Greene Jr., C. R., Malme, C. I. and Thomson, D. H.** (1995). Marine
30 Mammals and Noise. San Diego: Academic.

- 1 **Ridgway, S. H., Carder, D. A., Kamolnick, T., Smith, R. R., Schlundt, C. E. and Elsberry,**
2 **W. R.** (2001). Hearing and whistling in the deep sea: depth influences whistle spectra but
3 does not attenuate hearing by white whales (*Delphinapterus leucas*) (Odontoceti,
4 Cetacea). *J. Exp. Biol.* **204**, 3829-3841.
- 5 **Schlundt, C. E., Finneran, J. J., Carder, D. A. and Ridgway, S. H.** (2000). Temporary shift in
6 masked hearing thresholds of bottlenose dolphins, *Tursiops truncatus*, and white whales,
7 *Delphinapterus leucas*, after exposure to intense tones. *J. Acoust. Soc. Am.* **107**, 3496-
8 3508.
- 9 **Southall, B. L., Bowles, A. E., Ellison, W. T., Finneran, J. J., Gentry, R. L., Greene Jr, C.**
10 **R., Kastak, D., Ketten, D. R., Miller, J. H., Nachtigall, P. E. et al.** (2007). Marine
11 mammal noise exposure criteria: Initial scientific recommendations. *Aquatic Mammals*
12 **33**, 411-521.
- 13 **Supin, A. Y. and Popov, V. V.** (2007). Improved techniques of evoked-potential audiometry in
14 odontocetes. *Aquat. Mamm.* **33**, 14-23.
- 15 **Supin, A. Y., Popov, V. V. and Mass, A. M.** (2001). The sensory physiology of aquatic
16 mammals. Boston: Kluwer Academic Publishers.
- 17 **Szymanski, M. D., Bain, D. E., Kiehl, K., Pennington, S., Wong, S. and Henry, K. R.** (1999).
18 Killer whale (*Orcinus orca*) hearing: Auditory brainstem response and behavioral
19 audiograms. *J. Acoust. Soc. Am.* **106**, 1134-1141.
- 20 **Turl, C. W., Penner, R. H. and Au, W. W. L.** (1987). Comparison of target detection
21 capabilities of the beluga and bottlenose dolphin. *J. Acoust. Soc. Am.* **82**, 1487-1491.
- 22 **Tyack, P. L. and Clark, C. W.** (2000). Communication and acoustic behavior of dolphins and
23 whales. In *Hearing By Whales and Dolphins*. (ed. W. W. L. Au, A. N. Popper and R. R.
24 Fay), pp. 157-224. New York: Springer.
- 25 **Webster, D. B., Fay, R. R. and Popper, A. N.** (1992). The evolutionary biology of hearing, pp.
26 591. New York: Springer-Verlag.
- 27 **White, J., M.J., Norris, J. C., Ljungblad, D. K., Barton, K. and di Sciara, G. N.** (1978).
28 Auditory thresholds of two beluga whales (*Delphinapterus leucas*). In *Hubbs/Sea World*
29 *Research Institute Technical Report*, pp. 78-109. San Diego, CA: Hubbs Marine
30 Research Institute.
- 31 **Yost, W. A.** (1994). Fundamentals of hearing: An introduction. New York: Academic Press.

1 **Yuen, M. M. L., Nachtigall, P. E., Breese, M. and Supin, A. Y.** (2005). Behavioral and
2 auditory evoked potential audiograms of a false killer whale (*Pseudorca crassidens*). *J.*
3 *Acoust. Soc. Am.* **118**, 2688-2695.

4
5

1 Table 1. Morphometric measurements, sex, hearing thresholds. sampling duration and physiological noise levels for all belugas.
2

	Beluga #1	Beluga #2	Beluga #3	Beluga #4	Beluga #5	Beluga #6	Beluga #7	
	female	male	female	male	male	female	male	
	subadult	adult	adult	adult	adult	adult	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 μ Pa)							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 (μ V, 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

3

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

7
8 Figure 2. AEP audiograms of all seven wild belugas and Bristol Bay background noise
9 spectrum. Sound pressure levels are in dB re 1 μ Pa.

10
11 Figure 3. (A) The mean audiogram \pm s.d and Bristol Bay background noise spectrum (grey
12 dashed line). (B) Composite audiograms constructed by plotting the thresholds of maximum
13 (black, diamonds) and minimum sensitivity (grey triangles) and Bristol Bay background noise
14 spectrum (grey dashed line). (C) The standard deviation (s.d.) difference of thresholds at
15 measured hearing frequencies and fitted power trend line. S.d. values increased with frequency.
16 Sound pressure levels are in dB re 1 μ Pa. (D) Fourth order polynomial trend curve ($y = -1E-06x^4$
17 $+ 0.0003x^3 - 0.0168x^2 - 0.2966x + 85.832$; $R^2 = 0.6919$) for all collected thresholds and
18 frequencies and Bristol Bay background noise spectrum (grey dashed line).

19
20 Figure 4. Mean wild beluga audiogram \pm s.d. (black, circles) compared to the audiograms (grey
21 and/or open symbols) from belugas held in laboratory conditions or in aquaria. Other audiograms
22 include: (White et al., 1978)-squares, (Awbrey et al., 1988)-stars, (Mooney et al., 2008)-circles,
23 (Klishin et al., 2000)-triangles, (Finneran et al., 2005a)-x's, (Ridgway et al., 2001)-dashes. The
24 audiogram (x shapes) which cuts off near 50 kHz was considered a result of aminoglycoside

1 antibiotic treatment. All other audiograms are similar to the wild belugas. Sound pressure levels
2 are in dB re 1 μ Pa.

3
4 Figure 5. (A) Beluga #1 during an auditory evoked potential (AEP) hearing experimental
5 session. The whale is facing right. The three suction-cup attached sensors (right to left are active
6 sensor, invert sensor and ground) are visible and attached to the typical locations on the animal.

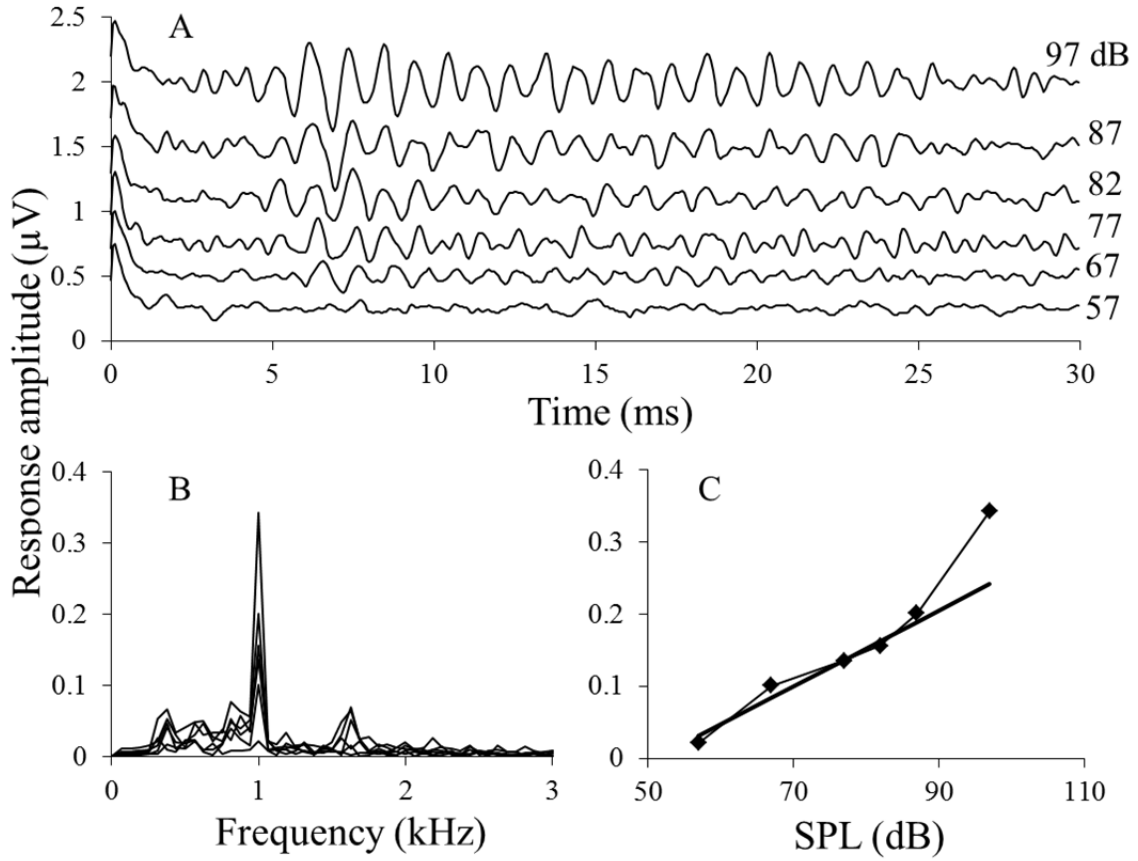
7 (B) The AEP equipment being operated in its ruggedized case in the small inflatable boat while
8 the whale is positioned adjacent to left (not visible).

9

1

2 Figure 1.

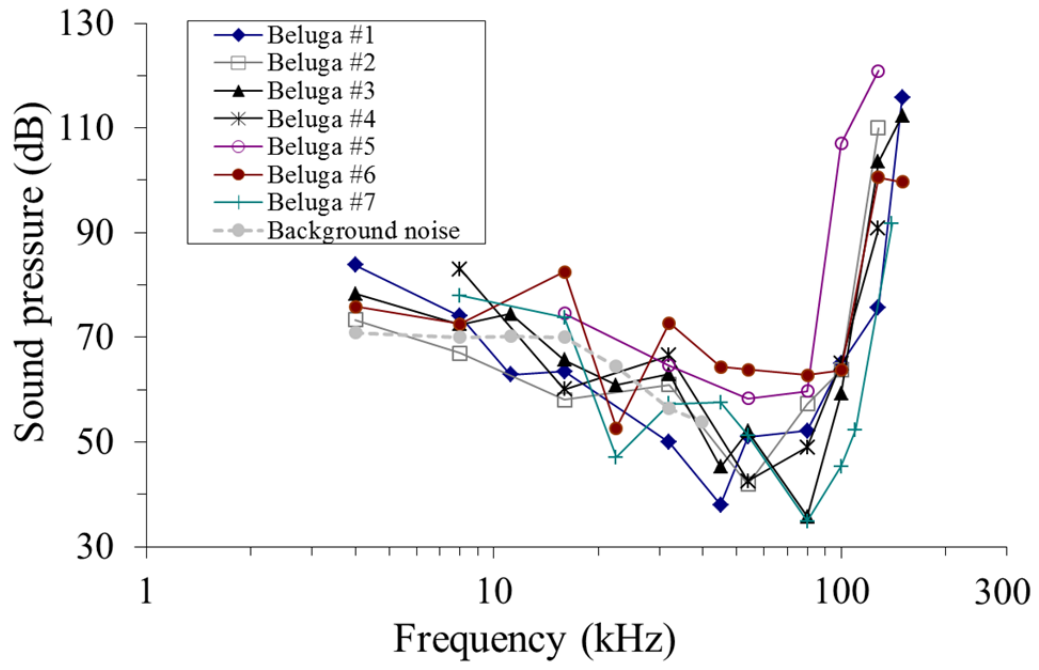
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1 Figure 2.

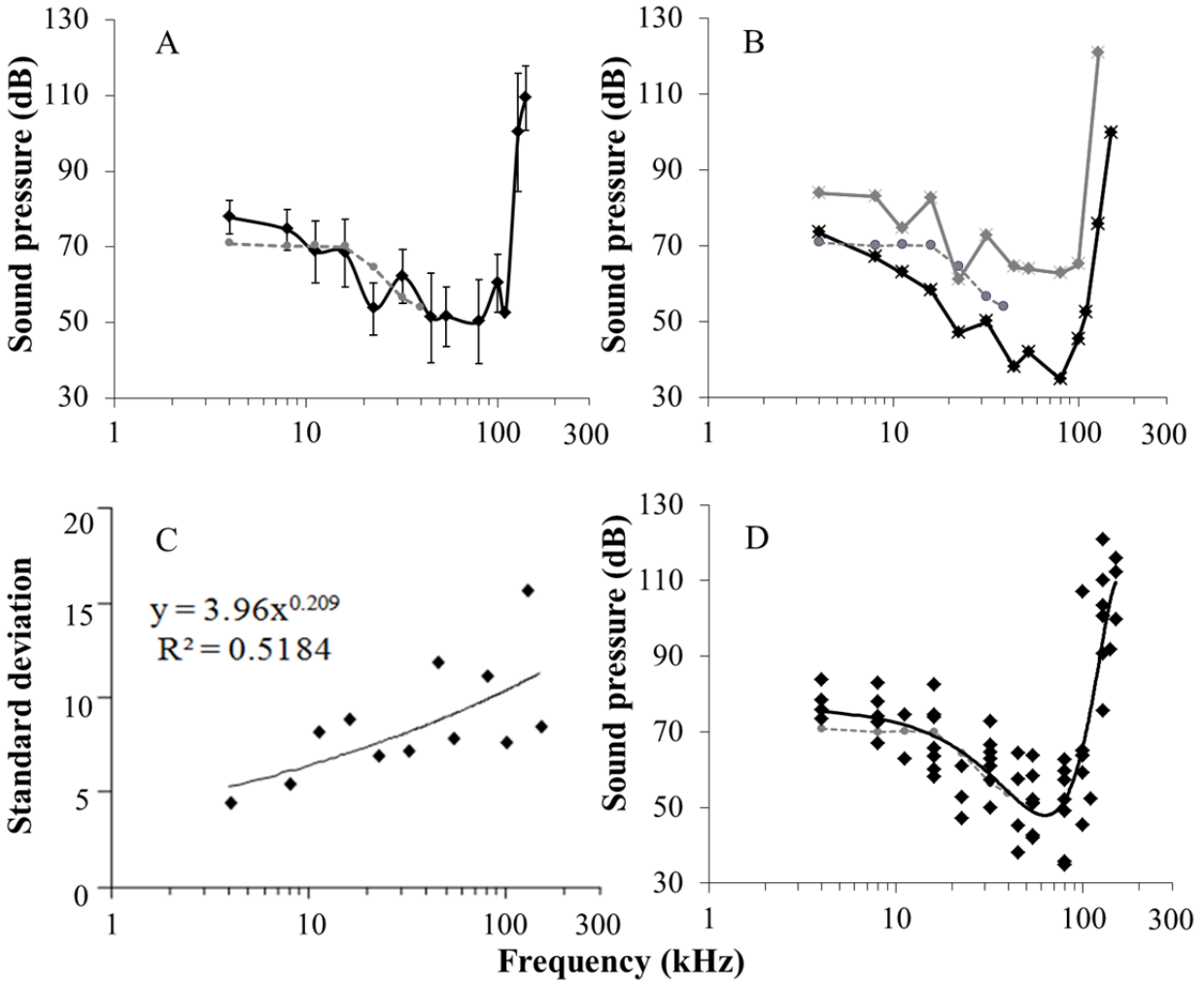
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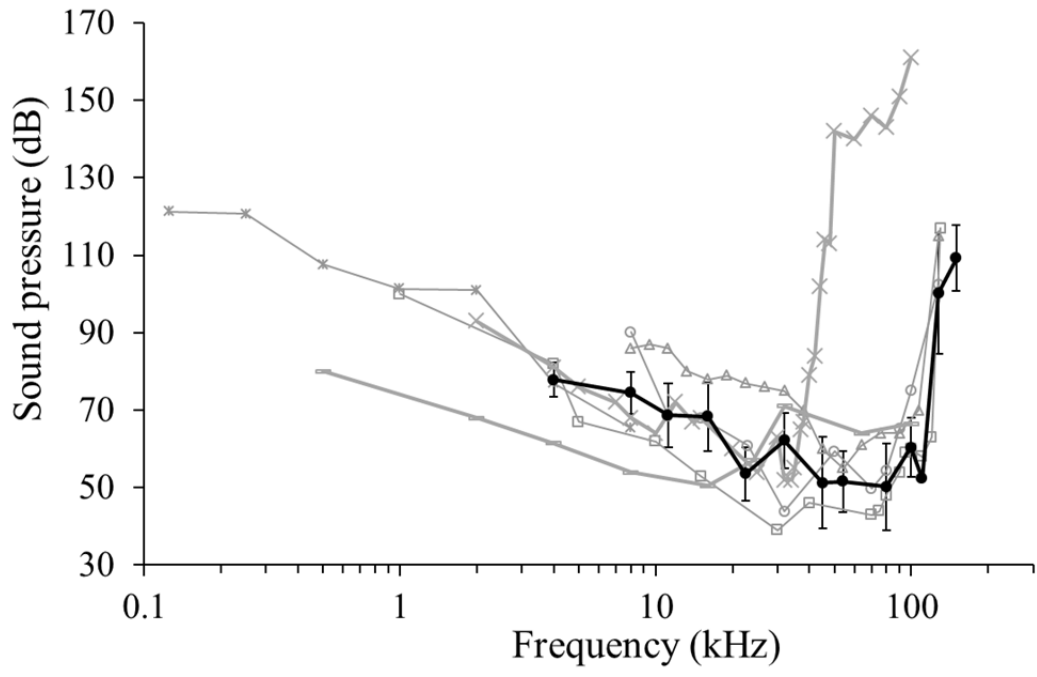
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1
2 Figure 3.



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2 Figure 4.



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1 Figure 1.

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