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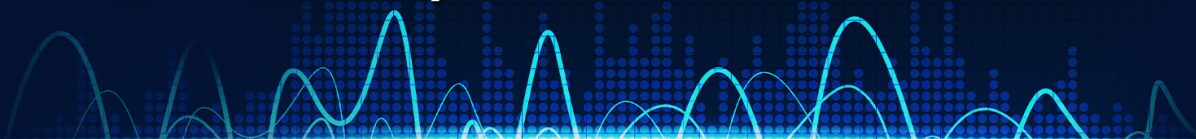
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Audiogram of a Cook Inlet beluga whale (*Delphinapterus leucas*)

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ABSTRACT:

Noise is a stressor to wildlife, yet the precise sound sensitivity of individuals and populations is often unknown or unmeasured. Cook Inlet, Alaska belugas (CIBs) are a critically endangered and declining marine mammal population. Anthropogenic noise is a primary threat to these animals. Auditory evoked potentials were used to measure the hearing of a wild, stranded CIB as part of its rehabilitation assessment. The beluga showed broadband (4–128 kHz) and sensitive hearing (<80 dB) for a wide-range of frequencies (16–80 kHz), reflective of a healthy odontocete auditory system. Data were similar to healthy, adult belugas from the comparative Bristol Bay population (the only other published data set of healthy, wild marine mammal hearing). Repeated October and December 2017 measurements were similar, showing continued auditory health of the animal throughout the rehabilitation period. Hearing data were compared to pile-driving and container-ship noise measurements made in Cook Inlet, two sources of concern, suggesting masking is likely at ecologically relevant distances. These data provide the first empirical hearing data for a CIB allowing for estimations of sound-sensitivity in this population. The beluga's sensitive hearing and likelihood of masking show noise is a clear concern for this population struggling to recover.

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I. INTRODUCTION

Belugas use sound rather than sight for many important functions. As a high latitude species they are often found in turbid coastal waters and waters where darkness and sea ice extends for many months. Thus, sound and hearing play crucial roles in navigation, feeding, predator avoidance, and social communication. Consequently, changes in ambient noise levels may have a large impact on their ability to thrive. Anthropogenic noise will increasingly threaten the northern high-latitude seas and habitats, as the Northwest Passage remains seasonally open due to shrinking ice coverage, which will allow significant increases in shipping along Alaska's North Slope as well as concurrent seismic exploration for hydrocarbon material concentrated offshore (Beauregard-Tellier, 2008).

The CIB population was listed as endangered under the U.S. Endangered Species Act in 2008 after a rapid decline attributed to the unregulated subsistence hunt (National

Marine Fisheries Service, 2008). Ten years later, this population of 279 whales continues with a steady decline of –2.3% per year, over the last 10 years (Shelden and Wade, 2019). Unless the factors that are impeding recovery are determined and mitigated, this population of whales will be lost (Hobbs *et al.*, 2006).

A considerable body of research documents anthropogenic noise impacts on odontocete communication and habitat use, specifically spatial displacement (Nowacek *et al.*, 2007), which cannot be excluded for CIBs (Small *et al.*, 2017). Cook Inlet is perhaps the most densely populated and fastest growing embayment in Alaska. Increased industrial and commercial activities, habitat degradation, and global warming/climate change are threatening the CIB population and its critical habitat. Massive infrastructure projects in Anchorage and other coastal towns typically have the potential to directly impact CIB's critical habitat. Oil and gas exploration and production, commercial fisheries, increasing commercial maritime traffic, industrial development, and the underwater noise generated by all these activities are damaging and modifying CIB habitat, contributing to its lack of recovery.

Man-made sources of noise in CI include large and small vessels, aircraft, oil and gas operations marine seismic

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surveys, pile driving, and dredging (Blackwell and Greene, 2002; Castellote *et al.*, 2018). This noise may result in disturbance and harassment, altering movements or avoidance of areas important to their life history, and/or causing chronic effects such as temporary or permanent damage to a whale's hearing (Southall *et al.*, 2008). Geophysical seismic activity has been described as one of the loudest man-made underwater noise sources, with the potential to harass or harm the CIBs, including temporary or permanent hearing loss (National Marine Fisheries Service, 2008). Permanent sources of noise in some areas of the CIB critical habitat, such as shipping, oil and gas production, or dredging, introduce significant noise with the potential for negative effects due to chronic exposure (Castellote *et al.*, 2018). Even low levels of noise may have biological impacts by "masking" (e.g., hiding or overwhelming) important communication signals, influencing communication behaviors and disrupting foraging (Jensen *et al.*, 2009; Lusseau *et al.*, 2009; Fouda *et al.*, 2018; Wisniewska *et al.*, 2018).

A major detraction to determine noise effects on this population is our poor understanding of hearing sensitivity for any CIB whale. With limited hearing data in many marine mammal populations, noise effects may be considered tenuous because insufficient information limits extrapolations between species and populations. Auditory differences among populations exist among some frog species. For example, auditory tuning (neuronal response amplitude at particular frequencies) is related to sexual selection, with increased responses for certain call frequencies and population-based female preference can play a role in selecting male call frequency (Ryan *et al.*, 1992). Similar population-based auditory sensitivity tuning differences appear based upon background noise conditions of local habitats (Witte *et al.*, 2005). Chemical pollution (and noise pollution) may impact auditory hair cells (Choi and Grandjean, 2008; Low and Higgs, 2015) and presumably this could also influence population-based differences. Such data suggests population based auditory studies are important to assess the sensory health of at-risk populations such as the CIB.

Studies on captive belugas established baseline auditory information, including the audiogram, masked hearing thresholds, temporary threshold shift phenomena, auditory filter shapes, and hearing pathways (Awbrey *et al.*, 1988; Klishin *et al.*, 2000; Finneran *et al.*, 2002; Mooney *et al.*, 2008). Hearing studies on wild, healthy adult Bristol Bay (BB) belugas offer a key comparison because these animals reside in somewhat pristine environment (Castellote *et al.*, 2014; Mooney *et al.*, 2016; Mooney *et al.*, 2018a; Mooney *et al.*, 2018b). The audiograms and variability in sensitivity offer a key baseline from which there may be comparisons to other belugas from other populations.

In 2017, we had the opportunity to diagnostically assess the hearing of a live stranded CIB calf. We used the same physiological auditory evoked potentials (AEPs) method as in the BB studies, which offers a direct comparison and evaluates for hearing loss. The AEP method involves

measuring small voltages generated by the peripheral and central auditory structures in response to various sound stimuli (Supin *et al.*, 2001). This technique compares to traditional behavioral methods (Yuen *et al.*, 2005), yet has the advantage of allowing rapid threshold measurements from untrained animals to diagnostically assess auditory health (Nachtigall *et al.*, 2005; Mooney *et al.*, 2006). Beluga responses are acquired non-invasively from the surface of the skin using electroencephalography (EEG) recording electrodes embedded in latex suction cups. AEPs have been applied to measure hearing in diverse taxa, including invertebrates, fish, birds, and mammals, as well as human infants (Kenyon *et al.*, 1998; Brittan-Powell *et al.*, 2002; Hall, 2007; Mooney *et al.*, 2010). In marine mammals, AEP systems are a well-established means to examine hearing, quickly and non-invasively.

The hearing results from the CIB calf were compared to BB beluga data (Castellote *et al.*, 2014; Mooney *et al.*, 2018a; Mooney *et al.*, 2018b). The hearing tests were diagnostic, one of several measurements collected that describes the health of the CIB calf. Similar hearing evaluations were completed on other odontocetes (Nachtigall *et al.*, 2005; Finneran *et al.*, 2009; Mann *et al.*, 2010; Pacini *et al.*, 2010). For this CIB calf, the hearing evaluation was done just days after the reported stranding to determine its immediate hearing abilities. A follow-up examination occurred two months after medical treatment, as part of his diagnostic evaluation for possible release.

II. METHODS

A. Stranding summary

Mid-afternoon, on 30 September 2017, a gray beluga whale was observed above the waterline in Trading Bay, south of McArthur River, on the west side of Cook Inlet (60.88 N, 151.73 W). Upon closer inspection, it was a live calf with moderate external physical trauma. Attempts to encourage swimming into the inlet were unsuccessful and no other belugas were visible in the area. The calf was assessed to be a new male calf with minor injuries, and because it was too young to survive on his own, the recommendation was to attempt rehabilitation. Rescue was authorized by the National Oceanographic and Atmospheric Administration. This calf was transported by helicopter to Anchorage and by truck to the Alaska SeaLife Center, Seward, for immediate care and rehabilitation; it was also the location of this study. Based on morphological characteristics (length 162 cm and 64 kg) and physiological parameters, the calf was aged at 17 days (anywhere between 10 to 30 days old). He was slightly malnourished, traumatized, and at risk to develop infections secondary to the stranding event due to aspirating sea water and his immature immune system. Pneumonia was confirmed by a computed tomography (CT) scan. While under care the beluga developed a pneumothorax and various gastrointestinal problems, including constipation and bloat. To treat these conditions the beluga was treated with several medications including

antibiotics (including an aminoglycoside), antifungals, and gastrointestinal support (Goertz *et al.*, 2019). After seven weeks, the beluga calf was considered recovered from problems present at admittance and all medications were stopped. Because of his age and inability to survive on his own, he was declared non-releasable by the National Marine Fisheries Service, National Oceanic and Atmospheric Administration and placed in another zoological facility (SeaWorld, Texas) with other beluga whales for long-term care.

Hearing measurements were made on 4 and 5 October, and 5 and 6 December, 2017 using AEP recording methods, a means to rapidly, passively, and non-invasively, examine hearing (Supin *et al.*, 2001; Houser and Finneran, 2006; Finneran *et al.*, 2007; Nachtigall *et al.*, 2007; Mooney *et al.*, 2012). All hearing examinations were conducted in-water, where the calf was gently secured by gently holding the animal with its head free (Fig. 1). Water depths were 1 m in both rehabilitation pools where the data were collected. Data acquisition, calibrations, and analyses were designed to follow the previous beluga AEP hearing studies and American National Standards Institute (ANSI) standards for measuring odontocete audiograms (Castellote *et al.*, 2014; ANSI, 2018; Mooney *et al.*, 2018b). During each set of tests, hearing data were collected for two consecutive days (a portion of the audiogram each day). This limited the examination time that the calf needed to be still. As the calf's health improved between October and December, he was moved on 7 November from the smaller, indoor rehabilitation pool to a larger outdoor pool (Fig. 1). Calibrations of the audiogram tones and background noise measurements were conducted in each test tank.

B. Stimulus presentation and evoked potential recording

Once the beluga calf was properly stationed, three custom-built silicone suction cups (KE1300T, Shin-Etsu, Tokyo, Japan) with embedded gold electrodes (Grass Technologies, Warwick, RI) were attached using conductive

electrode gel (Signagel, Parker Laboratories, Fairfield, NJ). An active (non-inverting) electrode was placed along the midline of the animal 3–4 cm behind the blowhole. A reference electrode was placed on the dorsal peduncle, and a ground electrode was placed on the animal's tail fin. The electrodes were connected to a biological amplifier (CP511, Grass Technologies) which amplified all responses 10 000-fold and bandpass filtered them from 300 to 3000 Hz. A second Krohn-Hite 300–3000 Hz bandpass filter further conditioned the signal. This bioamplifier was connected to a BNC Breakout Box (2110, National Instrument, Austin, TX) and a PCMCIA-6062E data acquisition card implemented in a laptop computer. Using a custom LabView program (National Instruments), the data acquisition card converted the analog signal to a digital record at a 16 kHz sampling rate. All data were stored on the laptop computer.

Acoustic stimuli were created using the same custom LabView program, laptop, and data acquisition card. Outgoing signals were produced at a 512 kHz update rate for signals at 100 kHz and 750 kHz, for higher frequency tones. Signals tested included: 4, 5.6, 8, 11.2, 16, 22.5, 32, 45, 54, 80, 100, 120, 128, and 150 kHz. At the October test, no responses were detected at 128 kHz and thresholds were clearly increasing (at 100 and 120 kHz), thus we did not test higher frequencies. Testing the lower frequencies allowed for comparisons to previous beluga studies. Signal amplitudes were controlled using a HP 350D (Palo Alto, CA) attenuator and projected to the animal through a custom "jawphone" or suction-cup transducer. This consisted of a Reson 4013 transducer (Slangerup, Denmark) implanted in a custom-built silicone suction cup. The transducer was attached to the whale using the electrogel to eliminate reflective air gaps between the cup and the calf's skin.

The CIB was presented with broadband clicks at the start of each examination. Subsequently, all stimuli were amplitude modulated tones. All signals were presented in 20 ms bouts at a 20 s⁻¹ rate. Carrier signals within the bout were modulated (tones) or presented (clicks) at a rate of 1 kHz. The individual click stimuli were 20 μs in duration.

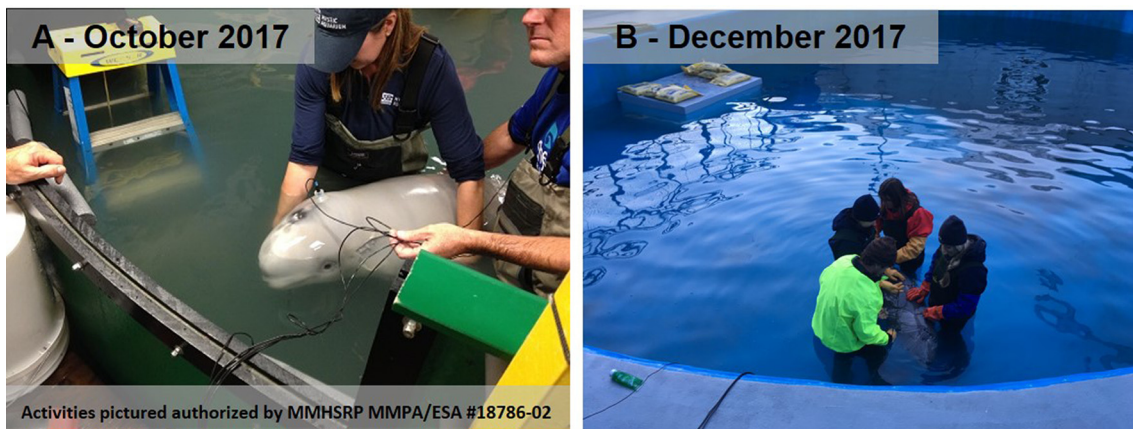


FIG. 1. (Color online) Diagnostic hearing evaluations of the Cook Inlet beluga. The studies were conducted (A) in an indoor rehabilitation facility in October 2017 and (B) a larger, outdoor facility in December 2017. In this photo the older beluga molted to a darker grey color. He is located between the rehabilitation staff, facing the wall.

Evoked response recordings were 30 ms duration and began to coincide with stimulus presentation. Stimuli were presented 1000 times for each sound level and a corresponding response was collected for each sound presentation. These 1000 responses were averaged using the custom software and stored for later data analyses. The total number of stimuli and sweeps is relatively standard for odontocetes because it establishes a robust response while averaging down the background noise to a relatively low level (Nachtigall *et al.*, 2007). A record for a particular frequency and sound level is then acquired in 50 s and thresholds are obtained in just a few minutes. These procedures were essentially identical to earlier beluga tests (Castellote *et al.*, 2014; Mooney *et al.*, 2018b).

C. Calibrations and data analysis

Suction-cup transducer stimuli were calibrated in the water in both test tanks. Received measurements were made using a DMON (Kaplan *et al.*, 2015) recording system. The projector and receiver were placed 35 cm apart at 15 cm depth. This was the approximate distance of auditory bulla to jawphone transducer placed at the rostrum tip, estimated from diagnostic CT scans. The use of the short-duration sinusoidally amplitude modulated (SAM) tone sweeps (i.e., several ms) and relatively low sound levels (i.e., reflections were significantly attenuated relative to the direct 1 m path) reduced the interference of overlapping standing waves and facilitated the calibration. For each frequency the received levels were calculated in dB_{RMS} re 1 μ Pa over the 20 ms tone pip duration at one attenuation setting. Proper attenuation was later checked by recording tones at multiple attenuation levels and verifying received levels matched predicted attenuation values. Sound pressure levels of the clicks were measured using V_{p-p} (Au 1993). Hereafter, dB levels of click sound pressure levels (SPLs) are presented in dB_{p-p} re: 1 μ Pa and SAM tone dB are presented in dB_{RMS} re: 1 μ Pa.

Response waveforms and power spectra were observed during the evaluation and sound levels were then decreased in steps of 5–10 dB, until responses were no longer visually detectable for 2–3 trials. However, actual threshold estimates were calculated offline, where a 16-ms portion of the response was fast-Fourier transformed (FFT) (256 sample points) and viewed in the frequency spectrum. The magnitude of the envelope following response was reflected by a peak in the FFT at the 1 kHz modulation rate (Supin and Popov, 1995). Thresholds were then calculated following two methods, a regression based method used in other beluga hearing-test studies (Mooney *et al.*, 2008; Mooney *et al.*, 2018b) and an *F*-statistic method (Dobie and Wilson, 1993; Finneran *et al.*, 2007). With respect to the regression based methods, briefly, the FFT-values at the modulation frequency were plotted as response intensity against SPL of the stimulus at a given frequency. A regression line addressing the data points was extended to hypothetical zero (horizontal axis intercept of the regression), the theoretical point where there would be no response to the stimulus. The

stimulus SPL value corresponding to the estimated zero response, 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. Similarly, the *F*-statistic method evaluates the response in the frequency domain, but here comparing the AEP amplitude at the modulation frequency (f_s) to a noise estimate obtained either from a control trial or concurrently with the AEP measurement; here we evaluated the noise power at adjacent frequencies. The ratio of the power at f_s to the noise power (P_n) provides the *F*-statistic which can be compared to a critical value (*F*-crit) using standard statistical tables. An AEP response is notable when the *F*-statistic is greater than the (*F*-crit). The threshold was taken as the last frequency at which such a response was observable.

D. Ambient noise recordings from Cook Inlet

We further compared the hearing thresholds of the CIBs to two types of anthropogenic noises frequently encountered in Cook Inlet. These recordings were not played to the beluga for masked hearing tests, rather they were recorded separately, prior to the hearing tests. They simply provided a comparison to estimate the potential for masking. Impact hammer pile driving noise was recorded by the mouth of Little Susitna River, May 3, 2016 at 7:39 pm. The noise was from a hydraulic impact hammer at the Port of Anchorage, 24 km away. The ship noise data were recorded near the mouth of Beluga River, on 16 August, 2010 at 10:25 am. The noise was likely a containership at an approximately 10 km. We selected 20 consecutive minutes of highest amplitude sound from the passing ship. The ambient data were assessed in power spectral density dB re 1 μ Pa²/Hz and in one-third octave bands, the approximate auditory filter bandwidth odontocete hearing (Erbe, 2008; Branstetter *et al.*, 2017).

III. RESULTS

Evoked potential response waveforms were quite similar in shape and latency to other belugas and odontocetes previously tested. Responses were clear and distinctly above the natural physiological noise at 20–30 dB, above an approximate threshold (Fig. 2). A series of early evoked potential waveforms were noted 3–5 ms after stimulus onset suggesting no obvious deficit to neurological structures. Primary responses to the later tones of a tone-pip train blended to form an envelope following response, which had a duration of approximately 20 ms, the same duration as the SAM tones, but ending about 5 ms after the tone quieted. Response amplitudes decreased as sound levels decreased. Response latency, noted in the earliest wave latencies, also appeared to decrease as stimulus level decreased.

The CIB audiogram was measured twice, first in October 2017, four days after it was brought to Seward and again in December 2017 [Fig. 3(A)]. Background noise conditions were measured for both test tanks. The audiograms

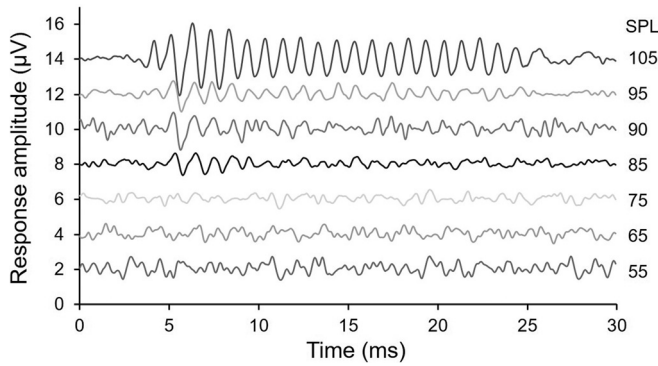


FIG. 2. Auditory evoked potential waveforms of a stranded Cook Inlet beluga calf. Test tone in this case was 80 kHz. Sound pressure levels used to evoke the responses are listed to the right.

were similar in shape and thresholds. Thresholds were lowest (below 70 dB) from 16 to 80 kHz. Although thresholds increased sharply at 80 and 100 kHz, responses were not detected at 128 kHz during the first evaluation, and not detected at 150 kHz during both exams. Thresholds increased at a lower rate in the lower frequencies, below 16 kHz. There seemed to be a small elevation in hearing at 45 kHz. Thresholds were calculated in two manners, using two comparable objective statistical methods [Fig. 3(B)]. Further, there was no substantial difference between the regression based methods and the F-statistic. In both cases, the mean seemed to smooth out some variability between the October and December measurements, reflecting and reinforcing the need to repeat audiogram measurements on rehabilitating or research animals, a task not often done.

The general shape of the audiogram for this CIB was similar to many other tested belugas. This includes wild belugas measured in Bristol Bay [Fig. 4(A)] and many belugas found in human-care facilities [Fig. 4(B)]. To enable comparisons, the CIB thresholds also were calculated using the regression-based methods, identical to those used in the

Bristol Bay studies. The lowest mean thresholds for both the Bristol Bay and CIBs were 54 and 80 kHz. Compared to the Bristol Bay adult belugas, the CIB calf thresholds were among the more sensitive thresholds but not the lowest beluga AEP hearing thresholds measured. At the frequencies of lowest mean thresholds, 54 and 80 kHz, 23% of Bristol Bay adult belugas had more sensitive thresholds than the CIB calf.

IV. DISCUSSION

Overall, the stranded CIB calf showed comparatively low thresholds and sensitive hearing (< 80 dB) at many frequencies across its two auditory evaluations. The audiogram shape and waveforms were generally reflective of a sensitive odontocete auditory system without substantial hearing loss. Yet the CIB calf was not quite as sensitive as some other wild belugas similarly measured. We are not certain why these differences exist. Given that the whale was quite young we did not expect presbycusis. Bristol Bay was generally a quiet test environment, free of noise from tank pumps and other laboratory features. The indoor tank did have somewhat elevated noise levels (Fig. 3), so it could be that the CIB hearing was masked by noise generated by the facility operations. However, the outdoor tank, during the December studies, was relatively quiet and the thresholds collected there were close to those measured in the indoor, noisier tank, suggesting this may be the CIB's actual hearing thresholds. Further, this variation falls within the natural variation of other hearing model taxa including bottlenose dolphins (*Tursiops truncatus*) and laboratory mice (*Mus musculus*) suggesting it is normal (Mooney *et al.*, 2009; Ohlemiller *et al.*, 2016).

We further compared the hearing thresholds of the CIB calf to measurements for two types of anthropogenic noise frequently encountered in upper Cook Inlet and the beluga's habitat (Fig. 5). These recordings were not played to the

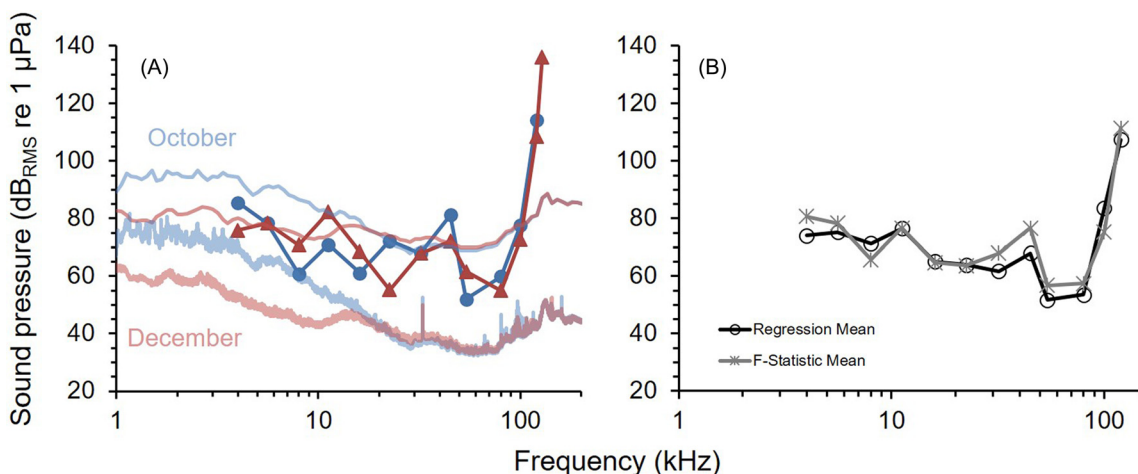


FIG. 3. (Color online) Comparison of two threshold detection methods. (A) Thresholds from October (blue circles) and December (red triangles) 2017 determined using the F-statistic method. Background noise of the test tanks were plotted in 1/12 octave bands (dB re: 1 μ Pa) thin blue and red lines and power spectral density dB re: 1 μ Pa²/Hz thick blue and red lines. (B) The mean of October and December thresholds determined using the F-statistic (grey, stars) and the regression of best fit (black, open circles). The mean audiograms greatly overlap in the thresholds determined.

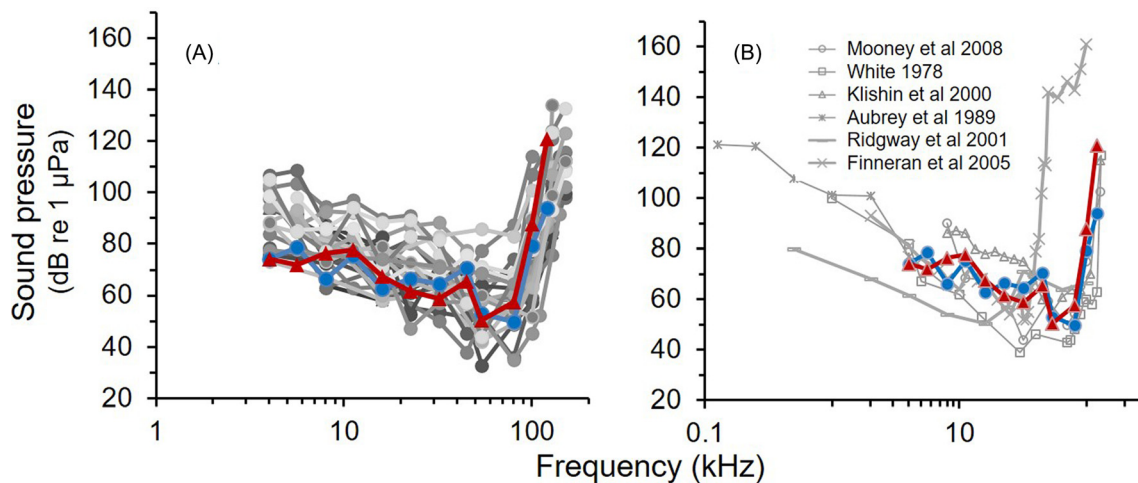


FIG. 4. (Color online) Comparison of Cook Inlet beluga audiograms to hearing thresholds of other belugas. The hearing of the CIB calf, was measured twice, in October (blue line and blue circles) and December (red line and red triangles) 2017 and are compared to the (A) hearing thresholds of wild beluga whales from Bristol Bay (Mooney *et al.*, 2018b) and (B) beluga whales housed in laboratory or public display facilities with respective references listed in the key and are plotted in faded grey. The CIB thresholds plotted here were calculated using regression methods.

beluga for potential masked hearing tests, rather they were recorded separately, and prior to, the hearing tests. While the power spectral density values were clearly below that of auditory thresholds, noise data in one-third octave bands were substantially higher than beluga thresholds, at least at the frequency range overlap between the two noise data-sets and the beluga auditory thresholds. While we lack true masking experimental data, this overlap suggests some level of masking of near-threshold sounds would likely occur from these noise sources even at distances of several kilometers, particularly in the frequencies of communication and navigation sounds. At the very least this overlap underscores the need for auditory masking studies to determine the extent of potential masking. We would need higher frequency sample rate recordings to address potential masking of echolocation signals, and the more sensitive ranges for beluga hearing. While it seems clear that CIBs can hear these sounds, masking is a complex phenomenon with many

aspects coming into play including animal orientation, temporal aspects of the masker, masking release mechanisms including abilities (or limitations) to achieve higher order processing such as discrimination (Branstetter and Finneran, 2008; Trickey *et al.*, 2010; Branstetter *et al.*, 2013; Dooling and Blumenrath, 2013; Erbe *et al.*, 2016). The overlap here provides initial insight into the likelihood of masking by these noise types. Even these limited data and their overlap raises concerns for noise limiting the acoustic communication space for CIBs. Beyond masking, this may cause other effects such as disturbance, displacement, and stress. Castellote *et al.* (2018) presents a more exhaustive analysis of the anthropogenic noise occurrence in CIB critical habitat. Castellote *et al.* (2018) concluded that permanently occurring activities (e.g., shipping, dredging) exceed hearing thresholds, as well as behavioral harassment levels, on a daily basis in a significant portion of CIB critical habitat. These results confirm CIB hearing thresholds are shaped by

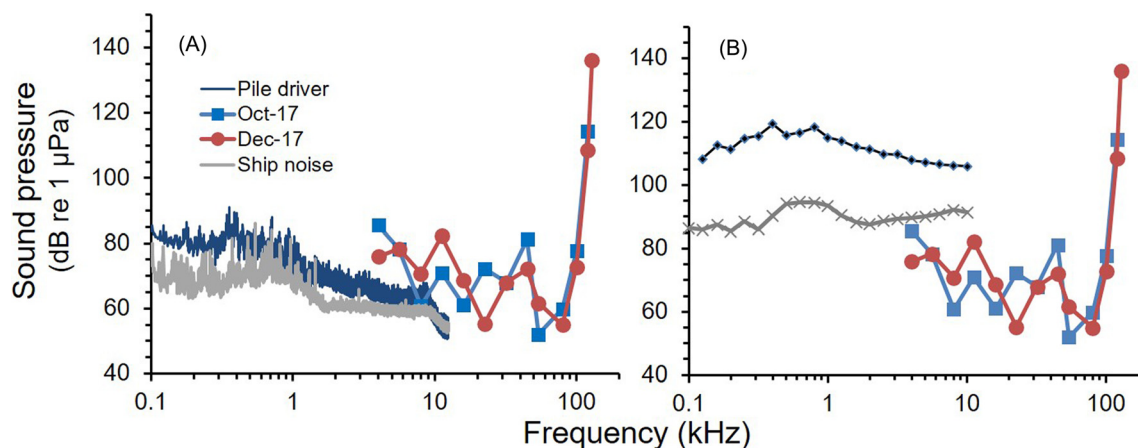


FIG. 5. (Color online) Hearing of the stranded Cook Inlet beluga compared to two types anthropogenic noise recorded in Cook Inlet. Noise is plotted in (A) 1-Hz and (B) one-third octave band noise. In this case, the F-statistic method was used to calculate these thresholds. The CIB calf was not actually exposed to these noises.

background noise levels across much of their hearing range, as in other beluga populations (Mooney *et al.*, 2018a). Anthropogenic increase in background noise levels (i.e., dredging, shipping, pile driving) will unavoidably alter CIB hearing abilities.

Anthropogenic noise is encroaching on wildlife in many terrestrial and marine habitats and has a diverse array of effects from displacement to inducing physiological stress and decreasing fitness. Data on hearing provides a key step to evaluating sound-sensitivity. The CIB calf hearing appeared healthy, but human-produced noise remains a concern for all CIBs. Similar to hearing tests in wild belugas, the CIB calf AEP data were collected non-invasively, with little impact to the animal. These auditory data are invaluable, providing the first step to empirically address sound-sensitivity in this endangered population. However, hearing sensitivities vary within a population. It is possible that other belugas have greater sensitivities, similar to what has been observed with Bristol Bay belugas. It is necessary to collect more audiograms of CIBs to compare to other wild and captive belugas, as well as other CIBs. This will allow us to better understand CIB hearing and their variability.

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