1	Measuring hearing in wild beluga whales
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## 1 Abstract

2	We measured the hearing abilities of seven wild beluga whales (Delphinapterus leucas)
3	during a collection-and-release experiment in Bristol Bay, AK, USA. Here we summarize the
4	methods and initial data from one animal, discussing the implications of this experiment.
5	Audiograms were collected from 4-150 kHz. The animal with the lowest threshold heard best
6	at 80 kHz and demonstrated overall good hearing from 22-110 kHz. The robustness of the
7	methodology and data suggest AEP audiograms can be incorporated into future collection-
8	and-release health assessments. Such methods may provide high-quality results for multiple
9	animals facilitating population-level audiograms and hearing measures in new species.
10	1. Introduction
11	Hearing is the primary sensory modality for odontocete marine mammals. They are
12	generally considered to have sensitive hearing and may detect a broad range of frequencies.
13	Relying on hearing can be particularly adaptive in the marine environment where light and
14	other cues are often limited and natural sounds are frequently abundant. Yet, these sensitive
15	auditory abilities may also be easily impacted by anthropogenic noise.
16	Human use of the Earth's oceans has steadily increased over the last century resulting in
17	an increase in anthropogenically produced noise (e.g., National Academy of Sciences 2003).
18	The Arctic is no exception to this increase (Blackwell & Greene 2003). Reductions of polar sea
19	ice and the opening of the Northwest Passage presumably will open up habitat for many top
20	predators. Yet, this decrease in sea ice provides greater human access to high latitude
21	environment and such a change poised to transform a relatively pristine environment into to
22	one saturated with human activities and associated noise. Sources are varied and include: naval
23	exercises, boundary definitions, shipping/movement along Alaska's North Slope, seismic
24	resources exploration, and the construction of infrastructure needed to support it (Wang &
25	Overland 2009; Titley & St. John 2010). These changes encompass habitats of Delphinapterus

*leucas* (beluga whales) and other top predators. Despite this obvious overlap of human-natural
 interests, there is a poor understanding of influences of these sound-associated changes. In
 order to estimate the impacts of this noise it is crucial to evaluate the natural hearing abilities
 and the variation with marine mammal populations.

5 Yet a primary challenge is that audiograms of odontocetes marine mammals have most 6 often been estimated from stranded animals or non-wild individuals (for a review see Mooney 7 et al. 2012). In many instances, these records have produced valuable data that is otherwise 8 unavailable. For example, hearing in several stranded beaked whale species have helped define 9 what these sound-sensitive animals hear (Finneran et al. 2009; Pacini et al. 2011). The 10 audiogram of a stranded infant Risso's dolphin helped redefine what the species actually 11 detects (Nachtigall et al. 2005). Work with trained odontocetes provides scientific data that is 12 likely unique to those settings and can address how animals hear or how they may be protected 13 from anthropogenic noise (Nachtigall & Supin 2008). Yet, in many instances health 14 compromised, stranded animals may not have normal auditory abilities, thus not necessarily 15 representative of wild populations. Further, without baselines to wild individuals it is difficult 16 to put differences and results of non-wild individuals in a relative context. Clearly, there is 17 value in increasing the number of animals within a species measured for hearing capabilities 18 whenever possible.

Here we describe methods and initial results for the measuring the hearing of wild *D*. *leucas* (Castellote et al. 2013). The goal of this study was to determine hearing sensitivity in
wild Bristol Bay *D. leucas*, during a planned collection-and-release operation. Monitoring of *D. leucas* has been recommended in recent years because this species is likely to be
negatively impacted by climate change, but also because such a broadly dispersed, high
trophic feeder can serve as an effective sentinel of the ecosystem(s) in which they live

(Moore 2008; Moore & Huntington 2008; Simpkins et al. 2009). Because noise may impact
 *D. leucas* in a variety of ways, it is essential to determine what these animals hear.

3 In view of the expected changes in the Arctic acoustic environment, expanding our 4 knowledge on *D. leucas* hearing is of central importance for an appropriate conservation 5 management framework. One of the five distinct stocks of *D. leucas* whales that are currently 6 recognized in U.S. waters, the Cook Inlet D. leucas population, is endangered and efforts for 7 its recovery to date have not been successful. The impact of anthropogenic noise has been 8 identified as a serious threat potentially impeding its recovery (NMFS 2008). On the 9 contrary, the Bristol Bay D. leucas population is increasing and is considered to be a healthy 10 population (NMFS 2008). The acoustic environment in Bristol Bay is different; many of the 11 chronic anthropogenic sources typically found in Cook Inlet D. leucas habitat are essentially 12 absent or seasonally present at lower intensities in Bristol Bay habitat. This suggests that 13 Bristol Bay D. leucas are a valuable asset to evaluate baseline hearing and health measures 14 for comparison to effected populations, such as Cook Inlet D. leucas.

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#### 16 **2. Temporary collection of beluga whales and hearing tests methods**

17 This study was conducted in September, 2012 in Bristol Bay AK, USA. The 18 audiograms were measured during an overall health assessment study that required the 19 collection-and-release of D. leucas. Audiograms were obtained on seven of seven belugas 20 tested. The procedures were similar to those followed by (Ferrero et al. 2000) and were 21 conducted under National Marine Fisheries Service marine mammal research permit #14245 22 and approved by the necessary Institutional Animal Care and Use Committees. The full 23 results are to be published elsewhere (Castellote et al. 2013); here we provide a summary of 24 the methods and preliminary results.

1	Bristol Bay is a generally shallow, muddy-bottomed estuary system that supports a
2	population of <i>D. leucas</i> . Using three 3.5 m aluminum skiffs and one soft-bodied inflatable
3	boat, we would search for an adult beluga. When a suitable animal was spotted (Fig. 1), one
4	of the skiffs would follow and gradually approach the whale to encourage it to swim into
5	shallow water (< 2 m). From one of the boats, a 125 m long by 4 m deep net, of 0.3 m $$
6	braided square mesh, was deployed around the whale. Once the deployment boat and net
7	encircled the whale the inflatable boat approached the outside of the net and three handlers
8	placed a soft tail-rope around the whale's peduncle. The rope's other end was fixed to the
9	inflatable boat to secure the whale. The large net was gradually recalled while a "belly-
10	band" stretcher was placed under the D. leucas. Handholds in this stretcher facilitated
11	adjusting the whale's position as the water depth changed with the tide. The animal was then
12	positioned parallel to the small inflatable boat. The D. leucas's head was typically rested on
13	or just above the soft mud bottom, keeping the lower jaw and primary hearing pathways
14	below the water surface. The animal's blowhole was generally above the surface. This setup
15	was consistent for all animals, except one for which the water level was too low and this test
16	was conducted partly out of the water. Animals were maintained in this position for the
17	audiogram and health exam. The AEP test equipment was outfitted in a ruggedized case; both
18	it and the operator sat in the small inflatable boat beside the D. leucas during the hearing tests
19	(Fig. 1).

#### **3. Discussion of results**

Audiograms were successfully collected on all seven adult *D. leucas* whales
temporarily collected and tested. Evoked response waveforms and envelope following
responses were generally easily identifiable and distinct from the background
electrophysiological noise. The inset in Figure 2 shows an EFR that was recorded using

1 stimuli ca. 20 dB about the hearing threshold at 32 kHz. Such a measurement would take ca. 2 30 sec to collect. Thus, overall thresholds at a particular frequency were obtained in 3-5 min. 3 This relatively rapid threshold measurement facilitated collecting multiple thresholds per 4 animal but also minimizing the "with-animal" time. For example, animal #7's audiogram 5 consisted of 12 frequencies tested. Two of these (4 and 150 kHz) did not induce measureable 6 AEPs. The entire data set was collected in 55 min which includes multiple breaks for other 7 measurements such as blood samples or repositioning the animal. Records were collected in 8 concert with a suite of other measurements with no discernible impact on the physiological 9 noise. This allowed for a relatively efficient of data collection when compared to behavioral 10 methods which require significant time to train animals and conduct experiments. It is also 11 relatively quick for other AEP audiograms which make take multiple days (sessions). Here 12 we collected seven audiograms over six field days (including one day which was poor 13 weather conditions and no whales were sighted). 14 Despite the potential challenges of the experiment (cold conditions, electrophysiology

15 close to the water, confined spaces, concurrent measurements potentially introducing noise, 16 safety and welfare of the people and animals) the audiograms were of very good quality. 17 They are of equal quality to the field-based collection-release audiometric data of Cook and 18 Mann (2004) for bottlenosed dolphins (*Tursiops truncates*) and of Nachtigall et al., (2008; 19 see also Mooney et al. 2009) for white-beaked dolphins (Lagenorhynchus albirostris) Our 20 success both in ease and safety of data acquisition and quality of the data suggest the methods 21 could easily be applied to other species in similar situations. This is of particular importance 22 for populations where anthropogenic noise is chronic and has been identified as a potential 23 stressor. Examples are the endangered Cook Inlet D. leucas or the threatened St. Lawrence D. 24 *leucas* populations. The prevalence of anthropogenic noise in their habitat and its cumulative 25 effects might be compromising the survival of both *D. leucas* populations (NMFS 2008;

1	DFO 2012). This assertion is based on current knowledge of the level and acuity of
2	anthropogenic noise in these ecosystems (e.g., Gervaise et al. 2012) and our understanding on
3	D. leucas hearing and acoustic communication. However, because of the inherent difficulties
4	in evaluating noise impact on cetaceans, there are no data supporting this hypothesis.
5	Audiograms using the method described here could be collected in Cook Inlet and in the St.
6	Lawrence Estuary to measure the hearing of <i>D. leucas</i> with greater exposed to anthropogenic
7	noise and be compared to the baseline audiogram described for Bristol Bay D. leucas.
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21	NWFSC2012-1) and WHOI IACUC protocols (ID number: BI166330).
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### 23 Figure captions

- Figure 1. (A) Spotting a *D. leucas* from the aluminum skiff. (B) The AEP audiogram setup.
- 25 The recording, reference and ground electrode are noted with from posterior to anterior (right

- 1 to left) by the yellow arrows. A measure of breath is also being taken concurrently. (C) The
- 2 AEP system in its case and (D) in the soft inflatable boat during data recording.
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- 4 Figure 2. The AEP audiogram and waveform (inset) of *D. leucas* #7. This animal had the
- 5 overall mean lowest threshold.
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# Figure 1









