Cetacean evoked potential audiometry by stranding networks enables more rapid accumulation of hearing information in stranded odontocetes

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ABSTRACT

Knowing the hearing range and sensitivity of a marine mammal is fundamental to determining its potential for being impacted by ocean noise. Enabling stranding responders to perform hearing tests on stranded odontocetes is the most likely means by which most odontocete species will be tested and by which population-level variability in hearing will be determined. A portable auditory evoked potential (AEP) system was modified for use by stranding response teams and optimised to test odontocete hearing. Stranding responders were trained on the system and deployed it to strandings from 2010–2013. Eighteen partial or complete audiograms from common dolphins (n = 15) and Atlantic white-sided dolphins (n = 3) were obtained. Both species demonstrated typically delphinid audiograms with upper frequency limits of hearing between 113–160kHz; however, the region of best sensitivity in the Atlantic white-sided dolphin (28–56kHz) was 18–28 dB less sensitive than that of the common dolphin. A single common dolphin presented with severe hearing loss consistent with presbycusis in delphinids, but with undefined etiology. The number of audiograms obtained during the study greatly increases our knowledge about hearing in these species, neither of which are common to managed care facilities. In the case of the common dolphin, the number of animals tested allows a first estimate of population-level variability. Continued use of AEP systems by stranding responders will expedite the collection of audiometric information for previously untested species and permit sufficient sample sizes to determine population-level variability in the hearing of tested species.

KEYWORDS: HEARING; NOISE; STRANDINGS; ACOUSTICS

INTRODUCTION

Awareness of issues caused by ocean noise and investigations into the potential impact of ocean noise on marine mammals has grown significantly since the 1990s (e.g. NRC, 2000; 2005; Southall *et al.*, 2007; Tyack, 2008). Most marine mammals rely on sound detection and localisation, to some degree, for purposes of foraging, predator avoidance, navigation and/or socialisation. For the odontocete cetaceans, and possibly all cetaceans, hearing is the primary sense by which information about the environment is obtained. With ocean noise continuing to increase, particularly at low frequencies (< 1000Hz), the impact of ocean noise on marine mammals has emerged as a leading conservation issue and the need to understand the hearing abilities of marine mammals has risen in importance.

Auditory evoked potentials (AEPs) are small voltages produced by the brain and auditory nervous system in response to sound. The measurement of AEPs is one method by which the hearing range and sensitivity of an animal can be tested. However, behavioural methods are the 'gold standard' for determining hearing range and sensitivity, as they present an integrated, whole-animal response (e.g. including cognitive processes). Within small odontocetes, audiometric studies have included both behavioural and AEP methods. Estimates of hearing sensitivity estimated by AEP methods underestimate unmasked, behaviourally-measured hearing sensitivities in odontocetes by an average of ~11dB (Finneran and Houser, 2006; 2007; Nachtigall *et al.*, 2008; Szymanski *et al.*, 1998; Yuen *et al.*, 2005), which is similar to differences between AEP and behavioural methods in humans (Lins *et al.*, 1995; Rance *et al.*, 1995; Vander Werff and Brown, 2005). Differences in the results obtained by behavioural and AEP methods are due to differences in stimulus durations, differences in sound presentation methods (e.g. free-field or contact transducer), differences in stimulus waveforms (e.g. pure tone or amplitude modulated tone), differences in test environments (e.g. background noise or testing 'in-water' versus 'in-air'), and how the hearing threshold (or sensitivity) is estimated. Nevertheless, evoked potential audiometry is advantageous to behavioural audiometry in that subjects need not be trained to participate in the test and it can be used in conjunction with anaesthesia or sedation, or while subjects are asleep. Due to these advantages, AEP hearing tests have gained wide acceptance in the clinical world for testing the hearing of infants and individuals that are otherwise incapable of participating in behavioural hearing tests.

The AEP method has been increasingly adopted by the marine mammal community for performing hearing tests in odontocete cetaceans and some pinnipeds (e.g. Cook *et al.*, 2006; Houser and Finneran, 2006a; Nachtigall *et al.*, 2005; Yuen *et al.*, 2005). The use of AEP methods increased, in large part, due to the availability of rugged portable systems capable of testing the frequency range across which marine mammals hear (e.g. Finneran *et al.*, 2009). Although the concept of a portable AEP system for testing marine mammal hearing was developed decades ago (Ridgway and Carder,

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1983), attempts to build portable systems for use on marine mammals did not begin in earnest until the 1990s (Carder and Ridgway, 1994; Helweg *et al.*, 1997). At this time, commercially available AEP systems were designed for clinical settings and for use on humans (e.g. limited to the human frequency range of hearing). More recently, improvements in the sampling rates of data acquisition cards (which increases the frequency range that can be tested), miniaturisation of computers and system components, and the availability of rugged portable computers have allowed AEP systems for testing marine mammal hearing to realised. This, in turn, has enabled the hearing of stranded marine mammals to be tested, which is the only means by which hearing information on many species of marine mammal can be obtained (Cook *et al.*, 2006; Finneran, 2009; Pacini *et al.*, 2011; Ridgway and Carder, 2001).

The number of live marine mammal strandings each year far exceeds the opportunities for performing AEP hearing tests. This has been due, in part, to the fact that the AEP systems used for testing require considerable training prior to use. For this reason, audiometric information obtained from stranded marine mammals has been collected by labs that focus on this type of research. However, relatively few tests by these labs on stranded animals have occurred because the stranding must either occur close to the location of the researchers (Pacini *et al.*, 2011) or the researchers must travel considerable distance to perform the test (Finneran, 2009). In the latter case, the decision to forego the test is often made because of the potential time delay in animal treatment or euthanasia.

Maximising the audiometric information that can be obtained from stranded marine mammals will require that stranding networks be enabled with the technology to perform AEP hearing tests. Since it is the stranding network that initially responds to the report of a stranded marine mammal, and because decisions about the disposition of the animal are often rapidly made, the technology must be placed in the hands of stranding responders so that testing is timely. However, the technology would necessarily need to be 'user friendly' in the sense that relatively little training should be required on its operation and maintenance. The system should also be relatively 'user proof' in its robustness to unforeseen user interaction. This paper reports on the outcome of an effort to enable stranding networks to perform AEP tests on stranded odontocete cetaceans through the development of a ruggedised, portable AEP system tailored for use by non-expert stranding responders.

A ruggedised, portable system for recording AEPs was modified for use by stranding responders and optimised for the testing of hearing in odontocetes. The original system has previously been described in detail (Finneran, 2009). In an arrangement with the stranding response team of the International Fund for Animal Welfare (IFAW), stranding response personnel were trained on the use of the system and then used the system over the course of several years to test the hearing of marine mammals. The results of this effort demonstrate that stranding responders can perform hearing tests using semi-automated AEP procedures and that enabling stranding responders with this technology significantly increases the rate at which marine mammal hearing information is collected.

MATERIALS AND METHODS

Equipment

A portable, field-rugged, semi-automated AEP system was created for the IFAW stranding response team. The hardware of the system was based on the Evoked Response Study Tool (EVREST), which has been used in a number of marine mammal hearing studies (Finneran, 2009; Finneran *et al.*, 2011; Houser *et al.*, 2008a; Houser *et al.*, 2007; Houser *et al.*, 2008b; Schlundt *et al.*, 2011). In brief, the system consisted of a rugged notebook computer with a multifunction data acquisition card (DAQ) and custom signal conditioning circuitry, a biopotential amplifier, a sound projector, and electrodes. For a detailed description of the hardware, see Finneran *et al.* (2009), in which the system hardware and software is comprehensively described. Modifications to the EVREST system were made to streamline it and make it deployable by users with minimal training. For the purposes of this paper, and to distinguish the modified version from the full version, the streamlined form of the software is henceforth referred to as EVREST^{LT}.

The EVREST control and analysis software was streamlined for use on mid-sized odontocetes in order to minimise the amount of training required for its operation. Modifications limited the number of options for user input and stimulus selection. Stimulus selections were limited to: (1) click waveform; (2) sinusoidal amplitude modulated (SAM) tones at single frequencies, which are used for testing hearing at individual frequencies; and (3) multiple SAM tones, which permit the testing of hearing at multiple frequencies simultaneously. Stimuli were selected based on their historical utility in measuring the click-evoked response and auditory steady-state response (ASSR) in odontocete cetaceans (Dolphin *et al.*, 1995; Finneran and Houser, 2007; Popov and Supin, 1998). These were presented on a drop-down, user-selectable menu. The specific waveform characteristics made available were as follows:

(1) Click – 100μs rectangular pulse with alternating polarity; presentation rate of ~51Hz;

(2) Single SAM tone – user-selectable frequency from a range of half-octave frequencies spanning 10 to 160kHz

(3) 4-component SAM tone – complex SAM tone consisting of individual frequency components of 14.1, 28.2, 56, and 113kHz; 62ms duration with components 100% amplitude modulated at rates of 950, 1,050, 1,150, and 1,250Hz, respectively;

(4) 5-component SAM tone – complex SAM tone consisting of individual frequency components of 10, 20, 40, 80, and 160kHz; 62ms duration with components 100% amplitude modulated at rates of 900, 1,000, 1,100, 1,200, and 1,300Hz, respectively; and

(5) 9-component SAM tone – complex SAM tone consisting of all frequency components of the 4- and 5-component signal; 62ms duration with components 100% amplitude modulated at rates given above.

Options for testing smaller odontocetes (e.g. harbour porpoise, *Phocoena phocoena*) were similar but were limited to a lower frequency of 20kHz.

The interface retained the user's ability to set the artifact rejection level and the stimulus sound pressure level (SPL, dB *re* 1µPa) for the click and single SAM tones. Threshold testing using SAM tones (i.e. determination of the minimum detectable SPL by the animal at a particular frequency) was automated. Individual components were initially produced at 120 or 110 dB SPL and were modified according to a staircase function, as previously described (Finneran and Houser, 2006). Initial step sizes were set as either 10 or 30dB; 10dB step sizes were assigned to the lowest and highest frequencies tested in order to prevent large increases in the SPL from producing distortion in the stimulus (lowest frequency) or rapid increases in the perceived loudness of the signal at the highest frequency resulting from rapid recruitment of sensory neurons. Step sizes were multiplied by 0.4 after each miss/hit reversal and by 0.45 after each hit/miss reversal. A 'hit' is defined as the detection of the steady-state AEP, whereas a 'miss' corresponds to the lack of a detected signal. Step size multipliers were set at their respective values to ensure that a particular stimulus level would not be tested more than once. Recorded evoked potential data were objectively assessed for AEP presence or absence using the magnitude-squared coherence (MSC) test described below. Thresholds were determined as the SPL intermediate of the lowest stimulus level resulting in a detectable evoked response and the highest stimulus level at which no evoked response was detected.

Training

Stranding network personnel from IFAW travelled to the US Navy Marine Mammal Program (MMP) for training with the EVREST^{LT} system prior to first use. Training was performed at the MMP because it holds bottlenose dolphins (*Tursiops truncatus*) trained to voluntarily beach themselves for AEP procedures, which allowed network personnel to deploy the system as they would during a stranding event. Over the course of one to several days, personnel were trained on equipment setup, operation, and maintenance, and were provided opportunities to perform evoked potential audiometry on the MMP dolphins. Yearly, after the initial training, refresher and more advanced training was provided to the previously-trained stranding network personnel and new personnel were trained on the system.

Field deployment

Throughout the course of a four-year evaluation period (2010–2013), stranding personnel deployed with the EVREST^{LT} system when responding to a live-stranded cetacean(s). The decision to test the hearing of the stranded cetacean(s) was made between the attending veterinarian and the senior on-site stranding responder, per the details of MMPA permit 932-1905-00/MA-009526. The permit included a disposition determination based on a standardised health assessment protocol. Disposition options included euthanasia, immediate release or relocation and release at a more suitable location. Once a subject was deemed suitable for testing, testing was performed either while stationary, during the transport to the release site, or at times, during both. Whether full or partial testing was completed was dictated by the disposition of the animal and other logistical circumstances or animal health concerns associated with the stranding event.

Testing on small odontocetes was performed as follows. Stimulus generation and evoked response recording were performed using EVREST^{LT}. Stimuli were digitally generated, converted to analogue with a 1MHz update rate and 16-bit resolution, low-pass filtered at 200kHz (eight-pole Butterworth, Krohn-Hite 3C series), and attenuated if necessary (custom hardware) before being applied to a 'jawphone' – a piezoelectric sound projector (either an ITC 1042 or a Reson TC 4013) embedded in a silicon rubber suction cup. The larger of the jawphones (ITC 1042) was designed for use on mid-sized and larger delphinids, whereas the smaller jawphone (Reson TC 4013) was developed for use on harbour porpoises or small delphinids (e.g. calves or sub-adults). Each jawphone was calibrated prior to delivery to the stranding network by measuring the underwater sound pressure produced at a distance equivalent to the distance between the skin surface over the pan region of the lower jaw (the outermost region of optimal high-frequency sound reception pathway) and the auditory bulla, as derived from anatomical measurements made from MRI images of the bottlenose dolphin and harbour porpoise, respectively. In dolphins, this calibration technique has produced reasonable agreement between AEP thresholds measured 'in-air' with jawphones and those behaviourally measured underwater (Finneran and Houser, 2006). Depending on the size of the animal, the appropriate jawphone was placed on the odontocete's lower jaw, over the pan region.

Stimuli consisted of clicks and sinusoidal amplitude modulated (SAM) tones. If applied, clicks were presented in order to: (1) ensure that the system was working and correctly connected to the subject; and (2) obtain a click-evoked auditory brainstem response (ABR). Clicks were positive, rectangular pulses with a duration of 100µs, designed to produce a broadband stimulus that would excite a large population of neurons and produce a relatively robust ABR. The polarity of the click was alternated on each presentation in order to eliminate stimulus artifacts from the click presentation. SAM tones evoke the ASSR, which is a periodic signal with a fundamental frequency related to the amplitude modulation frequency of the tone and which may be analysed in the frequency domain using established techniques for objective, statistically-based response detection (Dobie and Wilson, 1989; 1996; Finneran and Houser, 2007). The SAM tones were 100% amplitude modulated with 1-ms cosine rise/fall envelopes. Based on the pre-configured test frequencies for EVREST^{LT}, carrier frequencies varied from 10–160kHz (ITC 1,042) to 20–160kHz (Reson TC 4013). Amplitude modulation frequencies and the duration of the SAM tone stimulus depended on whether a single SAM tone was used during testing or whether multiple SAM tones were presented simultaneously. Provided sufficient frequency spacing in the modulation rates of individual tones exists, multiple SAM tones can be presented simultaneously because of the brain's ability to independently resolve the component signals. The use of multiple SAM tones has been demonstrated as an effective means for reducing the amount of time required for hearing assessments in odontocetes (Finneran and Houser, 2007).

AEPs were measured using three 10mm gold cup surface electrodes embedded in silicon suction cups and placed on the head and back. The noninverting (+) electrode was located on the dorsal midline approximately 10cm posterior to the blowhole. A ground (com) electrode was placed near the anterior insertion of the dorsal fin on the animal's back. The inverting (–) electrode was located either midway between the (+) and (com) electrodes, or was placed next to the external auditory meatus contralateral of the lower jaw being acoustically stimulated. Electrodes were coupled to the skin surface using conductive paste and electrode signals were passed into a biopotential amplifier (Grass ICP-511), which amplified ($\times 10^5$) and filtered (0.3–3kHz) the voltage between the (+) and (–) electrodes. The output of the amplifier was digitised with 16-bit resolution and then synchronously averaged over the sweep duration (i.e. synchronised with the stimulus onset). Click-evoked ABRs were digitised at a rate of 20kHz and SAM tones were digitized at 10kHz. Sweeps with peak instantaneous voltages above a user-defined artifact rejection level were excluded from analysis artifacts. The artifact rejection threshold was set according to the electrical noise conditions unique to each data collection, but typically ranged between 10–20 µV.

Click-evoked potentials were averaged over 1,024 sweeps. For threshold measurements with SAM stimuli, the presence or absence of an evoked response was determined after every 256 sweeps were collected, up to a maximum of 1,024 sweeps. If a response was detected, the measurement was complete; if not, an additional 256 sweeps were collected and the process repeated until the maximum number of sweeps was reached. At each integral multiple of 256 sweeps, a magnitude-squared coherence (MSC) test was performed using the total number of sweeps collected. If the MSC, defined as the ratio of the power in the grand average to the average power of the sub-averages (see discussion in: Dobie and Wilson, 1989; 1996; Finneran and Houser, 2007), was greater than the 'critical' value of 0.01 developed from a theoretical perspective by (Brillinger, 1978), the response at a particular modulation frequency was assumed to be detected.

Analysis

Stranding network personnel performed all audiometric procedures at the stranding site and/or during transport between the stranding site and a pre-determined release site. Data collected by stranding network personnel were then submitted to a quality assurance (QA) by either D. Houser or J. Finneran, where data of poor quality were discarded from further use. Following the QA, the threshold analysis procedure was repeated.

Latencies and amplitudes of click-evoked ABR waveforms were determined from the averaged ABR for each animal in which the click-evoked ABR was measured. Peaks were categorised in accordance with a previously established taxonomy for delphinid ABRs (Popov and Supin, 1985; 1990). Audiograms were qualitatively judged as being 'unremarkable' or reflective of compromised hearing. This was first done by applying the criteria previously used by Mann *et al.* (2010) for characterising severe hearing loss (70–90dB loss) and profound hearing loss (> 90dB loss) in odontocetes. For the common dolphins, this was performed using the previously determined AEP thresholds in this species (Popov and Klishin, 1998). Since no baseline audiometric information exists for the Atlantic white-sided dolphin, this process was only applied to the common dolphin. Audiograms determined to demonstrate severe or profound hearing loss were removed and each remaining audiogram was compared to the mean of the remaining audiograms. If thresholds across three or more consecutively increasing frequencies were > 30dB above the mean threshold, these audiograms were considered as having 'elevated thresholds'. The > 30dB criteria is an arbitrary decision point for determining thresholds as elevated, but is sufficiently large as to result in the functional decrement of hearing ability relative to the mean threshold of normal-hearing subjects. The requirement for three or more consecutively increasing frequencies demonstrating the elevation ensures that the determination for elevated thresholds is not made solely on the basis of a spurious measure at a single frequency. Audiograms with elevated thresholds were removed from the data set and all remaining audiograms were deemed as unremarkable, i.e. they had a typically delphinid audiogram shape and thresholds fell within an acceptable variation from the mean. Other peculiarities (e.g. a notch in the audiogram, indicating a region of elevated sensitivity) were identified on an individual basis. A composite audiogram was subsequently constructed for each species tested by calculating a mean threshold (±1 standard deviation) for each test frequency from the thresholds of the animals whose audiograms were deemed unremarkable.

RESULTS

A total of 18 partial or full audiograms were obtained from stranded odontocetes in the Cape Cod region from 2010–2013 (Table 1; see Fig. 1 for an example of equipment attachment to the dolphins). Fifteen complete or partial audiograms were collected from stranded common dolphins (*Delphinus delphis*) and three from Atlantic white-sided dolphins (*Lagenorhynchus acutus*). Click-evoked ABRs

Fig. 1. Electrode and jawphone attachment to a stranded common dolphin during the collection of an AEP audiogram. (All stranding activities conducted under NMFS permit 18786).

Table 1

Meta-data, degree of audiogram completion, results of the audiometric assessment, and disposition of subject animals following AEP testing.

Stranding ID	Length (cm)	Sex	Audiogram	Qualitative assessment	Disposition
Common dolphin					
10-018Dd	218	M	Full	Elevated thresholds	Euthanased
10-060Dd	159	F	Full	Unremarkable	Relocated
10-062Dd	165	F	Partial	Elevated thresholds	Euthanased
11-252Dd	177.2	M	Partial	Unremarkable	Relocated, restranded and later euthanased
11-253Dd	209.5	M	Partial	Severe hearing loss	Relocated
12-192Dd	232	M	Partial	Unremarkable	Relocated, restranded and relocated again
12-194Dd	229	M	Full	Unremarkable	Relocated, restranded and relocated again
12-200Dd	157	M	Full	Unremarkable	Euthanased
12-202Dd	211	F	Partial	Unremarkable	Relocated
12-204Dd	171.5	F	Partial	Unremarkable	Relocated
12-216Dd	203.2	M	Full	Unremarkable	Relocated
12-227Dd	161	M	Partial	Unremarkable	Relocated
12-229Dd	181	M	Full	Unremarkable	Euthanased
12-290Dd	*		Full	Unremarkable	Relocated
12-384Dd	187	F	Full	Notch at 40kHz	Relocated
Atlantic white-sided dolphin					
10-073La	162	M	Partial	Unremarkable	Relocated
10-112La	218	F	Partial	Unremarkable	Relocated
13-066La	203	M	Full	Unremarkable	Relocated

*Sex and length not reported on stranding report.

Table 2

Click-evoked ABR wave amplitudes and latencies for each common dolphin tested. Peaks correspond to those depicted in Fig. 2.

	Amplitude (μV)					Latency (ms)				
Animal ID	p1	n2	p3	p4	n5	p1	n2	p3	p4	n5
11-252Dd	0.81	–2.02	3.21	1.55	–2.92	1.4	1.9	2.4	2.9	3.2
11-253Dd**	0.52	–1.13	1.58	0.72	–1.56	1.5	2.0	2.3	2.9	3.3
12-192Dd	1.73	–4.76	5.70	3.65	–6.32	1.4	1.9	2.4	3.0	3.3
12-194Dd	4.57	–7.38	4.92	4.78	–5.27	1.4	1.9	2.3	2.9	3.3
12-200Dd	1.01	–2.15	2.61	3.43	–6.09	1.8	2.3	2.9	3.7	4.1
12-202Dd	3.31	–7.39	6.46	5.65	–6.64	1.5	2.0	2.5	3.1	3.4
12-204Dd	3.28	–9.26	8.60	7.77	–9.31	1.3	1.8	2.3	2.9	3.2
12-215Dd	1.25	–4.56	4.19	3.48	–5.74	1.4	1.9	2.4	3.0	3.3
12-216Dd	2.44	–7.01	6.85	5.44	–8.28	1.4	1.9	2.2	2.8	3.1
12-219Dd	1.87	–4.64	4.87	3.91	–6.89	1.5	1.9	2.3	2.9	3.3
12-227Dd	0.68	–1.74	4.56	1.25	–5.92	1.4	1.8	2.1	2.7	3.1
12-229Dd	*	–1.26	1.68	1.93	–3.65	*	2.0	2.5	3.2	3.5
12-290Dd	0.69	–2.60	5.90	1.77	–6.32	1.6	1.9	2.2	2.8	3.1
12-348Dd	1.47	–2.69	6.84	2.22	–9.06	1.2	1.8	2.3	2.8	3.2
Mean	2.1	–4.8	5.1	3.9	–6.4	1.5	1.9	2.4	3.0	3.3
SD	1.3	2.7	1.9	1.9	1.5	0.1	0.1	0.2	0.3	0.3

*The p1 wave could not be ascertained. **Dolphin presented with severe hearing loss.

were collected from fourteen stranded common dolphins. No attempts to record click-evoked responses were made in the white-sided dolphins. Click-evoked responses were also not always collected from the stranded animals, particularly in the first two years of the study, so the number of click-evoked measures does not equate to the number of audiograms measured. Five attempts to collect audiograms failed due to either user error or equipment failure (e.g. electrical short in a test cable). Three additional attempts acquired data, but the audiograms were considered questionable and were discarded following QA.

A representative click-evoked ABR waveform and spectra for the common dolphin is presented in Fig. 2. The peaks and latencies are typical of values and ranges for odontocetes and the large amplitudes reflect the relatively small size of the species (Table 2). The spectrum of the click-evoked response took the form of a low-pass filter with peaks at ~700Hz and ~1200Hz.

Eleven of the common dolphin audiograms were deemed as unremarkable, two were deemed potentially to have elevated thresholds across the range of hearing, one presented with severe high-frequency hearing loss, and one presented with a notch in its hearing range (see Fig. 3a for examples). The three white-sided dolphin audiograms were all deemed as unremarkable. The composite audiogram for both species is shown in Fig. 3b. Except at the lowest frequencies, where AEP threshold estimates are more variable, the audiograms were qualitatively similar in shape between the two species. However, the lowest mean thresholds for the common dolphin (from 28–56kHz) were

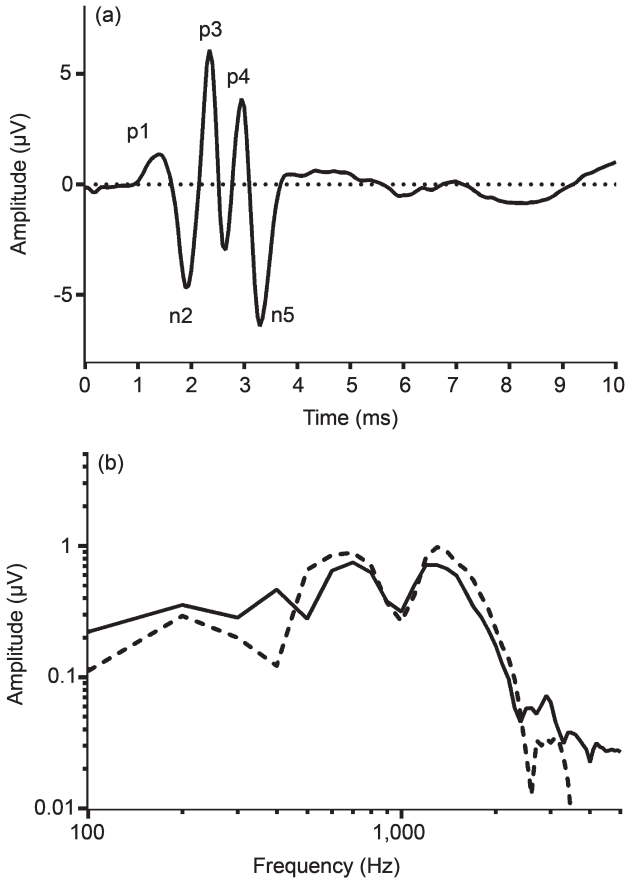

Fig. 2. (a) Click-evoked ABR waveform from a stranded common dolphin. Data are plotted with positive deflections upward. Positive peaks are designated as p1, p3 and p4. Negative peaks are designated as n2 and n5. (b) Spectra of the click-evoked response from two common dolphins.

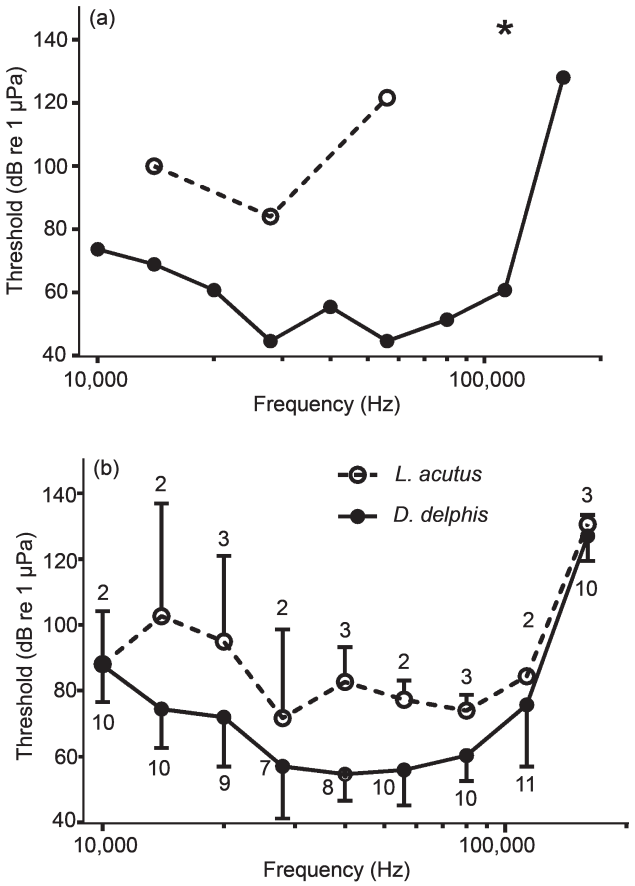

Fig. 3. (a) Audiogram comparison of a common dolphin with unremarkable hearing (solid line and symbols) and one with severe hearing loss (dashed line and open symbols). The asterisk indicates that no evoked response was observed at the highest stimulus level that could be generated at 113 kHz. (b) Comparison of the composite audiogram between common dolphins (*D. delphis*) and Atlantic white-sided dolphins (*L. acutus*). The composite audiogram was created as the mean of frequency-specific thresholds obtained from individual animals with unremarkable audiograms. The number of samples for each measurement is presented next to each threshold.

55–60 dB SPL, which was 18–28dB less than the best sensitivities obtained in the white-sided dolphins across the same frequencies.

DISCUSSION

Impact

A major outcome of this study was the demonstration that stranding network personnel can perform audiometry studies when provided with the tools and training to do so. Stranding responders trained in the use of AEP methods and supplied with streamlined equipment for the AEP testing of odontocetes, collected audiograms from odontocetes that would have otherwise been untested. Fifteen complete or partial audiograms were obtained from common dolphins and three from Atlantic white-sided dolphins over a time-frame of several years. The audiometric information obtained is either the first reported for this species (Atlantic white-sided) or it significantly increases the sample size of representative audiograms for the species (common dolphins). The number of audiograms compiled by the IFAW stranding responders over the course of the study far exceeds the numbers that could realistically be expected from behavioural procedures with captive specimens of these species, i.e. there are no or few individuals of these species maintained at marine mammal facilities. The fact that most cetacean species are not represented at marine mammal facilities means that the testing of most species will likely be limited to opportunities created through stranding events. In many instances, the ability to perform AEP tests is limited to a short period of time following a stranding but prior to death, euthanasia or being put back to sea. It is unlikely in nearly all such cases that researchers specialising in AEP work would be able to respond in a timely manner to perform audiometric testing (see Finneran, 2009). However, the success of stranding networks in collecting AEP audiograms, as demonstrated here, holds promise for first responders being able to capitalise on stranding opportunities to collect audiometric information for untested species and bolster audiometric sample sizes such that an understanding of population-level variability might someday be achieved. Both of these goals have particular importance to providing fundamental information on the potential for ocean noise to affect marine mammals, i.e. a knowledge of hearing range and sensitivity is the first step in determining what types of noise are most likely to impact an animal.

Findings

The audiograms of both the Atlantic white-sided dolphins and common dolphins appeared to be typically delphinid in nature for those suspected of not having compromised hearing. The frequency range of hearing is similar to that previously reported for dolphin species (Houser and

Finneran, 2006b; Houser *et al.*, 2008b; Nachtigall *et al.*, 2005; Nachtigall *et al.*, 2008; Popov and Klishin, 1998; Tremel *et al.*, 1998), with the upper limit of hearing occurring between 113–160kHz. The audiograms were also of a skewed U-shape with gradually increasing thresholds at lower frequencies and sharply increasing thresholds at the upper limit of hearing. However, there were notable differences in the threshold estimates of the two species (see below).

One common dolphin presented with severe high-frequency hearing loss; specifically, the threshold at 80kHz was > 130 dB SPL and an evoked response at 160kHz could not be measured at the highest levels of acoustic stimulation. Thresholds at 20 and 40kHz were also elevated. The audiogram is consistent with sensorineural hearing loss, as has been hypothesised for other dolphins with similar audiograms (Houser and Finneran, 2006b). Another dolphin presented with a notch in hearing sensitivity at 40kHz, a pattern which has been observed in bottlenose dolphins (Houser and Finneran, 2006a), but for which a causative mechanism has yet to be identified. In all cases presented here, the underlying cause of hearing loss can only be speculated upon, although potential causative factors include age (*presbycusis*), disease, parasites, exposure to intense noise, and congenital hearing impairment. Of these, the former is probably the most documented. In bottlenose dolphins, high-frequency hearing loss typically begins at ages in the mid-twenties and the onset of hearing loss occurs in males at slightly younger ages than in females (Houser and Finneran, 2006a). Unfortunately, it was not possible to age animals in the current study and the aetiology of hearing loss remains unknown. In most stranding situations, it is unlikely that the aetiology can be determined unless the stranded odontocete is sent to a rehabilitation facility where more testing and observation can be performed, or it dies or is euthanised such that a pathological examination of the auditory system can be conducted.

Depending upon whether only the animal with severe hearing loss is included in the calculation, or whether animals with elevated thresholds are also included (Table 1), from 7–20% of the stranded common dolphins tested had hearing loss (the dolphin with the notch in hearing is excluded from the calculation). The percentage is substantially less than the incidence of severe to profound hearing loss previously reported for stranded bottlenose dolphins (57%) and rough-toothed dolphins (36%) (Mann *et al.*, 2010). There is an inherent uncertainty in making broad conclusions based on the small sample sizes reported here and in Mann *et al.* (2010), but there are also a number of other factors to consider when comparing these findings. First, the species are different, and although it might seem unlikely that there would be large differences between delphinids in the incidence of hearing loss, it cannot be ruled out. Second are the differences in the manner of data collection, or more specifically, where the data were collected. Most of the animals reported by Mann *et al.* (2010) were tested at rehabilitation facilities and do not represent the broader number of stranding incidents where animals were either quickly released back to sea or were euthanised. The animals reported here were all either returned to sea (n = 13), or less commonly, euthanised (n = 5, including one dolphin that was released, re-stranded and was subsequently euthanised). Thus, the proportion of the population that each study represents is not the same. Furthermore, the degree to which the incidence of hearing loss observed here, or in other studies, can be extrapolated to wild populations as a whole remains uncertain.

Hearing in the common dolphin has previously been studied using evoked potential methods (Popov and Klishin, 1998). The findings of Popov and Klishin with respect to the waveform structure of the ABR are consistent with the findings in all common dolphins tested here. The spectra of the click-evoked response is also typical of findings in odontocetes (Finneran *et al.*, 2009; Finneran *et al.*, 2007). The results, however, highlight the error in an important assumption of the methods employed in this study. The peaks of the click-evoked ABR spectra can be used as estimates of the optimal amplitude modulation rates for SAM tone stimuli used during threshold testing (Finneran *et al.*, 2007; Supin and Popov, 1995). In the case of the common dolphin, estimates of the optimal amplitude modulation rates would be ~700 and ~1200Hz. The amplitude modulation rates in this study were 1000Hz for single SAM stimuli, and centred around 1000Hz for multiple SAM tone combinations. This rate of amplitude modulation falls within the valley of the spectrum between the 700 and 1200Hz peaks and it is not an optimal amplitude modulation rate for this species based upon this approach. However, to verify that this is truly the situation, a modulation rate transfer function, which describes the relationship between the SAM tone amplitude modulation frequency and the resulting ASSR amplitude and phase, should be determined for this species (and every novel species that is tested). Modifications to EVREST^{LT} are currently being explored to allow stranding responders to change the amplitude modulation rate of the signal following the collection of click-evoked responses for new species. Use of optimal modulation rates should increase the quality of the evoked response records and the quality of audiograms obtained with SAM tones.

No click stimuli were presented to the Atlantic white-sided dolphins and the optimal modulation rates for SAM tone stimuli are unknown. It is interesting to note that, although the audiograms were typically delphinid in nature for both species, there were significant differences between the best auditory thresholds for the common dolphin and the Atlantic white-sided dolphin; mean thresholds from the common dolphins were 18–28dB better than that of the Atlantic white-sided dolphins between 28–56kHz. Several reasons may exist for this. One explanation is that the results are real and that significant differences in hearing between the species exist. However, it may be that use of a suboptimal amplitude modulation rate resulted in overestimates of hearing thresholds in the Atlantic white-sided dolphins. Other explanations include the potential that the Atlantic white-sided dolphins were larger, such that evoked responses could not be tracked to the same low stimulus levels, that the effective stimulus level was lower at the auditory bullae due to the size difference, or that the few white-sided dolphins tested had hearing issues and were not representative of the species as a whole.

It will be critical to resolving the uncertainty in the estimates of the Atlantic white-sided dolphin hearing

sensitivity to better estimate optimal amplitude modulation rates for SAM tone stimuli. It seems unlikely that the size of the white-sided dolphins is the sole explanation of the discrepancies in the audiograms, although it may be a contributing factor. It is well known that as body size increases in delphinids, that the brain to body mass ratio becomes less favourable to evoked potential recording (Supin *et al.*, 2001), i.e. signals traveling farther from the brain to the surface where they are recorded become increasingly attenuated. However, lower thresholds closer to that observed in the common dolphin have been obtained in bottlenose dolphins (Houser and Finneran, 2006a), which are larger than both species tested here, suggesting that body size does not fully explain the observation. It is feasible that some of the Atlantic white-sided dolphins tested were older animals with elevated hearing thresholds. As previously stated, age has been related to hearing loss in delphinids (Houser and Finneran, 2006), and mammals in general. Nevertheless, as the current sample size limits the ability to interpret the white-sided dolphin audiograms, broadening the sample size and including animals of calf and sub-adult age classes will better inform the contribution of size and age to the results obtained from the Atlantic white-sided dolphin so far.

The future of AEPs in stranding response

Ideally, experts in the field of evoked potential audiometry would be onsite to perform AEP hearing tests in stranded whales; it is expected that the highest quality audiograms would be obtained in such situations. However, optimisation and automation of hearing test systems, as shown here, offer another option. Successful data collections may occur less often with this approach, but the number of successes given the number of opportunities available will ultimately result in a more rapid acquisition of audiometric information from odontocetes. The end result will be information on the frequency range of hearing and frequency-specific sensitivities from animals which would otherwise remain untested, as well as a gradual increase in sample sizes such that population-level variability in hearing abilities can be determined. Further, there is now a growing group of stranding responders who can collect AEP data during mass strandings and other anomalous events to aid in determining if acoustic disturbance could have played a role in the stranding event.

The eventual incorporation of AEP testing as a regular tool in assessing stranded marine mammals will depend upon AEP system availability and whether AEP methods are flexible, intuitive, and robust across species and situations. Lessons learned from the study described here are informing changes that need to be made in order to achieve these goals. For example, modifications to EVREST^{LT} are being made so that stranding responders can change the rate at which tones are amplitude modulated. As noted above, the use of a sub-optimal amplitude modulation rate can affect the amplitude of the evoked response and potentially affect threshold measurements. As the optimal amplitude modulation rate varies across species, the ability to change the amplitude modulation rate so that the optimal rate can be determined will be necessary for obtaining reliable hearing sensitivity measurements. As more stranding groups become involved in the collection of AEP audiograms, there is also a growing need to standardise procedures so all groups collect and analyse data the same way. As standardisation occurs, AEP systems will need to be modified to make standardised test and analysis procedures available. Nevertheless, progress on the broader use of AEP systems by stranding networks is encouraging, and the potential for using AEP systems to determine hearing abilities of untested species and estimate population-level variability in hearing is gradually being realised.

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