

RESEARCH ARTICLE

Possible age-related hearing loss (presbycusis) and corresponding change in echolocation parameters in a stranded Indo-Pacific humpback dolphin

Songhai Li^{1,2,*}, Ding Wang², Kexiong Wang², Matthias Hoffmann-Kuhnt¹, Nimal Fernando³, Elizabeth A. Taylor¹, Wenzhi Lin⁴, Jialin Chen⁴ and Timothy Ng⁵

¹Tropical Marine Science Institute, National University of Singapore, 18 Kent Ridge Road, Singapore 119227, ²Key Laboratory of Aquatic Biodiversity and Conservation of the Chinese Academy of Sciences, Institute of Hydrobiology of the Chinese Academy of Sciences, 7 South Donghu Road, Wuhan 430072, China, ³Ocean Park Corporation, 180 Wong Chuk Hang Road, Aberdeen, Hong Kong SAR, China, ⁴The Pearl River Estuary Chinese White Dolphin National Nature Reserve, 23 South Road Zhuhai Tangjia, Guangdong, 519080, China and ⁵Ocean Park Conservation Foundation, Hong Kong, 180 Wong Chuk Hang Road, Aberdeen, Hong Kong SAR, China

*Author for correspondence at present address: Marine Mammal and Marine Bioacoustics Laboratory, Sanya Institute of Deep-sea Science and Engineering of the Chinese Academy of Sciences, 62 Fenghuang Road, Sanya 572000, China (lish@sidsse.ac.cn)

SUMMARY

The hearing and echolocation clicks of a stranded Indo-Pacific humpback dolphin (*Sousa chinensis*) in Zhuhai, China, were studied. This animal had been repeatedly observed in the wild before it was stranded and its age was estimated to be ~40 years. The animal's hearing was measured using a non-invasive auditory evoked potential (AEP) method. Echolocation clicks produced by the dolphin were recorded when the animal was freely swimming in a 7.5 m (width)×22 m (length)×4.8 m (structural depth) pool with a water depth of ~2.5 m. The hearing and echolocation clicks of the studied dolphin were compared with those of a conspecific younger individual, ~13 years of age. The results suggested that the cut-off frequency of the high-frequency hearing of the studied dolphin was ~30–40 kHz lower than that of the younger individual. The peak and centre frequencies of the clicks produced by the older dolphin were ~16 kHz lower than those of the clicks produced by the younger animal. Considering that the older dolphin was ~40 years old, its lower high-frequency hearing range with lower click peak and centre frequencies could probably be explained by age-related hearing loss (presbycusis).

Key words: biosonar, high-frequency hearing, marine mammals, auditory evoked potential.

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INTRODUCTION

Extensive studies have described hearing loss from congenital defects, ageing, noise exposure and ear infections in humans (Petit et al., 2001; Morzaria et al., 2004; Nelson and Hinojosa, 2006; Daniel, 2007). Hearing impairment with increasing age (presbycusis) has long been recognised in human populations and presbycusis is the most common cause of hearing loss in older individuals. However, non-experimentally induced hearing loss in other species, particularly age-related hearing loss in marine mammals, has not been extensively studied.

Most marine mammals have an excellent ability to produce, receive and interpret sound (Richardson et al., 1995; Au et al., 2000), which makes them well suited to an aquatic lifestyle. In particular, odontocetes (toothed whales, including porpoises and dolphins) have evolved highly developed high-frequency sound production systems and hearing capabilities for echolocation or biosonar (Nachtigall and Moore, 1988; Au, 1993). Odontocetes typically produce ultrasonic pulse signals (echolocation clicks) varying in frequency between 20 and 150 kHz according to species (Au, 1993), and perceive signals with frequencies higher than (Au et al., 2000; Supin et al., 2001; Nachtigall et al., 2007) or, in a few species (Pacini et al., 2010; Pacini et al., 2011), close to 100 kHz. Compared with most other mammals including humans, the auditory system of odontocetes is hypertrophied and characterised by a large volume

of auditory nerve fibres, a high auditory ganglion cell count, and a high auditory innervation density in the inner ear (Ketten and Wartzok, 1990; Ketten, 1997). The hypertrophy of the auditory system in odontocetes might well arise from the importance of hearing in echolocation analysis in these animals (Ketten, 1997). However, the inner ear of the odontocetes possesses the basic structure and function of an inherently mammalian inner ear (Ketten, 1997). Therefore, the auditory system of odontocetes might reasonably be expected to be subject to impairment/hearing losses in a manner similar to humans. In recent decades, concerns about the impact of anthropogenic acoustics on odontocete species have become particularly acute (Richardson et al., 1995; Popper and Hawkins, 2012). It follows that hearing loss in odontocetes is an increasingly important consideration. While there is some information on the fundamental hearing ability of many odontocete species (Au et al., 2000; Mooney et al., 2012), hearing loss has only been investigated and demonstrated in a few species in captivity (Ridgway and Carder, 1997; Brill et al., 2001; Finneran et al., 2005b; Yuen et al., 2005; Houser and Finneran, 2006; Houser et al., 2008) or while stranded (Mann et al., 2010). Little is known about the incidence and cause of the reported hearing loss in odontocetes, and differences across species and habitat conditions.

Presbycusis and sex differences in the onset of presbycusis have been documented in captive populations of bottlenose dolphins

(*Tursiops truncatus*) (Ridgway and Carder, 1997; Brill et al., 2001; Houser and Finneran, 2006; Houser et al., 2008). Typically, presbycusis begins to occur around the age of 20–30 years for bottlenose dolphins kept in captivity, with a progressive loss of high-frequency hearing with age and an earlier onset of hearing loss in males than in females (Houser and Finneran, 2006). Although it has been assumed that loss of high-frequency hearing might result in a change in signal parameters of the echolocating dolphin (Houser and Finneran, 2006; Ibsen et al., 2007), a directly empirical test indicating that the animal changed its echolocation signals to match its range of best hearing has only been performed on a false killer whale (*Pseudorca crassidens*) in captivity (Kloepper et al., 2010a). It is therefore not clear whether presbycusis and active spectral compensation of echolocation clicks for the hearing deficits are common in all odontocete species and also for animals in the wild. Understanding how hearing and echolocation parameters change with age in odontocetes in natural contexts is important in order to understand how hearing and echolocation function in survival and exploration of the environment by animal populations, and also to assess how anthropogenic sound affects the populations.

This paper describes a study of hearing and echolocation clicks of a recently stranded Indo-Pacific humpback dolphin, *Sousa chinensis* (Osbeck 1765). The hearing and echolocation clicks of the subject were compared with those of a conspecific younger individual that was recently investigated using the same methods (Li et al., 2012). The comparisons of hearing and echolocation click

parameters between the two individuals provide a unique opportunity to understand and interpret the hearing capabilities of the recently stranded dolphin, and to understand how changes in hearing might affect echolocation click production in the Indo-Pacific humpback dolphin.

MATERIALS AND METHODS

Ethical statement

This research was conducted under China's Wildlife Protection Act, 1989, Implementation By-law on Aquatic Wildlife Conservation.

Subject

The subject of the present study (Fig. 1A) was a male Indo-Pacific humpback dolphin that was rescued from stranding in an inland river of Foshan, China, ~200 km upstream from the Pearl River Estuary, on 12 March 2012. On the same day, the dolphin was transported to the rescue centre of the Pearl River Estuary Chinese White Dolphin National Nature Reserve, Zhuhai, China (Fig. 1B), for further assessment and rehabilitation. With the support of Ocean Park Hong Kong and Ocean Park Conservation Foundation, Hong Kong, the animal's health stabilised after a few weeks. The dolphin was not administered any ototoxic drugs during the rehabilitation process. The dolphin was 2.43 m in length and 213 kg in mass at the time of the stranding. This stranded animal was photo-identified in 1996 for the first time in Hong Kong waters, the Pearl River Estuary, and since then identified in Hong Kong waters

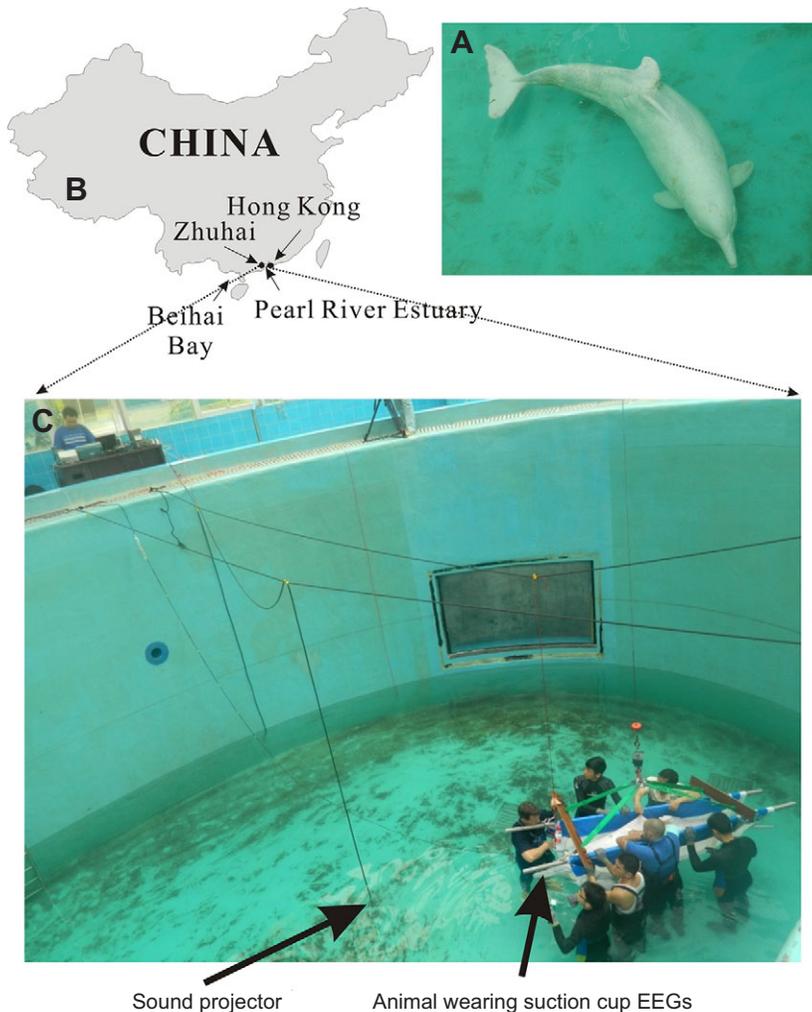


Fig. 1. Experimental site and facilities. (A) The present subject (the older dolphin). (B) Experimental site in the rescue centre of the Pearl River Estuary Chinese White Dolphin National Nature Reserve, Zhuhai, China. (C) The dolphin was positioned in a stretcher at the water surface while wearing electroencephalography (EEG) electrodes attached to the skin by soft silicone suction cups. The sound projector was positioned ~2 m from the animal's 'acoustic windows', where the sound is assumed to travel to the animal's inner ear (Norris, 1968; Popov et al., 2008).

approximately twice a year until November 2010 (Hung, 2011). Analysis of the teeth, particularly the wear on them, and the skin colour based on the life history information of the Indo-Pacific humpback dolphin (Jefferson et al., 2012) suggested that the dolphin was around 40 years old at the time of the study.

A hearing test has been deemed an important part of the health assessment of stranded dolphins. The present subject's hearing was measured on 16 April 2012. To better evaluate the dolphin's hearing, its echolocation clicks were also recorded on 15 April 2012. Both the hearing and echolocation clicks of the present subject were compared with those of a conspecific younger male, ~13 years old, which was rescued from stranding on the coast of Beihai Bay, China (Fig. 1B), in August 2007 (see Li et al., 2012). The methodology and equipment used for the two dolphins was the same or comparable.

Hearing measurement

The hearing measurement of the older dolphin was conducted in the main pool (Fig. 1C) of the rescue centre using a non-invasive auditory evoked potential (AEP) method. The pool was a kidney-shaped concrete structure 7.5 m in width, 22 m in maximum length and 4.8 m in depth, filled with artificial seawater. In order to facilitate a basic medical examination and the hearing measurement, the water depth in the pool was lowered to ~1.3 m before the measurement was performed.

During the hearing measurement, the method for sound stimulus presentation and calibration, AEP recording and hearing threshold determination was the same as previously described (Li et al., 2012) and is briefly described here. The sound stimuli were rhythmic pip trains composed of cosine-enveloped 0.25 ms tone pips with a 1 kHz pip rate and a variable carrier frequency. The 1 kHz pip rate was chosen based on an estimated modulation rate transfer function (MRTF) of the experimental subject, which was established right before the hearing measurement. Each pip train was 20 ms in duration followed by 30 ms silence so that the pip trains were

presented at a rate of 20 s⁻¹. The stimuli were digitally synthesised using a customised LabVIEW (National Instruments, Austin, TX, USA) program at an update rate of 512 kHz, and the digital-to-analog conversion was accomplished by a data acquisition card (DAQ, NI USB-6251 BNC, National Instruments) connected to a laptop computer (PC). The analog signals were then attenuated by a HP-350D attenuator (Hewlett Packard, Palo Alto, CA, USA) and amplified by a HP-465A power amplifier (Hewlett-Packard). The power amplifier had a fixed gain of 20 dB and the attenuator was used to vary the signal amplitude. The signals were monitored using an oscilloscope (Tektronix TDS1002C, Beaverton, OR, USA) before being projected through a Reson TC-4040 hydrophone (Reson, Slangerup, Denmark), which was positioned at a distance of ~2 m and a depth of 0.5 m in front of the subject's 'acoustic windows' along the body axis. The 'acoustic windows' are located at the lower jaw area of the subject, through which sound is assumed to travel to the inner ear (Norris, 1968; Popov et al., 2008). Sound pressure levels (SPLs, dB re. 1 µPa) of the projecting sound stimuli were measured and calibrated in root mean square (r.m.s.) of the whole pip train, including both the pips and inter-pip pauses (Li et al., 2012), by positioning a calibrated receiving hydrophone at the same location as the animal's lower jaw area. Carrier frequencies varied from 5.6 to 128 kHz, and were chosen to be 5.6, 11.2, 32, 38, 45, 54, 64, 76, 90, 108 and 128 kHz. In Fig. 2, the waveforms (left) and corresponding spectra (right) of the pip trains with carrier frequencies of 5.6, 11.2, 38, 54 and 108 kHz are presented as examples of the received stimuli at the animal's lower jaw area. The frequencies of the received stimuli were fairly centred at the expected carrier frequencies for all the stimuli.

The experimental setup for the hearing measurement is shown in Fig. 1C and the data flow chart is presented in Fig. 3. During the hearing measurement, the water depth in the pool was kept at ~1.3 m, and the dolphin was positioned in a stretcher (Fig. 1C) in such a way that the dorsal fin and the dorsal surface of the head with the blowhole remained above the water surface, while the lower jaw

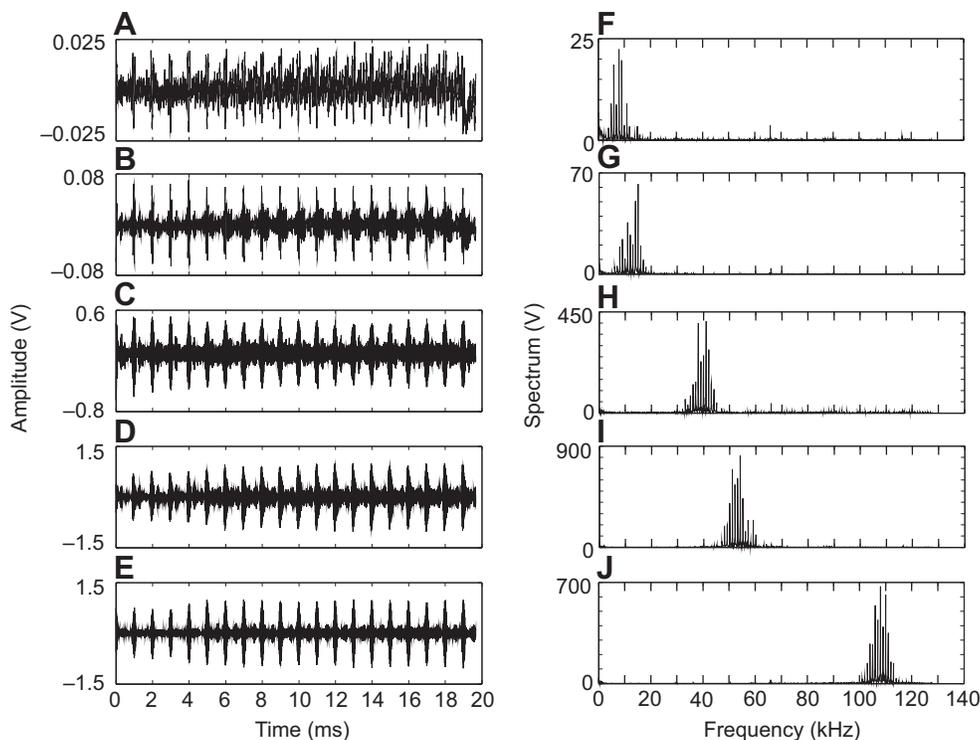


Fig. 2. Examples of waveforms (left) and spectra (right) of the stimuli (pip trains) received by the calibrated hydrophone at the subject's acoustic windows. Stimulus waveforms are presented with carrier frequencies of (A) 5.6 kHz, (B) 11.2 kHz, (C) 38 kHz, (D) 54 kHz and (E) 108 kHz. (F–J) The corresponding power spectra of the stimuli in A–E.

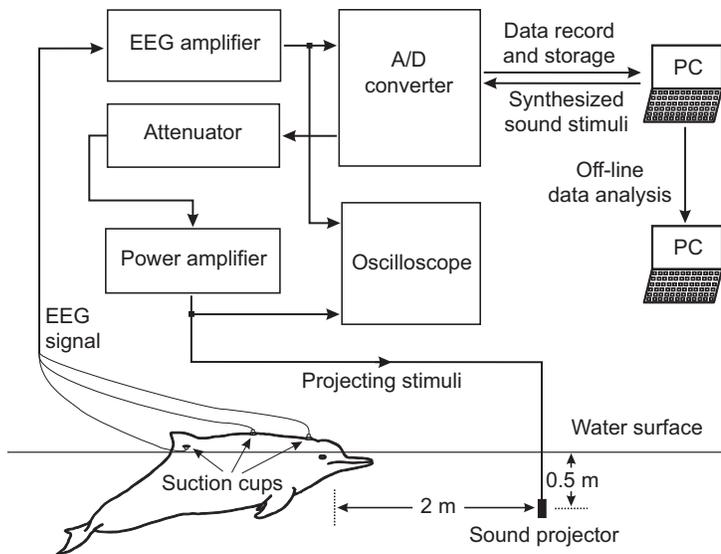


Fig. 3. Schematic diagram of the dolphin's relative position, data recording equipment and flow chart (from Li et al., 2012). A/D converter, analog-to-digital converter.

was maintained underwater and open to the sound stimuli throughout the experiment. Three suction-cup electroencephalography (EEG) electrodes were non-invasively attached to the back of the dolphin for the AEP recording. Examination of electrical noise before the AEP recording was comparable to that in our previous study (Li et al., 2012).

The background acoustic noise in the experimental pool was recorded before the hearing measurement commenced and when the water depth in the pool was ~ 2.5 m. Ambient noise was received using a Reson TC-4013-13 hydrophone (-216 dB re. $1 \text{ V } \mu\text{Pa}^{-1}$), and then amplified with a 50 dB gain within a frequency range of 0.1 to 200 kHz, via an EC6081 pre-amplifier (VP2000; Reson). The amplified noise was input to a 16 bit analog-to-digital (A/D) converter of the DAQ and recorded by the PC running a program designed using LabVIEW software with a sampling rate of 512 kHz. The recorded noise was analysed and averaged using a MATLAB algorithm (MathWorks, Natick, MA, USA). The experimental pool was drained from a water depth of 2.5 to ~ 1.3 m to facilitate the experimental operation during hearing measurements. However, for animal safety and comfort, the pool was quickly refilled after the hearing measurement and consequently we did not have the opportunity to measure the background acoustic noise in the experimental pool when the water depth was ~ 1.3 m. Therefore, the measured background acoustic noise level at a water depth of 2.5 m was adopted to represent the ambient noise environment when the hearing of the subject was measured, by assuming that the background acoustic levels of the pool would not change dramatically with a water depth change from 2.5 to 1.3 m.

The animal's AEP responses to the sound stimuli (i.e. envelope-following responses, EFRs) were received by the three suction-cup EEG electrodes (Grass Technologies, West Warwick, RI, USA): gold-plated discs 10 mm in diameter mounted within latex suction cups 60 mm in diameter. The recording electrode was attached using a suction cup to the dorsal surface of the head, ~ 5 – 7 cm behind the blowhole, using conductive gel to ensure conductivity. The reference electrode was attached in a similar way to the animal's dorsal fin. The third electrode acted as a grounding device and was positioned on the back of the animal between the recording and reference electrodes (Fig. 3). The AEP responses were conducted by shielded cables to an EEG amplifier (Grass CP511 AC Amplifier, Grass

Technologies) and amplified 20,000 times within a frequency band of 300 to 3000 Hz. The amplified signal was monitored by a Tektronix TDS1002C oscilloscope and input to a 16 bit A/D converter of the same DAQ that generated the synthesised sound stimuli (Fig. 3). The AEP response triggered by the sound stimulus onset was then digitised at a sampling rate of 25 kHz and transmitted to the laptop computer. To extract the AEP response from noise, AEPs were collected by averaging 1000 individual AEP records, each of which was 30 ms in duration, using the same LabVIEW program that synthesised the sound stimuli.

To estimate a hearing threshold for each carrier frequency, typically six to nine AEP records with a series of stimulus SPLs in 5–10 dB steps were recorded and measured (Li et al., 2012). For each frequency and stimulus SPL, a 15 ms (375 point) window of the EFR to the rhythmic sound stimulus, from 5 to 20 ms in the AEP record (Li et al., 2012), was fast Fourier transformed (FFT) to obtain a frequency spectrum. The magnitude at 1 kHz in the spectrum was used to estimate the response of the subject to the sound stimulus (Nachtigall et al., 2007; Li et al., 2012). For each frequency, the magnitudes at 1 kHz were measured and plotted as a function of stimulus SPLs, and the near-threshold portion of the plot was approximated by a linear regression line (Supin et al., 2001; Nachtigall et al., 2007). The intersection of the regression line with the zero crossing point of the response input–output function was adopted as a threshold estimate (Supin et al., 2001; Nachtigall et al., 2007; Li et al., 2012). Fig. 4 shows an example of the AEP records (i.e. EFR) for sound stimuli with a carrier frequency of 38 kHz (Fig. 4A), the corresponding FFT spectra (Fig. 4B), and the response input–output function (Fig. 4C) with data from the present study.

The resulting audiogram (a function of hearing threshold *versus* the corresponding stimulus carrier frequency) acquired from the older dolphin was compared with that of the younger dolphin (Li et al., 2012).

Echolocation recording and analysis

Echolocation clicks of the older dolphin were recorded before the hearing measurement when the animal was freely swimming in the main pool with a water depth of approximately 2.5 m. The sound was received by the Reson TC-4013-13 hydrophone at a depth of 0.5 m below the water surface, and then amplified with a 50 dB gain

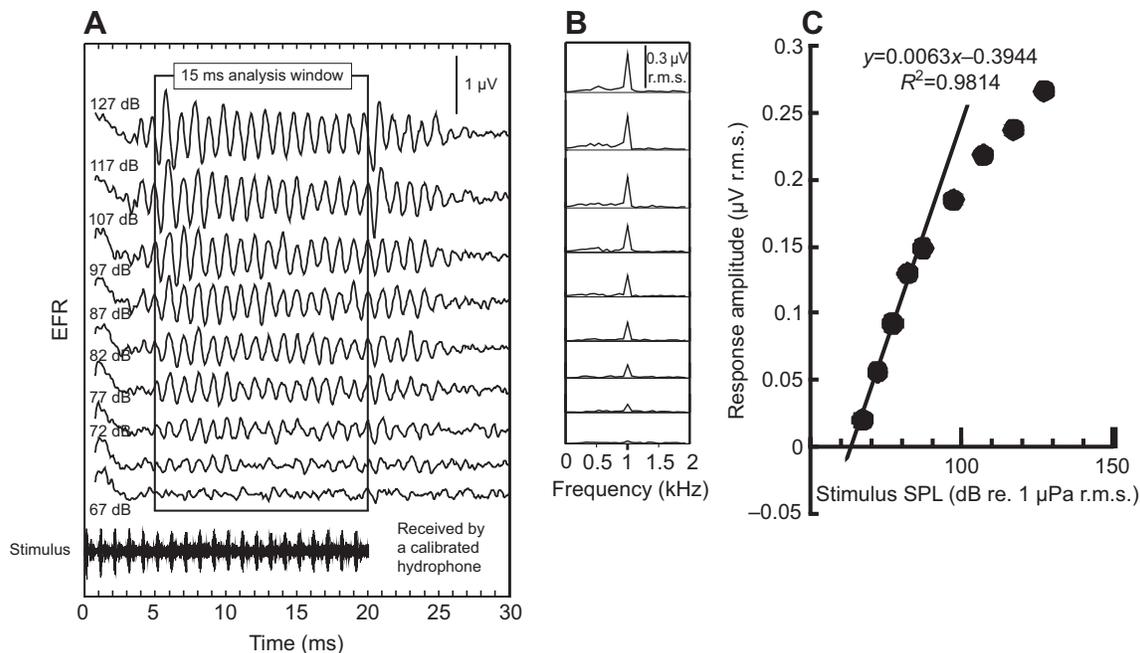


Fig. 4. (A) Examples of auditory evoked potential (AEP) response (envelope-following response, EFR) waveforms recorded at various sound pressure levels (SPLs) [as indicated in dB re. 1 μ Pa root mean square (r.m.s.) on the left of each EFR waveform] of the stimulus with 38 kHz carrier frequency; the stimulus was a rhythmic 20 ms pip train composed of cosine-enveloped 0.25 ms tone pips with a 1 kHz pip rate. (B) Frequency spectra of the corresponding EFR waveforms with fast Fourier transform (FFT) of a 15 ms analysis window. (C) Function of the EFR amplitude versus stimulus SPL for 38 kHz sound stimuli as an example. The function was approximated by a linear regression (bold line) at the near-threshold portion with SPLs between 67 and 87 dB. The threshold was defined as the intersection of the regression line with the hypothetical zero-response value. The threshold at the present stimulus frequency was estimated to be 63 dB.

within a frequency range of 0.1 to 200 kHz by the EC6081 preamplifier. The amplified sounds were input to a 16 bit A/D converter of the DAQ and recorded by the PC through a LabVIEW program at a sampling rate of 512 kHz. The echolocation clicks from the younger dolphin were previously collected in the same way with the same instruments or instruments of the same model (Li et al., 2012).

For the older and younger dolphins, the recorded sounds were analysed using customised MATLAB algorithms in the same way. To increase the chance that the analysed clicks were recorded from the on-axis transmission beam of the dolphin (Au, 1993), only the sounds recorded when the head of the dolphin was observed pointing towards the hydrophone were included for off-line analysis. In the off-line analysis, the sounds were continuously monitored by a customised MATLAB algorithm to extract the echolocation clicks. Each time the signal had a signal-to-noise ratio higher than 20 dB, a click extraction was triggered and a time window consisting of 37 sample points (approximately 70 μ s), including 10 pretrigger points (approximately 20 μ s), was extracted for further analysis. Because reverberations often followed and even overlapped with the click itself, it was necessary to make a subjective decision to differentiate the actual echolocation click, which has a clear sinusoidal wave-shape and is smoothly enveloped, from the reverberations. After the manual selection of the analysed clicks, parameters (see Au, 1993) for both time and frequency characteristics were extracted or calculated from each click. The click time duration was described by three parameters: -3 dB duration ($\tau_{-3\text{dB}}$), -10 dB duration ($\tau_{-10\text{dB}}$) and 95% energy duration ($\tau_{95\%E}$) (Fig. 5A,B). The click frequency characteristics were described in five parameters: peak frequency (f_p), centre frequency (f_c), -3 dB bandwidth ($BW_{-3\text{dB}}$), -10 dB bandwidth ($BW_{-10\text{dB}}$) and

r.m.s. bandwidth (BW_{rms}) (Fig. 5C). Because of uncertainties in estimating the distance between the hydrophone and the animal that was freely swimming in the pools, the source levels of the clicks were not calculated. Additionally, the selected clicks were categorised and sorted into four types (types I–IV) by spectrum shape based on similar spectral classification used elsewhere (Au et al., 1995; Kloepper et al., 2010a). Type I clicks contained a single spectrum peak with peak frequencies below 70 kHz. Type II clicks had peak frequencies lower than 70 kHz, but a secondary peak within 3 dB of the primary peak at high frequencies above 70 kHz. Type III clicks contained a primary spectrum peak at frequencies above 70 kHz, and a secondary peak within 3 dB of the primary peak at frequencies below 70 kHz. Type IV clicks contained a single spectrum peak at frequencies above 70 kHz.

The statistical software package PASW Statistics 19.0 (SPSS Institute Inc., Chicago, IL, USA) was used for the descriptive (mean, standard deviation) and comparative statistical analysis of the click parameters. After it was verified that the data distribution was not normal, the non-parametric Mann–Whitney test was used to determine whether parameters varied between the older dolphin and the younger dolphin.

RESULTS

Hearing

The measured audiogram of the older dolphin is presented in Fig. 6, together with the audiogram from the previously investigated younger dolphin (Li et al., 2012). Fig. 6 indicates that the audiogram of the older dolphin had a U-shape, generally similar to that of the previously investigated younger dolphin but shifted towards lower frequencies and higher thresholds. The lowest threshold (highest hearing sensitivity) of 63 dB re. 1 μ Pa r.m.s. was measured at 38 kHz,

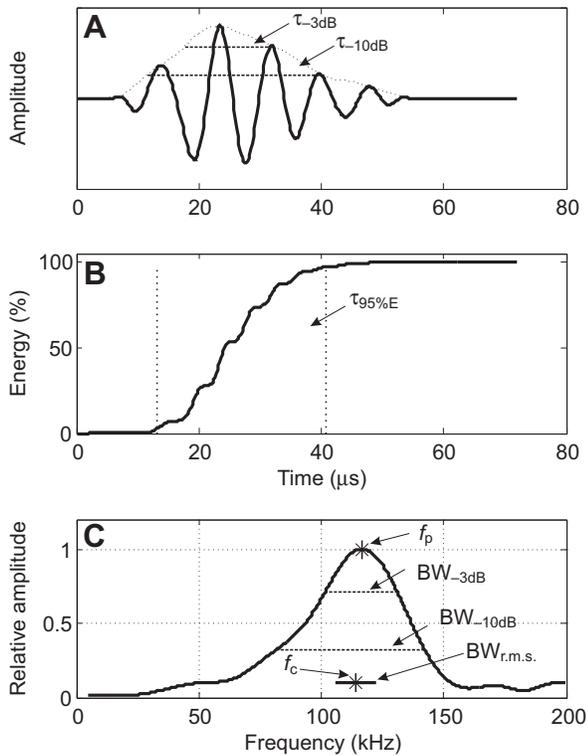


Fig. 5. Examined parameters of the echolocation clicks. (A) Click with signal envelope and -3 dB ($\tau_{-3\text{dB}}$) or -10 dB duration ($\tau_{-10\text{dB}}$); (B) accumulated energy content in the click over time and the 95% energy duration ($\tau_{95\%E}$) between the dotted lines; (C) normalized frequency spectrum of the click with peak frequency (f_p), centre frequency (f_c), -3 dB bandwidth ($BW_{-3\text{dB}}$), -10 dB bandwidth ($BW_{-10\text{dB}}$), and r.m.s. bandwidth ($BW_{\text{r.m.s.}}$).

and the frequency region with relatively high hearing sensitivity (within 20 dB of the lowest threshold) was identified between approximately 8 and 64 kHz for the older dolphin. While the hearing thresholds at frequencies of 5.6 and 11.2 kHz for the older dolphin were slightly lower than those of the younger dolphin, the hearing thresholds in the frequency range of 32 to 76 kHz for the older dolphin were approximately 10–20 dB higher. At frequencies higher than 76 kHz, hearing thresholds of the older dolphin increased steeply with a rate of approximately 107 dB/octave, up to 115 dB re. $1 \mu\text{Pa}$ r.m.s. at 90 kHz, which was over 50 dB higher than that of the younger dolphin at the same frequency.

The power spectrum density of background acoustic noise in the experimental pools (means \pm s.d.; dB re. $1 \mu\text{Pa}^2 \text{Hz}^{-1}$), which was calculated by performing a FFT of 10 ms noise windows for each sample and averaged over 1000 samples, is also shown in Fig. 6 for both the older dolphin and the younger dolphin. It was noted that the background acoustic noise in the two experimental pools inhabited by the two dolphins was obviously different. The background acoustic noise levels in the experimental pool for the older dolphin were approximately 20–30 dB higher than those in the experimental pool for the younger dolphin, and the power spectrum density of the background acoustic noise for the older dolphin was as high as, or higher than the thresholds for the younger dolphin for all frequencies between 20 and 108 kHz.

Echolocation clicks

From approximately 30 min of sound recordings for each dolphin, 574 clicks were selected for measurement for the older dolphin, and

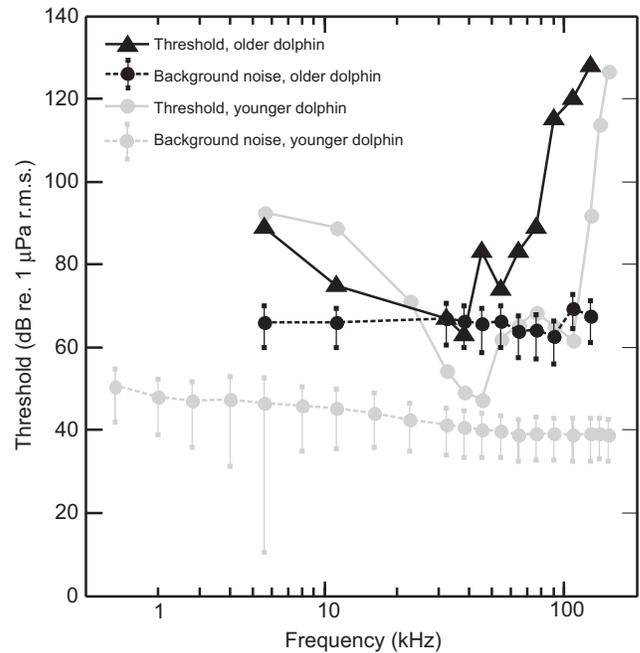


Fig. 6. Audiograms of the older dolphin (present study) and a conspecific younger individual, ~ 13 years of age. The power spectrum density (mean \pm s.d., dB re. $1 \mu\text{Pa}^2 \text{Hz}^{-1}$, $N=1000$) of the background noise in the experimental pools for both the older dolphin and the younger dolphin is also presented. The audiogram and noise power spectrum density for the younger dolphin were adapted from our previous study (Li et al., 2012).

316 clicks were selected for the younger dolphin. All the selected clicks were categorised by spectrum shape according to the click category criteria described above. Representative waveforms and spectra together with their time and frequency parameters for each click type are shown in Fig. 7A for the older dolphin, and Fig. 7B for the younger dolphin. For the older dolphin, 7.5% of the analysed clicks contained a single spectrum peak at low frequencies (type I); 6.6% of the clicks had a frequency peak at low frequencies but a secondary peak at high frequencies (type II); 14.1% contained a primary spectrum peak at high frequencies above 70 kHz and a secondary peak at low frequencies (type III); and most clicks (71.8%) contained a single high-frequency peak (type IV). The low-frequency peaks were typically at ~ 50 kHz, and the high-frequency peaks were typically at ~ 100 kHz (Fig. 7A). In the younger dolphin, no clicks were observed containing a single spectrum peak at frequencies lower than 70 kHz (type I); only 0.3% of the analysed clicks contained a primary spectrum peak at low frequencies and a secondary peak at high frequencies (type II); 6.0% of the clicks had a primary spectrum peak at high frequencies and a secondary peak at low frequencies (type III); and the overwhelming majority of the clicks (93.7%) contained a single high-frequency peak above 110 kHz (type IV) (Fig. 7B). For both animals, no apparent spectral peaks at frequencies above 150 kHz were observed.

Descriptive and comparative statistical analysis of the click parameters between the older dolphin and the younger dolphin are shown in Table 1. Except for $\tau_{-3\text{dB}}$ and $\tau_{-10\text{dB}}$, all other measured or calculated click parameters were significantly different between the two dolphins (Mann–Whitney test, $P < 0.05$). In particular, f_p and f_c of the older dolphin were 16.5 kHz lower (Mann–Whitney test, $N=890$; $Z=-17.6$; $P < 0.05$) and 16.1 kHz lower (Mann–Whitney test, $N=890$; $Z=-3.7$; $P < 0.05$), respectively, compared with those of the

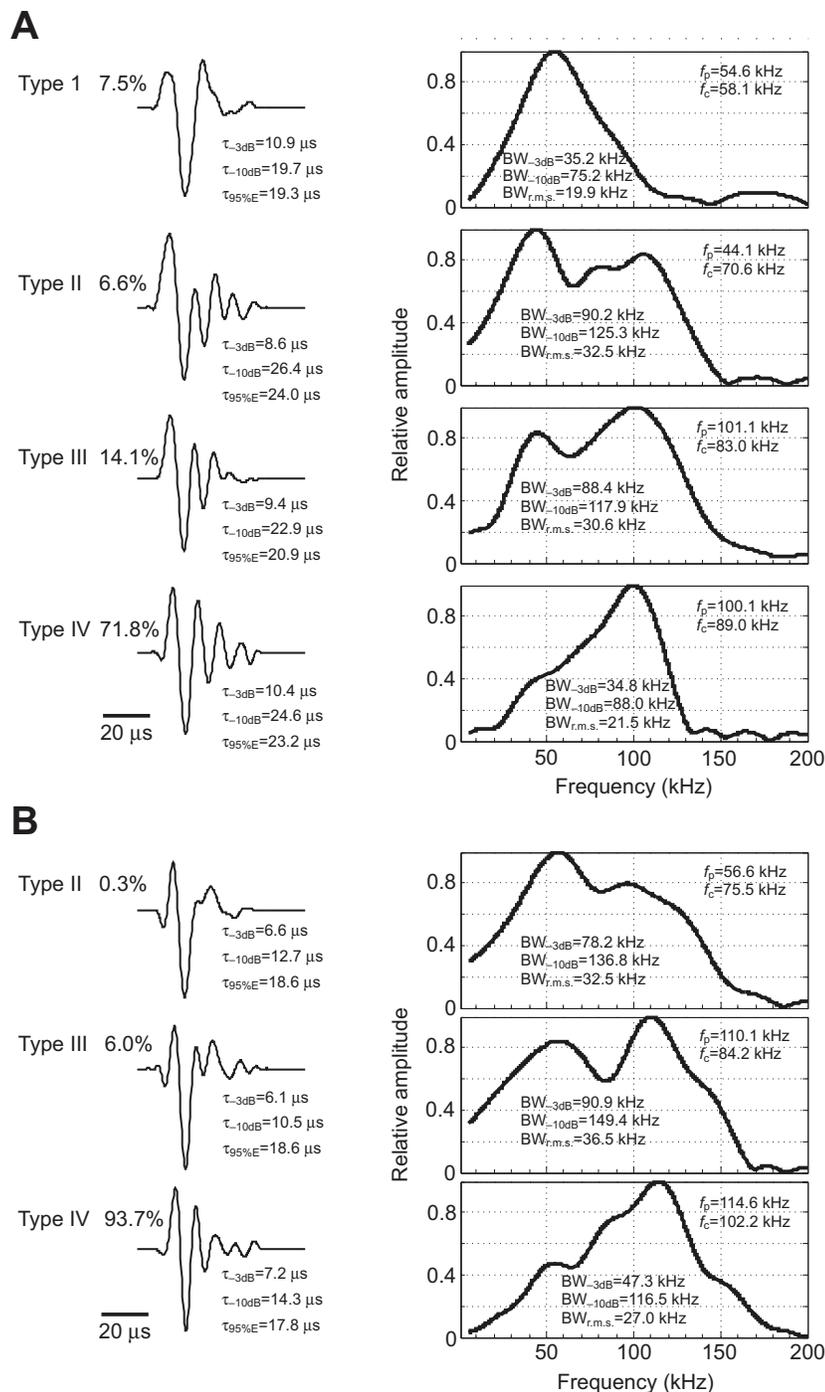


Fig. 7. Examples of the different click types produced by the older dolphin (A) and the younger dolphin (B). The representative waveform is shown on the left with the time parameters and the relative percentage of each click type, and the representative normalized frequency spectrum is on the right with frequency parameters.

younger dolphin. Histograms of the f_p indicated that the most preferred click f_p for the older dolphin was around 105 kHz, while the most preferred click f_p for the younger dolphin was around 115 kHz (Fig. 8A). Histograms of the f_c showed that most clicks produced by the older dolphin had a f_c between 80 and 100 kHz, while the younger dolphin appeared to have a preference for emitting clicks with f_c between 100 and 120 kHz (Fig. 8B).

DISCUSSION

While the method for hearing measurement and overall experimental procedure for the present subject (the older dolphin) was kept as similar (or comparable) as possible to that for the hearing measurement of the younger dolphin, the hearing of the two animals

was measured in different situations and different experimental pools. The hearing of the older dolphin was measured when the animal was positioned in a stretcher at the water surface of a pool with a water depth of ~ 1.3 m. The hearing of the younger dolphin was measured when the animal was trained to voluntarily position at the water surface of a pool with a water depth of ~ 5 m. The background acoustic noise levels in the experimental pool for the older dolphin were higher than those in the experimental pool for the younger dolphin throughout the examined frequencies (Fig. 6).

Despite the higher background noise levels, the older dolphin demonstrated slightly lower hearing thresholds at 5.6 and 11.2 kHz relative to the younger dolphin (Fig. 6). This seems to indicate that the masking effects on the older dolphin's hearing even at the relatively

Table 1. Descriptive and comparative statistical analysis for click parameters from the younger dolphin and the older dolphin

	Younger dolphin (N=316)			Older dolphin (N=574)			Z	P
	Mean \pm s.d.	Range	CV (%)	Mean \pm s.d.	Range	CV (%)		
τ_{-3dB} (μ S)	10.5 \pm 4.0	4.9-20.7	38.4	9.8 \pm 3.2	5.7-24.2	32.8	-0.3	0.792
τ_{-10dB} (μ S)	21.8 \pm 5.2	9.8-38.9	23.8	22.1 \pm 6.1	10.4-40.4	27.7	-0.1	0.921
$\tau_{95\%E}$ (μ S)	23.0 \pm 4.2	15.6-36.5	18.5	24.7 \pm 5.0	11.3-37.3	20.2	-5.8	0.000
f_p (kHz)	114.1 \pm 9.6	56.6-142.1	8.4	97.6 \pm 16.4	43.5-129.5	16.8	-17.6	0.000
f_c (kHz)	106.9 \pm 10.9	68.0-134.2	10.2	90.8 \pm 10.5	57.0-113.5	11.5	-3.7	0.000
BW_{-3dB} (kHz)	41.8 \pm 14.3	21.1-98.4	34.2	47.2 \pm 18.7	21.4-125.7	39.6	-3.7	0.000
BW_{-10dB} (kHz)	94.1 \pm 28.7	48.1-161.4	30.4	101.4 \pm 19.4	46.7-157.4	19.2	-17.5	0.000
BW_{rms} (kHz)	24.7 \pm 6.0	14.6-43.1	24.3	25.8 \pm 3.9	16.7-39.4	15.1	-3.8	0.000

*Significant difference ($P < 0.05$).

high acoustic noise levels in the pool were negligible at these low frequencies. The older dolphin perhaps had a comparable or even slightly better hearing than the younger dolphin at frequencies of 5.6 and 11.2 kHz. Nevertheless, in the frequency range of 32 to 76 kHz, the ~ 10 – 20 dB higher hearing thresholds for the older dolphin relative to the younger dolphin were quite possibly a result of the 20–30 dB higher background noise levels (Fig. 6). To quantitatively evaluate the masking effects of noise on the dolphin's hearing, information on hearing filter shape and receiving directivity index of the dolphin hearing system is essential (Au, 1993). Unfortunately, studies on the hearing filter shape and receiving directivity index of the hearing

system of the Indo-Pacific humpback dolphin have not yet been performed. Therefore, direct evaluation on masking effects of the background acoustic noise on the present subject's hearing thresholds was not possible. Assuming that the present subject had a hearing filter shape and receiving directivity index comparable with those of the bottlenose dolphin (Au and Moore, 1982; Au and Moore, 1990; Lemonds et al., 2012), it was very likely that the background acoustic noise in the frequency range between 32 and 76 kHz was audible to the older dolphin and almost certainly masked its true hearing thresholds. At high frequencies above 76 kHz, the hearing thresholds of the older dolphin increased steeply while the background acoustic noise levels were rather stable (Fig. 6). The steep increase in thresholds with a threshold of 115 dB re. 1 μ Pa r.m.s. at 90 kHz, which is over 50 dB higher than the background noise level and the corresponding threshold of the younger dolphin, might represent a natural feature of the older dolphin's hearing. This suggests that the cut-off frequency of the high-frequency hearing of the older dolphin was between 76 and 90 kHz, which is ~ 30 – 40 kHz lower than that of the younger dolphin (Fig. 6) (Li et al., 2012). Therefore, although the higher hearing thresholds at the frequency range of 32 to 76 kHz for the older dolphin might be a result of the masking effect, the older dolphin seemed to demonstrate a high-frequency hearing loss relative to the younger dolphin.

Hearing loss has previously been documented in odontocete species in both captive (Ridgway and Carder, 1997; Brill et al., 2001; Finneran et al., 2005b; Yuen et al., 2005; Houser and Finneran, 2006; Houser et al., 2008) and stranded conditions (Mann et al., 2010). Assuming that the auditory system of odontocetes is similarly subject to impairment/hearing loss as reported in humans and other mammals, hearing loss in odontocetes can be caused by intense chronic noise, transient intense noise exposure, congenital hearing impairment, physical trauma, infections of the inner ear, ototoxic drug treatment and presbycusis (Tarter and Robins, 1990). Acoustic trauma has been suggested as a factor leading to the stranding of odontocetes (Balcomb and Claridge, 2001). Unfortunately, we do not know the noise exposure history of the present subject, but based on the location of the animal's stranding and its home range observed between 1996 and 2010, it is possible that the older dolphin had been exposed to chronic environmental noise from boating and shipping and other natural and anthropogenic sources of noise. However, controlled experiments with odontocetes have shown that high levels of exposure are needed to induce temporary threshold shifts (Finneran et al., 2002; Finneran et al., 2005a; Nachtigall et al., 2003; Mooney et al., 2009). During the post-stranding treatment and rehabilitation, the older dolphin was not given ototoxic medicines such as aminoglycosidic antibiotics, which might damage the hair cells of the cochlea and result in dolphin hearing loss (Finneran et al., 2005b). Considering that the U-shaped audiogram

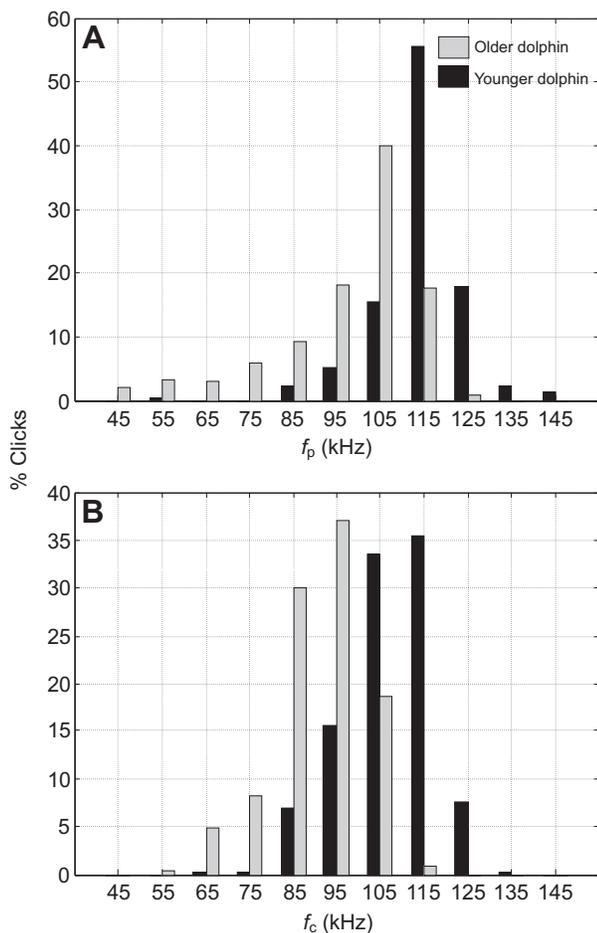


Fig. 8. Histograms of (A) the peak frequency (f_p) and (B) the centre frequency (f_c) of all the measured clicks for both the older and younger dolphin.

of the older dolphin was generally similar to that of the younger (13 year old) dolphin but shifted towards lower frequencies (Fig. 6), and the older dolphin was estimated to be ~40 years of age, the high-frequency hearing loss of the older dolphin relative to the younger dolphin was likely to be the result of presbycusis.

Presbycusis has been demonstrated in captive populations of bottlenose dolphins (Ridgway and Carder, 1997; Brill et al., 2001; Houser and Finneran, 2006; Houser et al., 2008) and a captive false killer whale (Yuen et al., 2005; Kloepper et al., 2010a; Kloepper et al., 2010b), although it might not be common in wild dolphins (Cook, 2006). For bottlenose dolphins studied in captivity, presbycusis generally begins to occur between the ages of 20 and 30 years, with an earlier onset of hearing loss in males than in females; and all animals over the age of 27 years had some degree of hearing loss (Houser and Finneran, 2006). However, measurement of hearing abilities in a free-ranging bottlenose dolphin population, ranging in age from 2 to 36 years, did not demonstrate substantial hearing loss in any of the measured dolphins (Cook, 2006). It is possible that older animals (>36 years old) in the wild do exhibit hearing loss, but have not been tested, or that wild individuals experiencing hearing loss have lower survival rates than individuals with normal hearing (Cook, 2006). Alternatively, it could be simply because the age structure of the sampled population in Cook's study does not substantially overlap with that of the animals in other studies. A female false killer whale in captivity demonstrated a loss of high-frequency hearing of about 70 kHz over a 16 year time period (Yuen et al., 2005; Kloepper et al., 2010a; Kloepper et al., 2010b). The f_p of the whale's echolocation clicks was correspondingly lowered by over 30 kHz, and f_c was lowered by about 10 kHz over the time period of the study (Kloepper et al., 2010a). This change in the echolocation clicks of the false killer whale was interpreted as matching the animal's range of best hearing (Kloepper et al., 2010a). Changes in click parameters over time were also observed in an echolocating female Atlantic bottlenose dolphin. The average f_p of this dolphin's echolocation clicks was down-shifted from 138 kHz in 1998, when the dolphin was 13 years old, to 40 kHz in 2003/2004 (Ibsen et al., 2007). Although the actual upper hearing limit of this dolphin in 1998 was unknown, the animal had an upper hearing limit of 45 kHz in 2001 and 2005, matching well with the click f_p of 40 kHz measured in 2003/2004. It is therefore possible that the observed down-shift in click f_p was an attempt by the bottlenose dolphin to compensate for a possible high-frequency hearing loss suffered between 1998 and 2003 (Ibsen et al., 2007). Assuming the high-frequency hearing loss of the present subject was due to presbycusis, and active frequency compensation of echolocation clicks for hearing deficits is common in odontocete species and also for dolphins in the wild, we expect that a similar pattern of change in click parameters over time occurred in the recently stranded Indo-Pacific humpback dolphin (i.e. the older dolphin).

Unfortunately, no echolocation clicks of the present subject were collected before the dolphin was stranded, so the way in which the subject's click parameters changed over time is unknown. However, a comparison of echolocation clicks between the older dolphin and the younger dolphin demonstrates significant differences in click frequency parameters and in some of the click time parameters (Table 1). The click spectrum shapes were obviously different for the two dolphins. The older dolphin produced more clicks with low-frequency peaks and fewer clicks with high-frequency peaks when compared with the younger dolphin (Fig. 7). The histograms of the f_p and f_c of the echolocation clicks produced by the older dolphin were shifted towards lower frequencies compared with those produced by the younger dolphin (Fig. 8). Average f_p and f_c values

for the older dolphin were ~16 kHz lower than those of the younger dolphin (Table 1). Large variations in echolocation click parameters, such as f_p , have been demonstrated in odontocetes between species, between populations within the same species, and even between clicks of the same individual under different behavioural contexts and acoustic environments (Au, 1993). Beluga whales have been shown to shift the f_p of their echolocation clicks upwards when moved to a noisier environment (Au et al., 1985). Bottlenose dolphins can be trained to adjust their average click f_p depending on reinforcement (Moore and Pawloski, 1990). However, in the present study, the echolocation clicks of both the older dolphin and the younger dolphin were recorded when the dolphins were freely swimming in their pools in a similar behavioural context. The older dolphin, in the pool with the higher background noise levels (Fig. 6), producing clicks with lower f_p and f_c would probably be at a disadvantage (Au, 1993) relative to the younger dolphin in a quieter pool. We know nothing about population differences in click parameters in the Indo-Pacific humpback dolphin. However, the stranding locations or home range of the older dolphin (Pearl River Estuary, Fig. 1B) and the younger dolphin (Beihai Bay, Fig. 1B) were geographically adjacent. Historically, the Indo-Pacific humpback dolphin was fairly continuously distributed in the coastal waters between Beihai Bay and the Pearl River Estuary (Fig. 1B) (Jefferson and Hung, 2004). The most logical explanation for the different click spectrum shapes between the two dolphins is that the older dolphin tended to shift the spectral content of its echolocation clicks downwards to where the animal's hearing is more sensitive to partially compensate for its high-frequency hearing loss. Thus, the lowered high-frequency cut-off and lowered click f_p and f_c in the older dolphin relative to the conspecific younger dolphin can probably be explained as a result of presbycusis.

As the older dolphin was a recently stranded animal, it seems reasonable to suggest that presbycusis in this animal occurred before it was stranded and the observed presbycusis and active frequency compensation of echolocation clicks for hearing deficits exists in old Indo-Pacific humpback dolphins in the wild. As presbycusis is common in human populations (Ries, 1982), it should not be surprising that other mammals in their natural habitats also share this development. A false killer whale in captivity demonstrated a concomitant reduction in echolocation discrimination ability with a loss of high-frequency hearing (Kloepper et al., 2010b). It is possible that wild dolphins experiencing high-frequency hearing loss also have compromised echolocation abilities, and thus lower survival rates compared with individuals with normal hearing. The stranding of the present subject might be partially ascribed to its potentially compromised echolocation ability with high-frequency hearing loss. However, in wild dolphin groups, individuals with hearing loss or even deafness/muteness could still have a robust and healthy physical condition and survive to an advanced age, probably due to mutual survival benefits accruing from living within a dolphin group (Ridgway and Carder, 1997). Individuals with hearing loss might learn from the other dolphins in the local group by not only observing but also using senses other than audition, to derive information that the conspecific individuals in the group receive through their acoustic sensory system (Ridgway and Carder, 1997).

LIST OF ABBREVIATIONS

A/D converter	analog-to-digital converter
AEP	auditory evoked potential
DAQ	data acquisition card
EEG	electroencephalography
EFR	envelope-following response

FFT	fast Fourier transform
r.m.s.	root mean square
SPL	sound pressure level

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AUTHOR CONTRIBUTIONS

S.L., D.W., K.W., M.H.K., N.F., W.L., J.C. and T.N. designed the study. S.L. analysed the data and interpreted the findings. All authors contributed to the writing and revising of the paper.

COMPETING INTERESTS

No competing interests declared.

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