Evoked-potential audiogram of an Indo-Pacific humpback dolphin (*Sousa chinensis*)

Songhai Li¹*¹, Ding Wang²*, Kexiong Wang², Elizabeth A. Taylor¹, Emilie Cros¹, Wenjing Shi², Zhitao Wang², Liang Fang², Yuefei Chen³, and Fanming Kong³

¹Marine Mammal Research Laboratory, Tropical Marine Science Institute, National University of Singapore, 18 Kent Ridge Road, Singapore, 119227, Singapore

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

³Dolphinarium of Nanning Zoo, Nanning, Guangxi, 530003, China

*Author for correspondence (tmsshl@nus.edu.sg; wangd@ihb.ac.cn)

Running title: Audiogram of *Sousa chinensis*

Key words: AEP response, hearing sensitivity, marine mammal, odontocete cetacean, stimulus, sound

SUMMARY

An evoked-potential audiogram was measured for an Indo-Pacific humpback dolphin (*Sousa chinensis*) living in the Dolphinarium of Nanning Zoo, China. Rhythmic 20 ms pip trains composed of cosine-enveloped 0.25 ms tone pips at a pip rate of 1 kHz were presented as sound stimuli. The dolphin was trained to remain still at the water surface and to wear soft latex suction-cup electroencephalography (EEG) electrodes used to measure the animal’s envelope-following evoked potentials to the sound stimuli. Responses to 1000 rhythmic 20 ms pip trains for each amplitude/frequency combination were averaged and analysed using a fast Fourier transform to obtain an evoked auditory response. The hearing threshold was defined as the zero crossing point of the response input-output function using linear regression. Fourteen frequencies ranging from 5.6 to 152 kHz were studied. The results showed that most of the thresholds were lower than 90 dB re. 1 µPa (root mean square, r.m.s.), covering frequency range from 11.2 to 128 kHz, and the lowest threshold of 47 dB was measured at 45 kHz. The audiogram, which is a function of hearing threshold-versus-stimulus carrier frequency, presented a ‘U’-shape with a region of high hearing sensitivity (within 20 dB of the lowest threshold) between approximately 20 and 120 kHz. At frequencies lower than this high-sensitivity region, thresholds increased at a rate of approximately 11 dB/octave,
up to 93 dB at 5.6 kHz. The thresholds at high frequencies above 108 kHz increased steeply with a rate of 130 dB/octave, up to 127 dB at 152 kHz.

**INTRODUCTION**

Odontocete cetaceans (toothed whales, including dolphins and porpoises) evolved highly developed sound production systems and hearing capabilities (Au, 1993; Au et al., 2000; Nachtigall and Moore, 1988), which enable them to effectively navigate, sense and communicate within their three-dimensional and often vision-limited underwater environment. Hearing is considered to be a primary sensory modality in odontocete cetaceans to aid in navigation, orientation, foraging and communication (Au, 1993; Nachtigall and Moore, 1988; Richardson et al., 1995). Since the first odontocete hearing was measured as a function of hearing threshold-versus-frequency of sound stimulus (i.e., an audiogram) in an Atlantic bottlenose dolphin, *Tursiops truncatus* (Johnson, 1967), audiograms of odontocete cetaceans have been measured in 16 species to date using either psychophysical or evoked-potential methods, including the harbour porpoise, *Phocoena phocoena* (Andersen, 1970; Kastelein et al., 2002), killer whale, *Orcinus orca* (Hall and Johnson, 1971), Amazon River dolphin, *Inia geoffrensis* (Jacobs and Hall, 1972), beluga or white dolphin, *Delphinapterus leucas* (White et al., 1978), Pacific bottlenose dolphin, *Tursiops truncatus gilli* (Ljungblad et al., 1982), false killer whale, *Pseudorca crassidens* (Thomas et al., 1988), Yangtze River dolphin, *Lipotes vexillifer* (Wang et al., 1992), Risso’s dolphin, *Grampus griseus* (Nachtigall et al., 1995), common dolphin, *Delphinus delphis* (Popov et al., 1998), Tucuxi, *Sotalia fluviatilis guianensis* (Sauerland and Dehnhardt, 1998), Yangtze finless porpoise, *Neophocaena phocaenoides asiaeorientalis* (Popov et al., 2005), Gervais’ beaked whale, *Mesoplodon europaeus* (Cook et al., 2006; Finneran et al., 2009), white-beaked dolphin, *Lagenorhynchus albirostris* (Nachtigall et al., 2008), long-finned pilot whale, *Globicephala melas* (Pacini et al., 2010), and Blainville’s beaked whale, *Mesoplodon densirostris* (Pacini et al., 2011). However, since there are more than 70 species of odontocete cetaceans, those species for which nothing is known about their hearing sensitivity are still an overwhelming majority. Some of these species, particularly those living in coastal or riverine ecosystems, are threatened by a wide variety of environmental factors, including climate change and anthropogenic activities. An example is the Indo-Pacific humpback dolphin (also called the Chinese white dolphin, *Sousa chinensis*). It is essential to obtain even basic information about
the hearing of these animals to better understand their biology and ecology, and guide effective conservation strategies such as mitigation of the potential effects of underwater noise, some of which fall under the term ‘noise pollution’.

The Indo-Pacific humpback dolphin is referred to as an 'inshore or near-shore' species, discontinuously distributed throughout coastal waters of the Indo-Pacific Oceans, from eastern Africa through the Arabian Sea, Bay of Bengal, southern China, Gulf of Thailand, Indonesia, to northern Australia (Corkeron et al., 1997; Jefferson and Leatherwood, 1997; Jefferson and Karczmarski, 2001). Since they inhabit shallow near-shore waters, humpback dolphins are particularly susceptible to human activities. The very significant recent increase in coastal development which is usually related to economic growth in China and South-east Asia has resulted in the influence of human activities permeating underwater. In consequence, marine mammals are being confronted with habitat degradation and destruction, and by factors including noise pollution, harassment, and overfishing of prey species (Jefferson and Hung, 2004). Recently, public knowledge and hence concern about the possible effects of anthropomorphic environmental noise, together with attempts to mitigate adverse effects on the humpback dolphin have steadily grown within scientific and conservation communities (Würsig et al., 2000; Jefferson and Hung, 2004; Jefferson et al., 2009). However, in order to propose effective and scientifically based measures for noise mitigation and animal conservation it is necessary to study their hearing and the possible effects of environmental noise on their hearing. Unfortunately, to date, nothing is known about the hearing sensitivity of the humpback dolphin.

To address this, in the present study, we measured the audiogram of a captive Indo-Pacific humpback dolphin by using an auditory evoked-potential (AEP) method. This enabled measurement of key audiometric variables within a short time (typically in a few days during approximately 100 minutes of recordings) and without the lengthy training of the animals which is required in traditional behavioural techniques using psychophysical procedures (Supin et al., 2001; Nachtigall et al., 2000, 2007). Previous studies of odontocete cetaceans suggested that accuracy and precision of the audiograms were comparable when they were obtained via audiometric measurements using the AEP method or traditional behavioural techniques (Yuen et al., 2005; Houser and Finneran, 2006). The AEP method has been widely used for audiometry in odontocete cetaceans. It has been successfully used for audiogram investigation of odontocetes in captive conditions (Popov et al., 2005; Pacini et al., 2010), catch-and-release scenarios (Nachtigall et al., 2008), and even wild conditions for stranded animals (Mann et al., 2010).
To provoke an AEP response during previous audiometric investigations of odontocetes, sinusoidally amplitude-modulated (SAM) signals were usually used as sound stimuli (Supin et al., 2001; Nachtigall et al., 2007). The SAM stimuli evoked a rhythmic sequence of auditory brainstem responses (ABRs), i.e., envelope-following response (EFR), following the modulation rate of the SAM stimuli, which was chosen to be approximately 600 to 1000 Hz in odontocetes (Supin et al., 2001; Nachtigall et al., 2007; Mann et al., 2010). While the SAM stimuli had many advantages and contributed efficient and fairly confident information about audiometry in odontocetes, there is a noteworthy disadvantage as demonstrated by Supin and Popov (Supin and Popov, 2007): the EFR evoked by the SAM stimuli with sound pressure levels (SPLs) within 20 dB of the hearing threshold was usually small and hardly visible. If measurements were made in an environment with high electrical background noise levels, the estimated hearing threshold could be false with an error of over 30 dB (Supin and Popov, 2007). The low response amplitude at the near-threshold SPLs was attributed to the narrow frequency bandwidth of the SAM stimuli, which were ±600 to ±1000 Hz (at the half-level), corresponding to 600 to 1000 Hz modulation rate (Popov and Supin, 2001; Supin and Popov, 2007). An effective solution to the problem is to enlarge the frequency bandwidth of the stimuli, which could be achieved by using rhythmic pip trains with each pip appropriately shorter than the modulation rate as the sound stimuli, instead of the SAM stimuli (Supin and Popov, 2007). In the present study, rhythmic pip trains composed of 0.25 ms pips were used as the sound stimuli, to provoke the AEP response of the subject.

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 experimental subject was a male Indo-Pacific humpback dolphin that was rescued from a stranding on the coast of Beihai Bay, China (Fig. 1) in August 2007. The animal was transported to the dolphinarium of Nanning Zoo, Nanning, China (Fig. 1A) on 25th August, 2007, approximately one week after the stranding, for further treatment and rehabilitation. Thanks to the great efforts of the veterinarians and other staff in the zoo, the animal’s health
became normal and stabilized within a few months. Subsequently, the dolphin was trained to perform in shows for the public. The hearing experiment and data collection were conducted between 18th and 22nd December, 2011. Prior to the experiment, the animal was trained (over a few days) to remain still at the water surface and wear soft latex suction cups in order to examine its hearing using the AEP method (Fig. 1B). During the time of the experiment the dolphin was fed four times per day with thawed small fish and also participated in two shows per day: 11:20-11:40 am; and 15:00-15:20 pm. Experimental sessions were conducted during the first feeding and last feeding, 8:10-8:30 am and 17:10-17:30 pm, respectively, well before or after the daily shows. The dolphin was approximately 2.25 m in length, 130 kg in weight, and estimated to be 13 years old at the time of the study.

**Experimental facility and background noise measurements**

The hearing experiment was conducted in the main pool (Fig. 1A, B) of the dolphinarium that was mainly used for dolphin training and shows. The pool was a kidney-shaped concrete structure 14 m in width, 30 m in length, and 5 m in depth, and filled with sea water transported from nearby coastal waters. Background noise in the pool was measured during the experiment using a Reson TC-4013 hydrophone (-212 dB re. 1V/µPa; Reson, Slangerup, Denmark) with 50 dB gain within a frequency range of 0.1 to 200 kHz by an EC6081 pre-amplifier (VP2000; Reson, Slangerup, Denmark). The amplified noise was input to a 16-bit analog-to-digital convertor of a data acquisition card (NI USB-6251 BNC, National Instruments, Austin, TX, USA) and recorded by a standard laptop computer (PC) with a custom-made program designed using LabVIEW software (National Instruments) with a sampling rate of 512 kHz. The recorded noise was analysed and averaged using a customised Matlab algorithm.

**Experimental set-up and sound stimuli presentation**

The experimental set-up is shown in Fig. 1B and the data flow chart is presented in Fig. 2. Each experimental session began with the primary trainer positioning the dolphin at the water surface parallel to the pool-side and approximately 80 cm away from the pool wall. The dolphin was positioned in such a way that the dorsal fin and the dorsal surface of the head with the blowhole remained above the water surface (Fig. 1B), while the ‘acoustic windows’ located at the lower jaw and/or external auditory meatus of the subject, where the sounds were assumed to travel to the inner ear (Norris, 1968; Popov et al., 2008), was maintained underwater throughout the session. Three suction-cup electrodes were then attached to the
back of the dolphin for AEP recording. Sound stimuli were presented using a Reson TC-4040 hydrophone (Reson, Slangerup, Denmark) as a projector, which was positioned at a distance of approximately 2 m and a depth of 50 cm in front of the subject’s ‘acoustic windows’.

The sound stimuli were rhythmic pip trains composed of cosine-enveloped 0.25 ms tone pips with 1 kHz pip rate and variable carrier frequency. Each pip train was 20 ms in duration followed by a silence of 30 ms so that the sound stimuli were presented at a rate of 20 s⁻¹. The 1 kHz pip rate was chosen based on previous publications for other odontocetes (Supin et al., 2001; Popov et al., 2005; Nachtigall et al., 2007; Supin and Popov, 2007; Pacini et al., 2010) and a pre-established modulation rate transfer function of the experimental subject. The stimuli were digitally synthesised using a customised LabVIEW programme at an update rate of 512 kHz, and the digital-to-analog conversion by the NI USB-6251 BNC (National Instruments, Austin, TX, USA) data acquisition card connected to a laptop computer. The analogue 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, Palo Alto, CA, USA) to vary the signal amplitude. The signals were monitored by an oscilloscope (Tektronix TDS1002C, Beaverton, OR, USA) before being projected by the Reson TC-4040 hydrophone. 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 (Supin and Popov, 2007) by positioning a calibrated receiving hydrophone at the same location as the animal’s ‘acoustic windows’. Carrier frequencies varied from 5.6 to 152 kHz, separated by 1-octave steps within a range of 5.6 to 22.5 kHz, 1/2-octave steps within a range of 22.5 to 32 kHz, 1/4-octave steps within a range of 32 to 128 kHz, and 1/8-octave steps within a range of 128 to 152 kHz, which are (rounded to 0.1 kHz): 5.6, 11.2, 22.5, 32, 38, 45, 54, 64, 76, 90, 108, 128, 139, and 152 kHz. The waveforms (left) and corresponding spectra (right) of the pip train segments with carrier frequencies of 5.6, 11.2, 45, 108, 152 kHz are presented in Fig. 3 as examples of the received stimuli at the animal’s ‘acoustic windows’. Fig. 3 showed that the frequencies of the received stimuli were fairly centered at the expected carrier frequencies even for the low-frequency stimuli, where the projector’s transmitting sensitivity is relatively low.

**AEP recording**

The animal’s AEP responses to the sound stimuli were picked up by three electroencephalography (EEG) electrodes (Grass Technologies, West Warwick, RI, USA): gold-plated disks 10 mm in diameter mounted within latex suction cups 60 mm in diameter.
The recording electrode was attached with conductive gel to the dorsal head surface, located on the midline, approximately 5-7 cm behind the blowhole. The reference electrode was also attached using conductive gel to the animal’s dorsal fin. The third EEG electrode acted as a grounding device and was positioned on the back of the animal between the recording and reference electrodes (Figs. 1B and 2). 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 the Tektronix TDS1002C oscilloscope and input to a 16-bit analog-to-digital converter of the same NI USB-6251 BNC data acquisition card that generated the synthesised sound stimuli (Fig. 2). 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, which were 30 ms in duration, using the same customised LabVIEW program that synthesized the sound stimuli.

**Hearing threshold determination**

To estimate a hearing threshold for each carrier frequency, typically 6-9 AEP records with a series of stimulus SPLs were recorded and measured. The initial stimulus SPL for each frequency was chosen based on previously published audiograms of other odontocetes (Supin et al., 2001; Popov et al., 2005; Nachtigall et al., 2007) and was usually 20-40 dB higher than the estimated threshold. The stimulus presentation level was then attenuated in 5-10 dB steps until no evoked potential was observed. 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, 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. For each frequency, the magnitudes at 1 kHz were measured and plotted as a function of stimulus SPLs, and a near-threshold part 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.

**Vocalisation recording of the subject**

For comparison of frequency range between hearing and biologically produced sounds, vocalizations by the experimental subject freely swimming (alone) in the main pool were recorded before or after the hearing study sessions. The animal’s vocalisations were recorded
in the same way as noise recording described above. In outline, the sound was picked up by
the Reson TC-4013 hydrophone with 50 dB gain within a frequency range of 0.1 to 200 kHz
by the EC6081 preamplifier. The amplified sounds were input to a 16-bit analog-to-digital
converter of the NI USB-6251 BNC data acquisition card and recorded by a standard PC with
a custom-made LabVIEW program at a sampling rate of 512 kHz. The recorded sounds were
analysed using a customised Matlab algorithm.

RESULTS

AEP response and hearing audiogram

Each hearing experimental session lasted approximately 20 mins with 10-15 AEP records
being collected. Examples of the recorded AEP responses to the rhythmic sound stimulus
(0.25 ms tone pips with carrier frequency of 108 kHz) are presented in Fig. 4A. The stimulus
SPL (dB re. 1µPa) calibrated near the animal’s ‘acoustic windows’ is indicated with the
corresponding AEP response. The zero point of the time scale in Fig. 4A corresponds to the
time point when the sound stimulus was projected and the AEP recording was triggered. The
tone pips evoked a sequence of evoked potentials following the 1 kHz pip rate, which was the
EFR. The EFR showed a temporal lag of around 3-4 ms compared to both the onset and
offset of the sound stimulus. This lag was contributed by both sound transmission time from
the projector to the animal’s ‘acoustic windows’, which was approximately 1.3 ms for a 2 m
distance (Fig. 2), and the 2-3 ms latency of the evoked potential following presentation of the
stimulus. The latter served as a predictable electrophysiological feature confirming that the
AEP recording occurred in direct response to the sound stimulus, and was not an artifact. The
EFRs were discernable well above the electrical noise level even during the near-threshold
part (Fig. 4A). As the stimulus SPL decreased, the EFR magnitude synchronously decreased
until the response disappeared in noise (Fig. 4A).

The frequency spectrum of the corresponding AEP response between 5 and 20 ms, which
contained a major part of the EFR record but did not contain the latency and the initial
transient part of the response, was calculated by FFT and is presented in Fig. 4B. The
consistent peak at 1 kHz reflected the animal’s EFR, and thus neurophysiological ‘following’
of the carrier tone pips at 1 kHz pip rate. The amplitude of the AEP response was reflected in
the magnitude of the peak at 1 kHz in the spectrum. As the stimulus level was attenuated, the
peak magnitude of the response decreased correspondingly. Fig. 4B showed that at the
stimulus SPL of 66 dB, the peak of the response spectrum was comparable to the electrical noise level which was typically lower than 0.04 $\mu$V rms at 1 kHz in the spectrum in the present experimental condition. The peak magnitude of each spectrum at 1 kHz was measured as an estimate of the EFR amplitude and plotted as a function of stimulus SPL. Examples for the stimuli with 45 and 108 kHz carrier frequency are presented in Fig. 5. The functions of EFR amplitude-versus-stimulus SPL in Fig. 5 showed that at a near-threshold part with stimulus SPL from 49 to 59 dB and 66 to 101 dB for the 45 and 108 kHz stimulus, respectively, the EFR amplitude increased fairly steeply; an inflection point appeared at stimulus SPL of 59 dB and 101 dB for the 45 and 108 kHz stimulus, respectively, after which the EFR amplitude increased at a reduced rate. In determining hearing threshold, the near-threshold part, up to the inflection point, was approximated by a linear regression line (Fig. 5). In most cases, the linear regression was satisfactory within a near-threshold part up to a range of 20-45 dB (35 dB for the 108-kHz stimulus in Fig. 5) with a high $r^2$ value typically from 0.96 to 1. The slope of the linear regression line was typically between 0.01 and 0.02 $\mu$V/dB. Theoretical zero-response SPL of the regression line was adopted as the hearing threshold for the corresponding carrier frequency, which was 47 and 62 dB in the shown examples in Fig. 5 for carrier frequencies of 45 and 108 kHz, respectively. Hearing thresholds determined for each of the fourteen examined carrier frequencies ranging from 5.6 to 152 kHz are presented in Table 1.

The resulting audiogram which is a function of hearing threshold-versus-stimulus carrier frequency is shown in Fig. 6. The spectrum density of the pool background noise (mean value ± s. d.; dB re. 1$\mu$Pa$^2$/Hz), which was calculated by FFT of 10 ms noise windows for each sample and averaged by 1000 samples, is also shown. The spectrum density indicated that the experimental pool had a quiet noise environment with a background noise level lower than 50 dB for all the examined frequencies and even lower than 40 dB for frequencies higher than 45 kHz. The quiet noise environment provided an excellent opportunity for hearing threshold measurement. The audiogram demonstrated that most of the thresholds were lower than 90 dB, covering the frequency range from 11.2 to 128 kHz, and the lowest threshold of 47 dB was measured at 45 kHz. The audiogram presented a ‘U’-shape with a region of high hearing sensitivity with thresholds below around 70 dB (within 20 dB of the lowest threshold) between approximately 20 and 120 kHz. For frequencies lower than this high-sensitivity region, thresholds increased at a rate of approximately 11 dB/octave, up to 93 dB at 5.6 kHz. The thresholds at high frequencies above 108 kHz increased steeply with a rate of 130
dB/octave, up to 127 dB at 152 kHz. Within the high-sensitivity region, there was a plateau at 64-76 kHz between the two regions of highest sensitivity at 32-54 and 90-108 kHz.

Vocalisation of the subject

Three sessions of sound recordings were conducted just before or after the hearing experimental session, when the dolphin was swimming freely and alone in the pool. Each session lasted approximately 10 mins. Most of the time, the dolphin was acoustically silent. Occasionally, the animal produced a short click train consisting of single clicks, probably exploring the experimental equipment deployed underwater. No ‘whistles’ were detected. Examples of two click trains are presented in Figs. 7A and B, respectively. The waveform and power spectrum of one of the clicks from the click train in Fig. 7A demonstrated that the clicks possess typical high-frequency (peak frequency >100 kHz) and short-duration (<50 µs) characteristics of odontocete cetacean echolocation clicks. However, the clicks from the click train in Fig. 7B had peak frequencies lower than 15 kHz and were of relatively long time duration. Three examples of click waveform and corresponding spectrum from the click train in Fig. 7B indicated that the click waveform and spectrum was changing from click to click.

DISCUSSION

Instead of using SAM signals, the present study used rhythmic pip trains with 0.25 ms pips, shorter than the 1 kHz modulation rate, as the sound stimuli. Previous work (Supin and Popov, 2007) demonstrated that the rhythmic pip trains composed of appropriately short tone pips as sound stimuli were capable of achieving a more reliable and confident estimation of the hearing threshold and thus audiogram measurement relative to the SAM sound stimuli. Our measurements showed that the present sound stimuli provoked robust AEP responses even in the near-threshold part (Figs. 4 and 5), which is ideal for reliable estimation of the hearing threshold. The robust AEP responses were supposed to be contributed by a wide bandwidth in the rhythmic pip trains composed of 0.25 ms pips with a 1 kHz modulation rate. In theory, the half-level (i.e., 6 dB) frequency bandwidth of the present stimuli in the spectra is ±4 kHz (1/0.25), wider than that of the SAM stimuli with a 1 kHz modulation rate, which is ±1 kHz. The 6 dB frequency bandwidth of approximately ±4 kHz was confirmed by monitoring and measuring the sound stimuli produced by the projector in the experimental
pool (Fig. 3). While shorter pips with a wider stimulus spectrum provoke higher AEP response amplitudes and result in steeper amplitude dependence on stimulus SPL in the near-threshold range and therefore more precise hearing threshold estimation (Supin et al., 2001; Supin and Popov, 2007), the wider the spectrum, the more ambiguous the estimated threshold attributed to a certain carrier frequency. In the present study, for the stimuli with carrier frequencies higher than 45 kHz, the approximately ± 4 kHz frequency bandwidth may be considered narrow enough to distinguish one from another. At the low frequency range, although the carrier frequencies were selected in frequency steps of 1 octave (from 5.6 to 22.5 kHz) or 1/2 octave (from 22.5 to 32 kHz), the spectra of the stimuli still slightly overlapped each other (see Figs. 3F and G). However, previous direct measurements indicated that the hearing thresholds of dolphins, estimated based on stimulus SPL in long-term r.m.s (computed throughout the stimulus duration, including both the pips and inter-pip pauses), were almost independent of pip duration, thus the stimulus spectrum bandwidth (Supin and Popov, 2007). Supin and Popov (2007) also demonstrated that the audiogram measured using a rhythmic pip train with 0.25 ms pips, shorter than the modulation rate, as the stimulus was comparable to but less scattered than that measured using SAM stimuli in an ideal background noise environment. Assuming that the present subject had a comparable auditory mechanism to the dolphin measured in Supin and Popov’s study, we would not expect that the present sound stimuli had introduced obvious ambiguity into the threshold estimation of a certain carrier frequency, even at the low frequency range.

For most of the tested carrier frequencies, the EFR amplitude increased steeply with the stimulus SPL within a near-threshold part up to a range of 20-45 dB. The functions of EFR amplitude-versus-stimulus SPL within the near-threshold part were approximated by linear regression lines with high $r^2$ values and fairly steep slopes, typically between 0.01 and 0.02 $\mu$V/dB. The examples in Fig. 5 showed that for the stimulus with a carrier frequency of 108 kHz, the EFR amplitude increased with the stimulus SPL in a rate of 0.0131 $\mu$V/dB within 35 dB of the near-threshold part up to the stimulus SPL of approximately 100 dB, after which the rate reduced. However, for the stimulus with carrier frequency of 45 kHz, the EFR amplitude steeply increased with the stimulus SPL only within a relative short range of the stimulus SPL, which was approximately 10 dB in the near-threshold part. The reason is unclear. Nevertheless, the function of EFR amplitude-versus-stimulus SPL featured two to three branches composed of a steeply rising near-threshold one, a quasi-horizontal one or/and
then an oblique one is a typical form of EFR amplitude dependence on stimulus SPL (Supin et al., 2001; Supin and Popov, 2007).

The ‘U’-shaped audiogram shown in Fig. 6 indicated that the studied dolphin had hearing abilities similar to those of many other odontocete species (Au et al., 2000; Supin et al., 2001). The main characteristics were that the animal had low hearing thresholds below approximately 70 dB for the sound stimuli with carrier frequencies ranging in a wide band from approximately 20 to 120 kHz. The lowest threshold of 47 dB at 45 kHz represented a fairly low threshold measured by the evoked-potential method (Supin et al., 2001; Popov et al., 2005). However, it’s comparable to the lowest thresholds in the hearing investigations of other odontocetes acquired by the same evoked-potential method, which were commonly close to or lower than 50 dB (Popov et al., 2005; Nachtigall et al., 2008; Pacini et al., 2010 and 2011). Such low thresholds indicated that the background noise environment in the present study was suitable for measurement of hearing and that masking effects of the animal’s hearing were negligible. The steep increase in hearing thresholds at high frequencies above 108 kHz suggested that the hearing cut-off frequency of the investigated animal was between approximately 110-130 kHz, similar to most investigated odontocetes (Au et al., 2000; Supin et al., 2001). While in all investigated odontoctes, the hearing cut-off frequency is higher than or, in a few cases (Pacini et al., 2010 and 2011) close to 100 kHz, in many species it does not exceed 120-130 kHz (Au et al., 2000; Supin et al., 2001). However, in some species, such as the harbour porpoise (Andersen, 1970; Kastelein et al., 2002), Yangtze finless porpoise (Popov et al., 2005), and white-beaked dolphin (Nachtigall et al., 2008), the hearing cut-off frequencies were over 150 kHz.

The extra high cut-off frequency in the hearing of the porpoise family and the white-beaked dolphin might be related to the high-frequency clicks they transmit, assuming that the animals could hear the sounds they produce. Both the harbour porpoise (Villadsgaard et al., 2007) and Yangtze finless porpoise (Li et al., 2005) produce echolocation clicks with peak frequencies typically over or close to 130 kHz. White-beaked dolphin clicks were reported to have a secondary energy peak at 250 kHz (Rasmussen and Miller, 2002) and contain energy up to 305 kHz (Mitson, 1990). Indo-Pacific humpback dolphin clicks recorded opportunistically from a group of animals in Hong Kong waters were shown to have spectral energy extending up to at least 200 kHz (Goold and Jefferson, 2004). However, sound recordings from the experimental subject of this paper indicated that typical high-frequency clicks of this humpback dolphin had a peak frequency around 120 kHz with no obvious
spectral energy above 150 kHz (Fig. 7A). The animal also produced click trains consisting of clicks with relatively longer duration and peak frequencies lower than 15 kHz (Fig. 7B). These may suggest that the experimental subject produced a variety of clicks with different frequency components. Alternatively, the low frequency clicks in Fig. 7B could be artifacts originating from ‘off-axis’ patterns of the ‘on-axis’ high-frequency clicks (Au, 1993). In either case, the measured audiogram of the experimental animal with a high-frequency hearing cut-off between 110-130 kHz approximately matched the frequency range of the animal’s high-frequency clicks that, as measured, had a peak frequency of about 120 kHz. At frequencies outside the high sensitivity region of 20-120 kHz, the animal would still be able to hear the sound stimuli but with relatively higher hearing thresholds (Fig. 6). Given that most mammalian audiograms are U-shaped, the hearing thresholds of stimuli with frequencies higher than 152 kHz were not measured. The plateau at 64-76 kHz between the two most sensitive regions of 32-54 and 90-108 kHz in the measured audiogram was similar to a phenomenon observed in the audiograms of the harbour porpoise (Popov et al., 1986), Amazon River dolphin (Popov and Supin, 1990), and Yangtze finless porpoise (Popov et al., 2005), where a plateau also appeared between two high-sensitivity regions. The reason or biological significance of this phenomenon remains unexplained.

The present data represent the first hearing measurements for the Indo-Pacific humpback dolphin. The investigated animal with an estimated age of 13 years old should be considered adult (Jefferson and Karczmarski, 2001). Medical records of the dolphin indicated that the animal had not received ototoxic medicines (as antibiotic medication) which might have adversely affected its hearing. The high-frequency click production with click peak frequency of around 120 kHz, and high-frequency hearing with cut-off frequency between approximately 110-130 kHz suggest a matching and healthy sound production and hearing capability. This audiogram of a healthy adult could form the baseline of hearing information for the Indo-Pacific humpback dolphin. However, although the measured audiogram had a ‘U’ shape and is similar to many odontocete audiograms, in which taxon the audiograms of each species were usually collected from only one or two individuals (Nachtigall et al., 2000), we should be cautious when interpreting and extending the present hearing data from one individual to represent the species. Many factors, including the age of the subjects, physical situation, medical administration, and background noise environment, could influence hearing measurements. Hearing measurements with a group of Atlantic bottlenose dolphins Tursiops truncatus (Popov et al., 2007) and Pacific bottlenose dolphins Tursiops truncatus
gilli (Houser et al., 2008) showed that intra-specific variations in hearing capability of the odontocetes does exist. While the present study provides basic hearing information for the Indo-Pacific humpback dolphin, it is essential, whenever possible, to measure hearing in more than one individual with different ages and under various environmental conditions to learn more about individual variation and better assess potential environmental effects on the hearing and behaviour of the species.

**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABR</td>
<td>auditory brainstem response</td>
</tr>
<tr>
<td>A/D Converter</td>
<td>analog-to-digital converter</td>
</tr>
<tr>
<td>AEP</td>
<td>auditory evoked-potential</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalography</td>
</tr>
<tr>
<td>EFR</td>
<td>envelope-following response</td>
</tr>
<tr>
<td>FFT</td>
<td>fast Fourier transform</td>
</tr>
<tr>
<td>PC</td>
<td>laptop computer</td>
</tr>
<tr>
<td>r.m.s.</td>
<td>root mean square</td>
</tr>
<tr>
<td>SAM</td>
<td>sinusoidally amplitude-modulated</td>
</tr>
<tr>
<td>s.d.</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SPL</td>
<td>sound pressure level</td>
</tr>
</tbody>
</table>

**ACKNOWLEDGEMENTS**

We are grateful to the staff and students at the Baiji Aquarium, Institute of Hydrobiology of the Chinese Academy of Sciences, the trainers and staff of the dolphinarium of Nanning Zoo, Nanning, China, for their assistance during data collection and travelling. The LabVIEW program used for stimuli synthesis and evoked potential recording was originally developed by Dr. Alexander Ya. Supin. The first author appreciates Drs. Alexander Ya. Supin and Paul E. Nachtigall for their continuous help and guidance on the hearing research of marine mammals. We thank Drs. Paul James Seekings and Brahim Hamadicharef and other staff in
Marine Mammal Research Laboratory of the Tropical Marine Science Institute for their encouragement and help during the research study. The study was supported by the National Natural Science Foundation of China (31070347), Ministry of Science and Technology of China (2011BAG07B05-3), State Oceanic Administration of China (201105011), and the MMRL, Tropical Marine Science Institute (TMSI), National University of Singapore.

REFERENCES


Nachtigall, P. E., Mooney, T. A., Taylor, K. A., Miller, L. A., Rasmussen, M. H.,
Shipboard measurements of the hearing of the white-beaked dolphin, Lagenorhynchus


measurements from a stranded infant Risso’s dolphin (*Grampus griseus*). *J. Exp. Biol.* **208**, 4181-4188.


Pacini, A. F., Nachtigall, P. E., Kloepper, L. N., Linnenschmidt, M., Sogorb, A. and


Popov, V. V., Ladygina, T. F. and Supin, A. Ya. (1986). Evoked potentials in the auditory

Popov, V. V. and Klishin, V. O. (1998). EEG study of hearing in the common dolphin,

Popov, V. V. and Supin, A. Ya. (1990). Electrophysiological investigation of hearing of the

Popov, V. V. and Supin A. Ya. (2001). Contribution of various frequency bands to ABR in

Popov, V. V., Supin, A. Ya., Klishin, V. O., Tarakanov, M. B. and Pletenko, M. G.

Popov, V. V., Supin, A. Ya., Pletenko, M. G. and Tarakanov, M. B. (2007). Audiogram


Table 1. Auditory evoked-potential (AEP) threshold for each carrier frequency tested.

<table>
<thead>
<tr>
<th>Carrier frequency (kHz)</th>
<th>AEP threshold (dB re. 1\mu Pa r.m.s.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.6</td>
<td>93</td>
</tr>
<tr>
<td>11.2</td>
<td>89</td>
</tr>
<tr>
<td>22.5</td>
<td>71</td>
</tr>
<tr>
<td>32</td>
<td>54</td>
</tr>
<tr>
<td>38</td>
<td>49</td>
</tr>
<tr>
<td>45</td>
<td>47</td>
</tr>
<tr>
<td>54</td>
<td>62</td>
</tr>
<tr>
<td>64</td>
<td>65</td>
</tr>
<tr>
<td>76</td>
<td>68</td>
</tr>
<tr>
<td>90</td>
<td>65</td>
</tr>
<tr>
<td>108</td>
<td>62</td>
</tr>
<tr>
<td>128</td>
<td>92</td>
</tr>
<tr>
<td>139</td>
<td>114</td>
</tr>
<tr>
<td>152</td>
<td>127</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

**Fig. 1.** Experimental site and facilities. (A) Experimental pool in the dolphinarium of Nanning Zoo, Nanning, China; (B). The subject maintained a stationary position at the water surface while wearing EEG electrodes held against the skin by soft silicone suction cups. The sound projector was positioned approximately 2 m away from the animal’s ‘acoustic windows’, where the sounds were assumed to travel to the inner ear of the animal (Norris, 1968; Popov et al., 2008).

**Fig. 2.** Schematic of the dolphin’s relative position, data recording equipment and flow chart. A/D Converter, analog-to-digital converter; PC, laptop computer.

**Fig. 3.** Examples of waveforms and spectra of the stimulus segments (pip trains) produced by the projector and received by the calibrating hydrophone at the subject’s ‘acoustic windows’. (A) stimulus waveform with carrier frequency of 5.6 kHz; (B) stimulus waveform with carrier frequency of 11.2 kHz; (C) stimulus waveform with carrier frequency of 45 kHz; (D) stimulus waveform with carrier frequency of 108 kHz; (E) stimulus waveform with carrier frequency of 152 kHz; (F) to (J) the corresponding power spectrum of the stimulus segment in (A) to (E), respectively.

**Fig. 4.** (A) Examples of envelope-following response (EFR) waveforms recorded at various stimulus sound pressure levels (SPLs), as indicated in dB re. 1 µPa r.m.s. on the left of each EFR waveform (108 kHz carrier frequency); the stimulus was a rhythmic 20 ms pip train composed of cosine-enveloped 0.25 ms tone pips with 1 kHz pip rate; (B) Frequency spectra of corresponding EFR waveforms with fast Fourier transform (FFT) of a 15 ms analysis window.

**Fig. 5.** Function of the EFR response amplitude-versus-stimulus sound pressure level (SPL) for the 45 and 108 kHz sound stimulus, respectively. The function was approximated by a linear regression at the near-threshold part with SPLs between 49 to 59 dB, and between 66 and 101 dB (bold lines) for the 45 and 108 kHz stimulus, respectively. The threshold was defined as the intersection of the regression line with the hypothetical zero response value. The threshold at the present stimulus frequencies was estimated to be 47 and 62 dB, for the 45 and 108 kHz stimulus, respectively.
**Fig. 6.** Audiogram of the Indo-Pacific humpback dolphin studied. Spectrum density (mean±s.d., dB re. 1µPa²/Hz, N=1000) of the background noise in the experimental pool is also presented.

**Fig. 7.** Examples of sounds produced by the dolphin subject. (A) A click train consisting of typical short-duration and high-frequency ‘dolphin’ echolocation clicks with a peak frequency of about 120 kHz; (B) a click train consisting of relatively long duration and low frequency clicks with peak frequency lower than 15 kHz. Note that the waveform and spectrum of individual clicks changed click to click within the same click train.
Fig. 1
Fig. 3
Fig. 4
Fig. 5

Response Amplitude ($\mu$V rms) vs. Probe Sound Pressure Level (dB re 1$\mu$Pa rms)

- 108 kHz stimulus
- 45 kHz stimulus

Equations:

- $y = 0.0199x - 0.9436$  
  $R^2 = 0.9737$
- $y = 0.0131x - 0.8105$  
  $R^2 = 0.9887$
Fig. 6
Fig. 7