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### Psychophysics vs. Auditory brainstem response

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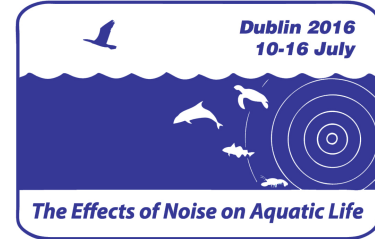
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## Testing auditory sensitivity in the great cormorant (*Phalacrocorax carbo sinensis*): Psychophysics vs. Auditory brainstem response

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Psychoacoustic and electrophysiological methods were used to measure the in-air hearing sensitivity of the great cormorant (*Phalacrocorax carbo sinensis*). One individual was used to determine the behavioral thresholds, which was then compared to previously collected data on the auditory brainstem response (ABR) thresholds from 11 individuals. The behavioral hearing sensitivities were measured at 500 Hz, 1 kHz, 2 kHz, 4 kHz, and 6 kHz, while the ABR hearing sensitivities were measured at 500 Hz, 1 kHz, 2 kHz, and 6 kHz. For both methods the sensitivities were found to be lowest at 2 kHz. The ABR hearing thresholds were found to be 23-53 dB higher than the hearing thresholds determined from the psychoacoustic results, but the audiograms derived with the two methods shared similarities in shape. The results from this study show that the hearing sensitivities of the great cormorant are more sensitive in the behavioral results than from the ABR measurements.



## 1. INTRODUCTION

More than 800 species of birds live on or close to water (Dooling & Therrien, 2012). Little is known about the hearing capabilities of aquatic birds and how the in-air hearing and aquatic hearing abilities compare. Such data will improve our understanding of the birds' acoustic behavior, and any possible impacts anthropogenic noise may have on their natural behavior (Crowell, 2016).

In this study, we have utilized two methods to determine the hearing thresholds of an animal: psychophysics and the auditory brainstem response (ABR). To determine the auditory sensitivity using psychophysics, an animal must first be trained to perform the required behavior. This process can take several weeks to several months. In comparison, no training is required for electrophysiological methods, but the response is measured at a very basic level of brain function and therefore does not take higher, cognitive centers into account. Therefore, comparing data derived from these two methods is non-trivial. Currently there are only a few examples of psychophysical and physiological data from the great cormorant to use for direct comparison (Johansen et al., 2016).

The goal of this study is to compare the psychophysical in-air hearing thresholds obtained for the great cormorant (*Phalacrocorax carbo sinensis*) with the thresholds obtained through ABR measurements.

## 2. MATERIALS AND METHODS

### A. Psychophysical Testing

The experimental bird was a male 6-year-old great cormorant, wild caught in September 2010 and housed at the University of Southern Denmark's Marine Biological Research Center in Kerteminde, Denmark, where the experiments were conducted.

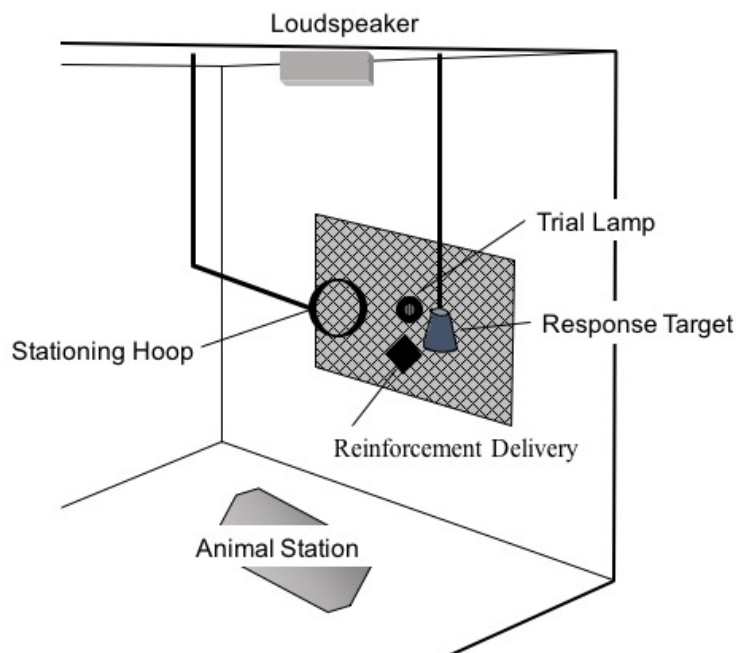
The cormorant was fed 200-400 g of fish (capelin, *Mallotus villosus*, and sprat, *Sprattus sprattus*) per day and trained two times a day using standard operant conditioning and positive reinforcement techniques. The testing was performed inside an acoustic chamber (1x2 m and 1 m high) directly attached to the animal's enclosure (Figure 1). The stimulus was delivered by a 20 cm diameter loudspeaker (X-sound 42943), and stimulus levels were regularly measured with a G.R.A.S. ½" free field microphone (Type 40AF) and preamplifier (Type 26AK) directly connected to a G.R.A.S. amplifier (Type 12AA), which was then connected to an Olympus LS-100 Multi-track Linear Recorder (16 bit, 96 kHz sampling rate). The stimulus level was measured by importing the recorded WAV file into MATLAB, and then estimating the root mean square (RMS) of the 95% duration of the stimulus (see Madsen and Wahlberg, 2007). The system was calibrated with a sound level calibrator (Type 4230; 94 dB re 20  $\mu$ Pa at 1000 Hz).

A custom-made Labview program was used to run the data acquisition system, controlled by a custom-built handheld console. The start of a trial began after the bird had positioned its head in a stationing hoop followed by the experimenter pushing a button on the handheld console, which then turned a lamp on in front of the animal. The lamp was lit for 2 s and indicated to the animal the period it should listen for the presence of a tone. Each session was chosen randomly from twelve sequences of thirty trials. Each series began with five warm-ups and ended with four cool-downs. In between the warm-ups and cool-downs the stimuli and catch trials were mixed in a

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random sequence (*sensu* Gellerman, 1933) of twenty-one data trials. Of these trials, sixteen had a stimulus present (four trials at each of the four intensity levels) and five trials with no stimulus present, which results in a 75:25 GO to NOGO ratio. For a GO trial, a 500 ms tone was presented to the animal at a specific frequency and at one of the four levels of intensity at 6 dB increments. The stimulus started with a 100 ms ramp-up and ended with a 100 ms ramp-down to avoid spectral smearing. The bird was trained to indicate whether a tone was present or not; by touching a target when a tone was present (GO), and remaining inside the stationing hoop when there was no tone (NOGO). If the bird remained in the stationing hoop when a tone was present it was recorded as a MISS, and if the bird touched the response target when a tone was not present, it was recorded as a FALSE ALARM.

For each trial, the response was recorded by the experimenter using the handheld console. Ten sessions were made at each frequency: 0.5, 1, 2, 4, and 6 kHz. Five of the ten sessions were run using a double-blind setup where the experimenter wore protective earmuffs and sunglasses to guarantee no accidental cueing of the trial type to the subject. The sunglasses prevented the animal from seeing eye movements, and the earmuffs prevented the experimenter from hearing the stimulus. In these trials, the experimenter relied on a small LED on the consol which would either light up right after the trial (and after the response of the bird) indicating a GO trial or remaining unlit indicating a NOGO trial. The LED was required so the experimenter knew when to reinforce the animal for a correct response (saying yes to a signal trial and no to a nonsignal trial) and not accidentally reinforce for an incorrect response. The spectral noise levels were estimated using a premade MATLAB code, using Welch's method of averaging (Proakis & Manolakis, 2006) with an FFT size of 2048 points, a Hanning window and 50% overlap between consecutive averages.



**Figure 1. Diagram of acoustic chamber, showing positioning of stationing hoop in relation to the loudspeaker, the response target, and the trial light. The hatched area indicates the window through which the reinforcement was delivered from outside the acoustic chamber.**

Ten sessions were made at five frequencies (Table 1). Thresholds were estimated at the sound level resulting in a 50% hit rate level of a probit function fitted to psychometric function (*sensu* e.g., Sills et al., 2015). Measurements of the ambient noise level inside the acoustic chamber were regularly taken after or before each research session. In addition, signal levels were tested every few weeks to assure that they varied by less than 1 dB throughout the data collection period. The spatial variations of the sound field within the space of where the bird positioned its head while listening for the signal was less than 3 dB.

**Table 1. Trial numbers and stimulus levels for each frequency, as well as individual frequency false alarm rate.**

Frequency (kHz)	Number of Trials per Received Level (N°)	Stimulus Levels (dB re 20 $\mu$ Pa)	False Alarm Rate (%)
0.5	3, 15, 38, 40, 34, 22	68, 62, 56, 50, 44, 38	24
1	8, 21, 38, 33, 28, 15	52, 46, 40, 34, 28, 22	22
2	14, 18, 29, 24, 29, 24, 14, 24, 10, 22	40, 34, 28, 25, 22, 19, 16, 13, 10, 7	24
4	8, 23, 40, 37, 31, 16	56, 50, 44, 38, 32, 26	33
6	5, 18, 38, 36, 31, 19	58, 52, 46, 40, 34, 28	31

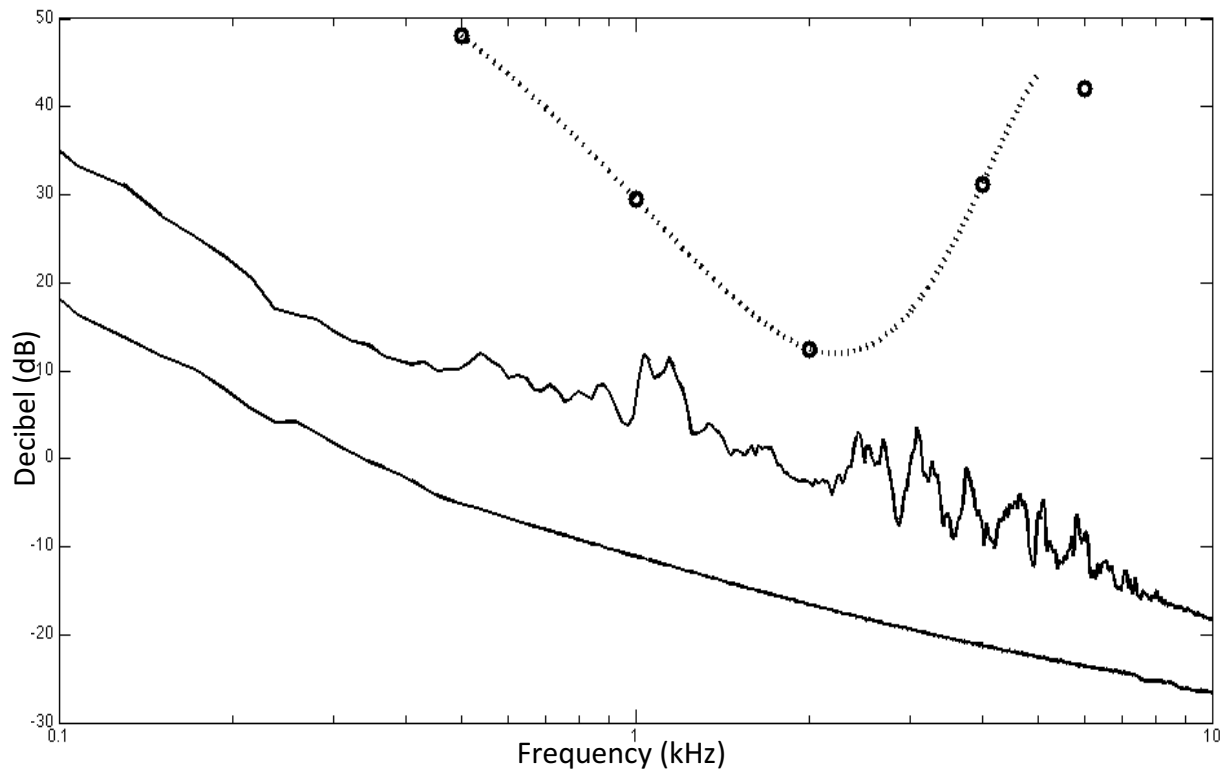
## B. Auditory Brainstem Response

To obtain ABRs, 13 wild-caught cormorant fledglings (6-8 weeks old) were lightly anaesthetized and placed on a heating blanket in a metal frame holder in an anechoic cage.

ABRs were recorded using three subdermal needle electrodes; differential electrodes placed right behind the left ear and over the brain stem, and a ground electrode placed in the neck region. The potentials were passed through a headstage, and a preamplifier (TDT; Tucker-Davis Technologies, Alachua, Florida, United States, models PA4 & RA4). Stimuli were 25 ms tone bursts at different intensities, which were emitted by a Creative (D100) loudspeaker. Calibration was done using a G.R.A.S. 1/2" microphone placed directly above the head of the bird. Stimulation, recording, and data analysis were performed using custom-made software (QuickABR), which was run on a digital signal processor (TDT RM2). Thresholds were evaluated by visual inspection of the ABR signals.

## 3. RESULTS

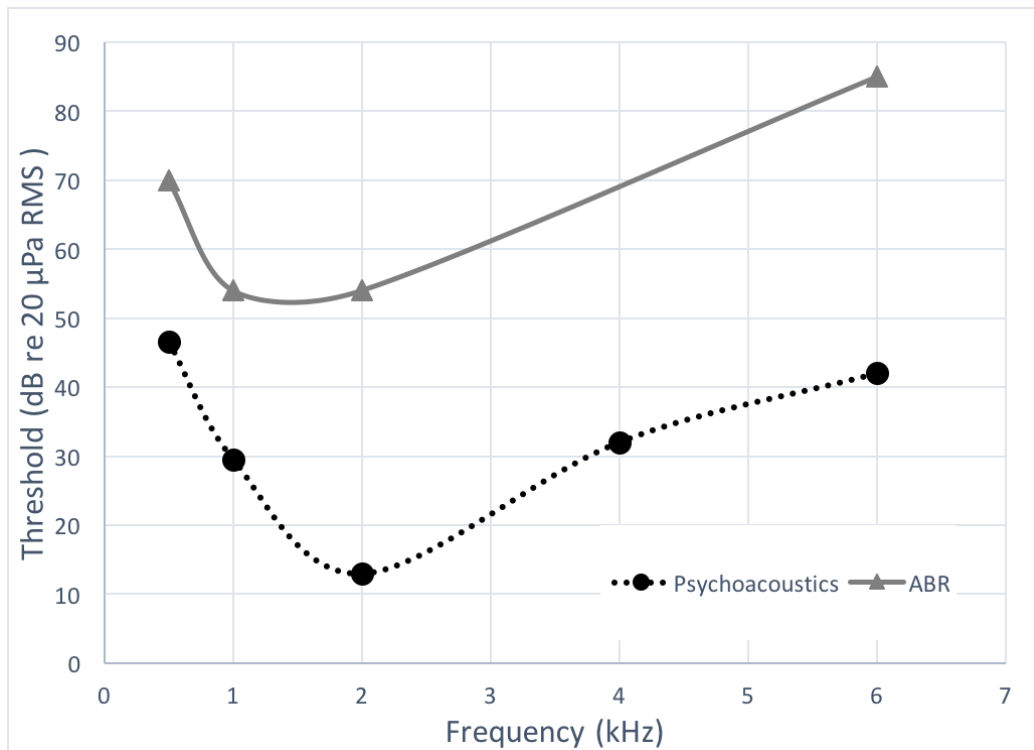
Figure 2 shows the behavioral hearing thresholds of the great cormorant at the five tested frequencies. The average false alarm rate for each frequency was calculated and showed that the false alarm rate for each of the five frequencies ranged from 24% to 28%. The average HIT rate for the five different frequencies was calculated at 62%, with the highest being 4 kHz at 73% and the lowest being 6 kHz at 52%. A HIT rate was determined for each dB level for each frequency. The thresholds were then determined by finding the 50% detection point on psychometric functions for each individual frequency. Ten series were made at 0.5, 1, 2, 4, and 6 kHz.



**Figure 2.** *In-air behavioral audiogram for one male great cormorant (open circles). The 50% detection thresholds obtained using psychophysical methods are shown for 5 frequencies from 0.5 to 6 kHz (dB re 20  $\mu\text{Pa}$  RMS). A spline interpolation was drawn from 0.5 to 5 kHz (the 6 kHz data point was not included in the interpolation, as it seems to be lower than predicted). Ambient noise levels measured in the acoustic chamber (power spectral density, dB re 20  $\mu\text{Pa}/\text{Hz}^{1/2}$ ) are plotted as two solid lines; the minimum and maximum of the recordings.*

Ambient noise files were collected and the spectral RMS was calculated for all files at each of the five tested frequencies. Variation in measurements were observed between each recording and between frequencies. The noise level varied as a function of frequency (Figure 2). The spectral noise level was measured to be between -13 and -2 dB re 20  $\mu\text{Pa}/\text{Hz}^{1/2}$  (mean values for the third octave band around the stimulus frequency) throughout the testing period.

The ABR audiogram had its highest sensitivity around 1-2 kHz and the lowest thresholds around 50 dB SPL.



**Figure 3.** In-air hearing threshold comparison of the psychoacoustic (circle) and ABR (triangle) hearing sensitivities of the great cormorant. The 50% detection thresholds obtained using psychophysical methods are shown for 5 frequencies from 0.5 to 6 kHz. ABR thresholds were evaluated by visual inspection and median values are shown for 4 frequencies from 0.5 to 6 kHz.

The ABR thresholds were 23 dB to 53 dB less sensitive than the psychophysical thresholds (Figure 3).

#### 4. DISCUSSION

Size has been found to play a significant role in a bird's sensitivity to sound (Fay, 1988). When the psychophysical and ABR thresholds from the great cormorant are compared to thresholds from birds of similar size (data from Fay, 1988), the psychophysical thresholds are within a similar range whereas the ABR thresholds are significantly higher. However, the shape of the ABR audiogram curve is more similar to that of other birds as compared to the psychophysical data. When the thresholds of the species of greatest sensitivity (lesser scaup *Aythya affinis*) from the ABR audiograms presented by Crowell et al. (2015) are compared with the ABR and psychophysical thresholds for the great cormorant, the great cormorant has a lower in-air hearing sensitivity. The species most similar to the great cormorant in terms of phylogenetics from Crowell et al. (2015) is the northern gannet (*Morus bassanus*). The region of greatest sensitivity measured by Crowell et al. (2015) for the northern gannet was 2 kHz, the same as for the great cormorant, but the northern gannet thresholds were 55 dB (psychophysics) and 14 dB (ABR) less sensitive than the great cormorant thresholds at 2 kHz.

It is not surprising that ABR and psychophysical thresholds vary as much as they do. Both the methodology and the definition of threshold are very different between these two methods. ABR gives a quick estimation of a bird's hearing capabilities (Brittan-Powell et al., 2002). However, the thresholds are often based on the experimenter's experience to determine whether



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a response is given or not (Pratt & Sohmer, 1978). For behavioral thresholds, the 50% HIT rate is usually used, but this is actually a very liberal choice, as the animal is missing half of the signals at this received level. Several studies on birds have shown the results from behavioral studies are more sensitive than the ABR measurements (Brittan-Powell et al., 2002; 2005; Crowell, 2016). Crowell also found a large difference (30 dB) between in-air psychophysics and ABR thresholds (Crowell, 2016). An undetermined factor of the different thresholds measured by the two methods is the difference in age between the birds used for the ABR and psychophysical trials. In the behavioral experiment performed here, the bird was 6 years of age, while the birds used to determine the ABR thresholds were fledglings. From the comparison of these two methods it is not possible to determine if age played a role in the results of the two studies. As the fledglings have not left the nest and not foraged under water, their internal hearing structures may not have been fully developed as in the adult male used in the behavioral studies. This may explain some of the differences between the ABR and psychophysical thresholds.

A main downfall with the behavioral study is the lack of data points. It is possible that with the addition of more sessions at each of the frequencies, specifically at 6 kHz, the shape of the behavioral audiogram will have a shape that better fits to what is observed in other bird audiograms.

In the previous attempts to obtain the full hearing spectrum of the great cormorant using psychophysics, the experiments were conducted outdoors (Johansen et al., 2016), whereas the present measurements were performed inside an insulated acoustic chamber. This may explain the discrepancy between these previous estimates of the in-air hearing thresholds and the ones presented here.

Despite there being a small observable difference in the shape between the two audiograms at the 6 kHz behavioral threshold and it not resembling the same sharp decrease in sensitivity as observed in the ABR 6 kHz threshold, the results show that great cormorants have a better in-air hearing sensitivity than previously believed (Johansen et al., 2016). This new information on the hearing sensitivity of the great cormorant is the first step in determining the effect that anthropogenic noise can have on this species, as well as understanding whether the cormorant is more adapted for hearing in-air or underwater sounds.

## 5. CONCLUSION

The findings in this comparison of the two methods show that there is a significant difference in the thresholds obtained between psychophysics and ABR, which has also been observed in other animals (Wolski et al., 2003). Both audiograms are similar in shape to birds of similar size, but the match is best with the ABR audiogram.

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