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RESEARCH ARTICLE

Amphibious hearing in a diving bird, the great cormorant (*Phalacrocorax carbo sinensis*)

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ABSTRACT

Diving birds can spend several minutes underwater during pursuit-dive foraging. To find and capture prey, such as fish and squid, they probably need several senses in addition to vision. Cormorants, very efficient predators of fish, have unexpectedly low visual acuity underwater. So, underwater hearing may be an important sense, as for other diving animals. We measured auditory thresholds and eardrum vibrations in air and underwater of the great cormorant (*Phalacrocorax carbo sinensis*). Wild-caught cormorant fledglings were anaesthetized, and their auditory brainstem response (ABR) and eardrum vibrations to clicks and tone bursts were measured, first in an anechoic box in air and then in a large water-filled tank, with their head and ears submerged 10 cm below the surface. Both the ABR waveshape and latency, as well as the ABR threshold, measured in units of sound pressure, were similar in air and water. The best average sound pressure sensitivity was found at 1 kHz, both in air (53 dB re. 20 µPa) and underwater (58 dB re. 20 µPa). When thresholds were compared in units of intensity, however, the sensitivity underwater was higher than in air. Eardrum vibration amplitude in both media reflected the ABR threshold curves. These results suggest that cormorants have in-air hearing abilities comparable to those of similar-sized diving birds, and that their underwater hearing sensitivity is at least as good as their aerial sensitivity. This, together with the morphology of the outer ear (collapsible meatus) and middle ear (thickened eardrum), suggests that cormorants may have anatomical and physiological adaptations for amphibious hearing.

KEY WORDS: Auditory brainstem response, Audiogram, Auditory adaptation, Auditory threshold curves, Bioacoustics, Underwater hearing

INTRODUCTION

Amphibious living requires that the senses function well both in air and underwater. The physical properties of air and water, however, are radically different. Underwater sound environments differ from terrestrial ones in terms of the speed and absorption of sound (e.g. Larsen and Wahlberg, 2017), and underwater light environments differ greatly from terrestrial ones regarding the spectral composition of light, luminance and turbidity (Tremblay et al., 2014). About 150 species of amphibious birds of seven orders are so-called pursuit-dive foragers, i.e. they forage by diving from a swimming position on the water surface to capture prey underwater

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at depths ranging from a few meters to much more than 100 m (White et al., 2007; Tremblay et al., 2014). At the same time, they must breed and nest on land, fly between nesting and foraging sites, and perform a range of other activities in air.

This amphibious lifestyle of diving birds requires adaptations of their visual system to function optimally in both terrestrial and aquatic environments. Terrestrial birds have excellent vision (Martin and Osorio, 2008). Most day-active birds of prey, for instance, primarily rely on vision to find their prey, whereas night-time hunters, such as owls, have acute hearing and vision abilities adapted for finding and catching prey in the dark (Payne, 1971; Kettler et al., 2016; Beatini et al., 2018). When entering the water, however, the eyes of terrestrially adapted birds lose the refractive power of their cornea (Katzir and Howland, 2003). This results in the picture on their retina becoming blurred and their visual field reduced, unless compensated for by anatomical adaptations such as thickened and flattened low-powered corneas, which serve to reduce the effects of loss of corneal refraction underwater (Martin and Brooke, 1991; Martin, 1999; Nelson, 2006). In addition, diving birds often have unusually well-developed intraocular muscles, which may help underwater accommodation by regulating the size of the pupil and altering the shape of the lens (Nelson, 2006; White et al., 2007).

Much less is known about possible adaptations of the auditory system of diving birds to the amphibious lifestyle. It is unknown whether they use their sense of hearing underwater at all; for instance, to avoid sound-emitting predators, to track down sound-emitting prey, to orient relative to ambient underwater sound sources, or perhaps even for underwater sound communication as suggested in a recent study (Thiebault et al., 2019). In-air auditory threshold curves of 8 species of diving birds have been determined using auditory brainstem responses (ABRs) (Crowell et al., 2015) but the underwater hearing ability of diving marine birds has so far been studied in only two species: the long-tailed duck (*Clangula hyemalis*; Therrien, 2014) performing only shallow dives and the great cormorant (*Phalacrocorax carbo*; Johansen et al., 2016; Hansen et al., 2017). The last two studies established that one diving cormorant specimen could detect underwater sounds, but the derived data were not sufficient to establish more objective psychophysical hearing thresholds that could be compared between the two media. This was probably due in large part to the methodology used in these studies, which required an enormous training effort and therefore time, and thus provided only limited data from one to two individuals of each species. However, these studies are very important as they demonstrated that underwater sounds are perceived and can be used behaviourally by the cormorant.

One way to obtain more data on diving birds' hearing abilities is to use physiological techniques such as auditory evoked potentials (AEPs). Such data collection has its own challenges, demanding the experimental bird to be anaesthetized not only during testing in air but also underwater, while breathing is maintained. Also, it is not always trivial to compare physiological and psychophysical

thresholds for birds (Brittan-Powell et al., 2002, 2005). Physiological techniques allow for several frequencies being tested carefully during a limited amount of time both in air and underwater, and therefore also a larger number of individual birds to be tested than when using psychophysical techniques (Beatini et al., 2018; Mooney et al., 2019a,b). However, the lower thresholds obtained by psychophysical techniques mean that these remain the 'gold-standard' for audiogram determination.

As a supplement to physiological data, anatomical investigations of the middle and outer ears of diving birds may reveal adaptations to underwater hearing. Aquatic mammals, turtles and frogs that are secondarily adapted to hear in the aquatic environment have special anatomical adaptations to cope with the high acoustic impedance of water as compared with air (Møhl, 1967; Christensen-Dalsgaard and Elepfandt, 1995; Christensen-Dalsgaard et al., 2012). Most notably, the presence of air cavities, such as air-filled middle ear cavities, has been shown to increase sensitivity of these ears (Christensen-Dalsgaard and Elepfandt, 1995; Christensen-Dalsgaard et al., 2012), and a recent modelling approach has shown that stiff eardrums, such as the tympanic discs in aquatic frogs and turtles, can increase the hearing efficiency of the underwater ear (Vedurmudi et al., 2018). If marine birds can hear well underwater, it is likely that similar anatomical features can be found in their outer and middle ears that will be different from those found in terrestrially adapted bird ears. Other anatomical adaptations, probably for protecting the eardrums underwater by passive closure of the auditory meatus, have been described in auks (Kartaschew and Iljitschow, 1964), and it is likely that there will be adaptations to protect the middle ear from the large hydrostatic pressures incurred while diving. However, because of the scarce hearing data available on these species, it is currently unclear whether such protective adaptations should be considered as being auditory or hydrostatic adaptations, or both.

Here, we report on the hearing abilities of the great cormorant in air and underwater using physiological methods. Great cormorants have been characterized as foot-propelled visually guided pursuit-dive foragers (Ashmole, 1971; Tremblay et al., 2014). They are globally distributed and have very flexible foraging habits, hunting wherever fish are available in coastal waters and freshwater lakes (Grémillet et al., 1998). Great cormorants hunt solitarily in waters where turbidity is high and light levels are low, hunting even at night and into the dark Arctic winter (Grémillet et al., 2006; Tremblay et al., 2014). They may also hunt socially when turbidity is very high, driving fish prey towards the surface (Van Eerden and Voslamber, 1995). Most of their dives last for about 30 s and reach depths of 6–10 m with 35 m as the extreme (Grémillet et al., 2006). They have highly pliable eye lenses and powerful intraocular muscles that may accommodate the loss of corneal refractive power underwater (Glasser and Howland, 1996). Their diving activity (frequency, duration and depth) is modulated by ambient light levels such that in Greenland waters, the deepest and longest duration dives are observed at midday, whereas duration and depth are lower at dawn and lowest during the night (White et al., 2008). Such a circadian entrainment is expected if their primary sense for hunting is vision and if potential prey performs vertical light-dependent migration. It is therefore highly surprising that great cormorants' visual acuity underwater is poor at the light levels that they are known to encounter during natural dives (White et al., 2007). Their visual acuity is comparable to unaided human vision underwater, which means that diving cormorants can detect individual prey only at distances of less than 1 m. This is surprising as they are known to be highly efficient hunters and are supposed to be visually guided.

This seeming contradiction could possibly be explained by the use of tactile cues (Voslamber et al., 1995) as in harbour seals, which use their vibrissae for sensing water currents (Dehnhardt et al., 2001). Close-quarter prey detection could then be combined with special foraging techniques, e.g. brief short-distance pursuit and/or rapid neck extension to capture prey at very short range (White et al., 2008). However, no vibrissae-like organ has been described in cormorants or other diving birds. Another possibility, not considered in the literature, is that great cormorants (and other diving birds) underwater may use supplementary information obtained through their sense of hearing.

To get an impression of the cormorants' underwater hearing ability compared with that in air, we measured AEPs, assumed to represent ABRs, to determine hearing thresholds using identical criteria, with the same specimens measured first in air and then underwater. In this way, it was possible to directly compare their hearing sensitivity in the two media, and judge to what extent cormorants have special adaptations for underwater hearing. It is unclear, however, how such specializations would be manifested in their outer and middle ear, and therefore we made some additional anatomical investigations to determine possible adaptations to the aquatic environment in these structures, as the anatomy of the cormorant ear has been described only in general terms (Saiff, 1978). Our observations were supplemented with recordings of eardrum vibrations in air and underwater to compare with observations in amphibious vertebrates (Christensen-Dalsgaard et al., 2012).

MATERIALS AND METHODS

In mid-to-late June in each of the years 2012, 2013, 2014 and 2015, five great cormorant (*Phalacrocorax carbo sinensis* Staunton 1796) fledglings were collected from treetop nests in the cormorant breeding colonies at Nørresø, (55°08′40″ N; 10°22′25″ E) and Brændegård Sø (55°07′52″ N; 10°23′03″ E), Funen, Denmark. The fledglings were subsequently transferred to a 6×3×2 m³ outdoor aviary at the Department of Biology, University of Southern Denmark, where they were provided with water and fish (capelin and sprat) *ad libitum* for the 4–16 days that they were kept until experiments were carried out. The fledglings were judged to be 4–6 weeks old and very close to leaving the nest when captured, based partly on frequent and regular observations of the colony (Jacob Sterup, personal information) and partly on their mass at the time of the experiment (see Table 1; Dunning, 2007). Collection of

Table 1. Overview of the cormorant fledglings used for auditory brainstem response (ABR) recordings in 2013–2015

Cormorant ID	Mass (kg)	Year	No. of frequencies in air	No. of frequencies underwater
2	2.2	2013	6	N/A
3	2.3	2013	4	N/A
4	2.1	2013	5	5
A	1.9	2014	6	N/A
B	1.8	2014	4	4
C	1.6	2014	6	5
D	2.3	2014	6	5
E	2.1	2014	6	N/A
F	1.8	2015	6	7
G	1.8	2015	8	3
H	2.0	2015	7	8
I	1.7	2015	5	9
Mean±s.d.	2.0±0.2		12 individuals	8 individuals

N/A indicates that no underwater ABR signals were recorded (either due to unknown technical problems or because the bird died during recording).

birds and experiments were carried out with permission from the Danish Nature Agency (J.nr. SNS-342-00056 and nst-342-00122 to O.N.L. and M.W.) and the Danish Animal Experimentation Inspectorate (J.nr. 2012-15-2934-00067 to O.N.L.) for using up to five cormorant fledglings per year. The birds collected in 2012 were used for pilot ABR measurements, for recording of eardrum vibrations in air and underwater, and for anatomical examinations, whereas the birds collected in 2013–2015 were used for the ABR experiments reported here. As a supplement, we dissected the ear region of the head of two adult cormorants: a 3 year old female cormorant that had been kept in captivity at the University of Southern Denmark's Marine Research Centre and had died from natural causes in 2017, and a wild cormorant found dead there in 2018 and frozen within a few hours of death.

Preparation for ABR experiments

For recording, each experimental bird was anaesthetized by intrapectoral injections of a Ketamine–Rompun mixture. The initial dose was $20\ \mathrm{mg\ kg^{-1}}$ ketamine hydrochloride and $5\ \mathrm{mg\ kg^{-1}}$ xylazine hydrochloride; supplementary doses of $10\ \mathrm{mg\ kg^{-1}}$ ketamine hydrochloride and $2.5\ \mathrm{mg\ kg^{-1}}$ xylazine hydrochloride were administered as needed to keep the birds anaesthetized for 3–5 h. We had to keep the dose this low, as higher doses such as $40\ \mathrm{mg\ kg^{-1}}$ ketamine hydrochloride plus $20\ \mathrm{mg\ kg^{-1}}$ xylazine hydrochloride normally used to anaesthetize small birds like starlings (e.g. Klump and Larsen, 1992) and budgerigars (e.g. Larsen et al., 2006) immediately overdosed the cormorants. At the end of experiments, each bird was overdosed with injection of pentobarbital i.p. before waking up, as required by the Danish Animal Experimentation Inspectorate.

To maintain core temperature, the anaesthetized bird was placed on a heating blanket (Heat Therapy pump model TP702, Gaymar Instruments Inc., Orchard Park, NY, USA) in a customized metal frame holder shaped such that the bird gently rested its head, kept in place with masking tape, in an upright, close to normal resting position. On each side of this metal frame holder, another metal rod shaped like an inverted V was welded such that the whole metal frame holder could be attached to an overhead holder like a gondola under a balloon. This arrangement was also used in the underwater setup (see Fig. 1). Then, three subdermal needle electrodes (16 mm, 27-gauge needles, isolated to the tip, 170 cm lead, Tucker-Davis Technologies, Alachua, FL, USA) were gently pushed through the skin parallel to the surface. Two differential electrodes were placed right behind the left ear meatus (the active electrode) and on the vertex of the head (the inverting electrode), respectively, with reference to the third ground electrode placed in the nape of the neck. Note that the placement of the active and inverting electrode here was opposite to that of Brittan-Powell et al. (2002, 2005), Crowell et al. (2015) and Beatini et al. (2018). Therefore, the ABR signals recorded here are inverted relative to the ones in those studies.

ABR recordings in air

For ABR recordings in air, the metal frame holder with the bird in sitting position on the heating blanket was transferred to a custom-built sound-attenuated and anechoic booth (inner dimensions $135\times105\times95\ \mathrm{cm}^3$; for specifications, see Jensen and Klokker, 2006). Here, the metal frame was placed on the floor such that the bird's head was located right below a ½ inch microphone (type 40AF, G.R.A.S. Sound and Vibration, Holte, Denmark) directed towards the speaker and calibrated with a sound level calibrator (type 4230, Brüel & Kjær Sound & Vibration Measurement a/s,
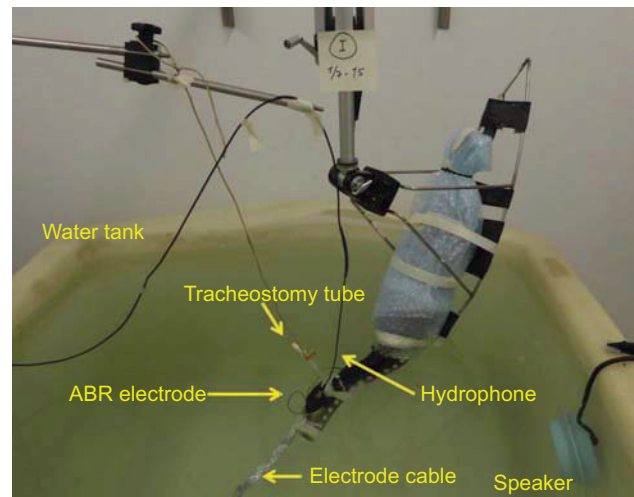

Fig. 1. Setup for recording auditory brainstem responses (ABRs) underwater. The cormorant's body rested on a metal frame attached to an upside-down photo tripod that could be lowered to place the bird's head underwater. The cormorant was lowered until the head was submerged to a depth where the ears were located 10 cm below the water surface and next to the hydrophone. The tracheostomy tube was 1–2 cm above the water surface. For further explanation, see Materials and Methods.

Nærum, Denmark). The microphone output used for system calibration was amplified and high-pass filtered (0 dB amplification; high-pass cut-off 20 Hz; power module, type 12AA, G.R.A.S. Sound and Vibration) and recorded on a laptop computer. Sound stimuli (click sounds or single-frequency tone bursts of 25 ms duration, including 2 ms long ramp-up and ramp-down segments at the start and end to avoid spectral smearing) were delivered at a repetition rate of 25 Hz by a battery powered loudspeaker (Creative D100, Creative Technology, Crawley, UK) located 90 deg to the left of the body–beak axis and 70 cm away from the left ear of the experimental bird.

Sound stimulation, recording and data analysis were performed using custom-made software (QuickABR) running on a digital signal processor (type TDT RM2, Tucker-Davis Technologies) and a PC as in previous studies from this laboratory (Christensen-Dalsgaard et al., 2011, 2012). AEPs, which are assumed to reflect the ABR, were passed from the subdermal needle electrodes via cables shielded with grounded aluminium foil through a low-impedance headstage and a preamplifier (type RA4LI and RA4PA, Tucker-Davis Technologies), where they were amplified (74 dB), digitized (sampling rate 25 kHz, 16 bits), acquired by the RM2 signal processor, and stored on a PC.

ABR recordings underwater

For subsequent ABR recordings underwater, the still deeply anaesthetized bird was first tracheostomized in the neck about 2 cm posterior to the corner of the mouth and an 8 mm diameter flexible plastic tube was inserted about 2 cm into the proximal part of the severed trachea. The trachea was tightly attached to the plastic tube and sealed with superglue; a piece of string was attached to the end of the 14 cm long plastic tube. By this arrangement, the bird breathed freely as before the operation. In addition, the bird's body was wrapped in bubble wrap to help maintain body temperature and the ABR electrodes were reattached. The anaesthetized bird was again placed in the customized metal frame holder, which was now attached like a gondola to a photo tripod hanging upside-down from the ceiling (Fig. 1) above the centre of a $100\times90\times60\ \mathrm{cm}^3$ water tank

used in a previous study when also the tank's sound field was investigated (for further information on the acoustics of the tank, see Christensen-Dalsgaard et al., 2012). The photo tripod could be turned and fixed such that the bird's body axis on the gondola frame holder was oriented about 40 deg relative to vertical with the bird's head facing downwards (see Fig. 1). Finally, the bird in the gondola frame holder was lowered by means of the photo tripod, until the ears of the experimental bird were positioned 10–11 cm below the water surface, while the end of the tracheostomy tube was kept above the water surface. In this position, the bird kept breathing normally. We chose to place only the bird's head below the water surface, as placing the whole body underwater lowered the body temperature unacceptably fast and released air bubbles, which raised the ambient noise level and interfered with the ABR measurements. ABR signals were recorded using the same stimuli and recording setup as for in-air recordings.

For sound stimulation, we used an underwater speaker (type UW30, Lubell Labs Inc., Columbus, OH, USA) hanging on the inner wall of the water tank about 45 cm from the bird's left ear (Fig. 1). It was powered by a power amplifier (type DD-8, Xelex AB, Stockholm, Sweden) that received signal input from the same digital signal processor and laptop used for stimulation in air. Sound pressure levels next to the bird's head were recorded with a small hydrophone (type TC4013, Reson, Slangerup, Denmark), amplified by a hydrophone amplifier (type A1105, Etec ApS, Frederiksværk, Denmark), and calibrated with a pistonphone calibrator (type 4223, Brüel & Kjær).

Experimental procedure for ABR recordings

Initially, both in air and underwater, intense click stimuli (half-cycle 4 kHz pulse with a relatively flat spectrum to above 4 kHz) presented at 25 Hz were used to produce clear ABRs that defined the duration of the post-stimulus response window (usually from about 3 ms to about 8 ms after calculated sound stimulus arrival at the left ear), within which to judge the threshold. Latencies from click-stimulus arrival at the exposed ear to maximum top of wave II in the ABR response (Fig. 2) were determined by correcting for transmission time in the two media (0.7 m/344 m s^{-1}=2.0 ms in air and 0.45 m/1500 m s^{-1}=0.3 ms underwater). To compare click response latencies underwater and in air, 26 dB was added to in-air dB values to transform the dB scale from a reference pressure of 20 µPa to 1 µPa (e.g. Larsen and Wahlberg, 2017). Click amplitudes were stated in peak equivalent dB (Fig. 2); this is the root mean

square (RMS) dB value of a sinusoid with the same peak amplitude as the click.

Subsequently, 400 single-frequency tone bursts were delivered at a repetition rate of 25 Hz for each of the frequencies 250, 500, 1000, 2000, 2500, 3000, 4000 and 6000 Hz, and the responses were averaged for each frequency. The phase was changed 180 deg for each burst to reduce microphonics. For each frequency, the initial sound pressure was set to produce clear ABR responses and then the sound pressure was reduced in a series of 5 dB steps. Like Brittan-Powell et al. (2010) and Beatini et al. (2018), we used the visual detection method, defining the ABR threshold as the sound pressure 2.5 dB below the lowest stimulus level at which a response could be visually detected on the trace between 4 and 8 ms (see also Brittan-Powell et al., 2002, 2005). Thresholds were determined independently by two of the authors (J.C.-D. and O.N.L.) as well as by T. Bojesen Lauridsen, an MSc student who had experience in judging ABR data from frog recordings.

Thresholds determined by this method critically depend on the recorded noise level both between air and water and across experimental animals. The noise level of the ABR recordings was estimated by dividing all recordings at each stimulus level into two batches by assigning every even numbered recording into batch 1 and every odd numbered recording into batch 2. Subsequently, each of the two batches was averaged. The difference between the two batch averages was taken as an estimate of the ABR noise, whereas the average of the two batches was an estimate of signal+noise. Measured in this way, the median ABR noise (RMS) was 0.19 µV in air and 0.23 µV in water (i.e. 1.7 dB higher in water than in air). Across birds, the ABR noise varied between 0.83 and 1.59 µV, or up to 5.6 dB. We therefore concluded that the noise levels in air and water were sufficiently similar to make threshold comparisons meaningful.

Statistics

To test whether the relationship between peak amplitude and latency differed in air and underwater, we conducted a random coefficient mixed model (RCMM) with peak amplitude, medium (air and water) and the interaction between them. The model had a common intercept, whereas the common slope for peak amplitude was omitted to enable model convergence. Individual was treated as subject. We used a Box–Cox transformation of latency (λ=−2) (Sokal and Rohlf, 1981) to enable residuals not to deviate from assumptions regarding normality and homoscedasticity.
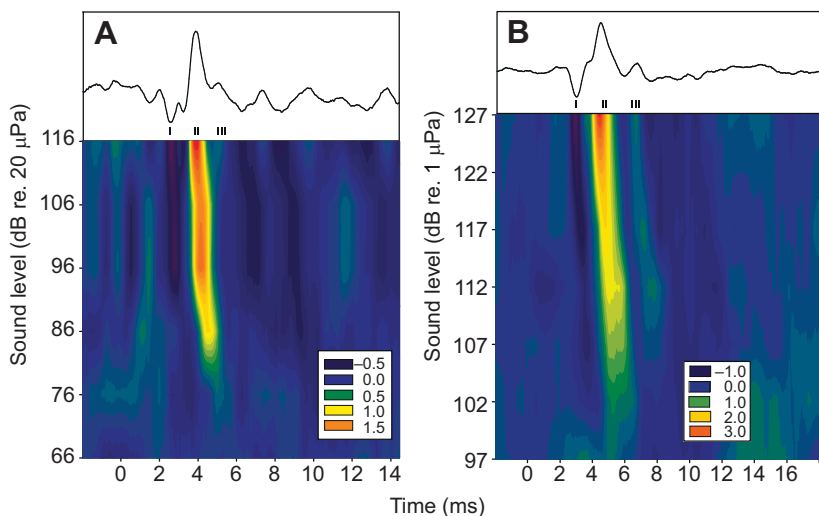

Fig. 2. ABR to clicks. (A) Typical in-air ABR to a click stimulus (individual F). With the active electrode at the exposed ear, three deflections, marked I–III, were always observed at the highest stimulus levels. The interpolated heat diagram below the click response shows the change in delay and peak amplitude for successive 5 dB reductions of stimulus sound level. The peak indicated by II in the response is clearly detected over a 30 dB range, whereas the trough indicated by I and the smaller peak indicated by III are detectable only over a range of up to about 20 dB. Colour coding −0.5 to +1.5 corresponds to amplitude peak–peak in µV. (B) Underwater ABRs from individual F. Colour coding −1.0 to +3.0 corresponds to amplitude peak–peak in µV.

To test whether the threshold curves in air differed from those underwater, we first related the in-air pressure–dB thresholds to the same reference value of 1 µPa as in water by adding 26 dB to each of the in-air data points. As thresholds may also be expressed in intensity units, we related the in-air to the underwater intensity thresholds by recalculating in-air and underwater thresholds to dB re. 1 pW m^{-2} (see Larsen and Wahlberg, 2017, for details). Note that the intensity is the far-field intensity calculated from the pressure measurements. As thresholds for individuals were measured at multiple frequencies in both air and water, we used a repeated measures ANOVA with individual as the repeated measure. Frequency and medium (air and water) were used as fixed effect discrete parameters in the model. The repeated measures analysis applied a first-order autoregressive covariance matrix. We tested the model first for differences between underwater and in-air pressure thresholds, and second for differences between underwater and in-air intensity thresholds. The residuals for both models abided with assumptions regarding normality and homoscedasticity. All analyses were made in SAS v. 9.4 (SAS Institute, Cary, NC, USA) using 'Proc mixed'.

Outer and middle ear anatomy

Upon termination of ABR experiments, the overdosed birds, and later the two deceased adult cormorants, were decapitated and the heads kept frozen at −80°C for later examination. Thawed heads were examined under a stereo microscope (Leica M60 with camera IC80 HD, Leica Microsystems GmbH, Wetzlar, Germany). The ear region was located, and dissection proceeded by removing feathers, skin and bone to reveal the overall anatomy of the ear region including eardrum and columella, to be photographed and measured using callipers (Mitutoyo, Japan) with a vernier scale.

Laser Doppler vibrometer recordings of eardrum vibrations in air and underwater

We investigated eardrum vibrations of three cormorants both in air and underwater. For the experiments in air, we placed the deeply anaesthetized birds in an anechoic room after surgically removing feathers, skin and muscle tissue posterior to the meatus to be able to direct a laser beam to the centre of the eardrum. We recorded eardrum vibrations with a laser Doppler vibrometer (OFV-505 sensor and OFV-5000 vibrometer, Polytec, Waldbronn, Germany) with the laser head placed approximately 1 m from the bird's ear. The bird was stimulated by sound from 12 loudspeakers (JBL 1G, Lansing, James B., Los Angeles, CA, USA) placed at 30 deg intervals on a circle 1 m from the bird's ear. The sound stimulus was a 100 ms frequency sweep from 200 to 8000 Hz repeated 10 times, and the loudspeakers were calibrated and centred acoustically by a ½ inch pressure microphone (type 4192, Brüel & Kjær) placed over the head of the animal. Stimulation, calibration and data acquisition were controlled by Tucker-Davis System 2 hardware (Tucker-Davis Technologies) and customized software ('DragonQuest'; Christensen-Dalsgaard and Manley, 2005). Sound at the ear opening was measured by a probe microphone (type 4082, Brüel & Kjær), allowing calculation of the transfer function between sound stimulation and eardrum vibration velocity.

In water, we used the same water tank as for the underwater ABR measurements, and the same method as reported earlier for underwater measurements in turtles (Christensen-Dalsgaard et al., 2012). An important modification, however, was that we measured eardrum vibrations in the severed heads of three freshly killed and decapitated birds, to have a cleaner sound field in the tank than the presence of the intact bird would allow, by avoiding, for instance,

re-radiated sound from the lungs. Briefly, the eardrum was surgically exposed and one small piece of reflecting film (3M, 3M Center, St Paul, MN, USA) was glued on the eardrum centre and another on the head for control measurements. The head was then tied to a PVC platform and suspended 13 cm below the water surface, approximately 45 cm above an underwater loudspeaker (type UW30, Lubell Labs Inc.). Sound was measured by a hydrophone (type 8103, Brüel & Kjær) located approximately 1 cm from the eardrum and conditioned by a conditioning amplifier (type Nexus 2, Brüel & Kjær). The laser Doppler vibrometer (model as above) was focused on the reflecting foil through a transparent window in the tank. The particle velocity in the direction of tympanum movement was measured from the pressure gradient between two closely spaced hydrophones (type 8103, Brüel & Kjær), as described previously (Christensen-Dalsgaard et al., 2012). Eardrum and particle velocity measurements were controlled by a digital signal processor (type TDT RM2, Tucker-Davis Technologies) and customized software. The speed of sound in the tank was 1370 m s^{-1} (Christensen-Dalsgaard et al., 2012). We compared the underwater laser measurements with a model of fish swim bladder vibration in an underwater sound field using coefficients for damping and stiffness taken from fish tissue (Alexander, 1966; see also Christensen-Dalsgaard et al., 2012). In SI units, the model states that:

$$v=p\left/\left(100\sqrt[3]{(3V/4\pi)} \cdot\omega^2\sqrt{\left(500\sqrt[3]{(3V/4\pi)}^{-2}\cdot\omega^{-2}-1\right)^2+200\sqrt[3]{(3V/4\pi)}^{-2}\cdot\omega^{-2}}\right),\right.$$

(1)

where v is vibration velocity, p is pressure, V is cavity volume and ω is angular frequency.

RESULTS
ABR and latency

The waveshape of the ABR, especially to click sounds, was similar in air and in water (Fig. 2). It typically had three phases, one negative and two positive ones, here denoted I, II and III. Phase II had the largest amplitude and was most prominent and consistent, and therefore it could reliably be detected over much larger stimulus ranges than phase I and III.

The latency from click stimulus arrival at the ear to time of highest amplitude of phase II in the ABR was determined as a function of stimulus amplitude after compensating for transmission time from the loudspeaker and underwater speaker to the ear (Fig. 3). Both in-air and underwater latencies seemed to follow the same general trend of decreasing latency with increasing peak equivalent sound pressure, as indicated by the non-significant interaction between peak amplitude and medium (RCMM $F_{1,53}=0.27$, $P=0.61$). The latencies ranged from about 6.0 ms to about 3.5 ms at the highest stimulus amplitudes. In this comparison, in-air stimuli had been presented at higher amplitudes than the underwater stimuli. Peak amplitude showed a significant negative relationship to latency, i.e. higher peak amplitude resulted in shorter latency (RCMM $F_{1,53}=85.94$, $P<0.0001$, slope=−0.00033). Medium (air versus water) did not affect latency time (RCMM $F_{1,53}=0.01$, $P=0.90$).

The similarity between in-air and underwater latencies may be interpreted to mean that the same physiological brain processes occur in the experimental bird in both media. However, three individuals (UW 4, UW C and UW G) displayed longer latencies at
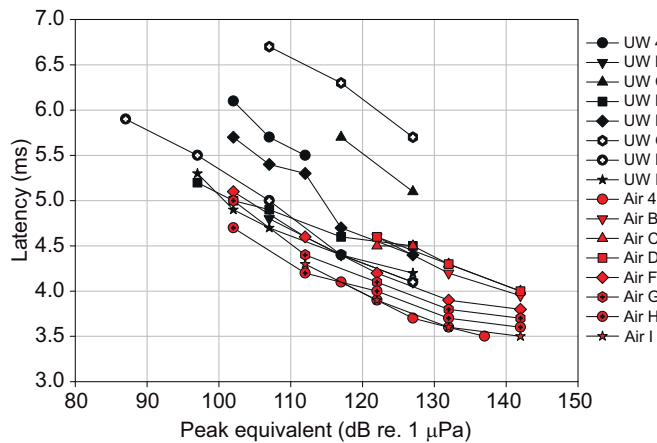
Fig. 3. Latency. Click response latency of the phase II deflection for the 8 birds whose ABRs were measured both in air and underwater (UW). Bird ID is indicated in the key (see Table 1).

very high stimulus amplitudes and were therefore measured at only 2–3 click sound amplitudes underwater. This might indicate that the general state of these experimental birds had deteriorated somewhat when they were transferred from the in-air to the underwater setup.

ABR thresholds in air

In-air hearing thresholds to pure tone bursts were obtained for 12 birds. Thresholds varied considerably (range 15–30 dB) from bird to bird and from frequency to frequency (Fig. 4A). Slightly different positions of the measuring electrode relative to the ear opening may account for some of the variation between individuals in the recorded ABR.

The average in-air ABR threshold curve was U-shaped and had best frequency (BF) at 1 kHz and a tuning Q_{10dB} of 2. The average threshold at BF was 53 dB re. 20 µPa. The 10 dB bandwidth (i.e. 10 dB above threshold at BF) of the average threshold curve was 2.0 kHz. The slope of the average threshold curve from BF towards lower frequencies was about 15 dB per octave.

ABR thresholds underwater

Underwater hearing thresholds were obtained from 8 of the 12 birds tested in air (Fig. 4B). Just as in air, there was considerable threshold variation from bird to bird, especially at frequencies below 2.5 kHz (range 10–40 dB). Just as in air, BF of the average threshold curve was at 1 kHz and Q_{10dB} was 2. The average of all underwater thresholds at BF was 84 dB re. 1 µPa. The 10 dB bandwidth of the average threshold curve was 2.0 kHz. The slope of the average threshold curve from BF towards lower frequencies was 8 dB per octave. The three individuals with longer latencies underwater also had higher underwater thresholds, again suggesting deteriorated conditions for these three birds. Therefore, a second average was calculated based on the data from the remaining five subjects. These two average curves were generally similar with only a few dB deviation.

Comparison of threshold curves in the two media

To directly compare the shape and sensitivity of ABR threshold curves in air and underwater, the sound pressure threshold values were all related to 1 µPa. This is equivalent to adding 26 dB to the in-air thresholds, which had been measured relative to 20 µPa (Fig. 4A). A repeated measures ANOVA found that the sound pressure thresholds underwater and in air did not differ significantly
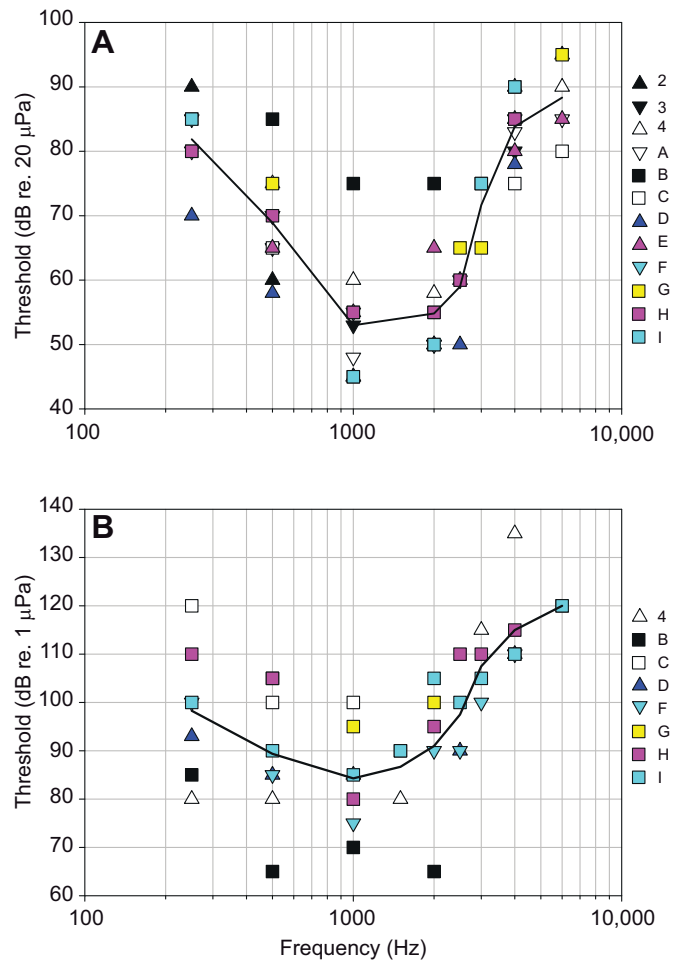

Fig. 4. Scatter plots of ABR audiograms from cormorant fledglings collected over 3 years. (A) In-air threshold curves of 12 cormorant fledglings. (B) Underwater threshold curves of 8 of the 12 fledglings. Solid line indicates average values. Bird ID is indicated in the key (see Table 1).

(Fig. 5A; $F_{1,7}=1.33$, $P=0.29$). However, the threshold differed significantly between frequencies ($F_{7,22}=18.93$, $P<0.0001$). Least square means indicated that thresholds were significantly lower for frequencies between 500 and 2500 Hz compared with those of higher and lower frequencies tested (Fig. 5A). There was a non-significant frequency–medium interaction effect ($F_{7,22}=0.66$, $P=0.71$).

Hearing thresholds may also be expressed in intensity terms by relating the dB values to 1 pW m^{-2} (for details, see Larsen and Wahlberg, 2017). The underwater intensity thresholds were significantly lower than those in air (Fig. 5B; $F_{1,7}=68.96$, $P<0.0001$). Frequencies also differed significantly, with those between 500 and 2500 Hz having lower thresholds than lower and higher frequencies ($F_{7,22}=18.30$, $P<0.0001$). Finally, the frequency–medium interaction was non-significant ($F_{7,22}=0.80$, $P=0.59$).

Anatomy of the outer and middle ear

The ear opening was often difficult to localize, even under a surgical microscope. In the two adult individuals, it was found about 40 mm from the centre of the ipsilateral eyeball and about 29 mm from the ipsilateral corner of the mouth (Fig. 6A). Here, the opening is covered with a dense layer of 15–16 mm long feathers indistinguishable from the other feathers covering the head. Thus, these feathers lacked the open structure of the ear coverts of, for instance, songbirds.
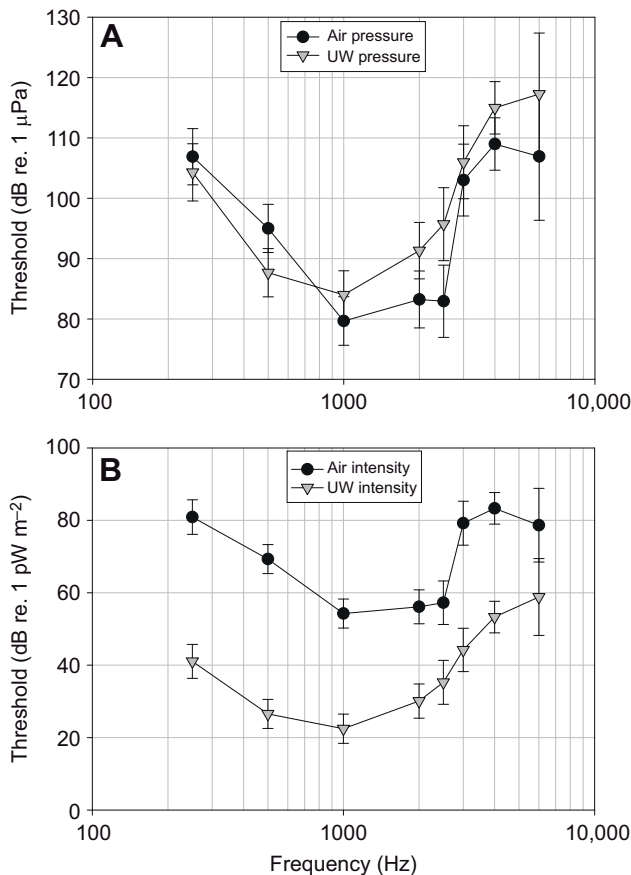
Fig. 5. Comparison of average ABR threshold in air and underwater predicted from a repeated measures ANOVA model (least means squares). (A) The model predicted average sound pressure curves (±s.e.) in air and underwater (dB re. 1 µPa) that were not significantly different. (B) When measured as average intensity thresholds (dB re. 1 pW m^{-2}), the model predicted statistically different curves (average±s.e.) in air and underwater.

In the adult cormorants, the external ear formed a lens-shaped naked recess (like a 'satellite dish', typically about 2.5 mm deep, 7.5 mm long and 4.0 mm wide), the long axis of which was almost parallel to the beak (Fig. 6B,C). The actual bean-shaped ear opening (about 2 mm by 1 mm; Fig. 6C) was located at the centre of the naked recess. The bottom of the naked recess was very resilient and applying a very slight pressure with two needles easily closed the ear opening along its long axis (Fig. 6D,E). The meatus was spoon-shaped at the bottom of the naked recess and led to a short passage posterior to the quadratum bone (Fig. 6C) that bent down into a pocket-like chamber, 7–8 mm high and 6–7 mm in diameter, i.e. with a volume of about 0.1 ml. The axis of the meatus chamber from surface to bottom was not perpendicular to the head surface but directed forward about 45–50 deg relative to the beak axis (Fig. 6C). Fledgling ears only differed from adult ears by the lens-shaped outer ear being partly covered with skin at its two ends.

The eardrum was located in the chamber's antero-medial wall and was not visible from the outside because of the curved meatus. In contrast to, for example, songbirds, the eardrum was not clearly protruding into the chamber but was rather flat and it was difficult to visually determine its exact rim because the eardrum proper was covered by a loosely fitting external epithelium continuous with the skin of the ear canal (named the 'outer eardrum' by Schwartzkopff, 1949). When the outer eardrum was peeled off, the extent and slight protrusion of the transparent 'inner eardrum' into the meatus was

clearly visible. The intact two-layered eardrum *in situ* appeared very thick and piston-like, especially around the three-pronged, about 2.0 mm by 1.3 mm, extra-columella's eccentric attachment (area about 4 mm^2; Fig. 6F,G) to the almost circular 'inner eardrum' (long diameter about 6.5 mm and short diameter about 5.5 mm; area about 28 mm^2); i.e. the extra-columella covered about 14% of the eardrum area. From the extra-columella, the slightly curved, about 4.5 mm long columella (Fig. 6F) led to the inner ear, to which it attached via the oval–triangular 1 mm diameter footplate (area about 0.8 mm^2; Fig. 6H). The ratio of eardrum area to footplate area therefore was about 35.

Eardrum vibrations in air
We collected eardrum responses to free-field air-borne sound in three anaesthetized cormorants. The transfer functions (i.e. the vibration spectrum divided by the sound spectrum recorded at the eardrum; see example in Fig. 7A) showed a maximum vibration amplitude around 1 kHz of about −21 dB re. 1 mm s^{-1} Pa^{-1} (0.1 mm s^{-1} Pa^{-1}) with ipsilateral stimulation (90 deg). Also, the eardrum transfer functions showed a systematic change in vibration amplitude with direction around 1 kHz, with a maximum difference of about 6 dB between ipsilateral and contralateral stimulus directions in the three birds.

Eardrum vibrations underwater
Underwater laser vibrometry measurements on three cormorant heads showed that the eardrum vibrated with a maximum amplitude of approximately −10 dB re. 1 mm s^{-1} Pa^{-1} (0.3 mm s^{-1} Pa^{-1}) at 1 kHz (Fig. 7B). The velocity amplitude was 20–30 dB higher than that of the surrounding tissue, and approximately 30 dB higher than underwater particle velocity amplitudes, which would be the source of bone conduction stimulation of the ear. The observed peak frequency corresponded to the peak frequency of a simple model (Fig. 7B) based on sound-induced vibration of fish swim bladders (Alexander, 1966; see Materials and Methods), assuming a cavity volume of 0.2 ml (see also Christensen-Dalsgaard et al., 2012, for details). This model, however, predicted lower vibration amplitudes than observed, approximately 15 dB lower at 1 kHz. These post-mortem measurements were performed approximately 10 min after the birds were killed and therefore were probably not affected by post-mortem changes in stiffness of the middle ear. Also, the responses were comparable to, or even larger than, the *in vivo* responses of the eardrum in air.

DISCUSSION
We have presented the first physiological measurements of underwater sound sensitivity in a diving bird. Evidence, derived from ABRs in air and underwater, from anatomy, and from laser vibrometry suggest that the auditory threshold curves of cormorant fledglings in both media are similar in shape and sensitivity to sound pressure. However, the sensitivity underwater is higher than in air in terms of intensity. Eardrum vibration amplitudes both in air and underwater are maximal at the most sensitive frequencies, suggesting that the underwater sensitivity is mediated by anatomical specialization of the middle ear for underwater hearing. These anatomical specializations will be detailed below.

Underwater and in air-sensitivity measured using the same ABR methodology
We used the same ABR methodology to derive both in-air and underwater hearing thresholds of the great cormorant. Compared with previous studies, the relatively large sample size of specimens
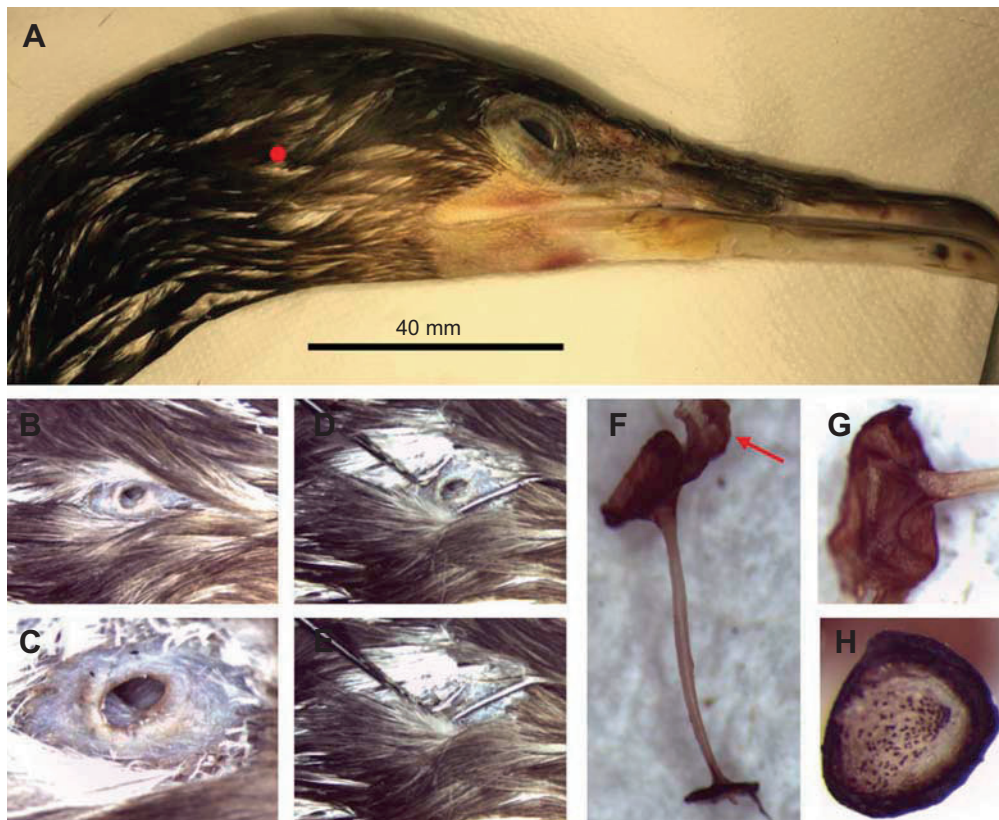
Fig. 6. Anatomy of the right ear of a 2 year old female cormorant. (A) Right side of the head. The red spot indicates the location of ear opening under the dense feather cover. (B) Close-up with cover feathers brushed aside. (C) The lens-shaped naked recess surrounding the bean-shaped ear opening. The black spot in the ear opening is the canal leading to the eardrum chamber below at a 45–50 deg angle relative to the head surface with the beak direction to the right. (D,E) Very light pressure applied with two needles closes the ear opening along its long axis. (F) The excised columella with the extra-columella at the top and the footplate at the bottom. Part of the excised eardrum is indicated with a red arrow. (G) Eardrum with eccentric extra-columella attachment seen from below (from the middle ear). (H) Footplate to the oval window seen from the inner ear.

tested as well as the consistency in methodologies of the measurements in air and underwater on the same individuals made it possible to more carefully analyse the differences in hearing threshold between the two media than was possible in previous studies on this species (Johansen et al., 2016; Maxwell et al., 2017; Hansen et al., 2017) as well as on other species of marine birds (Therrien, 2014; Crowell, 2016).

The cormorant in-air ABR threshold curve is shaped like in-air audiograms of other birds of similar size (see Maxwell et al., 2017) and shows similar sensitivity and shape to those of other diving birds in air (Crowell et al., 2015). This gives confidence to the ABR method for determining the general shape of the threshold curves, even though the hearing thresholds derived with this method are 20–40 dB higher than those derived by psychophysics (Fig. 8A). A large discrepancy between ABR thresholds and psychophysical thresholds is also found in other species of birds (Brittan-Powell et al., 2002) as well as in marine mammals (Wolski et al., 2003; Houser and Finneran, 2006). The difference is usually explained by the large methodological differences in the two approaches: ABR measurements essentially tap into the nerve signals funnelled through the animal's brainstem from synchronous activity in many auditory nerve fibres, whereas the psychophysical thresholds reflect activity in only the most sensitive fibres and make use of the animal's entire decision process.

In addition, we used 25 ms duration pulse tones in the ABR experiments. The peripheral auditory system is often modelled as a leaky energy detector with a certain integration time, T_i (Plomb and Bouman, 1959). In this model, thresholds will remain constant for stimulus tone durations longer than T_i but increase when stimulus duration is progressively shorter than T_i. Therefore, with 25 ms tones, much higher stimulus sound amplitude is required to deliver sound energy than the 500 ms duration tones used in the

psychoacoustic experiments (Dooling and Searcy, 1985; Klump and Maier, 1990). With an expected integration time for birds of about 200 ms, this accounts for 10–15 dB of the difference between ABR thresholds and psychoacoustic thresholds (Pohl et al., 2009, 2013). Therefore, even though the shape of average ABR threshold curve mimics the psychophysically derived audiogram, it is expected that the curves will not be comparable in terms of sensitivity. However, ABR-derived threshold curves do allow comparison between hearing sensitivity in the two media.

Finally, it might be argued that the relatively high thresholds in the present study could be a consequence of using fledglings and not adult birds, as avian auditory sensitivity increases during ontogeny. However, this is not supported by data from other species of birds: the increase in budgerigar (*Melopsittacus undulatus*) audiogram sensitivity was found to be most marked during the first 3 weeks of development, becoming indistinguishable from adult values at the age of fledging (see fig. 3 in Brittan-Powell and Dooling, 2004). As the cormorant chicks were judged to be 4–6 weeks old and very close to fledging, we expect their threshold curves to be close to those of adult birds. Any difference in threshold between juvenile and adult birds is therefore expected to be smaller than the measurement accuracy of this study, which is estimated to be about 6 dB.

The large variation in threshold obtained from individual birds of the present study (Fig. 4) is readily explained by the ABR methodology, which in humans has been shown to exhibit inherent amplitude variation of 10–15 dB (e.g. Picton et al., 2003). In addition, it is well known that small shifts in electrode position can produce different signal amplitudes, which will have a tremendous influence on the derived thresholds. The fact that in the present study the birds needed to be anaesthetized, and in the case of the underwater trials were subject to radical surgery, may also have caused variation in the birds' responses depending on their general
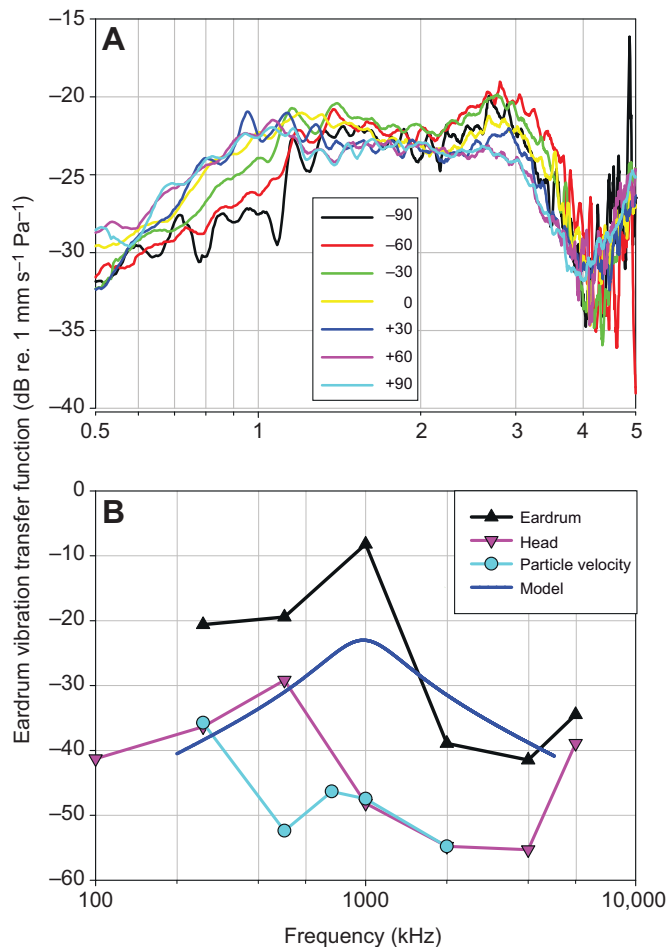
Fig. 7. Cormorant eardrum vibrations in air and underwater measured with laser Doppler vibrometry. (A) In-air eardrum vibration velocity transfer function as a function of sound source direction in the frontal hemi-field: 0 deg corresponds to the beak direction, whereas +90 deg corresponds to the ipsilateral source location. (B) Underwater vibration velocity transfer functions of the eardrum and the surrounding skin on the head compared with the measured particle velocity of the medium, close to the head, and the vibration output of a simple model.

Fig. 8. Comparison between ABR thresholds and thresholds obtained with behavioural methods. (A) Average ABR threshold curve (±s.e.) in air from 12 cormorant fledglings compared with the psychophysical threshold curve of a single adult cormorant in air (Maxwell et al., 2017). (B) Average ABR threshold curve (±s.e.) underwater from 8 of the 12 cormorant fledglings compared with the psychophysical threshold curve of a single cormorant underwater (Hansen et al., 2017).

physiological state and well-being, which may be mirrored in the different latency dependence on peak pressure (Fig. 3).

The fact that in-air ABR thresholds and psychophysical audiograms are so similar in shape (Fig. 8A) gives us confidence that the same would also be the case for the underwater hearing data. However, comparison of the average underwater ABR threshold curve with previously derived psychophysical data (Hansen et al., 2017) showed a large difference in the shape of the two threshold curves (Fig. 8B). The ABR threshold curve has its BF at 1 kHz and tapers off at lower frequencies at a rate similar to that found in the in-air audiogram. At higher frequencies, the underwater thresholds are also monotonically increasing, but at a lower slope than for the in-air audiogram (Fig. 4A). This is in great contrast to the underwater psychophysical data (Hansen et al., 2017), where the hearing thresholds varied by only 5 dB in the tested range of 1–4 kHz, whereas there was a 31 dB difference in the average ABR threshold in the same frequency interval (Fig. 8B). It should be remembered, however, that the psychophysical thresholds were derived from a single individual and with a limited dataset because of methodological difficulties (Hansen et al., 2017). It is therefore conceivable that more psychophysical data on several individuals
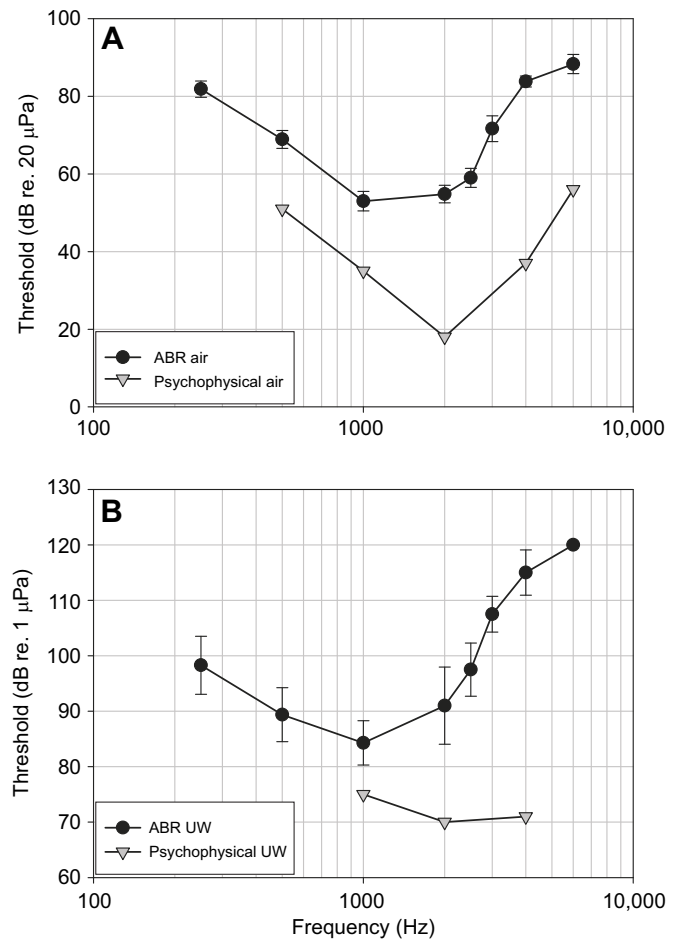
would result in underwater hearing thresholds more like the ones derived here by the ABR method.

Comparing threshold curves in air and underwater

Despite these discrepancies, it seems safe to compare the in-air and underwater ABR threshold curves in terms of both their shape and their sensitivity, as they were derived from a relatively large dataset using several individuals (8 of which produced both in-air and underwater data) and very similar methodologies. The cormorant average underwater ABR threshold curve seems slightly displaced towards lower frequencies relative to the average in-air ABR threshold curve (Fig. 4) but the statistical model predicts curves that are not significantly different for the two media (Fig. 5A). True seals, which are considered to be fully adapted for hearing in both media, have their underwater BF above the in-air BF, and the high-frequency slope is steeper in the underwater than in the in-air audiogram (Reichmuth et al., 2013; Sills et al., 2014, 2015). In fact, the weaker slopes of the average underwater threshold curve and its slightly lower frequency emphasis are more like those of another not aquatically adapted vertebrate, namely humans (Parvin and Nedwell, 1995). So, judging from the shape of the average

underwater threshold curve, cormorants may not have developed fully aquatic hearing abilities and are more adapted to hearing in air.

Another way to judge whether cormorant hearing is adapted or not for in-air and underwater hearing is to compare the magnitude of the hearing thresholds obtained in the two media. Previous comparisons between in-air and underwater thresholds have focused on true seals and sea lions (Kastak and Schusterman, 1998, 1999; Møhl, 1968; Lombard et al., 1981; Mulsow and Reichmuth, 2010; Reichmuth et al., 2013). Depending on whether the acoustic signals were assumed to be detected in units of sound pressure (in pascals) or intensity (in watts m^{-2}), the authors reached quite different conclusions as to whether the animals' hearing abilities were in-air or water adapted, or both. This counter-intuitive result can be elucidated with an example. Consider an animal with a hearing threshold at a frequency of 1 mPa in air (or 34 dB re. 20 µPa) and 1 mPa (or 60 dB re. 1 µPa) underwater, with decibels defined as:

$$dB = 20\log_{10}\left(\frac{p}{p_0}\right), \qquad (2)$$

where p_0 is the reference pressure of 20 µPa and 1 µPa in air and water, respectively. It is evident that the in-air and underwater decibels cannot be readily compared because of the different reference units (this would result in us erroneously reporting a higher threshold in water than in air, for identical sound pressures). By comparing decibels in pressure units, 26 dB must be added to the in-air decibels, as was done in Fig. 5A. This is a direct result of the definition of air decibels:

$$\begin{aligned} dB &= 20\log_{10}\left(\frac{p}{20 \times 10^{-6}}\right)\\ &= 20\log_{10}\left(\frac{p}{10^{-6}}\right) - 20\log_{10}(20)\\ &= 20\log_{10}\left(\frac{p}{10^{-6}}\right) - 26. \qquad (3) \end{aligned}$$

To complicate matters further, it is often assumed that animals detect acoustic energy or intensity, rather than acoustic pressure. In the free acoustic field, the acoustic intensity (I) is defined as:

$$I = \frac{p^2}{Z_0}$$

or

$$dB = 10\log_{10}\left(\frac{I}{I_0}\right), \qquad (4)$$

where Z_0 is the characteristic acoustic impedance of the medium (density multiplied by speed of sound). The reference acoustic intensity is often chosen as $I_0 = 1$ pW m^{-2}, mainly taken from audiology, where it is close to the human hearing threshold in air at 1 kHz.

Coming back to our example, the acoustic intensity of 1 mPa is 2200 pW m^{-2} (or +34 dB re. 1 pW m^{-2}) in air and 0.67 pW m^{-2} (or −2 dB re. 1 pW m^{-2}) in water. In other words, for the same acoustic pressure, the signal will carry 36 dB more intensity in air than underwater. This also means that the same acoustic intensity will result in acoustic pressures that are 36 dB higher in water than in air (see Larsen and Wahlberg, 2017, for a thorough discussion of this topic). Thus, our imagined animal would have identical hearing thresholds in air and underwater when measured in units of pressure, and we would probably conclude that the hearing system is adapted

to function equally well in both media. However, when measured in units of intensity (or energy), the hearing thresholds would be much higher in air than in water, and we would conclude that the ear would be more adapted for underwater than for aerial hearing.

A similar analysis can be done on the results from the present study. Assuming the cormorant detects acoustic pressure (Fig. 5A), the hearing thresholds can be directly compared and there is no difference between thresholds in the two media. Assuming the cormorant detects acoustic intensity or energy (Fig. 5B), however, the cormorant hearing thresholds are 20–40 dB lower underwater than in air. Thus, without a better understanding of whether the adequate stimulus is pressure or intensity, it is difficult to use hearing thresholds to conclude whether the cormorant hearing system is adapted for underwater hearing.

There is only one experiment known to us that has attempted to determine what the adequate stimulus unit actually is: a study by Finneran et al. (2002) on dolphins (*Tursiops truncatus*). Finneran et al. (2002) determined that pressure (or rather, pressure-squared) is the relevant unit for dolphins in water at low frequencies. Further experimentation in other species and at other frequencies (Finneran et al., 2002, only investigated the relatively low 100–300 Hz frequency range) would be crucial for understanding not only what the relevant stimulus unit is but also whether or not the hearing thresholds of an animal are lower or higher in water than in air.

Until the dilemma of unit confusions is resolved, we cannot resolve whether the cormorant's hearing thresholds were lower in water than in air. We can conclude, however, that the underwater hearing thresholds are at least as low as, and possibly lower than, the in-air ones. In addition, the results of Johansen et al. (2016) and of Hansen et al. (2017), although somewhat preliminary and based on just one animal, suggest that at the tested frequencies, the cormorant's hearing thresholds in water were similar to what is found in animals that are considered to have fully water-adapted hearing, such as seals and porpoises at low frequencies. All this makes it plausible that cormorants do have some adaptations for hearing underwater as have many other secondarily adapted aquatic vertebrates (summarized by Christensen-Dalsgaard and Manley, 2013).

The non-significant frequency–medium interaction suggests that the thresholds did not differ between air and water. However, this does not necessarily mean that the frequency response in the two media was equal. It should be remembered that for practical reasons the measurements were made only 10 cm below the water surface. It is conceivable that any in-water adaptation of the hearing system is not fully exploited that close to the water surface, and that the magnitude of such effects could vary depending on depth. One may also conceive more complicated interactions with the hearing system and a more complicated acoustic field close to a water surface that can only be resolved by further measurements.

Potential anatomical adaptations for underwater hearing

The sensitivity to underwater sound may be caused by special anatomical features of the middle ear. The eardrum vibration measurements (Fig. 7B) suggest that resonance of the air in the middle ear cavity is important in driving tympanic vibrations. One obvious difference between the cormorant middle ear and the middle ear of, for instance, songbirds is the thick plate-shaped extra-columella that covers about 14% of the double-layered tympanic area (Fig. 6F). This is reminiscent of the tympanic disc structure found in two aquatic-adapted middle ears, the ears of the clawed frog *Xenopus laevis* (Christensen-Dalsgaard and Elepfandt, 1995; Vedurmudi et al., 2018) and the turtle *Trachemys elegans* (Christensen-Dalsgaard et al., 2012). The cormorants' eardrum

vibration sensitivity to underwater sound measured by the transfer function (peak sensitivity 0.3 mm s^{-1} Pa^{-1}) is similar to the peak sensitivity of turtles (0.3 mm s^{-1} Pa^{-1}) and clawed frogs (0.05–0.25 mm s^{-1} Pa^{-1}). Possibly, some of these adaptations entail modifications of the middle ear, such as stiffening of the tympanic membrane, which in turn may explain the reduced sensitivity to air-borne sound shown by both audiograms and eardrum vibrations. An analytical model of the *Xenopus* ear suggests that a plate-like tympanum is more efficient than a protruding thin membrane in coupling sound energy in water to the inner ear (Vedurmudi et al., 2018). Alternatively, a more solid eardrum could have evolved as a protection of the ear against the increased pressures incurred when the bird is diving.

Other characteristics of the outer and middle ear anatomy described here are much like those for cormorants reported by Saiff (1978) and for auks reported by Kartaschew and Iljitschow (1964). The dense feather cover, the easily compressed ear opening and the bent meatus may all protect the ear during the relatively shallow cormorant dives rather than increase its sensitivity to underwater sound. The fine structure of the eardrum–columella complex may be an adaptation to underwater hearing but awaits comparative studies using modern high-resolution techniques such as micro-CT scanning (see e.g. Muyshondt et al., 2018).

Data availability
The data are stored at the University of Southern Denmark and are available on request to the authors.

References
Alexander, R. McN. (1966). Physical aspects of swimbladder function. *Biol. Rev.* **41**, 141-176. doi.org/10.1111/j.1469-185X.1966.tb01542.x

Ashmole, N. P. (1971). Sea bird ecology and the marine environment. In *Avian Biology*, Vol. 1 (ed. D. S. Farner and J. R. King), p. 223-286. New York, London: Academic Press.

Beatini, J. R., Proudfoot, G. A. and Gall, M. D. (2018). Frequency sensitivity in Northern saw-whet owls (*Aegolius acadicus*). *J. Comp. Physiol. A* **204**, 145-154. doi:10.1007/s00359-017-1216-2

Brittan-Powell, E. F. and Dooling, R. J. (2004). Development of auditory sensitivity in budgerigars (*Melopsittacus undulatus*). *J. Acoust. Soc. Am.* **115**, 3092-3102. doi:10.1121/1.1739479

Brittan-Powell, E. F., Dooling, R. J. and Gleich, O. (2002). Auditory brainstem responses in adult budgerigars (*Melopsittacus undulatus*). *J. Acoust. Soc. Am.* **112**, 999-1008. doi:10.1121/1.1494807

Brittan-Powell, E. F., Lohr, B., Hahn, D. C. and Dooling, R. J. (2005). Auditory brainstem responses in the Eastern screech owl: an estimate of auditory thresholds. *J. Acoust. Soc. Am.* **118**, 314-321. doi:10.1121/1.1928767

Brittan-Powell, E. F., Christensen-Dalsgaard, J., Tang, Y., Carr, C. and Dooling, R. J. (2010). The auditory brainstem response in two lizard species. *J. Acoust. Soc. Am.* **128**, 787-794. doi:10.1121/1.3458813

Christensen-Dalsgaard, J. and Elepfandt, A. (1995). Biophysics of underwater hearing in the clawed frog, *Xenopus laevis*. *J. Comp. Physiol. A* **176**, 317-324. doi:10.1007/BF00219057

Christensen-Dalsgaard, J. and Manley, G. A. (2005). Directionality of the lizard ear. *J. Exp. Biol.* **208**, 1209-1217. doi:10.1242/jeb.01511

Christensen-Dalsgaard, J. and Manley, G. A. (2013). The malleable middle ear: an underappreciated player in the evolution of hearing in vertebrates. In *Insights from Comparative Hearing Research* (ed. C. Köppl, G. A. Manley, R. R. Fay and A. N. Popper), p. 157-191: Springer Handbook of Auditory Research, New York: Springer.

Christensen-Dalsgaard, J., Brandt, C., Wilson, M., Wahlberg, M. and Madsen, P. T. (2011). Hearing in the African lungfish (*Protopterus annectens*): pre-adaptation to pressure hearing in tetrapods? *Biol. Lett.* **7**, 139-141. doi:10.1098/rsbl.2010.0636

Christensen-Dalsgaard, J., Brandt, C., Willis, K. L., Christensen, C. B., Ketten, D., Edds-Walton, P., Fay, R. R., Madsen, P. T. and Carr, C. E. (2012). Specialization for underwater hearing by the tympanic middle ear of the turtle, *Trachemys scripta elegans*. *Proc. Roy. Soc. B* **279**, 2816-2824. doi:10.1098/rspb.2012.0290

Crowell, S. C. (2016). Measuring in-air and underwater hearing in seabirds. In *The Effects of Noise on Aquatic Life II* (ed. N. A. Popper and A. Hawkins), pp. 1155-1160. New York, NY: Springer-Verlag.

Crowell, S. E., Wells-Berlin, A. M., Carr, C. E., Olsen, G. H., Therrien, R. E., Yannuzzi, S. E. and Ketten, D. R. (2015). A comparison of auditory brainstem responses across diving bird species. *J. Comp. Physiol. A* **201**, 803-815. doi:10.1007/s00359-015-1024-5

Dehnhardt, G., Mauck, B., Hanke, W. and Bleckmann, H. (2001). Hydrodynamic trail-following in harbour seals (*Phoca vitulina*). *Science* **293**, 102-103. doi:10.1126/science.1060514

Dooling, R. J. and Searcy, M. H. (1985). Temporal integration of acoustic signals by the budgerigar (Melopsittacus undulatus). *J. Acoust. Soc. Am.* **77**, 1917-1920. doi:10.1121/1.391835

Dunning, J. B. (2007). *CRC Handbook of Avian Body Masses*. 2nd edn. Boca Raton: CRC Press.

Finneran, J. J., Carder, D. A. and Ridgway, S. H. (2002). Low-frequency acoustic pressure, velocity, and intensity thresholds in a bottlenose dolphin (*Tursiops truncatus*) and white whale (*Delphinapterus leucas*). *J. Acoust. Soc. Am.* **111**, 447-456. doi:10.1121/1.1423925

Glasser, A. and Howland, H. C. (1996). A history of studies of visual accommodation in birds. *Q. Rev. Biol.* **71**, 475-509. doi:10.1086/419554

Grémillet, D., Argentin, G., Schulte, B. and Culik, B. M. (1998). Flexible foraging techniques in breeding cormorants *Phalacrocorax carbo* and shags *Phalacrocorax aristotelis*: benthic or pelagic feeding? *Ibis* **140**, 113-119. doi:10.1111/j.1474-919X.1998.tb04547.x

Grémillet, D., Enstipp, M. R., Boudiffa, M. and Liu, H. (2006). Do cormorants injure fish without eating them? An underwater video study. *Mar. Biol.* **148**, 1081-1087. doi:10.1007/s00227-005-0130-2

Hansen, K. A., Maxwell, A., Siebert, U., Larsen, O. N. and Wahlberg, M. (2017). Great cormorants (*Phalacrocorax carbo*) can detect auditory cues while diving. *Sci. Nat.* **104**, 45. doi:10.1007/s00114-017-1467-3

Houser, D. S. and Finneran, J. J. (2006). A comparison of underwater hearing sensitivity in bottlenose dolphins (*Tursiops truncatus*) determined by electrophysiological and behavioral methods. *J. Acoust. Soc. Am.* **120**, 1713-1722. doi:10.1121/1.2229286

Jensen, K. K. and Klokker, S. (2006). Hearing sensitivity and critical ratios of hooded crows (*Corvus corone cornix*). *J. Acoust. Soc. Am.* **119**, 1269-1276. doi:10.1121/1.2159431

Johansen, S., Larsen, O. N., Christensen-Dalsgaard, J., Seidelin, L., Boström, M., Lunneryd, S.-G. and Wahlberg, M. (2016). In-air and underwater hearing in the great cormorant (*Phalacrocorax carbo*). In *The Effects of Noise on Aquatic Life II* (ed. A. N. Popper and A. Hawkins), pp. 505-512. New York, NY: Springer New York.

Kartaschew, N. N. and Iljitschow, W. D. (1964). Über das gehörorgan der alkenvögel. *J. Ornithol.* **105**, 113-136. doi:10.1007/BF01670985

Kastak, D. and Schusterman, R. J. (1998). Low-frequency amphibious hearing in pinnipeds: methods, measurements, noise, and ecology. *J. Acoust. Soc. Am.* **103**, 2216-2228. doi:10.1121/1.421367

Kastak, D. and Schusterman, R. J. (1999). In-air and underwater hearing sensitivity of a northern elephant seal (*Mirounga angustirostris*). *Can. J. Zool.* **77**, 1751-1758. doi:10.1139/z99-151

Katzir, G. and Howland, H. C. (2003). Corneal power and underwater accommodation in great cormorants (*Phalacrocorax carbo sinensis*). *J. Exp. Biol.* **206**, 833-841. doi:10.1242/jeb.00142

Kettler, L., Christensen-Dalsgaard, J., Larsen, O. N. and Wagner, H. (2016). Low frequency eardrum directionality in the barn owl induced by sound transmission through the interaural canal. *Biol. Cybern.* **110**, 333-343. doi:10.1007/s00422-016-0689-3

Klump, G. M. and Larsen, O. N. (1992). Azimuthal sound localization in the European starling (*Sturnus vulgaris*): I: physical binaural cues. *J. Comp. Physiol. A* **170**, 243-251. doi:10.1007/BF00196906

Klump, G. M. and Maier, E. H. (1990). Temporal summation in the European starling (*Sturnus vulgaris*). *J. Comp. Psychol.* **104**, 94-100. doi:10.1037/0735-7036.104.1.94

Larsen, O. N. and Wahlberg, M. (2017). Sound and sound sources. In *Comparative Bioacoustics: An Overview* (ed. C. H. Brown and T. Riede), pp. 3-62. Sharjah, UME: Bentham Science Publishers.

Larsen, O. N., Dooling, R. J. and Michelsen, A. (2006). The role of pressure difference reception in the directional hearing of budgerigars (*Melopsittacus undulatus*). *J. Comp. Physiol. A* **192**, 1063-1072. doi:10.1007/s00359-006-0138-1

Lombard, R. E., Fay, R. R. and Werner, Y. L. (1981). Underwater hearing in the frog, *Rana catesbeiana*. *J. Exp. Biol.* **91**, 57-71.

Martin, G. R. (1999). Eye structure and foraging in king penguins *Aptenodytes patagonicus*. *Ibis* **141**, 444-450. doi:10.1111/j.1474-919X.1999.tb04413.x

Martin, G. R. and Brooke, M. D. L. (1991). The eye of a Procellariiform seabird, the Manx shearwater, *Puffinus puffinus*: visual fields and optical structure. *Brain Behav. Evol.* **37**, 65-78. doi:10.1159/000114347

Martin, G. R. and Osorio, D. (2008). Vision in birds. In *The Senses: A Comprehensive Reference*, Vol. 1, Vision I (ed. R. Masland and T.D. Albright), pp. 25-52. San Diego: Academic Press.

Maxwell, A., Hansen, K. A., Ortiz, S. T., Larsen, O. N., Siebert, U. and Wahlberg, M. (2017). In-air hearing of the great cormorant (*Phalacrocorax carbo*). *Biol. Open* **6**, 496-502. doi:10.1242/bio.023879

Mooney, T. A., Smith, A., Larsen, O. N., Hansen, K. A., Wahlberg, M. and Rasmussen, M. H. (2019a). Field-based hearing measurements of two seabird species. *J. Exp. Biol.* **222**, jeb190710. doi:10.1242/jeb.190710

Mooney, T. A., Smith, A., Hansen, K. A., Larsen, O. N., Wahlberg, M. and Rasmussen, M. (2019b). Birds of a feather: hearing and potential noise impacts in puffins (*Fratercula arctica*). *Proc. Mtgs. Acoust.* **37**, 010004. doi: 10.1121/2.0001037

Mulsow, J. and Reichmuth, C. (2010). Psychophysical and electrophysiological aerial audiograms of a Steller sea lion (*Eumetopias jubatus*). *J. Acoust. Soc. Am.* **127**, 2692-2701. doi:10.1121/1.3327662

Muyshondt, P. G. G., Claes, R., Aerts, P. and Dirckx, J. J. J. (2018). Quasi-static and dynamic motions of the columellar footplate in ostrich (*Struthio camelus*) measured ex vivo. *Hear. Res.* **357**, 10-24. doi:10.1016/j.heares.2017.11.005

Møhl, B. (1967). Seal ears. *Science* **157**, 99. doi:10.1126/science.157.3784.99

Møhl, B. (1968). Hearing in seals. In *The Behavior and Physiology of Pinnipeds* (ed. R. J. Harrison), pp. 172-195. New York: Appleton-Century-Crofts.

Nelson, J. B. (2006). *Pelicans, Cormorants, and Their Relatives: The Pelecaniformes*. Oxford, UK: Oxford University Press.

Parvin, S. J. and Nedwell, J. R. (1995). Underwater sound perception and the development of an underwater noise weighting scale. *Underwater Technol. Summer* **21**, 12-19. doi:10.3723/175605495783328836

Payne, R. S. (1971). Acoustic localization of prey by barn owls (*Tyto alba*). *J. Exp. Biol.* **54**, 535-573.

Picton, T. W., John, M. S., Dimitrijevic, A. and Purcell, D. (2003). Human auditory steady-state responses: Respuestas auditivas de estado estable en humanos. *Int. J. Audiol.* **42**, 177-219. doi:10.3109/14992020309101316

Plomp, R. and Bouman, M. A. (1959). Relation between hearing threshold and duration for tone pulses. *J. Acoust. Soc. Am.* **31**, 749-758. doi:10.1121/1.1907781

Pohl, N. U., Slabbekoorn, H., Klump, G. M. and Langemann, U. (2009). Effects of signal features and environmental noise on signal detection in the great tit, *Parus major*. *Anim. Behav.* **78**, 1293-1300. doi:10.1016/j.anbehav.2009.09.005

Pohl, N. U., Slabbekoorn, H., Neubauer, H., Heil, P., Klump, G. M. and Langemann, U. (2013). Why longer song elements are easier to detect: threshold level-duration functions in the great tit and comparison with human data. *J. Comp. Physiol. A* **199**, 239-252. doi:10.1007/s00359-012-0789-z

Reichmuth, C., Holt, M. M., Mulsow, J., Sills, J. M. and Southall, B. L. (2013). Comparative assessment of amphibious hearing in pinnipeds. *J. Comp. Physiol. A* **199**, 491-507. doi:10.1007/s00359-013-0813-y

Saiff, E. I. (1978). The middle ear of the skull of birds: the Pelecaniformes and Ciconiiformes. *Zool. J. Linn. Soc.* **63**, 315-370. doi:10.1111/j.1096-3642.1978.tb02099.x

Sills, J. M., Southall, B. L. and Reichmuth, C. (2014). Amphibious hearing in spotted seals (*Phoca largha*): underwater audiograms, aerial audiograms and critical ratio measurements. *J. Exp. Biol.* **217**, 726-734. doi:10.1242/jeb.097469

Sills, J. M., Southall, B. L. and Reichmuth, C. (2015). Amphibious hearing in ringed seals (*Pusa hispida*): underwater audiograms, aerial audiograms and critical ratio measurements. *J. Exp. Biol.* **218**, 2250-2259. doi:10.1242/jeb.120972

Schwartzkopff, J. (1949). Über sitz und leistung von gehör und vibrationssinn bei vögeln. *Z. vergl. Physiol.* **31**, 527-608. doi:10.1007/BF00348361

Sokal, R. R. and Rohlf, F. J. (1981). *Biometry: The Principles and Practice of Statistics in Biological Research*, 3rd edn, pp. 887. New York: W.H. Freeman.

Therrien, S. (2014). In air and underwater hearing of diving birds. *PhD thesis*, University of Maryland.

Thiebault, A., Charrier, I., Aubin, T., Green, D. B. and Pistorius, P. A. (2019). First evidence of underwater vocalisations in hunting penguins. *PeerJ* **7**, e8240. doi:10.7717/peerj.8240

Tremblay, Y., Thiebault, A., Mullers, R. and Pistorius, P. (2014). Bird-borne video-cameras show that seabird movement patterns relate to previously unrevealed proximate environment, not prey. *PLoS ONE* **9**, e88424. doi:10.1371/journal.pone.0088424

Van Eerden, M. R. and Voslamber, B. (1995). Mass fishing by cormorants *Phalacrocorax carbo sinensis* at lake Ijsselmeer, the Netherlands: a recent and successful adaptation to a turbid environment. *Ardea* **83**, 199-212.

Vedurmudi, A. P., Christensen-Dalsgaard, J. and van Hemmen, J. L. (2018). Modeling underwater hearing and sound localization in the frog *Xenopus laevis*. *J. Acoust. Soc. Am.* **144**, 3010-3021. doi:10.1121/1.5079647

Voslamber, B., Platteeuw, M. and Van Eerden, M. R. (1995). Solitary foraging in sand pits by breeding cormorants *Phalacrocorax carbo sinensis*: does specialised knowledge about fishing sites and fish behaviour pay off? *Ardea* **83**, 213-222.

White, C. R., Day, N., Butler, P. J. and Martin, G. R. (2007). Vision and foraging in cormorants. More like herons than hawks? *PLoS ONE* **2**, e609. doi:10.1371/journal.pone.0000639

White, C. R., Butler, P. J., Grémillet, D. and Martin, G. R. (2008). Behavioural strategies of cormorants (Phalacrocoracidae) foraging under challenging light conditions. *Ibis* **150**, 231-239. doi:10.1111/j.1474-919X.2008.00837.x

Wolski, L. F., Anderson, R. C., Bowles, A. E. and Yochem, P. K. (2003). Measuring hearing in the harbor seal (*Phoca vitulina*): comparison of behavioral and auditory brainstem response techniques. *J. Acoust. Soc. Am.* **113**, 629-637. doi:10.1121/1.1527961