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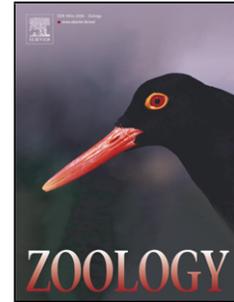
Reference:

Claes Raf, Muyshondt Pieter, Dirckx Joris, Aerts Peter.- Do high sound pressure levels of crowing in roosters necessitate passive mechanisms for protection against self-vocalization?
Zoology : analysis of complex systems - ISSN 0944-2006 - 126(2018), p. 65-70
Full text (Publisher's DOI): <https://doi.org/10.1016/J.ZOOL.2017.12.002>
To cite this reference: <https://hdl.handle.net/10067/1525160151162165141>

Accepted Manuscript

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PII: S0944-2006(17)30197-6
DOI: <https://doi.org/10.1016/j.zool.2017.12.002>
Reference: ZOOL 25619

To appear in:

Received date: 6-9-2017
Revised date: 22-11-2017
Accepted date: 5-12-2017

Please cite this article as: Claes, Raf, Muyshondt, Pieter G.G., Dirckx, Joris J.J., Aerts, Peter, Do high sound pressure levels of crowing in roosters necessitate passive mechanisms for protection against self-vocalization?. *Zoology* <https://doi.org/10.1016/j.zool.2017.12.002>

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Do high sound pressure levels of crowing in roosters necessitate passive mechanisms for protection against self-vocalization?

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Keywords: Domestic chicken; sound pressure level; crow, outer ear canal

Highlights

1. Sound pressure levels of a crowing rooster can reach a damaging 140 dB
2. A passive protective mechanism in roosters against self-vocalization is proposed
3. Hens have no need for the proposed protective mechanism

Abstract

High sound pressure levels (> 120 dB) cause damage or death of the hair cells of the inner ear, hence causing hearing loss. Vocalization differences are present between hens and roosters. Crowing in roosters is reported to produce sound pressure levels of 100 dB measured at a distance of 1m. In this study we measured the sound pressure levels that exist at the entrance of the outer ear canal. We hypothesize that roosters may benefit from a passive protective mechanism while hens do not require such a mechanism. Audio recordings at the level of the entrance of the outer ear canal of crowing roosters, made in this study, indeed show that a protective mechanism is needed as sound pressure levels can reach amplitudes of 142.3 dB. Audio recordings made at varying distances from the crowing rooster show that at a distance of 0.5m sound pressure levels already drop to 102 dB. Micro-CT scans of a rooster and chicken head show that in roosters the auditory canal closes when the beak is opened. In hens the diameter of the auditory canal only narrows but does not close completely. A morphological difference between the sexes in shape of a bursa-like slit which occurs in the outer ear canal causes the outer ear canal to close in roosters but not in hens.

1. Introduction

Noise induced hearing impairment can be caused by exposure to loud sounds. A 3 dB increase in sound pressure level (SPL) represents a doubling of the sound intensity and above 120 dB, sound may even result in immediate damage in both mammal and bird (Maassen et al., 2001; Smolders, 1999). Guidelines for humans set by the National Institute for Occupational Safety and Health (NIOSH) recommend noise exposure limits of 85 dB for a period of 8 hours as being safe. Exposures at and above this level are considered hazardous. Increased SPLs are safe for a shorter span of time (e.g. 100 dB is safe for less than 15 min; SPL above 130 dB can cause hearing damage at exposure times of less than 1 s) (NIOSH, 1998). This hearing loss is mostly the result of damage to, or death of, the hair cells of the inner ear (Wang et al., 2002).

In chickens (*Gallus gallus domesticus*) a drastic difference in vocalization is present between the two sexes. Alarm calls in hens reach 76 dB (Brumm et al., 2009), while crowing roosters are reported to produce sound levels of 100 dB, measured at a 1m distance, which recalculates (inverse square distance law) to approximately 126 dB close to the source (Brackenburry, 1977; Claes et al., 2016). Borg and Counter (1989) reported, quasi anecdotally, (i.e. no reference or details on how this measure is obtained) that the SPL can reach 130 dB measured directly next to the head of the rooster. These high SPL values suggest the necessity for active or passive mechanisms to protect the animals from self-generated vocalizations. It has been shown that the stapedius muscle in chicken middle ear activates in response to self-vocalizations, possibly influencing sound transmission through the middle ear (Borg and Counter, 1989). Attenuations, as a result of reflexive stapedius activation, of up to 20 dB for maximal contraction at 800 Hz are reported. Oeckinghaus and Schwartzkopff (1982) observed a frequency specific attenuation in tawny owls (*Strix aluco*). Higher levels of attenuation (10 dB) are present at lower frequencies than at higher frequencies. In starlings the songs and calls fall into the most sensitive hearing range (Konishi, 1970; Dooling et al., 1971). The stapedius muscle exerts little influence at these frequencies. This implies that the stapedius muscle probably only provides limited protection against self-vocalization. However, also passive mechanisms play a role in hearing protection. Claes et al. (2016) showed that wide upper beak elevation in roosters (as present during crowing) causes, through its coupling with the moving quadrate bone, relaxation of the tympanic membrane. Interestingly, however, this was not observed in hens suggesting that the effect of craniokinesis on the tympanic membrane in roosters represents a passive protective mechanism against loud self-vocalization. *Ex vivo* experiments by Muyschondt et al. (2017) show that beak opening in roosters results in reducing the power transferred from the tympanic membrane to the inner ear by approximately half (3.5 dB), while in hens, beak opening barely affects the transfer. As mentioned earlier, attenuation by stapedius activity reaches a maximum of 20 dB at 800 Hz. However, the findings of Oeckinghaus and Schwartzkopff (1982), that attenuation is frequency dependent, may imply that less attenuation is present at higher frequencies in chickens. This implies that, despite the passive attenuation found by Claes et al. (2016) and measured by Muyschondt et al. (2017) and if the above reported SPL at the level of the rooster's head are confirmed, the sound pressure reaching the inner ear still remains close to the threshold level at which immediate damage can, potentially, be caused. Even if active protection by reflexive stapedius activation occurs damage can still take place. In this case the question can be posed whether additional passive protection related to wide beak opening during crowing exists.

Former measurements of crowing roosters were taken in the far field (materials and methods). No doubt, however, that at close proximity, a rooster should be considered a highly directional sound source. Thus, measurements at a distance do not actually give any information about what is perceived by the animal itself. In this paper, we present the results of measurements of the sound level directly

perceived at the entrance of the external auditory canal during self-vocalization in roosters. Additionally, SPL in the proximity of the crowing rooster were also recorded at different distances and from multiple directions to compile the geometry of the sound field.

We hypothesize that SPLs at the rooster's ear are so high that the animal may indeed benefit from (passive) protective mechanisms, but that the decrease of the SPL in the environment is such that hens, even when relatively close to the rooster, are not in immediate danger of hearing impairment. This would explain the formerly reported functional anatomical difference in the coupling between craniokinesis and middle ear function in both sexes (see above; Claes et al., 2016). Moreover, we hypothesize that passive protective mechanisms related to wide beak opening additional to the tympanic membrane relaxation are likely present in roosters, but not in hens. If present, further passive attenuation must occur at the level of the external auditory canal (i.e. part of the path of the sound waves from the periphery to the tympanic membrane), as our former functional morphological study (Claes et al., 2016) assessed the passive SPL-reduction in the remaining part (i.e. by the entire middle ear, from the tympanic to the inner ear). Therefore, the effect of beak opening on the geometry of the external auditory canal is studied by means of micro-CT.

2. Materials and methods

2.1 Sound recording

Audio recordings of the crows of three adult roosters (different breeds of *Gallus gallus domesticus*: Welsummer, R1; Barnevelder, R2 and mix breed, R3) were made at the level of the entrance of the outer ear canal. For this purpose, a small audio recorder (Wristband Voice Recorder, J&R Electronics, Hong Kong, China) was strapped around the neck of the animals such that the microphone was positioned immediately adjacent to the ear opening (fig. 1a). The sensitivity of the device was modified to be able to measure sound pressure levels well over 140 dB SPL. Animals were instrumented the night before recording to get them accustomed to the device before the recording sessions the next day at dawn. Recordings were made in a large outside enclosure to ensure free-field conditions. R1 recordings were used in Muyschondt et al. (2017). The first 11 crows of each rooster were used to calculate the mean SPL for each time step. To assess variability between the individual crows of each individual, the mean and standard deviation of the maximal dB of each crow was calculated.

Audio recordings were also made at different distances from one rooster (R1). The audio recorder was strapped on a wooden pole in the chicken's enclosure, approximately 35 cm above the ground (approximately the height of a hen's outer ear when standing upright). The microphone opening was placed above the pole to ensure that traveling sound waves were not obstructed (fig. 1b). The distance from the rooster, wandering around in the enclosure, to the audio recorder during crowing was assessed by using markings on the ground and the directionality of the rooster towards the recorder was noted for each crow (front, front-right, right, back-right, back ...). When no data points were available on the left or right side of the rooster head the same SPL was taken from the corresponding direction on the contralateral side, as it can be expected that SPL on both sides of the rooster are the same. The recorder was not attenuated for sound pressure sensitivity for these recordings.

The audio recorder sampled at 16 kHz with a depth of 16 bit. It was calibrated twice (i.e. when attenuated for sound pressure sensitivity and when not; cf. above). The calibration was achieved by measuring the SPL of stepwise pure-tone sound signals (in free field) at a distance of 20 cm from a loudspeaker by means of a precalibrated microphone (Probe Microphone Type 4182, Brüel & Kjær, Nærum, Denmark). The frequency dependent calibration curve of the audio recorder was obtained by comparing the magnitudes of both signals. The resulting calibration curves are shown in fig. 2a. To determine the total SPL of the rooster's crow as a function of time, a spectrogram of the uncalibrated

vocalization signal was first calculated by means of a short-time Fourier transform. To correct the spectrogram for the frequency-dependent sensitivity of the microphone, the spectral decomposition of each time instant in the spectrogram was multiplied with the microphone's calibration curve. A spectrogram for a single crow of rooster R1 is shown in fig. 2b. Then, peaks of the vocalization signal were identified in the spectrogram, and the harmonic contributions of all peaks (i.e. sines with particular frequencies, amplitudes and phases) were summed. Subsequently, the root mean square of this reconstructed signal was calculated to obtain the total SPL.

2.2 Geometry of the external auditory canal

During manipulation experiments where the beak of chickens was maximally opened and closed it could be observed that the external auditory canal is compressed. However, visual inspection alone is inadequate to assess whether the auditory canal closes completely. To study this the μ CT scans of a hen and rooster used to study the effect of beak opening on the middle ear (Claes et al., 2016) were re-examined to reconstruct the geometry of the external auditory canal when the beak is closed and when opened widely. One fresh rooster and one chicken head (species: Dekalb white) were obtained from a chicken farm. The heads were stored in a refrigerator during one day and taken out the night before scanning. The heads were scanned with the beak closed and fully opened. μ CT-scans were made by the Centre for X-ray Tomography at Ghent University with the High Energy CT system Optimized for Research (HECTOR) (Masschaele et al., 2013). The samples were scanned over an angle of 360° in a closed container with the X-ray source set at 120 kV and 250 μ A. The exposure time was set at 667 ms and the total scanning time per sample was 30 min. A three-dimensional image processing software package (Amira 5.4.4; 64-bit version, VSG systems) was used to assign the voxels corresponding to the outer ear canals and the vestibular system. A surface model was created after smoothing the outlines. The outer ear canals were superimposed by aligning the vestibular systems so the geometry of the external auditory canals could be compared before and after beak opening.

3. Results

3.1 Sound recording

In figure 2 the SPL of the individual crows and mean are plotted as a function of time. R1 and R3 show only small fluctuations in SPL between the individual crows, being 1.4 dB and 1.3 dB respectively. R2 shows larger variability, 2.3 dB (fig. 3). However, individual performances are very comparable. The crows of rooster 1 and 2 took approximately 2 s. The SPL was over 130 dB for approximately 50% of this time period for R1 and approximately 25% for R2. For the remaining duration of the crow, the level in most crows stayed well above 110 dB (R1) and 120 dB (R2). SPL never dropped below 100 dB. For R3, crows were shorter in duration (approximately 1.3 s), but the SPL for approximately 10% of this time stays over 140 dB and never drops below 120 dB. When looking at the individual crows of each rooster maximal SPLs are 136.1 dB, 141.2 dB and 143.8 dB for the crows of individual R1, R2 and R3 respectively. The mean SPLs of the individual crows were calculated for each rooster. Maximal mean SPLs are 133.5 dB, 131.8 dB and 142.3 dB respectively.

The SPL declines when the audio recorder is located further away from the source. At shorter distance from the rooster (0.5 – 1.5 m) the SPL is higher when being in front, front-left and front-right of the rooster. This effect of directionality diminishes and eventually disappears when the distance increases (fig. 4).

3.2 Geometry of the auditory canal

In the rooster, μ CT-reconstruction shows that the external auditory canal closes completely when the beak opens maximally as during crowing (fig. 5A). Closing of the auditory canal occurs over a length of 1 mm, with the total length of the canal being approximately 4 mm. When the beak opens, soft tissue of the auditory canal comes into contact with almost 50% of the eardrum. For the hen the auditory canal compresses as a result of beak opening but does not close completely (fig. 5B). When the beak of the hen was closed a cross-sectional diameter of 4.5 mm was measured. When the beak was opened the diameter was reduced to 1.9 mm.

4. Discussion

The audio recordings of the crows of R2 and R3 confirm the previously measured SPL of R1 used in Muysshondt et al. (2017) and show that crowing in roosters is at levels that can cause immediate damage. The maximal recorded peak SPL (143.8 dB) even exceed the 126 dB as recalculated from the measurements by Claes et al. (2016) or the 130 dB suggested by Borg and Counter (1989) and are as loud as a jet taking off (Rabinowi, 1989). These vocalizations take only a few seconds, however, the rooster produces such vocalizations over and over again. In mammals it has been shown that short duration exposure to loud sounds may damage the inner ear (NIOSH, 1998).

As hair cells of the inner ear in birds are very similar to the mammalian hair cells (Köpl, 2011), it seems very probable that protection of the inner ear in roosters against the impressive SPL of self-vocalization is required. Relaxation of the eardrum due to maximal upper bill elevation was put forward as a passive protective mechanism against self-vocalization in roosters Claes et al. (2016). Sound attenuation measurements were conducted in Muysshondt et al. (2017) which showed that when roosters open their beak maximally 3.5 dB attenuation is present. Our anatomical reconstructions reveal that beak opening (craniokinesis) not only relaxes the eardrum, but also entirely closes the external auditory canal over a large distance in roosters, even such that approximately half the tympanic membrane is externally cushioned by soft tissue. Functionally, this compares in a way to humans inserting custom molded anatomical earplugs reported to achieve noise reductions up to several tens of dB's. Therefore, the attenuation of 3.5 dB as measured by Muysshondt et al. (2017) most likely only refers to the passive effects at the level of the middle ear described by Claes et al. (2016). Indeed, Muysshondt et al. (2017) report that, in their experiments where sound pressure were introduced at the entrance of the ear canal and modelling clay was used to acoustically seal the canal entrance, it could not be observed that the opening of the ear canal became effectively smaller.

In Claes et al. (2016) it was found that hens do not possess a passive protective mechanism. In the present study we also show that the auditory canals in hens do not close when the beak is opened. Only a small reduction in diameter is present. Our audio recordings in the far field support the hypotheses that hens do not need a mechanism to protect them from the loud crow of a rooster nearby, except when they are positioned directly in front of the beak of the rooster. The SPL already drops to 102 dB at a distance of 50 cm. It is also contra-intuitive that hens should anticipate by opening their beak when a rooster starts crowing.

The question remains as to why beak opening in roosters, and not in hens, causes complete closure of the auditory canal. This may well be due to the morphological difference in shape of the bursa-like slit which occurs at the fibrous plate which attaches to the depressor mandibulae (Pohlman, 1921). This bursa-like slit is more developed in roosters than in hens. The depressor mandibulae connects to the posterior articular process of the mandible and to the occipital bone and lies next to the auditory canal.

Contraction of this muscle will displace the bursa-like slit forward and outward and seal the external ear canal completely in roosters but will only reduce the diameter of the outer ear canal in hens. The need for protection against self-vocalization explains this sexual difference in anatomy as a result of selection.

Still other passive mechanisms may be present. Bray and Thurlow (1942) observed a 20 dB attenuation in pigeons due to changes in inner ear pressure. These changes can only be caused by displacements of the columella footplate, however, in Claes et al. (2016) no displacements were found when the beak of the rooster was opened. Muyshondt et al. (2017) suggested that roosters may open their pharyngotympanic tubes while crowing which allows sound to pass through, thus potentially balancing the pressure differential over the membrane. Whether such reflexive coupling exist is actually unknown. Borg and Counter (1989) showed that also active mechanisms can play a role in protection against loud self-vocalization. They found that stapedius muscle activity in roosters may cause attenuations up to 20 dB. Therefore, taking everything into account it seems very likely, sufficient protection against loud self-vocalization is present in crowing roosters. Moreover, unlike mammals, birds have the ability to regenerate hair cells of the inner ear after noise-induced hearing damage. When accidental overload of SPL occurs, birds have the ability to restore their hearing capacity in a relatively short amount of time (100 hours) (Smolders, 1999).

Acknowledgements

This research was funded by the Research Foundation of Flanders (FWO), grant numbers G049414N and 11T9316N.

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Figure legends

Figure 1. A) Position of the wristband with audio recorder around the neck of R1. B) Setup for audio recordings at distance. White arrow indicates position of the audio recording device.

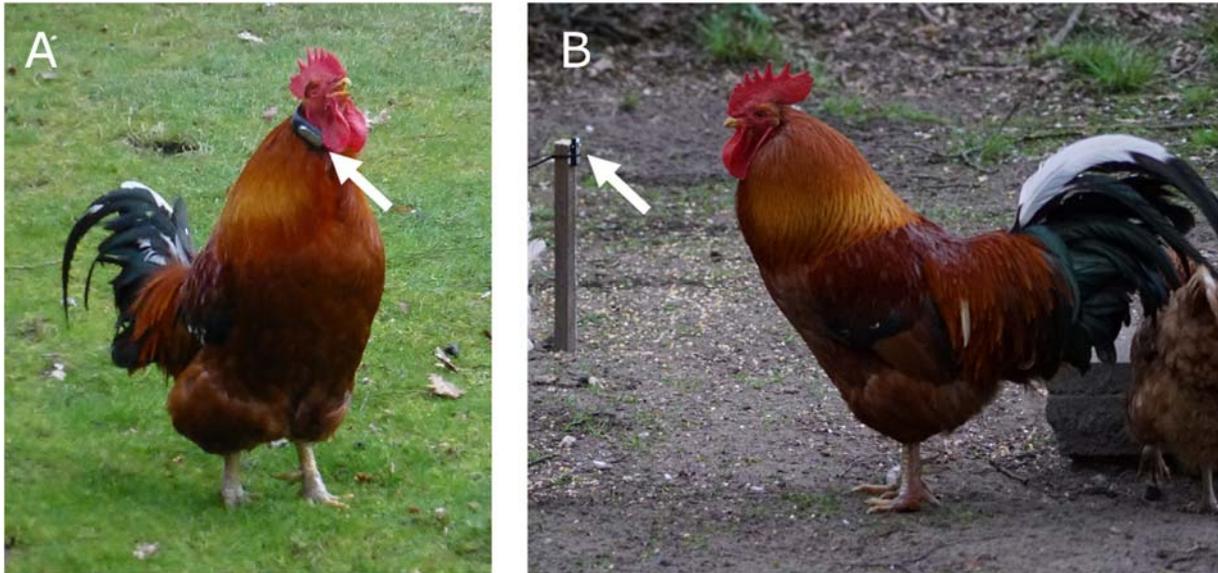


Figure 2. A) Calibration curve of the audio recorder when it was attenuated for sound pressure sensitivity (grey) and when it was not attenuated for sound pressure sensitivity (black). B) Calibrated spectrogram of a single crow of rooster R1, showing the spectral decomposition of the rooster's crow over time.

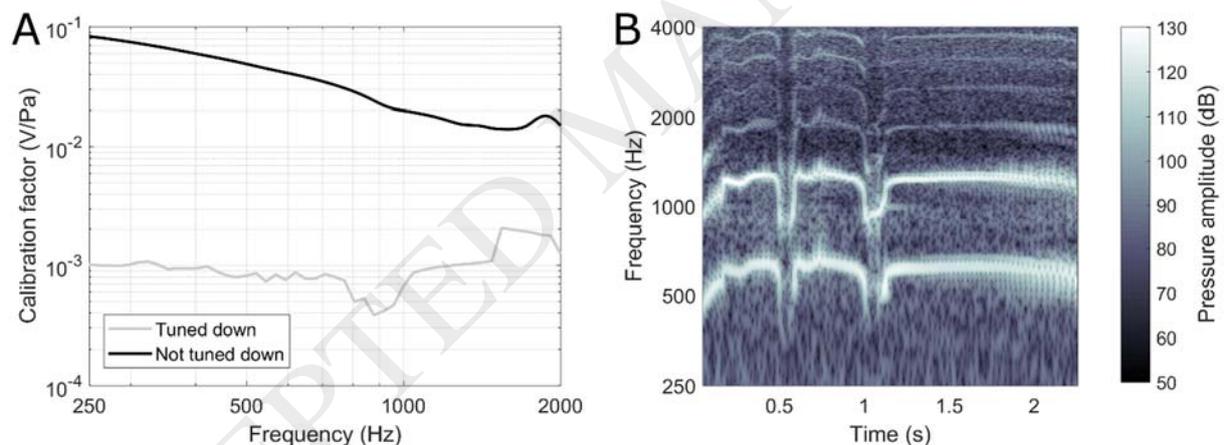


Figure 3. Graphic representation of 11 crows (grey) of rooster 1 (A), 2 (B) and 3 (C) with mean (black). Sound pressure level (dB) as a function of time (s).

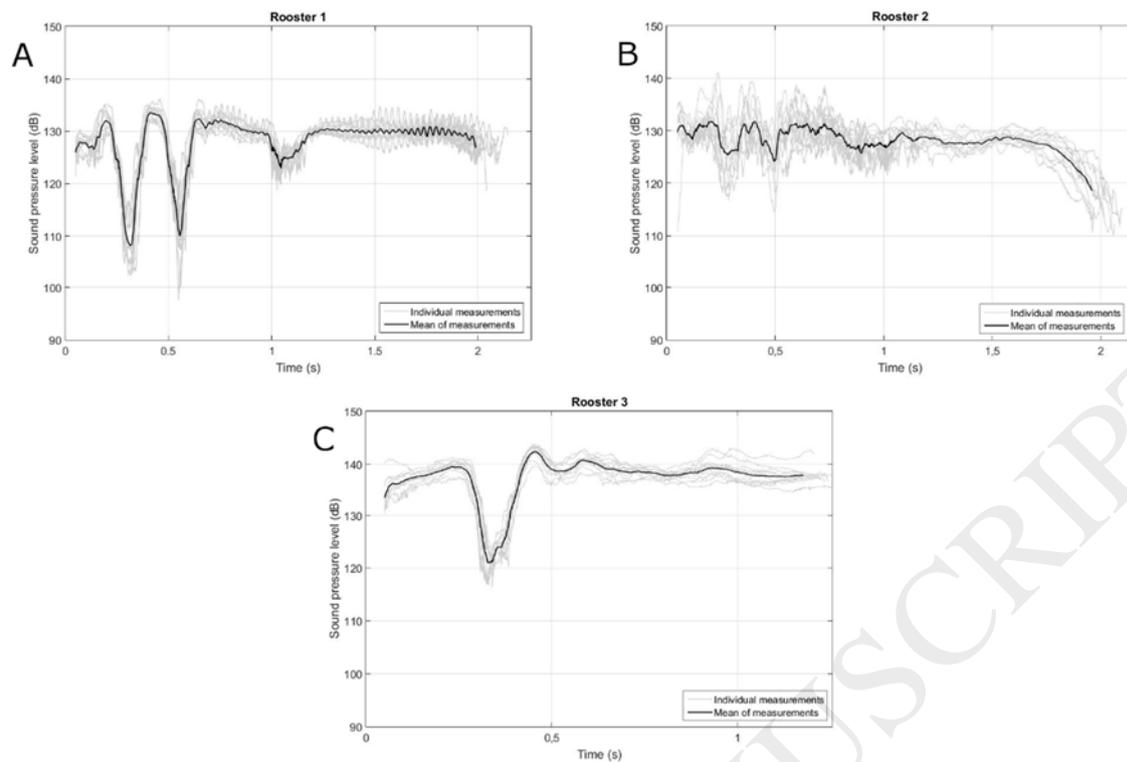


Figure 4. Schematic overview of the position of the audio recorder in relation to the head of the rooster (beak directed towards Front). Distance between the grey dotted circles is 1 m. Different colors indicate different SPL. Blue dots indicate at which position data was available. SPL (dB) is included (mean \pm standard deviation; when multiple crows were available; number between brackets indicate the number of recordings when more than one recording was present).

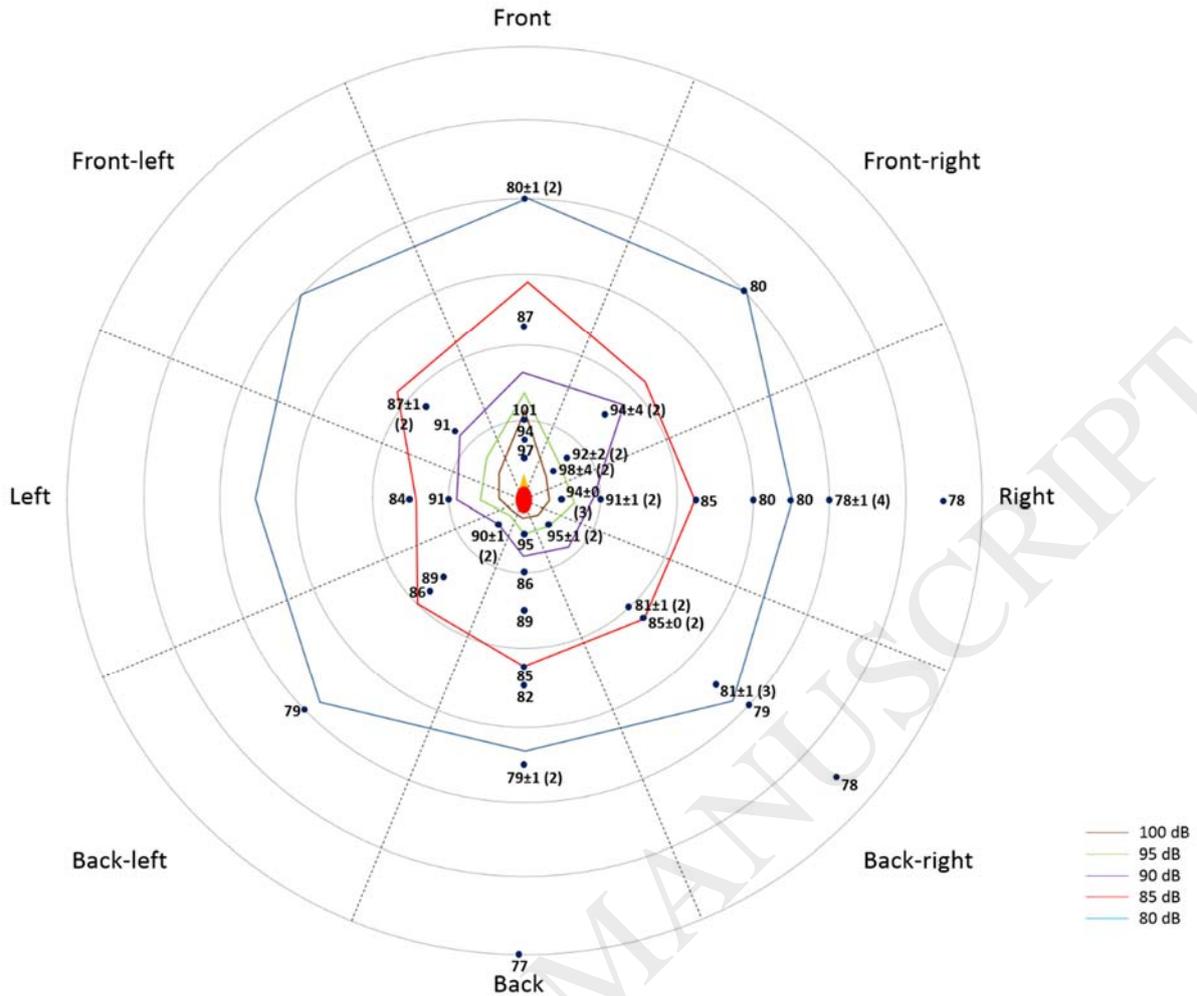


Figure 5. Reconstruction of the auditory canal. Green outlines indicate the outer wall of the auditory canal. μ CT-scan of the auditory canal of a rooster with closed (A) and opened (B) beak and a hen with closed (C) and opened (D) beak. 3 dimensional model of the closing of the auditory canal when the beak is closed and opened in a rooster (E) and in a hen (F). Transparent indication outlines the outer wall of the auditory canal with a closed beak (including the opaque indication). Opaque indication outlines the outer wall of the auditory canal with an opened beak. Scale bar: 2 mm.

