

## Old and young female voices: effects of body weight, condition and social discomfort on the vocal aging in red deer hinds (*Cervus elaphus*)

Ilya A. Volodin<sup>a,b,\*</sup>, Olga V. Sibiryakova<sup>a</sup>, Nina A. Vasilieva<sup>c</sup>,  
Elena V. Volodina<sup>b</sup>, Vera A. Matrosova<sup>d</sup>, Andrés J. Garcia<sup>e</sup>,  
Francisco J. Pérez-Barbería<sup>e</sup>, Laureano Gallego<sup>e</sup> and  
Tomás Landete-Castillejos<sup>e</sup>

<sup>a</sup> Department of Vertebrate Zoology, Faculty of Biology, Lomonosov Moscow State University, Vorobievsky Gory, 12/1, Moscow 119991, Russia

<sup>b</sup> Scientific Research Department, Moscow Zoo, B. Gruzinskaya, 1, Moscow 123242, Russia

<sup>c</sup> Severtsov Institute of Ecology and Evolution RAS, Leninskii pr. 33, Moscow 119071, Russia

<sup>d</sup> Engelhardt Institute of Molecular Biology RAS, Vavilov str., 32, Moscow 119991, Russia

<sup>e</sup> Instituto de Desarrollo Regional, Universidad de Castilla-La Mancha, 02071 Albacete, Spain

\*Corresponding author's e-mail address: volodinsvoc@gmail.com

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### Abstract

In female terrestrial mammals, vocal aging has only been studied in humans and pandas. In cervids displaying convergent sex dimorphism of vocal apparatus with humans, vocal aging is only investigated in males. This cross-sectional study examined acoustic variables of nasal (closed-mouth) and oral (open-mouth) contact calls of 32 farmed Iberian red deer hinds (*Cervus elaphus hispanicus*) aged of 4–18 years and their relationships with caller's age, weight, social discomfort score (bites of other hinds on hind pelt) and body condition score (fat reserves). Decrease of fundamental frequency was associated with age in both oral and nasal calls, but more prominently in the nasal calls. An increase in call duration, peak frequency and power quartiles was associated with a higher degree of bites due to social aggression. Weight and body condition weakly influenced acoustic traits. We discuss that vocal aging of hinds parallels that of vocal aging in human females.

### Keywords

animal vocal aging, female mammal, ungulate contact calls, discomfort acoustic correlates.

## 1. Introduction

Aging affects voice characteristics of both humans (Verdonck-de Leeuw & Mahieu, 2004; Lortie et al., 2015) and nonhuman mammals (Reby & McComb, 2003; Fischer et al., 2004; Vannoni & McElligott, 2008; Briefer et al., 2010; Hari Kumar et al., 2016). Vocal aging is strongly related to levels of hormones (Gugatschka et al., 2010; Lortie et al., 2015; Hari Kumar et al., 2016). At any age, the laryngeal tissues are highly receptive to steroid hormones in both humans (Gerritsma et al., 1994; Newman et al., 2000; Schneider et al., 2007; Voelter et al., 2008) and in other mammals (Aufdemorte et al., 1983). Processes of aging also affect the vocal apparatus, resulting e.g. in the vocal fold atrophy or oedema (Honjo & Isshiki, 1980; Kahane, 1983; Bloch & Behrman, 2001; Ximenes Filho et al., 2003; Kersing & Jennekens, 2004; Pontes et al., 2005, 2006).

In humans, acoustic traits of aging are remarkably gender-specific (Honjo & Isshiki, 1980; Linville, 1996; Stathopoulos et al., 2011; Lortie et al., 2015). In women, voice fundamental frequency ( $f_0$ ) reflecting the rate of vibration of the vocal folds (Titze, 1994), significantly decreases with senescence (Honjo & Isshiki, 1980; Torre & Barlow, 2009; Ma & Love, 2010; Da Silva et al., 2011; Stathopoulos et al., 2011; Dehqan et al., 2013; Goy et al., 2013; Lortie et al., 2015). These changes are related to the age-related decrease in levels of estrogen (Boulet & Oddens, 1996; Abitbol et al., 1999; Caruso et al., 2000).

By contrast, in elderly men, the voice  $f_0$  either increases (Honjo & Isshiki, 1980; Harnsberger et al., 2008; Torre & Barlow, 2009; Gugatschka et al., 2010; Ma & Love, 2010; Dehqan et al., 2013) or remains unchanged (Gugatschka et al., 2010; Stathopoulos et al., 2011; Goy et al., 2013; Lortie et al., 2015; Fouquet et al., 2016). A study by Gugatschka et al. (2010) showed the presence or lack of changes of  $f_0$  in elderly male voices depend on men's estrogen status, with a significant increase of mean  $f_0$  at decreased levels of estrogen compared to that at normal levels (Gugatschka et al., 2010). At the same time, the age-related decrease of levels of testosterone with age has no impact on  $f_0$  of old male humans (Gugatschka et al., 2010), although in younger male humans, testosterone has a negative effect on the  $f_0$  (Dabbs & Mallinger, 1999; King et al., 2001; Evans et al., 2008).

In nonhuman mammals, the effects of aging on voice properties were investigated in both male and female giant pandas (*Ailuropoda melanoleuca*) and only in males of ruminant and primate species. In the panda, no call

f0 variables significantly correlate to male or female age (Charlton et al., 2009). In old male ruminants, as in humans, call f0 either increases, as was reported for fallow deer bucks *Dama dama* (Vannoni & McElligott, 2008; Briefer et al., 2010), or remains unchanged, as was reported for red deer stags *Cervus elaphus* (Reby & McComb, 2003). In old male nonhuman primates, the baboons *Papio cynocephalus ursinus*, call f0 decreases with age, in contrast to elderly male humans (Fischer et al., 2004). Based on this limited evidence, vocal aging processes seem to be more similar between males of phylogenetically distant taxa (humans and ruminants) than between related taxa (humans and nonhuman primates).

This similarity in trends of f0 with male aging expands also to sexual dimorphism of vocal anatomy. The larynx rests lower in the neck in males than in females in both humans (Negus, 1949; Lieberman, 1973; Davidson, 2003) and in four species of ruminants: fallow deer (McElligott et al., 2006), Mongolian gazelle *Procapra gutturosa* (Frey & Riede, 2003; Frey et al., 2008), goitred gazelle *Gazella subgutturosa* (Frey et al., 2011; Efremova et al., 2016) and red deer *Cervus elaphus* (Fitch & Reby, 2001; Frey et al., 2012). Published research so far shows a lack of similar sex dimorphism in vocal anatomy in nonhuman primates. Sex dimorphism of vocal anatomy and vocal traits develops under effects of steroid hormones in both humans and ruminants (Lieberman, 1973; Davidson, 2003; Verdonck-de Leeuw & Mahieu, 2004; Frey et al., 2011, 2012; Lortie et al., 2015). In male Mongolian and goitred gazelles, the size of the larynx increases remarkably prior to each rut (Frey et al., 2008, 2012; Efremova et al., 2016).

The aging-related trends of decreased oestrogen levels are similar between female red deer (Šperanda et al., 2012) and female humans (Boulet & Oddens, 1996; Abitbol et al., 1999; Caruso et al., 2000). It seems that parallel evolution of similar vocal anatomies between red deer and humans (Fitch & Reby, 2001; Frey et al., 2012) can provide information about similar processes of vocal aging. We can predict therefore that steroid hormones that regulate development of similar vocal anatomy and vocal traits would also regulate similar processes of vocal aging in red deer.

To our best knowledge, the effects of aging on acoustic traits have not been investigated in females of ruminants. The research focus of our cross-sectional study is on voice properties in contact calls of ageing female red deer. Red deer hinds produce two types of contact calls: oral calls that are produced through a widely opened mouth, and nasal calls that are produced

through the nose with a closed mouth (Sibiryakova et al., 2015; Volodin et al., 2016). Commonly, the oral calls of ruminants are emitted in situations of higher emotional arousal compared to the nasal calls (Sebe et al., 2010; Volodin et al., 2011, 2017), but can be produced by callers in the same series (Volodin et al., 2011, 2015; Sibiryakova et al., 2015). The  $f_0$  of the oral calls is commonly higher than  $f_0$  of the nasal calls in many species of ruminants (Sebe et al., 2010; Volodin et al., 2011, 2014; Sibiryakova et al., 2017) and in red deer calves (Sibiryakova et al., 2015). However, hinds of the native Iberian populations of red deer are distinctive in that  $f_0$  does not differ between the oral and nasal calls (Sibiryakova et al., 2015; Volodin et al., 2015). Thus, we can predict that effects of aging might be similar on both the nasal and oral call types in the Iberian red deer hinds. In particular, we hypothesize that red deer hinds will display similar changes in  $f_0$  as female humans. The aim of this study is to estimate the effect of age in red deer hinds on the acoustic variables of their oral and nasal contact calls. Further, it aims to explore the effects of body weight, social discomfort (related to aggression by other females) and body condition (reflecting animal fat reserves).

## **2. Materials and methods**

### *2.1. Study site and subjects*

Calls of red deer hinds were recorded from 10 June 2011 to 27 June 2011 and from 14 June 2012 to 23 June 2012 at the experimental farm of the University of Castilla-La Mancha (Albacete, Spain, 38°57'10"N, 1°47'00"W, 690 m a.s.l.). The population originated in 1994 from 15 male and 50 female Iberian red deer, phylogenetically representing the Western European line according to Skog et al. (2009); or the central Iberian–Western European line according to Carranza et al. (2016), from a nearby Las Dehesas public game reserve in Alpera (Albacete) and from Cabañeros National Park (Toledo). The animals used in this study were born and kept in four 10 000 m<sup>2</sup> enclosures on an irrigated pasture. The age of each hind was known. All study hinds had calves younger than one month of age during data collection. They were fed ad libitum with a diet of barley straw and meal from barley, alfalfa, oats and sugar beets (Landete-Castillejos et al., 2003).

All hinds were kept together with their calves in permanent groups (4 groups in 2011 and 3 groups in 2012) separately from adult stags and yearlings. The groups ranged in size from 6 hinds and 2 calves to 30 hinds and 24

calves (mean  $\pm$  SD =  $17.4 \pm 7.6$  hinds and  $15.0 \pm 7.7$  calves per group). The entire population of animals from which we collected contact calls counted 61 hinds in 2011 and 61 hinds in 2012 (45 hinds were the same in both years). All hinds were individually labelled with both Allflex (Palmerston North, New Zealand) plastic ear tags and with Allflex colour collars with numbers.

## 2.2. Data collection

Data included acoustic recordings of hind oral and nasal contact calls and three measures that could potentially affect the acoustics: body weight as proxy of body size, discomfort score (reflecting number of bites on hind pelt because of aggression of other hinds) and body condition score (reflecting fat reserves). For acoustic recordings (48 kHz, 16 bit), we used solid state recorders Marantz PMD-660 (D&M Professional, Kanagawa, Japan) with Sennheiser K6-ME66 cardioid electret condenser microphones (Sennheiser Electronic, Wedemark, Germany). The distance from the hand-held microphone to the animals was between 5 and 35 m, the level of recording was adjusted during the recordings accordingly to the intensity of the produced calls.

We recorded calls daily, for 28 days in total (18 days in 2011 and for 10 days in 2012), from 6:00–7:00 to 12:00–13:00, often with synchronous video for documenting the oral or nasal vocal emission, using a digital camcorder Panasonic HDC-HS100 (Panasonic, Kadoma, Japan). During recordings, individual identities of callers producing calls through the mouth and through the nose were labelled by voice. Contact calls were elicited by brief separation of individually identified hinds from their calves by a distance over 10 m by different reasons; the animals remained in visual contact and wanted to join but something prevented the joining. The particular contexts included everyday routine activity, when mothers searched for their offspring, which were hidden in the enclosures; at translocations to small paddocks, from where the animals were taken for weightings; during temporal separations of hinds and calves after the weighing; and at short separations evoked by appearance of researchers between a mother and her calf. All these contexts were routine for the animals, and all only moderately stressful, as they regularly occurred during everyday management and all individuals were approximately equally tolerant to this degree of separation stress. Both nasal and oral calls occurred within the same series. Recordings have been conducted both inside and outside the outdoor enclosures. In total, in 2011 and

2012, we collected 30 h of audio recordings (16 h in 2011 and 14 h in 2012) from 32 individual hinds (21 hinds in 2011 and 11 hinds in 2012). Samples of animals and calls did not overlap between 2011 and 2012; that is, each hind was only recorded in one of the years, either in 2011 or in 2012.

All animals were weighed one time with Mettler-Toledo ID1 scales (Mettler-Toledo S.A.E., Barcelona, Spain) as the part of routine farm management (Landete-Castillejos et al., 2001) during the periods of acoustic recordings. All animals were scored for body condition. The Condition Score represented a standard body condition index, varying from 1 to 5, scored from 1 = emaciated to 5 = obese (Carrión et al., 2008; Zeiler et al., 2017).

All animals were scored for social discomfort using the Discomfort Score representing an index related to being recipient of social aggression. This score has been developed by A. Garcia based on over 20 years of experience in handling deer in the experimental farm. Such aggression among animals feeding in concentration points is similar to those reported in the wild in other cervids, e.g., in small snow craters in reindeer *Rangifer tarandus*: (Collins & Smith, 1991, Hansen et al., 2010), which supports its use in the present study. The Discomfort Score was a proxy of the number of bites on the pelt of the animal, from 1 = no bites to 5 = extensive upper half of body skin lacking fur as a result of frequent bites. Score 1 = all the hair of the deer is intact. Score 2 = occasional lack of hair, mainly in the sides and rear quarters. Less than 10% naked (bald) skin. Score 3 = substantial lack of hair on sides and rear quarters. Less than one third of the skin naked. Score 4 = substantial lack of hair on sides, rear quarters and also in neck. Less than two thirds of naked skin. Score 5 = lack of hair very substantial. Less than 10% of the skin with hair considering neck, sides and rear quarters including upper part of the four legs, from elbow/knew upwards. The bites can reach in the neck very high, up to nearly the head. Percentages of the naked skin are calculated from the surface of part of the body which can be bitten (excluding the belly part): upper part of the four legs, back and neck. From Score 4 onwards the skin is totally naked, although at Score 2 bites leave undercoat fur visible, so the skin is not bald. At Score 3 there is a combination in bitten areas with those showing undercoat fur (most) with some parts showing the naked skin. The animals on the farm are never allowed to reach such high Discomfort Score as 4 or 5 because it means they have also difficulties accessing food and for humane reasons in those cases they are transferred to

another pen with calves or less aggressive animals. In this study, all measurements of the Discomfort Score and the Condition Score have been conducted by the same researcher, A. Garcia.

### 2.3. Acoustic analyses

For acoustic analyses, we only used calls of good quality, sufficient for analysis of all acoustic variables, measured in this study, that were not disrupted by wind, overlapped by calls of other animals or saturated with very high amplitude in the recording. We analysed only individually identified calls of known call type (nasal or oral). Calls were classified to nasal and oral based on voice comments of researchers made during recording; by video clips, made synchronously with the recordings; by the obvious nasal quality of sound within a recording and by the difference in call energy distribution, which shifted towards higher frequencies in oral calls due to the shortening of the vocal tract when the mouth is open. These methods of classification to nasal and oral call types were previously applied for the Iberian red deer (Sibiryakova et al., 2015; Volodin et al., 2015), for goitred gazelles (Volodin et al., 2011; Lapshina et al., 2012) and for saiga antelopes *Saiga tatarica* (Volodin et al., 2014; Sibiryakova et al., 2017). Two researchers (OS and IV) independently classified all calls, and we took for analysis only calls where both researchers were concordant in their judgments concerning their type. To avoid pseudo-replication, we took calls from different recording sessions per animal and from different parts within session, because calls from the same sequence are commonly more similar in their acoustic structure than calls from different sequences (Durbin et al., 1998). The mean  $\pm$  SD number of sessions per animal was  $5.3 \pm 4.4$ . We took from 1 to 23 (on average  $11.07 \pm 6.80$ ) high-quality oral calls per individual from 28 hinds and from 1 to 20 (on average  $11.91 \pm 5.71$ ) high-quality nasal calls per individual from 32 hinds for further acoustic analyses and calculating the average values of acoustic variables per individual hind. Three individuals provided only one oral call and two individuals provided only one nasal call. From the 32 hinds, 28 provided both the oral and the nasal calls. In total, we analysed 691 calls (310 oral and 381 nasal).

Acoustic analyses were conducted in the same way for the oral and nasal calls. For each nasal and each oral call, we measured the same set of nine acoustic variables, following Sibiryakova et al. (2015). We measured the duration, the start (f0beg), maximum (f0max) and end (f0end) fundamental

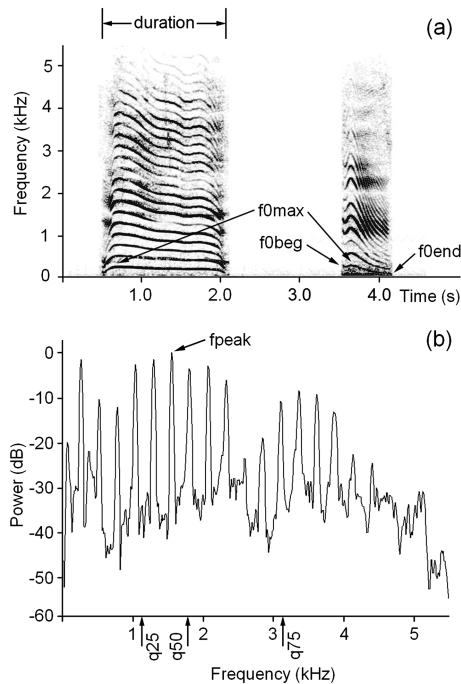
frequencies, the depth of modulation of the  $f_0$  ( $df_0 = f_{0\max} - f_{0\min}$ ),  $f_{\text{peak}}$ , representing the value of the frequency of maximum amplitude, and the  $q_{25}$ ,  $q_{50}$  and  $q_{75}$ , representing the lower, medium and upper quartiles, covering 25, 50 and 75% of the energy of the call spectrum, respectively.

Before measurements, the calls were down-sampled to 11 025 Hz and high-pass filtered at 50 Hz, to increase frequency resolution and to reduce the low-frequency background noise. We measured the duration of each call manually on the screen with the reticule cursor in the spectrogram window (Hamming window, FFT 1024 points, frame 50% and overlap 96.87%) by using Avisoft SASLab Pro software (Avisoft Bioacoustics, Berlin, Germany). Then we performed manual measurements on the screen with the standard marker cursor of the start ( $f_{0\text{beg}}$ ), maximum ( $f_{0\max}$ ) and end ( $f_{0\text{end}}$ ) fundamental frequencies of each call (Figure 1). In a 0.05 s call fragment symmetrical about  $f_0$  (comprising about 5–10% of average call duration), we created the power spectrum, from which we automatically measured  $f_{\text{peak}}$ ,  $q_{25}$ ,  $q_{50}$  and  $q_{75}$  (Figure 1). Measurements were exported automatically to Microsoft Excel (Microsoft, Redmond, WA, USA). In addition, for each call we selected the minimum  $f_0$  ( $f_{0\min}$ ) as the minimum value between  $f_{0\text{beg}}$  and  $f_{0\text{end}}$  and calculated the depth of frequency modulation  $df_0$  as the difference between  $f_{0\max}$  and  $f_{0\min}$ . For subsequent acoustic analyses, we calculated the average values of acoustic variables per individual hind respectively for oral and nasal calls.

#### 2.4. Statistical analyses

Most acoustic variables for nasal and oral contact calls were normally distributed (Shapiro–Wilk's  $W$ -test,  $p > 0.05$ ), but for some variables errors for the linear relationships slightly violated normality and were not perfectly homoscedastic. Therefore the effects on the acoustic variables of factors Age, Weight, Discomfort Score and Condition Score were analysed using generalized linear models (GLZ, the method that is more robust to minor violations of normality than general linear models) for normal distribution with identity link function. Linear relationships between the factors were estimated using Pearson correlation. Statistical analyses were conducted using STATISTICA v. 13.0 (StatSoft, Tulsa, OK, USA). Means are given as mean  $\pm$  SD, all tests were two-tailed, and differences were considered significant whenever  $p < 0.05$  and marginally significant whenever  $p < 0.06$ .





**Figure 1.** Measured acoustic variables. (a) Spectrogram of hind oral (left) and nasal (right) calls. (b) Mean power spectrum of a 0.05 s fragment of an oral call. Designations: duration, call duration;  $f_{0max}$ , the maximum fundamental frequency;  $f_{0beg}$ , the fundamental frequency at the onset of a call;  $f_{0end} = f_{0min}$ , the fundamental frequency at the end of a call;  $f_{peak}$ , the frequency of maximum amplitude within a call;  $q_{25}$ ,  $q_{50}$ ,  $q_{75}$ , the lower, the medium and the upper quartiles, covering 25, 50 and 75% energy of a call spectrum, respectively. The spectrogram was created with a Hamming window; 11 025 kHz sampling rate; FFT 1024 points; frame 50%; and overlap 93.75%.

### 3. Results

#### 3.1. Descriptive statistics of contact calls

Separately for samples of oral and nasal contact calls, we calculated means of mean values of acoustic variables in order to use them for estimating the effects of factors Age, Weight, Discomfort Score and Condition Score on the acoustics (Table 1). For either oral or nasal call samples, Age ranged of 4–18 years, Weight varied from 84.5 to 121.5 kg, Discomfort Score varied from 1.0 to 3.5 (from little to moderate number of bites on the hind pelt), and Condition Score varied from 3.0 to 4.5 (the larger the score the better condition) (Table 1).

**Table 1.**  
Descriptive statistics (mean ± SD and min–max) of averaged per caller values of acoustic variables for oral and nasal contact calls and the meanings for factors Age, Weight, Discomfort Score and Condition Score.

	Oral calls ( <i>N</i> = 28 callers)	Nasal calls ( <i>N</i> = 32 callers)
Acoustic variables		
Duration (s)	0.74 ± 0.23 (0.37–1.20)	0.77 ± 0.26 (0.33–1.41)
f0beg (kHz)	0.14 ± 0.03 (0.08–0.23)	0.14 ± 0.03 (0.09–0.22)
f0max (kHz)	0.18 ± 0.03 (0.13–0.26)	0.18 ± 0.03 (0.14–0.25)
f0end (kHz)	0.09 ± 0.02 (0.06–0.13)	0.09 ± 0.01 (0.06–0.12)
df0 (kHz)	0.09 ± 0.03 (0.03–0.14)	0.08 ± 0.03 (0.02–0.14)
fpeak (kHz)	1.47 ± 0.53 (0.31–2.55)	1.05 ± 0.54 (0.15–2.67)
q25 (kHz)	0.95 ± 0.27 (0.31–1.55)	0.72 ± 0.23 (0.31–1.27)
q50 (kHz)	1.72 ± 0.24 (1.20–2.13)	1.63 ± 0.24 (0.94–2.27)
q75 (kHz)	2.46 ± 0.24 (2.02–2.96)	2.57 ± 0.28 (2.15–3.20)
Factors		
Age (years)	11.25 ± 3.68 (4–18)	11.00 ± 3.61 (4–18)
Weight (kg)	101.94 ± 11.32 (84.5–121.5)	102.58 ± 10.86 (84.5–121.5)
Discomfort Score	1.61 ± 0.70 (1.0–3.5)	1.59 ± 0.67 (1.0–3.5)
Condition Score	3.59 ± 0.39 (3.0–4.5)	3.64 ± 0.38 (3.0–4.5)

3.2. Relationships between factors

Pearson correlation for relationship between the four factors revealed negative correlations between factors Age and Discomfort Score and between factors Discomfort Score and Condition Score, and revealed a positive correlation between factors Weight and Condition Score (Table 2). No other correlations between factors were found.

**Table 2.**  
Pearson’s correlation for relationship between factors Age, Weight, Discomfort Score and Condition Score.

Examined factors	Oral calls ( <i>N</i> = 28 callers)	Nasal calls ( <i>N</i> = 32 callers)
Age–Weight	<i>r</i> = 0.01; <i>p</i> = 0.97	<i>r</i> = −0.06; <i>p</i> = 0.75
Age–Discomfort Score	<i>r</i> = −0.58; <i>p</i> = 0.001*	<i>r</i> = −0.47; <i>p</i> = 0.007*
Age–Condition Score	<i>r</i> = 0.16; <i>p</i> = 0.41	<i>r</i> = 0.07; <i>p</i> = 0.71
Weight–Discomfort Score	<i>r</i> = −0.09; <i>p</i> = 0.65	<i>r</i> = −0.17; <i>p</i> = 0.37
Weight–Condition Score	<i>r</i> = 0.47; <i>p</i> = 0.01*	<i>r</i> = 0.47; <i>p</i> = 0.007*
Discomfort Score–Condition Score	<i>r</i> = −0.42; <i>p</i> = 0.03*	<i>r</i> = −0.47; <i>p</i> = 0.007*

\*Significant values.

### 3.3. Effects of age, weight, discomfort score and condition score on the acoustics

The Generalized Linear Models (GLZs) revealed a negative effect of Age on the variables of fundamental frequency: on f0max of the oral calls and on f0beg, f0max and df0 of the nasal calls (Tables 3, 4, Figure 2). Factor Age also had a negative effect on q75 of the oral calls (Figure 2). Factor Weight had a negative effect on q75 of the oral calls and the positive effect on df0 and fpeak of the nasal calls. Factor Discomfort Score had a positive effect on call duration and on df0 of the oral and of the nasal calls, as well as additional positive effects on q50 of the oral calls and q25 and fpeak of the nasal calls (Tables 3, 4, Figure 3). Factor Condition Score did not influence on the acoustic variables of the oral calls and had a positive effect only on q75 of the nasal calls (Tables 3, 4).

## 4. Discussion

### 4.1. Acoustic correlates of voice aging

This study examined, for the first time, the effect of aging on female vocal variables in a nonhuman mammal, red deer. Fundamental frequency characters of hind oral and nasal contact calls decreased with age. Body weight and amount of fat resources (body condition) of hinds weakly affected the acoustics. At the same time, there was an effect of increased call duration, peak frequency, and power quartiles with increasing social distress, scored as damaged area on hind's pelt because of bites received as a result of aggression of other females.

Our results on vocal aging in red deer hinds are consistent with reported data about decrease of fundamental frequency with senescence in woman (Honjo & Isshiki, 1980; Torre & Barlow, 2009; Ma & Love, 2010; Da Silva et al., 2011; Stathopoulos et al., 2011; Dehqan et al., 2013; Goy et al., 2013; Lortie et al., 2015). The obtained results for red deer hinds were predicted based on parallelism of sex differences of vocal anatomy between ruminants and humans (Frey et al., 2011, 2012) and consistently similar profiles of hormonal aging between female red deer (Šperanda et al., 2012) and female humans (Boulet & Oddens, 1996; Abitbol et al., 1999; Caruso et al., 2000).

Against expectations that effects of aging might be similar on both the nasal and oral call types in the Iberian red deer hinds, the effect of aging

**Table 3.**  
Results of Generalized Linear Models (GLZs) for effects of factors Age, Weight, Discomfort Score and Condition Score on variables of hind oral contact calls.

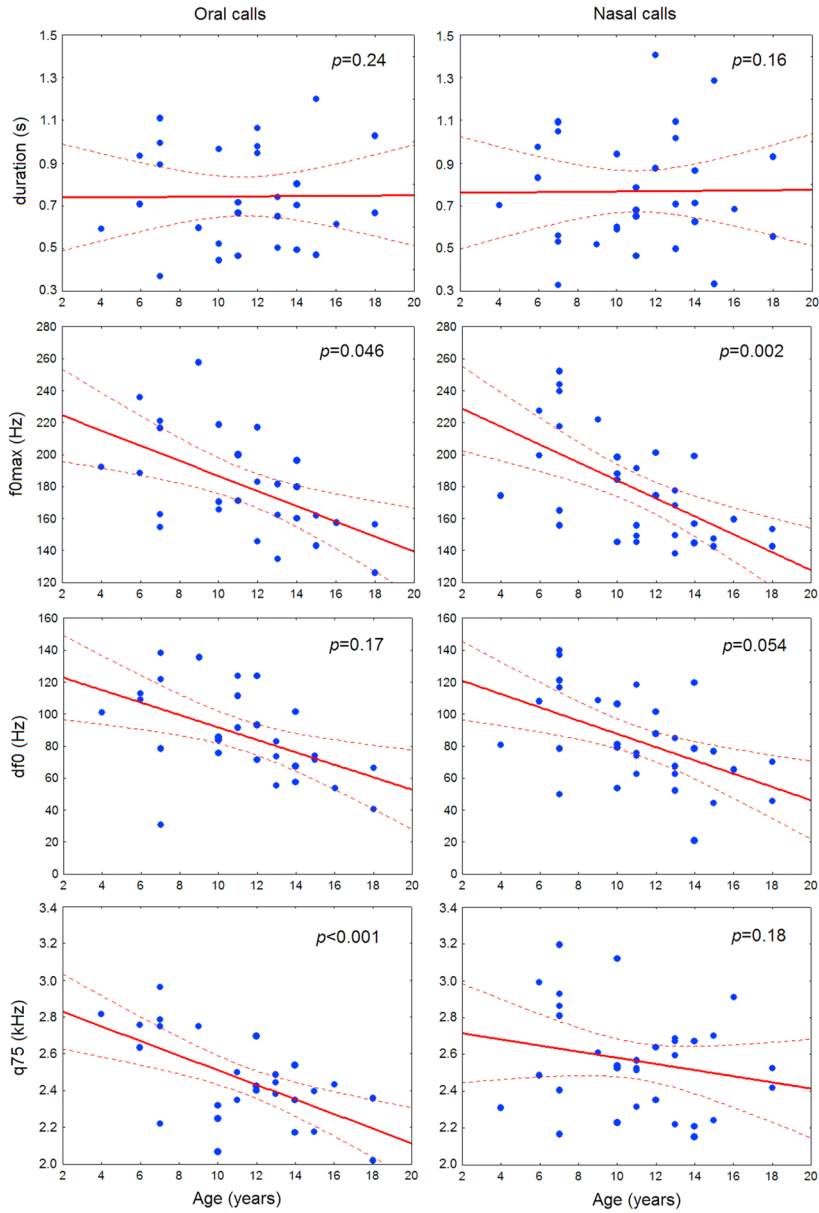
Acoustic variable	Factor and its effect on acoustic variables of oral calls											
	Age			Weight			Discomfort Score			Condition Score		
	B	$\chi^2$	p	B	$\chi^2$	p	B	$\chi^2$	p	B	$\chi^2$	p
Duration	0.02 ± 0.01	1.41	0.24	0.01 ± 0.01	2.04	0.15	0.14 ± 0.08	3.59	0.058	0.03 ± 0.13	0.06	0.81
f0beg	-2.68 ± 1.87	2.06	0.15	-0.39 ± 0.56	0.49	0.49	14.53 ± 10.78	1.81	0.18	5.47 ± 17.93	0.09	0.76
f0max	-3.19 ± 1.60	3.99	0.046*	-0.02 ± 0.48	0.01	0.97	15.34 ± 9.24	2.76	0.10	6.82 ± 15.36	0.20	0.66
f0end	-1.80 ± 1.11	2.65	0.10	-0.37 ± 0.34	1.19	0.28	-1.73 ± 6.40	0.07	0.79	-2.27 ± 10.64	0.05	0.83
df0	-1.85 ± 1.34	1.90	0.17	0.47 ± 0.41	1.32	0.25	18.29 ± 7.75	5.56	0.02*	-3.36 ± 12.89	0.07	0.79
fpeak	26.83 ± 32.31	0.69	0.41	-0.03 ± 9.77	0	1	192.21 ± 186.68	1.06	0.30	-59.26 ± 310.34	0.04	0.85
q25	18.47 ± 16.18	1.30	0.25	-4.63 ± 4.89	0.90	0.34	136.63 ± 93.47	2.14	0.14	29.15 ± 155.38	0.04	0.85
q50	6.11 ± 13.19	0.21	0.64	-3.89 ± 3.99	0.95	0.33	148.96 ± 76.22	3.82	0.051	-11.46 ± 126.71	0.01	0.93
q75	-37.67 ± 9.45	15.90	0.001***	-10.47 ± 2.86	13.42	0.001***	23.25 ± 54.59	0.18	0.67	45.42 ± 90.75	0.25	0.62

$\chi^2$  corresponds to Wald statistics and  $B \pm SE$  corresponds to parameter estimates in GLZs: \*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$ .

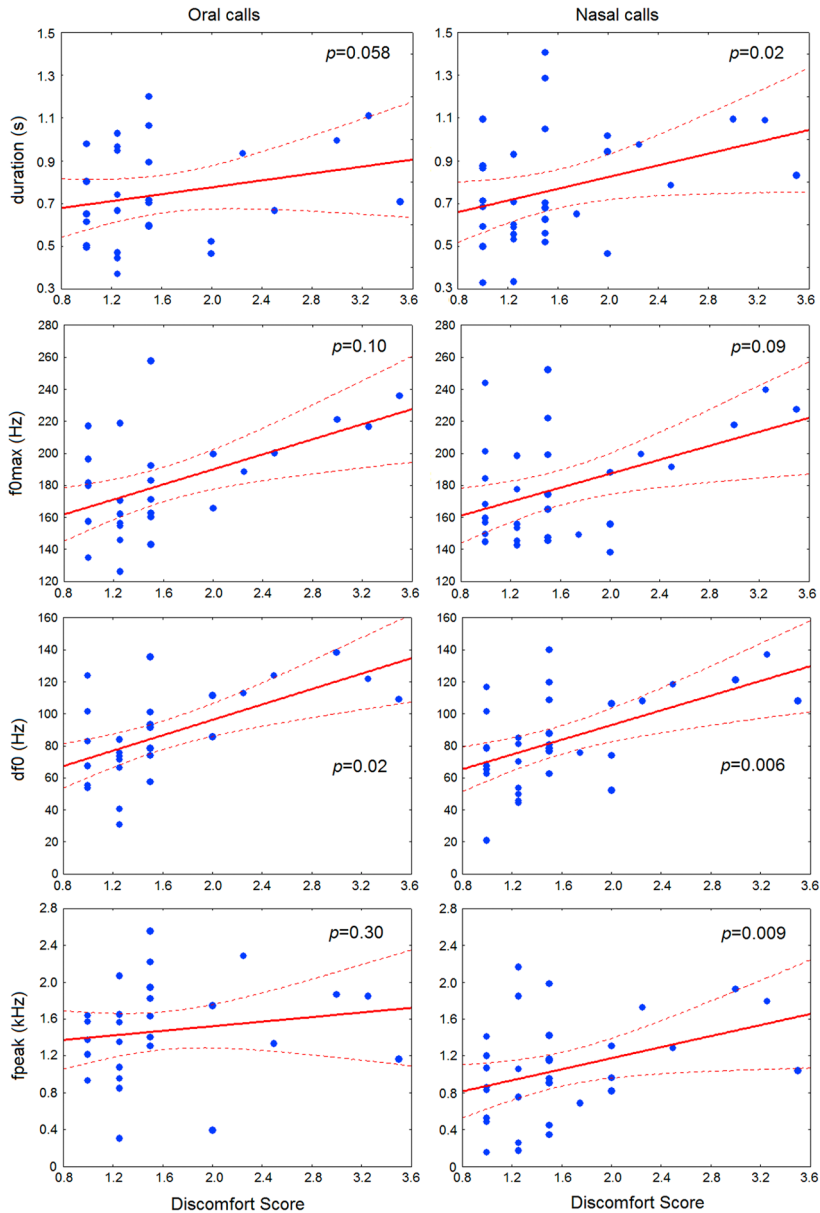
**Table 4.**  
Results of Generalized Linear Models (GLZs) for effects of factors Age, Weight, Discomfort Score and Condition Score on variables of hind nasal contact calls.

Acoustic variable	Factor and its effect on acoustic variables of oral calls											
	Age			Weight			Discomfort Score			Condition Score		
	<i>B</i>	$\chi^2$	<i>p</i>	<i>B</i>	$\chi^2$	<i>p</i>	<i>B</i>	$\chi^2$	<i>p</i>	<i>B</i>	$\chi^2$	<i>p</i>
Duration	0.02 ± 0.01	1.93	0.16	0.01 ± 0.01	2.35	0.13	0.18 ± 0.08	5.19	0.02*	−0.07 ± 0.14	0.26	0.61
f0beg	−3.57 ± 1.40	6.53	0.01**	0.19 ± 0.46	0.16	0.69	4.98 ± 8.47	0.35	0.56	−12.33 ± 14.61	0.71	0.40
f0max	−4.33 ± 1.42	9.34	0.002**	0.43 ± 0.46	0.87	0.35	14.56 ± 8.60	2.87	0.09	9.82 ± 14.84	0.44	0.51
f0end	−1.00 ± 0.80	1.55	0.21	−0.15 ± 0.26	0.32	0.57	−0.74 ± 4.86	0.02	0.88	−0.59 ± 8.39	0.01	0.94
df0	−2.31 ± 1.20	3.73	0.054	0.76 ± 0.39	3.81	0.051	19.77 ± 7.25	7.44	0.006**	2.31 ± 12.50	0.03	0.85
fpeak	40.72 ± 25.85	2.48	0.12	18.56 ± 8.46	4.82	0.03*	408.35 ± 156.77	6.79	0.009**	−169.35 ± 270.52	0.39	0.53
q25	10.91 ± 12.18	0.80	0.37	1.05 ± 3.98	0.07	0.79	151.75 ± 73.85	4.22	0.04*	142.93 ± 127.44	1.26	0.26
q50	4.24 ± 13.29	0.10	0.75	0.37 ± 4.35	0.01	0.93	98.28 ± 80.61	1.49	0.22	134.12 ± 139.10	0.93	0.33
q75	−19.34 ± 14.27	1.84	0.18	−7.51 ± 4.67	2.59	0.11	8.30 ± 86.54	0.01	0.92	288.65 ± 149.34	3.74	0.053

$\chi^2$  corresponds to Wald statistics and *B* ± SE corresponds to parameter estimates in GLZs: \**p* ≤ 0.05, \*\**p* ≤ 0.01, \*\*\**p* ≤ 0.001.



**Figure 2.** Scatterplots illustrating the relationships between hind age and acoustic variables of oral and nasal contact calls: duration, call duration; f0max, the maximum fundamental frequency; df0, the depth of frequency modulation; q75, the upper quartile of a call spectrum. Linear regression lines with 95% confidence intervals are shown.  $p$ -values indicate the effects of Generalized Linear Models.



**Figure 3.** Scatterplots illustrating the relationships between hind age and acoustic variables of oral and nasal contact calls: duration, call duration; f0max, the maximum fundamental frequency; df0, the depth of frequency modulation; fpeak, the frequency of maximum amplitude within a call. Linear regression lines with 95% confidence intervals are shown.  $p$ -values indicate the effects of Generalized Linear Models.

on fundamental frequency was more prominent in the nasal calls. A greater number of variables related to the fundamental frequency decreased in the nasal calls, whereas only the maximum fundamental frequency decreased in the oral calls. Probably, the effects of aging were better expressed in the nasal than in the oral contact calls because of the effect of the higher emotional arousal of the hinds during emission of the oral calls (Sibiryakova et al., 2015, 2018). The energy shifts towards higher frequencies (and respective increase of power quartile values) represent a common rule of acoustic changes associated with increased emotional arousal of mammalian callers (Volodin et al., 2009; Gogoleva et al., 2010a, b; Briefer, 2012) and callers across other taxa of vertebrates (Lingle et al., 2012; Filippi et al., 2017). Consistently, elevated emotional arousal results in increase of fundamental frequency in cervid species: red deer (Volodin et al., 2015; Golosova et al., 2017; Sibiryakova et al., 2018) and fallow deer (Charlton & Reby, 2011). We conclude therefore that the effect of emotional arousal on call fundamental frequency ( $f_0$  increase) was the opposite to the effects of aging ( $f_0$  decrease).

In this study, hind age did not influence call duration. At the same time, a recent study of vocal aging of a marine mammal with laryngeal sound production, the North Atlantic right whale, *Eubalaena glacialis*, showed a remarkable increase of duration in calls of this species with age (Root-Gutteridge et al., 2018). The authors interpret these clear age-related voice cues as indicators of increased stamina or condition in older adults (Root-Gutteridge et al., 2018).

#### 4.2. Acoustic correlates of social discomfort, body weight and condition

An increased level of social discomfort related to the frequency of bites received from other females resulted in an increase of call duration, depth of frequency modulation and values of power variables for both the oral and the nasal calls. The effect of discomfort on  $f_0$  was a shift toward high frequencies, as is common in the vocalizations of many animals with elevated arousal levels (Volodin et al., 2009; Gogoleva et al., 2010a, b; Briefer, 2012). The finding that social discomfort has a measurable effect on the acoustic properties of a red deer female's contact calls might be important to researchers interested in maximizing animal welfare.

Effect of hind body weight on the acoustics was very weak; calls of the heavier hinds had lower upper quartile of the oral calls and increased depth of frequency modulation and the peak frequency of the nasal calls. These



results are consistent with evidences that body weight is a poor predictor of call frequency in both red deer (Volodin et al., 2013, 2015; Sibiryakova et al., 2015) and in humans (Fitch & Giedd, 1999).

The negative correlation found between age and social discomfort suggests that the index is actually an indirect measure of social aggression (a proxy of dominance), with older hinds being more often among dominate individuals whereas younger hinds are more often subordinate. The negative correlation between social discomfort and condition suggests that individuals in better condition are less subjected to social distress because of bites of dominant hinds. Alternatively, individuals that are subjected to social distress may subsequently suffer from decreased body condition. The positive correlation found between body weight and condition suggests that proxies of body size and fat reserves relate to each other and might together reflect animal fitness. We did not find a correlation between age and body weight in females. However, another study conducted with the same red deer population revealed a correlation between age, body weight, body condition and social rank and their effects on milk production (Landete-Castillejos et al., 2010). In addition, other studies have found a positive relationship between age and body weight in male Scottish red deer (Reby & McComb, 2003) and between dominance and body weight in cows *Bos taurus* (Phillips & Rind, 2002).

#### 4.3. Comparative aspects of vocal aging

Parallel trends of  $f_0$  changes in elderly female humans and red deer hinds suggest that the human speech model of voice aging predicts the age-related changes of non-verbal vocalizations in a Cervidae species. At the same time, female human model of vocal aging does not predict the lack of  $f_0$  changes reported in the ageing female giant pandas (Charlton et al., 2009). However, it remains unclear, whether the predictive power of this model in cervids may be expanded to other aspects of vocal aging in red deer hinds. For example, a recent study of male and female humans revealed that the voice aging is less expressed in frequently singing human persons compared to non-singers (Lortie et al., 2017). Consistent with this human model of aging, it may be worthwhile exploring the idea that aging processes less affect more vocal hinds than less vocal hinds.

In human speakers, the age-related voice and speech disorders evoke discomfort, anxiety and depression, and can cause seniors to avoid telephone

conversations and other social communications because of the lowered self-esteem due to their voice problems (Verdonck-de Leeuw & Mahieu, 2004; Lortie et al., 2015). So far, no nonverbal model of human vocal aging is available. All research studies of the age-related human vocal disorders including invasive hormonal therapy, irreversible affecting the structure of vocal folds, are conducted with human volunteers and patients (Gerritsma et al., 1994; Lortie et al., 2015). However, further research is necessary to investigate at what extent the hind vocal aging model may be applied for modelling the processes of the age-related changes of non-verbal vocalizations in women. Although 18 years of age approaches to the upper limit of reproduction in hinds of *Cervus elaphus* (e.g. Fedosenko, 1980) all red deer hinds in this study were not indeed old animals. The study hinds were mothers vocalizing in the contexts of separation with their calves, whereas few true senescent hinds without calves only vocalized to a small extent occasionally and unpredictably and therefore could not be recorded (own observations of the authors). At the same time, voice problems in female humans mostly arise in late non-reproductive age after menopause (Lortie et al., 2015). Therefore, the unmatched age categories (e.g. of mature hinds in this study and of *senex* age category of women after menopause) impose limitations on applying both human-deer and deer-human models of vocal aging.

Potentially, hinds of other populations of European red deer may also demonstrate the same trends of vocal aging as female Iberian red deer. All studied European red deer populations share the same, based on the vocal fold vibration, mechanism of vocal emission (Titze, 1994; Frey et al., 2012; Volodin et al., 2013). This mechanism results in low  $f_0$  values, ranging of 40–380 Hz in male and female red deer (see discussions in Volodin et al., 2015; Golosova et al., 2017). This is in spite of their origin from the three different European isolates of the last Pleistocene glacial maximum: Iberian Peninsula/Southern France, North Italy and the Balkans (Zachos & Hartl, 2011) and their respectively different Cytochrome *b* haplogroups (Skog et al., 2009; Zachos & Hartl, 2011).

In contrast, distinctive trends of vocal aging are expected from hinds of Asian and American wapiti (including all currently accepted Asian subspecies of *Cervus elaphus* and *Cervus hanglu* and all currently accepted subspecies of *Cervus canadensis*) (Lorenzini & Garofalo, 2015; Kumar et al., 2017). In wapiti, all age and sex-classes produce very high-frequency vocalizations over 1000 Hz (Volodin et al., 2013, 2016; Reby et al., 2016;

Golosova et al., 2017) potentially by using a distinctive vocal production mechanism compared to European red deer; of an aerodynamic whistle produced as air flows rapidly through a narrow supraglottic constriction (Reby et al., 2016). Because of the potentially distinct mechanism of vocal production, the human model of vocal aging seems inapplicable for explaining the effects of vocal aging in wapiti.

We should mention however that the proposed mechanism for vocalization in wapiti is not a fact, as Reby et al. (2016) analysed calls from only four different adult males and one of these animals was a hybrid between a red deer and a wapiti. So, this mechanism has only been proposed for adult males, not for all age groups of wapiti and was hypothesised based on calculations rather than proved by direct experiments. Nevertheless, a wapiti model might be convenient for comparative investigation of vocal aging between cervid taxa with different vocal production mechanisms.

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## References

- Abitbol, J., Abitbol, A. & Abitbol, B. (1999). Sex hormones and the female voice. — *J. Voice* 13: 424–446.

- Aufdemorte, T.B., Sheridan, P.J. & Holt, G.R. (1983). Autoradiographic evidence of sex steroid receptors in laryngeal tissues of the baboon (*Papio cynocephalus*). — *Laryngoscope* 93: 1607-1611.
- Bloch, I. & Behrman, A. (2001). Quantitative analysis of videostroboscopic images in presbylarynges. — *Laryngoscope* 111: 2022-2027.
- Boulet, M.J. & Oddens, B.J. (1996). Female voice changes around and after the menopause — an initial investigation. — *Maturitas* 23: 15-21.
- Briefer, E., Vannoni, E. & McElligott, A.G. (2010). Quality prevails over identity in the sexually selected vocalisations of an ageing mammal. — *BMC Biol.* 8: 35. DOI:10.1186/1741-7007-8-35.
- Briefer, E.F. (2012). Vocal expression of emotions in mammals: mechanisms of production and evidence. — *J. Zool.* 288: 1-20.
- Carranza, J., Salinas, M., de Andrés, D. & Pérez-González, J. (2016). Iberian red deer: paraphyletic nature at mtDNA but nuclear markers support its genetic identity. — *Ecol. Evol.* 6: 905-922.
- Carrión, D., García, A.J., Gaspar-López, E., Landete-Castillejos, T. & Gallego, L. (2008). Development of body condition in hinds of Iberian red deer during gestation and its effects on calf birth weight and milk production. — *J. Exp. Zool.* 309: 1-10.
- Caruso, S., Roccasalva, L., Sapienza, G., Zappala, M., Nuciforo, G. & Biondi, S. (2000). Laryngeal cytological aspects in women with surgically induced menopause who were treated with transdermal estrogen replacement therapy. — *Fertil. Ster.* 74: 1073-1079.
- Charlton, B.D. & Reby, D. (2011). Context-related acoustic variation in male fallow deer (*Dama dama*) groans. — *PLoS ONE* 6: e21066. DOI:10.1371/journal.pone.0021066.
- Charlton, B.D., Zhihe, Z. & Snyder, R.J. (2009). The information content of giant panda, *Ailuropoda melanoleuca*, bleats: acoustic cues to sex, age and size. — *Anim. Behav.* 78: 893-898.
- Collins, W.B. & Smith, T.S. (1991). Effects of wind-hardened snow on foraging by reindeer (*Rangifer tarandus*). — *Arctic* 44: 217-222.
- Da Silva, P.T., Master, S., Andreoni, S., Pontes, P. & Ramos, L.R. (2011). Acoustic and longterm average spectrum measures to detect vocal aging in women. — *J. Voice* 25: 411-419.
- Dabbs, J.M. & Mallinger, A. (1999). High testosterone levels predict low voice pitch among men. — *Pers. Individ. Diff.* 27: 801-804.
- Davidson, T.M. (2003). The great leap forward: the anatomic basis for the acquisition of speech and obstructive sleep apnea. — *Sleep Med.* 4: 185-194.
- Dehqan, A., Scherer, R.C., Dashti, G., Ansari-Moghaddam, A. & Fanaie, S. (2013). The effects of aging on acoustic parameters of voice. — *Folia Phoniatr. Logop.* 64: 265-270.
- Durbin, L.S. (1998). Individuality in the whistle call of the Asiatic wild dog *Cuon alpinus*. — *Bioacoustics* 9: 197-206.
- Efremova, K.O., Frey, R., Volodin, I.A., Fritsch, G., Soldatova, N.V. & Volodina, E.V. (2016). The postnatal ontogeny of the sexually dimorphic vocal apparatus in goitred gazelles (*Gazella subgutturosa*). — *J. Morphol.* 277: 826-844.

- Evans, S., Neave, N., Wakelin, D. & Hamilton, C. (2008). The relationship between testosterone and vocal frequencies in human males. — *Physiol. Behav.* 93: 783–788.
- Fedosenko, A.K. (1980). The maral (ecology, behaviour, management). — Nauka, Alma-Ata. (In Russian).
- Filippi, P., Congdon, J.V., Hoang, J., Bowling, D.L., Reber, S.A., Pasukonis, A., Hoeschele, M., Ocklenburg, S., de Boer, B., Sturdy, C.B., Newen, A. & Gunturkun, O. (2017). Humans recognize emotional arousal in vocalizations across all classes of terrestrial vertebrates: evidence for acoustic universals. — *Proc. Roy. Soc. Lond. B: Biol. Sci.* 284: 20170990. DOI:10.1098/rspb.2017.0990.
- Fischer, J., Kitchen, D., Seyfarth, R. & Cheney, D. (2004). Baboon loud calls advertise male quality: acoustic features and relation to rank, age, and exhaustion. — *Behav. Ecol. Sociobiol.* 56: 140–148.
- Fitch, W.T. & Giedd, J. (1999). Morphology and development of the human vocal tract: a study using magnetic resonance imaging. — *J. Acoust. Soc. Am.* 106: 1511–1522.
- Fitch, W.T. & Reby, D. (2001). The descended larynx is not uniquely human. — *Proc. Roy. Soc. Lond. B: Biol. Sci.* 268: 1669–1675.
- Fouquet, M., Pisanski, K., Mathevon, N. & Reby, D. (2016). Seven and up: individual differences in male voice fundamental frequency emerge before puberty and remain stable throughout adulthood. — *Roy. Soc. Open Sci.* 3: 160395. DOI:10.1098/rsos.160395.
- Frey, R. & Riede, T. (2003). Sexual dimorphism of the larynx of the Mongolian gazelle (*Procapra gutturosa* Pallas, 1777) (Mammalia, Artiodactyla, Bovidae). — *Zool. Anz.* 242: 33–62.
- Frey, R., Gebler, A., Olson, K.A., Odonkhuu, D., Fritsch, G., Batsaikhan, N. & Stuermer, I.W. (2008). Head anatomy of male and female Mongolian gazelle — a striking example of sexual dimorphism. — In: *Anatomical imaging — towards a new morphology* (Endo, H. & Frey, R., eds). Springer, Tokyo, p. 1–13.
- Frey, R., Volodin, I.A., Volodina, E.V., Carranza, J. & Torres-Porras, J. (2012). Vocal anatomy, tongue protrusion behaviour and the acoustics of rutting roars in free-ranging Iberian red deer stags (*Cervus elaphus hispanicus*). — *J. Anat.* 220: 271–292.
- Frey, R., Volodin, I.A., Volodina, E.V., Soldatova, N.V. & Juldashev, E.T. (2011). Descended and mobile larynx, vocal tract elongation and rutting roars in male goitred gazelles (*Gazella subgutturosa* Gldenstaedt, 1780). — *J. Anat.* 218: 566–585.
- Gerritsma, E.J., Brocaar, M.P., Hakkesteegt, M.M. & Birkenhager, J.C. (1994). Virilization of the voice in postmenopausal women due to the anabolic-steroid nandrolone decanoate (decadurabolin) — the effects of medication for one-year. — *Clin. Otolaryngol.* 19: 79–84.
- Gogoleva, S.S., Volodina, E.V., Volodin, I.A., Kharlamova, A.V. & Trut, L.N. (2010a). The gradual vocal responses to human-provoked discomfort in farmed silver foxes. — *Acta Ethol.* 13: 75–85.
- Gogoleva, S.S., Volodin, I.A., Volodina, E.V., Kharlamova, A.V. & Trut, L.N. (2010b). Sign and strength of emotional arousal: vocal correlates of positive and negative attitudes to humans in silver foxes (*Vulpes vulpes*). — *Behaviour* 147: 1713–1736.

- Golosova, O.S., Volodin, I.A., Isaeva, I.L. & Volodina, E.V. (2017). Effects of free-ranging, semi-captive and captive management on the acoustics of male rutting calls in Siberian wapiti *Cervus elaphus sibiricus*. — Mammal. Res. 62: 387-396.
- Goy, H., Fernandes, D.N., Pichora-Fuller, M.K. & van Lieshout, P. (2013). Normative voice data for younger and older adults. — J. Voice 27: 545-555.
- Gugatschka, M., Kiesler, K., Obermayer-Pietsch, B., Schoekler, B., Schmid, C., Groselj-Strele, A. & Friedrich, G. (2010). Sex hormones and the elderly male voice. — J. Voice 24: 369-373.
- Hansen, B.B., Aanes, R. & Sæther, B.-E. (2010). Feeding-crater selection by high-Arctic reindeer facing ice-blocked pastures. — Can. J. Zool. 88: 170-177.
- Hari Kumar, K.V.S., Garg, A., Ajai Chandra, N.S., Singh, S.P. & Datta, R. (2016). Voice and endocrinology. — Ind. J. Endocrinol. Metab. 20: 590-594.
- Harnsberger, J.D., Shrivastav, R., Brown, W.S., Rothman, H. & Hollien, H. (2008). Speaking rate and fundamental frequency as speech cues to perceived age. — J. Voice 22: 58-69.
- Honjo, I. & Isshiki, N. (1980). Laryngoscopic and voice characteristics of aged persons. — Arch. Otolaryngol. 106: 149-150.
- Kahane, J.C. (1983). A survey of age-related changes in the connective tissues of the human adult larynx. — In: Vocal fold physiology: contemporary research and clinical issues (Bless, D. & Abbs, J., eds). College-Hill, San Diego, CA, p. 44-49.
- Kersing, W. & Jennekens, F.G. (2004). Age-related changes in human thyroarytenoid muscles: a histological and histochemical study. — Eur. Arch. Otorhinolaryngol. 261: 386-392.
- King, A., Ashby, J. & Nelson, C. (2001). Effects of testosterone replacement on a male professional singer. — J. Voice 15: 553-557.
- Kumar, V.P., Thakur, M., Rajpoot, A., Joshi, B.D., Nigam, P., Ahmad, K., Kumar, D. & Goyal, S.P. (2017). Resolving the phylogenetic status and taxonomic relationships of the Hangul (*Cervus elaphus hanglu*) in the family Cervidae. — Mitochondrial DNA Part A 28: 835-842.
- Landete-Castillejos, T., Garcia, A. & Gallego, L. (2001). Calf growth in captive Iberian red deer (*Cervus elaphus hispanicus*): effect of birth date and hind milk production and composition. — J. Anim. Sci. 79: 1085-1092.
- Landete-Castillejos, T., García, A., Gómez, J.A., Molina, A. & Gallego, L. (2003). Subspecies and body size allometry affect milk production and composition, and calf growth in red deer: comparison of *Cervus elaphus hispanicus* and *C. e. scoticus*. — Physiol. Biochem. Zool. 76: 594-602.
- Landete-Castillejos, T., Ceacero, F., Garcia, A.J., Estevez, J.A. & Gallego, L. (2010). Direct versus indirect effects of social rank, maternal weight, body condition and age on milk production in Iberian red deer (*Cervus elaphus hispanicus*). — J. Dairy Res. 77: 77-84.
- Lapshina, E.N., Volodin, I.A., Volodina, E.V., Frey, R., Efremova, K.O. & Soldatova, N.V. (2012). The ontogeny of acoustic individuality in the nasal calls of captive goitred gazelles, *Gazella subgutturosa*. — Behav. Process. 90: 323-330.
- Lieberman, P. (1973). On the evolution of language: a unified view. — Cognition 2: 59-94.

- Lingle, S., Wyman, M.T., Kotrba, R., Teichroeb, L.J. & Romanow, C.A. (2012). What makes a cry a cry? A review of infant distress vocalizations. — *Curr. Zool.* 58: 698-726.
- Linville, S.E. (1996). The sound of senescence. — *J. Voice* 10: 190-200.
- Lorenzini, R. & Garofalo, L. (2015). Insights into the evolutionary history of *Cervus* (Cervidae, tribe Cervini) based on Bayesian analysis of mitochondrial marker sequences, with first indications for a new species. — *J. Zoolog. Syst. Evol. Res.* 53: 340-349.
- Lortie, C.L., Thibeault, M., Guitton, M.J. & Tremblay, P. (2015). Effects of age on the amplitude, frequency and perceived quality of voice. — *Age* 37: 117. DOI:10.1007/s11357-015-9854-1.
- Lortie, C.L., Rivard, J., Thibeault, M. & Tremblay, P. (2017). The moderating effect of frequent singing on voice aging. — *J. Voice* 31: 112.e1-112.e12. DOI:10.1016/j.jvoice.2016.02.015.
- Ma, E.P.M. & Love, A.L. (2010). Electroglottographic evaluation of age and gender effects during sustained phonation and connected speech. — *J. Voice* 24: 146-152.
- McElligott, A.G., Birrer, M. & Vannoni, E. (2006). Retraction of the mobile descended larynx during groaning enables fallow bucks (*Dama dama*) to lower their formant frequencies. — *J. Zool.* 270: 340-345.
- Negus, V.E. (1949). The comparative anatomy and physiology of the larynx. — Grune and Stratton, New York, NY.
- Newman, S.R., Butler, J., Hammond, E.H. & Gray, S.D. (2000). Preliminary report on hormone receptors in the human vocal fold. — *J. Voice* 14: 72-81.
- Phillips, C.J.C. & Rind, M.I. (2002). The effects of social dominance on the production and behavior of grazing dairy cows offered forage supplements. — *J. Dairy Sci.* 85: 51-59.
- Pontes, P., Brasolotto, A. & Behlau, M. (2005). Glottic characteristics and voice complaint in the elderly. — *J. Voice* 19: 84-94.
- Pontes, P., Yamasaki, R. & Behlau, M. (2006). Morphological and functional aspects of the senile larynx. — *Folia Phoniatr. Logop.* 58: 151-158.
- Reby, D. & McComb, K. (2003). Anatomical constraints generate honesty: acoustic cues to age and weight in the roars of red deer stags. — *Anim. Behav.* 65: 519-530.
- Reby, D., Wyman, M.T., Frey, R., Passilongo, D., Gilbert, J., Locatelli, Y. & Charlton, B.D. (2016). Evidence of biphonation and source-filter interactions in the bugles of male North American wapiti (*Cervus canadensis*). — *J. Exp. Biol.* 219: 1224-1236.
- Root-Gutteridge, H., Cusano, D.A., Shiu, Y., Nowacek, D.P., Van Parijs, S.M. & Parks, S.E. (2018). A lifetime of changing calls: North Atlantic right whales, *Eubalaena glacialis*, refine call production as they age. — *Anim. Behav.* 137: 21-34.
- Schneider, B., Cohen, E., Stani, J., Kolbus, A., Rudas, M., Horvat, R. & van Trotsenburg, M. (2007). Towards the expression of sex hormone receptors in the human vocal fold. — *J. Voice* 21: 502-507.
- Sebe, F., Duboscq, J., Aubin, T., Ligout, S. & Poindron, P. (2010). Early vocal recognition of mother by lambs: contribution of low- and high-frequency vocalizations. — *Anim. Behav.* 79: 1055-1066.
- Sibiryakova, O.V., Volodin, I.A., Matrosova, V.A., Volodina, E.V., Garcia, A.J., Gallego, L. & Landete-Castillejos, T. (2015). The power of oral and nasal calls to discriminate individual

- mothers and offspring in red deer, *Cervus elaphus*. — Front. Zool. 12: 2. DOI:10.1186/s12983-014-0094-5.
- Sibiryakova, O.V., Volodin, I.A., Frey, R., Zuther, S., Kisebaev, T.B., Salemgareev, A.R. & Volodina, E.V. (2017). Remarkable vocal identity in wild-living mother and neonate saiga antelopes: a specialization for breeding in huge aggregations? — Sci. Nat. 104: 11. DOI:10.1007/s00114-017-1433-0.
- Sibiryakova, O.V., Volodin, I.A. & Volodina, E.V. (2018). Advertising individual identity by mother and adolescent contact calls in Siberian wapiti *Cervus elaphus sibiricus*. — Ethology 124: 733-742.
- Skog, A., Zachos, F.E., Rueness, E.K., Feulner, P.G.D., Mysterud, A., Langvatn, R., Lorenzini, R., Hmwe, S.S., Lehoczy, I., Hartl, G.B., Stenseth, N.C. & Jakobsen, K.S. (2009). Phylogeography of red deer (*Cervus elaphus*) in Europe. — J. Biogeogr. 36: 66-77.
- Šperanda, M., Florijančić, T., Šperanda, T., Mandić, S., Bošković, I., Đidara, M., Ozimec, I., Horvat, V., Šefer, D. & Marković, R. (2012). Steroid hormones profile during an ovarian synchronization procedure in different age categories of red deer hinds (*Cervus elaphus* L.). — Acta Vet. 62: 67-75.
- Stathopoulos, E.T., Huber, J.E. & Sussman, J.E. (2011). Changes in acoustic characteristics of the voice across the life span: measures from individuals 4–93 years of age. — J. Speech Lang. Hear. Res. 54: 1011-1021.
- Titze, I.R. (1994). Principles of voice production. — Prentice-Hall, Englewood Cliffs, NJ.
- Torre, P. & Barlow, J.A. (2009). Age-related changes in acoustic characteristics of adult speech. — J. Commun. Disord. 42: 324-333.
- Vannoni, E. & McElligott, A.G. (2008). Low frequency groans indicate larger and more dominant fallow deer (*Dama dama*) males. — PLoS ONE 3: e3113. DOI:10.1371/journal.pone.0003113.
- Verdonck-de Leeuw, I.M. & Mahieu, H.F. (2004). Vocal aging and the impact on daily life: a longitudinal study. — J. Voice 18: 193-202.
- Voelter, C., Kleinsasser, N., Joa, P., Nowack, I., Martinez, R., Hagen, R. & Voelker, H.U. (2008). Detection of hormone receptors in the human vocal fold. — Eur. Arch. OtoRhino-Laryngol. 265: 1239-1244.
- Volodin, I.A., Matrosova, V.A., Volodina, E.V., Garcia, A.J., Gallego, L., Márquez, R., Llusia, D., Beltrán, J.F. & Landete-Castillejos, T. (2015). Sex and age-class differences in calls of Iberian red deer during rut: reversed sex dimorphism of pitch and contrasting roars from farmed and wild stags. — Acta Ethol. 18: 19-29.
- Volodin, I.A., Lapshina, E.N., Volodina, E.V., Frey, R. & Soldatova, N.V. (2011). Nasal and oral calls in juvenile goitred gazelles (*Gazella subgutturosa*) and their potential to encode sex and identity. — Ethology 117: 294-308.
- Volodin, I.A., Sibiryakova, O.V., Frey, R., Efremova, K.O., Soldatova, N.V., Zuther, S., Kisebaev, T.B., Salemgareev, A.R. & Volodina, E.V. (2017). Individuality of distress and discomfort calls in neonates with bass voices: wild-living goitred gazelles (*Gazella subgutturosa*) and saiga antelopes (*Saiga tatarica*). — Ethology 123: 386-396.



- Volodin, I.A., Sibiryakova, O.V., Kokshunova, L.E., Frey, R. & Volodina, E.V. (2014). Nasal and oral calls in mother and young trunk-nosed saiga antelopes, *Saiga tatarica*. — Bioacoustics 23: 79-98.
- Volodin, I.A., Sibiryakova, O.V. & Volodina, E.V. (2016). Sex and age-class differences in calls of Siberian wapiti *Cervus elaphus sibiricus*. — Mammal. Biol. 81: 10-20.
- Volodin, I.A., Volodina, E.V., Frey, R., Carranza, J. & Torres-Porras, J. (2013). Spectrographic analysis points to source-filter coupling in rutting roars of Iberian red deer. — Acta Ethol. 16: 57-63.
- Volodin, I.A., Volodina, E.V., Gogoleva, S.S. & Doronina, L.O. (2009). Indicators of emotional arousal in vocal emissions of the humans and nonhuman mammals. — J. Gen. Biol. 70: 210-224. (In Russian).
- Ximenes Filho, J.A., Tsuji, D.H., do Nascimento, P.H.S. & Sennes, L.U. (2003). Histologic changes in human vocal folds correlated with aging: a histomorphometric study. — Ann. Otol. Rhinol. Laryngol. 112: 894-898.
- Zachos, F.E. & Hartl, G.B. (2011). Phylogeography, population genetics and conservation of the European red deer *Cervus elaphus*. — Mammal. Rev. 41: 138-150.
- Zeiler, G.E. & Meyer, L.C.R. (2017). Captive management of wild impala (*Aepyceros melampus*) during intensive immobilization and general anesthesia study trials. — J. Zoo. Wildl. Med. 48: 1058-1071.