



Vocal changes accompanying the descent of the larynx during ontogeny from neonates to adults in male and female goitred gazelles (*Gazella subgutturosa*)

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ABSTRACT

The pronouncedly enlarged and descended larynx in male goitred gazelles (*Gazella subgutturosa*), Mongolian gazelles (*Procapra gutturosa*) and fallow deer (*Dama dama*) represents an interesting parallel to the 'Adam's apple' of human males. Goitred gazelles, as humans, are not born with a descended larynx. Therefore the sexual dimorphism of larynx size and position develops during ontogeny. In this study, the vocal ontogeny of male and female goitred gazelles was investigated across five age classes from neonates to adults. The acoustic variables of nasal contact calls were measured in 53 (24 male, 29 female) individuals, body mass and neck dimensions in 63 (31 male, 32 female) live individuals and nasal vocal tract and vocal fold lengths in 26 (16 male, 10 female) anatomical specimens. Call fundamental frequency (f_0), the acoustic correlate of the ontogenetically enlarging larynx, decreased significantly in either sex. Call formants (second, third and fourth), the acoustic correlates of the ontogenetically elongating vocal tract, did not differ significantly between sexes up to early adulthood, but clearly diverged in adults. Significant differences between sexes in neck circumference at the level of the larynx emerged already at 2–3 months of age, whereas body mass, neck circumference at the neck-body transition and the degree of larynx descent significantly differed in adults only. We discuss that, in contrast to humans, the accelerated enlargement of the larynx in male goitred gazelles starts early in ontogeny. A moderate descent of the larynx develops equally in both sexes before early adulthood, whereas the additional prominent descent of the larynx in males is shifted to late ontogeny. This might avoid selective disadvantages of this sexually dimorphic trait on males during their period of growth. As has been previously proved for humans, the emergence of the strong male-specific descent of the larynx in goitred gazelles may go along with the increasing social status and the males' increasing chances of siring offspring. Similar to the Adam's apple of human males, this may indicate the important role of the enlarged and descended larynx for signaling male status via masculine voice.

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1. Introduction

In polygynous goitred gazelles (*Gazella subgutturosa*), Mongolian gazelles (*Procapra gutturosa*), fallow deer (*Dama dama*) and in humans (*Homo sapiens*), the male larynx is larger and has a lower resting position than in females (goitred gazelles: Frey et al., 2011; Mongolian gazelles: Frey and Gebler, 2003; Frey and Riede,

2003; Frey et al., 2008a, 2008b; fallow deer: McElligott et al., 2006; humans: Fitch and Giedd, 1999). In humans, the sexual dimorphism of larynx size and position develops during puberty (Negus, 1949; Fitch and Giedd, 1999; Fitch, 2000) and correlates with a pronounced decrease of fundamental and formant frequencies in boys (Lee et al., 1999). In adult male humans, the combination of the enlarged and descended larynx (Negus, 1949; Fitch, 2000; Rendall et al., 2005) provides masculine attributes to their voice, particularly a low fundamental frequency (f_0) and low formants (Cartei et al., 2014; Pisanski et al., 2014) which are attractive for potential mates (Collins, 2000; Saxton et al., 2006). Similarly, in adult male

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goitred gazelles, the combination of the enlarged and descended larynx provides masculine attributes to their orally produced types of rutting calls: roars, growls and grunts (Frey et al., 2011).

As in human newborns (Negus, 1949; Fitch and Giedd, 1999; Fitch, 2000), a sexual dimorphism of larynx size and position is lacking in newborn goitred gazelles (Efremova et al., 2011a, 2011b). In goitred gazelles, during the ontogeny from newborns to adolescents, call f_0 and formants steadily descend at similar rates in both sexes (Efremova et al., 2011a, 2011b) while body variables (body mass and neck circumferences), vocal tract length and vocal fold size steadily increase at similar rates in males and females (Efremova et al., 2011a, 2011b). This phase roughly corresponds to the minor laryngeal descent in both sexes of humans occurring from 3 months to approximately 3 years of age that enables to relax the tongue for fine movements that are necessary during speech (Negus, 1949; Lieberman, 1984; Fitch and Giedd, 1999; Fitch, 2000).

In boys at puberty the larynx additionally enlarges and further descends by 10–15 mm, creating the “Adam’s apple”, whereas the female larynx size increases at slow speed (Kahane, 1978, 1982; Fitch and Giedd, 1999). Acoustic correlates of this stage, termed “voice breaking” in human boys and in some non-passerine birds (Radford, 2004; Klenova et al., 2010), have not yet been investigated in any nonhuman mammal. For goitred gazelles, the vocal ontogeny has been investigated from two weeks to adolescence (6 months of age, Efremova et al., 2011a, 2011b). So, the stage approximately corresponding to the male-specific descent of the larynx of humans has not yet been studied in this species.

In nature, the onset of maturation for both sexes of goitred gazelles occurs at 6 months; at one year males start to display mating postures and at two years they display sexual behavior, but participate in the rut only when they reach ‘social maturity’ at 3 years of age. In contrast, more than one fourth of the yearling females participate in the rut by the age of 7 months, while in the following year all females become involved in breeding (Blank, 1998). Rutting calls (roars, growls and gurgles) were only heard or recorded from mature males in nature (Frey et al., 2011; Blank et al., 2014).

Juvenile goitred gazelles (both males and females) regularly produce open-mouth (oral) and closed-mouth (nasal) contact calls (Volodin et al., 2011). Oral contact calls disappear from the vocal repertoire of goitred gazelles before adolescence, whereas nasal contact calls occur in either sex at any age (Efremova et al., 2011a; Volodin et al., 2011, 2014b; Lapshina et al., 2012). Therefore, this call type is appropriate for a comparative study of vocal ontogeny across age classes in goitred gazelles.

According to the source-filter theory of voice production, the entire source signal, i.e. the call fundamental frequency (f_0) and its harmonics, resulting from vocal fold vibrations, is filtered by the vocal tract. This filtering selectively accentuates resonance frequencies of the vocal tract (formants) and attenuates anti-resonances (Fant, 1960; Titze, 1994; Taylor and Reby, 2010). In humans, the f_0 is inversely related to mass and length of the oscillating portions of the vocal folds (Titze, 1994). In bovids, such as takins (*Budorcas taxicolor*), saiga antelopes (*Saiga tatarica*) and goitred gazelles, their very low f_0 values are probably related not only to the length of vocal folds but also to large connective tissue pads attached to their vocal folds (Frey and Hofmann, 2000; Frey et al., 2007, 2011; Volodin et al., 2014a). Formant frequencies are inversely related to the vocal tract length (Titze, 1994; Fitch and Hauser, 2002; Taylor and Reby, 2010). Therefore, physical correlates of the enlarged and descended larynx are larger vocal folds and longer vocal tracts. Their respective acoustic correlates are lower fundamental and formant frequencies.

An elongation of the vocal tract in live mammals can be achieved by a permanently low resting position of the larynx, as, e.g., in humans (Negus, 1949; Lieberman, 1984), large pantherine felids

(Weissenrubler et al., 2002) and the koala (*Phascolarctos cinereus*) (Charlton et al., 2013). A further, non-permanent elongation of the vocal tract can be achieved by active retraction of the larynx from its permanent low resting position down towards the sternum, as, e.g., in red deer (*Cervus elaphus*) (Fitch and Reby, 2001; Frey et al., 2012; Frey and Riede, 2013), fallow deer (McElligott et al., 2006), the Mongolian gazelle (Frey et al., 2008a) and the goitred gazelle (Frey et al., 2011; Efremova et al., 2016). The purpose of the present study was to document the ontogenetic emergence of the strong sexual dimorphism of vocal organs and their acoustic correlates in goitred gazelles. This study follows the entire period of the vocal and physical development of goitred gazelles from neonates to mature adults. In particular, we investigate the ontogeny of sexual divergence of acoustic variables of nasal contact calls along to changes of vocal tract and vocal fold lengths, body mass and neck dimensions in goitred gazelles.

2. Materials and methods

2.1. Site, subjects and dates of work

Calls as well as measurements of body mass and neck dimensions were collected from captive goitred gazelles (*Gazella subgutturosa subgutturosa*) at the EcoCenter “Djeiran” (Uzbekistan, Bukhara region, Kagan district, 39°41’N, 64°35’E) in 2008–2013. The EcoCenter “Djeiran” is located on a fenced 5000 ha semi-desert area with 600–1200 free-ranging goitred gazelles and includes a complex of enclosures housing up to 40 captive goitred gazelles of different ages each year (for details, see Efremova et al., 2011a; Frey et al., 2011). Anatomical specimens of captive and free-ranging animals which had died of natural causes were collected at the EcoCenter “Djeiran”.

Captive gazelles were raised in the enclosures either by their own mothers or by humans. The animals were individually marked with black p-phenylenediamine (Rhodia, Paris, France) and with ear-tags. The human-raised animals were kept in matched-age groups of 5–7 individuals in a few enclosures of 2 m × 4 m in a row with indoor shelters made of dried reeds. The small enclosures had exits to a common passageway 1.5 m wide, leading to a large enclosure 25 m × 18 m, where all animals stayed together during the day. The mother-raised animals were kept in larger enclosures together with their mothers, non-reproductive adult females and adult males. The animals were fed one to three times a day, depending on the age (for housing, keeping and management details, see Volodin et al., 2011).

Only one call type was included in the analyses: nasal calls produced with closed mouth, as only nasal contact calls were present in all age classes from neonates to adults. Distinction between the nasal and oral calls (produced with open mouth) was made as described by Volodin et al. (2011). Nasal contact calls were collected from 53 (24 male, 29 female) captive goitred gazelles of different ages. Measurements of body mass and neck dimensions were taken from 63 (31 male, 32 female) captive goitred gazelles of different ages. Anatomical whole-body or head-and-neck specimens of 26 (16 male, 10 female) free-ranging or captive goitred gazelles have been collected at the EcoCenter “Djeiran”. Vocal tract and vocal fold measurements of the anatomical specimens were taken during dissections conducted either at the EcoCenter “Djeiran” or at the Leibniz Institute of Zoo and Wildlife Research (IZW), Berlin, Germany.

2.2. Age classes

All animals and anatomical specimens were assigned to five age classes: 1–7-d neonates, 2–3-mo juveniles, 6–9-mo adolescents,

Table 1

Samples of subjects included in different analyses of this study. Designations of age classes: 1 = 1–7-d neonates, 2 = 2–3-mo juveniles, 3 = 6–9-mo adolescents, 4 = 12–23-mo yearlings and 5 = ≥ 24 -mo adults. n trials = n weighing & measuring trials. Distinctive samples of animals are given in brackets: (n) for body mass, [n] for skull basal length and {n} for larynx maximum height.

Age class	Number of animals				Number of anatomical specimens	
	Body mass & neck dimensions		Nasal contact calls		Vocal tract & vocal fold lengths	
	males	females	males	females	males	females
1 = Neonates	5 (2)	6 (1)	5	7	2	2
2 = Juveniles	5	7	5	7	2	2
3 = Adolescents	13	10	9	6	3	3 [2]
4 = Yearlings	4	4	3	4	3	0
5 = Adults	4 (3)	5	2	5	6 [5] {4}	3
Total subjects	31	32	24	29	16	10
Total data	n trials = 63		n animals = 53; n calls = 737		n specimens = 26	

12–24-mo yearlings and ≥ 24 -mo adults (Table 1). For all live animals, the age at the dates of call collection, body mass and neck measurements was known. The age at death was known for animals that had died in captivity in 2008–2011 (9 of 19 male and 5 of 11 female specimens). In contrast, the age was unknown for the free-ranging 10 males and 6 females. For these specimens we estimated the age by comparing their skull basal lengths and state of dentition with those of reference animals of known age (see supplementary material 1). In addition, we used published data on the skull basal length of 61 goitred gazelle museum specimens (Mambetjumaev, 1970) and data on age-related changes of the dentition in domestic goats (*Capra hircus* f. *domestica*) (Nickel et al., 1987) as reference values for age estimation (see supplementary material 1).

2.3. Call recording and analysis

Nasal contact calls (hereafter “calls”) were recorded before the morning or evening feeding. For acoustic recordings (48 kHz, 16 bit), we used a Marantz PMD660 (D&M Professional, Kanagawa, Japan) and a Zoom-H4 (Zoom Corp., Tokyo, Japan) digital recorder with a Sennheiser K6-ME64 or a Sennheiser K6-ME66 cardioid electret condenser microphone (Sennheiser electronic, Wedemark, Germany). Distance from a caller to the microphone varied from 1 to 10 m. During recordings, the calls of the focal animal were labeled on the recorder by voice.

For 28 animals we could record vocalizations in only one age class. Therefore, we used data from a single age class per animal throughout, i.e. for all the 53 animals (Table 1). For individuals where calls were available from several age classes, we used data from a single age class per animal making equal or similar sized samples of males and females per age class (Table 1). We measured from 2 to 25 calls (mean \pm SD = 13.91 ± 8.67) per individual and calculated the average value of each measured acoustic variable per caller. In total, acoustic variables were measured in 737 calls (Table 1).

In each call, we measured 6 acoustic variables (Fig. 1). With Avisoft SASLab Pro software (Avisoft Bioacoustics, Berlin, Germany), we measured the duration with the standard marker cursor in the spectrogram window (sampling frequency 48 kHz, Hamming window, Fast Fourier Transform 1024 points, frame 50% and overlap 93.75%). With the “Autocorrelation” option of Avisoft, we measured the mean fundamental frequency (f_0). This option recognizes periodic components in the sound signal and measures their period with 0.25-ms precision. All measurements were exported to Excel (Microsoft Corp., Redmond, WA, USA).

With Praat DSP package v. 4.3.21 (Boersma and Weenink, 2013), we measured the mean values for the first four formants (F1–F4) (Fig. 1). The Linear Prediction Coding settings were Burg analysis, window length 0.04 s, time step 0.01 s, maximum number of formants 4. For newborn calls (age class 1), the maximum for-

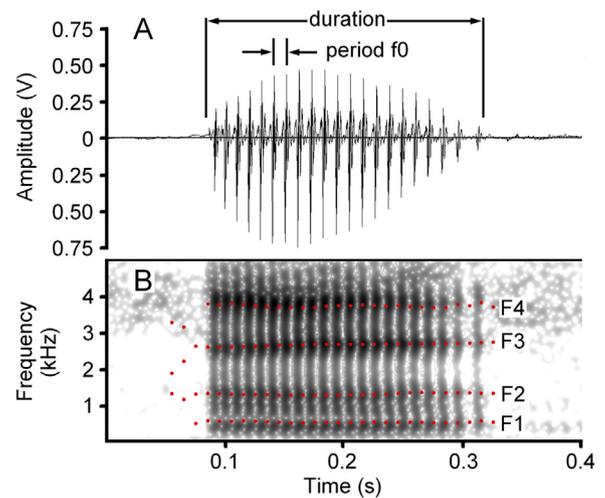


Fig. 1. The nasal call of a juvenile female goitred gazelle: (A) waveform and (B) spectrogram. Measured variables: duration, fundamental frequency period (period f_0) and tracks of the first 4 formants (F1–F4), created with Praat software. The Linear Prediction Coding settings were: Burg analysis, window length 0.04 s, time step 0.01 s, maximum number of formants 4, and maximum formant frequency 4000 Hz.

mant frequency was 5000–5500 Hz. For juvenile calls (age class 2), the maximum formant frequency was 4000–5000 Hz. For adolescent calls (age class 3), the maximum formant frequency was 2900–3500 Hz. For yearling calls (age class 4), the maximum formant frequency was 2900–3400 Hz. For adult calls (age class 5), the maximum formant frequency was 2500–2800 Hz. The values of the maximum formant frequency were adjusted based on visual inspection of the spectrograms, because the formant values varied among individuals and sexes. We took formant frequencies from a call portion where the formant tracks were nearly horizontal, i.e. where the vocal tract length was not subjected to rapid change (Fig. 1). Positions of formants were verified by superposition on the spectrogram. We extracted point values of the formant tracks, exported them to Excel and calculated the value of each formant of a given call as the average value from the point values.

Applying the model of a uniform tube closed at one end, we calculated formant dispersion (DF) using linear regression (Reby and McComb, 2003; Frey et al., 2011). Based upon the formant frequencies of the calls, the nasal vocal tract length (vtl) during calls was calculated by the equation: $vtl = c/2DF$, where c is the speed of sound in air, approximated as 350 ms^{-1} .

2.4. Nasal vocal tract and larynx

Nasal vocal tract length and larynx dimensions were measured using anatomical dissections of dead specimens. Prior to the

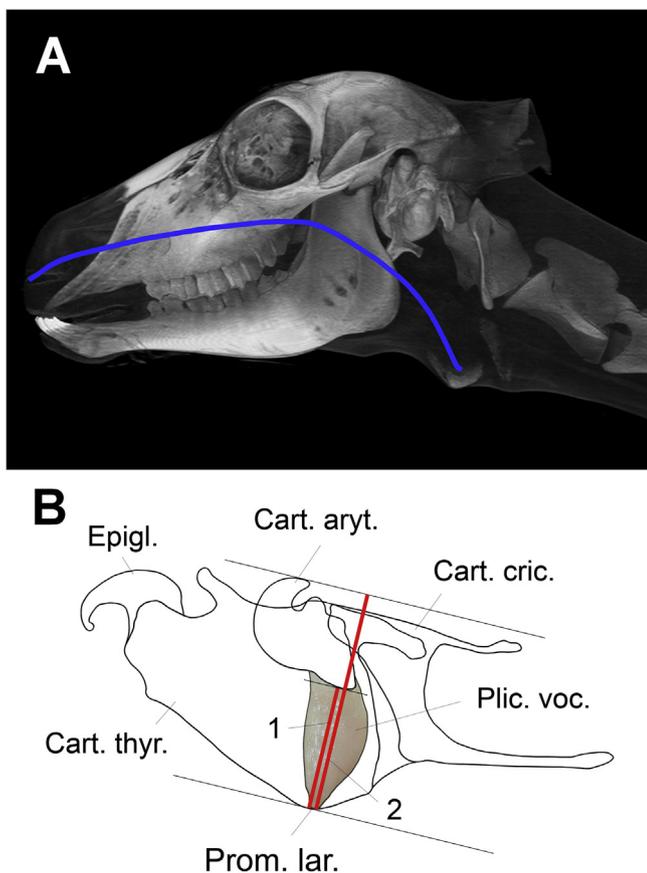


Fig. 2. (A) The nasal vocal tract length (curved line) and (B) the larynx measurements, left lateral view: (1) the dorsoventral length of the vocal folds, (2) the maximum height of the larynx. Abbreviations: Cart. aryt. = arytenoid cartilage; Cart. cric. = cricoid cartilage; Cart. thy. = thyroid cartilage; Epigl. = epiglottis; Plic. voc. = vocal fold; Prom. lar. = laryngeal prominence.

anatomical dissections the specimens were thawed at +5 °C for the duration of 24–48 hours. We began the dissections on the left side, removing the anatomical structures layer by layer, and then the right side was dissected in an identical manner (for details see Efremova et al., 2016). A comparison of photos and videos, taken from live and dead animals of the same age and sex in lateral profiles, suggested that the post-mortem position of the larynx approximately corresponded to its resting position in live animals. During nasal calls, the larynx did not change its position pronouncedly. As the number of anatomical specimens was limited, we took data of vocal tract and vocal fold measurements from all available specimens, including two adult males that died during the rut (Table 1).

After exposure of the left half of the lower jaw, the nasal vocal tract length was taken using a string accurate to 1 mm from the approximate position of the vocal folds along the pharyngeal and nasal cavities up to the nostrils (Fig. 2). The larynx measurements were taken on the excised larynges. The dorsoventral height of the larynx was measured from the laryngeal prominence to the most dorsal part of the larynx by using electronic calipers (Aerospace, Brüder Mannesmann Werkzeuge GmbH, Remscheid, Germany) accurate to 0.5 mm. The dorsoventral length of the vocal folds was measured along their medial surface, facing the glottis, from their ventral attachment to the thyroid cartilage dorsally up to the vocal process of the arytenoid cartilage (Fig. 2). Two persons (one after the other) repeated each measurement three times for each specimen and then we calculated the mean value of each measurement. For 24 of the 26 specimens (15 males, 9 females) whose skulls

were available we measured the skull basal length as an additional variable indicating body size and age (see supplementary material 1).

2.5. Body mass and neck dimensions

Body mass and neck measurements were taken from the live study animals before the evening feeding. For 35 animals we could only take measurements of one age class. Therefore, we used data from a single age class per animal throughout, i.e. for all the 63 animals (Table 1). For individuals where calls were available from several age classes, we used data from a single age class per animal making equal or similar sized samples of males and females per age class (Table 1). For adult males, we included in the analyses only data of body mass and neck dimensions taken outside of the rutting period. During the rutting period, the muscles and connective tissues around the larynx of adult males increase considerably in size (Frey et al., 2011).

For weighing neonates, juveniles and adolescents, we used Kern De 36K10NL electronic scales (Kern & Sohn GmbH, Balingen-Frommern, Germany), accurate to 10 g. For weighing yearlings and adults, we used VEU-150C electronic scales (Tulin Instrument Plant, Tambov, Russia), accurate to 100 g. Neonates, juveniles and adolescents were weighed in a cotton bag, whereas the older animals were weighed in a wooden box. During handling of all animals, we applied a cotton mask covering the eyes to decrease anxiety.

The neck measurements were taken with a tape measurer accurate to 1 mm, from animals lying in a close-to-natural posture, with legs bent under the body. In this posture, the neck was in an upright position and the head was kept at an angle of 90° to the neck. For each individual, four measurements were taken (Fig. 3): (1) “rostral neck circumference” = neck circumference at the laryngeal prominence; (2) “caudal neck circumference” = neck circumference at the neck/body transition; (3) “larynx descent” = distance between the angle of the lower jaw and the most prominent point of the larynx (perpendicular to the rostral neck circumference); (4) “neck length” = distance between the angle of the lower jaw and the sternal manubrium (Fig. 3). Two persons (one after the other) repeated each measurement three times for each specimen and then we calculated the mean value of each measurement.

2.6. Statistics

Statistical analyses were made with STATISTICA (StatSoft, Tulsa, OK, USA). Means are given as mean ± SD. All tests were two-tailed, and significance levels were set at 0.05. We used two-way ANOVA with a Tukey post hoc test to estimate the effects of sex and age on body mass, neck dimensions and call variables. We had no measurements for the females at the age class 4 for the length of the nasal vocal tract and the length of the vocal folds (Table 1), so we applied a two-factor GLMM with age included as random factor and sex included as fixed factor. The main problem for conducting a statistical analysis of our data on vocal tract and vocal folds were small sample sizes for either sex in each age class (Table 1). So, we checked GLMM results by applying to the same values a non-parametrical Mann–Whitney U-test, considered to be less sensitive to sample size (Zar, 1999). As the results of the two tests were very similar, we present here only the GLMM results. We also used Pearson’s correlation to compare the vocal fold dorsoventral length with the fundamental frequency of calls and to compare the vocal tract length with the formant frequencies of calls.

2.7. Ethics

The audio recordings, body mass and neck measurements were conducted in tight cooperation with administration, managers and

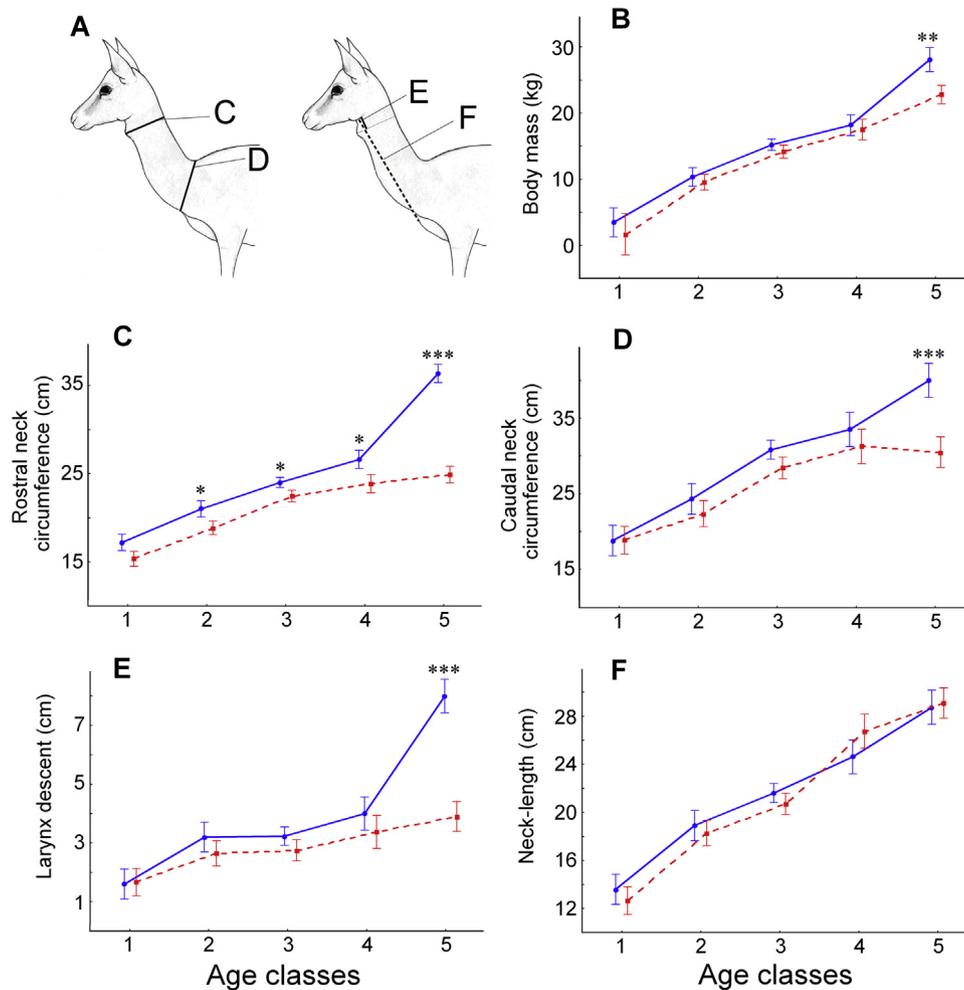


Fig. 3. (A) Neck measurements and age-related changes in (B) body mass, (C) rostral neck circumference, (D) caudal neck circumference, (E) larynx descent and (F) neck length in males (solid line) and females (dashed line). Designations of age classes as in Table 1. Central points show means, whiskers show 0.95 confidence intervals. Significant differences between sexes: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; Tukey post hoc test.

the working staff of the EcoCenter “Djeiran”. During our work we strictly followed the special welfare instructions developed by the EcoCenter for work with goitred gazelles. We adhered to the “Guidelines for the treatment of animals in behavioural research and teaching” (doi:10.1016/j.anbehav.2005.10.001) and to the laws on animal welfare for scientific research of the Republic of Uzbekistan, where the study was conducted. The study was approved by the Committee of Bio-ethics of Lomonosov Moscow State University (protocol # 2011-36).

3. Results

3.1. Sex and age effects on body mass and neck dimensions

Two-way ANOVA revealed strong and significant effects of age and sex on body mass, rostral neck circumference, caudal neck circumference and larynx descent (Table 2). Neck length was significantly affected by age, but not by sex (Table 2). Significant sex differences in the rostral neck circumference emerged as early as 2–3 mo of age (age class 2), whereas significant sex differences in body mass, caudal neck circumference and larynx descent could be detected only in adults (age class 5) (Fig. 3). The neck length did not differ significantly between sexes at any age class (Fig. 3).

3.2. Sex and age effects on the acoustics

Two-way ANOVA revealed significant effects of age class on the mean f_0 , duration and all four formants (Table 3). Significant sex differences were found on f_0 , the third formant and the fourth formant. Sex had no significant effect on duration and the first two formants (Table 3). With age, the f_0 and all the four formants decreased, whereas the duration slightly increased (Figs. 4 and 5). Although f_0 was always higher in females than in males, it did not differ significantly between sexes in all age classes. Duration and the first formant (F1) also did not differ significantly between sexes in all age classes, whereas F2, F3 and F4 showed significantly lower values in adult males than in adult females (Fig. 5).

3.3. Age and sex effects on vocal tract, vocal fold, and skull basal lengths

GLMM (with age included as random factor and sex included as fixed factor) revealed significant effects of age class and sex on skull basal length, nasal vocal tract length and dorsoventral vocal fold length (Table 4). The age effect on the maximum height of the larynx was significant, whereas the sex effect only approached significance (Table 4). Values of all anatomical variables increased with age (Fig. 6). Significant sex differences in nasal vocal tract length, maximum height of the larynx and dorsoventral vocal fold length

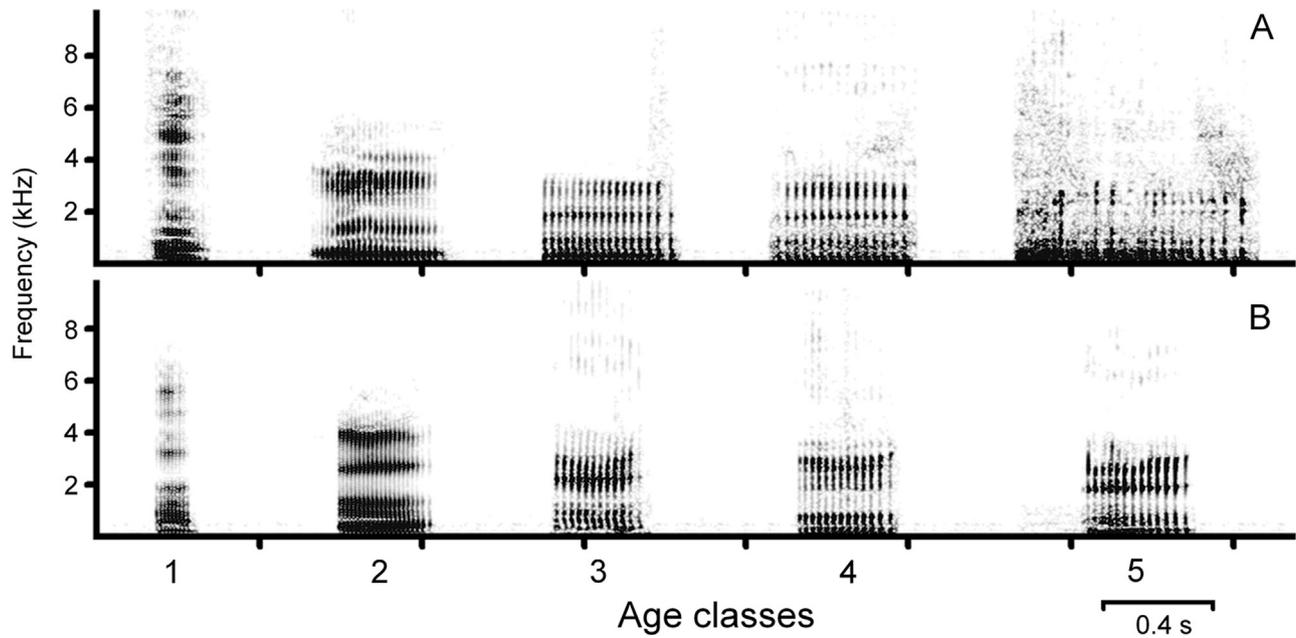


Fig. 4. Spectrogram illustrating nasal contact calls of goitred gazelles across age classes: (A) males, (B) females. Calls of ten different individuals are given, one call per sex per age class. Designations of age classes as in Table 1. The spectrogram was created with Hamming window; 32 kHz sampling rate; FFT 1024 points; frame 50%; and overlap 93.75%. Calls are available as online supplementary material 2.

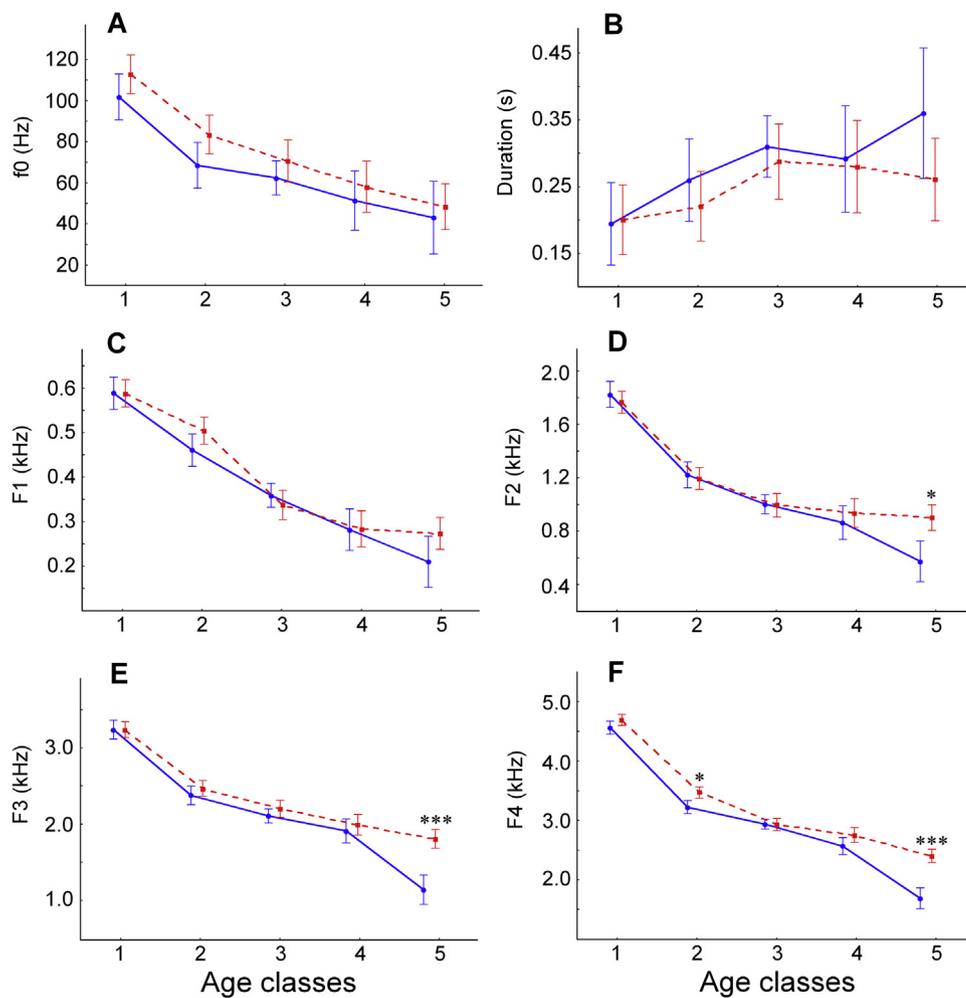


Fig. 5. Age-related changes in (A) f_0 = fundamental frequency, (B) duration, (C) F1 = first formant, (D) F2 = second formant, (E) F3 = third formant and (F) F4 = fourth formant in males (solid line) and females (dashed line). Designations of age classes as in Table 1. Central points show means, whiskers show 0.95 confidence intervals. Significant differences between sexes: * $P < 0.05$, *** $P < 0.001$; Tukey post hoc test.

Table 2

Values (mean ± SD) for body mass and neck dimensions of goitred gazelles across sex and age classes and ANOVA results for effects of age and sex. Designations of age classes as in Table 1.

Age class	Body mass (kg)		Rostral neck circumference (cm)		Caudal neck circumference (cm)		Larynx descent (cm)		Neck length (cm)	
	male	female	male	female	male	female	male	female	male	female
1	3.5 ± 0.1	1.7	17.2 ± 0.4	15.3 ± 1.2	18.8 ± 1.5	18.8 ± 1.3	1.6 ± 0.2	1.7 ± 0.3	13.6 ± 0.6	12.7 ± 1.6
2	10.4 ± 0.9	9.6 ± 0.8	21.0 ± 0.6	18.9 ± 0.4	24.3 ± 1.4	22.4 ± 1.1	3.2 ± 0.3	2.6 ± 0.5	18.9 ± 1.5	18.3 ± 1.3
3	15.2 ± 1.3	14.2 ± 1.5	24.0 ± 1.1	22.5 ± 1.2	30.8 ± 2.3	28.4 ± 2.2	3.2 ± 0.6	2.8 ± 0.4	21.6 ± 1.5	20.7 ± 1.0
4	18.2 ± 2.7	17.5 ± 0.8	26.6 ± 2.1	23.9 ± 0.5	33.5 ± 5.1	31.3 ± 3.5	4.0 ± 1.3	3.4 ± 0.3	24.6 ± 0.5	26.8 ± 1.6
5	28.1 ± 1.7	22.8 ± 2.7	36.4 ± 0.8	24.9 ± 1.0	40.0 ± 1.8	30.5 ± 1.7	8.0 ± 0.7	3.9 ± 0.8	28.8 ± 2.9	29.1 ± 0.9
Age	$F_{4,44} = 165.7; P < 0.001$		$F_{4,53} = 269.6; P < 0.001$		$F_{4,53} = 90.4; P < 0.001$		$F_{4,53} = 76.0; P < 0.001$		$F_{4,53} = 185.9; P < 0.001$	
Sex	$F_{1,44} = 12.7; P < 0.001$		$F_{1,53} = 198.3; P < 0.001$		$F_{1,53} = 27.5; P < 0.001$		$F_{1,53} = 55.1; P < 0.001$		$F_{1,53} = 0.0; P = 1.0$	

Table 3

Values (mean ± SD) for acoustic variables of nasal contact calls of goitred gazelles across sex and age classes and ANOVA results for effects of age and sex. Designations of age classes as in Table 1.

Age class	f0 (Hz)		Duration (s)		F1 (Hz)		F2 (Hz)		F3 (Hz)		F4 (Hz)	
	male	female	male	female	male	female	male	female	male	female	male	female
1	101.8 ± 9.2	112.8 ± 23.8	0.19 ± 0.06	0.20 ± 0.08	592 ± 69	591 ± 51	1825 ± 155	1766 ± 183	3237 ± 187	3237 ± 226	4563 ± 97	4693 ± 192
2	68.5 ± 7.3	83.5 ± 7.1	0.26 ± 0.09	0.22 ± 0.07	464 ± 13	508 ± 43	1222 ± 74	1195 ± 79	2376 ± 100	2467 ± 149	3223 ± 90	3471 ± 150
3	62.4 ± 8.8	70.6 ± 9.6	0.31 ± 0.06	0.29 ± 0.06	362 ± 39	341 ± 35	1002 ± 60	994 ± 106	2106 ± 106	2200 ± 35	2938 ± 79	2931 ± 155
4	51.3 ± 17.5	58.0 ± 14.0	0.29 ± 0.09	0.28 ± 0.06	285 ± 21	287 ± 23	864 ± 34	936 ± 46	1909 ± 144	1991 ± 89	2567 ± 65	2755 ± 75
5	43.0 ± 6.4	48.4 ± 2.2	0.36 ± 0.03	0.26 ± 0.07	213 ± 7	277 ± 23	575 ± 20	901 ± 108	1139 ± 7	1805 ± 57	1688 ± 21	2405 ± 88
Age	$F_{4,43} = 34.1; P < 0.001$		$F_{4,43} = 4.8; P = 0.003$		$F_{4,43} = 117.6; P < 0.001$		$F_{4,43} = 141.7; P < 0.001$		$F_{4,43} = 207.7; P < 0.001$		$F_{4,43} = 563.9; P < 0.001$	
Sex	$F_{1,43} = 6.2; P < 0.05$		$F_{1,43} = 2.6; P = 0.11$		$F_{1,43} = 2.1; P = 0.15$		$F_{1,43} = 3.5; P = 0.06$		$F_{1,43} = 21.1; P < 0.001$		$F_{1,43} = 47.5; P < 0.001$	

Table 4

Values (mean ± SD) for anatomical variables of goitred gazelles across sex and age classes and GLMM results for effects of age and sex. Designations of age classes as in Table 1.

Age class	Skull basal length (mm)		Nasal vocal tract length (mm)		Larynx maximum height (mm)		Dorsoventral vocal fold length (mm)	
	male	female	male	female	male	female	male	female
1	96 ± 8	99 ± 9	127 ± 13	141 ± 5	20 ± 1	21 ± 1	7 ± 1	8 ± 2
2	138 ± 1	123 ± 14	205 ± 7	165 ± 21	29 ± 1	29 ± 1	14 ± 2	14 ± 0
3	153 ± 7	145 ± 10	218 ± 7	199 ± 19	33 ± 4	31 ± 0	14 ± 3	12 ± 0
4	179 ± 9	no data	251 ± 16	no data	44 ± 2	no data	20 ± 2	no data
5	192 ± 8	179 ± 2	315 ± 30	255 ± 6	54 ± 6	43 ± 3	28 ± 5	17 ± 2
Age	$F_{4,15} = 95.8; P < 0.001$		$F_{4,17} = 44.4; P < 0.001$		$F_{4,15} = 53.2; P < 0.001$		$F_{4,15} = 20.5; P < 0.001$	
Sex	$F_{1,15} = 5.4; P = 0.03$		$F_{1,17} = 9.0; P = 0.008$		$F_{1,15} = 4.2; P = 0.06$		$F_{1,15} = 6.2; P = 0.02$	

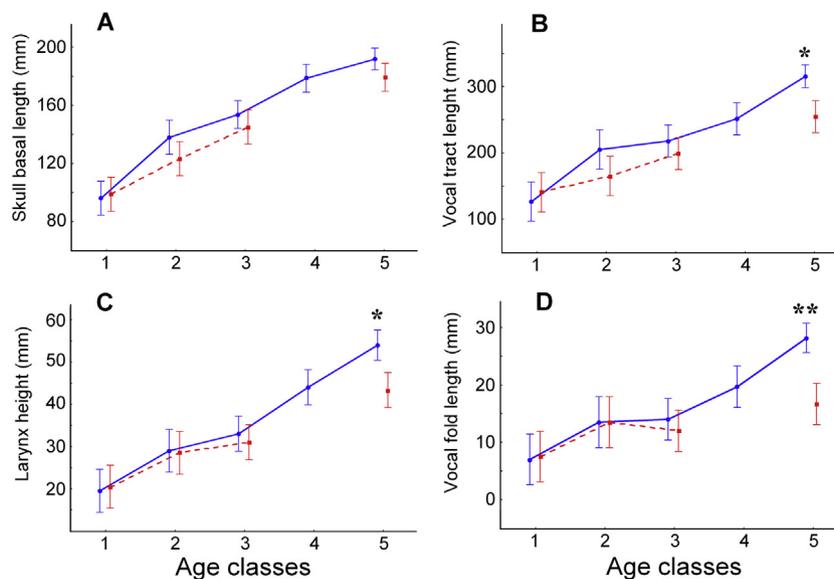


Fig. 6. Age-related changes in (A) skull basal length, (B) nasal vocal tract length, (C) maximum height of the larynx and (D) dorsoventral vocal fold length in males (solid line) and females (dashed line). Designations of age classes as in Table 1. Central points show means, whiskers show 0.95 confidence intervals. Significant differences between sexes: * $P < 0.05$, ** $P < 0.01$; Tukey post hoc test.

Table 5
Comparison of values for the nasal vocal tract length measured directly from the anatomical specimens and calculated by linear regression based on formant dispersion. Designations of age classes as in Table 1.

Age class	Nasal vocal tract length (mm)					
	Measured anatomically		Calculated by formant dispersion		Ratio calculated/measured	
	male	female	male	female	male	female
1	127 ± 13	141 ± 5	136	134	1.1	0.95
2	205 ± 7	165 ± 21	191	180	0.93	1.1
3	218 ± 7	199 ± 19	213	211	0.98	1.1
4	251 ± 16	no data	242	227	0.96	no data
5	315 ± 30	255 ± 6	378	255	1.2	1.

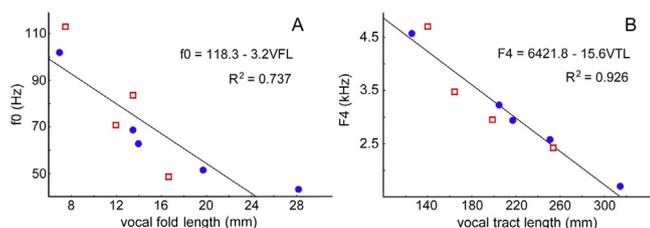


Fig. 7. Correlations between (A) the dorsoventral length of the vocal folds (VFL) and the fundamental frequency (f_0) and (B) the length of the nasal vocal tract (VTL) and the fourth formant (F_4). Mean values per age class for each sex are given. Solid circles indicate males, empty squares indicate females.

were detected only in adults (age class 5) (Fig. 6). The skull basal length did not differ significantly between sexes at any age class (Fig. 6).

3.4. Relationship between anatomy and the acoustics

We compared nasal vocal tract lengths measured directly from the anatomical specimens with those calculated by linear regression based on formant dispersion of call formants (Table 5). Unlike the vocal tract length obtained during the anatomical dissections, the measure based on formant dispersion reflects the nasal vocal tract length during vocal emission. In adult males (age class 5), differences between calculated and measured values exceeded 20%, whereas in all other sex and age classes the differences did not exceed 10% (Table 5).

For both sexes together across age classes, we compared the dorsoventral length of the vocal fold with respective values of the fundamental frequency of the nasal contact calls as well as the nasal vocal tract length with the fourth formant values (Fig. 7). Significant negative correlations were found in both comparisons ($r = -0.86$; $P < 0.01$; $n = 9$, and $r = -0.96$; $P < 0.001$; $n = 9$, respectively). For each sex, samples were $n = 5$ for males and $n = 4$ for females, which was insufficient for conducting regression analyses.

4. Discussion

This study for the first time follows the vocal ontogeny from birth to adulthood in both sexes of a ruminant species: the goitred gazelle. We found that sexual dimorphism is not prominent for most vocal and physical variables before reaching adulthood. While significant sex differences in the rostral neck circumference emerged as early as at 2–3 mo of age (age class 2), significant sex differences in body mass, caudal neck circumference, larynx descent, nasal vocal tract length, maximum height of the larynx and vocal fold length could only be detected in mature adults. Both fundamental and formant frequencies of the nasal calls gradually decreased with age, but significant differences in formant frequencies did not emerge prior to adulthood. The neck length and basal skull length did not differ significantly between sexes at any age.

Although the measured values of the mean fundamental frequency of male nasal contact calls were lower than the respective values in females in any age class, statistically significant sex differences were only detectable between adult males and adult females. However, the achievable fundamental frequencies are not exclusively determined by the length of the vocal folds in the larynx but also by their tension, which is affected, for instance, by the thyroarytenoid muscle, which shortens the vocal folds, and the cricothyroid muscle, which lengthens the vocal folds (Titze, 1994). Interestingly, the measured mean f_0 of adult male goitred gazelles can vary considerably. The mean f_0 of the nasal contact calls of adult males (43 Hz) was close to that of the nasal contact calls of adult females (48 Hz, Table 3). However, the mean f_0 of the nasal contact calls of adult males was twice as high as the mean f_0 of male rutting calls (22 Hz, Frey et al., 2011), which are produced orally. These data suggest that adult males do not produce their nasal contact calls with the lowest possible f_0 . Presumably, pronounced larynx retraction favours maximal relaxation of the vocal folds during production of the rutting calls to achieve lowest possible f_0 (Frey et al., 2011). In contrast, maximal larynx retraction is not necessary during production of the nasal contact calls. Accordingly, the dominating influence of the extrinsic retractor muscles on vocal fold state diminishes and the tension of the vocal fold appears to increase to a medium level, mainly controlled by integrated action of the intrinsic thyroarytenoid and cricothyroid muscles (Efremova et al., 2016). This would allow the f_0 of adult male nasal contact calls to remain close to that of adult females.

When goitred gazelles reach adulthood (age class 5), significant sexual differences arise regarding nasal vocal tract length and formant frequencies, especially in the third and fourth formants. According to our data, significant sexual differences in skull basal length and in neck length do not occur in goitred gazelles at any age, including adults. At the same time, larynx descent was found to be significantly more expressed in male than in female adults. Therefore, the difference in nasal vocal tract length between adult males and adult females appears to be a direct consequence of the more pronounced descent of the larynx in males.

In female goitred gazelles, the values of the nasal vocal tract length, calculated on the basis of the nasal call formant frequencies, corresponded well to those measured in the anatomical specimens within each age class. This also applied to age classes from neonates to yearlings of males. In adult males (age class 5), however, we observed substantial disparities. The adult male nasal vocal tract length as based on nasal call formants exceeded the nasal vocal tract length taken from the anatomical specimens by 20%. This result suggests that adult males moderately retract the larynx during nasal call production, and this may entail a limited additional elongation of the vocal tract and a corresponding decrease of the formant frequencies of the nasal calls compared to the expected frequencies if the larynx remained stationary at its resting position. And this dynamic vocal tract elongation would of course escape notice in measurements taken during dissections. In contrast, adult male rutting roars are produced orally under strong retraction of

the larynx (Frey et al., 2011; Blank et al., 2014, 2015). This results in vocal tract extension by 47%, causing a more pronounced decrease of formants F1–F4 (adult male nasal contact calls: 213, 575, 1139 and 1688 Hz, this study; adult male rutting roars: 253, 490, 982 and 1401 Hz, respectively, Frey et al., 2011). Consistently, the elongation of the nasal vocal tract by 20% in adult male saigas due to extension of the nose during production of their nasal rutting calls entailed a corresponding decrease of formants by 21% (Frey et al., 2007).

We did not find a sexual dimorphism of neck length in goitred gazelles whereas a significant sex difference of neck length has been documented in humans, although it is small (~12 mm) in absolute terms (Zheng, 2011). In contrast to goitred gazelles, the human neck is much shorter and the double-descended larynx of adult male humans is not located in a mid-neck resting position, but towards the caudal end of the neck at the level of the clavicle (Fitch and Giedd, 1999; Davidson, 2003). As a consequence, formant decrease in adult male humans cannot be enhanced by larynx retraction as much as in male goitred gazelles because the larynx position is already very close to the origins of the laryngeal retractor muscles, dorsally from the sternal manubrium and the sternoclavicular articulation (Schünke et al., 2009).

In a few nonhuman short-necked species with a descended larynx, however, drastic anatomical changes have evolved, probably for achieving larynx retraction in spite of having a short neck. In the 'roaring felids' of the genus *Panthera* and in the marsupial koala, parts of the suspensory elements of the hyoid apparatus transformed into highly resilient ligaments and the main retractor muscles of the larynx extended their origins down into the thorax (Weissengruber et al., 2002; Charlton et al., 2013). Presumably, these profound anatomical changes allow for larynx retraction despite an already low larynx position in these short-necked species. As a consequence, call-synchronous vocal tract extensions are expected to occur in these species. Humans also evolved a resilient stylohyoid ligament but pronounced larynx retraction down into the thoracic inlet might be counter-selected.

Other species of ruminants with a descended larynx, such as Mongolian gazelle, red and fallow deer, have relative neck lengths comparable to that of goitred gazelles and their main retractor muscles of the larynx, as in goitred gazelles, do not extend down into the thorax but take their origin from the sternal manubrium. Accordingly, these species convergently evolved call-synchronous larynx retraction, vocal tract extension and formant decrease in a way similar to goitred gazelles (Fitch and Reby, 2001; Frey and Gebler, 2003; McElligott et al., 2006; Frey et al., 2008a, 2011, 2012; Efremova et al., 2016).

The primary function of the larynx is protective; it prevents solid food particles and liquids from entering the trachea by reflexive closure. If this action fails and a particle or a small amount of liquid has entered the infraglottic cavity or the upper trachea, it may be expelled by coughing (Wadie et al., 2013; Hall, 2016). The importance of its protective function might explain why humans and goitred gazelles are born with a non-descended larynx. In human newborns, a descended larynx would interfere with the safe expelling of amniotic fluid from the oral and nasal vocal tract and the fast milk suckling during the first weeks of life (Bolisetty et al., 2001; Iwadata et al., 2001; Kibayashi et al., 2004; Hermansen and Lorah, 2007; Warren and Anderson, 2010). In addition, a shorter oral vocal tract can be more easily cleared of foreign substances by coughing compared to an elongated vocal tract. Therefore, a descended larynx in neonate and juvenile goitred gazelles and humans is probably counter-selected.

In goitred gazelles, liquid and solid food material can pass around the laryngeal entrance during swallowing or regurgitation without the danger of choking. To retain this separation during development, laryngeal descent in both female and male goitred

gazelles involves an elongation of the soft palate, a corresponding caudal shift of the intra-pharyngeal ostium and an increased mobility of the larynx (Efremova et al., 2016). For deglutition and regurgitation the larynx is protracted. Protraction involves a shortening of the soft palate by contraction of its intrinsic muscles including the constrictor muscles of the intrapharyngeal ostium. In adult male goitred gazelles with descended larynx, the intra-pharyngeal ostium is narrow and tightly surrounds the laryngeal entrance most of the time (Frey et al., 2011; Efremova et al., 2016).

In contrast, in human ontogeny the larynx loses contact with the soft palate (Fitch and Giedd, 1999). The larynx of adult male and female humans is permanently located inside the oral portion of the pharynx and the intrapharyngeal ostium becomes extremely wide. This results in high risk of suffocation in humans (Fitch and Giedd, 1999). In adolescent boys, this risk is three times higher than in adolescent girls because of the male-specific larynx descent at puberty (Baker et al., 1992).

Data from the present study are in good agreement with those of previous short-term longitudinal studies on the vocal ontogeny in goitred gazelles from soon after birth up to 6 months-old adolescents, corresponding to age class 3 of the present study (Efremova et al., 2011a, 2011b). In our previous studies of early vocal ontogeny, sexual differences in acoustic and body mass variables were detected as early as at 2.5 months of age (Efremova et al., 2011a) and in neck variables as early as at 1.5 months of age (Efremova et al., 2011b), whereas the first and second formants did not differ even at 6 months of age (Efremova et al., 2011a). The differences between the two studies possibly arose because in the previous study we used 23 goitred gazelles of matched age, which were repeatedly tested in all age classes, resulting in rather small and similar parameter variations during the first 6 months of ontogeny (Efremova et al., 2011a, 2011b). In the present study, each live animal and each anatomical specimen was included at only one time in only one of the 5 age classes, which resulted in a more uneven distribution of parameters and increased values of standard deviation.

Together with previous studies (Efremova et al., 2011a, 2011b, 2016; Frey et al., 2011), the results presented here suggest that the sexually divergent development of the vocal organs in goitred gazelles begins as early as at 2–3 months of age and proceeds at a slow rate towards early adulthood in both sexes. Then the ontogenetic transformation of the vocal organs accelerates in males but flattens in females. The most pronounced changes of the male vocal organs occur in late ontogeny when males reach social sexual maturity, i.e. at an age older than 2 years, when their chance of siring offspring distinctly increases (Blank, 1998; Frey et al., 2011; Blank et al., 2014, 2015; Efremova et al., 2016). This indicates the important role of the enlarged and descended larynx for signaling male status via masculine voice in the context of male reproductive success (Blank, 1998; Frey et al., 2011; Blank et al., 2014), as has been previously reported for humans. In humans, the sexual dimorphism of the larynx and vocal anatomy results in male-specific voice characteristics: a lower f_0 (Titze, 1994; Evans et al., 2008) and decreased formants (Rendall et al., 2005). In industrial communities, both these acoustic characteristics affect the impression of dominance of men's voices as perceived by other men and by women (Puts et al., 2006, 2007) and they are also attractive for women (Collins, 2000; Feinberg et al., 2005, 2006; Puts, 2005; Saxton et al., 2006). Among African hunter-gatherers, men with low-frequency voices have a higher reproductive success compared to men with higher-frequency voices, apparently due to female choice (Apicella et al., 2007; Apicella and Feinberg, 2009). Similar to the Adam's apple of human males, this may indicate the important role of the enlarged and descended larynx for signaling male status via masculine voice in goitred gazelles.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.zool.2016.09.001>.

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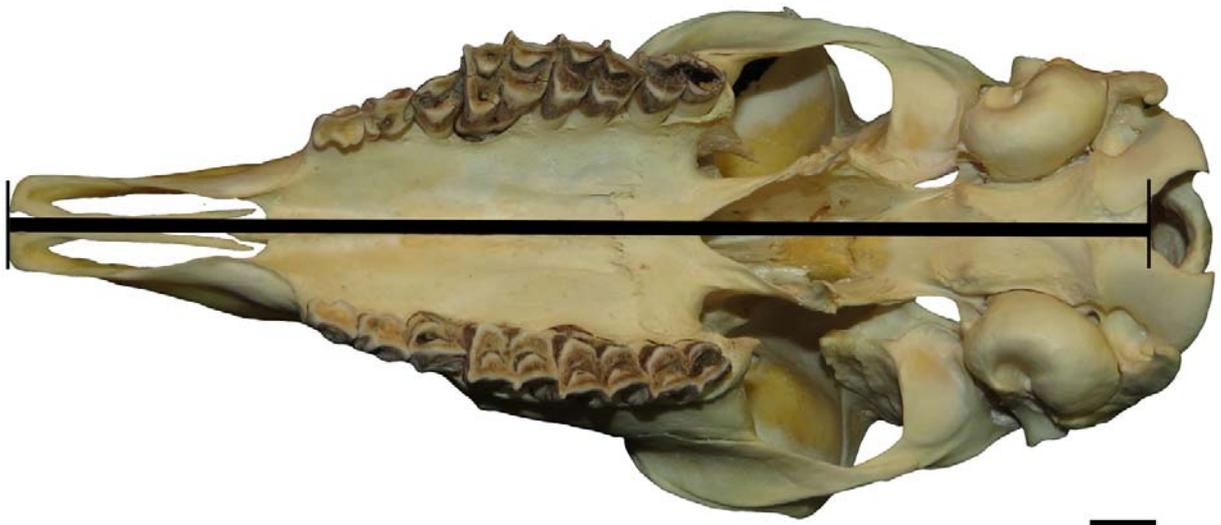
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Supplementary material 1

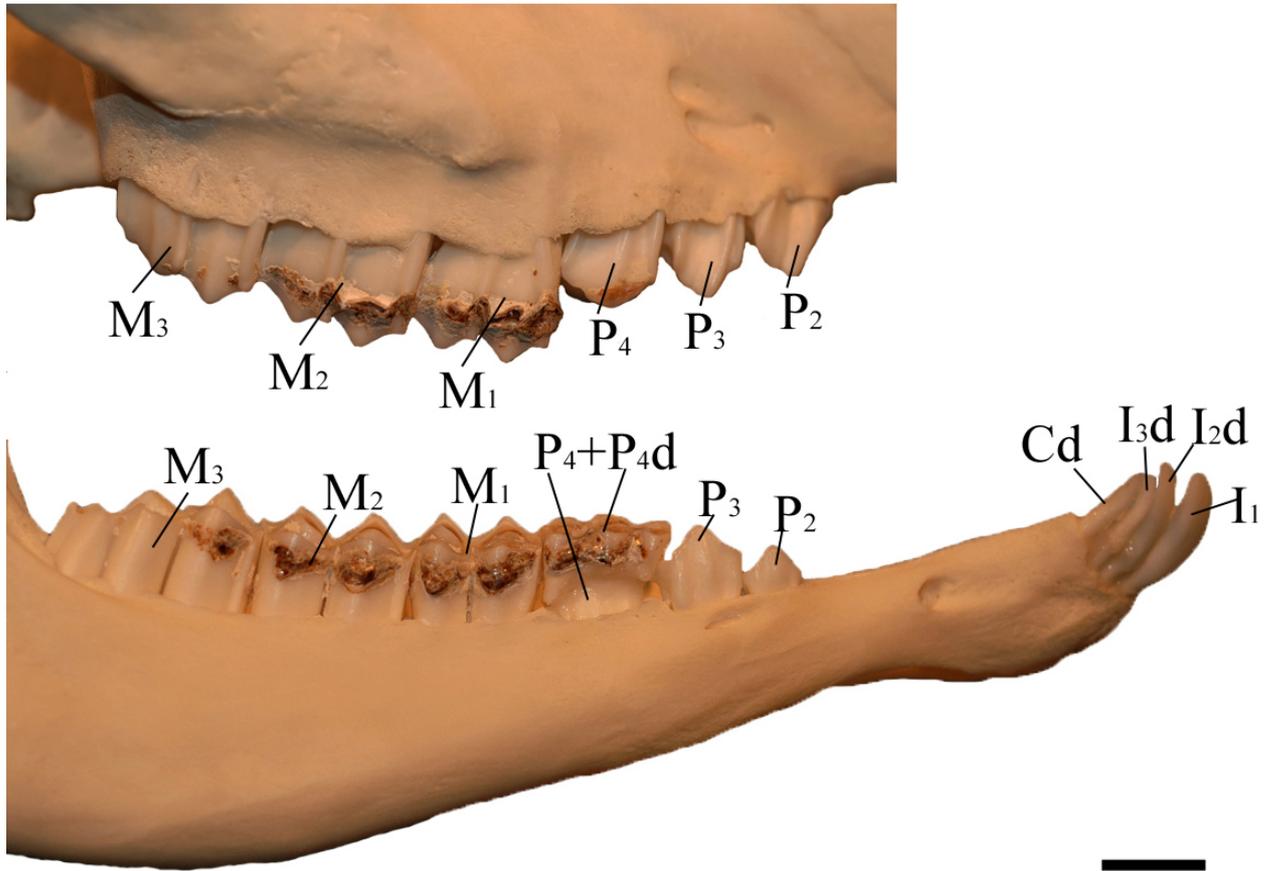
Age estimates for anatomical specimens of unknown age

We could estimate the age of goitred gazelle specimens whose age at death was not documented by using: 1) the skull basal lengths (Supplementary Fig. 1) and dentition (Supplementary Fig. 2) of goitred gazelles of known age (9 of the 19 males and 5 of the 11 females) of this study; 2) the skull basal lengths of 61 skulls of goitred gazelles of different ages from museum collections (Supplementary Table 1) (Mambetjumaev, 1970); 3) a table of dentition changes along ontogeny in domestic goats (Nickel et al., 1987). For male skulls, we also estimated the length of the horns.

To describe the state of dentition, we used the number and ratio of deciduous and permanent teeth (Supplementary Fig. 2): incisors (*dentes incisivi*), canines (*dentes canini*), premolars (*dentes premolares*) and molars (*dentes molares*) and the stages of their eruption. The deciduous and permanent teeth could be distinguished, because the crowns of the deciduous teeth were always smaller and whiter compared to the permanent teeth. By degree of teeth appearance over the jaw bones we established three stages of teeth eruption: not yet erupted, in the state of eruption, fully erupted.



Supplementary Fig. 1. Skull basal length (black line). Photo of the skull of an adult female goitred gazelle, ventral view. Scale bar 10 mm.



Supplementary Fig. 2. The right upper and lower jaws of a 20-month-old adolescent male illustrating the dentition (lateral view). The change of deciduous to permanent teeth is shown. I = incisors, C = canines, P = premolars, M = molars, d = deciduous. Scale bar 10 mm.

Supplementary Table 1. Ranges of age-classes for male and female goitred gazelles based on the dentition and the basal length of the skull (mean±SD). I = incisors, C = canines, P = premolars, M = molars, d = deciduous, (d) = degree of eruption of permanent teeth at this age varied among individuals, 0 = the tooth has not yet erupted. Basal skull lengths were measured in the specimens of this study and in the study of Mambetjumaev (1970).

Age class	Dentition	Basal skull length (mm)			
		This study		Mambetjumaev (1970)	
		male	female	male	female
1	P ₂ d P ₃ d P ₄ d M ₁ 0 M ₂ 0 M ₃ 0	96±8	99±9	105	109
	I ₁ d I ₂ d I ₃ d Cd P ₂ d P ₃ d P ₄ d M ₁ 0 M ₂ 0 M ₃ 0	n=2	n=2	n=1	n=1
2	P ₂ d P ₃ d P ₄ d M ₁ M ₂ 0 M ₃ 0	138±1	123±14	127±14	130±4
	I ₁ d I ₂ d I ₃ d Cd P ₂ d P ₃ d P ₄ d M ₁ M ₂ 0 M ₃ 0	n=2	n=2	n=5	n=3
3	P ₂ d P ₃ d P ₄ d M ₁ M ₂ M ₃ 0	154±6	145±10	157±6	158±5
	I ₁ d I ₂ d I ₃ d Cd P ₂ d P ₃ d P ₄ d M ₁ M ₂ M ₃ 0	n=3	n=2	n=14	n=8
4	P ₂ (d) P ₃ (d) P ₄ (d) M ₁ M ₂ M ₃	179±9	no data	173±1	168±9
	I ₁ (d) I ₂ (d) I ₃ (d) C(d) P ₂ P ₃ P ₄ M ₁ M ₂ M ₃	n=3		n=2	n=11
5	P ₂ P ₃ P ₄ M ₁ M ₂ M ₃	192±8	179±2	191±5	181±6
	I ₁ I ₂ I ₃ C P ₂ P ₃ P ₄ M ₁ M ₂ M ₃	n=5	n=3	n=9	n=7

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