



Original Investigation

Sex and age-class differences in calls of Siberian wapiti *Cervus elaphus sibiricus*

Ilya A. Volodin^{a,b,*}, Olga V. Sibiryakova^a, Elena V. Volodina^b^a Department of Vertebrate Zoology, Faculty of Biology, Lomonosov Moscow State University, Vorobievsky Gory, 1/12, Moscow 119991, Russia^b Scientific Research Department, Moscow Zoo, B. Gruzinskaya, 1, Moscow 123242, Russia

ARTICLE INFO

Article history:

Received 20 April 2015

Accepted 9 September 2015

Handled by Luca Corlatti

Available online 21 September 2015

Keywords:

Acoustic communication

Gender differences

Developmental pathway

Farmed animals

Subspecies vocal indices

ABSTRACT

Stag rutting calls are strongly different among subspecies of red deer *Cervus elaphus*. Studying sex-, age- and subspecies-related vocal variation may highlight the forces driving the evolution of vocal communication in this species after their expansion from Central Asia to Europe and North America, however, this information was lacking so far for any Asian subspecies of *Cervus elaphus*. We analysed frequency, temporal and power variables of contact and bugle calls, collected from 63 Siberian wapiti *Cervus elaphus sibiricus*, the most abundant Asian subspecies of red deer. The open-mouth (oral) and closed-mouth (nasal) contact calls were registered in all sex and age-classes, whereas the open-mouth bugles were found in both stags and hinds but not in the calves. The maximum fundamental frequency (f_{0max}) of contact calls was similar between calves and hinds. Similarly to American subspecies, the small differences of f₀ between calls of the young and adults in *C. e. sibiricus* suggests only a minor ontogenetic decrease of call fundamental frequency compared to European subspecies of red deer. At the same time, the call f₀ of all sex and age-classes of *C. e. sibiricus* was substantially higher compared to those of European subspecies of red deer (*C. e. hippelaphus*, *C. e. corsicanus*, *C. e. italicus* and *C. e. hispanicus*), although lower than in any studied American subspecies (*C. e. roosevelti* and *C. e. canadensis*). These findings provide vocal cues to indicate subspecies of *Cervus elaphus*, in addition to existing molecular and morphological traits.

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Introduction

Genetic studies suggest that *Cervus elaphus* originated in Middle Asia and then slowly spread to Asia (Siberia, Kazakhstan, India, China) and further to Northern America (Mahmut et al. 2002; Ludt et al. 2004; Kuznetsova et al. 2012; Mukesh et al. 2015), and to Europe (Zachos and Hartl 2011; Meiri et al. 2013). This global geographic radiation over the Holarctic region resulted in a continuum of subspecies or recent species, showing a strong divergence of vocal characteristics between European, Asian and American subspecies of *Cervus elaphus* (e.g. Frey and Riede 2013; Volodin et al. 2013a, 2015a). Studying vocal divergence across subspecies and sex and age-classes of *Cervus elaphus* might help in tracing the evolution of vocal communication in this species. However, current data are insufficient for the general synthesis, as sex- and

age-related acoustic variation is poorly investigated in American (Bowyer and Kitchen 1987; Feighny et al. 2006) and only scarcely studied in Asian subspecies (Volodin et al. 2013b, 2015b). This study is intended to partially fill this gap in the knowledge, by studying the acoustic variation within one of the Asian subspecies, the Siberian wapiti *C. e. sibiricus*.

Red deer adult males (stags) use rutting calls for deterring rival males and for attracting receptive females (Clutton-Brock and Albon 1979; Reby et al. 2005; Charlton et al. 2007; Frey et al. 2012), whereas adult females (hinds) and calves use their contact calls for mother-offspring communication (Vankova et al. 1997; Kidjo et al. 2008; Teichroeb et al. 2013; Sibiryakova et al. 2015). Very rarely, red deer hinds are capable of producing call patterns that are indistinguishable from stag rutting calls (Feighny et al. 2006), whereas stag contact calls were not registered to date in any subspecies.

The call fundamental frequency (f₀), reflecting the rate of vibration of vocal folds in the larynx, represents the main demarcating acoustic trait between European and Asian/American branches of *Cervus elaphus*. Whereas European subspecies of *Cervus elaphus* produce calls with relatively low maximum f₀, ranging of 52–270 Hz (Reby and McComb 2003; Kidjo et al. 2008; Frey et al.

* Corresponding author at: Department of Vertebrate Zoology, Faculty of Biology, Lomonosov Moscow State University, Vorobievsky Gory, 1/12, Moscow 119991, Russia.

E-mail address: volodinsvoc@gmail.com (I.A. Volodin).

2012; Bocci et al. 2013; Passilongo et al. 2013), the Asian and American subspecies produce calls with very high values of maximum f₀, ranging of 660–2080 Hz (Struhsaker 1968; Bowyer and Kitchen 1987; Feighny et al. 2006; Volodin et al. 2013b, 2015a,b). In stags and hinds, f₀ values are closer within than between subspecies (Volodin et al. 2015a).

Stag rutting calls represent low-frequency roars in European subspecies and high-frequency bugles in Asian/American subspecies. Patterns of stag rutting roars were described in many European subspecies: *C. e. scoticus* (Long et al. 1998; Reby and McComb 2003), *C. e. corsicanus* (Kidjo et al. 2008), *C. e. hispanicus* (Frey et al. 2012; Passilongo et al. 2013) and *C. e. italicus* (Della Libera et al. 2015). Patterns of stag rutting bugles were described in three American subspecies: *C. e. canadensis* (Struhsaker 1968; Feighny et al. 2006); *C. e. roosevelti* (Bowyer and Kitchen 1987) and *C. e. nelsoni* (Frey and Riede 2013) and in three Asian subspecies: *C. e. sibiricus* (Nikol'skii 2011; Volodin et al. 2013b), *C. e. bactrianus* (Nikol'skii 1975; Volodin et al. 2013a) and *C. e. xanthopygus* (Volodin et al. 2015b). Hind bugles, perfectly imitating stag rutting bugles, were described only for hinds of *C. e. canadensis* in non-rut (calving) period (Feighny et al. 2006).

The voice fundamental frequency, generated by vocal folds in the larynx, is commonly higher in the young than in the adults within species, with minor exclusions primarily among small mammals (e.g. Matrosova et al. 2007, 2011; Volodin et al. 2015c). This is because acoustic differences in f₀ result from the differences in sizes of sound-producing structures (Fitch and Hauser 2002) and their biomechanical properties (Riede and Brown 2013). Within subspecies of *Cervus elaphus*, the maximum f₀ varies inconsistently among age classes. Between calf and hind calls of European subspecies of *Cervus elaphus*, the maximum f₀ is higher in calf than in hind calls (*C. e. hippelaphus*: Vankova et al. 1997; *C. e. corsicanus*: Kidjo et al. 2008; *C. e. hispanicus*: Sibiryakova et al. 2015; Volodin et al. 2015a). For American subspecies, some scarce information suggests that maximum f₀s are similar between hinds and calves (Feighny 2005). In Asian subspecies, the differences in call f₀ between hinds and calves were not yet studied to date.

The species *Cervus elaphus* seems unique among mammals, with lower f₀ in smaller subspecies (Volodin et al. 2015a). Within subspecies, the maximum f₀ varies inconsistently among sex classes of adults. Between hind and stag calls of European subspecies of *Cervus elaphus*, the maximum f₀ is slightly lower in hinds than in stags in *C. e. hispanicus* (Volodin et al. 2015a) and is higher in hinds than in stags in *C. e. corsicanus* (Kidjo et al. 2008). In an American subspecies (*C. e. canadensis*), the maximum f₀ has the same values in hinds and stags (Feighny et al. 2006). In Asian subspecies, the ratios of male and female f₀ are unknown so far. So, one general focus of this study was to investigate the ratios of fundamental frequencies between stag, hind and calf calls in Asian subspecies of *Cervus elaphus*, represented here by *C. e. sibiricus*.

Contact calls of ungulates, including red deer, may be produced at separation of animals from group members (review: Lingle et al. 2012; Padilla de la Torre et al. 2015) or during everyday activity, e.g. food anticipation (e.g. Volodin et al. 2011). Contact calls are made either through an opened mouth (oral calls), or through the nose, with a closed mouth (nasal calls). The oral and nasal modes of vocal production were previously reported for the young of white-tailed deer *Odocoileus virginianus* (Richardson et al. 1983), goitred gazelles *Gazella subgutturosa* (Efremova et al. 2011; Volodin et al. 2011), for mother domestic sheep *Ovis aries* (Sebe et al. 2010) and domestic cattle *Bos taurus* (Padilla de la Torre et al. 2015), for mother and young saiga antelopes *Saiga tatarica* (Volodin et al. 2014) and red deer *C. e. hispanicus* (Sibiryakova et al. 2015; Volodin et al. 2015a). Oral calls are produced at situations of higher arousal (Volodin et al. 2011; Padilla de la Torre et al. 2015) and more individualized compared to the nasal calls (Volodin et al. 2011; Sibiryakova et al. 2015).

In red deer, oral and nasal contact calls may be produced in the same sequences (Sibiryakova et al. 2015; Volodin et al. 2015a).

Vocalizations of ungulates have been proposed as potential indicators of animal welfare (Briefer 2012; Manteuffel et al. 2004; Briefer et al. 2015; Padilla de la Torre et al. 2015). The *C. e. sibiricus* is the most important cervid species among farmed production animals of Russia, China and Kazakhstan, as it is intensively bred for velvet antlers and meat since 40s years of 19th century to nowadays (Lunitsin and Borisov 2012). In Korean markets, the velvet antlers of this subspecies are considered to be of particularly good quality and command the highest prices (Kim et al. 2015).

Welfare standards are not yet established for red deer of *C. e. sibiricus* that are kept at the deer farms. This study of basic vocal variation of *C. e. sibiricus*, living in captivity in good conditions, provides important reference information, representing a startpoint, against which further research would compare vocal parameters recorded under conditions of poor or good welfare. The objectives of this study were (1) to compare the acoustic structure of contact calls among Siberian wapiti calves, hinds and stags; (2) estimate the effect of nasal versus oral vocal emissions for variables of contact calls; (3) compare the acoustic structure of bugle calls produced by Siberian wapiti stags and hinds.

Material and methods

Study sites and subjects

Calls were collected in 2004–2015 at three zoos (Tierpark Berlin, Germany, Novosibirsk Zoo, Russia, and St. Petersburg Zoo, Russia) and two farms (Kazakhstan farm, located at 49°16'N, 86°07'E and Kostroma farm, located at 58°24'N, 43°15'E), from the total of 63 Siberian wapiti. Fifty-eight animals were identified as being distinct animals during recordings with hand-held microphones (15 calves, 36 hinds and 7 stags) and 5 stags were not identified as being distinct animals from automated recordings without a researcher present (Table A.1). At Tierpark Berlin, animals were kept in a harem group, including one stag, 3 hinds and 3 calves (aged 3–4 months); all these animals provided calls for this study. At Novosibirsk Zoo, animals were kept in a harem group, including one adult stag, 3 hinds and a few calves; the stag and all the hinds provided calls for this study. At Saint Petersburg Zoo, one stag was kept together with one hind; only the stag provided calls for this study. At Kazakhstan deer farm, animals were free-ranging in a large herd of unknown number of animals; one hind provided calls for this study. At Kostroma deer farm, animals were free-ranging in a herd of 132 animals including 37 stags, 60 hinds and 35 calves (aged 1–45 days); the stags were kept separately from hinds and calves and were mixed with them during rut period in September–November. Twelve calves, 27 hinds and four stags identified as being distinct animals and five individually indistinguishable stags of Kostroma deer farm provided calls for this study (Table A.1).

Call collection

For acoustic recordings (48 kHz, 16 bit), we used a solid state recorder Marantz PMD-660 (D&M Professional, Kanagawa, Japan) with a AKG-C1000S cardioid electret condenser microphone (AKG-Acoustics GmbH, Vienna, Austria) or a Sennheiser K6-ME66 cardioid electret condenser microphone (Sennheiser electronic, Wedemark, Germany). The distance from the hand-held microphone to the animals was 5–100 m. We used AKG-C1000S for recordings at distances of 5–30 m and Sennheiser K6-ME66 for recordings at distances 30–100 m. Certain types of calls typically were not recorded from different distances than other types of calls; the variation in recording distance could not influence the

temporal and frequency acoustic characteristics of calls, measured in this study. We recorded calls in light time of day, often with synchronous video, using a digital camcorder Panasonic HDC-HS100 (Panasonic Corp., Kadoma, Japan). During recordings, individual identities of callers producing calls through the mouth and through the nose were labelled by voice. Recordings have been conducted both inside and outside the outdoor enclosures. In addition, for recordings of stag bugle rutting calls at Kostroma deer farm, we used recordings (22.05 kHz, 16 bit) made in the absence of researchers with an automated recorder system SongMeter SM2+ (Wildlife Acoustics Inc., Maynard, MA, USA) mounted on trees at 2 m above the ground in places where stags were most active during the rut, and programmed to record 5 min per hour, 24 h per day. The recording system was equipped with two omni-directional microphones, fixed horizontally at 180° to each other. All the three sets of recording equipment provided comparable qualitative recordings, perfectly covering the range of frequencies produced by study animals. In all cases the contact calls were made as part of the animals' normal activities within a social group; no artificial manipulation or isolation was applied during the recording process.

Call samples

For acoustic analyses, we took only calls of good quality that were not disrupted by wind or the calls of other animals or overloaded during the recordings and with signal-to-noise ratios sufficient for analysis of all acoustic variables measured in this study. Contact calls were classified to nasal and oral types based on voice comments of researchers made during recording, by video clips, made synchronously with the recordings, or by the nasal quality of sound within a recording. 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. 2015a), for goitred gazelles (Volodin et al. 2011; Lapshina et al. 2012) and for saiga antelopes (Volodin et al. 2014). Calls, starting with closed mouth and ending orally, with approximately equal in duration nasal and oral parts, were excluded from analysis. Calls with short nasal initial part (less than 10% of the total call duration) were included with the sample of oral calls. 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.

Contact calls of calves and stags were collected in non-rut periods, in June and in December. Contact calls of hinds were recorded either out of rut periods in June, July and December or during rut period lasting from August to November (Table A.1). We included in the analyses of contact calls animals which provided at least two measurable contact calls. Only 5 calves, 11 hinds and 2 stags provided both oral and nasal contact calls. Five calves, 17 hinds and 1 stag provided only oral contact calls, whereas another 5 calves, 6 hinds and 2 stags provided only nasal contact calls. In total, we included in the analyses 443 contact calls, 288 oral contact calls: 71 from 10 calves (mean \pm SE = 7.1 ± 2.5), 195 from 28 hinds (7.0 ± 0.8) and 22 from 3 stags (7.3 ± 2.9); and 155 nasal contact calls: 49 from 10 calves (4.9 ± 1.2), 83 from 17 hinds (4.9 ± 1.3), 23 from 4 stags (5.8 ± 1.4).

Orally produced hind bugles were collected in non-rut period in June, whereas the orally produced stag bugles were recorded in rut period in August–October (Table A.1). In total, we included in the analyses 11 bugles from 2 hinds, 2 and 9 bugles respectively; and 22 bugles from 2 stags (6 and 16 bugles respectively). In addition, 48 stag bugles, recorded from 5 stags, individually unidentified from automated recordings, were selected from 1080 sound files, evenly distributed among recordings collected between 13 September and 25 October 2013, which reduced the possibility

of over-representation of certain individuals. In total, we took for analyses 524 calls: 288 oral contact calls, 155 nasal contact calls and 81 bugle calls (Table A.1).

Call analysis

Acoustic analyses were conducted in the same way for calves, hinds and stags and for all types of calls. For each call, we measured the same set of 13 acoustic variables: 2 temporal, 6 variables of fundamental frequency (f_0) and 5 power variables. Before analysis, the calls were downsampled to 11.025 kHz for better frequency resolution and high-pass filtered at 50 Hz to reduce the low-frequency background noise. We measured the duration of each call and the duration from call onset to the point of maximum f_0 (dur-to-max) manually on the screen with the reticule cursor in the spectrogram window (Hamming window, FFT = Fast Fourier Transform 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 initial ($f_{0\text{beg}}$), maximum ($f_{0\text{max}}$) and end ($f_{0\text{end}}$) fundamental frequencies of each call (Fig. 1). Measurements were exported automatically to Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

In a 50-ms call fragment symmetrical about f_0 maximum, we created the power spectrum, from which we automatically measured f_{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 (Fig. 1). In addition, we

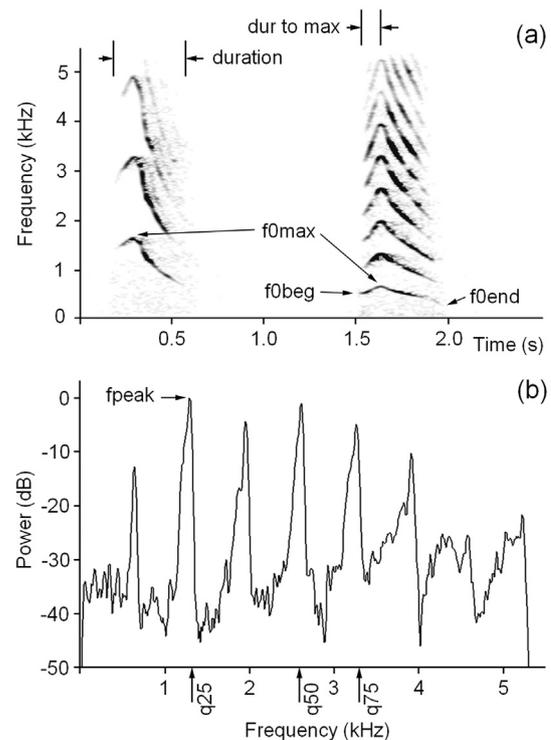


Fig. 1. Measured acoustic variables of Siberian wapiti calls. (a) Spectrogram of a calf oral call (left) and a hind nasal call (right). (b) Mean power spectrum of 50-ms fragment of a hind call. Designations: duration – call duration; dur-to-max – duration from call onset to the point of the maximum fundamental frequency; $f_{0\text{max}}$ – the maximum fundamental frequency; $f_{0\text{beg}}$ – the fundamental frequency at the onset of a call; $f_{0\text{end}}$ – 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, medium and upper quartiles, covering respectively 25%, 50% and 75% energy of a call spectrum. The spectrogram was created with Hamming window, 11.025 kHz sampling rate, FFT 1024 points, frame 50%, and overlap 96.87%.

recorded the peak-harm, representing the order number of the frequency band with the maximum energy.

We measured the f_0 variables following Reby and McComb (2003) by using the Praat DSP package (Boersma and Weenink 2013). The f_0 contour was extracted by using a cross-correlation algorithm (to Pitch (cc) command in Praat). The time steps in the analysis were 0.005 s for calves and 0.01 s for hinds and stags; the lower and upper limits of the f_0 range were 100–2000 Hz. A preliminary visual analysis of the spectrograms in Avisoft showed that the lower limit was lower than the minimum f_0 for calls of either hinds or calves. Spurious values and octave jumps in the f_0 contour were corrected manually on the basis of the spectrograms. Values of f_{0min} , f_{0max} , the depth of frequency modulation f_0 ($\Delta f_0 = f_{0max} - f_{0min}$) and average f_0 of a call (f_{0mean}) were taken automatically by using the Pitch info command in the Pitch edit window.

Two different methods of measuring f_{0max} (one using Avisoft and another using Praat) applied to the same calls, resulted in very similar values. Coefficients of correlation, calculated separately for the oral, for the nasal and for the bugle calls, ranged between 0.996 and 0.999 ($0.993 < R^2 < 0.999$). Thus, for subsequent acoustic analyses we could select between these methods and we used the f_0 values measured with Avisoft. We did not measure formants, as they cannot be measured in high-frequency calls with widely spaced harmonics (Taylor and Reby 2010; Sibiryakova et al. 2015).

Statistical analyses

Statistical analyses were made with STATISTICA, v. 6.0 (StatSoft, Tulsa, OK, USA); all means are given as mean \pm SD. Significance levels were set at 0.05, and two-tailed probability values are reported. Distributions of 124 parameter values of 132 distributions did not depart from normality (Kolmogorov–Smirnov test, $p > 0.05$), what allowed us to apply parametric tests.

For 18 individuals (5 calves, 11 hinds and 2 stags) which provided both the oral and the nasal contact calls, measurements of calls from a single animal were averaged separately for oral and for nasal calls. Then we applied a repeated measures ANOVA controlled for individuality, to compare the mean parameter values between contact oral and nasal calls. To compare the acoustics among calves, hinds and stags, we calculated average values of acoustic variables for each individual, separately for nasal and oral calls. Then we used a two-way factorial ANOVA with a Tukey honestly significant difference (HSD) test to assess whether acoustics differed between contact oral and nasal calls of calves, hinds and stags. As we had

only two hinds which provided bugles, we could not use average values per individual for comparisons of hind and stag oral contact calls and bugles. So, to compare the acoustic variables among hind and stag oral contact and bugle calls we used a nested design of ANOVA with a Tukey HSD test with an individual nested within sex/call type combination (with sex/call type combination as fixed factor and individual as random factor).

Ethics statement

All acoustic recordings were made during routine deer management conducted by the staff of zoo and farm facilities. Disturbance was kept to a minimum and social structure was not altered for the purpose of this study. We adhered to the ‘Guidelines for the treatment of animals in behavioural research and teaching’ (Anim. Behav., 2006, 71, 245–253) and to the laws on animal welfare for scientific research of the Russian Federation, Kazakhstan and Germany, where the study was conducted. Data collection protocol # 2011–36 was approved by the Committee of Bio-ethics of Lomonosov Moscow State University.

Results

Oral and nasal contact calls

All sex/age classes (calves, hinds and stags) produced both oral and nasal contact calls (Fig. 2). A chevron-shaped contour of f_0 was very similar among the sex and age-classes and between oral and nasal calls (Fig. 3). The f_{0beg} always exceeded the f_{0end} , and the f_{0end} was equal to the f_{0min} . The point of maximum f_0 was shifted towards the start of a call, being located at the distance of 20.0–24.9% of the total call duration for both oral and nasal calls of all sex/age classes (Fig. 3).

In oral contact calls, the band with the maximum energy was never higher than the 4th frequency band (considering f_0 as the first frequency band) in all sex and age-classes. The f_0 was the band with the maximum energy in 59% of oral contact calls of calves, in 85% of oral contact calls of hinds and only in 41% of oral contact calls of stags. In nasal contact calls, the highest band with the maximum energy could be the 8th frequency band in calves, the 7th frequency band in hinds and the 8th frequency band in stags. The f_0 was the band with the maximum energy in 43% of nasal contact calls of calves, in 48% of nasal contact calls of hinds and in 74% of nasal contact calls of stags.

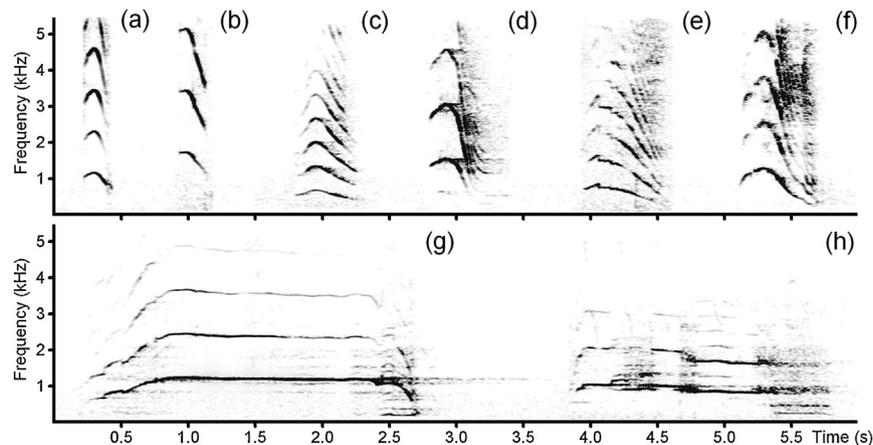


Fig. 2. Spectrogram of Siberian wapiti calls. (a) Calf nasal contact call. (b) Calf oral contact call. (c) Hind nasal contact call. (d) Hind oral contact call. (e) Stag nasal contact call. (f) Stag oral contact call. (g) Stag bugle rutting call. (h) Hind bugle call. The spectrogram was created with a Hamming window, 11.025 kHz sampling rate, FFT 1024 points, frame 50%, and overlap 96.87%. Calls are available in Supplementary Audio 1.

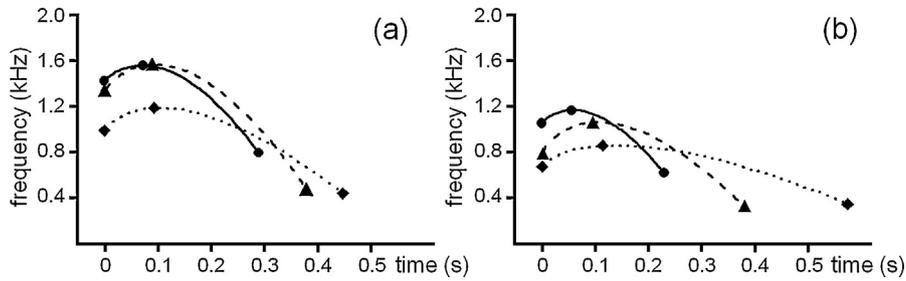


Fig. 3. Fundamental frequency (f_0) contours of Siberian wapiti contact calls. (a) Oral contact calls. (b) Nasal contact calls. Solid lines with circles indicate calves, dashed lines with triangles indicate hinds; dotted lines with rhombs indicate stags. The circles, triangles and rhombs label positions of call start, maximum and end fundamental frequencies.

Table 1
Contact call frequency variables (mean \pm SD) and results for their comparison with two-way ANOVA. Column and row headings: Oral – oral contact calls; Nasal – nasal contact calls; n – number of averaged calls (one per animal); f_0 mean – the mean fundamental frequency; f_0 max – the maximum fundamental frequency; f_0 min = f_0 end – the minimum fundamental frequency; f_0 beg – the initial fundamental frequency; Δf_0 – the depth of fundamental frequency modulation.

Sex/age class	Call type	n	f_0 mean, kHz	f_0 max, kHz	f_0 min, kHz	f_0 beg, kHz	Δf_0 , kHz
Calves	Oral	10	1.29 \pm 0.19	1.56 \pm 0.18	0.79 \pm 0.26	1.43 \pm 0.24	0.74 \pm 0.26
	Nasal	10	0.98 \pm 0.35	1.17 \pm 0.40	0.62 \pm 0.30	1.06 \pm 0.37	0.52 \pm 0.25
Hinds	Oral	28	1.19 \pm 0.27	1.57 \pm 0.27	0.47 \pm 0.19	1.34 \pm 0.36	1.06 \pm 0.28
	Nasal	17	0.73 \pm 0.15	1.06 \pm 0.24	0.32 \pm 0.10	0.79 \pm 0.22	0.72 \pm 0.27
Stags	Oral	3	0.90 \pm 0.24	1.18 \pm 0.27	0.43 \pm 0.17	0.98 \pm 0.13	0.67 \pm 0.28
	Nasal	4	0.64 \pm 0.15	0.86 \pm 0.24	0.34 \pm 0.05	0.67 \pm 0.15	0.51 \pm 0.20
ANOVA between sex/age classes			$F_{2,65} = 6.42$ $p = 0.003$	$F_{2,66} = 4.19$ $p = 0.02$	$F_{2,65} = 16.7$ $p < 0.001$	$F_{2,65} = 5.00$ $p = 0.01$	$F_{2,65} = 8.46$ $p < 0.001$
ANOVA between oral/nasal calls			$F_{1,65} = 19.6$ $p < 0.001$	$F_{1,66} = 22.5$ $p < 0.001$	$F_{1,65} = 4.83$ $p = 0.03$	$F_{1,65} = 18.5$ $p < 0.001$	$F_{1,65} = 8.37$ $p = 0.005$

We compared average values of acoustic variables of oral and nasal calls among 18 animals (5 calves, 11 hinds and 2 stags), from which both oral and nasal contact calls were available. Repeated measures ANOVA showed the lack of differences between oral and nasal contact calls regarding the duration ($F_{1,17} = 0.65$, $p = 0.43$) and dur-to-max ($F_{1,17} = 0.49$, $p = 0.49$). All f_0 variables were significantly higher in oral than in nasal contact calls: f_0 mean ($F_{1,17} = 29.91$, $p < 0.001$), f_0 max ($F_{1,17} = 43.91$, $p < 0.001$), f_0 min = f_0 end ($F_{1,17} = 5.36$, $p = 0.03$), f_0 beg ($F_{1,17} = 16.19$, $p \leq 0.001$), Δf_0 ($F_{1,17} = 20.85$, $p < 0.001$). The values of f_{peak} ($F_{1,17} = 0.02$, $p = 0.89$) and of all quartiles q_{25} ($F_{1,17} = 0.05$, $p = 0.83$), q_{50} ($F_{1,17} = 0.04$, $p = 0.84$) and q_{75} ($F_{1,17} = 1.28$, $p = 0.27$) did not differ between oral and nasal contact calls.

Two-way ANOVA for average values of acoustic variables of oral and nasal contact calls calculated for each individual, showed a significant effect of sex/age class for all variables of f_0 , for temporal variables, and for one of the four power variables (Tables 1 and 2). At the same time, two-way ANOVA showed a significant effect of call type (oral vs. nasal contact calls) only for all variables of fundamental frequency, but not for temporal or power variables

(Tables 1 and 2). These results were very similar with results of repeated measures ANOVA for comparison between oral and nasal contact calls in the 18 animals.

In calves, all the five f_0 variables of oral contact calls were higher than respective variables of nasal contact calls, but significant differences were found only in f_0 max (Table 1, Fig. 4). The duration, the dur-to-max and all power variables did not differ between oral and nasal contact calls (Table 2, Fig. 4). In hinds, all f_0 variables with the exception of f_0 min, were significantly higher in the oral than in the nasal contact calls (Table 1, Fig. 4). All temporal and power variables did not differ between oral and nasal contact calls (Table 2, Fig. 4). Stag oral and nasal contact calls did not differ significantly by any measured variable (Tables 1 and 2, Fig. 4).

Contact calls of calves, hinds and stags

In the oral contact calls, among f_0 variables only f_0 min was significantly higher in calves than in hinds, whereas Δf_0 was significantly lower in calves than in hinds (Table 1, Fig. 4). The values of all other f_0 , temporal and power variables did not differ among sex/age

Table 2
Contact call temporal and power variables (mean \pm SD) and results for their comparison with two-way ANOVA. Column and row headings: Oral – oral contact calls; Nasal – nasal contact calls; n – number of averaged calls (one per animal); duration – call duration; dur-to-max – the duration from call onset to the point of the maximum fundamental frequency; f_{peak} – frequency of maximum amplitude; q_{25} , q_{50} , q_{75} – the lower, medium and upper quartiles.

Sex/age class	Call type	n	Duration, s	dur-to-max, s	f_{peak} , kHz	q_{25} , kHz	q_{50} , kHz	q_{75} , kHz
Calves	Oral	10	0.29 \pm 0.10	0.07 \pm 0.04	2.55 \pm 0.60	1.15 \pm 0.36	2.21 \pm 0.60	3.38 \pm 0.40
	Nasal	10	0.23 \pm 0.06	0.06 \pm 0.02	2.21 \pm 0.54	1.12 \pm 0.33	2.13 \pm 0.41	3.12 \pm 0.46
Hinds	Oral	28	0.38 \pm 0.12	0.09 \pm 0.05	1.78 \pm 0.45	1.22 \pm 0.30	2.02 \pm 0.42	2.91 \pm 0.51
	Nasal	17	0.38 \pm 0.14	0.10 \pm 0.03	2.01 \pm 0.72	1.30 \pm 0.53	2.24 \pm 0.56	3.33 \pm 0.49
Stags	Oral	3	0.45 \pm 0.23	0.09 \pm 0.05	1.89 \pm 0.91	1.28 \pm 0.68	2.08 \pm 1.03	3.06 \pm 0.91
	Nasal	4	0.58 \pm 0.18	0.12 \pm 0.03	1.33 \pm 0.30	0.83 \pm 0.20	1.76 \pm 0.08	2.96 \pm 0.31
ANOVA between sex/age classes			$F_{2,65} = 11.9$ $p < 0.001$	$F_{2,65} = 4.27$ $p = 0.02$	$F_{2,66} = 6.79$ $p = 0.02$	$F_{2,66} = 1.26$ $p = 0.29$	$F_{2,66} = 0.67$ $p = 0.51$	$F_{2,66} = 0.77$ $p = 0.47$
ANOVA between oral/nasal calls			$F_{1,65} = 0.37$ $p = 0.55$	$F_{1,65} = 0.07$ $p = 0.79$	$F_{1,66} = 1.59$ $p = 0.21$	$F_{1,66} = 1.15$ $p = 0.29$	$F_{1,66} = 0.14$ $p = 0.71$	$F_{1,66} = 0.03$ $p = 0.87$

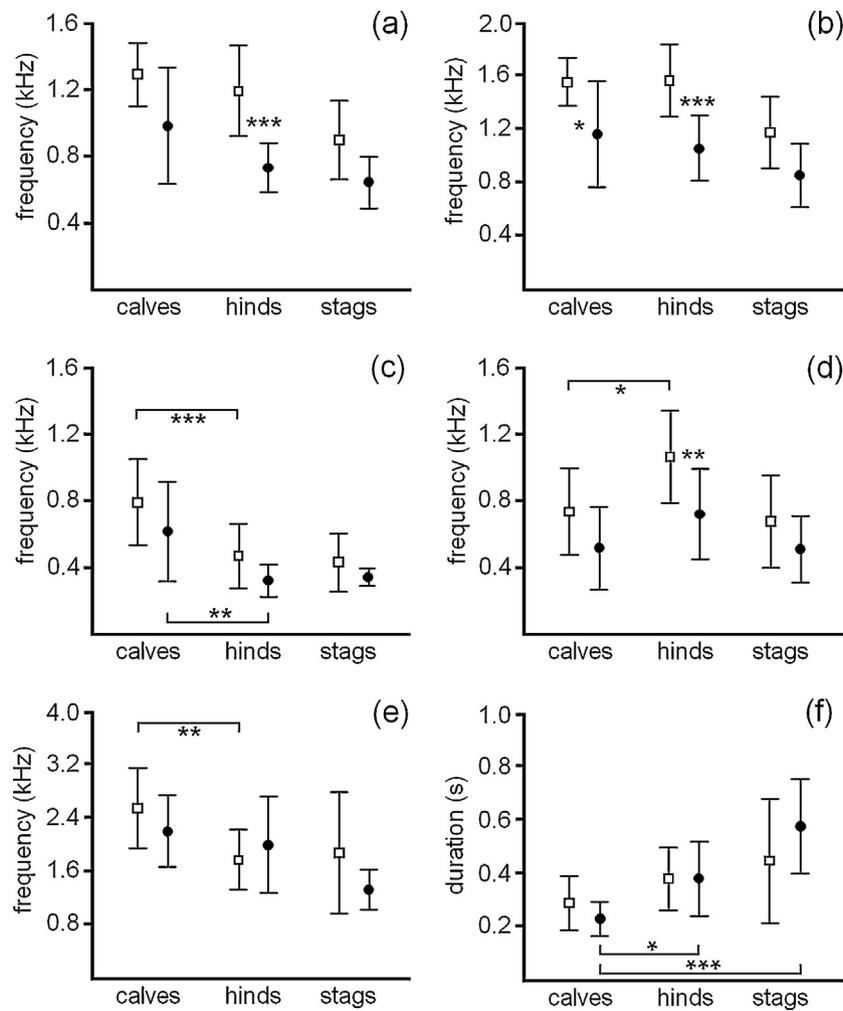


Fig. 4. Acoustic variables of oral and nasal contact calls of Siberian wapiti calves, hinds and stags. Central points (white = oral calls; black = nasal calls) indicate mean values; whiskers show \pm SD: (a) f_0 mean – the mean fundamental frequency; (b) f_0 max – the maximum fundamental frequency; (c) f_0 min – the minimum fundamental frequency; (d) Δf_0 – the depth of fundamental frequency modulation; (e) f_{peak} – the frequency of maximum amplitude; (f) duration – call duration. Tukey post hoc results significant differences: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; stars with brackets indicate significant differences between sex and age-classes; stars without brackets indicate significant differences between oral and nasal contact calls.

classes, with the exception of f_{peak} , which was found significantly higher in calves than in hinds (Tables 1 and 2, Fig. 4).

Similarly, in the nasal contact calls, only f_0 min was significantly higher in calves than in hinds (Table 1, Fig. 4). The values of all other f_0 , temporal and power variables did not differ among sex/age classes, with the exception of duration, which was shorter in calves than in either hinds or stags (Tables 1 and 2, Fig. 4).

Oral contact calls and bugle calls of stags and hinds

Nested ANOVA revealed that values of all frequency variables and all power variables, with the exception of f_0 beg, did not differ

significantly between bugle calls of stags and hinds (Tables 3 and 4, Fig. 5). The duration and the dur-to-max were greater in stag bugles compared to hind bugles.

In hinds, all f_0 variables with the exception of f_0 min, were significantly lower in the bugles than in the oral contact calls (Table 3, Fig. 5). The values of q_{50} and q_{75} were significantly lower in the bugles than in the oral contact calls, whereas f_{peak} and q_{25} did not differ significantly (Table 4). The duration of bugles was a few times longer compared to the duration of oral contact calls, whereas the dur-to-max did not differ significantly between these call types.

In stags, the values of f_0 mean, f_0 max and Δf_0 did not differ between bugles and oral contact calls (Table 3, Fig. 5). The values

Table 3

Oral contact and bugle call frequency variables (mean \pm SD) and results for their comparison with nested ANOVA (with an individual nested within sex/call type combination). Column and row headings: Oral – oral contact calls; Bugle – bugle calls; n – number of calls; f_0 mean – the mean fundamental frequency; f_0 max – the maximum fundamental frequency; f_0 min = f_0 end – the minimum fundamental frequency; f_0 beg – the initial fundamental frequency; Δf_0 – the depth of fundamental frequency modulation.

Call type	Sex class	n	f_0 mean, kHz	f_0 max, kHz	f_0 min, kHz	f_0 beg, kHz	Δf_0 , kHz
Oral	Hinds	195	1.19 \pm 0.29	1.55 \pm 0.28	0.45 \pm 0.22	1.37 \pm 0.38	1.07 \pm 0.27
	Stags	22	0.95 \pm 0.18	1.29 \pm 0.17	0.45 \pm 0.20	1.02 \pm 0.21	0.80 \pm 0.26
Bugle	Hinds	11	0.99 \pm 0.19	1.30 \pm 0.26	0.38 \pm 0.24	1.09 \pm 0.32	0.90 \pm 0.23
	Stags	70	0.96 \pm 0.18	1.20 \pm 0.25	0.30 \pm 0.13	0.62 \pm 0.26	0.82 \pm 0.27
ANOVA results			$F_{3,263} = 40.5$ $p < 0.001$	$F_{3,263} = 58.4$ $p < 0.001$	$F_{3,263} = 19.8$ $p < 0.001$	$F_{3,263} = 178.0$ $p < 0.001$	$F_{3,263} = 19.3$ $p < 0.001$

Table 4
Oral contact and bugle call frequency variables (mean \pm SD) and results for their comparison with nested ANOVA (with an individual nested within sex/call type combination). Column and row headings: Oral – oral contact calls; Bugle – bugle calls; *n* – number of calls; duration – call duration; dur-to-max – the duration from call onset to the point of the maximum fundamental frequency; *f*_{peak} – frequency of maximum amplitude; *q*₂₅, *q*₅₀, *q*₇₅ – the lower, medium and upper quartiles.

Call type	Sex class	<i>n</i>	duration, s	dur-to-max, s	<i>f</i> _{peak} , kHz	<i>q</i> ₂₅ , kHz	<i>q</i> ₅₀ , kHz	<i>q</i> ₇₅ , kHz
Oral	Hinds	195	0.38 \pm 0.13	0.09 \pm 0.06	1.76 \pm 0.59	1.20 \pm 0.49	1.96 \pm 0.58	2.85 \pm 0.68
	Stags	22	0.52 \pm 0.29	0.12 \pm 0.05	2.30 \pm 1.04	1.58 \pm 0.65	2.50 \pm 0.85	3.42 \pm 0.71
Bugle	Hinds	11	1.94 \pm 0.35	0.32 \pm 0.40	1.43 \pm 0.37	0.99 \pm 0.34	1.58 \pm 0.45	2.15 \pm 0.65
	Stags	70	3.04 \pm 0.89	1.24 \pm 0.66	1.43 \pm 0.58	0.95 \pm 0.38	1.51 \pm 0.56	2.05 \pm 0.65
ANOVA results			$F_{3,263} = 567.7$ $p < 0.001$	$F_{3,263} = 131.5$ $p < 0.001$	$F_{3,263} = 3.96$ $p = 0.008$	$F_{3,263} = 2.12$ $p = 0.10$	$F_{3,263} = 7.82$ $p < 0.001$	$F_{3,263} = 17.6$ $p < 0.001$

of *f*_{0min}, *f*_{0beg} and all power variables were significantly lower in bugles than in oral contact calls. As in hinds, the duration of bugles was a few times longer compared to the duration of oral contact calls, and the dur-to-max was significantly greater in bugles than in oral contact calls (Table 4).

In hind and stag bugle calls, the band with the maximum energy in a call spectrum never exceeded the 2nd frequency band. The *f*₀-band was the maximum energy band in 64% of hinds and in 76% of stag bugle calls.

Discussion

This is the first study reporting the emission of contact calls in red deer stags and the second study (after Feighny et al. 2006) reporting the emission of bugles by red deer hinds. Previously, contact calls were reported only for hinds and calves. We found that Siberian wapiti hind contact calls had very high values of fundamental frequency, 3–4 times higher than hinds of all studied European subspecies. Our data show that Siberian wapiti calf

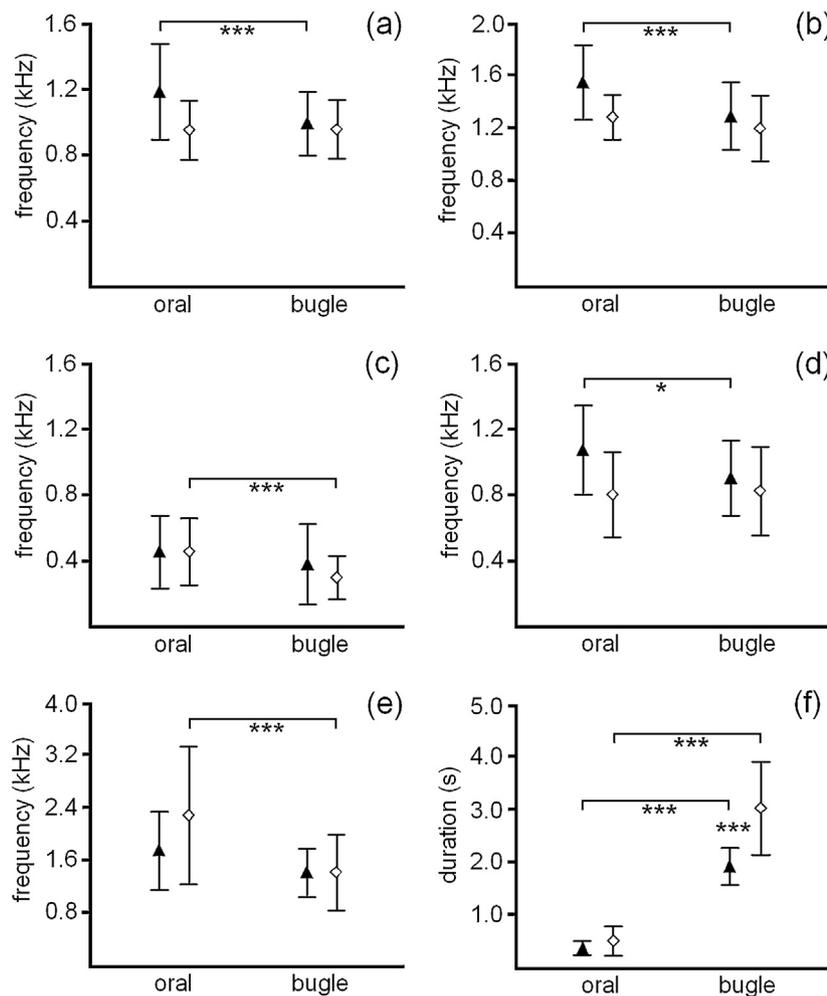


Fig. 5. Acoustic variables of oral contact and bugle calls of Siberian wapiti hinds and stags. Central points (black = hinds; white = stags) indicate mean values; whiskers show \pm SD: (a) *f*_{0mean} – the mean fundamental frequency; (b) *f*_{0max} – the maximum fundamental frequency; (c) *f*_{0min} – the minimum fundamental frequency; (d) Δf_0 – the depth of fundamental frequency modulation; (e) *f*_{peak} – the frequency of maximum amplitude; (f) duration – call duration. Tukey post hoc results significant differences: * $p < 0.05$; *** $p < 0.001$; stars with brackets indicate significant differences between oral contact and bugle calls; stars without brackets indicate significant differences between hind bugles and stag bugles.

contact calls (both oral and nasal) have the same maximum fundamental frequency as hinds, and are two times higher than in calves of any European subspecies. Also, hind bugles had the same fundamental frequency as stag bugles. We showed that the fundamental frequency of Siberian wapiti hind and stag contact calls was in the same range as Siberian wapiti stag bugles, which is consistent to our earlier proposals (Volodin et al. 2015a).

Call type repertoire in *Cervus elaphus*

Contact calls (nasal and oral) were found in all sex and age-classes in Siberian wapiti. Our findings of contact calls in stags and bugle calls in hinds are complementary to the findings of bugle calls in *C. e. canadensis* hinds, indistinguishable by the acoustic structure of definitive stag bugle rutting calls (Feighny et al. 2006). Together, these data suggest that in *Cervus elaphus*, both hinds and stags produce the same call types (contact and bugle), but they differ drastically in their occurrence. Contact calls are very rare for stags (this study), whereas the bugle calls are similarly rare for hinds (this study; Feighny et al. 2006). This suggests the role of sexual selection for call usage rates or for the context in which the call is used rather than for call pattern per se in *Cervus elaphus*. In other mammals, it is common that one sex, but not the other, produces vocalizations or that each sex produces distinctive calls (Rendall et al. 2004; Bouchet et al. 2011), thus, these vocalizations are considered as sex-specific.

Subspecies-specific acoustic features of Siberian wapiti

Contact calls of Siberian wapiti calves were substantially higher-pitched compared to calves of European subspecies. The maximum fundamental frequency of the contact calls in 1 day–4-month Siberian wapiti calves (1.56 kHz in the oral and 1.17 kHz in the nasal calls, Table 1), was higher compared to calls of 1–2 day calves *C. e. hippelaphus* (0.74 kHz, Vankova and Malek 1997), 4-month calves of *C. e. corsicanus* (0.71 kHz, Kidjo et al. 2008), calls of 1–52 day calves of *C. e. hispanicus* (0.88 kHz in oral and 0.78 kHz in nasal calls, Sibiryakova et al. 2015), or calls of 4-month calves of *C. e. hispanicus* (0.57 kHz in oral and 0.47 kHz in nasal calls, Volodin et al. 2015a). At the same time, the maximum fundamental frequency for 1 day–3-month calf calls of an American subspecies *C. e. canadensis* (1.48–1.52 kHz, Feighny 2005), are similar to Siberian wapiti calves.

Similarly, contact calls of Siberian wapiti hinds were a few times higher-pitched compared to hinds of European subspecies of *Cervus elaphus*. The maximum fundamental frequency of the contact calls in Siberian wapiti hinds (1.57 kHz in the oral and 1.06 kHz in the nasal calls, Table 1) was much higher compared to contact calls of hinds of *C. e. hippelaphus* (0.11 kHz, Vankova and Malek 1997), *C. e. corsicanus* (0.10 kHz, Kidjo et al. 2008) and *C. e. hispanicus* (0.17–0.21 kHz, Sibiryakova et al. 2015; Volodin et al. 2015a). At the same time, the maximum fundamental frequency for hind oral contact calls of an American subspecies *C. e. canadensis* (1.41–1.59 kHz, Feighny 2005) and *C. e. roosevelti* (more than 1.5 kHz, Bowyer and Kitchen 1987) close in values to Siberian wapiti hinds. Among Asian subspecies of red deer, no comparative data for the acoustics of calves and hinds are available.

The maximum fundamental frequency of the Siberian wapiti stag bugles (1.20 kHz, Table 3) was intermediate between European and American subspecies of *Cervus elaphus*. Among the European subspecies, the maximum fundamental frequency of the rutting roars were reported of 0.05 kHz in *C. e. corsicanus* (Kidjo et al. 2008), 0.09 kHz in *C. e. italicus* (Della Libera et al. 2015), 0.14–0.21 kHz in *C. e. scoticus* (Long et al. 1998; Reby and McComb 2003), 0.27 kHz in *C. e. hippelaphus* (Bocci et al. 2013) and 0.21–0.27 kHz in *C. e. hispanicus* (Frey et al. 2012; Passilongo et al. 2013; Volodin et al.

2015a). Among the American subspecies, the maximum fundamental frequency of the rutting bugles was reported of 2.08 kHz in *C. e. canadensis* (Feighny et al. 2006) and over 1.5 kHz in *C. e. roosevelti* and *C. e. nelsoni* (Bowyer and Kitchen 1987; Frey and Riede 2013). However, the maximum fundamental frequency of the Siberian wapiti stag rutting bugles was higher than in another Asian subspecies, *C. e. xanthopygus* (0.66 kHz, Volodin et al. 2015b). We did not find any differences in the structure of rutting bugles of Siberian wapiti stags, recorded in captivity (Tables 3 and 4) and in the wild ($f_{0max} = 1.23$ kHz, $f_{0min} = 0.29$ kHz, duration = 3.07 s, Volodin et al. 2013b). Similar to stag bugles, the maximum fundamental frequency of the Siberian wapiti hind bugles (1.30 kHz, Table 3) was lower than in *C. e. canadensis* hind bugles (about 1.90 kHz, Feighny et al. 2006).

Summarizing, we conclude that the high-frequency quality of calls of Siberian wapiti *C. e. sibiricus* represents a characteristic feature of vocalizations in all sex and age-classes of this subspecies. As compared to other subspecies of *Cervus elaphus*, contact calls of calves and hinds as well as bugles of stags and hinds in Siberian wapiti were closer in fundamental frequency to American subspecies than to European subspecies, being substantially higher than in any European subspecies of *Cervus elaphus* and higher than in an Asian subspecies *C. e. xanthopygus*. These acoustic differences are consistent with molecular phylogenetics data on closer relatedness of Siberian wapiti to American than to European subspecies of *Cervus elaphus* (Mahmut et al. 2002; Ludt et al. 2004; Kuznetsova et al. 2012; Mukesh et al. 2015) and prominent genetic variation between *C. e. sibiricus* and *C. e. xanthopygus* (Mahmut et al. 2002; Kuznetsova et al. 2012). Therefore, as for European subspecies (Frey et al. 2012), acoustic traits can be used as subspecies indices also in Asian subspecies in red deer, in addition to morphological and genetic traits (Geist 1998; Mahmut et al. 2002; Kuznetsova et al. 2012; Kim et al. 2015).

Non-descending ontogeny of fundamental frequency in Siberian wapiti

The ranges of fundamental frequency of hind and stag contact calls (0.32–1.57 kHz in hinds and of 0.34–1.18 kHz in stags, Table 1) and hind and stag bugles (0.38–1.30 kHz in hinds and of 0.30–1.20 kHz in stags, Table 3) overlapped. This is consistent to our earlier proposals that the fundamental frequencies of stag and hind calls are more similar within subspecies than they are among subspecies (Volodin et al. 2015a). Previously, overlapped ranges of fundamental frequency between stags and hinds were found in a European subspecies *C. e. hispanicus* (Volodin et al. 2015a). This overlap is consistent with the fact that size of the larynx is similar between males and females in *C. e. hispanicus* (Frey et al. 2012) and in *C. e. nelsoni* (Riede and Titze 2008). However, the probable similarities of the larynx size in male and female *C. e. sibiricus* and between sexes in other Asian subspecies of *Cervus elaphus* still have to be investigated.

Although the source-filter theory predicts that the fundamental frequency should not depend on the length of the vocal tract (Fant 1960; Fitch and Hauser 2002; Taylor and Reby 2010), we found the higher f_0 in oral than in nasal contact calls in all sex and age classes (Table 1, Fig. 4), although in stags differences were non-significant, probably because of small call sample (Table 1). Among cervids, the higher f_0 values in oral than nasal contact calls were found also in calves but not in hinds of *C. e. hispanicus* (Sibiryakova et al. 2015; Volodin et al. 2015a). Among bovids, the higher f_0 values in oral than nasal contact calls were found in mother and offspring goitred gazelles (Volodin et al. 2011), saiga antelopes (Volodin et al. 2014), and in mother domestic sheep (Sebe et al. 2010) and domestic cows (Padilla de la Torre et al. 2015). Potential mechanics for production of the higher f_0 in the oral than in the nasal calls are discussed

in detail in Volodin et al. (2011). At the same time, the study of mother and offspring saiga antelopes demonstrated invariability of the fundamental frequency at transition from the closed-mouth to the opened-mouth vocal emission within calls, which started nasally and ended with widely opened mouth (Volodin et al. 2014).

Calf calls of European subspecies of *Cervus elaphus* show the higher fundamental frequency compared to adults (Vankova and Malek 1997; Kidjo et al. 2008; Sibiryakova et al. 2015; Volodin et al. 2015a). This decrease of fundamental frequency with age e.g. in *C. e. hispanicus*, might be account by the age-related increase of the vocal folds, as the dorsoventral length of the vocal fold was found 9 mm in 1-day-old male and 13 mm in 12-days-old male (our unpublished data), and about 30 mm in adult stags *C. e. hispanicus* (Frey et al. 2012). The vocal fold characteristics are responsible for production of fundamental frequency in mammals (Fitch and Hauser 2002; Riede and Brown 2013). Decreasing values of fundamental frequency with calf age represent a usual ontogenetic pathway in ungulates (Briefer and McElligott 2011; Efremova et al. 2011; but see Padilla de la Torre et al. 2015). At the same time, the overlapping or even lower-frequency calls of offspring than in mother were reported in three species of ground squirrels (Matrosova et al. 2007; Volodina et al. 2010; Swan and Hare 2008) and two species of shrews (Schneiderová 2014; Volodin et al. 2015c; Zaytseva et al. 2015) and may be inferred from similar f_0 values between cows and calves in domestic cattle (Padilla de la Torre et al. 2015).

In this study, contact calls of hinds and calves were very close in values of maximum and mean fundamental frequencies and stag

contact calls were non-significantly lower in frequency variables compared to calls of hinds and calves (Fig. 4). This is consistent with data for *C. e. canadensis*, reporting a coincidence of maximum fundamental frequencies between calves and hinds (Feighny 2005). We infer therefore that Siberian wapiti display a distinctive ontogenetic trajectory of fundamental frequency, practically non-descending with age from calves towards adults. This is the first study indicating that different ontogenetic trajectories of fundamental frequency are possible among subspecies within a mammalian species.

Acknowledgements

We thank the Kostroma farm owners N. Romanenko, O. Semenkov and farm manager A. Ermolaev for their help and support, and V. Matrosova and O. Golosova for their help with data collection. We are sincerely grateful to the two anonymous reviewers for their valuable comments and for the correction of grammar. The research was funded by grants from the Russian Scientific Foundation, grant no. 14-14-00237.

Appendix A.

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.mambio.2015.09.002>.

Table A.1

Distributions of included in the analyses oral and nasal contact calls and rutting calls by animals, sites and dates. Oral = oral contact calls; Nasal = nasal contact calls; Bugle = bugle calls.

Animals	Sites	Dates of recording	Calls			
			Oral	Nasal	Bugle	Total
Calf 1	Tierpark Berlin	Dec. 2012		3		3
Calf 2	Tierpark Berlin	Dec. 2013	13	3		16
Calf 3	Tierpark Berlin	Dec. 2013	27			27
Calf 4	Kostroma farm	Jun. 2015	8	6		14
Calf 5	Kostroma farm	Jun. 2015	2			2
Calf 6	Kostroma farm	Jun. 2015		5		5
Calf 7	Kostroma farm	Jun. 2015	4			4
Calf 8	Kostroma farm	Jun. 2015	9	11		20
Calf 9	Kostroma farm	Jun. 2015	2			2
Calf 10	Kostroma farm	Jun. 2015		4		4
Calf 11	Kostroma farm	Jun. 2015		3		3
Calf 12	Kostroma farm	Jun. 2015		12		12
Calf 13	Kostroma farm	Jun. 2015	1	1		2
Calf 14	Kostroma farm	Jun. 2015	1	1		2
Calf 15	Kostroma farm	Jun. 2015	4			4
Hind 1	Tierpark Berlin	Dec. 2013	6	11		17
Hind 2	Tierpark Berlin	Dec. 2012; Dec. 2013; Nov. 2014	16	21		37
Hind 3	Tierpark Berlin	Dec. 2012; Dec. 2013	1	11		12
Hind 4	Novosibirsk Zoo	Jul. 2004	9			9
Hind 5	Novosibirsk Zoo	Aug. 2007	13			13
Hind 6	Novosibirsk Zoo	Aug. 2007	5	3		8
Hind 7	Kazakhstan farm	Sep. 2014	12	1		13
Hind 8	Kostroma farm	Jun. 2013	8			8
Hind 9	Kostroma farm	Jun. 2013	7			7
Hind 10	Kostroma farm	Jun. 2015	6			6
Hind 11	Kostroma farm	Jun. 2015	6	2		8
Hind 12	Kostroma farm	Jun. 2015	11			11
Hind 13	Kostroma farm	Jun. 2015	1	3		4
Hind 14	Kostroma farm	Jun. 2015	1	3		4
Hind 15	Kostroma farm	Jun. 2015	8	3		11
Hind 16	Kostroma farm	Jun. 2015		2		2
Hind 17	Kostroma farm	Jun. 2015	2	1		3
Hind 18	Kostroma farm	Jun. 2015		2		2
Hind 19	Kostroma farm	Jun. 2015	5			5
Hind 20	Kostroma farm	Jun. 2015	4			4
Hind 21	Kostroma farm	Jun. 2015	6			6
Hind 22	Kostroma farm	Jun. 2015	15	2		17
Hind 23	Kostroma farm	Jun. 2015	5			5
Hind 24	Kostroma farm	Jun. 2015		6		6

Table A.1 (Continued)

Animals	Sites	Dates of recording	Calls			
			Oral	Nasal	Bugle	Total
Hind 25	Kostroma farm	Jun. 2015		7		7
Hind 26	Kostroma farm	Jun. 2015	6			6
Hind 27	Kostroma farm	Jun. 2015	9			9
Hind 28	Kostroma farm	Jun. 2015	6			6
Hind 29	Kostroma farm	Jun. 2015	5			5
Hind 30	Kostroma farm	Jun. 2015		2		2
Hind 31	Kostroma farm	Jun. 2015	4			4
Hind 32	Kostroma farm	Jun. 2015	6			6
Hind 33	Kostroma farm	Jun. 2015		3		3
Hind 34	Kostroma farm	Jun. 2015	12			12
Hind 35	Kostroma farm	Jun. 2015			9	9
Hind 36	Kostroma farm	Jun. 2015			2	2
Stag 1	Tierpark Berlin	Dec. 2012	2	9		11
Stag 2	Kostroma farm	Jun. 2015	8			8
Stag 3	Kostroma farm	Jun. 2015		7		7
Stag 4	Kostroma farm	Jun. 2015	12	3		15
Stag 5	Kostroma farm	Jun. 2015		4		4
Stag 6	Novosibirsk Zoo	Aug. 2007			6	6
Stag 7	St. Petersburg Zoo	Oct. 2010			16	16
5 stags	Kostroma farm	Sep.–Oct. 2013			48	48
Total			288	155	81	524

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