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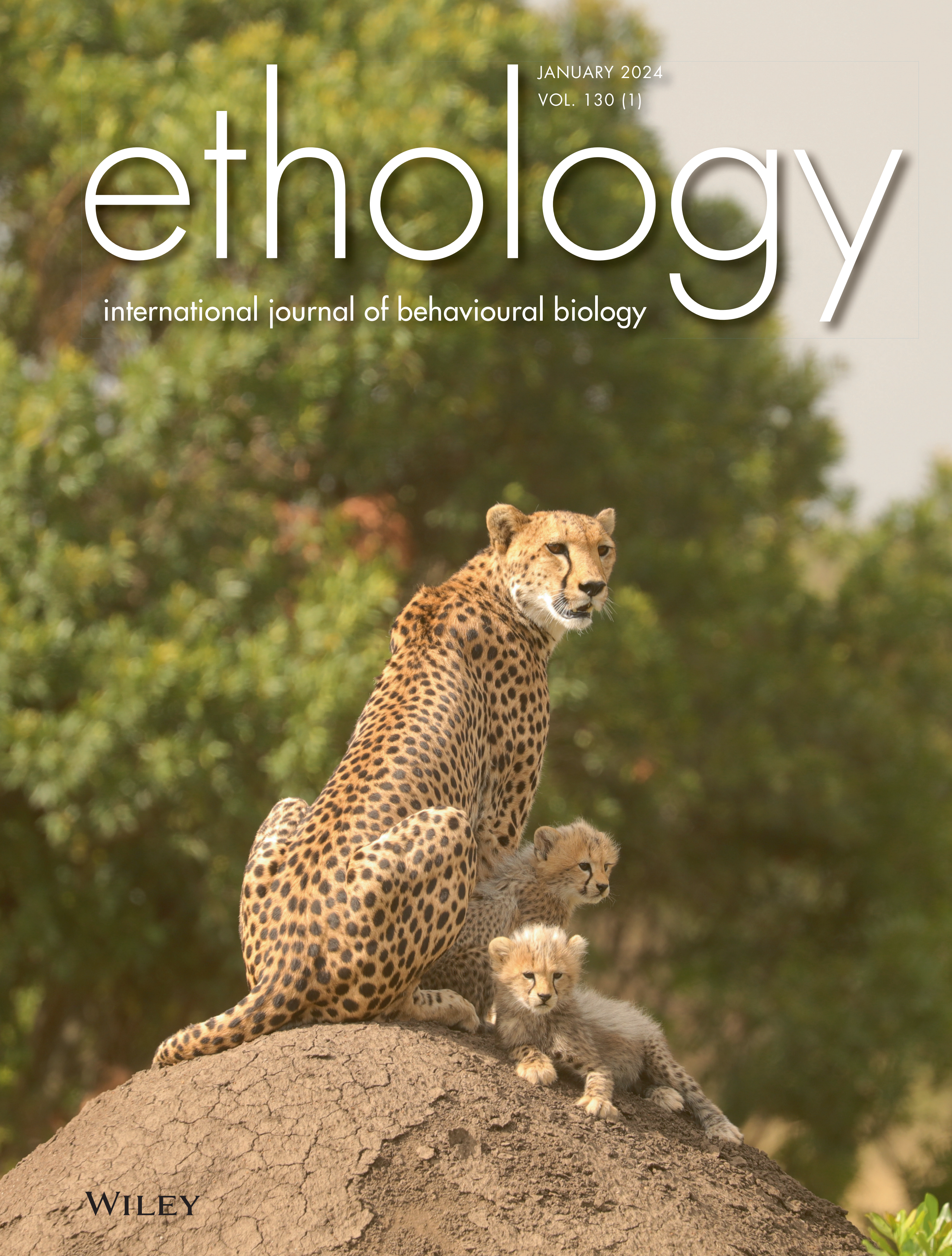

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RESEARCH ARTICLE

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Acoustic features of long-distance calls of wild cheetahs (*Acinonyx jubatus*) are linked to the caller age from newborns to adults

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Abstract

Wild cheetahs *Acinonyx jubatus* of all age classes, from newborns to adults, use their long-distance chirps for communication with conspecifics. We investigated the ontogenetic changes of eight acoustic parameters of the chirps produced by wild-living cheetahs across 14 age classes in Kenya. Chirp maximum fundamental frequency (f0max) was found to be best acoustic correlate of cheetah age. The f0max was the highest in neonates (up to 10 kHz), then decreased steadily across age classes and reached a plateau of about 1 kHz in mature adults older than 4 years. Based on a close relationship of f0max with age, we fitted polynomial models for estimating cheetah age by their chirps. We discuss that gradual changes of chirp f0max suggest the gradual development of cheetah vocal apparatus. Model for age estimation by chirps in the cheetah proposed in this study may provide conservationists a non-invasive bioacoustic tool for estimating cheetah age, particularly at ages younger than 4 years. However, introducing more data from cheetahs of precisely known age would be necessary for obtaining more accurate results of age determination by voice for the older individuals.

KEYWORDS
Acinonyx jubatus raineyi, acoustic communication, age-related parameters, far-distant calls, vocal development

1 | INTRODUCTION

Studying vocal ontogeny in wild-living animals is difficult, as it demands collecting large call samples from many individuals across age classes. Thus, the ontogenetic changes of acoustic parameters were primarily investigated in captivity for small-sized mammals: rodents (Campbell et al., 2014; Volodin et al., 2021; Yurlova et al., 2020), shrews (Schneiderová, 2014; Zaytseva et al., 2015), and small primates (Elowson et al., 1992; Hammerschmidt et al., 2000, 2001; Newman, 1995).

For large-sized mammals, investigations of full vocal ontogeny from newborns to adults were only conducted for humans *Homo sapiens* (Fitch & Giedd, 1999; Lee et al., 1999) and for goitred gazelles *Gazella subgutturosa*, the Artiodactyla species displaying remarkable anatomical parallelisms of the descended larynx with humans (Efremova et al., 2011; Volodin, Efremova, et al., 2017). In both humans and goitred gazelles, the early ontogenetic descent of the larynx occurs in both sexes, whereas with maturation the larynx descends further only in male sex, which results in abrupt changes of acoustic parameters (Efremova et al., 2016; Fitch & Giedd, 1999; Lee et al., 1999; Volodin, Efremova, et al., 2017).

In large "roaring" felids of the genus *Panthera*, the larynx of adults is also descended in both sexes (Hast, 1989; Klemuk et al., 2011; Peters & Hast, 1994; Weissengruber et al., 2002, 2008). In jaguar *Panthera onca*, the only felid in which larynx position was investigated in both newborn and adult individuals, only adult individuals had a descended larynx (Weissengruber et al., 2002). This indicates that, in felids of the genus *Panthera* the vocal traits may change rapidly following the descent of the larynx along ontogeny, as in human boys, displaying "voice breaking" at puberty (Lee et al., 1999).

In contrast, in adult pumas *Puma concolor*, cheetahs *Acinonyx jubatus*, and domestic cats *Felis catus* the larynx is not descended (Hast, 1989; Peters & Hast, 1994; Weissengruber et al., 2002). Therefore, it is expectable that in the cheetah and other felids without laryngeal descent, the developmental vocal changes may occur more smoothly and steadily.

In addition to the descent of the larynx, the difference in vocal ontogeny between the "roaring" felids of the genus *Panthera* and other Felidae species might be affected by development of the large pads of fibro-elastic tissue which constitute the rostral portions of the vocal folds (Hast, 1989; Klemuk et al., 2011; Peters & Hast, 1994; Weissengruber et al., 2002). In *Panthera* species, their intense distant calls are low-frequency roars (Ji et al., 2013; Peters, 2011; Pfefferle et al., 2007; Wijers, Trethowan, et al., 2021). In contrast, in pumas and cheetahs, in which the vocal pads are lacking and the larynx is not descended (Hast, 1989; Peters & Hast, 1994; Weissengruber et al., 2002, 2008) the distant calls are tonal and high-frequency. In the puma, the distant calls are high-frequency meows (Allen et al., 2016; Macarrão et al., 2012; Peters, 2011; Potter, 2005); in the cheetah, the distant calls are high-frequency chirps (Chelysheva et al., 2023; Ruiz-Miranda et al., 1998; Smirnova et al., 2016; Volodina, 2000). For all age categories of cheetahs, a common context for the production of their long-distance chirps is loss of contact with conspecifics and advertising own location to resume spatial proximity or to establish a novel contact (Chelysheva et al., 2023; Ruiz-Miranda et al., 1998; Smirnova et al., 2016; Volodina, 2000).

Previous studies point to the ontogenetic decrease of maximum fundamental frequency (f0max) of cheetah chirps. The f0max was about 4 kHz in one 2-day-old newborn male and on average 5.85 kHz (varying from 3.2 to 8.0 kHz) in 1.5–3-month-old cubs (Volodina, 1998). In 1-year-old adolescents, the f0max was 2.29–3.00 kHz (Nagorzanski, 2018; Stoeger-Horwath & Schwammer, 2003). In 2-year-old and older cheetahs, the f0max was 1.19–1.81 kHz (Smirnova et al., 2016; Volodina, 2000). In cheetahs of 4 years and older, the f0max was on average 0.76 kHz in males and 0.98 kHz in females (Chelysheva et al., 2023). However, a detailed pathway of changes in acoustic parameters of the chirps across ontogeny has yet to be investigated for cheetahs.

Preliminary exploration by the authors of acoustic parameters in the cheetahs across ages suggested a monotonic way of the vocalization-age relationship. Thus, knowledge of detailed changes of acoustic variables of long-distant chirps across ontogeny in the cheetah might open a door of determining the cheetah age by voice traits. The possibility to determine the age of cheetahs from audio recordings might provide to conservationists a non-invasive tool for monitoring age categories in natural populations. However, so far, no good mathematical models were developed for estimating age of mammals by vocal traits. Currently, the most appropriate methods for age estimating in mammals are based on analysis of morphological traits (dental and skeletal). These invasive methods are commonly inappropriate for alive animals (Barratclough et al., 2023; Herrman et al., 2020).

Generally, methods of age estimation by morphological traits (e.g., Read et al., 2018) only enable approximation of age class (juvenile, subadult, adult), although have proved to be useful for lions *Panthera leo* (White & Belant, 2016). Age class approximation by morphological traits can usually be done using simple statistical approaches, such as discriminant analysis (Karels et al., 2004). In contrast, estimating chronological age by morphological traits is much more challenging. Currently available methods for age estimating in absolute values (in years, with about 1-year precision) were also developed based on analysis of morphological traits, as bone and dental growth layers or earplug layers (Hohn, 2009; Klevezal, 1995; Read et al., 2018).

New approaches of age determination are based on analysis of pulp/tooth area ratios. These approaches were initially developed for estimating age of humans in medicine, anthropology and in forensic science (Cameriere et al., 2004; Tardivo et al., 2014). These techniques are based on quantitative parameters and allow relatively accurate estimation of chronological age using regression models. Pulp/tooth area ratio decreases non-linearly but gradually and monotonically with age, thus providing a basis for developing analytical approaches for age determination in humans (Tardivo et al., 2014) and animals (Barratclough et al., 2023; Herrman et al., 2020; Read et al., 2018), including a felid species, the domestic cat (Fleming et al., 2021). Our preliminary analyses of acoustic parameters of cheetah chirps suggested that their relationship with age was very similar to the age dynamics of the pulp/tooth ratio in canines (Cameriere et al., 2004; Tardivo et al., 2014). Thus, the mathematical algorithms developed for these methods of age estimation can be potentially extended to cheetah chirps.

Currently, systems of passive acoustic monitoring develop intensely, for purposes of ecoacoustics (calculating the indexes of acoustic variation and estimation of landscape state) and for searching and monitoring different animal species (both rare and common, as e.g., cattle grazing at conservation areas, Pérez-Granados & Schuchmann, 2023). For felids, some such systems are already available (e.g., CARACAL, Wijers, Loveridge, et al., 2021). For their extending to cheetahs, new referential data are necessary, for validating the blind recordings made by automated recorders in the field. Our research is intended to provide such kind of data for far-distant calls of cheetahs of different ages, from newborns to adults.

The aim of this study was to describe the changes in the acoustic structure of the long-distant chirps in wild-living cheetahs, from newborn cubs to mature adults. In addition, the practical task of this study was to develop an algorithm for estimating the age of cheetahs by acoustic parameters of the long-distance chirps, on the basis of a potential relationship between cheetah age and the acoustics.

2 | MATERIALS AND METHODS

2.1 | Study area and dates

Long-distance chirps were recorded from 2014 to 2022 in southwestern Kenya from wild-living cheetahs *Acinonyx jubatus raineyi* (Prost et al., 2022), aged from newborns (3–5 days old) to mature adults (up to 10 years old). The study area covers about 2300 km^2 and belongs to the Maasai Mara Ecosystem (centered at 1°S, 35°E, elevation ca. 1700 m). This area comprises the Maasai Mara National Reserve (1510 km^2), the adjoining conservancies and pastoralist communities in the north and east, and borders with the Serengeti National Park (Tanzania) in the south (Chelysheva et al., 2023; Jacobson et al., 2015; Ogutu et al., 2009). The wildlife is free-ranging over the entire area, where the habitat types are represented by plains covered by dwarf shrubs/short grassland, tall grassland, shrubland, forest, and woody/shrubby grassland (Chelysheva et al., 2023; Oindo et al., 2003).

2.2 | Audio recording

For audio recordings (48 kHz, 16-bit resolution, frequency range 40–20,000 Hz; wav-format), a Marantz PMD-661MKII solid state recorder (D&M Professional) with a Sennheiser K6-ME67 microphone (Sennheiser electronic) was used. All cheetah vocalizations were spontaneous and no additional stimulation for eliciting the calls from the researcher collecting the audio recordings (EVC) was applied. Methods of audio recording were the same as described in the study by Chelysheva et al. (2023). Cheetahs were searched between 06:00 and 18:00 when in a vehicle, stopping at the elevated points and scanning the surroundings with binoculars. When the cheetahs were located, the research vehicle approached them up to 25 m and remained on the designated roads with the engine turned off. The researcher (EVC) documented geographic coordinates and recorded cheetah vocalizations from the vehicle, with a microphone mounted on the window and pointed toward the animals being recorded. Cheetahs in the Mara are habituated to the presence of tour vehicles and let to be followed and observed from a distance. During hours of observations, while cheetahs were moving, they sometimes were passing by the researcher's car at 10 m or moving away by 75 m or more. Thus, calls were recorded from 10 to 75 m distance.

In litters of small dependent cubs, the callers were individually unrecognizable, and the recordings were litter-based, that is, collected from the entire litter together, without individual identification of the callers. Older callers were recognizable by their unique spot patterns on their front and back limbs, and spots and rings on the tail (Chelysheva, 2004), so the collected chirps were identified as belonging to particular individuals. Chirps were recorded in the following contexts: offspring produced the chirps toward their mothers and each other, adult males produced the chirps toward coalition members or toward potential mates; adult females produced the chirps toward potential mates and toward offspring (Chelysheva et al., 2023).

2.3 | Cheetah litters and age classes

The number of cubs per litter at recording varied from 1 to 7 (Table A1). In total, for the 8-year-long recording period, the long-distance chirps were collected from 56 different litters (Table A1), which included cubs with mothers, independent adolescent littermates, and adult individuals lonely or in groups (male coalitions, partners in courtship, mothers with cubs). Cubs which were first recorded collectively with their littermates and then were recorded at individual basis at later age classes retained their litter identity when being included in the statistical models for age determination by chirps.

For 7 of the 56 litters, the age was known with 2–7 days precision because mothers have been observed before and after delivery. For 35 of the 56 litters, the age was known with about 1-month precision, because these litters were detected with their mothers, and cub growth and development could be used for the accurate estimation of age (following Bell et al., 2012; Laurenson, 1995; Wack et al., 1991). For the remaining 14 litters which were first seen as adolescents without their mothers, only the year of birth was known, and the month of birth was estimated based on data from the literature that cheetah cubs reach independence at the age of about 18 months (Durant et al., 2004; Kelly et al., 1998).

We split the chirps recorded from the 56 different litters to 14 age classes: age class 1 (below 1 month); age class 2 (1–2 months); age class 3 (2–3 months); age class 4 (3–4 months); age class 5 (4–5 months); age class 6 (5–6 months); age class 7 (6–9 months); age class 8 (9–12 months); age class 9 (12–18 months); age class 10 (18–24 months); age class 11 (24–36 months); age class 12 (36–48 months); age class 13 (4–6 years); age class 14 (6 years and older) (Table 1). Of the 56 different litters, 33 litters provided the chirps only at one age class, 11 litters provided the chirps at two age classes, 7 provided the chirps at three age classes, 4 litters provided the chirps at four age classes, and only 1 litter provided the calls at 5 age classes. Thus, in total in the 14 age classes we included in the statistical analysis the chirps from 97 litters*age class (Table 1, Table A1).

2.4 | Call samples

We selected for acoustic analyses only high-quality chirps with high signal-to-noise ratios, not disrupted by wind or overlapped

TABLE 1 Numbers of cheetah litters and chirps included in analyses for each of the 14 age classes, from newborns to mature adults.

Age class	Age	*N* litters	*n* chirps
1	Below 1 month	5	101
2	1–2 months	2	52
3	2–3 months	10	186
4	3–4 months	11	203
5	4–5 months	10	179
6	5–6 months	8	123
7	6–9 months	5	68
8	9–12 months	7	104
9	12–18 months	6	99
10	18–24 months	7	143
11	24–36 months	3	80
12	36–48 months	6	184
13	4–6 years	7	186
14	6 years and older	10	269
Total	All ages	97	1977

Note: Of the 56 unique litters, 33 litters provided the chirps only at one age class, 11 litters provided the chirps at two age classes, 7 litters provided the chirps at three age classes, 4 litters provided the chirps at four age classes, and only 1 litter provided the calls at 5 age classes.

with extraneous noises (e.g., from water stream, airplane, car engine, birds, crickets, other animals, or human voices). We took the best quality calls regardless of distance, in which all necessary acoustic parameters could be measured. From 97 litters*age class (2–11 litters per age class, 14 age classes in total), we included in acoustic analyses 1977 chirps (Table 1, Table A1). For most litters, the recordings contained about 20 measurable chirps per litters*age class, on average, 20.4 ± 10.3 chirps per litters*age class, minimum 2 and maximum 60 chirps per litters*age class (Table A1). If more than one recording per litter per age class was available, we selected for analyses, where possible, the chirps from different recordings. To decrease potential pseudoreplication by taking consecutive chirps, the chirps were primarily selected from different parts of audio files.

Most chirps recorded from cubs at the younger age classes 1–9 were collected from individually unidentified callers. Nevertheless, if any cub within litter was recognizable and its chirps could be distinguished among others, we tried to uniformly present the chirps of this caller in the total call sample of the litter. In contrast, at the older age classes 10–14, chirps were collected from particular individuals. For these individuals, we tended to select for analyses equal numbers (usually 20) of chirps per individual per age class. Samples of chirps for cheetahs at 13–14 age classes overlapped with samples of chirps from a previous study: 444 of 455 chirps were used previously (Chelysheva et al., 2023), and 11 chirps were new. Samples of chirps for cheetahs of age classes 1–12 (in total, 1522 chirps) were new.

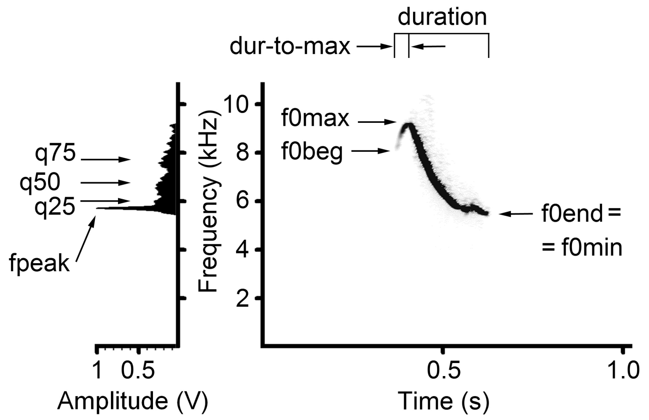


FIGURE 1 Measured parameters for cheetah chirps. Spectrogram (right) and mean power spectrum (left) of the chirp call from cheetah cub below 1 month of age (age class 1) are given. Designations: call duration (duration); duration from call onset to the point of maximum f0 (dur-to-max); the fundamental frequency at the onset of a call (f0beg); the fundamental frequency at the end of a call (f0end); the maximum fundamental frequency (f0max); the minimum fundamental frequency (f0min); the frequency of maximum amplitude within a call (fpeak); the lower, medium and upper quartiles (q25, q50 q75), covering respectively 25%, 50% and 75% energy of a call spectrum. The spectrogram was created at 22,050 Hz sampling frequency, FFT length 1024, Hamming window, frame 50%, overlap 96.87%.

2.5 | Call measurements

For acoustic analyses, we used Avisoft SASLab Pro software (Avisoft Bioacoustics). Before the analyses, the acoustic files (all recorded with sampling frequency of 48 kHz) were downsampled to 22,050 Hz, and high-pass filtered at 200 Hz for removing the background noise. The filtering did not affect the calculated values of fundamental frequency (f0), because the values of f0 parameters were higher than 200 Hz (Chelysheva et al., 2023). For call analyses, we used 22,050 Hz sampling frequency, the Hamming window, FFT (Fast Fourier Transform) length 1024 points, frame 50%, and overlap 96.87%. These settings allowed a frequency resolution 22 Hz and time resolution 1.45 ms. All measurements were made manually and exported to Microsoft Excel (Microsoft Corp).

For each chirp, we manually measured two temporal parameters from the screen with the standard marker cursor in the spectrogram window: call duration and the duration from call onset to the point of maximum f0 (dur-to-max) (Figure 1). We calculated dur-to-max% as ratio dur-to-max to the total duration of each chirp. We measured, with the reticule cursor, four frequency parameters: the initial (f0beg), end (f0end), maximum (f0max), and minimum (f0min) fundamental frequencies of each chirp (Figure 1). In addition, we measured four power parameters: the maximum amplitude (= peak) frequency (fpeak) and three quartiles (q25, q50 and q75), covering, respectively, 25, 50, and 75% of call energy (hereafter the lower, medium, and upper quartiles) from the mean power spectrum of each chirp.

2.6 | Statistical analysis

Statistical analyses were performed using R 4.2.2 (R Core Team, 2022). All means are given as mean ± SD. Significance levels were set at 0.05, and two-tailed probability values are reported.

To investigate the structure of acoustic data and reveal linear relationships, we calculated Pearson correlation coefficients between acoustic parameters and age classes. To examine variation among age classes for each measured acoustic parameter, we performed a separate linear mixed-effect model (LMM) and fitted each parameter in the model as a response variable using R package *lme4* (Bates et al., 2015). Because of many zero values, one parameter (dur-to-max%) was analyzed as binary: zero/non-zero. For this parameter, we performed Generalized Linear Models (GLZ) for binomial error structure implemented in package *lme4*. Litter identity was fitted in all LMM and GLZ models as random effect. To assess the significance of fixed effects, we used Likelihood Ratio Tests (LRT). To determine the performance of the models and estimate contribution of litter identity to the total variation, we calculated the conditional and marginal coefficients of determination for all LMMs using the *MuMIn* package (Barton, 2018). Post hoc comparisons between age classes were performed with Tukey HSD (Honest Significantly Different) test using *emmeans* package (Lenth et al., 2023).

To identify the parameters which had the strongest correlation with caller age in months and were related to age in a monotonic way, we calculated Spearman rank-order correlation coefficients between the acoustic parameters and age. To develop a reliable method for age estimation of cheetahs based on the acoustic parameters of the chirps, we followed the statistical approaches developed for human age determination using the pulp/tooth volume ratio as the tooth morphological age predictors (Cameriere et al., 2004; Tardivo et al., 2014). Relationships of the acoustic parameters of cheetah chirps and caller age in month were visualized using the smoothed conditional means lines constructed using stat_smooth() function from the *ggplot2* package (Wickham, 2016).

For two acoustic parameters (f0max and fpeak), we fitted several linear regression models: simple linear regression model, polynomial model, and log-linear regression model, with caller age as a response. We included litter identity as a random term in all models and, therefore, used the mixed-effect modeling approach in the package *lme4*. To compare the performance of the models, to evaluate the models fit and to select the best model, we calculated the mean absolute error (MAE) using *Metrics* package (Hamner & Frasco, 2018), marginal (R^2_m), and conditional (R^2_c) coefficients of determination as well as Akaike's information criterion (AICc) using the *MuMIn* package for each model. To construct the prediction intervals incorporating the random effects for each model, we used predictInterval() function for mixed-effect models from the *merTools* package (Knowles & Frederick, 2023).

2.7 | Ethical note

This study was a part of the long-term monitoring program of the Mara-Meru Cheetah Project. Permissions for data collection in the field were granted to EVC by the Kenya Wildlife Service (Permit No.: KWS/BRM/5001), National Commission for Science, Technology and Innovations (Permit No.: NACOSTI/P/19/0995/27656), Narok County Government (Permit No.: NCG/MMNR/R/R/VOL. VII/53) and the management of the conservancies. We adhered to the “Guidelines for the treatment of animals in behavioural research and teaching” (Animal Behaviour, 2020, 159, I–XI) and to the Wildlife Conservation and Management Act 2013 – Protecting the welfare of wild animals (Kenya), where the study was conducted. During observations, researchers prioritized welfare of animals, maintaining a distance recommended by the local rules to minimize disturbance.

3 | RESULTS

3.1 | Chirp general acoustic structure

At all ages, cheetah chirps were tonal calls with well-visible fundamental frequency (f0) and harmonics (Figure 2). Generally, the contour of the f0 band was descending, so the start fundamental frequency f0beg was higher than the end fundamental frequency f0end, in 95.2% of the chirps (Figure 2). The location of the maximum point of f0 was strongly skewed toward call onset, so that in 52.4% of the chirps, the value of f0max coincided with value of f0beg. Correlation between f0max and f0beg among all age classes was very high ($r = .995$). At age classes 2–11, in over than 60% of chirps the point of f0 maximum coincided with call onset (Figure 3). In contrast, the f0beg was lower than the f0max and contour had a slight ascend at call onset in 80% of the chirps at age classes 1 and 12 and in 98% of the chirps at age classes 13 and 14 (Figures 2 and 3).

The minimum point of f0 was strongly skewed toward a call end, so the value of f0min coincided with the value of f0end in 79.8% of the chirps. Correlation between f0min and f0end among all age classes was very high ($r = .998$). At all age classes, for the exclusion of age classes 4 and 13, the location of f0min coincided with call end in over than 70% of the chirps (Figure 3). At age class 4, there were 68% chirps of such structure; at age class 13, there were 59% chirps of such structure (Figure 3).

3.2 | Chirp acoustic structure across age classes

Factor “age class” affected significantly all the eight acoustic parameters of the chirps (Table 2). Chirp duration was the longest in the cheetahs below 1 month old (age class 1), then shortened twice from age class 2 to age class 5 (between 1 and 5 months old), slightly increased at age class 6 (between 5 and 6 months old), then decreased again from age class 7 to age class 12 (between 6 and 48 months old) and then increased at age classes 13–14 (4 years old and older) (Table 2, Figure 4). Chirp durations at age classes 1, 6, 13, and 14 did not differ significantly ($p > .05$, Tukey post hoc) and were longer than in all other age classes ($p < .05$, Tukey post hoc).

The values of parameters of f0 of the chirps were the highest at age class 1 (below 1 month old) and steadily decreased up to adulthood, not differing between age class 13 (between 4 and 5 years old) and 14 (6 years old and older) (Table 2, Figure 4). The values of peak frequency and quartiles repeated the pathways of f0 parameters: they steadily decreased from age class 1 (below 1 month old) to age class 13 (between 4 and 5 years old) and slightly increased to age class 14 (6 years old and older) (Table 2, Figure 4). Both marginal (R^2_m) and conditional (R^2_c) coefficients of determination were high (up to 0.91 and 0.95, respectively, Table 2) in all frequency parameters. Therefore, the high percentage of variation in these parameters could be explained by age class. The difference between R^2_m and R^2_c for chirp duration was high, suggesting a strong random effect (i.e., among-litter variability) on this parameter.

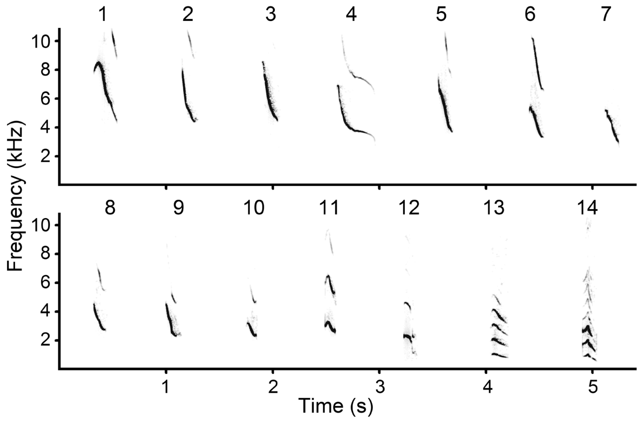


FIGURE 2 Spectrogram illustrating the changes of cheetah chirps from newborns to adults, one chirp per age class. Designations: 1–14—age classes. The spectrogram was created at 22,050 Hz sampling frequency, FFT length 1024, Hamming window, frame 50%, overlap 93.75%. The audio file of these calls is available as Audio S1.

3.3 | Modeling relationships of f0max and fpeak with cheetah age in months

To evaluate an approach for age determination using acoustic cues we initially selected the acoustic parameters that were the most stable. The Spearman rank-order correlation with age in months was significant for all studied acoustic parameters and was very strong and negative for the frequency estimations (Table 3). Based on simplicity, accuracy and robustness of measurements, and additionally on Spearman correlation coefficients, reflecting monotonicity of the relationships, we selected two parameters (measured in kHz) for constructing the age prediction models: the maximum fundamental frequency f0max and the frequency of maximum amplitude fpeak (Figure 5). Both parameters correlated strongly and negatively

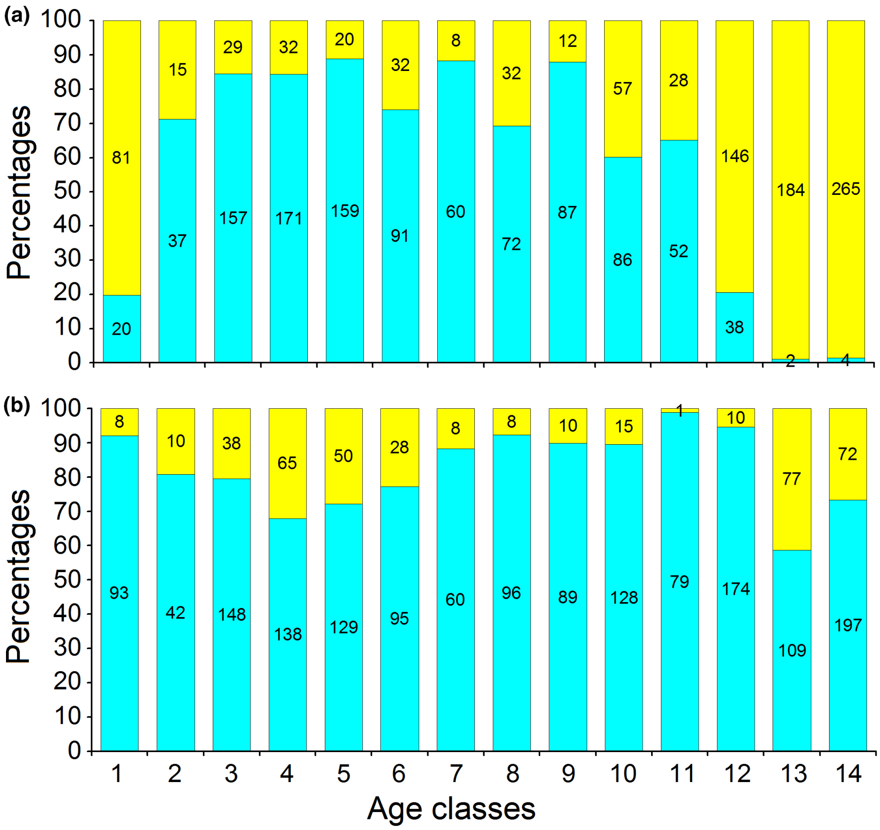


FIGURE 3 (a) Percentages of chirps at different age classes, in which the value of maximum fundamental frequency coincides (blue bars) or not (yellow bars) with the value of fundamental frequency at the onset of a call; (b) Percentages of chirps at different age classes, in which the value of minimum fundamental frequency coincides (blue bars) or not (yellow bars) with the value of fundamental frequency at the end of a call. Digits indicate the absolute numbers of chirps.

TABLE 2 Values (mean ± SD) of chirp frequency and temporal parameters for 14 age classes, marginal (R^2_m), and conditional (R^2_c) coefficients of determination, and results of likelihood ratio tests (LRT) for linear mixed-effect models (LMM) estimating the effects of age class on these parameters.

		Chirp acoustic parameters									
Age class	*n* chirps	Duration (s)	Dur-to-max%	f0beg (kHz)	f0end (kHz)	f0max (kHz)	f0min (kHz)	fpeak (kHz)	q25 (kHz)	q50 (kHz)	q75 (kHz)
1	101	0.23 ± 0.08	0.10 ± 0.08	7.56 ± 0.90	4.57 ± 0.92	7.92 ± 0.82	4.55 ± 0.91	6.29 ± 1.32	5.23 ± 1.06	6.24 ± 0.90	7.27 ± 0.69
2	52	0.12 ± 0.03	0.03 ± 0.06	7.46 ± 1.00	4.43 ± 0.43	7.54 ± 0.99	4.41 ± 0.44	5.49 ± 0.70	5.08 ± 0.36	5.62 ± 0.34	6.39 ± 0.52
3	186	0.11 ± 0.03	0.02 ± 0.06	7.10 ± 0.95	4.10 ± 0.46	7.20 ± 0.85	4.07 ± 0.44	4.86 ± 0.78	4.50 ± 0.61	5.08 ± 0.55	5.77 ± 0.61
4	203	0.12 ± 0.03	0.02 ± 0.06	6.78 ± 0.87	3.90 ± 0.55	6.86 ± 0.81	3.82 ± 0.51	4.41 ± 0.54	4.14 ± 0.57	4.69 ± 0.47	5.44 ± 0.63
5	179	0.12 ± 0.03	0.01 ± 0.04	6.60 ± 0.91	3.88 ± 0.55	6.63 ± 0.89	3.84 ± 0.53	4.38 ± 0.66	4.18 ± 0.54	4.64 ± 0.54	5.28 ± 0.59
6	123	0.17 ± 0.06	0.04 ± 0.08	5.62 ± 0.81	3.53 ± 0.57	5.78 ± 0.73	3.51 ± 0.56	4.03 ± 0.55	3.69 ± 0.63	4.32 ± 0.52	5.15 ± 0.84
7	68	0.14 ± 0.06	0.02 ± 0.05	4.73 ± 0.68	2.86 ± 0.42	4.76 ± 0.65	2.85 ± 0.40	3.43 ± 0.80	3.12 ± 0.47	3.71 ± 0.49	4.64 ± 1.01
8	104	0.13 ± 0.03	0.05 ± 0.08	4.57 ± 0.80	2.80 ± 0.55	4.66 ± 0.75	2.79 ± 0.55	3.33 ± 0.54	3.15 ± 0.46	3.52 ± 0.49	4.18 ± 0.71
9	99	0.12 ± 0.06	0.02 ± 0.06	3.83 ± 0.47	2.35 ± 0.33	3.87 ± 0.44	2.32 ± 0.30	2.82 ± 0.55	2.66 ± 0.26	3.17 ± 0.48	4.14 ± 1.09
10	143	0.11 ± 0.03	0.09 ± 0.12	3.47 ± 0.78	2.30 ± 0.48	3.61 ± 0.72	2.28 ± 0.46	2.76 ± 0.50	2.63 ± 0.40	2.90 ± 0.46	3.56 ± 0.88
11	80	0.12 ± 0.02	0.08 ± 0.13	2.47 ± 0.58	1.31 ± 0.62	2.73 ± 0.53	1.30 ± 0.62	2.23 ± 0.50	2.02 ± 0.47	2.31 ± 0.42	2.72 ± 0.37
12	184	0.17 ± 0.07	0.20 ± 0.13	1.36 ± 0.76	0.76 ± 0.35	1.77 ± 0.73	0.75 ± 0.35	1.88 ± 0.64	1.63 ± 0.41	2.16 ± 0.50	3.20 ± 0.84
13	186	0.20 ± 0.07	0.25 ± 0.12	0.67 ± 0.21	0.62 ± 0.14	0.87 ± 0.20	0.59 ± 0.14	1.65 ± 0.83	1.45 ± 0.46	2.22 ± 0.51	3.10 ± 0.55
14	269	0.18 ± 0.06	0.27 ± 0.13	0.78 ± 0.22	0.63 ± 0.17	0.97 ± 0.20	0.62 ± 0.15	1.75 ± 0.69	1.52 ± 0.35	2.28 ± 0.70	3.39 ± 1.31
R^2_m		$R^2_m = 0.18$		$R^2_m = 0.89$	$R^2_m = 0.85$	$R^2_m = 0.91$	$R^2_m = 0.86$	$R^2_m = 0.74$	$R^2_m = 0.80$	$R^2_m = 0.81$	$R^2_m = 0.66$
R^2_c		$R^2_c = 0.71$		$R^2_c = 0.94$	$R^2_c = 0.94$	$R^2_c = 0.95$	$R^2_c = 0.94$	$R^2_c = 0.84$	$R^2_c = 0.90$	$R^2_c = 0.89$	$R^2_c = 0.81$
LRT results	df = 13	$\chi^2 = 406.1$; $p < .001$	$\chi^2 = 263.7$; $p < .001$	$\chi^2 = 2203.1$; < 0.001	$\chi^2 = 2101.8$; $p < .001$	$\chi^2 = 2451.8$; $p < .001$	$\chi^2 = 2173.7$; $p < .001$	$\chi^2 = 1121.0$; $p < .001$	$\chi^2 = 1523.3$; $p < .001$	$\chi^2 = 1641.9$; $p < .001$	$\chi^2 = 1005.1$; $p < .001$

Note: Designations: duration, chirp duration; dur-to-max%, ratio of the duration from chirp onset to the point of maximum f0 to chirp duration; f0beg, the fundamental frequency at the onset of a chirp; f0end, the fundamental frequency at the end of a chirp; f0max, the maximum fundamental frequency; f0min, the minimum fundamental frequency; fpeak, the frequency of maximum amplitude; q25, lower quartile; q50, medium quartile; q75, upper quartile.

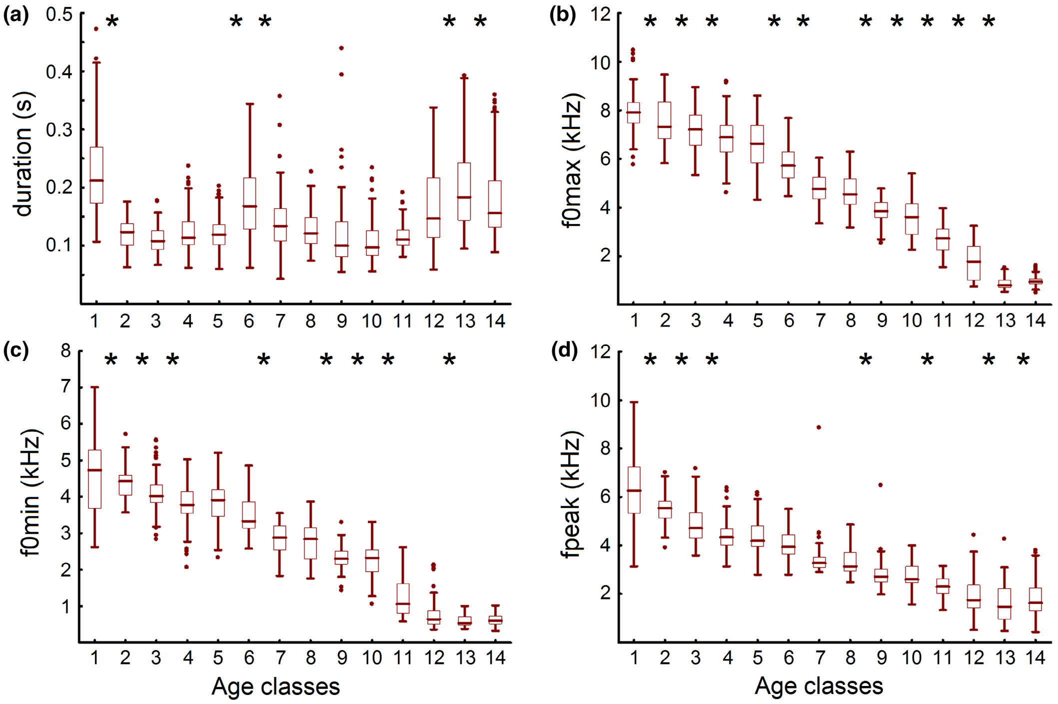


FIGURE 4 Changes in values of acoustic parameters of cheetah chirps across 14 age classes. (a) call duration; (b) the maximum fundamental frequency; (c) the minimum fundamental frequency; (d) the frequency of maximum amplitude. Central lines indicate medians, boxes indicate quartiles, whiskers indicate min and max values, circles indicate outliers. Asterisks indicate neighboring age classes, which are significantly different by the given acoustic parameter ($p < .05$, Tukey post hoc).

TABLE 3 Spearman rank-order correlation coefficients (r_s) between the acoustic parameters of cheetah chirps and caller age in months. Designations see in the legend to Table 2.

Acoustic parameter	Statistics
Duration	$r_s = .24$, $p < .0001$
f0beg	$r_s = -.92$, $p < .0001$
f0end	$r_s = -.91$, $p < .0001$
f0max	$r_s = -.93$, $p < .0001$
f0min	$r_s = -.91$, $p < .0001$
fpeak	$r_s = -.89$, $p < .0001$
q25	$r_s = -.90$, $p < .0001$
q50	$r_s = -.89$, $p < .0001$
q75	$r_s = -.79$, $p < .0001$

Note: *N* calls = 1977.

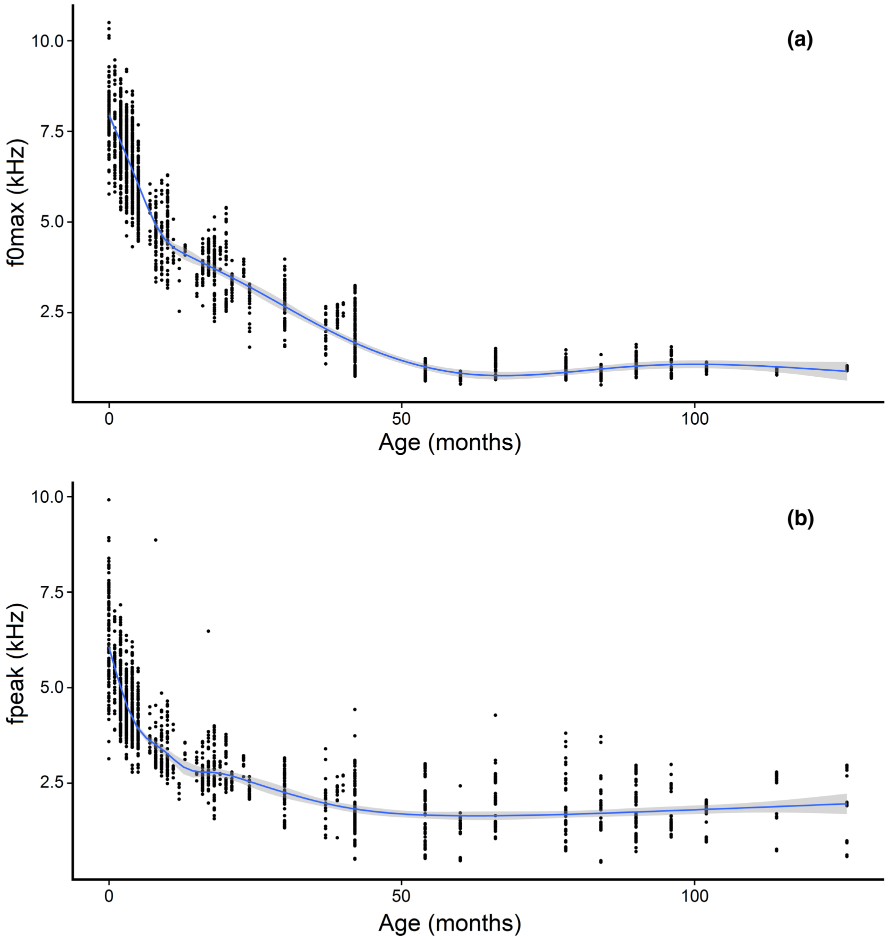
with age (Table 3), and in both parameters, the shape of the point clouds showed signs of non-linearity and heteroscedasticity. Due to the non-linear profile of the graphs for the both variables, we fitted four initial models for each acoustic parameter (Table 4): (1) the simple lineal regression model; (2) the model with the linear and the quadratic terms $[\mathrm{f0max}]^2$ or $[\mathrm{fpeak}]^2$; (3) the model with the linear, quadratic and cubic terms $[\mathrm{f0max}]^3$ or $[\mathrm{fpeak}]^3$; (4) the model with logarithmic term log[f0max] or log[fpeak].

Initially, we fitted all models for the full set of data (Table 4), but then we performed the modeling of age for individuals below 48 months of age to reduce heteroscedasticity and increase accuracy of the age determination. Low values of both f0max and f0peak occurred at a range of ages (Figure 5), thus, applicability of these parameters for the accurate age prediction in individuals older than 48 months was questionable.

We compare the performance of the models (both for the full and reduced subsets) using Akaike information criterion (AICc), mean absolute error (MAE) and marginal (R^2_m) and conditional (R^2_c) coefficients of determination (Table 4). The best model with the lowest AICc and the highest percentage of the explained variance for the full dataset was the cubic model for f0max. In the reduced subset the cubic model for f0max was the most parsimonious model with the lowest AICc (Figure 6a), but the marginal coefficient of determination was the largest ($R^2_m = 0.69$) for the quadratic model for f0max; moreover, the cubic model for f0max demonstrated poor fit for the youngest age classes in all models (the modeled age tended to be overestimated for the youngest juveniles). In addition, the cubic model for f0max was less appropriate for practical use, because it included four terms and therefore was too complicated. Therefore, we selected the second-best model for the age estimation, the quadratic model for f0max (Figure 6b).

Separately for fpeak the best models were as well as the cubic and quadratic models, but all fpeak models (Figure 6c,d) fitted the data much worse than f0max models (Table 4), and therefore we did not use them for the age prediction. Thus, the quadratic model for f0max created using the reduced set of individuals below 48 months of age was selected as the model for age prediction. The 95% fluctuation interval associated with age prediction for age below 48 month was ±4.3 months.

FIGURE 5 Scatterplot illustrating the changes in values of (a) maximum fundamental frequency (f0max) and (b) frequency of maximum amplitude (fpeak) of cheetah chirps across ages in months. Each point indicates the values for one chirp. The blue lines correspond to the smoothed conditional means lines.



The average age of callers with f0max <1.5 kHz was 73.9 ± 22.5 months (mean ± SD), and no individual younger than 37 months had f0max below 1.5 kHz; there were 70 calls from 521 with f0max <1.5 kHz with age = 42 months (*N* = 65) and age = 37 months (*N* = 5). The average age of the cheetahs with f0max ≥1.5 kHz was 10.7 ± 12.3 months and only four calls from 1456 with f0max above 1.5 kHz were from individuals older than 4 years. Therefore, based on the full set of data and the constructed models we suggest that the calls with the maximum fundamental frequency (f0max) below 1.5 kHz should be classified as adult individuals with the age >4 years.

4 | DISCUSSION

This study showed that, in wild-living cheetahs, the f0max of the long-distance chirps monotonously decreased, from 7.92 kHz in neonates to about 0.90 kHz in mature cheetahs of 4 years and older (Table 2). After 4 years of age (age classes 13–14), the f0max reached a plateau and then remained unchanged (Table 2, Figure 5). Duration of the chirps did not differ between neonate and mature cheetahs, whereas in cheetahs from 2 months to 3 years of age, the duration of the chirps was two times shorter (Table 2, Figure 4). Non-linear but monotonous decrease of f0max of the chirps allowed advancing a voice-based method for estimating cheetah age, which might be a useful tool for conservationists, for non-invasive monitoring of cheetah ages in nature.

4.1 | Comparison with vocal ontogeny of other felids

This study is the first which estimated the full-scale vocal ontogeny from neonate cubs to mature adults in a felid species. Earlier studies on felids reported only results of cross-sectional comparisons of calls of cubs and adults and sometimes adolescents.

Meows of captive cheetah cubs aged from 2 days to 3 months of age have an average f0max of 3.89 kHz; the average duration of cub meow was 0.56 s (Volodina, 1998). With increasing age, the f0 of cheetah meows decreases, and meows of adult cheetahs have an average f0max of 0.94–1.09 kHz and duration of 0.32 s (Smirnova et al., 2016; Volodina, 2000).

In felids, the vocal ontogeny was previously investigated in domestic cat. Kittens from neonates to 1 month of age produce the meows with f0max of 1.3–1.55 kHz and duration of 0.44–1.0 s (Banszegi et al., 2017; Brown et al., 1978; Haskins, 1979; Hubka et al., 2015; Romand & Ehret, 1984; Scheumann et al., 2012). From 1 to 3 months of age, the f0max of the meows decreases to 0.7 kHz, whereas duration slightly increases to 0.9 s (Banszegi et al., 2017;

TABLE 4 Equations for age determination for each model (LMM; the litter identity was fitted in all models as a random term).

Model	Dataset	Equation: Age =	Coefficients with SE	R^2_m	R^2_c	AIC$_c$	MAE
Max linear	Full	$48.6 - 4.8X_1$	48.6 ± 3.3, −4.8 ± 0.2	0.20	0.91	14,308	5.00
Max quadratic	***Full***	***$28.0 - 776.0X_1 + 237.9X_1^2$***	***28.0 ± 2.5, −776.0 ± 22.7, 237.9 ± 12.7***	***0.45***	***0.92***	***13,973***	4.36
Max cubic	Full	$28.0 - 780.4X_1 + 243.5X_1^2 - 17.6X_1^3$	28.0 ± 2.5, −780.4 ± 22.8, 243.5 ± 13.0, −17.6 ± 9.2	**0.46**	**0.92**	**13,965**	4.32
Max logarithmic	Full	$51.0 - 19.7 \log X_1$	51.0 ± 2.8, −19.7 ± 0.6	0.37	0.91	14,092	4.61
Peak linear	Full	$40.5 - 3.9X_1$	40.5 ± 4.1, −3.9 ± 0.3	0.20	0.92	14,686	5.43
Peak quadratic	Full	$27.4 - 284.6X_1 + 47.5X_1^2$	27.4 ± 3.9, −284.6 ± 18.7, 47.5 ± 12.5	0.20	0.91	14,658	5.36
Peak cubic	Full	$27.5 - 307.6X_1 + 43.1X_1^2 + 73.2X_1^3$	27.5 ± 3.9, −307.6 ± 18.8, 43.1 ± 12.3, 73.2 ± 10.5	0.20	0.92	14,606	5.27
Peak logarithmic	Full	$36.1 - 8.3 \log X_1$	36.1 ± 4.2, −8.3 ± 0.7	0.20	0.92	14,757	5.46
Max linear	Reduced	$30.2 - 3.5X_1$	30.2 ± 1.1, −3.5 ± 0.08	0.49	0.89	8325	2.47
Max quadratic	Reduced	$11.5 - 336.1X_1 + 80.6X_1^2$	11.5 ± 0.8, −336.1 ± 6.7, 80.6 ± 5.1	**0.69**	0.91	8083	2.24
Max cubic	Reduced	$11.5 - 318.8X_1 + 56.3X_1^2 + 52.7X_1^3$	11.5 ± 0.9, −318.8 ± 6.4, 56.3 ± 5.0, 52.7 ± 3.8	0.62	**0.92**	**7902**	2.07
Max logarithmic	Reduced	$35.3 - 15.4 \log X_1$	35.3 ± 1.0, −15.4 ± 0.4	0.62	0.88	8472	2.50
Peak linear	Reduced	$26.2 - 3.9X_1$	26.2 ± 1.4, −3.9 ± 0.1	0.20	0.86	8884	2.85
Peak quadratic	Reduced	$11.4 - 234.9X_1 + 78.9X_1^2$	11.4 ± 1.1, −234.9 ± 7.0, 78.9 ± 5.4	0.20	0.86	8677	2.71
Peak cubic	Reduced	$11.4 - 235.9X_1 + 71.8X_1^2 + 29.6X_1^3$	11.4 ± 1.2, −235.9 ± 7.0, 71.8 ± 5.5, 29.6 ± 4.6	0.20	0.87	8634	2.70
Peak logarithmic	Reduced	$28.8 - 13.9 \log X_1$	28.8 ± 1.3, −13.9 ± 0.5	0.20	0.85	8867	2.83

Note: X_1 = f0max (kHz), X_2 = fpeak (kHz). Full dataset included all measurements; reduced dataset included calls from individuals younger than 4 years old. Mean absolute error (MAE), Akaike's information criterion (AIC$_c$), marginal (R^2_m), and conditional (R^2_c) coefficients of determination are presented as measurements of the model fit. R^2_m considered the percentage of variance explained by fixed terms only. AIC$_c$ were calculated for full and reduced sets of models separately. The lowest AIC$_c$ and the highest R^2_m are marked with bold for the full and reduced subsets of the data. The best model selected for the age prediction is marked with bold and italic.

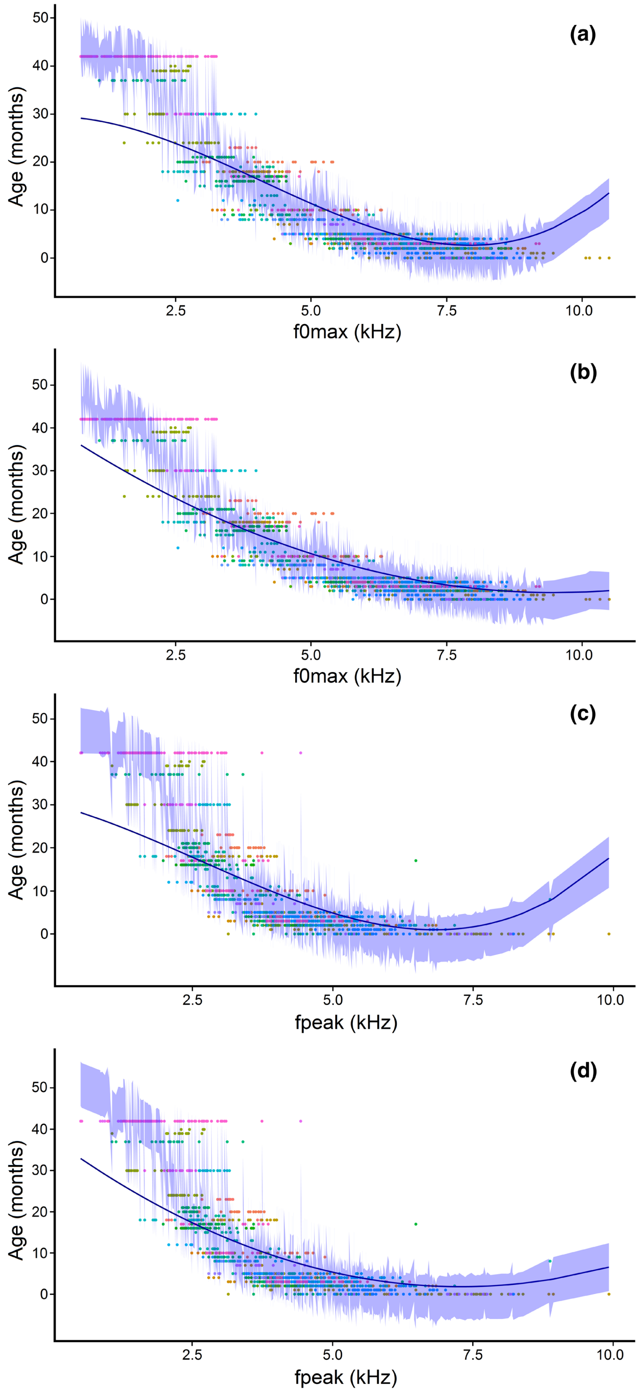


FIGURE 6 Fit and prediction intervals of the linear regression analysis for the acoustic parameters and cheetah age. Dots indicate individual chirps; colors indicate litter identities. All plots were created for the reduced subset with caller age <48 months. (a) the cubic model for f0max; (b) the quadratic model for f0max; (c) the cubic model for fpeak; (d) the quadratic model for fpeak.

Hubka et al., 2015). Furthermore, from 5 weeks to 6 months of age, the meows f0 substantially decreases, whereas duration remains the same (Romand & Ehret, 1984; Urrutia et al., 2022). Young adult (18-months) domestic cats produce meows of 0.54–0.70 s in duration and with mean f0 of 1.19 kHz (Schötz, 2012) or 0.30–0.35 kHz (Urrutia et al., 2022); differences in these data were probably related to the differences in the recording contexts. The meows of adult domestic cats, recorded in various contexts, ranged from 0.50 to 0.88 kHz in f0max and from 0.60 to 1.02 s in duration (Farley et al., 1992; Nicastro, 2004; Nicastro & Owren, 2003; Schnaider et al., 2022; Sedova et al., 2023; Yeon et al., 2011). Adult feral domestic cats produced the meows with f0max of 0.40 kHz and a prolonged duration of 1.7–1.8 s (Yeon et al., 2011). Thus, in the vocal ontogeny of the domestic cat meows, the f0 monotonously decreases, whereas the duration remains unchanged, similar to cheetah chirps in this study. However, the f0 of cat meows is much lower than those of chirps and meows of cheetahs along ontogeny, although cheetahs are much larger in body size than domestic cats.

In contrast to domestic kittens and similar to cheetahs, puma cubs produce very high-frequency long-distance calls. From birth to approximately 4 months of age, the f0max of the calls in captivity is about 4.1–5.5 kHz (Peters, 2011). For wild pumas, there is a description of the call (probably produced by a cub), with f0max of 4.5 kHz and duration of 0.4 s, however, the caller was out of sighting during the recording (Allen et al., 2016). Adult 7–10-year-old captive pumas produced high-frequency long-distance calls, belonging to three call types: meows, main calls and whistle by classification of Peters (1978), with f0max of 1.09–1.45 kHz and duration of 0.30–0.70 s (Potter, 2005). However, in the wild, pumas responded to playbacks of their own calls by calls with f0max of 0.43 kHz and duration of 0.60 s (Macarrão et al., 2012).

Other felids with high-frequency cub calls are the Eurasian lynx *Lynx lynx* and the bobcat *L. rufus*. In contrast to cheetahs, these calls can be more complex in the acoustic structure, because, in both *Lynx* species, cubs and adults are capable of producing the biphonic calls with two independent fundamental frequencies (Peters, 1987; Rutovskaya et al., 2009). In the calls of the Eurasian lynx cubs at the age from 4 days to 1 month, there are two well-detectable fundamental frequencies, the high fundamental frequency about 3 kHz and the low fundamental frequency about 1 kHz (Peters, 1987). In newborn cubs of Eurasian lynx, the maximum values of the high fundamental frequency can reach as high as 5–6 kHz during the first week of life (our unpublished data). In adult Eurasian lynxes, 43% of their intense long-distance meows, produced by males during the mating season, contain two close-in-values fundamental frequencies, 0.65–0.92 kHz and 0.90–1.16 kHz, respectively (Rutovskaya et al., 2009). In the bobcat cubs, the meows also have a high fundamental frequency (about 3 kHz), but biphonations are not present on the published spectrograms (Peters, 1987). In adult bobcats, their long-distance meows are biphonic, as in the Eurasian lynx, with the high maximum fundamental frequency of about 1.5 kHz (Peters, 1987).

Among large felids of the genus *Panthera*, cub calls are only described for the tiger *Panthera tigris* (Kong et al., 2022). At isolation in captivity, tiger cubs from birth to 10 months of age produce “ar” calls, with f0max of 0.59 kHz and duration of 0.80 s, which are very similar by spectrogram to meows of other felids (Kong et al., 2022). In the long-distance roars of captive adult tigers, the f0max is about 0.32 kHz and f0mean is about 0.16–0.17 kHz (Ji et al., 2013; Walsh et al., 2010). For adult captive tigers of 3–10 years old, meow-like moans with f0mean 0.18 kHz were also described (Rose et al., 2018). The lack of data on longitudinal vocal ontogeny in felids of the genus *Panthera* with descended larynx in adults (Hast, 1989; Klemuk et al., 2011; Peters & Hast, 1994; Weissengruber et al., 2002, 2008) prevents understanding, whether the ontogenetic decrease in fundamental frequency occurs smoothly or abruptly in these species, as the result of descent of the larynx with maturation.

We conclude that vocal ontogeny of cheetahs and other investigated felid species follows a common rule for mammals, that calls of small offspring with their small vocal folds have a higher fundamental frequency than analogous calls of large adults with their large vocal folds (Ey et al., 2007; Fitch & Hauser, 2003; Matrosova et al., 2007), but see exclusions from this rule found in shrew (Schneiderová, 2014; Volodin et al., 2015), rodents (Matrosova et al., 2007, 2011), and artiodactyls (Padilla de la Torre et al., 2015; Volodin et al., 2016). However, in mammals, additionally to calls produced with phonation mechanism based on air flow-induced vibrations of the vocal folds (Berke & Long, 2010; Herbst, 2014; Herbst et al., 2012) there are calls produced with another mechanism, the aerodynamic whistle, based on airflow vorticities in the vocal tract (Håkansson et al., 2022; Mahrt et al., 2016; Riede et al., 2017, 2022). Calls of the same individual animal produced with phonation mechanism have a substantially lower fundamental frequency than those produced with whistle mechanism (carnivores: Frey et al., 2016; Sibiryakova et al., 2021, artiodactyls: Reby et al., 2016; Volodin, Volodina, & Frey, 2017, rodents: Dymskaya et al., 2022; Fernández-Vargas et al., 2022). In addition to cubs of cheetahs, cubs of other felids, as lynxes and pumas are capable of producing very high-frequency calls, up to 5 kHz (Allen et al., 2016; Peters, 1987, 2011). For cheetahs in this study, we tracked the monotonous decrease of the very high-pitched fundamental frequency from newborns to adults. We did not find in the vocal ontogeny of the cheetah any signs of sudden fast changes of f0, which are characteristic of mammals with descent of the larynx in ontogeny, as humans and goitred gazelles (Fitch & Giedd, 1999; Lee et al., 1999; Volodin, Efremova, et al., 2017), or of birds with voice breaking, as cranes (Klenova et al., 2010). Biphonic calls with two fundamental frequencies, common in cubs and adults Eurasian lynx and bobcat (Peters, 1987; Rutovskaya et al., 2009), lacked in adult cheetahs and were only observed a few times in cubs.

Thus, we can propose that cheetah cubs produce their very high-frequency chirps (up to 10 kHz in newborns, Figures 4 and 5) with phonation mechanism based on vibration of the vocal folds. Producing such high-frequency calls with vocal folds is very unusual for such large-sized animals as cheetahs, because even for Norway rats *Rattus norvegicus*, a possibility of producing vocalizations higher than 6 kHz with vocal folds was experimentally rejected (Riede et al., 2011). Findings of very high-frequency calls in cheetah and puma cubs and of the biphonic calls in the Eurasian lynx and bobcat encourage more detailed studying of physiological mechanisms for producing calls in felids.

4.2 | Voice-based method for estimating cheetah age

In this study, we designed for the first time for a wild-living mammalian species, the technique of age determination based on parameters of vocalizations. We modified the computational approaches developed for the quantitative estimation of age in humans (Cameriere et al., 2004; Tardivo et al., 2014) by including in our models a random term. This made the modeling more complicated. Almost all estimations of frequency parameters of cheetah chirps decreased strongly and monotonously with age and therefore all of them could be used as indicator of caller age with various degrees of precision. It was fortunate to find for this charismatic carnivorous species an age indicator that can be easily and non-invasively measured in the wild without even watching an animal. Despite the fact that all our models, including the best one, did not fit the data perfectly, we obtained a tool that could be used for aging individuals with precision ±4.3 month and for distinguishing mature individuals from the younger animals below 4 years of age. Such tool can be useful for some applied conservational tasks.

Limitations of our model for age determination by voice in the cheetah based on partly from the character of our data: heteroscedasticity and the deficiency of audio recordings and acoustic measurements for cheetahs between the 40th and 60th months of age (Figure 5). Thus, imprecise age prediction was obtained for chirps with f0max below 2.5 kHz. In addition, it was impossible to include in the current model caller sex due to difficulties of sex determination in small cheetah cubs. The modeling can be improved if data on caller sex are available for all age classes. Caller sex was known only for animals older than 1 year and number of chirps from animals with known sex (*n* = 907 chirps) comprised less than half of chirps used in our study (*n* = 1977 chirps, Table 1). Nevertheless, even without data about sex, the relationship of f0max with age was evident. Animal sex as a source of dispersion does not change the found relationship. For conservation purposes, it is important that while in field sex often cannot be determined, the age of cheetahs can anyway be estimated by chirps.

For mature cheetahs, we estimated the effect of sex on the f0 of chirps in a previous study (Chelysheva et al., 2023). In cheetahs of 4 years old and older, the f0max of the chirps was on average 0.76 kHz in males and 0.98 kHz in females (Chelysheva et al., 2023). These differences are consistent with sex dimorphism of body size in this species, up to 15% in captivity (Wildt et al., 1993) and from 15% to 22% in the wild (Caro, 1994; Marker & Dickman, 2003). For comparison, distant meows of male domestic cats produced in mating season are much lower in f0max than female meows, 0.37 and 0.61 kHz, respectively (Sedova et al., 2023), although in free-ranging domestic cats sex differences in body mass are 15%–20%, with males heavier than females (Naidenko et al., 2020).

Some limitations of our model based on the used computational approaches developed for the quantitative estimation of age in humans. We tested numerous different models used for estimating human age based on pulp/tooth volume ratio to find the best fit model for cheetah age estimation by voice. Difficulties with applying some of these models suggest that lack of fit could be expected not only for our model, but also for pulp/tooth volume ratio models for humans. Actually, in some such studies, a simple linear regression was used as the main instrument for prediction (Elgazzar et al., 2020; Kazmi et al., 2019), and in some other works polynomial and linear-log regression (Herrman et al., 2020) analyses were used, all of which we tried in our work. This modeling can be potentially refined by applying the models based on logistic function (Cavallini, 1993), since the general form of curves in our study was more or less similar to inverse logistic curve (Haddon et al., 2008).

AUTHOR CONTRIBUTIONS

Ilya A. Volodin: Conceptualization; methodology; software; validation; resources; data curation; writing – review and editing; writing – original draft; project administration. **Anna V. Klenova:** Conceptualization; methodology; software; formal analysis; visualization; writing – review and editing. **Elena V. Chelysheva:** Conceptualization; methodology; investigation; formal analysis; resources; writing – review and editing; project administration; funding acquisition. **Nina A. Vasilieva:** Conceptualization; methodology; software; formal analysis; visualization; writing – review and editing; writing – original draft. **Elena V. Volodina:** Conceptualization; methodology; resources; writing – review and editing; writing – original draft; validation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A

TABLE A1 Number of chirps included in the analyses for each of the 14 age classes and for each of the 56 cheetah litters.

					Age classes													
Litter N	Mother ID	Cubs ID[a]	Litter birth	N cubs per litter[b]	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	F3	M30, F50	06.2012	2														35(2)
2	F3	F55	09.2013	1												20(1)		
3	F3	F73, F74	02.2016	2										35				
4	F3	M108, F82, F83	04.2018	3						20					40(2)	20(1)		
5	F5	F3	12.2009	1														20(1)
6	F7	M44, M45	11.2014	2												20(1)		
7	F7		07.2016	5	20	35	20					2						
8	F8	M65, F68, F69	06.2015	3									20				40(2)	
9	F8		02.2019	1					22									
10	F10	F13	10.2007	1														20(1)
11	F13	M46, F57	07.2014	2					9									
12	F13	M86, M87	06.2016	2			22					20		20				
13	F16	F42	05.2012	1														20(1)
14	F18	F8	10.2008	1														20(1)
15	F19	F64	07.2014	1													20(1)	
16	F26	F59	12.2014	1														20(1)
17	F42		12.2018	1				20										
18	F50	M62, M63, F67	05.2015	3								4					20(1)	20(1)
19	F50		01.2018	3			20		8									
20	F50		10.2020	3				20	20									
21	F50		06.2021	2			15											
22	F51	M109, M110, F84	09.2018	5	7					20	4			20				
23	F53	F78	06.2016	1													20(1)	
24	F55		02.2017	2		17	9											
25	F55		08.2018	5	40													
26	F56		08.2021	1						20								
27	F59	M111, M112, M113	11.2018	3								18		3				
28	F59		06.2021	5						4	40							
29	F63		06.2018	2			20											
30	F63		09.2019	7			20	8										
31	F63		04.2021	4			20	20	20				20					

(Continues)

APPENDIX A (Continued)

Litter N	Mother ID	Cubs ID[a]	Litter birth	N cubs per litter[b]	Age classes 1	2	3	4	5	6	7	8	9	10	11	12	13	14
32	F67		08.2019	2					20				20					
33	F67		11.2021	2					20	20								
34	F68	M120, F98, F99	11.2020	3				20				20	20	40(2)				
35	F69		11.2018	1	20													
36	F69	M117, F89	05.2020	2			20	20		20			14	9				
37	F69		03.2022	3	14													
38	F74		06.2019	6				20			10	20						
39	F74		07.2021	3			20											
40	F76	M116	01.2020	1										16(1)				
41	F78	F95	01.2020	3				20							20(1)			
42	F78		06.2021	3					20									
43	F80		05.2020	3							4							
44	F82		03.2021	3					20	9			5					
45	F83		05.2020	2				20	20									
46	F83		10.2021	3								20						
47	F84		05.2022	4				15										
48	F87		08.2020	3						10								
49	F90		10.2021	1							10							
50	F95		04.2022	3				20										
51	Unknown	M58, F63	end 2014	2												24(2)	20(1)	34(2)
52	Unknown	M107	beg 2018	1											20(1)			
53	Unknown	M51, M52	end 2014	2												40(2)		
54	Unknown	M70, M71, M72, M73	10.2014	4												60(3)	40(2)	60(3)
55	Unknown	M66, M67	beg 2016	2													26(2)	
56	Unknown	M4	09.2009	1														20(1)
Total[c]					101/5	52/2	186/10	203/11	179/10	123/8	68/5	104/7	99/6	143/7	80/3	184/6	186/7	269/10

Note: The number of individually identified animals at the age of 18 months and older (age classes 10–14) in a given litter is indicated in brackets. M, male, F, female.

[a] Cubs ID are only indicated for animals for which recordings were available at the age of 18 months and older (age classes 10–14).

[b] Number of cubs in the litter, found during the first observation.

[c] Number of calls/litters per age class in total.