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## Siberian crane chick calls reflect their thermal state

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### ABSTRACT

Chicks can convey information about their needs with calls. But it is still unknown if there are any universal need indicators in chick vocalizations. Previous studies have shown that in some species vocal activity and/or temporal-frequency variables of calls are related to the chick state, whereas other studies did not confirm it. Here, we tested experimentally whether vocal activity and temporal-frequency variables of calls change with cooling. We studied 10 human-raised Siberian crane (*Grus leucogeranus*) chicks at 3–15 days of age. We found that the cooled chicks produced calls higher in fundamental frequency and power variables, longer in duration and at a higher calling rate than in the control chicks. However, we did not find significant changes in level of entropy and occurrence of non-linear phenomena in chick calls recorded during the experimental cooling. We suggest that the level of vocal activity is a universal indicator of need for warmth in precocial and semi-precocial birds (e.g. cranes), but not in altricial ones. We also assume that coding of needs via temporal-frequency variables of calls is typical in species whose adults could not confuse their chicks with other chicks. Siberian cranes stay on separate territories during their breeding season, so parents do not need to check individuality of their offspring in the home area. In this case, all call characteristics are available for other purposes and serve to communicate chicks' vital needs.

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Cold-induced calling;  
Siberian crane;  
thermoregulation; signals of  
need; acoustic signalling

## Introduction

It is well known that avian hatchlings can convey information about their demands and fitness with vocalizations (e.g. Hamer et al. 1999; Leonard and Horn 2006). Not only do parents perceive such information, but moreover, they adjust their investment into hatchlings on the basis of it (e.g. Leonard and Horn 2001a; Quillfeldt et al. 2004; Gladbach et al. 2009). However, it is still unclear if birds have any universal ways to express chick needs with acoustic clues.

The vast majority of parent–offspring communication studies have focused on food begging in altricial birds. Almost all of them have shown significant increase in overall vocal activity (e.g. N of begging calls per minute) with the increase of nutritional needs (e.g. Leonard and Horn 2001b; Royle et al. 2002; Sacchi et al. 2002; Marques et al. 2008, 2009).

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However, reported changes in the acoustic structure of calls, e.g. fundamental frequency modulation or frequency spectrum, were inconsistent in different studies: some of them have shown correlation of acoustic structure with hunger level (Leonard and Horn 2001a; Gladbach et al. 2009; Marques et al. 2009; Goncharova and Klenova 2015; Klenova 2015), whereas others have not (Träger et al. 2006; Duckworth et al. 2009; Quillfeldt et al. 2010).

Warmth is another basic need of chicks at an early age. But it is still unclear how young birds encode information about need for warmth in their acoustic signals and if they use the same acoustic variables to solicit feeding and brooding. Reports about the effect of warmth deprivation on overall vocal activity and time-frequency parameters are scarce. It has been shown that bird embryos could convey information about their thermal needs (either warming or cooling) as early as they peck through the air sac (e.g. Evans 1990; Bugden and Evans 1991; Brua et al. 1996; Berlin and Clark 1998; Abraham and Evans 1999). Interestingly, the reaction of altricial birds to cooling becomes stronger when the moment of hatching approaches (e.g. Berlin and Clark 1998), whereas precocial species' embryos react equally strong regardless of time prior to hatching (e.g. Bugden and Evans 1991; Evans et al. 1994; Brua et al. 1996; Gräns and Altimiras 2007). In addition, altricial birds' embryos increase their call rates in response to both cooling and heating above optimal temperature, and the effect of heating is stronger (Evans 1990; Berlin and Clark 1998).

Studies of the impact of temperature on single call parameters of bird embryos are particularly scarce. For instance, cooling of altricial species embryos increases their call duration and amplitude (Evans 1990), whereas no correlation has been found between ambient temperature and vocal parameters of precocial species embryos (Gräns and Altimiras 2007).

The studies focusing on altricial hatchlings of different age revealed that the older the chick is, the weaker the effect of ambient temperature on the vocal behaviour (e.g. Evans 1994; Leonard and Horn 2001b; Dakin et al. 2016). However, the effect of cooling on call rate of newly hatched altricial chicks is mixed: in some species the call rate increases with cooling (Evans 1994), but it declines in others (Choi and Bakken 1990). Moreover, the effect of cooling on the call rate and other vocal parameters of tree swallow *Tachycineta bicolor* vary among chicks of same age depending on their body mass (Leonard and Horn 2001b; Dakin et al. 2016). For instance, 3-day-old low-body-mass chicks decrease their call rate and average call duration with cooling, whereas high-body-mass chicks of same age increase them (Dakin et al. 2016). However, the peak frequency declines with cooling in both low-weight and high-weight groups of 6-day-old chicks (Leonard and Horn 2001b).

In all studied precocial species, cooling increases call rate (e.g. Conover and Miller 1981; Bugden and Evans 1991; Awam et al. 2011). Furthermore, in some cases older chicks can be more sensitive to cold than embryos and newly born hatchlings despite that the former have better thermoregulation (Bugden and Evans 1991; Awam et al. 2011).

Comparative observations of acoustic indicators of both vital needs, namely cooling and food deprivation, have been rarely done. For example, Tree swallow nestlings can react to cooling and food deprivation in different ways: nestlings increased the call rate, call length and amplitude after food deprivation, but decreased the call rate and call frequency after cooling (Leonard and Horn 2001b). Unfortunately, the narrow taxonomic scope of research on this topic limits our knowledge about how widespread, typical and universal acoustic indicators of chicks' hunger and thermal state are.

Here we studied the acoustic properties of calls in relation to warmth deprivation of Siberian crane chicks (*Grus leucogeranus*, Gruidae). Cranes are interesting subjects for such

research, because as a rule they rear just one chick per season and have prolonged parental care lasting up to the next breeding season (Johnsgard 1983; Archibald and Lewis 1996). Therefore, crane chicks usually do not face intra-brood competition; however they still need to attract constant and stable parent attention to maximize their fitness during first year of life. Despite the fact that crane chicks hatch downy and start to follow their parents around a pair territory a few hours after hatching (Johnsgard 1983; Archibald and Lewis 1996), cranes are not strictly precocial birds because adults feed their young from bill to bill and brood them during the first weeks of life (Ricklefs et al. 1986). Crane chicks' vocal behaviour has been studied thoroughly. Previous studies described chick vocal repertoires and development for different cranes species (Niemeier 1979; Gebauer and Kaiser 1998; Budde 2001; Klenova et al. 2010, 2014), as well as pronounced vocal individuality and the lack of sex differences in chick calls (Klenova et al. 2009; Goncharova et al. 2015). The effect of hunger on vocal activity and acoustic variables of calls has also been studied recently in Siberian crane chicks. Compared to satiated chicks, hungry ones emit calls with higher frequency parameters and value of entropy, longer duration and shorter gaps between single calls (Goncharova and Klenova 2015). However, the effect of cooling on vocal behaviour has never been studied in any crane chicks before. In the present study, we test this effect on frequency and temporal variables of Siberian crane chick calls. We also compare these findings with the previous ones to answer the question whether conspecific chicks use the same keys to signal about cooling and hunger.

## Materials and methods

### *Study objects and site*

Our subjects were 10 Siberian crane chicks (5 males, 5 females) aged 3–15 days. All the chicks were human-raised in the Oka Crane Breeding Centre of Oka Biosphere State Nature Reserve (Ryazan region, Russia) in 2014 (4 chicks, 1 male and 3 females) and 2015 (6 chicks, 4 males and 2 females). According to previous studies of vocal development in cranes, the temporal-frequency parameters of calls remain relatively stable in the first months of life (Niemeier 1979; Gebauer and Kaiser 1998; Budde 2001; Hudiakova 2002; Klenova et al. 2007, 2010). Therefore, although we used a wide range of ages, we believe that in the scope of our study the parameters of chick calls are unaffected by age. The staff removed the chicks from their parents' nests just after hatching. That provoked parent birds to lay a new clutch, whereas the hatchling imprinted on human as its parent and started following its keeper on walks (which was twice a day for a 1–1.5 h) and beg for food as chicks do in a regular crane family. During the first 1.5 months of life, chicks were kept in individual aviaries with constant visual and auditory contact with neighbouring chicks. Each chick aviary had an indoor pen (about 5 m<sup>2</sup> each) with an infrared warmer and outdoor enclosure with vegetation outside (about 20 m<sup>2</sup> each). The chicks were kept in the indoor pen to prevent overcooling between 21.00 and 6.00. All the chicks were individually marked with colour leg rings and sexed independently by two laboratories with DNA PCR-amplification (Griffiths et al. 1998).

### **Call recordings**

We recorded calls on 25–28 May in 2014 and 13–17 June in 2015 (in 2015 adult cranes started the breeding season later than usual, so in both years we worked with chicks of the same age). We performed the experiments at dawn (4.30–5.30), when the chicks were already active but it was still cold outside. No keepers were inside the enclosures and no technical procedures were performed next to the aviaries at that time (the first feeding happened at 6.00; the other feedings at 9.00, 12.00, 15.00, 18.00 and 21.00). As one of the study goals was to compare how crane chicks convey information about different needs, the present protocol was similar to a protocol used by Goncharova and Klenova (2015) to study food deprivation effect on acoustic parameters of Siberian crane chicks calls. Each chick under study was recorded twice, each time for 15 min. The first recording session (control recording) was performed in the indoor pen at a normal temperature that was always kept at 25° C. We placed a recorder in the pen and quickly left a focal chick to minimize a researcher's influence on the focal chick behaviour. After 15 min, we moved the recorder and the focal chick to the outdoor enclosure, started the second recording session (experimental recording) that lasted 15 min as well and similarly left the focal chick. During the study dates, the outdoor temperature registered just after recording session in outdoor enclosure was 7–17° C. After 15 min of experimental recording, each chick was returned into the warm indoor pen. We did not notice any signs of overcooling in all the birds under study. Therefore, we believe that conditions of experimental recording sessions were uncomfortable but not dangerous for the chicks. During both recording sessions the recorder was placed on the tripod; the distance between it and a chick was usually 1–2 m. We moved chicks from indoor pen to outdoor aviary with our hands. Every tactile contact lasted for less than a minute. According to our experience, we assume that although short handling period is stressful for chicks, they calm down in few minutes after the handling is over.

These two recording sessions were conducted in one day for each chick and followed each other in the same order (experimental after control). We used Marantz PMD-660 (D&M Professional, Kanagawa, Japan) and Zoom H5 (Zoom North America, Ronkonkoma, NY, USA) digital recorders with an AKG C1000S (AKG-Acoustics GmbH, Vienna, Austria) microphone.

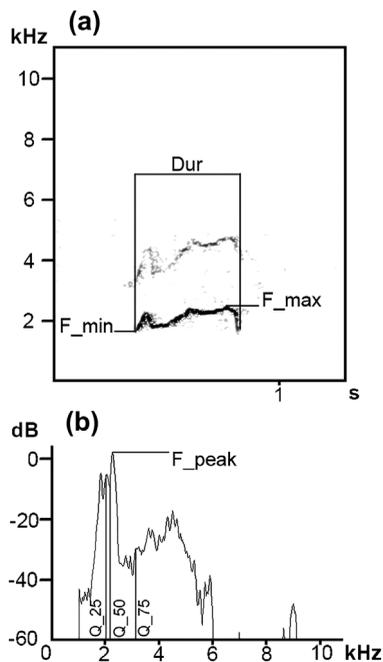
### **Call analysis**

For further analysis we used only 5-min recording intervals that were randomly selected from the second to the fifteenth minute of each recording session to decrease the effect of handling or a researcher presence on chicks' vocal behaviour.

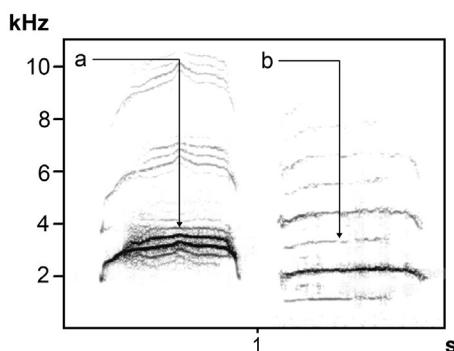
We performed the acoustic analysis with Avisoft SASLabPro v.5.1.23 (Avisoft Bioacoustics, Berlin, Germany). To increase the frequency resolution of sound presentation in the spectrogram window of the program, chick calls were downsampled from 48 to 22 kHz with a simultaneous anti-aliasing filtration. We created spectrograms using a 1024-point Hamming window, frame 50% and overlap 93.75%, providing a time resolution of 2.9 ms and a frequency resolution of 22 Hz. Since the fundamental frequency of Siberian crane chick calls usually is higher than 1.5 kHz and there are no low-frequency components in the calls in the age period under the study (Goncharova et al. 2015), we also applied a high-pass filter at 1 kHz to cut out the background noise.

We analysed calls regardless of their type, duration, etc. We measured 20 calls in a row, counted from the beginning and up to 20 (8–20, 18.3 average) calls in a row counted from the end of every selected 5-min recording interval ('first' and 'last' calls, respectively) to test the effect of prolonged cold exposure. If there were less than 40 calls per 5-min recording interval, we did not count any call twice: firstly we picked up calls for a sample of 'first' calls, and then added remaining calls to a sample of 'last' calls. All chicks except three emitted over 40 calls during each 5-min recording interval. In total, we analysed 786 calls of 10 Siberian crane chicks. Also we prepared so-called 'joint calls' by cutting out all silent gaps between single calls within the 5-min recording interval. The 'joint calls' allowed us to assess the effect of deprivation on overall vocal activity and frequency parameters in a given recording session (independently of total call number emitted).

For each single call we measured nine variables: maximum fundamental frequency (F\_max), minimum fundamental frequency (F\_min), peak fundamental frequency (F\_peak), three energy quartiles of the mean power spectrum of the call (Q25, Q50 and Q75), total call duration (Dur) and duration of the inter-call interval from the end of the measured call to the beginning of the next one (Dur\_gap) (Figure 1). We also measured the entropy of the mean power spectrum (Entropy), which reflects how chaotic a sound is (pure tones have low entropy, while broadband sounds have high entropy). For all the measured calls we also registered a presence or absence of non-linear phenomena – sidebands (Figure 2a) and subharmonics (Figure 2b) (e.g. Wilden et al. 1998; Frommolt 1999; Riede et al. 2004; Zollinger et al. 2008). For the joint calls we measured five variables: peak frequency (F\_peak\_total), three energy quartiles (Q25\_total, Q50\_total and Q75\_total) and total duration (Time\_total), which is equivalent to the total time spent on vocalizing



**Figure 1.** The spectrogram (a) and mean power spectrum (b) of Siberian crane chick call (male 8, 12 days old). The measured acoustic parameters (except Dur\_gap and Entropy) are shown.



**Figure 2.** The spectrograms of Siberian crane chick calls with non-linear phenomena: sidebands (a, male 13, 8 days old) and subharmonics (b, male 9, 8 days old). The corresponding non-linear phenomena marked with arrows.

within the selected 5-min recording interval. To measure the fundamental frequencies and durations we used the reticule cursor in the spectrogram window of Avisoft; the energy variables were measured in the power spectrum window of Avisoft.

### **Statistical analysis**

We conducted statistical analyses with STATISTICA 8.0 (StatSoft, Inc., Tulsa, OK, USA). All tests were two-tailed; significance was set at  $p < 0.05$ , values are reported as mean  $\pm$  SD. Measurements on all acoustic variables were normally distributed for each chick ( $p > 0.05$ , Kolmogorov–Smirnov test) except Dur\_gap because of its high dispersion. Therefore, in the further analysis we used the natural logarithms of Dur\_gap values.

As some acoustic variables were correlated with each other, we also derived four principal components (PC1–PC4) from values of nine measured call variables. PC1–PC4 had eigenvalues of 4.229, 1.424, 1.091 and 0.964, respectively. A number of fundamental frequency variables (namely, maximum and peak fundamental frequency), as well as the first and the second quartiles of the power spectrum contributed most to PC1 (47% of variance). Minimum fundamental frequency, the third quartile of power spectrum and the total duration of call contributed most to PC2 (15.8% of variance). PC3 was represented mainly by maximum fundamental frequency and duration of inter-call intervals, and, to a lesser extent, with entropy, and explained 12.1% of variance. PC4 explained 10.7% of variance and was represented by the third quartile, entropy, total duration of a call and inter-call intervals.

For ‘first’ and ‘last’ calls we used a single general linear model (GLMM) to test the effects of factors ‘sex’, ‘individual’ (nested within the sex) and ‘experiment’ (with ‘sex’ and ‘experiment’ as fixed factors and ‘individual’ as a random factor) and the effect on interaction of sex and experiment on the PC1–PC4 along with the 9 measured acoustic variables of chick calls. For the ‘joint calls’ we conducted one-way ANOVA to test the effects of a factor ‘experiment’ on the acoustic variables.

To estimate the occurrence of non-linear phenomena in Siberian crane chicks’ calls we calculated a percentage of calls containing non-linear phenomena in the ‘first’ and ‘last’ call samples in each recording session. To evaluate the differences in the occurrence of

non-linear phenomena between experiment and control recordings, between ‘first’ and ‘last’ call samples within each recording session and between sexes, we performed a series of pairwise comparisons via Yates’ chi squared test ( $df = 1$  in all cases).

## Results

GLMM analysis revealed the significant effect of cooling on all principal components and measured acoustic variables of calls, except Entropy (Table 1). According to  $F$ -ratios, the effect of the ‘experiment’ factor was stronger for the ‘last’ call sample than for the ‘first’ one for most of the measured variables (Table 1). At the same time, the factors ‘individuality’ and ‘sex’ had a significant effect on both acoustic variables and principal components as well (with the exception of Entropy for ‘sex’ factor). However, although the effect of the ‘sex’ factor was significant for most of measured variables, the values of such variables broadly overlapped between sexes (e.g. for minimal fundamental frequency and total call duration; Table 1, Figure 3). For the ‘first’ call sample for four measured variables of nine the comparison of  $F$ -ratios could indicate that the effect of cooling was stronger than the effect of an individual. For five of nine measured variables, the effect of cooling could be stronger than the effect of sex.

For the ‘last’ call sample the  $F$ -ratio for cooling was greater than the effect of the individual for seven of nine measured variables and greater than the effect of sex for six of nine measured variables, which could indicate that with prolonged cooling the effect of deprivation became even stronger in comparison with effects of individuality and sex. The interaction of experiment and sex was the weakest for both call samples, unless it was significant in

**Table 1.** Results of GLMM for the separate effects of sex, individual and experiment and the interaction of experiment and sex on the acoustic variables of Siberian crane chick’ calls from the first half and from the second half of recording session (‘first’ and ‘last’ calls, respectively),  $F$ -ratio and  $p$  value are shown.

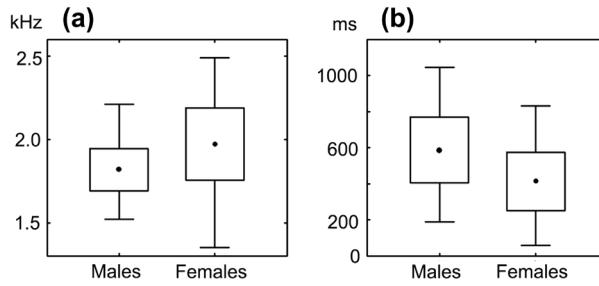
	First calls				Last calls			
	$n = 400$				$n = 386$			
	Experiment	Individuality <sup>a</sup>	Sex	Experiment and sex	Experiment	Individuality <sup>a</sup>	Sex	Experiment and sex
	$F_{1,388}$	$F_{8,388}$	$F_{1,388}$	$F_{1,388}$	$F_{1,374}$	$F_{8,374}$	$F_{1,374}$	$F_{1,374}$
F_min	6.7*	92.0***	<b>330.6***</b>	5.6*	9.1*	70.7***	<b>177.0***</b>	9.1*
F_max	88.16***	<b>120.03***</b>	113.82***	38.05***	<b>143.68***</b>	116.01***	56.55***	22.35***
F_peak	<b>142.39***</b>	80.05***	99.73***	24.74***	<b>322.49***</b>	101.33***	81.88***	6.47*
Q25	<b>201.74***</b>	106.97***	90.80***	55.44***	<b>442.01***</b>	141.87***	38.09***	11.46***
Q50	<b>110.33***</b>	80.23***	26.49***	25.94***	<b>211.87***</b>	74.22***	4.79*	9.91*
Q75	11.98***	12.96***	<b>64.73***</b>	12.89***	42.71***	8.49***	<b>76.45***</b>	0.135 ns
Entropy	0.01 ns	<b>47.42***</b>	0.32 ns	11.70***	0.77 ns	<b>22.78***</b>	2.69 ns	21.12***
Dur	<b>491.64***</b>	20.47***	289.27***	6.42*	<b>612.11***</b>	30.59***	320.20***	1.17 ns
ln(Dur_gap) <sup>b</sup>	15.66***	<b>20.91***</b>	8.89***	3.48 ns	<b>10.12*</b>	5.66***	3.56 ns	0.02 ns
PC1	<b>188.82***</b>	126.50***	47.69***	55.87***	<b>393.83***</b>	119.53***	15.47***	15.74***
PC 2	96.23***	21.56***	<b>442.45***</b>	2.50 ns	52.41***	14.19***	<b>388.60***</b>	0.92 ns
PC 3	30.58***	<b>38.18***</b>	14.94***	0.94 ns	0.03 ns	<b>20.06***</b>	7.54*	6.00*
PC 4	<b>53.01***</b>	22.46***	9.99***	0.45 ns	16.80***	<b>26.11***</b>	6.01*	3.63 ns

Note: The largest values for  $F$ -ratios are highlighted in bold for each acoustic variable.

<sup>a</sup>‘Individuality’ was used as a random factor and was nested within the factor ‘sex’.

<sup>b</sup>We used the natural logarithm from Dur\_gap to normalize the sample of this highly diverse acoustic variable.

\*\*\* $p < 0.001$ ; \* $p < 0.05$ ; ns:  $p > 0.05$ .



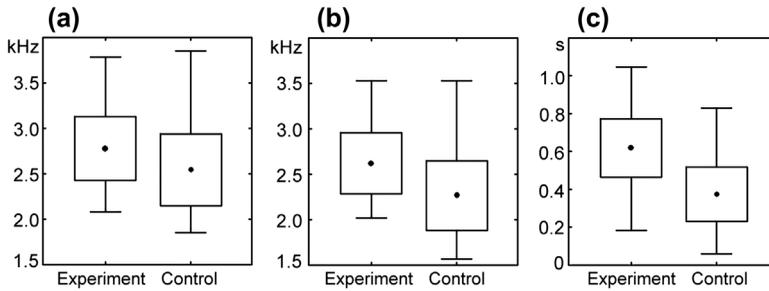
**Figure 3.** Comparison of values of the minimum fundamental frequency (a) and the total call duration (b) between sexes in the 'first' call sample. Note: The middle points show the averages; box – SD; whiskers – minimum and maximum values; all differences between values for males and females of both variables are significant (GLMM,  $p < 0.05$ ).

most cases. It had no significant effect on PC3 in the 'first' call sample, on the third quartile of power spectrum and call duration in the 'last' call sample, and on inter-call interval, PC2 and PC4 in both call samples (Table 1).

The calls of the warmth-deprived chicks differed from those of the control chicks in most of the acoustic variables, for both 'first' and 'last' calls within the recording session (Table 2). According to our results, the warmth-deprived chicks emitted vocalizations with higher  $F_{min}$ ,  $F_{max}$  and  $F_{peak}$ , as well as Q25, Q50 and Q75; also their calls had on average 1.5 times longer duration than in control conditions ( $Dur$ ) and shorter inter-call interval ( $Dur_{gap}$ ). At the same time, the values of Entropy for calls in the warmth-deprived and control recording sessions were almost equal. All mentioned relations were observed both for 'first' and 'last' call samples (Table 2). Nevertheless, the values of all measured acoustic variables broadly overlapped between the recording sessions (Table 2, Figure 4).

**Table 2.** Descriptive statistics (Mean  $\pm$  SD and Min–Max) for the acoustic variables of Siberian crane chicks calls recorded in the normal temperature condition (control) and during warmth-deprivation (experiment).

	Control			Experiment		
	'First' calls <i>n</i> = 200	'Last' calls <i>n</i> = 186	All calls <i>n</i> = 386	'First' calls <i>n</i> = 200	'Last' calls <i>n</i> = 200	All calls <i>n</i> = 400
$F_{min}$ (kHz)	1.87 $\pm$ 0.20 (1.50–2.43)	1.87 $\pm$ 0.22 (1.35–2.49)	1.88 $\pm$ 0.21 (1.35–2.49)	1.90 $\pm$ 0.15 (1.59–2.41)	1.91 $\pm$ 0.16 (1.59–2.39)	1.91 $\pm$ 0.15 (1.59–2.41)
$F_{max}$ (kHz)	2.53 $\pm$ 0.39 (1.83–4.06)	2.54 $\pm$ 0.39 (1.85–3.85)	2.54 $\pm$ 0.39 (1.83–4.06)	2.73 $\pm$ 0.44 (2.08–4.24)	2.78 $\pm$ 0.35 (2.08–3.78)	2.76 $\pm$ 0.40 (2.80–4.24)
$F_{peak}$ (kHz)	2.25 $\pm$ 0.37 (1.65–3.78)	2.27 $\pm$ 0.38 (1.57–3.53)	2.26 $\pm$ 0.38 (1.57–3.78)	2.52 $\pm$ 0.39 (1.95–3.61)	2.63 $\pm$ 0.37 (2.02–4.80)	2.57 $\pm$ 0.38 (1.95–4.80)
Q25 (kHz)	2.19 $\pm$ 0.28 (1.67–2.86)	2.18 $\pm$ 0.29 (1.63–2.95)	2.19 $\pm$ 0.29 (1.63–2.95)	2.42 $\pm$ 0.34 (1.95–3.23)	2.50 $\pm$ 0.32 (1.85–3.20)	2.46 $\pm$ 0.33 (1.85–3.23)
Q50 (kHz)	2.37 $\pm$ 0.33 (1.72–3.66)	2.37 $\pm$ 0.33 (1.67–3.33)	2.37 $\pm$ 0.33 (1.67–3.66)	2.60 $\pm$ 0.40 (2.00–4.30)	2.70 $\pm$ 0.37 (2.06–4.34)	2.65 $\pm$ 0.39 (2.00–4.34)
Q75 (kHz)	3.15 $\pm$ 0.90 (1.78–5.10)	3.07 $\pm$ 0.85 (1.76–5.03)	3.11 $\pm$ 0.88 (1.76–5.10)	3.40 $\pm$ 0.83 (2.04–5.18)	3.57 $\pm$ 0.84 (2.21–5.38)	3.49 $\pm$ 0.84 (2.04–5.38)
Entropy	0.31 $\pm$ 0.10 (0.12–0.66)	0.33 $\pm$ 0.11 (0.13–0.70)	0.32 $\pm$ 0.10 (0.12–0.70)	0.31 $\pm$ 0.08 (0.16–0.57)	0.34 $\pm$ 0.09 (0.15–0.60)	0.33 $\pm$ 0.08 (0.15–0.60)
$Dur$ (ms)	379 $\pm$ 134 (67–941)	374 $\pm$ 144 (58–830)	377 $\pm$ 139 (58–941)	599 $\pm$ 155 (197–915)	619 $\pm$ 154 (184–1045)	609 $\pm$ 155 (184–1045)
$Dur_{gap}$ (s)	3.89 $\pm$ 7.59 (0.26–70.55)	7.75 $\pm$ 16.75 (0.24–127.06)	5.75 $\pm$ 12.97 (0.24–127.06)	3.53 $\pm$ 3.75 (0.49–35.56)	3.78 $\pm$ 7.78 (0.34–102.62)	3.66 $\pm$ 6.10 (0.34–102.62)



**Figure 4.** Shifts in maximum fundamental frequency (a), peak frequency (b) and call duration (c) across experimental conditions in the ‘last’ calls of Siberian crane chicks. Note: The middle points show the averages; box – SD; whiskers – minimum and maximum values; all differences between control and experiment values of each variable are significant (GLMM,  $p < 0.05$ ).

The means  $\pm$  SD, minimum and maximum values of measured acoustic variables of the ‘joint calls’, as well as one-way ANOVA results, are shown in Table 3. We found that all frequency variables of the warmth-deprived chicks’ ‘joint calls’ were higher than in the control chicks. At the same time, the warmth-deprived chicks vocalized more during the recording session. One-way ANOVA revealed the significant effect of cooling on all measured acoustic variables except the upper energy quartile (Q75; Table 3).

The occurrence of non-linear phenomena differed neither between call samples within each recording session, nor between control and experiment recording sessions (75.5 and 77.0% for ‘first’ and ‘last’ calls, respectively in experimental recording; 80.5 and 78.5% for ‘first’ and ‘last’ calls, respectively in control recording;  $p > 0.05$  for all comparisons, Yates’ chi square test). However, there was a difference in occurrence of non-linear phenomena between males and females: 66 and 94% for total call sample in control recording for males and females, respectively (22.78,  $p < 0.001$ , Yates’ chi square test). During warmth deprivation there was no difference between sexes (80.5 and 72% correspondingly; 1.43,  $p = 0.23$ , Yates’ chi square test).

**Table 3.** The values of acoustic variables (mean  $\pm$  SD and min–max) of the ‘joint’ calls recorded in the normal temperature conditions (control) and during warmth-deprivation (experiment), as well as one-way ANOVA results ( $F$ -ratio and  $p$  value are shown) for the effects of experiment on the acoustic variables of Siberian crane chick ‘joint’ calls.

	Control	Experiment	Factor ‘experiment’
	$n = 10$	$n = 10$	$F_{1,18}$
F_peak_total (kHz)	2.24 $\pm$ 0.28 (1.72–2.69)	2.57 $\pm$ 0.36 (2.15–3.20)	5.25*
Q25_total (kHz)	2.15 $\pm$ 0.24 (1.74–2.45)	2.49 $\pm$ 0.32 (2.15–3.01)	7.27*
Q50_total (kHz)	2.38 $\pm$ 0.28 (1.87–2.78)	2.70 $\pm$ 0.32 (2.32–3.20)	5.63*
Q75_total (kHz)	3.31 $\pm$ 0.80 (2.02–4.34)	3.65 $\pm$ 0.66 (2.69–5.03)	1.06 ns
Time_total (s)	25.42 $\pm$ 15.19 (11.34–54.08)	53.15 $\pm$ 21.21 (28.79–82.00)	11.30*

\* $p < 0.05$ ; ns:  $p > 0.05$ .

## Discussion

Our results indicate that during warmth deprivation, Siberian crane chicks emitted calls with a higher fundamental frequency. The cooled chicks' call energy was shifted towards higher frequencies as well. Cooled chicks spent more time vocalizing; their calls were longer and followed each other through shorter gaps than in control conditions. Therefore, according to the previous study of effect of food deprivation on Siberian crane chicks' vocal behaviour, we can conclude that they react to both hunger and cooling deprivation in the same way (Goncharova and Klenova 2015). The only other species for which an effect of both food and warmth deprivations was observed is the Tree swallow, and the results of that study are inconsistent with our findings. Tree swallow nestlings' peak frequency was affected by cooling, but not by food deprivation (Leonard and Horn 2001b).

### *Vocal activity*

An increase of vocal activity (for example, call rate) with hunger has been shown in many studies focused on begging behaviour (e.g. Leonard and Horn 2001b; Hamer et al. 2006; Marques et al. 2008; Quillfeldt et al. 2010), and we suggest that it is a universal indicator of nutritional needs in chicks. However, results of the effect of cooling on vocal activity are not uniform. Chicks of some altricial species, being ectothermic at an early age after hatch, react according to their body mass. Low body mass chicks decrease vocal activity with cooling, while chicks and embryos of high body mass increase it (e.g. Choi and Bakken 1990; Evans 1992, 1994; Abraham and Evans 1999; Dakin et al. 2016). At the same time, chicks of precocial and semi-precocial species, which develop endothermy in late embryogenesis (e.g. Tzschentke and Rumpf 2011), increase their call rate regardless of body mass if ambient temperature drops (for instance, Bugden and Evans 1991; Evans et al. 1994; Brua et al. 1996; Gräns and Altimiras 2007; Awam et al. 2011). Crane chicks are semi-precocial as well. They are relatively active and mature compared with altricial nestlings, but have poor thermoregulation at an early age (Ricklefs et al. 1986). Consistent with other studies, we found that Siberian cranes increase vocal activity level with cooling. We suggest that in precocial birds vocal activity is a universal indicator of need for warmth.

### *Acoustic variables*

The effects of hunger or cooling on acoustic variables of calls are inconsistent. Some species change temporal-frequency parameters with food deprivation or cooling (Abraham and Evans 1999; Leonard and Horn 2001b; Gladbach et al. 2009; Marques et al. 2009; Klenova 2015; Dakin et al. 2016) but others do not (Leonard and Horn 2001b; Träger et al. 2006; Gräns and Altimiras 2007; Duckworth et al. 2009; Quillfeldt et al. 2010; Awam et al. 2011). This inconsistency could be partly explained by different temperature conditions used in different studies, because vocal behaviour could dramatically change when body temperature range goes beyond limits which occur in natural incubation conditions (Abraham and Evans 1999).

It is interesting to investigate which biological traits affect the chick's ability to convey information about its state and needs via vocal activity only or both vocal activity and temporal-frequency call variables. Quillfeldt et al. (2010) suggested that coding of needs

and condition in temporal-frequency parameters of chick calls is related to intra-brood competition for resources, therefore chicks of uniparous species have no need for such encoding. However, chicks of several uniparous colonial seabirds (Gladbach et al. 2009; Quillfeldt et al. 2010; Klenova 2015), as well as Siberian crane (Goncharova and Klenova 2015; present study), which almost always raises one chick per season (Archibald and Lewis 1996; Shao et al. 2014), code information about their needs in the acoustic structure of calls. Therefore, the usage of temporal-frequency call variables to convey information about chick state apparently is not related to brood size.

Another hypothesis is that temporal-frequency parameters of chick calls can be tuned either to convey vocal individual recognition or vital needs (Klenova 2015). Thus, when adults are very likely to confuse their chicks with others, chicks need to communicate vocally not only hunger, but also their individuality. In such cases temporal-frequency call variables usually encode individuality and are largely unaffected by a nestling's state (Quillfeldt et al. 2010; Reers and Jacot 2011). At the same time, when adults have reliable clues to distinguish their chicks, the latter can use all possible variables to communicate their needs (Klenova 2015). Siberian crane families stay on separate territories during a breeding season, and usually raise only one chick from a two-egg clutch, so each chick normally does not encounter others until the autumn migration (Archibald and Lewis 1996). In this study, we showed that warmth deprivation had a significant effect on most studied acoustic variables of calls and cooling impacted most of the variables relatively stronger than individuality. Thus, our findings partially support the hypothesis that coding of needs via temporal-frequency parameters can compete with vocal individual recognition.

The present study has a number of limitations that could mask the real picture or add additional noise to results. For example, the study was carried on a sample of birds of different ages (3–15 days old) and the temperature range in experimental conditions was quite wide due to natural weather conditions (7–17 °C). So it is essential to additionally test the effect of different values of ambient temperature on vocal behaviour of crane chicks, as well as the effect of certain chick age. It is also necessary to reconsider the study protocol to avoid potential biases of hunger and handling stress which chicks could feel being tested before the first feeding in the given day.

### ***Non-linear phenomena***

It is widely assumed that during the period of parental care, the offspring attempt to get more investment than parents prefer to give (Trivers 1974; Krebs and Dawkins 1984). As an example of offspring manipulation, juveniles can imitate signals of younger stages to exaggerate their helplessness (Trivers 1974). At the same time, non-linear phenomena could strongly increase call diversity. For instance, in colonial seabirds (e.g. Aubin et al. 2000; Aubin and Jouventin 2002; Mathevon et al. 2003) and some mammals (e.g. Volodina et al. 2006) non-linear phenomena in calls create important basis of individual coding. Also, one of the possible functions of this vocal feature is avoiding habituation to chick calls in the parent birds because adult cranes habituate and stop reacting to invariant signals very soon (Fitch and Kelley 2000). However, if the signal frequency spectrum changes, the attention of cranes restores immediately (Fitch and Kelley 2000). Interestingly, it has been also shown that after the start of autumn migration, the occurrence of non-linear phenomena in crane chick calls dramatically increases. Such changes could be associated with provoking

additional care from parents by a relatively independent chick during foraging stops and at wintering sites (Goncharova et al. 2015).

Therefore, we expected that in uncomfortable conditions of warmth deprivation Siberian crane chicks would increase the per cent of calls with non-linear phenomena to evoke more attention to their needs. However, although the occurrence of non-linear phenomena in cooled chicks was quite high, it did not differ significantly from those in control conditions.

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