#### **ORIGINAL PAPER**



# Altai pika (Ochotona alpina) alarm calls: individual acoustic variation and the phenomenon of call-synchronous ear folding behavior

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#### Abstract

Non-hibernating pikas collect winter food reserves and store them in hay piles. Individualization of alarm calls might allow discrimination between colony members and conspecifics trying to steal food items from a colony pile. We investigated vocal posture, vocal tract length, and individual acoustic variation of alarm calls, emitted by wild-living Altai pikas *Ochotona alpina* toward a researcher. Recording started when a pika started calling and lasted as long as possible. The alarm call series of 442 individual callers from different colonies consisted of discrete short (0.073–0.157 s), high-frequency (7.31–15.46 kHz), and frequency-modulated calls separated by irregular intervals. Analysis of 442 discrete calls, the second of each series, revealed that 44.34% calls lacked nonlinear phenomena, in 7.02% nonlinear phenomena covered less than half of call duration, and in 48.64% nonlinear phenomena covered more than half of call duration. Peak frequencies varied among individuals but always fitted one of three maxima corresponding to the vocal tract resonance frequencies (formants) calculated for an estimated 45-mm oral vocal tract. Discriminant analysis using variables of 8 calls per series of 36 different callers, each from a different colony, correctly assigned over 90% of the calls to individuals. Consequently, Altai pika alarm calls are individualistic and nonlinear phenomena might further increase this acoustic individualization. Additionally, video analysis revealed a call-synchronous, very fast (0.13–0.23 s) folding, depression, and subsequent re-expansion of the pinna confirming an earlier report of this behavior that apparently contributes to protecting the hearing apparatus from damage by the self-generated high-intensity alarm calls.

**Keywords** Vigilance behavior  $\cdot$  Pika short call  $\cdot$  Individual differences  $\cdot$  Source and filter acoustic variables  $\cdot$  Disturbance vocalization  $\cdot$  Hearing protection

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# Introduction

Individualistic vocal traits are found in all investigated vertebrate taxa (Terry et al. 2005; Yorzinski 2017), including amphibians (Zhang et al. 2017), birds (Klenova et al. 2008), and mammals (Bouchet et al. 2012; Jansen et al. 2012; Déaux et al. 2016; Schneiderová et al. 2017). Essential individual vocal traits are predetermined by size variation of the sound-producing structures, particularly the larynx with its vocal folds and the vocal tract (Efremova et al. 2011, 2016; Lapshina et al. 2012; Furuyama et al. 2016; Gamba et al. 2017). Additional vocal identity beyond this anatomy-based "default-settings" is achieved by individual dynamics of the vocal apparatus (Klenova et al. 2009; Gamba et al. 2017; Syrová et al. 2017) that affect several acoustic variables (Briefer and McElligott 2012; Lapshina et al. 2012; Volodin et al. 2014). Nonlinear vocal phenomena (Wilden et al. 1998; Fitch et al. 2002; Volodin et al. 2005; Klenova et al. 2009) additionally

increase vocal individuality (Volodina et al. 2006; Papale et al. 2015; Zhang et al. 2017).

Individual identity of vigilance-related calls was extensively studied in colonial rodents (Hare 1998; McCowan and Hooper 2002; Matrosova et al. 2011; Schneiderová 2012; Schneiderová et al. 2017) and lagomorphs of the genus *Ochotona* (Lutton 1975; Conner 1984, 1985; Trefry and Hik 2009, 2010) comprising 28 species (Lissovsky 2014). Individualistic traits in these calls allow the individual recognition of conspecifics (Conner 1984, 1985; Trefry and Hik 2009, 2010). The individual recognition enables discriminating between reliable callers from those producing false alarms (Hare and Atkins 2001; Blumstein et al. 2004) and communicating directional predator movement by multiple personalized callers (Thompson and Hare 2010).

In different studies, the vigilance-related vocalizations of species of the genus Ochotona are either termed "alarm calls" (Lutton 1975; Lissovsky and Lissovskava 2002; Lissovsky 2005; Trefry and Hik 2009) or "short calls" (Somers 1973; Conner 1984, 1985) (thereafter "alarm calls"). Seasonal onset of extensive alarm calling of pikas coincides with the construction of hay piles of harvested vegetation (Lutton 1975; Nikolskii and Srebrodolskaya 1989; Nikolskii and Mukhamediev 1996). The alarm calls are used by territorial pikas for defending these hay piles (Huntly et al. 1986; Holmes 1991), which are vitally important for winter survival of these non-hibernating animals (MacArthur and Wang 1973; Conner 1984). The alarm calling activity is concentrated around the largest hay piles (Nikolskii and Srebrodolskaya 1989). Most (up to 79%) of the alarm calls (Nikolskii and Srebrodolskaya 1989) are emitted toward conspecific pikas (Markham and Whicker 1973; Lutton 1975; Ivins and Smith 1983; Nikolskii and Srebrodolskaya 1989), trying to rob stored vegetable parts from the piles of neighboring pikas (Markham and Whicker 1973; Lutton 1975).

Pika alarm calls, encoding caller individual identity (Lutton 1975; Conner 1985; Trefry and Hik 2009), are effective against the intrusion of conspecifics (Conner 1984). Experimental removal of a resident results in significant increase of intrusions by adjacent pikas and the persistence of the intruder within the vacant territory (Conner 1984). However, replacing a removed resident with its alarm calls effectively prevents such intrusions (Conner 1984). The high rates of alarm calls might help avoiding direct fights and chases that are rarely observed in pikas (Conner 1984, 1985). Alarm calls are also emitted in response to conspecific alarm calls (Nikolskii and Srebrodolskaya 1989) and toward various heterospecific animals that potentially threat the pikas themselves or their hay piles, as bear, lynx, large birds (Broadbooks 1965; Markham and Whicker 1973), mustelids (Lutton 1975; Ivins and Smith 1983), horses, and humans (observations of the authors). Despite this extensive research, referential, i.e., predator- or intruder-specific alarm communication has never been reported for pikas, suggesting that the same species-specific alarm calls are produced toward any kind of threat to the animals themselves or to their food reserves, e.g., predators, other animals and humans, or alien conspecifics.

Alarm calling might be a costly display, as in the pre-winter period pikas produce alarm calls at very high rates (Nikolskii and Srebrodolskaya 1989) and at very high intensities (Lissovsky 2005). The high intensity might request a special ear folding behavior during the emission of the alarm calls in two pika species, the Altai pika and the northern pika *Ochotona hyperborea* (Lissovsky 2005). The vocalizing pika raises its head, bends the ear pinnae twice longitudinally, and presses them to the neck, so that, at the moment of call emission, the auditory meatus is closed by the walls of the pinna. This behavior was considered as an adaptation for protecting the middle or inner ear of the caller from potential damage by its own loud vocalizations (Lissovsky 2005). So far, although based on video recordings, this calling posture and the ear folding were only described verbally.

The *Ochotona* species lack sex dimorphism in body size (Liao et al. 2006; Lin et al. 2008; Hafner and Smith 2010). The alarm calls of *Ochotona* species are emitted by both sexes (Somers 1973; Conner 1982; Ivins and Smith 1983; Smith and Ivins 1984; Lissovsky 2014) and sex differences are lacking in either calling behavior (Ivins and Smith 1983) or the acoustics (Lissovsky 2005). Their one-note calls may be repeated at intervals of a few seconds for as many as 150 calls in series (Somers 1973). In different *Ochotona* species, the number of calls with nonlinear phenomena (biphonations and subharmonics) varies from 7.7 to 54.5% (Lissovsky 2005).

As other pikas (Lutton 1975; Ivins and Smith 1983; Conner 1984, 1985; Lissovsky 2005; Trefry and Hik 2009, 2010), the Altai pikas (Lissovsky 2003; Lissovsky et al. 2007; Lissovsky 2014) are very vocal animals that are known to produce two main call types: the male and female advertising song and the alarm call (Nikolskii and Mukhamediev 1998; Nikolskii et al. 1999; Lissovsky 2005). In the Altai pika, acoustic variation has been investigated only for male and female advertising songs (Nikolskii and Mukhamediev 1998; Nikolskii et al. 1999). For the alarm calls of the Altai pikas, geographic variation has been described, example spectrograms representing nonlinear phenomena in the alarm calls were presented, and a comparison of the acoustic structure of the alarm calls between the Altai and northern pikas and their hybrids was conducted (Lissovsky 2005). However, the acoustic individuality of the alarm calls was not yet investigated in the Altai pika.

For Altai pikas of the West Sayan Mountains (Khakasia and Altai regions of Siberia), the family group comprises a parental pair with young, which, at the start of September, mostly have dispersed to the periphery of the parental territories or to free territories between occupied pika territories (Nikolskii and Mukhamediev 1995, 1996, 1997; Lissovsky 2003). In this study, we examine acoustic variables, individual identity, and the amount of nonlinear phenomena in the Altai pika alarm calls. In addition, based on video single frames analysis, we describe the Altai pika calling posture and specifically the ear folding behavior as a potential adaptation for protecting the hearing apparatus of a caller from damage by its own high-intensity calls.

# Methods

# Study site, dates, and animals

Altai pika alarm calls were collected during daytime from unmarked and unsexed wild-living adult or full-grown young animals from 13th to 29th September, 2012, in the buffer zone of Khakasskiy State Nature Reserve, Republic of Khakasia (52° 07' N, 89° 32' E), Russia. This study site is located in the West Savan Mountain taiga at an altitude of 1600-1800 m covered by Abies sp. and Pinus sp. forest with large clearings. The study area represented a long narrow valley with a spring and a small creek originating from it. Pika colonies were located on the bottom of the valley and on the mountain slopes. Neighboring valleys were also inhabited by Altai pikas so that the study area represented a continuous habitat extending for many kilometers, with a large, practically unrestricted number of pika colonies available for research. Each colony of Altai pikas was represented by a territorial pair, but sometimes, more than two animals were observed in a colony despite reported evidence that until the second half of September the offspring has usually dispersed from the family colonies (Nikolskii and Mukhamediev 1996). During the period of data collection, the minimal distance between family colonies was only 20-30 m and the pikas inhabited all appropriate places: talus slopes, naked rocks, stumps, fallen logs, and the branched roots of large trees, primarily of Pinus sibirica. Aside the clearings, the pikas occupied also places under tree cover and even small islands among wet and swampy plots. The pikas foraged and collected vegetation in hay piles for the winter, mobbed conspecific and heterospecific intruders and, between periods of foraging activity, took a rest, at which they commonly remained vigilant, although they sometimes relaxed and were sunbathing with closed eyes.

#### Acoustic recording

For acoustic recording (48 kHz, 16 bit), we used two solid state digital recorders Marantz PMD-660 (D&M Professional, Kanagawa, Japan) with handheld microphones Sennheiser K6-ME66 (Sennheiser electronic, Wedemark, Germany). Pikas produced alarm calls toward a slowly moving researcher, commonly from a distance of 10–30 m to him. Closer approach to a caller evoked its freezing and (at 4–5 m) escape to a burrow or another hide (e.g., under a log), either silently or

with a short squeak. Pikas also produced alarm calls in response to alarm calls of conspecifics and toward raptors flying over the pika colonies. Commonly, vocalizing pikas were stationary and only rarely produced calls when moving.

Pika colonies from which the alarm calls were collected were labeled by GPS coordinates using Garmin GPSmap 60CS (Garmin Int., Olathe, KS, USA) at a point close to the supposed colony area, to avoid repeated inclusion of calls from individuals of the same colonies in the analyses. Calls were considered as belonging to the focal caller when the calls came from a single point (hay pile or stump) and were all of the same intensity. After hearing the first call, a researcher stopped, directed the microphone to the sound source, and labeled the calls by voice (one, two, three, ...), trying to record alarm call series as long as possible. The recording continued until the animal escaped to a hide or when the researcher could no longer identify the calls of the focal animal because the animal had moved. After the end of the recording, the researcher moved slowly to another colony. The lengths of continuous recording of a focal individual ranged from 0.5 to 4-5 min; an alarm call series included from 2 to 27 calls. Thus, each alarm call series represented a recording session from one individual pika and was associated with a particular pika colony.

Two researchers (IAV and EVV), working independently, collected 22.5 h of recordings containing 544 series with a total of 2734 single alarm calls. Previously, a similar approach of recording individual alarm call series from unmarked animals was used for ground squirrels (McCowan and Hooper 2002).

# **Call samples**

To minimize a potential bias in call selection and analyses, all acoustic analyses were performed using a blind approach by researchers (VAM and JDK) who were not involved in data collection. Selection of calls for analysis and subsequent spectrographic analysis was done using Avisoft SASLab Pro software (Avisoft Bioacoustics, Berlin, Germany). Only calls of good quality, not disrupted by wind and not overlapped by noise or other calls, were included in the spectrographic analyses.

For spectrographic analysis, we created two call samples. The first sample, for estimating the acoustic diversity of the alarm calls, included 442 good-quality series of 442 individual callers with at least two alarm calls per series. As the microphone had to be moved and directed to a caller while hearing the first call of a series, only the second calls from these 442 series (442 single calls in total) were included in the analysis. Another sample, for estimating the individual identity of the alarm calls, included 36 series (= 36 individual callers) with at least eight (from 8 to 27) calls per series, 458 calls in total. In both samples, we took only one call series per colony. The samples of calls and individuals were mostly independent; only 26 calls were the same in both samples.

#### Acoustic analysis

Before analysis, the calls were high-pass filtered at 0.5 kHz by using Avisoft, to reduce the low-frequency background noise, as preliminary visual analysis of the spectrograms showed that call fundamental frequency always exceeded 1 kHz. In each call, we measured 10 acoustic variables: 2 temporal, 4 variables of fundamental frequency (f0), and 4 power variables (Fig. 1). We measured the duration of each call and the duration from call onset to the point of maximum f0 (dur-to-max) manually on the screen with the reticule cursor in the spectrogram window (sampling frequency 48 kHz, Hamming window, FFT 1024 points, frame 50% and overlap 96.87%) by using Avisoft SASLab Pro. Then, we performed manual measurements on the screen with the standard marker cursor of the start (f0beg), end (f0end), maximum (f0max), and minimum (f0min) fundamental frequencies of each call (Fig. 1). We created the entire call power spectrum, from which we automatically measured fpeak, representing the value of the frequency of maximum amplitude, and the q25, q50, and q75, representing the lower, medium, and upper quartiles, covering 25, 50, and 75% of the energy of the call spectrum, respectively (Fig. 1). All measurements were exported automatically to Microsoft Excel (Microsoft Corp., Redmond, WA, USA). In addition, for each call, we calculated the df0 as the difference between f0max and f0min, and calculated the dur-tomax% as ratio dur-to-max to the call duration (in percents).

In each call, we also scored presence/absence of nonlinear vocal phenomena, primarily biphonations and subharmonics (Wilden et al. 1998; Volodina et al. 2006; Frey et al. 2016). We



**Fig. 1** Measured acoustic variables of the Altai pika alarm calls: mean power spectrum of the entire call (left) and spectrogram of the call (right). Nonlinear phenomena are lacking in this call spectrum. To the right of the fundamental frequency contour, a weak echo contour can be traced. Designations: duration—call duration; dur-to-max—duration from call onset to the point of the maximum fundamental frequency; f0beg (=f0min)—the fundamental frequency at the onset of a call; f0end—the fundamental frequency at the end of a call; fpeak—the frequency of maximum amplitude within a call; q25, q50, q75—the lower, the medium and the upper quartiles, covering respectively 25, 50, and 75% energy of a call spectrum. The spectrogram was created at 48 kHz sampling frequency, Hamming window, FFT 1024, frame 50%, overlap 96.87%

scored the presence of nonlinear vocal phenomena only in cases where the total duration of the call portions bearing these nonlinear phenomena was over 10% of the total call duration. We also scored whether the nonlinear phenomena covered less than half of call duration or more than half of call duration.

#### Video recording

Video recording of Altai pika behavior was conducted at distances of 5–10 m to the focal animal independently of audio recording using a Panasonic HC-X909EG-K Camcorder with a Leica Dicomar objective (Panasonic Marketing Europe, Wiesbaden, Germany), 25 frames per second. One researcher (RF) collected over 10 h of video recordings of pika behavior. Despite this rich supply of video material, only very few alarm calls were recorded and still fewer in an appropriate lateral position for analysis. In many cases, the pikas produced alarm calls at the approaching researcher when video recording was not yet possible but quickly accustomed to the quietly sitting researcher and then stopped producing alarm calls. And often the alarm calls were produced by callers from different sorts of hide so that they were not visible to the researcher during call emission.

# Video analysis

Frame-by-frame analysis of the Altai pika vocal posture has been conducted using Adobe Premiere Pro, 1.5 AOO License ALL (Adobe Systems Inc., San Jose, CA, USA). We selected footage displaying lateral views of calling animals. In these videos, we considered animal posture during alarm call emission, ear movements, the degree of mouth opening (gape), and externally visible contractions of the ventral neck musculature and of the abdominal musculature. Representative single frames at maximum effort during call emission were used for reconstructing the lengths of the oral and nasal vocal tracts.

# Museum skull specimens

To improve the quality of the reconstruction of the nasal and oral vocal tract lengths, we inserted a photo of a skull and lower jaw of an Altai pika into the single frame of a calling Altai pika. Several skulls (three males, one female) with corresponding lower jaws of Altai pikas from the collection of the Zoological Museum of Moscow University (ZMMU) were photographed in lateral view together with a millimeter scale using a Canon SX60HS digital camera (Canon Inc., Tokyo, Japan). According to the skull measurements done by Lissovsky (2004), three skulls (two males, one female) were from adult animals and one skull was from a younger male. The skulls had all been collected during October 1940 in the Sayano-Shushensky Nature Reserve, Russia, a locality that is only about 150 km from our study site. For estimating potential sex differences in size, we measured the greatest length of skull (GLS) of the four museum skull specimens as a proxy of male and female body size. The GLS represented the distance between the most caudal and the most rostral points of the skull, excluding the teeth.

Two photos of one adult male skull (ZMMU number S-44477) were selected as best lateral views of upper skull and lower jaw and size-adjusted with Adobe Photoshop CS4 (Adobe Systems Inc., San Jose, CA, USA) to fit the head size of the calling Altai pika in the single frame. Together with the externally visible landmarks, nostril, mouth opening, eye position, and ear position, this facilitated the reconstruction of the nasal and oral vocal tract lengths and allowed the introduction of an absolute measure in the single frame.

#### Larynx position

Information on larynx position in pikas was obtained from CT scans of an adult male Daurian pika (Ochotona daurica), which had died in Tierpark Berlin, Germany, and was transferred to the Leibniz Institute for Zoo and Wildlife Research (IZW), Berlin, Germany, for routine postmortem investigation (postmortem no: 252/2017). The CT scans were done in the IZW using a 64-slice spiral Computer Tomograph Aquilion CX (Toshiba Medical Systems Corp., 1385 Shimoishigami, Otawara-shi, Tochigi 324-8550, Japan). For reconstruction of the larynx position in the Altai pika, we selected a CT image of the Daurian pika in which the larynx was visible. The body area of the Daurian pika was cut into several pieces and virtually adjusted with Photoshop to fit the larger body silhouette and the posture of the calling Altai pika in the selected video single frame. Tacitly assuming that there are no pronounced changes of larynx position during call emission, this allowed a reconstruction of the approximate larynx position in the calling Altai pika. In addition, we used published CT images of intubated domestic rabbits (Oryctolagus cuniculus) as a comparative anatomical information of larynx position in lagomorphs (Engbers et al. 2017).

#### Vocal tract length estimation

Two video single frames of calling Altai pikas in almost perfect lateral view were selected. After virtual insertion of skull and lower jaw, inferring of the approximate larynx position and two-dimensional graphical reconstruction of the vocal tract lines, the known size of the museum skull allowed the estimation of absolute nasal and oral vocal tract lengths in the Altai pika.

We had to select an indirect three-step approach of vocal tract length estimation as we did not have an Altai pika specimen for anatomical measurements at our disposal. Luckily, we received an adult male Daurian pika. However, Daurian pikas are of considerably smaller body size than Altai pikas. Therefore, we virtually enlarged a CT image of the Daurian pika to fit the body contour of an Altai pika in a video single frame (step one). Then, we marked the larynx position in an overlay of the size-adjusted CT image of a Daurian pika and the single frame of an Altai pika (step two), inserted the sizeadjusted museum skull of an Altai pika (step three) and estimated the vocal tract length. Alternatively, we could have estimated vocal tract length in the size-adjusted CT image of the Daurian pika but as we wanted to demonstrate vocal tract length in the Altai pika, we did not use this approach. However, as the CT section of the Daurian pika was sizeadjusted to the body contour of an Altai pika, the vocal tract length estimates should be matching along both lines.

# Statistics

Statistical analyses were conducted using STATISTICA v. 8.0 (StatSoft, Tulsa, OK, USA) and R v. 3.0.1 (R Foundation for Statistical Computing 2009). Means are given as mean  $\pm$  SD, all tests were two-tailed, and differences were considered significant whenever p < 0.05. Distributions of 362 measured parameter values of 370 distributions did not depart from normality and distributions of all mean parameter values did not depart from normality (Kolmogorov-Smirnov test, p > 0.05). As parametric ANOVA and discriminant function analysis (DFA) are relatively robust to departures from normality (Dillon and Goldstein 1984), this was not an obstacle to the application of these tests.

We used a one-way ANOVA to compare within and between individual variations of acoustic parameter values. We used DFA to calculate the probability of the assignment of calls to the correct individual. We included 10 call variables in the DFA, excluding f0min, because it was used for calculating another variable. Differences between the first, the last, and the other alarm calls within a series can occur, as was demonstrated in the alarm calls of rodents (Randall and Rogovin 2002). Therefore, we considered this in our analyses and conducted three ANOVAs and three DFAs, respectively, with the first 8 calls, the last 8 calls, and 8 randomly selected calls of 36 alarm call series.

We used Wilks' Lambda values to estimate how strongly acoustic variables of calls contribute to the discrimination of individuals. With a  $2 \times 2$  Yates' chi-squared test, we compared the values of correct assignment of calls to the correct individual. To validate our DFA results, we calculated the random values of correct assignment of calls to individual by applying randomization procedure with macros, created in R. The random values were averaged from DFAs performed on 1000 randomized permutations on the data sets as described by Solow (1990). For example, to calculate the random value of classifying alarm calls to individual pikas, each permutation procedure included the random permutation of 288 calls among 36 randomization groups corresponding to the 36 individual pikas, which were examined, followed by DFA standard procedure built-in in STATISTICA. All other permutation procedures were made similarly. Using a distribution obtained by the permutations, we noted whether the observed value exceeded 95, 99, or 99.9% of the values within the distribution (Solow 1990). If the observed value exceeded 95, 99, or 99.9% of values within this distribution, we established that the observed value did differ significantly from the random one with a probability p <0.05, p < 0.01, or p < 0.001, respectively (Solow 1990; Matrosova et al. 2010a, b; Sibiryakova et al. 2015).

# Results

# Calling posture, ear folding behavior, and vocal tract length

For the emission of an alarm call, Altai pikas extend the head and neck region and, thereby, poke out the head forward and upwards. The mouth is widely opened during call emission but the incisors and the tongue are not visible in a lateral view. The typical calling posture of Altai pika is illustrated in Fig. 2. The externally visible contractions of the ventral neck musculature and of the abdominal musculature are presented in Fig. 2a, b. A call-synchronous contraction of the ventral neck musculature was inferred from the constriction of the throat and ventral neck region. It was not equally obvious in all calling individuals but is clearly visible in Fig. 2b. A momentary constriction of the abdominal cavity during call emission, indicating strong contraction of the abdominal musculature, is clearly visible in the videos (Electronic Supplementary Material 1 and 2). In video single frames, it can be inferred by comparing the ventral body contour between fore and hind paws in the resting position and at maximal effort during a call (Fig. 2a, b).

The typical resting shape of the external ears, the pinnae, of Altai pikas is slightly oval to almost circular exposing a laterally directed, large surface. A tuft of caudally pointing hairs covers the rostral section of the pinna leading into the external acoustic meatus (Fig. 2a). Amazingly, the pinnae are pronouncedly folded during a call (Figs. 2 and 3). The ventral edge of the pinna is pulled upwards and the dorsal edge pulled downwards so that the exposed surface is dramatically reduced and the resulting flaps cover the entrance to the external auditory meatus. The observed mechanism involves a longitudinal double-folding of the pinna (Figs. 2b and 3b). Often, the folded ears are additionally pressed against the lateral neck region. This might reduce the incoming amplitude of the own loud call. Judging from the single frames, the ear folding occurs about three frames (0.1-0.12 s) earlier than the beginning of the call while the pinna expands again with the end of the



**Fig. 2** Resting (**a**) and calling posture (**b**) of the Altai pika, almost lateral view. During call emission head and neck are extended and the mouth is widely opened. The ears are strongly folded and adducted to the lateral neck region. In the resting position, the entrance to the external acoustic meatus is covered by a tuft of hairs (red asterisk). The ventral neck muscles and the abdominal muscles strongly contract as inferred from the constriction of the throat and ventral neck region (yellow arrow) and of the abdominal region (green arrow); for the latter compare ventral body contour between fore and hind paw in subfigures **a** and **b** 

call. This ear folding movement is very fast and impossible to resolve during real time observation. The duration of the ear folding was about 0.13–0.23 s, i.e., slightly longer than the duration of an alarm call.

Based on the reconstruction of the vocal tract (Fig. 3), the estimated Altai pika oral vocal tract length was approximately 45 mm, whereas the estimated nasal vocal tract length was approximately 50 mm. As Altai pika alarm calls were produced via a widely opened mouth, we approximated the formant frequencies of the pika oral vocal tract by calculating the first three formants F1–F3 using a simple model of the mammalian vocal tract (a pipe closed at one end) (Riede and Fitch 1999). The respective calculated formant frequencies of the 45-mm pipe were 1.94 kHz (first formant, F1), 5.83 kHz (second formant, F2), and 9.72 kHz (third formant, F3), according to the formula  $Fi = (2i - 1) \times c / 4vtl$ , where *i* is formant number, *c* is the speed of sound in moist air (350 m/s), and vtl is the vocal tract length (Riede and Fitch 1999). Formant dispersion (dF, i.e., the difference between neighboring formants) for the



**Fig. 3** Estimation of the oral and nasal vocal tract lengths in a calling Altai pika. **a** With inserted and size-adjusted museum skull that allowed to introduce an absolute measure in the single frame (scale of skull not shown). **b** Without skull to demonstrate call-synchronous ear folding behavior (red arrows). Larynx position was reconstructed by using a CT scan of a Daurian pika. Designations: turquoise dot—eye position; red dot—position of the external acoustic meatus; yellow ellipse—larynx position; blue line—nasal vocal tract; green line—oral vocal tract. Scale bar 10 mm

45-mm pipe model, calculated by using the formula dF = c / 2vtl, was 3.89 kHz.

For the four museum skull specimens, the values of the greatest length of skull (GLS) were 51 mm (adult male S-44477), 55 mm (adult male S-44494), 48.5 mm (subadult male S-44496), and 52 mm (adult female S-44483), respectively. The close values for the four specimens (all collected in October 1940) suggest that the estimated vocal tract length (45 mm) of one pika sample applies to all individuals of approximate adult size and can be used for estimating potential formant frequencies in all calls analyzed in this study.

# **Alarm call acoustics**

The acoustic analyses of a call sample of 442 alarm calls, all recorded from different individuals, revealed that Altai pika alarm calls were short (duration from 0.073 to 0.157 s) and high frequency (f0max from 7.31 to 15.46 kHz). As both the start (from 1.31 to 4.35 kHz) and end (from 1.40 to 4.21 kHz) fundamental frequencies were low, the calls were deeply frequency modulated (df0 from 5.58 to 13.59 kHz) (Table 1). The time span from call onset to the point of maximum fundamental frequency ranged from 13.1 to 71.0% of total call duration (Table 1).

The peak frequency had a three-humped distribution with maxima at 3–3.5, 6–6.5, and 10.5–11.5 kHz (Fig. 4). These maxima approximately correspond to the calculated values of the three first formants (F1 = 1.94 kHz; F2 = 5.83 kHz; F3 = 9.72 kHz) based on a 45-mm Altai pika oral vocal tract. The mean difference between the neighboring maxima of the peak frequency comprises 4.0 kHz. This value fits well the calculated value of formant dispersion of 3.89 kHz. Therefore, these peaks might indicate formants.

Table 1 Values (mean  $\pm$  SD) of acoustic variables measured in alarm calls (442 calls from 442 different individuals) and results of three one-way ANOVAs for individual differences (36 individual pika callers, one series per caller), using the first 8 calls, the last 8 calls, and 8 randomly selected calls of the alarm call series

Acoustic variable	Value	ANOVA				
	(n = 442)	First 8 calls $(n = 288)$	Last 8 calls ( <i>n</i> = 288)	8 randomly selected calls $(n = 288)$		
duration (s)	$0.105\pm0.014$	$F_{35,252} = 32.43*$	$F_{35,252} = 37.62^*$	$F_{35,252} = 27.27*$		
dur-to-max% (%)	$29.1\pm 6.3$	$F_{35,252} = 43.37^*$	$F_{35,252} = 42.83^*$	$F_{35,252} = 38.95^*$		
f0max (kHz)	$12.28\pm1.18$	$F_{35,252} = 129.06*$	$F_{35,252} = 124.66*$	$F_{35,252} = 118.32*$		
f0min (kHz)	$2.09\pm0.33$	$F_{35,252} = 27.22*$	$F_{35,252} = 38.33^*$	$F_{35,252} = 35.65^*$		
f0beg (kHz)	$2.16\pm0.45$	$F_{35,252} = 44.88*$	$F_{35,252} = 45.47*$	$F_{35,252} = 44.03*$		
f0end (kHz)	$2.49\pm0.32$	$F_{35,252} = 21.26*$	$F_{35,252} = 29.74*$	$F_{35,252} = 23.22*$		
df0 (kHz)	$10.19\pm1.15$	$F_{35,252} = 130.85*$	$F_{35,252} = 124.03*$	$F_{35,252} = 117.93*$		
fpeak (kHz)	$6.79\pm3.19$	$F_{35,252} = 10.57*$	$F_{35,252} = 16.31^*$	$F_{35,252} = 10.05*$		
q25 (kHz)	$4.80\pm0.91$	$F_{35,252} = 27.18^*$	$F_{35,252} = 34.42^*$	$F_{35,252} = 19.04*$		
q50 (kHz)	$7.25 \pm 1.61$	$F_{35,252} = 22.88*$	$F_{35,252} = 30.26^*$	$F_{35,252} = 18.56*$		
q75 (kHz)	$10.34\pm1.58$	$F_{35,252} = 41.33*$	$F_{35,252} = 60.14*$	$F_{35,252} = 38.48*$		

\**p* < 0.001



**Fig. 4** Distribution of alarm calls of 442 individual Altai pika callers (one call per individual) according to peak frequency. Boxes with spectrograms and power spectra of example alarm calls display the three peak frequency maxima (the left at 3–3.5 kHz, the middle at 6–

6.5 kHz, and the right at 10.5–11.5 kHz) that fit to the first three formants calculated for a 45-mm oral vocal tract model. Arrows indicate the three peak frequency maxima on the distribution

#### Nonlinear phenomena

Of a total of 442 alarm calls, 196 (44.34%) lacked nonlinear phenomena. In 31 (7.02%) alarm calls, nonlinear phenomena covered less than half of call duration, whereas in the remaining 215 (48.64%) alarm calls, they covered more than half of call duration (Fig. 5). The nonlinear phenomena were primarily biphonations (Fig. 5a, b), i.e., two independent fundamental frequencies f0 and g0 in a call spectrum, interacting with each other and causing the appearance of combinatory frequency bands (Wilden et al. 1998; Volodina et al. 2006; Frey et al. 2016). Subharmonics (Fig. 5e, f), representing frequency bands of 1/2, 1/3, or 1/4 of fundamental frequency (Wilden et al. 1998; Gogoleva et al. 2008; Stoeger et al. 2012), were observed but more rarely. In some calls, we observed transitions from the biphonic mode to the subharmonic mode (Fig. 5c, d). Deterministic chaos, even if potentially present in the Altai pika alarm calls, was undistinguishable from echo; moreover, we never observed calls, in which call parts with potential deterministic chaos were interrupted by frequency windows, an effect that would definitely prove the presence of deterministic chaos in these calls (Wilden et al. 1998; Gogoleva et al. 2008). So, the presence of deterministic chaos in the alarm calls of Altai pikas remained questionable.

#### Individual identity of alarm calls

Results of three one-way ANOVAs for individual differences (based on the first 8 calls, the last 8 calls, and 8 randomly selected calls of the 36 selected individual alarm call series) showed that factor "individual" significantly influences all measured acoustic variables (Table 1). Comparison of *F*-ratios revealed that f0max and df0 had the highest potential for encoding individual identity in the alarm calls.

Three DFAs, based on the first 8 calls, the last 8 calls, and 8 randomly selected calls of the 36 individual alarm call series, assigned calls to individuals with an accuracy of 93.06, 91.67, and 91.33%, respectively (Fig. 6). Classifying accuracy did not differ between the three DFAs (p > 0.5 in all comparisons, chi-square test). In all three DFAs, the results of correct classification were significantly higher than chance levels (16.9  $\pm$  $1.7, 16.8 \pm 1.8, 17.2 \pm 1.8\%, p < 0.001$  in all comparisons, permutation text, 1000 permutations) (Fig. 6). The first two discriminant functions in the three DFAs, based on the first 8 calls, the last 8 calls, and 8 randomly selected calls, accounted for only 59.39, 54.52, and 57.90% of variation, respectively. For the complete discrimination, 10 discriminant functions were necessary in all three DFAs. The eigenvalues over 1 had eight, nine, and eight functions, respectively, in the three DFAs. Contrary to ANOVA results, Wilks' Lambda values (Table 2) showed that variables mainly contributing to discrimination were dur-to-max, duration, and f0end (in order of decreasing importance). The fpeak was the variable least contributing to individual discrimination, although in 17 of the 36 focal callers, all calls throughout the series retained a uniform (individual-dependent) peak frequency maximum.

Among the 36 focal callers, 17 (47.2%) individuals lacked nonlinear phenomena in their calls. Seven (19.4%) individuals had nonlinear phenomena covering less than half of call duration, 7 (19.4%) individuals had nonlinear phenomena covering more than half of call duration, and 5 (13.9%) individuals had nonlinear phenomena but not in all their calls. Thus,



**Fig. 5** Spectrogram illustrating nonlinear phenomena in Altai pika alarm calls. **a** Call with two fundamental frequencies f0 and g0 and their combinatory frequency band f0-g0. **b** Call with two fundamental frequencies f0 and g0 and their four combinatory frequency bands. **c** Call representing a transit from the biphonic mode in its first part to a 1/4f0 subharmonic mode in its second part. **d** Call representing a transit from the biphonic mode in its second part. **d** Call with 1/2f0 subharmonics. Designations: f0—high fundamental frequency; g0—low fundamental frequency; f0-g0, 2g0-f0, 2f0-2g0, 3g0-2f0—linear combinations of f0 and g0; 1/4f0—the 1/4 subharmonic of f0; 1/2f0—the 1/2 subharmonic of f0. The spectrogram was created at 48 kHz sampling frequency, Hamming window, FFT 1024, frame 50%, overlap 96.87%. The audio file of these calls, all recorded from different individuals, is available as Electronic Supplementary Material 3

the presence/absence of nonlinear phenomena was consistent within individuals for 31 of the 36 callers (86.1%).

#### Discussion

We found that Altai pika alarm calls are strongly individualistic. The abundant nonlinear phenomena might further increase their individualization. Time and frequency variables of the alarm calls allowed assigning over 90% of the calls to the correct callers, whereas the role of formants in encoding individual identity was evidently low. The potential of encoding vocal identity did not differ for alarm calls emitted at the start or at the end of a series, or randomly selected along



**Fig. 6** Individual discrimination of 36 Altai pika callers. Three discriminant function analyses (DFAs) were based respectively on the first 8 alarm calls (series start), the last 8 alarm calls (series end), and 8 randomly selected alarm calls (series random). Red bars indicate DFA values and yellow bars indicate random values, calculated with the randomization procedure. Comparisons between observed and random values are shown by brackets above

a series. The degree of individual identity encoded by time and frequency variables in the Altai pika alarm calls resembled that found in the alarm calls of ground-dwelling sciurids lacking nonlinear phenomena (Matrosova et al. 2009, 2010a, 2011; Schneiderová 2012; Schneiderová et al. 2017). Video analysis confirmed an earlier report (Lissovsky 2005) that Altai pikas briefly fold the ears and press them to the neck while emitting their high-intensity alarm calls, probably to avoid damaging the own hearing apparatus by these selfgenerated vocalizations. We found that this movement was very fast and that it was only slightly longer than the duration of the alarm call.

This study revealed that all alarm calls of the Altai pikas were short, high frequency, and deeply frequency modulated. Some authors supposed that such strictly species-specific patterns of pika alarm call structure might result from constraints on individual variations imposed by specific sound propagation features of the habitat (Conner 1982; Hayes and Huntly 2005). However, this hypothesis did not receive support neither for pikas (Trefry and Hik 2010) nor for ground-dwelling sciurids (Matrosova et al. 2016).

The high amount of calls with nonlinear phenomena found in this study might provide an extra source for increasing individual identity in the Altai pika alarm calls. Nonlinear phenomena add qualitative changes (Fitch et al. 2002) to the quantitative (time and frequency) acoustic variation (Lapshina et al. 2012) and have been shown to play a role in encoding individual identity in different vertebrate taxa, as, e.g., amphibians (Zhang et al. 2017), carnivores (Volodina et al. 2006), and cetaceans (Papale et al. 2015).

The estimates of the Altai pika oral vocal tract length, obtained in this study, supported interpretation of the three peak

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Table 2DFA results for Altai pika alarm calls. The Wilks' Lambdavalues and call variable effects are presented for each acoustic variableincluded in the three independent DFAs for call assignment to individualon the basis of the first 8 calls, the last 8 calls, and 8 randomly selected

calls from 36 alarm call series (one series per individual). The smaller the Wilks' Lambda value, the greater the contribution of the given call variable to the overall discrimination. For each DFA, the four variables that contributed most to discrimination are highlighted in bold

Acoustic variable	First 8 calls		Last 8 calls		8 randomly selected calls	
	Wilks' Lambda	Variable effect	Wilks' Lambda	Variable effect	Wilks' Lambda	Variable effect
duration	0.19063	F=29.479	0.15939	F=36.617	0.19419	F=28.810
dur-to-max%	0.15305	F = 38.421	0.15377	F = 38.209	0.16105	F=36.168
f0max	0.47772	F = 7.591	0.32288	F = 14.560	0.37591	F=11.527
f0beg	0.34047	F=13.449	0.26479	F = 19.278	0.29376	F=16.692
f0end	0.28377	F = 17.524	0.26499	F = 19.258	0.30053	F = 16.159
df0	0.51337	F = 6.581	0.32554	F = 14.385	0.38329	F=11.171
fpeak	0.64677	F = 3.792	0.65708	F = 3.623	0.70435	F=2.914
q25	0.32985	F = 14.106	0.28549	F = 17.376	0.36883	F=11.881
q50	0.40182	F = 10.336	0.45778	F = 8.224	0.44019	F=8.830
q75	0.28177	F = 17.697	0.27216	F = 18.567	0.30146	F=16.088

frequency maxima as the first three formant frequencies (F1-F3). These supposed formants appear to add little to identity of the Altai pika alarm calls. We suggest therefore that formant frequencies might not be reliable cues to vocal identity in animals as small as Altai pikas, with a body mass of only 113-171 g (Lissovsky and Lissovskaya 2002) and correspondingly short vocal tracts of about 45 mm (this study). In larger-sized mammals with individualistic calls, as, e.g., goitred gazelle Gazella subgutturosa and saiga antelope Saiga tatarica, the formant frequencies play an important role in encoding individual identity, along with variables of fundamental frequency (Volodin et al. 2011; Lapshina et al. 2012; Sibiryakova et al. 2017). The prevailing role of formants over frequency acoustic variables in encoding and recognizing individuals was also shown in a primate species, the Japanese macaque Macaca fuscata (Furuyama et al. 2016).

For estimating individual vocal identity in this study, we used only one alarm call series per focal caller, produced during a single intrusion event. This single call series per individual was sufficient for detecting vocal individual identity and this fits natural conditions, where an animal should discriminate between the voices of other surrounding conspecific individuals based on a single series of calls (McCowan and Hooper 2002; Matrosova et al. 2009, 2010a; Sibiryakova et al. 2017). However, in studies of rodents (ground squirrels of the genus Spermophilus), individual vocal signatures of alarm calls remained stable only in about half of the individuals over a period of a few days (Matrosova et al. 2009; Schneiderová et al. 2017). Thus, ground squirrels should permanently update their knowledge of the individual vocal signatures of their territorial neighbors. No data is available yet about the temporal stability of individual vocal signatures in the vocalizations of pikas.

In this study, we only recorded and analyzed Altai pika alarm calls in a frequency range audible to humans. Ultrasonic alarm calls or their components have never been described for lagomorphs, although some acoustic components higher than 20 kHz were reported along the ontogeny of distress calls in the American pika Ochotona princeps in a laboratory colony (Conner and Whitworth 1985). Ultrasonic components of alarm calls have been found in some grounddwelling sciurids (Wilson and Hare 2004; Wilson and Hare 2006; Matrosova et al. 2012). Our study setup was not designed for examining potential ultrasonic alarms of Altai pikas, as ultrasonic components of alarm calls are always very faint and so, the distance from the microphone to a caller should be less than 0.5 m (Conner and Whitworth 1985; Wilson and Hare 2004; Matrosova et al. 2012; Murrant et al. 2013). In this study, however, a researcher spotted a caller based on hearing and conducted recordings at distances of 10-30 m from a free-ranging focal caller.

Our video analysis confirmed an earlier report (Lissovsky 2005) that Altai pikas briefly fold the ears and press them to the neck while emitting their alarm calls involving strong contractions of the abdominal musculature. Probably, the ear folding serves to avoid damage of the inner ear hair cells by their own high-intensity vocalizations. Ear folding as a hearing protection mechanism has also been reported for the bat *Myotis lucifugus* (Wever and Vernon 1961). However, in contrast to the Altai and northern pikas, in which the pinna is double-folded longitudinally (Lissovsky 2005; this study), the pinna of *M. lucifugus* is simply folded transversely. Despite incomplete closure of the ear's orifice, the acoustic barrier effect of the folded pinna has been demonstrated in *M. lucifugus*, particularly for high frequencies (Wever and Vernon 1961). In addition, *M. lucifugus* use the ear folding

in response to external loud sounds (Wever and Vernon 1961), whereas in the pikas, the ear folding occurs during the emission of their self-generated high-intensity calls (Lissovsky 2005; this study).

As both sexes of pikas emit high-intensity alarm calls (Ivins and Smith 1983; Lissovsky 2005), ear folding behavior and ear protection probably occur to a similar extent in males and females. In contrast, there is a pronounced sexual dimorphism of call production in domestic fowl (Gallus gallus domesticus). Roosters produce high-intensity crows of up to 140 dB, whereas the volume of the moderate clucking of hens is only 61-76 dB (Brumm et al. 2009). Correspondingly, hearing protection mechanisms are more effective in males than in females (Brumm et al. 2009; Claes et al. 2017, 2018). In roosters, hearing protection mechanisms involving craniokinesis comprise the considerably reduced transmission of acoustic energy to the inner ear due to relaxation of the eardrum at elevation of the upper jaw and the closure of the auditory canal when the beak is opened (Pohlmann 1921; Claes et al. 2017, 2018; Muyshondt et al. 2017). In contrast, craniokinesis in hens is less pronounced than in roosters, and therefore, these hearing protection mechanisms (eardrum relaxation and auditory canal narrowing) are less efficient than in roosters (Claes et al. 2017, 2018). Another mechanism of hearing protection is the contraction of the stapedius muscle, that in birds always contracts during self-generated loud vocalizations but not in response to loud external sounds (Borg and Counter 1989).

Mammalian skulls do not allow craniokinesis and, as a consequence, mammalian hearing protection mechanisms, aside the pinnae movements that were reported in bats (Wever and Vernon 1961) and documented in pikas (Lissovsky 2005; this study), to a large extent rely on callsynchronous contractions of the middle ear muscles: the stapedius, an analogue of the stapedius muscle in birds (Takechi and Kuratani 2010) and the tensor tympani, lacking in birds (Henson 1965; Suga and Jen 1975; Jen and Suga 1976; Fenton et al. 1995; Borg et al. 1984; Borg and Counter 1989). Importantly, the reflexive contractions of the middle ear muscles increase as vocalizations become louder and also occur in response to external loud sounds (Borg and Counter 1989). This "acoustic reflex" considerably dampens the sound energy reaching the inner ear (Borg et al. 1984; Borg and Counter 1989). Together, we expect that protection of the inner ear in Altai pikas from the damaging effect of their self-generated high-intensity alarm calls comprises the observed ear folding mechanism and, most probably, also callsynchronous contractions of the middle ear muscles.

Whether Altai pikas, similar to several bird species, further protect their hearing by closure of the auditory canal involving auricular muscle contractions remains to be investigated. In any case, findings of ear folding behavior in pikas might promote research for similar self-protective acoustic mechanisms in other species producing very intense alarm calls, as, e.g., many species of ruminants (Volodin et al. 2017; Volodina et al. 2018).

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#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This study has been conducted in collaboration with the staff of the Natural State Reserve "Khakassian," in accordance with its research projects, with its ethical and animal welfare standards and with the Guidelines for the treatment of animals in behavioral research and teaching (Anim Behav, 2006, 71:245–253). Animal disturbance was kept at a minimum, and no animal was injured as a result of our work. The conservation status of the Altai pika is "Least Concern," so there is no need for special conservation measures across its distribution area. The data collection protocol no. 2011-36 was approved by the Committee of Bioethics of Lomonosov Moscow State University.

Informed consent Informed consent was not required.

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