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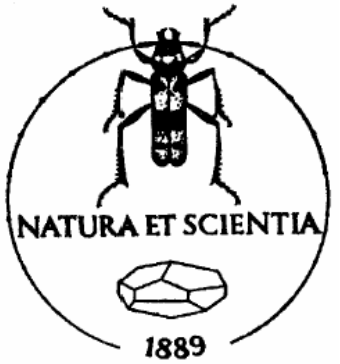

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A preliminary investigation into the alarm-calling behaviour of European sousliks (*Spermophilus citellus)**

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CONTENTS

ABSTRACT

European souslik (*Spermophilus citellus*) alarm-calling behaviour is observed in several naturally occurring predation episodes during a field study in North-eastern Bulgaria. The stimulus that triggered them, however, is not positively ascertained in all cases. Attempts to elicit alarm-calling by playbacks of predators and other methods mostly fail. Playbacks of souslik alarm-calls, however, succeed in increasing the vigilance behaviour of focal animals. Recordings of audible calls are made. Their frequency is approx. 8 KHz. During several ultrasonic recording attempts no evidence for souslik vocalizations outside human hearing range is obtained. The sample size of data collected in all instances, however, is too small to allow any valid conclusions to be drawn. The results of this preliminary investigation would be of value to a future study into the alarm-calling behaviour of European sousliks.

Key words: European souslik, *Spermophilus citellus*, alarm-calling, vocalization

1. INTRODUCTION

Alarm calls have been widely documented for many ground-dwelling sciurid rodents and for the European souslik, *Spermophilus citellus,* in particular (Betts 1976, Owings *et al* 1977, Leger *et al* 1984, Hoffman 1995, Blumstein & Armitage 1997, Popov & Sedefchev 2003). All of these calls are within the hearing range of humans but Wilson & Hare (2004) report the first evidence for an ultrasonic alarm call in a North American rodent – Richardson's ground squirrel, *Spermophilus richardsonii*. According to their work these 'whisper calls' serve the function of highly directional warning signals to conspecifics. Alarm calling in social rodents has been proven to be nepotistic behaviour – one that favours kin (Sherman 1977, Hoogland 1983, 1996). This behaviour is potentially risky to the caller in that it can draw the attention of a predator. Therefore it would pay the signallers if they can employ an effective strategy of alarm signalling that is less costly to them. The use of ultrasonic alarms, it is hypothesized by Wilson & Hare (2004), can serve precisely these purposes since most of the ground squirrel predators are not capable of hearing the 'whisper calls'. Furthermore, even those predators that *could* potentially perceive these high-frequency sounds, would not be able to do so in reality, since the 'whisper calls' documented for Richardson's ground squirrels fade down quickly with increased distance from the caller and also show high directionality. Because of this directionality of the 'whisper calls', according to Wislon & Hare (2004), the ground squirrels would be able to target precisely the recipients of their warnings (philopatric kin) and avoid detection at the same time.

While the presence of such calls has not been recorded in other similar species so far, it is believed that the phenomenon is more widespread than previously thought (James Hare, Vladimir Stefanov, personal communications).

From an evolutionary perspective it would be interesting to examine whether the European species of ground squirrel also uses ultrasounds in its anti-predator behaviour. The presence of such vocalizations in *Spermophilus citellus* will show that this phenomenon is not an oddity of the vocal repertoire of one particular species but a more broadly expressed adaptation in a wide array of species experiencing ecological pressures similar to those, which predators impose on Richardson's ground squirrels.

In this study we set out to collect preliminary data on general alarm-calling behaviour of the European souslik and specifically to examine the possibility for use of ultrasonic warning signals by this species.

2. MATERIALS & METHODS

2.1. Study site & animals

European sousliks (*Spermophilus citellus*) were studied near Kacelovo village in Northeastern Bulgaria. The colony was located on a hill slope. For our observations we concentrated on a small part of the colony (30.75 m x 37.75 m or approximately 1160.81 m^2), demarcated by sign-posts (sticks with white tape attached). A fixed viewpoint was used - a natural hillock at the bottom of the souslik hill slope (4.60 m away from the demarcated study area).

2.2 Scoring vigilance behaviour

To score the changes in vigilance levels in sousliks the following observational criteria were applied for focal animals:

- *Crouch with head down*: standing on four feet, head below horizontal plane.
- *Crouch with head up*: standing on four feet, head above horizontal plane. Denotes a higher alertness than the preceding category.

- *Slouch* (*sensu* Hare & Atkins 2001): hind feet on ground, head above a curved back.
- *Alert* (*sensu* Hare & Atkins 2001): standing up on hind feet with head raised above back, stance is perpendicular to the ground). This indicates a high vigilance level.

2.3. Naturally occurring potential predation episodes

Any events when sousliks alarm-called without any prior interference by the observers were considered to be *naturally occurring potential predation episodes*. Any instance when a potential predator was present in the area was also classified as a naturally occurring potential predation episode (whatever the reaction of the sousliks was). Behavioural sampling (Altmann 1974) was applied to observed animals during such episodes.

2.4. Experimental simulations of potential predation episodes.

Since naturally occurring predation episodes are infrequent (Barash 1975 in Sherman 1977, Hoogland 1983) and also the presence of three human observers on site could inhibit predation, response of sousliks to potential predation situations were tested under experimental conditions. Three main methods were used:

- *Direct approach by observers*: walking slowly towards a focal animal.
- *Playback of animal calls*: broadcasting vocalizations of potential souslik predators (dogs, wolves, jackals, cats and long-legged buzzards). These were used to test for situation-specificity in souslik response (whether they react differently to different types of danger).
- *Playback of conspecific calls:* souslik alarm-calls recorded at the same site were used as samples.

2.5. Recording audible calls

Souslik alarm calls within the human hearing range were recorded with a *Sennheisser* microphone and a SONY tape recorder mounted on a tripod (distance from burrows approx. 0.5 m), with the observers sitting on the view-point, away from the recording equipment.

2.6. Ultrasounds recording trials

A custom-made ultrasound sensitive microphone (University of Tuebingen) was used to test production of ultrasonic vocalizations. The microphone was set up on a tripod within 0.5 – 1.0 m of a burrow's entrance and two observers/recorders sat uphill at around 10 m distance in a way that their presence is not immediately obvious to an animal emerging from below ground. Once an animal was sighted coming out, several recordings were conducted. During one of the recording trials a slow direct approach was initiated towards the focal souslik in order to elicit possible 'whisper calls' (*sensu* Wilson & Hare 2004).

2.7. Sound analysis

Sound recordings were digitized and analyzed with the colour sonograph software *Selena* (custom-made, University of Tuebingen). FFT Filter Length of 512 for audible range recordings and 256 for the ultrasound recordings was used. We choose a Hann window option for visualation. The frequency resolution was 0.15625 kHz for the audible recordings and 0.375 kHz for the ulrasound recordings.

3. RESULTS

3.1. Naturally occurring potential predation episodes

Several such episodes were witnessed and they are summarized in *Table 1*.

TABLE 1. Observed potential predation episodes.

#	Predator involved	Details	Souslik reaction
1	Dog (*Canis lupus domesticus*)	a) Passing through the colony	a) Run down burrow
		b) Walking on opposite hill.	b) Increase in vigilance & alarm-calling
2	Long-legged buzzard (*Buteo rufinus*)	Circling and calling above the colony.	No increase in vigilance levels.
3	Goshawk (*Accipiter gentilis*)	Flying very low over the hill (attack mode)	Intensive alarm-calling (from mainly one indiv.)
4	Threats unknown to the observers	No threat was identified.	Notable increase in vigilance levels and alarm-calling.

3.2. Researcher-simulated predation episodes

The results of the field experiments are summarized in *Table 2*.

TABLE 2. Reaction of sousliks to experimental simulations.

#	Type of simulation	Souslik reaction
1	Direct approach by observers	Retreat to burrows without alarm calling mostly; some distant alarm calls heard occasionally but no visual confirmation of their origin achieved.
2	Playback of animal calls (see *Materials & methods*)	No significant increase in vigilance levels; no alarm-calling.
3	Playback of souslik alarm calls (from the same site)	Notable increase in vigilance. No alarm-calling.

None of the animal call playbacks succeeded in triggering alarm calling although during the playback of dogs barking some of the observed sousliks appeared to pause their activity and show low levels of vigilance (e.g. *Slouch* posture). The most obvious change in souslik behaviour was observed during simulation № 3.

3.3. Audible alarm calls

Sixteen alarm calls from one individual were recorded. Table 3 shows the sonic parameters of those samples.

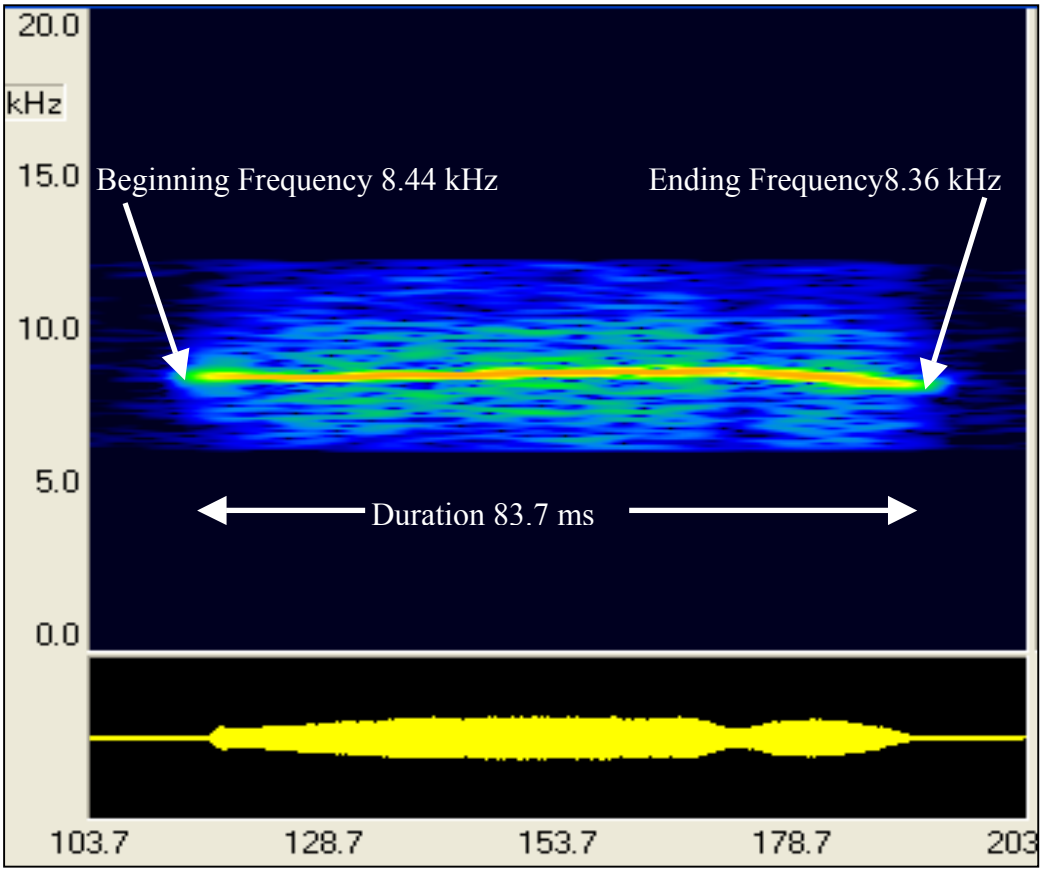
TABLE 3.

Souslik call characterics. Based on a data set of 16 separate calls from one individual.

Call Characteristic	Mean	SD
Beginning Frequency	8455.7 Hz	120.9
Ending Frequency	8421.5 Hz	198.2
Mean of Beginning and Ending Frequency	8438.6 Hz	162.4
Call duration	81.26 ms	3.68

Figure 1 displays a typical call from the sample (note that kHz are used on the Figure). There are no multi harmonic components.

FIGURE 1. Typical souslik call (one from the sample).



3.4. Ultrasonic trial recordings

Of all 41 recordings in the ultrasound range none showed evidece for 'whisper calling' in European sousliks.

4. DISCUSSION

Alarm calling behaviour is hypothesized to serve two main functions: warn conspecifics of impeding danger and communicate to predators that they have been spotted (Shelley & Blumstein, in press). This seems to be true in the case of the European souslik as well, although because of the limited time in the field our data is too insufficient to allow any definite conclusions. The apparent lack of vocalizations in the several cases when potential predators were seen close to the colony, might be due to either that:

1) the predators were not seen by the animals (strongly backlit by the sun long-legged buzzards circling high above the colony). The lack of reaction of sousliks when the buzzards called might be explained by inexperience (point 4);
2) the predators (dogs) were too close and it must have been more beneficial for the sousliks to escape urgently without vocalizing,
3) there were too few animals around and those were not related to each other thus making alarm calling more costly (since alarm calling in Sciurids is considered to be nepotistic behaviour, Sherman 1977) or
4) the animals, which were present were young and not experienced enough with predators (at this time of the year it's mostly juveniles that are out, while older individuals are already hybernating, Popov & Sedefchev 2003).

The only case when there was strong souslik respone to a predator was in the goshawk situation (see Table 1). The fact that the souslik did not evade but stayed above ground calling intensively is consistent with the hypotheses of animals alarm-calling in order to communicate to predators that they have been spotted and thus discourage pursuit (Hasson 1991 in Shelley & Blumstein 2004 in press).

The results from the experimental simulations are also not conclusive. The direct approach method, although successful in eliciting alarm calling in other Sciurids (Greene & Meagher 1998), was not effective in our case. Focal animals during approaches would not alarm-call but would just escape underground when the human observer approached closer. This might be because humans are not perceived as predators (thus no need to signal), and evasive action is only taken if proximity is great. This explanation is not very satisfactory, though, since in other cases we repeatedly heard (but did not see) sousliks alarm calling when we moved through the colony.

The lack of obvious reaction of the sousliks to playbacks of potential predators might be explained by several factors:

1) lack of experience on the part of the sousliks with the playback predators
2) for the dog and cat playbacks – habituation to these calls since there is a village nearby
3) need of visual confirmation of danger (not just audible)
4) design of playback experiments that is not realistic enough

To test the sousliks' reaction to predator cues further, more experiments must be done, involving sousliks of different age and sex classes and predator dummies (to serve as visual stimuli).

The souslik sound recordings show little variation in frequency and time length. The slight frequency modulations might result from the different body postures, which

were observed when the individual was calling. The calls lack any multi harmonic components, leading to the conclusion that these sounds are whistles. Playbacks made with these recordings at the same site indicate clearly that these whistles denote danger to conspecifics. Whether they could be used to communicate semantic information in the way that alarm calls are used by other animals (reviewed in Macedonia & Evans 1993; also Zuberbuhler 2000) is arguable. Since the frequency range and call length show very little variation, it seems there is not much scope for situation specificity in Europeans souslik alarm call production. However, considerably more alarm calls from *different* individuals in *different* situations are needed to confirm this.

The lack of ultrasound recordings does not necessarily preclude the existence of ultrasonic alarm calls in this species. According to James Hare (personal communication) during the study of Richardson's ground squirrels only a part of the animals that were tested actually produced ultrasonic alarm calls. This means that our sample size of 2 individuals on four separate recording attempts is far too small to allow us to make any conclusions about the occurrence of such vocalizations in the species in general. There is evidence that this behaviour is adaptive for the Richardson's ground squirrel (Wilson & Hare 2004) so it makes sense for it to be present in other related *Spermophilus* species, too. We believe the possibility for ultrasonic communication in sousliks to be significant enough to demand further exploration. It would be advantageous that such work is carried out during the breeding season for then, there will be more animals present and much more opportunities to observe natural alarm-calling episodes, arrange playbacks and record vocalizations.

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