Olfactory communication and hunting behaviour of Eurasian lynx *Lynx lynx* in the Northwestern Swiss Alps

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Today I think
Only with scents, - scents dead leaves yield,
And bracken, and wild carrot's seed,
And the square mustard field;
Odours that rise
When the spade wounds the root of tree,
Rose, currant, raspberry, or goutweed,
Rhubarb or celery;

The smoke's smell, too,
Flowing from where a bonfire burns
The dead, the waste, the dangerous,
And all to sweetness turns.
It is enough
To smell, to crumble the dark earth,
While the robin sings over again
Sad songs of autumn mirth.

- Edward Thomas, *Digging*

To MILA & EYWA
for letting me see glimpses of four
generations of lynx family life

and to Akela
for his never-ending enthusiasm

Contents

Summary

Chemical signalling in the form of scent-marking with urine, faeces or gland secretions is widespread in mammals and its role in territoriality, competition or mate choice is widely recognised for many species. Mammals regularly check and renew their scent-marks and place their own scent-marks on top of those left by others. Such over-marking is essential for communication with neighbouring territory holders or group members and high scent-marking rates increase reproductive success. However, chemical signals are not only perceived by the intended receivers but can be intercepted and exploited by competitors, predators or parasites (eavesdropping). In wild felids, the occurrence of scent-marking behaviour and the chemical compositions of different scent sources have been described for an increasing number of species. However, the role of over-marking in social organisation of wild felid populations, the factors influencing spatial and temporal distribution of scent-marks in territories (e.g. eavesdropping by prey), and the information content of scent-marks have not yet been studied extensively in wild felids. The aim of my thesis was to explore the possible functions of scent-marking in felid social organisation using the Eurasian lynx *Lynx lynx* as a model species and focusing on the above-mentioned research topics.

In my first study (chapter 1), I investigated scent-marking behaviour and its role in communication among resident and non-resident Eurasian lynx using infrared camera traps. I could show that communal marking sites play an important role in communication between male and female lynx and in competition between males. I demonstrated that lynx were able to discriminate between self and non-self and that over-marking does not mask the underlying scent-mark. These results support the function of communal marking sites as “chemical bulletin boards”.

In chapter 2, we describe a robust method for identifying kill sites from movement patterns by analysing GPS location clusters (GLCs) generated by GPS-collared lynx. We were able to find large as well as small prey items and could show that the majority of the kills (92%) were found in GLCs lasting ≥ 9h. The method was then used in the next chapter to relate lynx scent-marking rates to hunting behaviour.

In my third study (chapter 3), I followed tracks of GPS-collared lynx in the snow and recorded scent-marks and evidence of hunting behaviour along these tracks. I was able to show that overall scent-marking rate was lower when lynx were hunting but that hunting lynx increased scent-marking rates in places, where there was a high chance of detection by conspecifics (along forest roads). Lynx also

increased scent-marking rates during mating season but only when they were not hunting. My results suggest that lynx face a trade-off between enhancing the detection probability of scent-marks by conspecifics and avoiding eavesdropping by prey.

Finally, in my last study (chapter 4), I collected urine from captive and wild Eurasian lynx and analysed volatile constituents of urine by means of solid phase microextraction and gas chromatography-mass spectrometry. I identified several carboxylic acids, aldehydes, ketones, and esters, as well as high amounts of cyclic octaatomic sulphur. I could show that lynx urine contains sex-specific information on reproductive state, as well as individual identity cues. Relative sulphur content in urine samples decreased with age of the urine sample and could serve as an indicator for the freshness of a scent-mark.

The patterns of scent-marking I observed during my studies suggest that urine marking plays an important role in communication between potential mates and rivals and, hence, in social and spatial organisation of Eurasian lynx populations. I was also able to chemically analyse the information content of lynx urine and show that urine marks are well-suited to fulfil the suggested functions in the wild. As such, my work contributes to a better understanding of the functions and constraints of chemical signalling in wide-ranging solitary predators.

Introduction

"For the sense of smell, almost more than any other, has the power to recall memories and it is a pity that we use it so little." – Rachel Carson

Communication by means of visual, acoustic or chemical signalling is the key to most social interactions in animals. Acoustic signalling, i.e. speech, is an integral part of human culture, although humans also use non-verbal ways of communication. We also possess a keen sense for interpreting body language (de Gelder 2006) which even enables us to communicate with other species (Miklósi et al. 2000; Tami & Gallagher 2009). Therefore, it is not surprising that humans are fascinated by the many ways in which other animals communicate. Especially conspicuous visual ornaments or acoustic displays involved in the communication between males and females have long since captured the attention of artists and researchers alike (e.g. Doupe & Kuhl 1999; Kettle 2013; Lal 2007; Nicoletto 1993). There is an almost ubiquitous appreciation for bird song in human cultures and the peacock's tail is arguably the most well-known textbook example for sexual selection. Probably because human beings mostly rely on vision and hearing, olfactory communication has sparked the interest of the scientific community only quite recently (Wyatt 2014). Chemical senses are among the oldest sensory systems and are shared by all organisms including bacteria. While the first studies on pheromones (chemical signals evolved for intra-specific communication) were conducted on insects, research on chemical signalling now brings together scientists with many different areas of expertise, from a rich diversity of chemists to biologists working on many different species and interested in a broad variety of research topics, such as sexual selection, social behaviour, predator-prey interactions, or pest control (Wyatt 2014). By now, chemical signalling has been found to be of central importance in various social contexts across taxa, including insects (e.g. Eisner & Meinwald 1995), amphibians (e.g. Kikuyama et al. 1995), fish (e.g. Brown et al. 2000), birds (e.g. Bonadonna et al. 2007), and mammals (Roberts et al. 2014; Wedekind et al. 1995).

In mammals, chemical signalling in the form of scent-marking with urine, faeces or gland secretions is widespread. Its role in territoriality, mate attraction, competition or mate choice has been studied most extensively in small rodents but is widely recognised for many species (Ferkin & Pierce 2007; Penn 2002; Roberts et al. 2014). Animals invest a lot of time in placing scent-marks throughout their home ranges and in receiving chemical signals left by conspecifics. This exchange of chemical information often involves placing your own scent-mark on top of, touching or adjacent to an already existing one which has been referred to as 'over-marking' by Ferkin & Pierce (2007). Empirical studies on the possible functions of over-marking provide support for several (not necessarily mutually

exclusive) hypotheses (reviewed in Ferkin & Pierce 2007). Over-marking has been suggested to mediate pair bonding (e.g. grey wolf *Canis lupus*, Peters & Mech 1975), to signal social dominance and resource holding potential (e.g. house mouse *Mus domesticus*, Hurst 1990; blackbuck *Antelope cervicapra*, Rajagopal et al. 2010), or to strengthen the cohesion of social groups (e.g. spotted hyena *Crocuta crocuta*, Burgener et al. 2008; banded mongoose *Mungos mungo*, Jordan et al. 2011). In several rodent species (e.g. golden hamster, *Mesocricetus auratus*: Johnston et al. 1994; meadow vole, *Microtus pennsylvanicus*: Johnston et al. 1997), females are able to distinguish the donor of the top-scent-mark from the underlying scent-marks and prefer to mate with donors of top-scent-marks.

Regularly checking and renewing scent-marks is essential for communication with neighbouring territory holders, potential mates or group members and eventually for reproductive success. However, chemical signals are not only perceived by the intended receivers but can be intercepted and exploited by competing conspecifics or even by other species for their own benefit. This phenomenon is known as `eavesdropping' and has been described in both intra- and inter-specific contexts (Hughes et al. 2010; Peake et al. 2001; Zuk & Kolluru 1998). The role of eavesdropping has been studied extensively in the context of predator-prey interactions (Apfelbach et al. 2005) and it has been shown that leaving and receiving chemical signals comes at the cost of increased predation risk (Hughes et al. 2010; Koivula & Korpimäki 2001). For example, the scent-marks of voles are visible in ultraviolet light and attract predatory birds such as rough-legged buzzards (Koivula & Viitala 1999). While prey scent-marks are attractive to predators, predator scent-marks have been shown to repel prey species (Apfelbach et al. 2005). The question whether scent-marking in predator species comes at the cost of decreased hunting success due to inter-specific eavesdropping by prey is not yet understood.

Scent-marking in felid species

In wild felid species, scent-marking is assumed to play an important role in territoriality, in reproductive behaviour, and in competition among same sex individuals (Allen et al. 2015; Sunquist & Sunquist 2002). Felids are well known to leave scent-marks at visually conspicuous sites (Macdonald 1985) involving a variety of distinct marking behaviours (Mellen 1993): urine spraying, head or cheek rubbing, defecating, scraping, claw raking, sniffing, licking, or `flehmen´ (use of the vomeronasal organ, Doving & Trotier 1998). Males mark generally more often than females and marking-frequency increases during the mating season (Allen et al. 2015; Mellen 1993). In recent years, a variety of compounds potentially involved in chemical communication have been characterized in urine and facial scent samples of several felid species by means of gas

chromatography-mass spectrometry (GCMS), e.g. hydrocarbons, ketones, aldehydes, fatty acids, alcohols, lactones, thioethers, sulphones, amines, and amides (e.g. Burger et al. 2008; Mattina et al. 1991; Soini et al. 2012). While the occurrence of scent-marking behaviours and the chemical compositions of different scent sources have been described for an increasing number of felid species, empirical evidence on the functions of scent-marking is still scarce. For example, not much is known about the role of over-marking in social organisation of wild felid populations, the factors influencing spatial and temporal distribution of scent-marks in territories (e.g. eavesdropping by prey), and the information content of scent-marks (Allen et al. 2015; Brahmachary & Poddar-Sarkar 2015; Soini et al. 2012; Sokolov et al. 1996).

The aim of my thesis was to explore the possible functions of scent-marking in felid social organisation choosing an interdisciplinary approach and using the Eurasian lynx *Lynx lynx* as a model species. I started with behavioural observations of wild lynx at the population level and then narrowed down to investigate factors influencing spatial and temporal distribution of scent-marks at the level of individual GPS-collared lynx. Finally, I zoomed in to the level of chemical composition of lynx urine and investigated the information content of individual scent-marks.

The study organism

The best-studied model organism for research on scent-marking and its role in social behaviour and sexual selection is the house mouse (Roberts et al. 2014). However, if we want to capture the variety of contexts in which scent-marking occurs, we should not restrict research on its functions to rodents alone but extend our interest to organisms belonging to different taxonomic groups, living in different social systems, or occupying different trophic niches. For example, scent-marking behaviour is very prominent in the felidae, where it has not yet been studied extensively and hardly any experimental research on the functions of scent-marking is available. Eurasian lynx have a social system representative for many felid species: they are solitary, territorial and occur at low densities (1-2 individuals per 100 km2, Zimmermann et al. 2012). Resident male and female lynx occupy large home ranges of more than 100 km^2 with little home range overlap between neighbouring animals of the same sex (Breitenmoser-Würsten et al. 2001). Resident males almost entirely overlap the home ranges of one or two resident females, which they try to monopolise. However, extra-territorial excursions of males occur during the mating season, indicating male-male competition and a potential for female choice (Breitenmoser & Breitenmoser-Würsten 2008). It is therefore crucial for a male lynx to find and guard the female when she is ready to mate and this could be facilitated by scent-marking. Part of a lynx population also consists of non-residents, which do not hold territories

but move among the home ranges of established residents (Zimmermann et al. 2005). Since encounter rates of a wide ranging species like the Eurasian lynx are low, it is likely that indirect communication via scent-marking plays an important role in maintaining their social and spatial organisation.

Eurasian lynx are also specialised predators of medium-sized ungulates like roe deer *Capreolus capreolus* and chamois *Rupicapra rupicapra*, which make up 84% of their diet in Switzerland on average. The remaining 16% consist of smaller prey species such as red foxes *Vulpes vulpes*, European brown hares *Lepus europaeus* or marmots *Marmotta marmotta* (Breitenmoser et al. 2010). Observations of prey animals investigating lynx marking sites have occasionally been made and a recent study has shown increased vigilance levels in roe deer after detection of experimentally applied lynx urine (Eccard et al. 2015). It is, however, still unclear how this may translate into changes in lynx hunting success.

Thesis outline

We first described chemical communication at the population level in **chapter 1 ("Scent-marking behaviour and social dynamics in a wild population of Eurasian lynx *Lynx lynx*")**, where we investigated scent-marking behaviour and its role in intra- and intersexual communication among resident and non-resident Eurasian lynx by observing interactions among wild lynx at natural marking sites using infrared camera traps. We especially focused on seasonal changes in marking frequency and on the potential functions of over-marking and discussed our findings in the light of different hypotheses related to mate attraction, competition, and territoriality (Vogt et al. 2014).

In **chapter 2 ("Suitability of GPS-telemetry for studying the predation of Eurasian lynx on small and medium-sized mammals in the Northwestern Swiss Alps")** we developed a method for identifying kill sites from lynx movement patterns by analysing GPS location clusters generated by radio-collared lynx (Vimercati et al., in preparation). The method was then used in the next chapter to relate scent-marking rates to lynx hunting behaviour.

We focused on the potential influences of inter-specific eavesdropping on lynx scent-marking behaviour in **chapter 3 ("Is there a trade-off between scent-marking and hunting behaviour in a stalking predator, the Eurasian lynx *Lynx lynx*?"),** where we investigated whether there was a trade-off between intra-specific communication through scent-marking and the risk of alerting prey in this

stalking predator. To this end, we followed tracks of GPS-collared lynx in the snow and recorded scent-marks and evidence of hunting behaviour along these tracks (Vogt et al., under revision).

Finally, in **chapter 4 ("Chemical composition of Eurasian lynx urine conveys information on reproductive state, individual identity, and urine age"),** we investigated whether chemical composition of Eurasian lynx urine was related to sex, reproductive state, individual identity, and dietary cues. We collected urine from captive and wild Eurasian lynx and analysed volatile constituents of urine by means of solid phase microextraction and gas chromatography-mass spectrometry (Vogt et al., in preparation).

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Chapter 1

Scent-marking behaviour and social dynamics in a wild population of Eurasian lynx *Lynx lynx*

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Scent-marking behaviour and social dynamics in a wild population of Eurasian lynx *Lynx lynx*

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ABSTRACT

Scent-marking is widespread among mammals and has been observed in many felid species. Although the behaviour is well-described, little is known about its function in wild felid populations. We investigated patterns of scent-marking and its role in intra- and intersexual communication among resident and non-resident Eurasian lynx *Lynx lynx* by observing interactions among wild lynx at natural marking sites by means of infrared camera traps. Marking activity of resident animals showed a peak during the mating season and was lowest during the time when females gave birth and lactated. Both sexes scent-marked, but male lynx visited marking sites much more often than females and marked relatively more often when visiting a site. Most visits to marking sites were by residents but we also observed scent-marking by non-residents. Juveniles were never observed marking. We found no evidence of lynx regularly renewing scent-marks after a certain 'expiry date' but the presence of a strange scent-mark triggered over-marking. Males responded similarly to the presence of another individual's scent-mark, irrespective of whether it was the top- or the underlying scent-mark in a mixture of scent-marks they encountered. Our results suggest that marking sites could serve as 'chemical bulletin boards', where male lynx advertise their presence and gain information on ownership relationships in a given area. Females placed their urine marks on top of the ones left by resident males, but further studies are needed to explain the functions of over-marking in females.

Scent-marking with faeces, urine or glandular secretions is widespread among mammals (reviewed in Gosling and Roberts, 2001a,b). For instance, felids are well known to leave scent-marks (i.e. urine, faeces, saliva) at visually conspicuous sites (Macdonald, 1985) including a variety of distinct marking behaviours (Mellen, 1993): urine spraying, head or cheek rubbing, scraping, claw raking, sniffing, licking, or 'flehmení' (use of the vomeronasal organ; Doving and Trotier, 1998). When mammals encounter a scent-mark of another individual, they often place their own scent-mark on top of, touching, or adjacent to it. This phenomenon has been referred to as 'over-marking' by Ferkin and Pierce (2007) and has been observed in many different species (reviewed in Ferkin and Pierce, 2007). Over-marking occurs among breeding pairs (e.g. grey wolf, *Canis lupus*: Peters and Mech, 1975), same-sex competitors (e.g. house mice, *Mus domesticus*: Hurst, 1990a,b) and within social groups (e.g. spotted hyena, *Crocuta crocuta*: Burgener et al., 2008; banded mongoose, *Mungos mungo*: Jordan et al., 2011a,b,c). The behaviour has also been observed in different felid species, most of which are solitary and territorial (i.e. tiger *Panthera tigris*: Smith et al., 1989; several small felid species: Mellen, 1993; cheetah, *Acinonyx jubatus*: Marnewick et al., 2006).

Several hypothesis for the behavioural function of over-marking have been proposed (reviewed in Ferkin and Pierce, 2007): Over-marking could create a mixture of odours ('scent blending'), such as a 'group odour' used for recognition in group-living species. Alternatively, over-marking could also cover the underlying odour of a conspecific ('scent masking'). Or finally, the information of both, the underlying and the overlying scent-mark, could remain available, thereby creating a 'chemical bulletin board' displaying information from multiple individuals. Several rodent species (e.g. golden hamster, *Mesocricetus auratus*: Johnston and Bhorade 1998; meadow vole, *Microtus pennsylvanicus*: Johnston et al., 1997; Ferkin, 1999) seem to be able to distinguish the donor of the top-scent-mark from the underlying scent-marks. Many studies have further investigated the possible functions of over-marking and the evidence

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provides support for several (not necessarily mutually exclusive) hypotheses. Ferkin and Pierce (2007) review 10 hypotheses related to over-marking and the supporting evidence. Here we focus on the first four, which are likely of high relevance for solitary and territorial carnivores such as felids: (1) Competition hypothesis: Over-marking animals gain an advantage over those individuals whose scent-marks they overlap, either by masking the underlying scent-mark or by demonstrating social dominance (Johnston et al., 1994; Rich and Hurst, 1999). Therefore, over-marking should occur most often among same-sex competitors (Ferkin and Pierce, 2007). (2) Chemical bulletin board hypothesis: Both the information from the bottom- and the top-scent-mark remains available, so that both donors can advertise their presence in an area (Wolff et al., 2002). Since scent-marks supposedly are costly and reliable signals of an animal's quality (Gosling and Roberts, 2001a), individuals could use information from 'chemical bulletin boards' to assess potential mates or same-sex competitors (Ferkin and Pierce, 2007). (3) Territoriality hypothesis: Scent-marking could play a role in competition between territory holders and potential intruders (Temeles, 1994; Sun and Müller-Schwarze, 1999; Sillero-Zubiri and Macdonald, 1998; Gosling and Roberts, 2001b). Under this hypothesis, only animals successfully defending an area can ensure that their own scent-marks predominate and are the ones most recently deposited. Thus, the consistent over-marking of scent-marks of rivals can potentially reflect the marker's resource-holding potential (Rich and Hurst, 1999). Resident individuals should most often over-mark the scent-marks of intruders, while intruders should be less likely to over-mark than residents. (4) Mate attraction hypothesis: Over-marking serves as a form of mate attraction and facilitates interactions between potential mates (Hurst, 1990c; Ferkin, 1999). It should therefore occur most often among conspecifics of opposite sex and during the time when females are receptive (Ferkin and Pierce, 2007).

Some studies on captive or domestic felids have provided the first insights on the information contained in scent-marks: Domestic cats (Natoli, 1985) and captive Eurasian lynx *Lynx lynx* (Sokolov et al., 1996) seem to distinguish different sexes, individuals and reproductive status from urine marks. In captivity, it has also been shown that males mark more often than females and marking frequency increases during the mating season (several small felid species: Mellen, 1993). However, only few studies have described scent-marking in the wild, e.g. leopard *Panthera pardus* (Bothma and Coertze, 2004), tiger (Smith et al., 1989), cheetah (Marnewick et al., 2006), Geoffroy's cat *Leopardus geoffroyi* (Soler et al., 2009) and Eurasian lynx (Hucht-Ciorga, 1988; Sokolov et al., 1995). These studies have focused mostly on describing the occurrence and frequency of scent-marking behaviour in few individuals, and not on observing interactions between different individuals. Here, we present the results from detailed observations on over-marking in a population of free-ranging felids. Eurasian lynx are solitary, territorial and occur at low densities (1–2 individuals per 100 km^2, Zimmermann et al., 2012a,b). Resident male and female lynx in our study area occupy large home ranges (males: 137 km^2 (mean Kernel 95%, $N = 11$), females: 76 km^2 (mean Kernel 95%, $N = 12$); Breitenmoser-Würsten et al., 2001), with little home range overlap between neighbouring animals of the same sex (males: 4.8% (mean overlap of Kernel 95%, $N = 5$), females: 12.8% (mean overlap of Kernel 95%, N = 12); Breitenmoser-Würsten et al., 2001). Resident males almost entirely overlap the home ranges of one or two resident females, which they try to monopolise (Breitenmoser and Breitenmoser-Würsten, 2008). However, extra-territorial excursions of males occur during the mating season (Haller and Breitenmoser, 1986; Breitenmoser and Breitenmoser-Würsten, 2008) and we have documented one case where a resident male sired the offspring of a neighbour's female (Breitenmoser-Würsten, unpublished results). Eurasian lynx are thought to be monoestrous (Kvam, 1990), although replacement litters are known to occur when the first litter is lost (Breitenmoser-Würsten et al., 2007). It is therefore crucial for the male to find and guard the female when she is ready to mate and this could be facilitated by scent-marking. Part of a lynx population consists of (mostly subadult) non-residents, which do not hold territories but move among the home ranges of established residents (Zimmermann et al., 2005). Since encounter rates of the wide ranging species lynx are low and aggressive conflicts among residents and non-residents have rarely been observed (Breitenmoser and Breitenmoser-Würsten, 2008), it is likely that indirect communication via scent-marking could play an important role in maintaining the social and spatial organisation of the lynx.

The first aim of this study was to describe the marking behaviour of Eurasian lynx at commonly used natural marking sites in a wild population. Since a communicative function of scent marking requires the repeated use of particular conspicuous structures and captive lynx of both sexes are known to scent-mark (Burmester, 2005), we predicted that both male and female adult lynx in the wild regularly head rub and spray urine to the same visually conspicuous structures (i.e. rocks, trees, woodpiles). Scent-marks of wide ranging mammals need to have a certain longevity in order to be picked up by conspecifics, which may not encounter them for several days or weeks. We therefore further expected that there would be an 'expiry date' to scent-marks in that resident lynx are more likely to refresh their own scent-marks as the marks become older.

The second aim of this study was to gain insight into whether over-marking at these sites has a function in communication among males and females and/or in competition among neighbouring residents and non-residents. We predicted that lynx marking activity increases before and during the mating season (mate attraction hypothesis), encountering the scent-mark of another individual triggers over-marking in resident lynx of both sexes (competition, chemical bulletin board or territoriality hypothesis), and non-resident lynx refrain from marking in order to avoid conflicts with residents (territoriality hypothesis).

1. Methods

This study was conducted in the north-western Swiss Alps, where spatial and social structure of the lynx population is well known from several previous radio telemetry studies (Haller and Breitenmoser, 1986; Breitenmoser and Haller, 1993; Breitenmoser-Würsten et al., 2001; Molinari-Jobin et al., 2007) and repeated camera trapping censuses (Laass 2001; Zimmermann et al., 2011, 2013; Pesenti and Zimmermann, 2013). The study area expands over 1424 km^2 and includes the Simmental, Diemtigtal and Saanenland in the Bernese Oberland, as well as the Pays d'Enhaut, the Haute Gruyère and the Jauntal in the pre-Alps of the cantons Vaud and Fribourg (Zimmermann et al., 2012a,b). During the camera trapping census in winter 2011/12, lynx density in the study area was estimated at 2.13 (1.73–2.53, 95% confidence interval) independent (subadult and adult) lynx/100 km^2 of suitable habitat (95.3% of total study area; Zimmermann et al., 2012a,b).

Marking sites were found along trails and forest roads frequently used by lynx and were identified either during snow tracking, radio tracking or while choosing sites for camera trapping censuses. Scent-marks are usually placed on visually conspicuous objects, where lynx hair can be found and urine marks can be smelled even by humans. The marked objects included wood piles, (cut) tree trunks, rocks, small spruce trees and the corner of a wooden shed. From December 2009 to July 2012, we observed a total of 22 marking sites by means of camera trapping (Fig. 1). Observation periods for different marking sites ranged from 4 months to 2.5

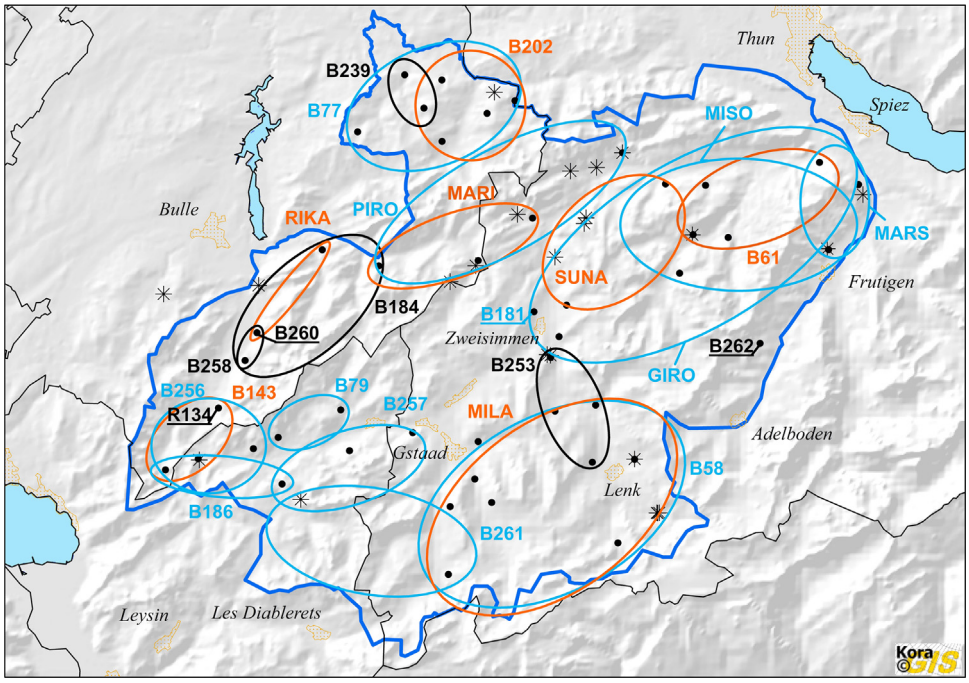

Fig. 1. Location of observed marking sites in relation to approximate lynx home ranges in the study area. Black lines—canton borders. Blue line—study area (1424 km^2). Black stars—observed marking sites. Black dots—sites where lynx were pictured during the photographic capture recapture census in winter 2011/12. Ellipses—approximate lynx home ranges according to camera trapping data (light blue—males, orange—females, black—sex unknown). Names of lynx are given next to the ellipses around all the sites where they were pictured. Names of lynx pictured only at a single site are underlined. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

years. 4 to 14 camera traps were operating at the same time. Each camera trap was adjusted to observe one marking site, that is one repeatedly marked object.

We used Reconyx PC90 and RC55 (Reconyx, Inc., Wisconsin) infrared camera traps set to take 10 pictures at each trigger and no delay between triggers. As opposed to infrared video camera models available at the time, these cameras had a fast trigger speed and image quality of at least one of multiple pictures was sufficient to recognize individuals by their fur patterns. Picture interval within one picture series ranged from 1 to 7 s under field conditions in our study. We changed batteries and SD-cards every two weeks in winter and once a month in summer and checked during each control visit whether the camera was still working. Individual lynx were identified by their unique coat pattern using the picture library of continuous camera trapping censuses and captures in the study area (Zimmermann et al., 2012a,b). Sex can be identified if testes are visible or when females are photographed together with their young. We considered a lynx a resident if it was photographed as an adult at overlapping camera trapping sites within the same area for at least two consecutive years. Non-residents included: (1) known subadults (previously pictured as juveniles with their mother and now in their second year of life) pictured outside their mother's home range in the approximate home range (estimated from camera trapping census data) of another known resident (2) previously unknown individuals, which were detected during only one year and subsequently vanished or settled somewhere else. Juveniles were cubs still following their mother. If none of the above criteria were applicable for an individual lynx, it was assigned unknown status. For each lynx visit to a marking site, we recorded date and time, observed behavioural patterns, identity, sex and residence status (resident, non-resident, dependent juvenile, unknown) of the visiting lynx. Over-marking was defined as scent-marking on the same area of an object (i.e. same corner of wood pile), including scent-marking on top, touching, as well as adjacent to the previous mark. We reconstructed the history of lynx visits and marking events for each site in order to generate a new parameter termed 'over-marking sequence'. For this parameter, we identified the donors of the two most recent scent-marks (top-scent-mark and underlying scent-mark) in a mixture of scent-marks encountered by an investigating lynx at a given marking site, thereby ignoring any older marks. In order to describe whether the underlying and the top-scent-mark had been left by the investigator on previous visits or by another individual, we established the following categories: ss, so, os, oo (s = scent-mark left by the investigating lynx, o = scent-mark left by another individual). Age of the most recent scent-mark was also recorded (days passed from the moment the most recent scent-mark was left until the investigating lynx was pictured).

1.1. Ethical note

We collected all data presented in this study by means of non-invasive methods (infrared camera traps). Furthermore, we could use additional knowledge gained from radio-collared lynx regarding information on their sex, residence status, location of home range borders and frequently used travel routes. In the study area, 12 lynx were radio-collared for a research project on lynx demography, genetics and predation (project by KORA; Carnivore Biology and Wildlife Management, www.kora.ch). As part of this larger project and survey of the lynx population, individual lynx were captured following established protocols (described in Breitenmoser et al., 1993; Ryser-Degiorgis et al., 2002; Ryser et al., 2005; Zimmermann et al., 2005). Lynx were equipped with GPS/GSM tracking units containing a break-off device allowing the unit to drop off after 1–2 years (GPS Plus Mini-1 C collars, Vectronic Aerospace GmbH, Berlin, Germany; Wild Cell SL/SD GPS–GSM collars, LoTek wireless, Ontario, Canada). KORA holds all necessary permits required according to Swiss legislation for using the described capture systems, capturing, immobilising, and radio-tagging lynx (capture permits from the Federal Office for the

Environment: Bewilligung_KORA_Luchsfang_BE_2010/2011/2006-03219/02/05/03, Bewilligung_KORA_Luchsfang_Kompartimente I, III und IV_2011–2015; animal experimentation permit from the Animal Welfare Commission of the Office for Agriculture and Nature of the Canton of Bern: 109/10).

1.2. Statistical analysis

To analyse the factors influencing marking activity and over-marking, we used generalized linear mixed models (GLMMs) fitted by maximum likelihood. We recorded a total number of 338 lynx observations but sample sizes in statistical tests vary depending on the kind of comparison carried out. We used a Wilcoxon each pair test to investigate changes in marking activity among different years. Fisher's exact tests were used for comparisons between sexes. We did not carry out quantitative comparisons between individuals of different residence status because most of our observations came from resident adult lynx. All calculations were performed in R (ver. 2.15.2, R Core Team 2012) and JMP Pro (ver. 10.0.1, SAS Institute Inc. 2012).

1.2.1. Seasonal patterns

For the analysis of seasonal variation in marking activity, we only used data from two periods of comparable length (15th of December to 14th of July 2010/11 and 2011/12). We calculated marking activity as the number of lynx scent-marking events/100 trap nights for each site per monthly period ($N = 138$). At two marking sites, boxtraps were set up for lynx captures during the course of the study and we did not consider these sites for analysis of seasonal changes in marking activity from the moment the boxtrap was in place. Each monthly period started at the 15th in order to match the different stages of the lynx life cycle based on reproductive season of females. Reproductive season was characterized as follows: 1 = pre-mating season (15/12–14/02), 2 = mating season (15/02–14/04), 3 = pregnancy (15/04–14/06), 4 = birth/denning (15/06–14/08), 5 = small cubs (15/08–14/10), 6 = big cubs (15/10–14/12) (adapted from Breitenmoser and Breitenmoser-Würsten, 2008). We calculated a GLMM with marking activity as the response variable, a Poisson error distribution and a log link function. We included reproductive season as fixed factor, year and marking site ID as random factors and an interaction term between reproductive season and year.

1.2.2. Over-marking

Due to low sample size of female lynx, we used only data from adult male lynx of resident or unknown status for the analysis of the factors influencing over-marking. We excluded all observations where it was impossible to determine whether a lynx over-marked or did not over-mark an already existing scent-mark, resulting in a final number of scent-marking events of $N = 128$. We calculated a GLMM with over-marking as the binary response variable, a binomial error distribution and a logit link function. We included lynx identity and marking site ID as random factors and over-marking sequence (origin of the the underlying and the top-scent-mark in a mixture of scent-marks encountered by the investigating lynx: ss, so, os, oo; s = scent-mark left by investigating lynx, o = scent-mark left by another individual), age of the most recent scent-mark (log-transformed) and reproductive season as fixed factors.

2. Results

We observed marking sites during a total of 8349 trap nights. Camera traps worked in 91% of all potential trap nights (number of trap nights cameras were working/total number of trap nights). 40% of picture series were shot with intervals of 2 s or less, 57% with intervals of 3–5 s, and 3% with intervals of >5 s. We recorded 338 lynx visits and identified 40 individual lynx (19 males, 10 females, 11 unknown) over the course of the study. The number of different lynx observed at any marking site ranged from 1 to 5. The median number of observations per lynx was 30 (ranging from 1 to 67). The animals visiting marking sites most often were males (244 observations). Female lynx were observed less often (47 observations) and for 47 observations, we could not identify the sex of the lynx. 242 observations came from adult resident lynx, 16 from dependent juveniles visiting a site together with their mothers, and only 9 from known non-residents. For 71 observations we could not establish the residence status of the lynx. From 2009 to late autumn 2011, territory ownership relations were stable and only 7% of observations belonged to lynx of unknown residence status. In winter 2011/12, however, there was a high population turn-over. Eight known residents died or vanished and 11 new individuals appeared in the study area (Table 1). As a consequence, we could not clearly define the residence status of the lynx for 28% of the observations from this time to the end of the study.

2.1. Behaviour, sex and status differences

Scent-marking of both male and female adult lynx is typically a behavioural sequence beginning with sniffing the object to be marked, followed by rubbing of cheeks and neck, and concluded by urine spraying. Head rubbing normally occurred above the spot that was marked with urine, but at the same time the neck and shoulders brushed the place where the urine mark was left and hairs could be found there (K. Vogt, pers. obs.). The duration of a typical scent-marking sequence was about 20 s but the sequence may also be repeated one or more times right on top or adjacent to the previous mark. Certain individuals spent up to several minutes in front of the camera. Such events of multiple marking ($N = 27$) accounted for 14% of all marking events. Multiple marking was displayed mostly by males (residents and non-residents) before and during the mating season ($N = 20$).

67% ($N = 193$) of all observed behavioural sequences involved scent-marking by means of urine-spraying and/or head rubbing or in four cases also elimination (defecation/urination) on the ground. Lynx passed by without any apparent behaviour directed towards the marking site in 30% of all cases ($N = 86$). Sniffing of the site without marking was observed in 2.5% of cases ($N = 7$). When comparing the behaviour of adult lynx of resident or unknown residence status, we found differences among the sexes: males scent-marked significantly more often than females, which passed by marking sites without marking relatively more often (Fisher's Exact Test: $N = 267$, $P < 0.001$). Contrary to our expectation, we found non-resident lynx scent-marking during five out of nine visits at marking sites usually visited by residents. Non-resident males accounted for seven of the visits at marking sites and in two cases the sex of the non-resident was unknown. Non-resident lynx visited marking sites throughout the year but all marking events occurred during the pre-mating and the mating season. Juvenile lynx still following their mother never marked. They often simply passed by ($N = 12$) but they also sometimes sniffed on the scent-mark left by their mother ($N = 4$).

2.2. Seasonal patterns

The relationship between reproductive season and marking activity was highly significant (Table 2). Marking activity showed a peak during the mating season (mid-February to mid-April) and was lowest during the denning period, when females gave birth and lactated (mid-May to mid-July, Fig. 2). There was no significant correlation between marking activity and camera trapping effort (realized trap nights/potential trap nights) (Spearman's

Table 1
Changes in population structure of resident lynx from 2008 to 2012. Rows show the presence of all lynx pictured at marking sites in the study area. Juveniles and known non-residents are excluded. Dark grey—pictured in this study, grey—detected during photographic capture recapture censuses or by chance photographs, light grey—not detected, but was present before and after this study period in the study area, white—not detected. Photographic capture recapture censuses in the study area took place in the winters 2009/10 and 2011/12. Summer—May–October, winter—November–April. Lynx names with numbers—animals known from camera trapping, 4-letter names—lynx was radio-collared. F—female, m—male, u—sex unknown. Born—born in this summer, juv—juvenile, X—resident, non—non-resident, ?—residence status unknown, †—reported dead, (†)—presumably dead (orphaned cub found).

Lynx Name	Sex	2008/09 winter	2009 summer	2009/10 winter	2010 summer	2010/11 winter	2011 summer	2011/12 winter	2012 summer
B53	f	X	X	X					
B107	m	X	X	X					
B61	f	X	X	X	X	X	X	X	X
PIRO	m	X	X	X	X	X	X	X	X
B58	m	X	X	X	X	X	X	X	X
MILA	f	X	X	X	X	X	X	X	X
B144	m	X	X	X	X	X	X	X	X
B189	f			X	X	X	X	X	X
GIRO	m			X	X	X	X	X	X
B202	f			X	X	X	X	X	X
MARI	f		born	juv	?	X	X	X	X
B177	m	X	X	X	X	X	X	X	X
B77	m	X	X	X	X	X	X	X	
SIBO	m	X	X	X	X	X	X	†	
B103	m	X	X	X	X	X	X	†	
NERO	m	X	X	X	X	X	X	†	
B129	f	X	X	X	X	X	(†)		
B94	m	X	X	X	X	X	X		
B106	f	X	X	X	X	X			
B261	m				born	juv	?	?	
EYWA	f						born	juv	?
SUNA	f							?	?
B253	u							?	?
B256	m							?	?
B257	m							†	
B300	u								?
B294	f							?	
B140	u	?	?	?	?	?	?	?	?
MARS	m							?	†
MISO	m						non	non	X
B279	u					non	?	?	X

rank correlation: $rs = 0.047$, $N = 138$, $P = 0.585$) indicating that changes in the number of observations were not due to variations in camera trapping effort or technical problems associated with season.

Seasonal patterns in lynx marking activity did not change before and after the high population turn-over in winter 2011/2012 (Wilcoxon each pair test: $N = 142$, $Z = 0.627$, $P = 0.531$).

2.3. Over-marking

Both male and female lynx over-marked their own old scent-marks as well as scent-marks left by other individuals. Female lynx tended to over-mark more often during the pre-mating and mating season (12 out of 27 visits) than before or after (3 out of 18 visits) (Fisher's Exact Test: $N = 45$, $P = 0.06$). In all cases where the origin of the underlying scent-mark was known they only over-marked the scent-marks left by the resident male ($N = 12$) or their own scent-mark ($N = 1$). Each marking site was visited by only one confirmed female lynx. New female lynx appeared at two marking sites previously used by other resident females only after the death of those two females in autumn 2011. We only included data of adult male lynx of resident or unknown residence status ($N = 128$) in the statistical analysis of over-marking due to low sample size of female observations. The relationship between over-marking sequence and rate of over-marking in male lynx was significant in the GLMM (Table 3).

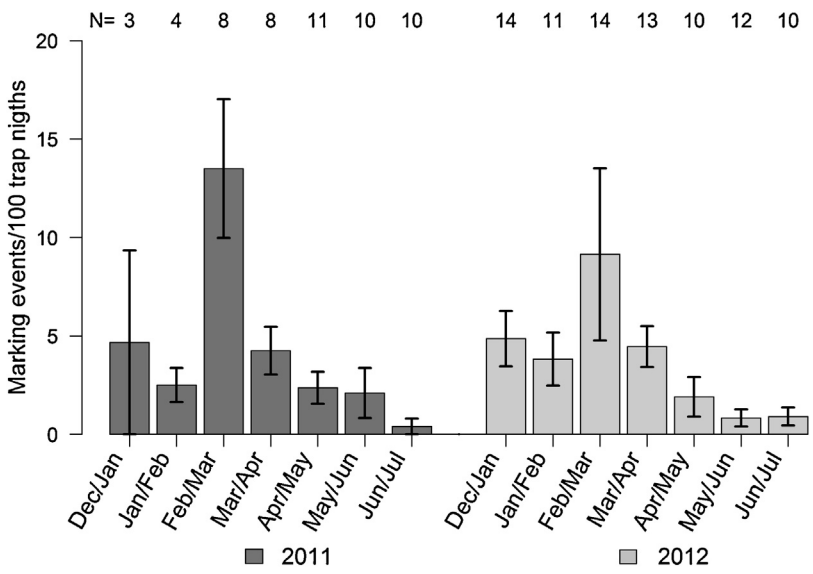

Fig. 2. Seasonal activity at lynx marking sites. Changes in mean number of marking events/100 camera trap nights are shown for two study periods of comparable length from 15th of December to 14th of July 2010/11 and 2011/12, respectively. Values were calculated for each marking site for monthly periods starting at day 15. Whiskers show standard errors. *N*—number of camera traps operating during each monthly period.

Table 2
Generalized linear mixed model on the effect of lynx reproductive season on marking activity.

	Estimate	SE	*z*-Value	*P*
Reproductive season	−0.519	0.055	−9.442	**<0.001**

Data were fitted by maximum likelihood to a poisson distribution with log-link function and a response variable (count data) indicating the number of lynx marking events/100 trap nights for each marking site per monthly period. The analysis was conducted on 138 mean monthly values for 20 marking sites. We used data from 15th of December to 14th of July 2010/11 and 2011/12, respectively. Each monthly period started at the 15th in order to match the different stages of the lynx life cycle based on reproductive season of females. Year (estimated variance component = 0.031, SD = 0.175), marking site ID (estimated variance component = 0.753, SD = 0.868) and an interaction term between reproductive season and year (estimated variance component = 0.074, SD = 0.273) were included as random effects.

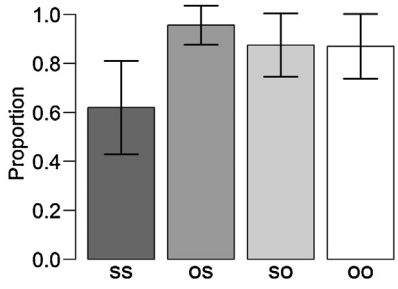

Fig. 3. Effect of over-marking sequence on rate of over-marking in male lynx. Over-marking sequence categories describe the origins of the top- and the underlying scent-mark in a mixture of scent-marks encountered by an investigating lynx. o—scent-mark left by other individual, s—scent-mark left by the investigator. Bars show the proportion of scent-mark mixtures which were over-marked. Whiskers show Confidence Intervals.

When male lynx encountered another individual's scent-mark, either as top- or underlying scent-mark in a mixture of scent-marks they investigated, they were more likely to over-mark. They over-marked significantly less often when they encountered a marking site where the last two scent-marks had been left by themselves (Fig. 3). Males over-marked the scent-marks of other lynx of both sexes throughout the year but over-marking occurred most often during the mating season and reproductive season was significant in the GLMM. In contrast, age of the most recent scent-mark had no influence on the rate of over-marking of male lynx, indicating that lynx did not over-mark older scent-marks more readily than fresher ones (Table 3).

Table 3
Generalized linear mixed model on the factors affecting the probability that adult male lynx over-mark encountered scent-mark mixtures.

	Estimate	SE	*z*-Value	*P*
Intercept	1.252	0.781	1.602	0.109
Over-marking sequence	0.574	0.236	2.426	**0.015**
Reproductive season	−0.483	0.229	-2.107	**0.035**
Age of scent-mark	0.014	0.011	1.219	0.223

Data were fitted by maximum likelihood to a binomial distribution with logit-link function and binary response variable (1 or 0) indicating whether an individual over-marked existing scent-mark mixtures. The analysis was conducted on data from 128 observations of adult male lynx of resident or unknown residence status. Lynx identity (estimated variance component = 0.220, SD = 0.469) and marking site ID (estimated variance component = 2.888e − 10, SD = 1.699e − 05) were included as random effects.

3. Discussion

The first aim of this study was to describe the scent-marking behaviour of Eurasian lynx at natural marking sites. We found that adult lynx of both sexes scent-marked but males visited marking sites more often and were also more likely to mark at any given visit. Dependent juveniles, however, were never observed marking, although they sometimes investigated the scent marks left by their mother. Our observations of the typical behavioural sequence at marking sites suggest that lynx leave messages in two different ways when-scent marking: one from saliva and/or cutaneous facial glands and one from urine marks. Especially the urine mark is very long-lasting and can be perceived even by a human for up to three weeks (pers. obs.). The combined information from body odour and urine mark when both are present might enable another individual to associate a lynx's urine mark directly to its owner upon a direct encounter ('scent matching', Gosling, 1982). Lynx sometimes repeat the marking sequence and rub adjacent to or right on top of their own fresh scent-marks (Hucht-Ciorga, 1988; Burmester, 2005; this study) but the function of this behaviour is still unclear. One explanation could be that brushing their neck and forequarters against the place where they previously left the urine spray

further enhances the association between urine mark and body odour. We also predicted that there is an 'expiry date' to scent-marks and that lynx would refresh older scent-marks more readily than newer ones. However, we could not confirm such a relationship. The most costly part of scent-marking is probably the time spent visiting a marking site and not the marking fluid itself. It may correspondinlgy pay for a lynx checking a marking site to renew its scent-marks irrespective of the time since the last marking.

The second aim of this study was to investigate the role of scent-marking in the social organisation of Eurasian lynx populations. We expected it to serve as a means for communication among resident males and females and for competition among neighbouring territory owners and non-residents. Our study is observational without experimental manipulation of over-marking and we cannot fully disentangle the different hypotheses for the behavioural function of over-marking. Nevertheless, our detailed observations of interactions in a wild population shed light on possible roles of the mate attraction hypothesis, the competition hypothesis, the chemical bulletin board hypothesis, and the territoriality hypothesis.

Our results are in line with our first prediction that both sexes scent-mark most frequently during the mating season (February–April). This has also been observed in other felid species (Mellen, 1993) and supports the hypothesis that over-marking may serve as a means for attracting mates. Eurasian lynx are thought to be monoestrous with induced ovulation (Kvam, 1990), although replacement litters are known to occur when the first litter is lost (Breitenmoser-Würsten et al., 2007). It is therefore crucial for the male to find and monopolise the female when she is ready to mate and receptive females could facilitate this by increasing their marking frequency. Indeed, females tended to over-mark most often during the pre-mating and mating season. Although male lynx typically encompass the entire home ranges of one or two females, extra-territorial excursions occur during the mating season (Breitenmoser and Breitenmoser-Würsten, 2008) and we could document one case of a resident lynx siring the offspring of his neighbour's female (Breitenmoser-Würsten, unpublished results). There is hence the potential of a certain amount of female choice and female over-marks could serve as an invitation to mate to high quality males. In our study, we only observed females over-marking the scent-marks of resident males. In turn, resident males who regularly use all parts of their home ranges are able to frequently over-mark scent-marks of both possible mates and same-sex competitors. In several rodent species, females prefer males which come out 'on top' of over-marking events, e.g. which have left the most recent scent-mark (Johnston et al., 1994, 1997; Ferkin, 1999).

Jordan et al. (2011b) found no evidence of female choice in banded mongoose but their results supported the competition hypothesis: higher over-marking scores by males were related to higher mating success through male–male competition. Males with the highest over-marking scores were also in good body condition and were able to mate-guard females at a younger age (Jordan et al., 2011b). One of the predictions of the competition hypothesis is that after inspecting an over-mark, individuals should respond more favourably to the top-scent donor's marks than to the bottom-scent donor's marks (e.g. spend more time investigating, place their own scent-mark in the vicinity; Rich and Hurst, 1999; Ferkin and Pierce, 2007). One explanation for this could be that the bottom-scent-mark is masked by the top-scent-mark. In our study, we could not find any evidence for scent-masking. As we expected, encountering another individual's top-scent-mark triggered over-marking in adult male lynx. Interestingly, the over-marking behaviour occurred at a similar rate when lynx encountered another individual's scent-mark which they had already over-marked during their last visit. Only when male lynx encountered a marking site where the last two scent-marks had been left by themselves did they over-mark less often. Another prediction of the competition hypothesis is that scent-marks of same-sex conspecifics should be over-marked more often than those of opposite-sex conspecifics. Although both sexes over-marked, most of the interactions at the observed marking sites took place between adult male lynx and we never observed more than one female per site. For our observations we chose most marking sites in zones of overlap between home ranges of neighbouring lynx, where we had photographed several different individuals during previous camera trapping censuses. From earlier telemetry studies we know that male and female home ranges can overlap in the same areas (Breitenmoser and Breitenmoser-Würsten, 2008). However, female lynx may mark more often in core areas where they spend most of the time ('hinterland marking', Gosling and Roberts, 2001b; Wyatt, 2003) as has also been observed by Hucht-Ciorga (1988) for lynx in the Bavarian Forest.

According to the 'chemical bulletin board' hypothesis, individuals should place their scent-marks along paths frequently travelled by conspecifics or on prominent features in a commonly used area (Ferkin and Pierce, 2007). The marking sites we observed match this description: prominent structures such as woodpiles located along travel routes frequently used by several individuals in areas where home ranges overlap. Although these sites only represent part of a lynx's scent-marking activity throughout its home range (Hucht-Ciorga, 1988), their regular use by different individuals still suggests a high importance for communication at least in male lynx. On a 'chemical bulletin board', no scent-masking occurs and both the information of the bottom- and the top-scent-mark remains available, allowing all scent-donors to advertise their presence. We found that male lynx responded similarly to the presence of another individual's scent-mark, whether it was the top- or the underlying scent-mark in a mixture of scent-marks they encountered. Only when both top- and underlying scent-mark were left by the investigator himself, the likelihood of over-marking decreased independent of the time that had passed since the most recent scent-mark was left. It therefore seems likely that communal marking sites in Eurasian lynx could serve as 'chemical bulletin boards'.

We also expected over-marking to serve as a means for competition among neighbouring territory owners and non-residents as predicted by the territoriality hypothesis. The 'dear enemy phenomenon' (Temeles, 1994) could potentially apply to the social structure of Eurasian lynx, as it is likely to be the most efficient for neighbouring territory owners to restrict aggression among themselves to a minimum and instead invest it in fending off unknown intruders. Indeed, only few territorial fights among Eurasian lynx have been reported in Scandinavia and most resulted in successful displacement of the resident (Mattisson et al., 2012). The rare observations of territorial fights in our study area always involved a subadult (non-resident) and an adult resident lynx (Breitenmoser and Breitenmoser-Würsten, 2008). In the present study, neighbouring resident male lynx over-mark each other's scent-marks as well as the scent-marks of non-residents throughout the year. Contrary to our prediction that non-resident lynx should refrain from sent-marking, we observed five cases in which non-residents scent-marked during the mating season in the same way residents would. These observations suggest that marking sites could serve as 'chemical bulletin boards' not only for resident territory owners, but also for transient non-residents.

Taking into account that social systems are dynamic and wild animals face changing conditions, it is conceivable that the functions of over-marking in natural populations may also depend at least partly on changes in population structure. During the course of our study, a marked disturbance in the social and spatial organisation of the lynx population occurred. Several resident lynx died or vanished and were replaced by new individuals in winter 2011/12, which allowed us to test if seasonal patterns in marking activity may be related to the population turn-over. However, there were

no statistically detectable changes in lynx marking activity before and after the disturbance. The mechanisms of how lynx determine which territories are vacant and how communication by means of scent-marking influences the outcome of direct encounters are not yet understood. Research on scent-marking in a social context has so far focused more on group living species and less is known about the social interactions among solitary species. The combination of methods from the field of movement ecology with observations of social behaviour by means of camera or video traps could allow for interesting new insights into the social organisation of wild populations of wide-ranging, solitary carnivores such as large cats.

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Chapter 2

Suitability of GPS-telemetry for studying the predation of Eurasian lynx on small and medium-sized mammals in the Northwestern Swiss Alps

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In preparation

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Suitability of GPS-telemetry for studying the predation of Eurasian lynx on small and medium-sized mammals in the Northwestern Swiss Alps

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Abstract

Predator diet composition and kill rates have to be known in order to understand the significance of predation. While ground-truthing of GPS location clusters (GLCs) is a reliable method for finding large and medium-sized prey items, finding the remains of small prey is still considered a major difficulty. In this study, we searched GPS location clusters of Eurasian lynx *Lynx lynx* in the Northwestern Swiss Alps in order to determine if GLC analysis is a suitable method for detecting kill sites of new-born ungulates and other small mammals. We checked 935 GLCs of 12 GPS-collared lynx and found 492 kills. We found significantly more juvenile ungulates and small mammals (53% of all prey items) than in earlier VHF-telemetry studies conducted in our study area. Lynx spent significantly more time in GLCs containing large prey (> 10kg) but no clear cut-off duration for distinguishing between large and small prey was apparent. The majority of the kills (92%) were found in GLCs lasting ≥ 9 h and the longer the duration of a GLC, the more likely it was to find a kill. Most checks of GLCs < 9h were unsuccessful, no matter if the observer had a lot of experience, checked the GLC shortly after formation, or was accompanied by a trained dog. However, 17% of the small prey items were found in GLCs < 9h. Checking GLCs shortly after formation had a positive effect on search success in GLCs lasting ≥ 9 h and also increased the chances of finding enough prey remains to determine sex and age class of the prey species. We conclude that GLC analysis can be a potent tool for exploring the impact of predation on new-born ungulates, mesopredators, and other smaller mammals. However, since search success in short-lasting GLCs is not easy to predict, substantial field effort has to be invested.

Introduction

Understanding the impact of predation on prey populations is crucial for the conservation of carnivores across the globe, particularly when the predators are competing for prey with human hunters and conflicts need to be mitigated through management (Breitenmoser et al. 2010). Predator diet composition and kill rates are two main factors that researchers have to take into account in order to understand the significance of predation (Breitenmoser et al. 2010). In this regard, the advent of GPS telemetry as a new field technology has substantially advanced our understanding of movement and feeding behaviour of large carnivores and has improved systematic quantification of predation (Blecha & Alldredge 2015). Ground-truthing of GPS location clusters (GLCs) is now replacing VHF telemetry and other methods for finding large and medium-sized prey items in the field (Bacon et al. 2011). However, detecting the remains of small prey items is still considered a major difficulty, since less evidence of the predation event is left and the few remains may disappear fast because of different scavenger species (Krofel et al. 2013; Martins et al. 2011; Mattioli et al. 2011; Palacios & Mech 2011; Svoboda et al. 2013). Consequentially, both VHF and GPS telemetry studies are often biased towards medium and large-sized prey items (Bacon et al. 2011; Webb et al. 2008). As GPS technology advances, it allows obtaining movement data at previously unknown resolutions (Bacon et al. 2011; Martins et al. 2011; Matthews et al. 2013; Svoboda et al. 2013). In theory, it is now possible to detect the presence of a predator also in places where it spent only a limited amount of time, just few hours needed to consume a small prey. However, for many types of GPS collars, limitations of battery weight still don't permit usage of high localisation rhythms over extended periods of time for small to medium-sized predators. Therefore, in order to balance the number of GPS locations taken per day with the collar longevity needed to capture a representative period of the animal's lifetime (i.e. to calculate home range size), a compromise between collecting data and saving battery life is usually sought (e.g. in Blecha & Alldredge 2015; Krofel et al. 2013; Martins et al. 2011; Ruth et al. 2010).

Since the effort and time invested in the field to ground-truth GPS locations and find prey remains may be considerable, models have been developed to predict predation events and reduce time and resources required to obtain reliable kill estimates. This has been achieved for example for leopards *Panthera pardus* (Martins et al. 2010), cougars *Puma concolor* (Blecha & Alldredge 2015; Knopff et al. 2010), wolves *Canis lupus* (Webb et al. 2008), bobcats *Lynx rufus* (Svoboda et al. 2013) and Eurasian lynx *Lynx lynx* (Krofel et al. 2013). Important factors allowing for discrimination between kill sites and non-kill sites in predictive models were GLC duration, the timespan between GLC formation (i.e. presumed time of predation) and investigation, and the distance from the kill site to the closest night location (compared to the closest day location) (Krofel et al. 2013; Svoboda et al. 2013). For example, Krofel et al. (2013) found 99% of kills in GLCs with a duration longer than 30h

and observed that the distances from the kill site to the closest night location were more than 3 times smaller than the distances to the closest day location. Svoboda et al. (2013) found that 82% of kill sites were detected less than 7 days after cluster formation and suggested that researchers should investigate GLCs within seven days following GLC initiation whenever possible. Other factors, such as daytime of GLC formation, land cover, and ground-truthing error have also been taken into account, but were of less significance (Blecha & Alldredge 2015; Svoboda et al. 2013). Generally, the model algorithm efficiency of identifying kill sites for large prey was higher than for small prey (Knopff et al. 2010, Svoboda et al. 2013, Webb et al. 2008). For example, the top logistic regression models applied by Webb et al. (2008) to predict wolf kill sites correctly classified 100% of kills of large prey species, whereas for small prey species, 40% were classified as non-kills. Thus, predicting kill sites of small prey items, which have a shorter handling time, seems to be more difficult (Palacios & Mech 2011; Svoboda et al. 2013; Webb et al. 2008).

In this study, we searched GPS location clusters of Eurasian lynx in the Northwestern Swiss Alps, where lynx are efficient stalking predators of roe deer *Capreolus capreolus* and chamois *Rupicapra rupicapra* (Breitenmoser & Breitenmoser-Würsten 2008; Molinari-Jobin et al. 2002). They usually feed for several days on adult ungulate kills, hiding during the day and returning to the carcass each evening (Breitenmoser & Breitenmoser-Würsten 2008; Krofel et al. 2013; Molinari-Jobin et al. 2002; Nilsen et al. 2009). This quite predictable pattern has made the Eurasian lynx a suitable species for the application of GLC analysis (Krofel et al. 2013; Mattisson et al. 2011). However, part of the lynx' diet is made up of small prey items weighing < 10kg (e.g. brown and mountain hares *Lepus europaeus* and *L. timidus*, Alpine marmot *Marmota marmota*, red fox *Vulpes vulpes* and new-born ungulates, Molinari-Jobin et al. 2004). Since previous studies on lynx diet conducted in Switzerland were mostly based on VHF-telemetry (e.g. Breitenmoser et al. 2010; Haller & Breitenmoser 1987; Jobin et al. 2000; Molinari-Jobin et al. 2002, 2004), their results were likely biased towards larger prey. If new-born ungulates and other small prey items are overlooked, this may lead to an underestimation of kill rates and may influence how predation impact of Eurasian lynx on their prey populations is interpreted.

The objectives of our study were (i) to determine if GLC analysis is a suitable method for detecting kill sites of new-born ungulates and other small mammals killed by Eurasian lynx, (ii) to characterise GLCs containing large prey items versus small prey items in order to understand when and where small prey items are found and (iii) to optimize field effort and search success especially for GLCs with a low chance of finding prey remains.

Methods

Study area

The study area is situated in the Northwestern Swiss Alps, expands over approximately 1500 km² and includes parts of the Bernese Oberland and the pre-Alps of the cantons Vaud and Fribourg (Vogt et al. 2014; Zimmermann et al. 2012a,b). The landscape is composed of a mixture of forests, fragmented by pastures and human settlements, with a human density of about 42/km^2 on average (Swiss Federal Statistical Office 2015). Altitudes range up to more than 2000 m a.s.l. Mean monthly temperatures in Adelboden (1320 m a.s.l.) range from -1.7°C in January to 14.2°C in July (1981-2010, Federal Office of Meteorology & Climatology MeteoSwiss). Snow depth during winter varies from 0 to over 100 cm depending on elevation, exposure and year. The Eurasian lynx is the only widespread large carnivore species in the study area, with an estimated density of 2.05 (1.50-2.60, 95% confidence interval) independent (subadult and adult) lynx/100 km^2 of suitable habitat (95.3% of total study area; Zimmermann et al. 2014). Lynx main prey in the area are roe deer and chamois (Breitenmoser & Breitenmoser-Würsten 2008; Molinari-Jobin et al. 2002), whereas other possible prey species with an adult body weight of ≥ 1 kg are red fox, badger *Meles meles*, pine marten *Martes martes*, stone marten *M. foina*, brown and mountain hare, Alpine marmot, black grouse *Tetrao tetrix*, and hazel grouse *Tetrastes bonasia* (Molinari-Jobin et al. 2007). Red deer, Alpine ibex *Capra ibex*, wild boar *Sus scrofa,* and capercaillie *T. urogallus* occur only locally and in low numbers.

Lynx captures

Between 2012 and 2014, we captured and radio-collared 12 Eurasian lynx (7 males, 5 females) and recaptured 3 of them, following established standard protocols (described in Ryser-Degiorgis et al. 2002; Ryser et al. 2005; Zimmermann et al. 2005) and with all permits required according to Swiss legislation. Two trapping techniques were used, i.e. double-door box traps (3 captures) and foot snares (12 captures). Unbaited double-door box traps made from solid wood were placed on forest roads used by lynx. They were equipped with a GSM-based alarm system allowing for 24-hour monitoring. In case of an alarm, the box trap was controlled within 30 min to 1 hour by one capture team member or by local game wardens. Any non-target species were directly released and the rest of the capture team was alerted if a lynx was in the trap. Foot snares made from light aluminium hoops and 3mm wire cables were placed around fresh lynx kills and connected to an alarm system. The cables were passed through aluminium tubes equipped with long springs to avoid leg injuries. The capture team, consisting of several experienced field biologists and a trained wildlife veterinarian, was always able to reach the capture site within less than 15 minutes. Trapping systems were operated from November to April, in order to avoid capturing pregnant or lactating females or

small kittens. Three single animals were captured between late July and October, after the absence of kittens had been confirmed by camera traps set for one night at the kills where they were captured with foot snares the following night. All lynx caught were examined by a veterinarian. After release, we tightly monitored the movements of all lynx and searched GPS location clusters until we could confirm that they were hunting successfully.

Lynx were immobilized with 0.1-0.15 mg/kg medetomidine hydrochloride (Domitor®, Orion Corporation, Espoo, Finland) and 3.2-5.5 mg/kg ketamine hydrochloride (Ketasol®, Graeub, Switzerland). Atipamezole hydrochloride (0.56-0.77 mg/kg; Antisedan®, Orion Corporation, Espoo, Finland) was used as an antagonist for medetomidine and was injected at least 1 hour after the last ketamine injection in order to assure that ketamine had been fully metabolised (Ryser-Degiorgis et al. 2002). Each individual was equipped with a GPS/GSM tracking unit (GPS Plus Mini-1 C collars, Vectronic Aerospace GmbH, Berlin, Germany; Wild Cell SL/SD GPS-GSM collars, LoTek wireless, Ontario, Canada) weighing 250-300 g. Collars contained a break-off device allowing the unit to drop off after 1-2 years. None of the captured lynx died due to capture procedures or problems with the collar or showed any skin abrasions caused by the collar.

Data collection

Similar as in other studies on wild felids (i.e. Blecha & Alldredge 2015; Krofel et al. 2013; Martins et al. 2011; Ruth et al. 2010), GPS collars were programmed to record 7-8 GPS fixes per day. In the first weeks after capture, collars were set to take a location every 3 hours in order to monitor lynx activity throughout the day and ensure normal behaviour after captures. From May onwards, most collars were set to a rhythm with a higher resolution during twilight and night hours, when lynx are most likely to feed on their kills (02:00, 05:00, 14:00, 18:00, 20:00, 21:00, and 22:00 CET time). The collar of one 17 years old female lynx was programmed to record only 6 locations per day (00:00, 05:00, 11:00, 18:00, 20:00, and 22:00 CET time) in order to prolong battery life and increase the chance of monitoring her until the end of her life. All GPS locations were received via GSM network and data was downloaded from a ground station. Kill sites were located by searching GPS location clusters (GLCs), similar to the procedures described in Bartnick et al. (2013), Krofel et al. (2013), Lone et al. (2014), and Palacios and Mech (2001). GPS data of radio-collared lynx was downloaded daily before field work and GLCs were identified by visual inspection (see section GLC analysis) using Google Earth software (Version 7.1.5.1557). We attempted to search all GLCs from 1st January 2013 to 31st December 2014 for 4 focal lynx individuals (2 males, 2 females) in each year (*N*=7, 1 female followed during both years). For 5 additional animals (2 females, 3 males), we searched at least one “large” (> 3 GPS fixes) and one “small” (2-3 GPS fixes) GLC per month. GLCs were not checked if they were inaccessible due to extreme steepness or high risk of avalanches. If time constraints did not permit us

to check all GLCs of our focal lynx, we gave priority to those GLCs containing at least one night location (between 18:00 and 06:00 CET). Each selected GLC was examined as soon as possible with the timespan between GLC formation and investigation ranging between 0.5 and 40 days (median= 4 days). GPS locations within GLCs were searched using a handheld GPS (Etrex vista HCx, Garmin, Olathe, KS, USA) with a mean accuracy of 1.9m (± 0.4m SE) in our study area (calculated from 71 test waypoints). We searched the area within a radius of 30 m of each fix and zigzagged the area between fixes until we found prey remains or for a minimum of one man hour (i.e. one person searching for one hour or 2 people for 30 minutes), when possible using a trained dog (in 38% of all checked GLCs). Even when a kill was found, we continued checking all remaining fixes and the surrounding area in case there were additional prey remains or a second kill in the same GLC. In 46% of all cases, not all GPS fixes in a GLC were accessible (e.g. fix within a rock face). We checked GLCs after the lynx had left the area whenever possible, although lynx in our study area live in close proximity to humans and are known to be tolerant to human activities near their kills from earlier studies (Breitenmoser & Breitenmoser-Würsten 2008; Molinari-Jobin et al. 2002). All checked GPS locations were recorded. Kill sites were logged with a handheld GPS and distance to nearest fix, species, sex, age class, as well as found body parts were noted for each kill. Although lynx can occasionally eat carrion (Ray et al. 2014), we considered found prey remains as kills for the purpose of this study as long as they matched the age of the GLC.

Statistical analysis

GLC analysis

For statistical analysis, GLCs were automatically generated in R (version 3.1.0, R Development Core Team 2013) using an adaptation of the cluster algorithm developed by Svoboda et al. (2013). We defined a GLC as a set of at least 2 GPS fixes within a maximum distance of 100 m from the centroid of the GLC and with a maximum time span of 48h between consecutive fixes for GLCs containing only 2 GPS fixes and 72 h for GLCs containing 3 or more GPS fixes. We chose these timespans, because lynx sometimes move up to several kilometres away from kill sites and interrupt use of their kills for one to several days (Breitenmoser & Breitenmoser-Würsten 2008). In order to reduce pseudoreplication caused by associating multiple GLC's with the same kill site, we considered a GPS fix as part of the same GLC if a lynx returned into this GLC within three days. This method was validated by double-checking exemplary kill sites after lynx had returned from an excursion and by calculating the mean duration of all excursions from GLCs. According to Ray et al. (2014), scavengers in temperate ecosystems completely deplete ungulate carcasses within 10 days in summer, while carcasses last considerably longer in winter. For calculation of mean excursion durations, we

considered excursions where the lynx was outside the 100 m radius from the GLC for >12 hours and < 14 days, assuming that lynx could still expect to find consumable prey remains after this time. The mean excursion duration was 26.6 ± 53.0 h *SD* (*N*= 732). Thus, setting the maximum time span between consecutive fixes in a GLC to three days was considered appropriate. For GLCs containing only 2 fixes, the maximum time span of 48h was more suitable, since this allowed us to exclude "false GLCs", i.e. areas repeatedly travelled through by lynx, such as ridges or forest roads, where locations may be taken in close spatial proximity without the lynx actually being stationary there. We attributed kill sites to checked GLCs if they were not further away than 150 m from the GLC centroid. In 10 cases, kills were found in between 2 GLCs formed within the same time period and were consequentially attributed to both.

Suitability for finding new-born ungulates and small mammals

In order to determine the suitability of GLC analysis for finding new-born ungulates and other small mammals, we compared kill rates and the number of prey items in each prey category to the results of two earlier studies using VHF telemetry in our study area. Prey selection and kill rates in Eurasian lynx depend on sex and social status and especially females with kittens have higher reported kill rates during winter time (Jobin et al. 2000). The proportion of females with and without kittens was comparable for all three studies, so we pooled kill rates for females. Kill rates were calculated as the number of days between two consecutive predation events using data of the 7 focal lynx (3 females, 4 males) for which series of continuously checked GLCs between at least 2 consecutive kills were available. Fisher's exact and Wilcoxon signed rank tests were used for comparisons between studies.

Characteristics of GLCs containing large versus small prey items

We tested how prey size correlated with environmental factors (serving as proxies for accessibility of the GLC) and the time a lynx spent in a given GLC in a Generalized Linear Mixed Model (GLMM). The GLMM was fitted to the data from 494 GLCs assuming a binomial error distribution with a probit link function and using Maximum Likelihood (Laplace Approximation). Prey size (small/large) was set as the binary response variable. Ungulates > 5 months of age were considered as large prey, ungulates ≤ 5 months and non-ungulate species (e.g. red foxes, hares, marmots, birds, small rodents) were considered as small prey. Between May and September, the juveniles of both roe deer and chamois still weigh less than 10-15 kg (chamois (Canton of Bern, late autumn): < 11 kg, Wandeler & Huber 1969; roe deer (our study area, late August): mean = 10.6 ± 0.3 kg *SE*, *N* = 18, M. Pewsner, unpublished data). The following factors were included as fixed effects: elevation, terrain ruggedness, duration of the GLC, and study period (1 = January to April, 2 = May to August, 3 = September to December). Elevation was calculated from a digital elevation model (DEM) for

Switzerland with a grid cell size of 25 m (BFS GEOSTAT, http://www.geostat.admin.ch). To quantify terrain ruggedness, we calculated a Terrain Ruggedness Index (TRI, Riley et al. 1999) for each GLC centroid. TRI calculates the sum change in elevation between a grid cell and its eight neighbour grid cells (Riley et al. 1999). Duration of each GLC in hours was calculated as described in Appendix 1 and values were log-transformed to match a normal distribution. Values of the factor ruggedness were divided by 10 and values of elevation were divided by 100 to fit the scale of the other parameters in the model. Lynx identity and GLC ID were included as random effects in order to account for variation in prey size selection of individual lynx and to avoid pseudoreplication (20 GLCs contained 2 prey items). All statistical analyses were conducted in R (version 3.1.0, R Development Core Team 2013) and ArcGIS (ArcGIS 10.1 SP for Desktop, ©1999-2012 Esri Inc.).

Optimizing field effort and search success

In order to optimize field effort and search success, we investigated the chance of finding prey items in GLCs of different duration categories. We further analysed factors affecting search success, taking into account observer experience and presence or absence of a trained dog as proxies for detection error. We constructed four Generalised Linear Mixed Models for two GLC duration categories (duration ≤ 9 h, > 9 h) and for two categories of observer experience (field experience ≤ 6 months, > 6 months). GLC duration categories were analysed in separate models because models including both categories did not converge. The distribution of the factor observer experience was bimodal and, therefore, the two categories of observer experience were also analysed separately. The GLMM was fitted to the data from 964 GLCs assuming a binomial error distribution with a logit link function and using Maximum Likelihood (Laplace Approximation). Success (kill found/ not found) was set as binary response variable. The following factors were included as fixed effects: duration of the GLC, field experience of the observer, the timespan between GLC formation and search, as well as presence or absence of a trained dog. Duration of each GLC in hours was calculated as described in Appendix 1, field experience of the observer was calculated as the time between start of field work and date of GLC search in days, and timespan between GLC formation and search was calculated as the number of days between the dates of the first point in the GLC and the GLC search. All timespans were log-transformed to match a normal distribution. Lynx identity and GLC ID were included as random effects in order to account for variation among individual lynx and to avoid pseudoreplication (20 GLCs contained 2 prey items, 12 GLCs were checked twice by different observers). All statistical analyses were conducted in R (version 3.1.0, R Development Core Team 2013).

Results

The mean collar accuracy calculated from a subsample of 46 GPS locations from 4 collars was 8.8m (± 1.3m *SE*) in our study area and there were only few missing GPS locations (mean percentage of successful GPS fixes for all collars= 90%). The collars of the 12 study animals recorded 23'952 GPS fixes and we obtained 2599 GLCs (males, *N*= 1597; females, *N*= 1002). We checked 935 GLCs (males, *N*= 528, 56%; females, *N*= 407, 44%), 12 of which were checked twice by different observers. 20 of the GLCs (checked once or twice) contained 2 kills. We found 492 kills, 10 of which were attributed to 2 GLCs. Search effort was intensified from May to August (Table 1), during the period when young ungulates are born and still weigh less than 10-15kg (Wandeler & Huber 1969; M. Pewsner, unpublished data).

Table 1. Seasonal search effort from 1st January 2013 to 31st December 2014.

Study period	Formed GLCs	Checked GLCs	% checked	Found kills
1. January - April	998	250	25	149
2. May - August	905	441	50	210
3. September - December	696	244	35	133

Suitability for finding new-born ungulates and small mammals

In order to determine the suitability of our method for finding new-born ungulates and other small mammals such as red foxes, marmots or hares, we compared the number of prey items in each prey category found during our study with the prey spectrum of Eurasian lynx in our study area described in two earlier studies using VHF telemetry to find kills (Table 2). Juvenile ungulates made up 26% of the prey items found in our study, while 25% of the prey items were comprised by red foxes, hares and marmots (small mammals). Very rarely, even smaller prey items like grouse, squirrels or voles (2%, *N*= 9) were detected. We found significantly more juvenile ungulates and small mammals than in the NWA II study (juvenile ungulates= 19%; small mammals= 10%) conducted in the same study area from 1997-2001 (Table 2; Fisher's Exact Test, $N = 935$, $p < 0.01$).

Table 2. Prey spectrum of Eurasian lynx described in three telemetry studies conducted in the Northwestern Swiss Alps.

Study	chamois adult	chamois kids	chamois indet.	roe deer adult	roe deer fawns	roe deer indet.	fox	marmot	hare spp.	other prey
NWA III	86	86	28	81	42	29	29	40	53	18
NWA II	48	33	6	137	51	29	16	8	19	96
NWA I	-	-	38	-	-	63	0	2	9	10

GPS telemetry: NWA III (2013-2014, our study); VHF-Telemetry: NWA II (1997-2001, Breitenmoser & Breitenmoser-Würsten 2008, A. Ryser, unpublished data); NWA I (1983-1988, Breitenmoser & Breitenmoser-Würsten 2008). Adult ≥ 1 year, juvenile < 1 year, indet.= age not determined. Hare spp. = European brown hare and mountain hare. Other prey species include domestic animals (sheep, goats, cats), undetermined wild ungulates, and very rare prey items (e.g. rodents, birds, mustelids, Alpine ibex *Capra ibex*).

We also estimated kill rates for male and female lynx calculating the mean number of days between two consecutive predation events. Comparing our results to earlier studies using VHF-telemetry in the same study area, we found that our kill rates were considerably higher (Table 3). When only the timespan between two ungulate kills in winter was considered, the mean kill rate of males in our study was significantly higher than in the NWA I study (Wilcoxon signed rank test, N= 23, p= 0.010), while the mean kill rate of females was similar (Wilcoxon signed rank test, N= 31, p= 0.564).

Table 3. Mean number of days (± Standard Error) between 2 consecutive predation events (all prey items considered) for male and female lynx in the Northwestern Swiss Alps.

Study	lynx sex *(no. lynx)*	summer *(no. kills)*	winter *(no. kills)*	winter/ only ungulate kills *(no. kills)*
NWA III (2013-2014)	males *(4)*	2.9 ± 0.3 *(83)*	3.8 ± 0.4 *(28)*	4.1 ± 0.5 *(12)*
	females *(3)*	3.4 ± 0.3 *(83)*	3.4 ± 0.7 *(19)*	5.0 ± 0.7 *(7)*
NWA II (1999-2000)	males *(3)*	-	6.2 *(28)*	-
	females *(7)*	-	5.25 *(43)*	-
NWA I (1984-1985)	males *(2)*	8.6 ± 1.7 *(11)*	6.5 ± 1.0 *(14)*	7.1 ± 1.0 *(13)*
	females *(1)*	-	4.8 ± 0.9 *(30)*	5.4 ± 1.1 *(27)*

NWA I (Breitenmoser & Haller 1987), NWA II (A. Ryser, unpublished results). Summer = May - October, winter = November - April. All prey items considered.

Characteristics of GLCs containing large versus small prey items

We tested how prey size correlated with environmental factors (serving as proxies for accessibility of the GLC) and the time a lynx spent in a given GLC in a GLMM (Table 4). Lynx spent significantly more time in GLCs containing large prey. However, no clear cut-off duration for distinguishing between large and small prey was apparent (Figure 1). Ruggedness of the terrain was not related to prey size

but GLCs with large prey items were located at significantly lower elevations than GLCs containing small prey items. The correlation between prey size and elevation was mainly caused by juvenile chamois and marmots, which were found at higher elevations than other prey items (Figure 2). We also found a higher proportion of small prey items in the time period from May to August compared to the rest of the year, which also corresponded to the availability of new-born chamois and marmots during this period.

Table 4. Generalized linear mixed model (GLMM) on the factors affecting the probability of finding small (ungulates ≤ 5 months, non-ungulate prey) instead of large prey items (ungulates > 5 months) in searched GLCs.

Fixed effects	Estimate	*SE*	z-value	*P*
intercept	0.711	0.540	1.316	0.188
ruggedness index (TRI)	0.006	0.006	1.110	0.267
elevation	0.069	0.031	2.218	**0.027**
log(GLC duration)	-0.807	0.127	-6.331	**< 0.001**
study period 2	1.198	0.222	5.386	**< 0.001**
study period 3	0.383	0.205	1.869	0.062

The GLMM was fitted to the data assuming a binomial error distribution with a probit link function and using Maximum Likelihood (Laplace Approximation). Prey size (small/large) was set as binary response variable. Ungulate prey includes roe deer, chamois and livestock. Non-ungulate prey includes red foxes, hares, marmots, birds, rodents and one badger. *SE*= Standard Error. Study period 1= January to April, 2= May to August, 3= September to December. Study period levels are compared against study period 1. Values of the factor ruggedness were divided by 10 and values of elevation were divided by 100 to fit the scale of the other parameters. The analysis was conducted on 494 GLCs which could be attributed to kills with known prey size. Lynx identity (σ = 0.539) and GLC ID (σ= 0.115) were included as random effects. Significant p-values are indicated in bold script.

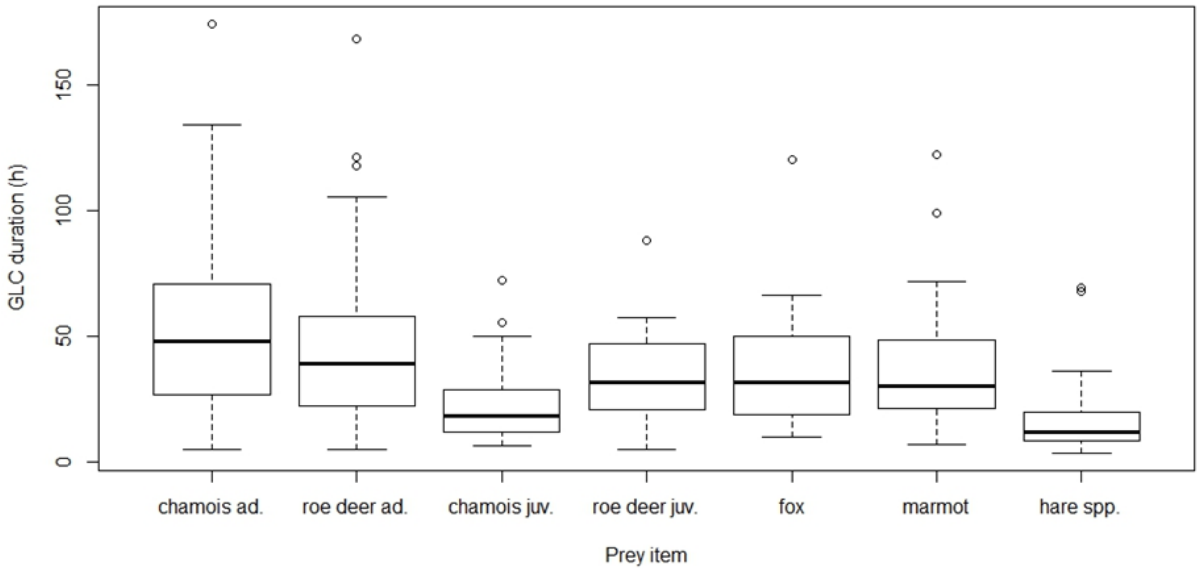

Figure 1. GLC duration for different prey types (*N*= 417 prey items). Ad.= adult (> 1 year), juv.= juvenile (< 1 year), hare spp.= European brown hare and mountain hare. Each box encompasses the 25th through 75th percentiles, with the median represented by an interior line. Whiskers denote maximum values or in case of outliers 1.5 times the interquartile range. Circles denote outliers.

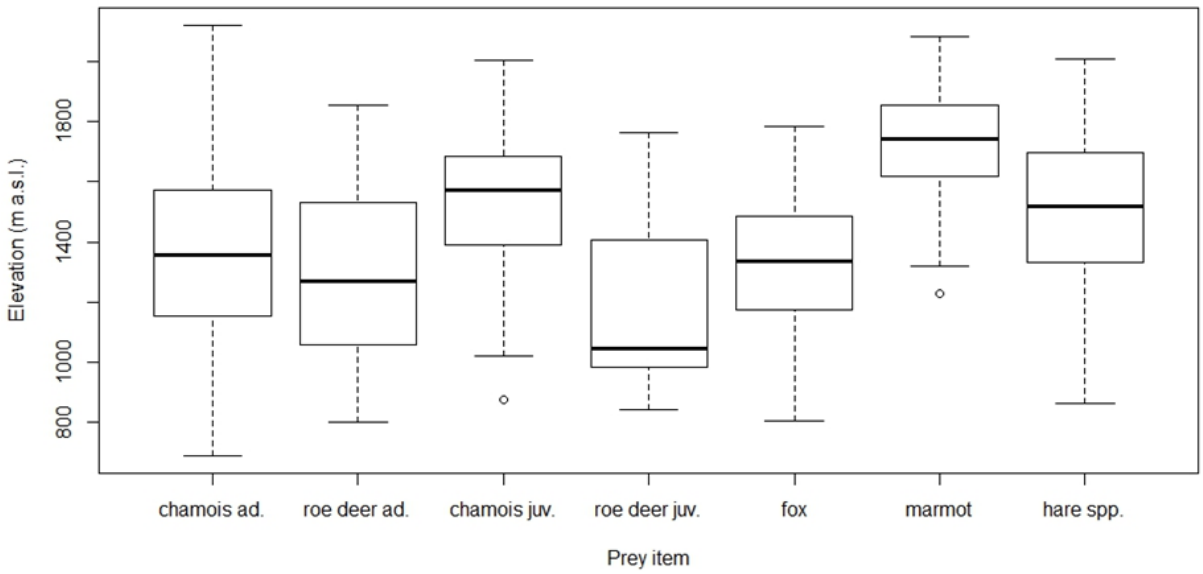

Figure 2. Elevation at GLC centroids for different prey types (*N*=417 prey items). Ad.= adult (> 1 year), juv.= juvenile (< 1 year), hare spp.= European brown hare and mountain hare. Each box encompasses the 25th through 75th percentiles, with the median represented by an interior line. Whiskers denote maximum values or in case of outliers 1.5 times the interquartile range. Circles denote outliers.

Optimizing field effort and search success

The proportion of GLCs in which we found prey remains ranged from < 10% for GLCs lasting < 6h to 100% for GLCs lasting between 60 and 72h (Figure 3). While the chances of finding prey remains strongly increased with GLC duration during the first 36h, GLCs lasting for > 36h had consistently high chances of containing prey remains (mean= 87%).

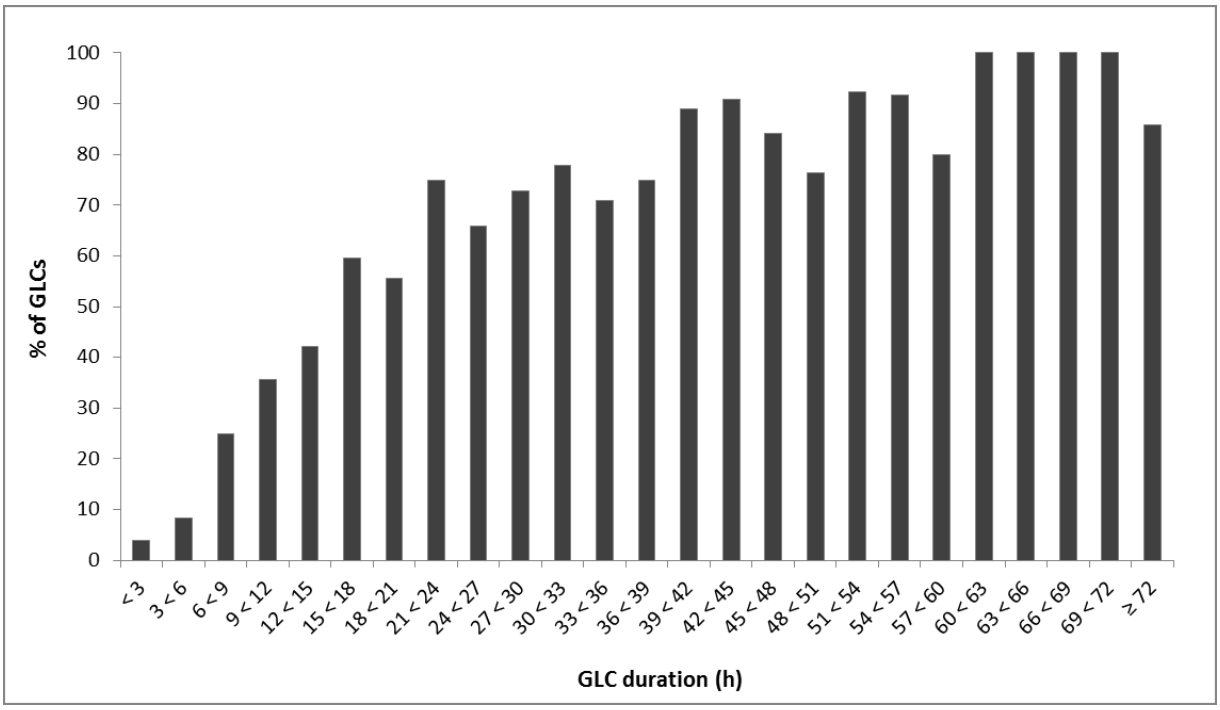

Figure 3. Proportion of GLCs containing prey remains in relation to GLC duration (*N*= 935).

In order to find an optimal method to balance invested field effort with the chances of missing (small) prey items and thereby biasing results of feeding studies, we investigated the number of found prey items in GLCs with ≤ 25% search success. We compared the categories of prey found in GLCs lasting < 9 h to GLCs lasting ≥ 9 h (Table 5). The majority of the kills (92%) were found in GLCs lasting ≥ 9 h. However, 17% of the small ungulates and other small prey items would have been lost, if short-lasting GLCs had not been checked.

Table 5. Prey categories found in short-lasting (< 9 h) and long-lasting (≥ 9 h) GLCs.

GLC duration	No. of kills	large ungulates	small ungulates	other small prey	prey size indet.
< 9 h	41	10	9	22	0
≥ 9 h	451	251	77	107	16
Total	492	261	86	129	16

Ungulates = chamois, roe deer and livestock (*N*= 5). Large ungulates > 5 months, small ungulates ≤ 5 months. Other small prey = red fox, marmot, European brown hare, mountain hare, and other rare small prey items (i.e. rodents, birds, mustelids, *N*= 11). Prey size indet.= ungulate prey where age class could not be determined.

We further investigated possible factors affecting search success and explored how our field protocol could be optimized for short-lasting GLCs with a low probability of finding prey remains. To this end, we constructed four GLMMs for both GLC duration categories and two categories of observer experience (Table 6).

Table 6. Generalized linear mixed models (GLMMs) on the factors affecting search success in short-lasting (< 9 h) and long-lasting (≥ 9 h) GLCs.

Short-lasting GLCs								
	Experienced personnel (N = 108)				*Unexperienced personnel (N = 148)*			
Fixed effects	Estimate	*SE*	z-value	*P*	Estimate	*SE*	z-value	*P*
intercept	-5.120	4.244	-1.207	0.228	-12.367	3.166	- 3.907	< 0.001
log(GLC duration)	0.753	0.856	0.879	0.379	4.441	1.117	3.978	**< 0.001**
log(obs. experience)	0.456	0.699	0.653	0.514	0.786	0.483	1.627	0.104
log(timelag formation)	-0.097	0.338	-0.289	0.773	-0.494	0.371	- 1.333	0.182
dog present	-1.161	0.584	-1.986	**0.047**	-0.301	0.844	-0.357	0.721
Random effects	GLC_ID: σ = 0, lynx_ID: σ = 0				GLC_ID: σ = 0, lynx_ID: σ = 0.381			
Long-lasting GLCs								
	Experienced personnel (N = 329)				*Unexperienced personnel (N = 379)*			
Fixed effects	Estimate	*SE*	z-value	*P*	Estimate	*SE*	z-value	*P*
intercept	-4.173	2.423	-1.723	0.085	-5.882	1.204	-4.886	< 0.001
log(GLC duration)	1.420	0.234	6.080	**< 0.001**	2.012	0.351	5.737	**< 0.001**
log(obs. experience)	0.087	0.388	0.224	0.823	0.260	0.137	1.903	0.057
log(timelag formation)	-0.421	0.173	-2.426	**0.015**	-0.622	0.218	-2.860	**0.004**
dog present	0.790	0.300	2.631	**0.009**	0.554	0.392	1.415	0.157
Random effects	GLC_ID: σ = 0, lynx_ID: σ = 0				GLC_ID: σ = 0.356, lynx_ID: σ = 0.004			

The GLMM was fitted to the data assuming a binomial error distribution with a logit link function and using Maximum Likelihood (Laplace Approximation). Success (kill found/ not found) was set as binary response variable. *SE*= Standard Error. Experienced personnel: ≥ 6 months of field experience. Unexperienced personnel: < 6 months of field experience. Significant p-values are indicated in bold script.

GLC duration was significantly correlated with search success in long-lasting GLCs as well as in short-lasting GLCs checked by unexperienced personnel. The latter finding reflected higher chances to find prey remains in GLCs with durations between 6 and 9 h as opposed to < 6 h (Figure 3). The number of field days of the observer did not play a role for personnel with more than 6 months of experience but there was a trend towards an increased search success with number of field days in unexperienced personnel. We found that new personnel reached the same search success in large GLCs as experienced personnel (71%) after 3 months of field experience (70%). The timespan between GLC formation and GLC control was not correlated to search success in short-lasting GLCs but was negatively correlated in long-lasting GLCs. The presence of a dog was only relevant for

experienced personnel, where it was negatively correlated to search success in short-lasting GLCs and positively correlated in long-lasting GLCs.

The timespan between GLC formation and GLC control was not only significantly related to search success, but was also important for the amount of information that could be inferred from detected prey remains. For all GLCs in which we found the remains of wild ungulates (*N*= 352), we noted whether the head of the prey animal was available for identification of sex and age class. GLCs in which the head of an ungulate could still be found were searched significantly earlier after their formation than GLCs in which we could not find the head of the prey animal anymore (Welch Two Sample t-test, t = 3. 671, df = 317.832, $p < 0.001$).

Discussion

In order to understand predator-prey systems and to evaluate the impact of predation on the prey population, studies on diet and kill rates of carnivores should also include predation on juvenile and alternative prey. Finding remains of small prey items < 10kg in the field is often considered as a major difficulty, as less evidence of the predation event is left and the few remains may disappear fast because of different scavenger species (Krofel et al. 2013; Martins et al. 2011; Mattioli et al. 2011; Palacios & Mech 2011; Svoboda et al. 2013). As a consequence, most studies based on VHF and GPS-telemetry are biased towards medium and large-sized prey items (Bacon et al. 2011; Webb et al. 2008). Scat analysis is considered to be better suited for detection of small prey items in the diet of carnivores, although other problems may arise using this method, such as difficulties in finding scats during summer, incorrect identification of the predator species leaving the scat, as well as incorrect identification of prey from scat samples (Bacon et al. 2011; Foran et al. 1997; Marucco et al. 2008).

The first objective of our study was to determine whether GLC analysis can be a suitable method for detecting kill sites of new-born ungulates and other small mammals. During our study, we rarely found prey items weighing ≤ 1kg such as black grouse *Tetrao tetrix*, hazel grouse *Tetrastes bonasia*, or red squirrels *Sciurus vulgaris*. While the remains of grouse are relatively easy to find, Eurasian lynx in Switzerland rarely prey upon them (Breitenmoser & Breitenmoser-Würsten et al. 2008). Smaller prey items like mice or voles are probably completely consumed in most cases, which makes scat analysis the only method to reliably detect their occurrence in lynx diet (Bacon et al. 2011; Krofel et al. 2011). For our analysis, we focused on juvenile ungulates and small mammals weighing between 2 and 10kg, i.e. new-born roe deer and chamois, red foxes, hares (European brown hare, mountain hare), and marmots. We found a higher proportion of juvenile ungulates and a more than twice as high proportion of small mammals in lynx diet than was reported from earlier VHF-telemetry studies conducted in the same study area (Breitenmoser & Breitenmoser-Würsten 2008). Consequentially, also our calculated kill rates were higher. This could either indicate that lynx

have potentially higher energy requirements than estimated in previous studies (Breitenmoser & Haller 1987; Jobin et al. 2000; Okarma et al. 1997) but could also reflect a change in lynx diet towards smaller prey occurring during the last decade in our study area. However, we would have expected a shift towards smaller prey to be more likely during the late 1990ies, when roe deer and chamois populations were thought to be declining while lynx densities were still high (Breitenmoser & Breitenmoser-Würsten 2008), as opposed to 2013-2014, when both lynx and wild ungulate densities were stable at a lower level (Federal Hunting Statistics 2015; Zimmermann et al. 2014). Moreover, when comparing only the mean number of days between kills of ungulates in winter, when juvenile ungulates approach adults in weight, our kill rates were similar (females) or even higher (males) than those reported in Breitenmoser & Haller (1987) considering only ungulate prey in winter. Thus, lynx in our study did not consume less large prey but we could detect additional small prey. Most studies on Eurasian lynx in Europe found that lynx kill an ungulate prey every 5-6 days (Andrén & Liberg et al. 2015; Jobin et al. 2000; Krofel et al. 2013; Okarma et al. 1997; Pedersen et al. 1999), as compared to every 4-5 days in our study during winter. However, our mean kill rate for male lynx in winter (considering only ungulate prey) was comparable to another study using GLC analysis to find lynx kills in areas with high reindeer densities in Scandinavia (Mattisson et al. 2011), although we found higher kill rates for females than reported in this study. It is possible that we mistook some incidents of scavenging for predation events when searching GLCs, which could have led to a slight overestimation of kill rates, as was recognized for cougars by Ruth et al. (2010). Although scavenging is considered to occur only rarely in Eurasian lynx (Breitenmoser & Breitenmoser-Würsten 2008), a recent study in Germany has documented frequent visits of lynx at layed-out carcasses (Ray et al. 2014). Future studies applying GLC analysis in combination with activity data, as was done for cougars in Williams et al. (2014) and Wang et al. (2015), could help to better identify true predation events and to shed more light on energy requirements and kill rates of Eurasian lynx living in different habitats with different prey communities.

The second objective of this study was to characterise GLCs containing large versus small prey items in order to understand when and where small prey items are found. Not surprisingly, the time a lynx spent in a GLC was a good indicator for prey size, although large prey items found in short-lasting GLCs did occur occasionally (Table 5) and there was no clear cut-off in GLC duration that would have allowed us to distinguish between large and small prey. Small prey was not found in more rugged terrain but at higher elevations than large prey and occurred more often in summer. In our pre-Alpine study area, there are many forest roads allowing easy access to high elevations in summer, which enabled us to track the lynx' utilization of juvenile chamois and marmots. In winter, however, many of these roads are not ploughed and accessing higher GLCs gets extremely time-consuming. Especially in remote study areas with few roads, short-lasting GLCs at high elevations

may be the first to be discarded as they require a huge effort while usually yielding a low search success. Even with a randomized sampling procedure, some GLCs may be discarded because they are inaccessible. Depending on the habitat requirements of potential prey species, prioritizing GLCs with certain characteristics may lead to an underestimation of predation impact. For example, killed chamois in our study area were found in more rugged terrain than roe deer (Welch Two Sample T-Test, t = 11.556, df = 364.74, p-value < 0.001) and extensive training of field personnel was necessary to ensure that such GLCs could be checked while security requirements were met.

The last objective of our study was to optimize field effort especially for short-lasting GLCs, which had ≤ 25% search success but still contained 17% of all small prey items. We found that the proportion of short-lasting GLCs containing prey items is generally low, although the chances of finding prey increased with GLC duration at least for unexperienced personnel. Even so, most checks of GLCs < 9h were unsuccessful, no matter if the observer had a lot of experience, checked the GLC shortly after formation, or was accompanied by a trained dog. Surprisingly, the presence of a dog actually had a negative influence on success rate in short-lasting GLCs, indicating that experienced observers without dog were more successful in finding prey in short-lasting GLCs than observers with dog. Especially under dry conditions in summer, it is possible that humans may be more efficient in visually locating dried out pieces of bone and hair, than dogs are olfactorily.

In long-lasting GLCs, GLC duration was the most important factor explaining search success. The longer the duration of a GLC, the more likely it was to find a kill, as previously shown by Martins et al. (2010), Palacios and Mech (2011), and Ruth et al. (2010). A short timelag between cluster formation and GLC search had a positive effect on search success, as has been shown in other recent studies (Blecha & Alldredge 2015; Svoboda et al. 2013), and also increased the chances of finding enough prey remains to still gain information on sex and age class of the prey species. Increasing field experience showed a positive trend in unexperienced personnel but was not related to search success of observers with more than 6 months of field experience. We found that the initial learning phase during which students and interns who are trained for field work still had to form a search image and learn how to navigate in difficult terrain lasted about 3 months. The presence of a trained dog had a positive effect, but was only significant in experienced personnel. This suggests that dogs are most efficient when deploying stable human-dog teams where the human observer knows how to read the dog. From our experience, we found the benefit of a trained dog most noticeable when prey remains had been scattered by scavengers and were located far from GPS fixes or when fresh snow had covered prey remains.

We conclude that GPS-telemetry provides a substantial improvement of the bias towards large prey items even for medium-sized predator species such as Eurasian lynx, for which the weight of the battery pack is still a limiting factor for the fix rates that can be acquired with GPS-collars

available to date. We could show that GLC analysis with a resolution of 7 fixes per day enabled us to find prey weighing between 2 and 10kg. Therefore, GPS location cluster analysis is a very potent tool for exploring the impact of predators on new-born ungulates and can help shed more light on energy requirements of large carnivores as well as on predation on mesopredators and other smaller mammals. However, since search success in short-lasting GLCs is not easy to predict, substantial field effort has to be invested and unsuccessful GLC searches have to be accepted. Discarding short-lasting GLCs in inaccessible terrain (i.e. GLCs formed at high elevations without road access) out of logistic reasons may indeed lead to an underestimation of small prey items in the diet of carnivores.

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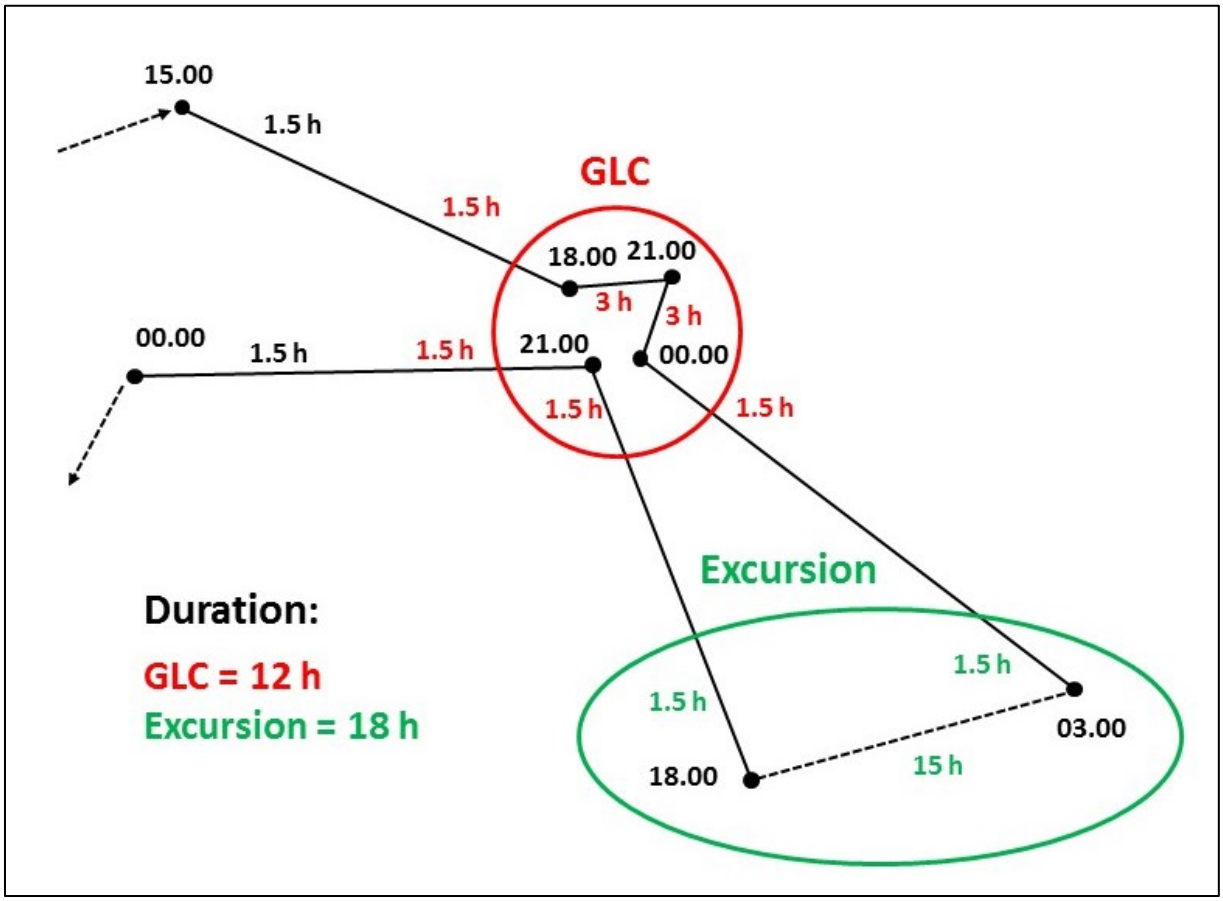
Appendix 1. Calculation of GLC duration.



The time differences between all fixes in a GLC were summed up, adding half the time difference to the next fixes outside the GLC. Excursions were excluded.

Chapter 3

Is there a trade-off between scent-marking and hunting behaviour in a stalking predator, the Eurasian lynx *Lynx lynx*?

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Animal Behaviour (2015), under revision

KV designed the study, conducted field work, analysed data and prepared the manuscript.

Is there a trade-off between scent-marking and hunting behaviour in a stalking predator, the Eurasian lynx *Lynx lynx*?

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Abstract

The costs of signalling are often expressed in terms of increased predation risk to the signaller; however, whether signalling predators also bear costs due to eavesdropping by prey and may attempt to reduce these costs is less well studied. In this study, we investigated whether there is a trade-off between intra-specific communication through scent-marking and the risk of alerting prey in a wild population of a stalking predator, the Eurasian lynx. We followed lynx-tracks in the snow and recorded scent-marks and evidence of hunting behaviour along these tracks. Lynx preferred conspicuous objects for marking and increased scent-marking rate when walking along linear structures, such as forest roads. This association was strongest when lynx were hunting, while there was only a weak correlation when no evidence of hunting could be detected. On tracks with evidence of hunting behaviour, lynx engaged less in scent-marking. The relationship was most evident during the mating season, when lynx increased scent-marking rates while they were not hunting. We further expected lynx to mark most in areas, where they had recently hunted successfully, but time and distance to the last kill were not associated with scent-marking rate. Our study supports the hypothesis that lynx face a trade-off between enhancing the detection probability of scent-marks by conspecifics and avoiding eavesdropping by prey, and indicates that scent-marking rate is influenced by several factors.

Introduction

Communication by means of visual, acoustic or chemical signalling is the key to most social interactions in animals. However, signals are often not only perceived by the intended receivers but can be intercepted and exploited by competing conspecifics or even by other species for their own benefit. This phenomenon is known as `eavesdropping' and has been described in both intra- and inter-specific contexts (Hughes et al. 2010a, b; McGregor & Dabelsteen 1996; Peake et al. 2001; Steinberg et al. 2014; Zuk & Kolluru 1998). The role of eavesdropping has been studied extensively in

the context of predator-prey interactions (Apfelbach et al. 2005; Conover 2007). Most of these studies have focused on the prey animal's perspective and have described either the costs of signalling in terms of increased predation risk (Hughes et al. 2010a,b; Koivula & Korpimäki 2001), or the reaction of prey animals to predator cues (Apfelbach et al. 2005; Kats & Dill 1998). The question whether a signalling predator may incur costs due to inter-specific eavesdropping by prey is less well understood. Several studies on echo locating predators have investigated how eavesdropping on ultrasounds by prey may influence the predators' hunting strategies and their communication (e.g. bats, Rydell et al. 1995; killer whales *Orcinus orca*, Deecke et al. 2005). Furthermore, it has been proposed that predatory mites *Amblyseius swirskii* 'chemically disguise' themselves to improve attack success on thrips larvae *Frankliniella occidentalis* (Van Maanen et al. 2015). These studies provide evidence that predators can prevent detection by their prey by modifying their hunting or communication behaviour, but we are not aware of any study investigating how mammalian predators may modulate chemical signalling in order to reduce the risk of inter-specific eavesdropping.

Many mammalian predator species use scent-marks for communication with neighbouring territory holders, mates or group members (i.e. wolves *Canis lupus*, Peters & Mech 1975; several felid species, Mellen 1993; spotted hyena *Crocuta crocuta*, Burgener et al. 2008; banded mongoose *Mungos mungo*, Jordan et al. 2011). In wild felid species, scent-marking is assumed to play an important role in territoriality, in reproductive behaviour, and in competition among same sex individuals (several felid species, Sunquist & Sunquist 2002; Eurasian lynx *Lynx lynx*, Vogt et al. 2014; bobcat *Lynx rufus*, Allen et al. 2015). Wide ranging predators such as large felids or canids are limited in the amount of scent-marks they can produce and the time they can invest in scent-marking behaviour (Wyatt 2014). In order to optimize scent-marking efficiency, they should leave scent-marks where they are most likely to be detected by conspecifics, e.g. along guiding topographic features such as paths or rivers (Wyatt 2014). Scent-marks are also more likely to be encountered when they are placed along a straight path (since a strongly wound path of the same length passes through a much smaller area, Conover 2007). It is conceivable that optimizing detection probability by conspecifics may also facilitate eavesdropping by other species. In fact, a variety of studies has demonstrated that prey animals react to predator scent-marks (Apfelbach et al. 2005). The observed responses range from changes in habitat use (e.g. avoidance of scent-marks, Forsman et al. 2013; Swihart et al. 1991) to behavioural adjustments (e.g. decreased movement rates, Borowski 1998) and physiological changes (e.g. delayed ovulation, Apfelbach et al. 2001). Wild ungulates are known to show anti-predatory behaviours in response to predator scent-marks: Kuijper et al. (2014) found that red deer *Cervus elaphus* showed a more than two-fold increase of vigilance levels when presented with olfactory cues of a predator (wolf scats). Roe deer *Capreolus capreolus* increased vigilance levels

when presented with Eurasian lynx urine (Eccard et al. 2015) and reduced visitation duration of sites where lynx scats were presented (Wikenros et al. 2015). Such eavesdropping responses by prey are of particular relevance for ambush predators as they have to remain undetected by their prey for successful hunting. At the same time, they have a need for social communication and marking of their territory and, thus, may face a trade-off between intra-specific communication and the risk of alerting prey. In this study, we investigated whether there is evidence for such a trade-off in a wild population of the Eurasian lynx, a stalking and ambush predator for which scent-marking has been formerly shown to play an important role in communication with competitors and mates (Vogt et al. 2014). To this end, we followed tracks of GPS-collared lynx in the snow across the study area and identified scent-marks along these tracks.

Eurasian lynx are specialised predators of medium-sized ungulates like roe deer and chamois *Rupicapra rupicapra*, which make up 84% of their diet in Switzerland on average. The remaining 16% consist of smaller prey species such as red foxes *Vulpes vulpes*, European brown hares *Lepus europaeus* or marmots *Marmotta marmotta* (Breitenmoser et al. 2010). The way lynx move throughout their large home ranges (males: 137 km^2, females: 76 km^2 (mean Kernel 95%), Breitenmoser-Würsten et al. 2001) follows a characteristic pattern: stationary phases during which a lynx remains in the vicinity of a fresh kill for up to several days are interspersed with phases of increased movement, when it uses larger parts of its home range and presumably searches for prey in new areas. Excursions from unfinished kills occur mostly in males during the mating season (Breitenmoser & Breitenmoser-Würsten 2008). During their movements, lynx of both sexes engage in scent-marking behaviour by means of urine marking at visually conspicuous objects such as rocks or young spruce trees (Vogt et al. 2014). Males mark generally more often than females, marking-frequency increases during the mating season and there is evidence that marking sites may serve as 'chemical bulletin boards' for competitors and mates (Hucht-Ciorga 1988; Vogt et al. 2014). Observations of prey animals investigating lynx marking sites have occasionally been made (roe deer, red deer, chamois, red fox; K. Vogt, unpublished results). Lynx could avoid detection of fresh scent-marks by potential prey by separating hunting from scent-marking activity either in space or in time. Under the hypothesis of a trade-off between lynx hunting and scent-marking behaviour, we made the following non-mutually exclusive predictions: 1) Lynx should leave their scent-marks where there is a high chance for them to be encountered by other lynx (e.g. along guiding topographical features or during long distance movements; Conover 2007). 2) They should increase scent-marking when the social benefits are high (e.g. during the mating season or when encountering other lynx). 3) Lynx should spatially and/or temporally separate areas with high scent-marking intensity from areas where the costs of being detected by potential prey are high, e.g. they should a) mark more in places where they have recently hunted successfully and b) avoid scent-marking where they intend to hunt.

4) When the benefits gained from scent-marking are high compared to the costs in terms of decreased hunting success (e.g. during the mating season), the separation between scent-marking and hunting behaviour should be less pronounced.

Methods

Ethical note

From 2012-2014, we captured and radio-tagged 15 Eurasian lynx (8 males, 7 females) and recaptured 4 of them to change their collars. Lynx were captured following established standard protocols (described in Breitenmoser & Haller 1993; Ryser-Degiorgis et al. 2002; Ryser et al. 2005; Zimmermann et al. 2005) and with all permits required according to Swiss legislation for capturing, immobilising, and radio-tagging lynx (capture permits from the Federal Office for the Environment: Bewilligung_KORA_Luchsfang_BE_2010/2011/2006-03219/02/05/03, Bewilligung_KORA_Luchsfang_Kompartimente I, III und IV_2011-2015; animal experimentation permit from the Animal Welfare Commission of the Office for Agriculture and Nature of the Canton of Bern: 109/10 and 111/13). The used capture methods included foot snares (14 captures), solid wooden box traps (3 captures), and a remote-controlled teleinjection system (1 capture). Most captures took place from November to April, in order to avoid capturing pregnant or lactating females or small kittens. 3 single animals (2 males, 1 nonreproductive female) were captured between July and October, after the absence of kittens had been confirmed by camera traps set for one night at the kills where they were captured with foot snares the following night (see below).

Foot snares made from light aluminium hoops (20.5cm diameter) and 3mm wire cables were placed around fresh kills and connected to an alarm system. The cables were passed through aluminium tubes equipped with long springs to avoid leg injuries. The capture team, consisting of several experienced field biologists and a trained wildlife veterinarian, was always able to reach the capture site within 15min of an alarm at most. Since foot snares were placed only at known lynx kills, the only bycatch were 2 dogs *Canis familiaris*, which were released immediately. All animals were thoroughly checked for injuries during handling and all results were documented. The only injuries reported were 2 cases with minor skin abrasions or swelling on one leg and 2 cases where lynx had bitten their own lips (no stitching needed). We tightly monitored the movements of all lynx after release and searched GPS location clusters until we could confirm that they were hunting successfully. Throughout this study, we regularly checked the movement patterns of all lynx and searched for prey remains. All lynx found dead in Switzerland are submitted to the Fish and Wildlife Health Centre at the University of Bern for examination (including histology). No capture-related injury (e.g. myopathy) has been detected during our study.

Unbaited double-door box traps made from solid wood (2 x 0.75 x 0.8 m) were placed on forest roads used by lynx. They were equipped with a GSM-based alarm system allowing for 24-hour monitoring. We checked the functioning of the alarm daily and controlled box traps physically every 4 to 5 days to ensure proper functioning. In case of an alarm, the box trap was controlled within 30 min to 1 hour by one capture team member or by local game wardens. Any non-target species were directly released and the rest of the capture team was alerted if a lynx was in the trap. Box traps were only operating during winter time when outside temperature was low. Lynx did not spend more than 4 hours in the box trap before immobilisation, hence providing food and water was not necessary. Since the traps were dark and closed at all sides, animals were safe from weather conditions and predators and generally behaved calmly. All lynx caught were examined by a veterinarian and the only injuries reported were in some cases several split or broken claws, which lynx regrow. 16 non-target animals were captured (4 red foxes, 2 dogs, 9 badgers *Meles meles*, 1 stone marten *Martes foina*), all of which were released without any visible injuries.

For one lynx capture, a minimally invasive capture system (MICS, Ryser et al. 2005) was used. The device consists of a blowgun remotely controlled by means of a built-in camera and a swivelling 2-way pan-tilt head. The MICS is a highly selective system and allows for relatively stress-free captures of lynx. However, its use is restricted in our study area, since it can only be used in safe terrain (no main roads, cliffs or white water nearby) as the darted animal is not physically restrained.

Lynx were immobilized with 0.1–0.15 mg/kg medetomidine hydrochloride (Domitor®, Orion Corporation, Espoo, Finland) and 3.2–5.5 mg/kg ketamine hydrochloride (Ketasol®, Graeub, Switzerland). Atipamezole hydrochloride (0.56–0.77 mg/kg; Antisedan®, Orion Corporation, Espoo, Finland) was used as an antagonist for medetomidine and was injected at least 1 hour after the last ketamine injection in order to assure that ketamine had been fully metabolised (Ryser-Degiorgis et al. 2002). The anaesthesia protocol is well established and no adverse long-term effects have been recorded during our study or in previous studies using the same protocol (Molinari-Jobin et al. 2002; Ryser-Degiorgis et al. 2002; Ryser et al. 2005; Zimmermann et al. 2005, 2012b). Vital parameters of anaesthetised animals were closely monitored by a wildlife veterinarian. Our capture kit included blankets and hot-water bottles to prevent hypothermia as well as medications to counter the possible adverse effects described for the used anaesthetics. The use of an antagonist for medetomidine further enabled us to interrupt anaesthesia in case of complications. However, this was never necessary during this study. Drugs were administered either using a blow dart (box trap, foot snare and MICS captures) or lynx were held down with a net and given a direct intramuscular injection (foot snare captures). To reduce stress, animals were left undisturbed after drug injection until they were unconscious. During the process of waking up animals were observed from a distance.

Lynx were equipped with GPS/GSM tracking units (GPS Plus Mini-1 C collars, Vectronic Aerospace GmbH, Berlin, Germany; Wild Cell SL/SD GPS-GSM collars, LoTek wireless, Ontario, Canada) weighing 250-300g (Ø 1.5% of lynx body weight, ranging from 1.25 to 2% for largest and smallest lynx, respectively). GPS-collars were secured with blunt screw nuts provided by the manufacturer. Each collar contained a break-off device allowing the unit to drop off after 1-2 years. The dropping of the unit has been documented for 3 of these individuals using camera traps. None of the captured lynx died due to capture procedures or problems with the collar or showed any skin abrasions caused by the collar. From earlier telemetry studies conducted in our study area using the same methods, radio-tagged lynx are known to reproduce normally and live up to old age (Zimmermann et al. 2005, 2012b).

Study area

The study area is situated in the north-western Swiss Alps, expands over approximately 1500 km² and includes parts of the Bernese Oberland and the pre-Alps of the cantons Vaud and Fribourg (Vogt et al. 2014; Zimmermann et al. 2012a). Altitudes range up to more than 2000 m a.s.l. The forested area covers 30% but is fragmented by human settlements and pastures. Human density is about 42/km2 on average (Swiss Federal Statistical Office 2015) and the area is intensively used for recreation, both in summer and winter (hiking, skiing). The most common ungulate species are chamois and roe deer (Breitenmoser & Breitenmoser-Würsten 2008; Breitenmoser & Haller 1993). Red deer, Alpine ibex *Capra ibex* and wild boar *Sus scrofa* occur only locally and in low numbers. During a camera trapping census in winter 2013/14, lynx density in the study area was estimated at 2.05 (1.50-2.60, 95% confidence interval) independent (subadult and adult) lynx/100 km2 of suitable habitat (95.3% of total study area; Zimmermann et al. 2014).

Snow-tracking

We followed the tracks of 15 GPS/GSM-collared lynx (8 males, 7 females) from November to April 2012/2013 and 2013/2014. GPS/GSM-collars were set to take 7 locations per day and collars attempted to send GPS-locations via SMS to a ground station at the office once per night. Locations were downloaded from the ground station every morning and 1-2 focal lynx were chosen from all successfully downloaded collars according to accessibility and snow conditions. We attempted to record a similar number of tracks per month for each lynx and a similar number of tracks corresponding to different movement patterns. Movement patterns were classified using GPS-data (long distance= lynx moved >1000m straight line within 6 hours, explorative= lynx moved 100m-1000m straight line within 6 hours, stationary= lynx moved <100m straight line within 6 hours). Since

GSM coverage of our study area was not continuous and not all GPS-collars were functioning during the whole study period, effective number of tracks for each lynx ranged from 1-17 tracks/individual (median= 6 tracks/individual). Moreover, snow cover during our study periods was not always continuous and some areas were inaccessible due to risk of avalanches, which resulted in our sample consisting of 20 stationary tracks, 33 explorative tracks and 64 long distance tracks.

We started tracking at a chosen GPS location of a collared lynx in order to assure proper assignment to the individual. The mean accuracy calculated from a subsample of 46 GPS-locations from 4 collars was 8.8m (± 1.3m *SE*) in our study area. Tracks were 6-48h old and were generally followed backwards. If lynx were known to have left the area, we could also track forwards without disturbing the animal. We attempted to track for a minimum of 1000m for long distance and explorative movements and 150m for stationary movements. Tracking was interrupted either when the tracks were lost or when they led into inaccessible areas. All tracks were logged using a handheld GPS (Etrex vista HCx, Garmin, Olathe, KS, USA) with a mean accuracy of 1.9m (± 0.4m *SE*) in our study area (calculated from 71 test points). We recorded date and time of the snow tracking event, date and time of track formation, track length, time since last snowfall, snow conditions, number of marking sites, number of scats, presence of other lynx, and evidence of hunting behaviour (see below). Track formation was derived from the GPS collar fix at the starting point. If the track passed several GPS locations taken by the collar, the median time point was calculated unless time since last snowfall provided additional information on track age. Snow conditions were described as follows: 1= continuous snow cover, 2= patchy (<50% of track), 3= very patchy (>50% of track), 4= no snow. Snow cover was 1 or 2 for 93% of the tracks. For the remaining 7% of tracks, we relied on a trained dog to detect and follow the lynx track. When scent-marking, lynx typically make a short detour from their direction of travel and turn their rear towards visually conspicuous objects in order to spray urine (Breitenmoser & Breitenmoser-Würsten 2008), thereby creating a distinct track pattern in the snow. 92% of marking sites were identified by the track pattern; 8% were detected by the trained dog and then confirmed by their typical smell. All marking sites and scats were logged with the handheld GPS and we recorded which objects were marked and whether scats were covered with snow, earth or plant material. Presence of other lynx was confirmed if the track of the focal lynx was crossing or following the track of another individual. Evidence of hunting was confirmed if prey remains were found or tracks showed that lynx were pursuing a prey animal unsuccessfully. We considered only cases where tracks of lynx and prey matched the following criteria: strides lengthened to full-out gallop, tracks parallel or overlapping, signs of a fast start, direct line of sight between starting point of chase and starting point of flight.

For each track, we searched the previous kill the focal lynx had made. In order to find kills, we searched GPS location clusters (GLC's), similar as in Krofel et al. (2012) and Svoboda et al. (2013).

A GLC was defined as a set of at least 2 GPS locations within 100m and a maximum time span of 72h between consecutive fixes in the same GLC. Within each GLC, we searched a radius of 30m around each fix for at least 1h using a trained dog whenever possible. We searched all accessible GLC's preceding the track of the focal lynx until we found prey remains. Inaccessible GLC's were not searched but were included in the analysis as potential kills, if the time the lynx spent in the GLC was ≥9 hours. Analysis of all searched GLC's in our study area during the two winter seasons 2012/2013 and 2013/14 shows that kills were found in 72% of GLC's with a duration of ≥9 hours (N= 259), while we could find kills in only 17% of the shorter GLC's (N= 70). We also found kills by chance on 6 tracks of uncollared lynx in the study area and included these tracks into our analysis.

Statistical analysis

To test for a possible relationship between lynx scent-marking rate and hunting behaviour we quantified the following parameters from the tracking data: time since last kill was calculated as the time difference in days between the last GPS location in the GLC containing the previous (potential) kill and the time of track formation. Distance to last kill (in km) was calculated as the median of the distances of all track points from the previous kill site (true kills) or from the geometric centre of the GLC (potential kills) and was log-transformed to fit a normal distribution. GLC's were identified using the cluster generator script in R (version 3.1.0, R Development Core Team 2013) kindly provided by N.J. Svoboda (Svoboda et al. 2013) and adapted by K. Vogt. Evidence of hunting was classified as 0 (no hunting detected), 1 (lynx attempted to hunt or made a kill during this tracking event) and 2 (lynx was still using a kill it had made before track formation).

In order to test for the relationship between social factors, lynx movement patterns and scent-marking rate we investigated the following parameters: reproductive season was defined by the date of track formation lying either before or during the mating season of Eurasian lynx (15th of February to 15th of April, Breitenmoser & Breitenmoser-Würsten 2008). Presence of other lynx was classified as 1= detected or 0= not detected. We assigned a structuredness index to each track, which was calculated as the proportion of a track's length intersecting with a 10m buffer laid around linear structures (i.e. forest edges, primary and secondary roads, hiking trails, and small watercourses or ravines). If a large portion of the track follows linear structures the index lies close to 1; tracks with only a small portion following linear structures give values close to 0. Linear structures were extracted as vector data from the SwissTLM3D geodatabase of the Swiss Federal Office of Topography (ESRI File Geodatabase 10.1, http://www.swisstopo.admin.ch). This database has an accuracy of 1-3m. In order to describe the movement patterns of lynx, we assigned a simple straightness index to each track. The index was calculated as the total track length divided by the distance between start and end point as described in Benhamou (2004). The index lies between 0

and 1, with values close to 1 representing very straight tracks and values close to 0 representing very curved or circular tracks.

We analysed factors influencing scent-marking rate using a Generalised Linear Mixed Model (GLMM) with a Poisson error distribution and log-link function fitted by Laplace approximation. The number of marking sites was set as the response variable and track length (log) was set as offset to take into account different track lengths. Evidence of hunting, time since last kill, distance to last kill, structuredness index, straightness index, mating season and presence of other lynx were included as fixed factors. We further added the interactions between evidence of hunting and mating season and between evidence of hunting and structuredness index. We considered all factors and interactions in a single model because these factors corresponded to our non-mutually exclusive predictions for our hypothesis of a trade-off between scent-marking and hunting. Sample size was too small to analyse tracks of male and female lynx separately or include sex and its interactions with other factors in the full model. Graphical visualisation showed that correlations among factors were similar for both sexes and, therefore, data from males and females was pooled. We included lynx identity as random factor to account for repeated measures (several tracks per individual) and for variation among individuals. Calculation of the scaling parameter using the function recommended by D. Bates (https://stat.ethz.ch/pipermail/r-sig-mixed-models/2011q1/015392.html) revealed slight overdispersion in our model (1.51). To account for overdispersion, we multiplied the standard errors of the model output with the scaling factor. The model before correction for overdispersion is presented in Appendix 1. We present the model results as parameter estimates with standard errors and use Bayesian approaches to compute Credible Intervals (CrI) and draw inference from the GLMM. This procedure has been described in Bolker et al. (2008) and has been implemented, for example, by Grüebler et al. (2010). For calculation of fitted values and Credible Intervals, we used the function sim() of the R package *arm* applying improper prior distributions (Gelman & Hill 2007). We obtained posterior distributions of fitted values by directly simulating 5000 values from the joint posterior distributions of the model parameters. The means and the 2.5% and 97.5% quantiles of these fitted values were used as point estimates with their lower and upper 95% credible interval boundaries, respectively. The strengths and uncertainties of correlations were evaluated based on estimates and CrI's of model parameters. All statistical analyses were conducted in R (version 3.1.0, R Development Core Team 2013) and ArcGIS (ArcGIS 10.1 SP for Desktop, ©1999-2012 Esri Inc.).

Results

We followed 111 tracks of 15 radio-collared lynx (1- 17 tracks/individual) and 6 tracks of uncollared individuals over a total distance of 185 km (mean ± *SE*= 1.6 ± 0.1 km, range= 0.2- 5.6 km). The mean

straightness value among all tracks was 0.447 (± 0.018 *SE*) and the mean structuredness index was 0.466 (± 0.021 *SE*). 64 tracks were followed outside and 53 during the mating season. Tracks of other lynx were encountered in 22 cases. We found evidence of hunting during 28 tracking events, in 65 cases no hunting behaviour was detected and during 24 tracking events lynx were still using a kill made before track formation. We were able to retrieve the last kill for 71% of the tracks; potential kills were assumed for 29%. Marking rate varied widely among tracks and individual lynx (mean ± *SE*= 3.4 ± 0.3 marks/km track, range= 0-18 marks/km track, *N*= 117) but was similar among tracks with continuous snow cover (mean ± *SE*= 3.5 ± 0.4 marks/km track, *N*= 78) and without continuous snow cover (mean ± *SE*= 3.3 ± 0.5 marks/km track, *N*= 39). We recorded a total of 594 marking sites. The different objects used by lynx for scent-marking with urine, and the frequency by which they were used, are shown in Figure 1.

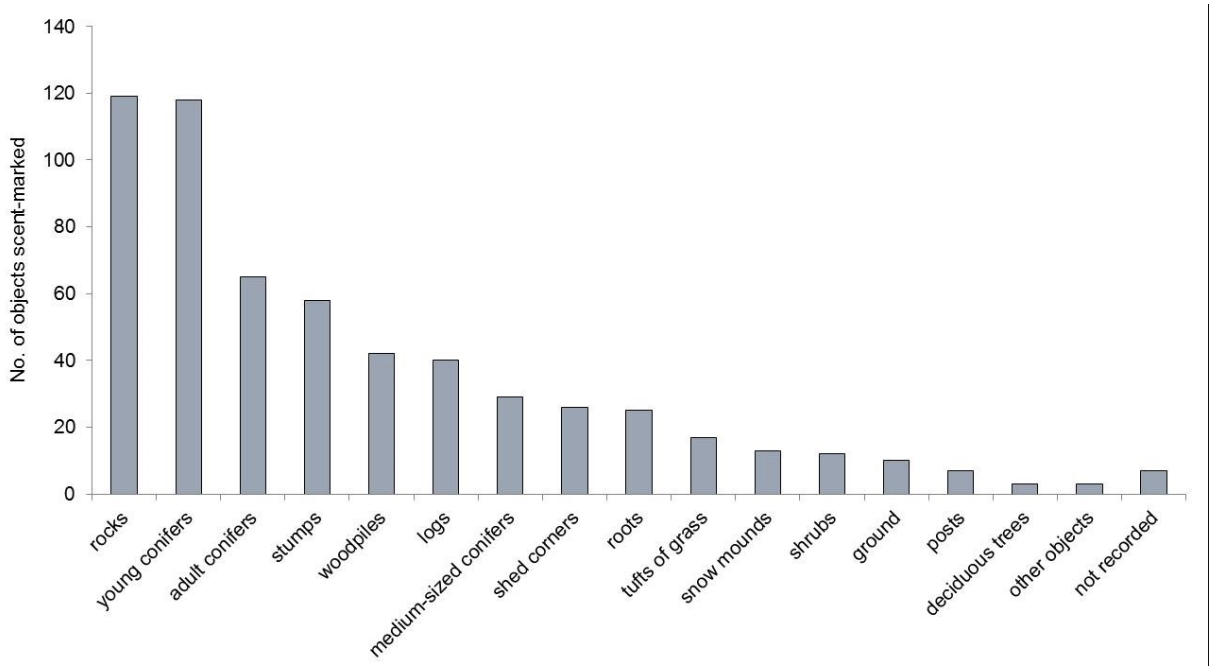

Figure 1. Objects used by lynx for scent-marking with urine. Bars show the occurrence of different object types in a total of 594 marking sites found during snow tracking. Rocks= vertical faces of rocky structures, young conifers= up to 1m in height, medium-sized conifers= up to 2m in height, adult conifers/deciduous trees= higher than 2m (trunk or lower branches marked). Stumps= cut or broken tree trunks, logs= cut or broken trunks lying on the ground, roots= root plates of uprooted trees. Tufts of grass/snow mounds= clearly elevated from ground, shrubs= bushes and young deciduous trees up to 2m in height, ground= not elevated. Man-made structures include woodpiles, shed corners, fence or power line posts and other objects. Not recorded= object type not recorded.

Factors influencing scent-marking rate

The correlations between structuredness index and scent-marking rate and between straightness of the track and scent-marking rate were weak and subject to a high level of uncertainty (Table 1).

Table 1. Parameter estimates (± standard errors) of the generalized linear mixed model (GLMM) for scent-marking rate.

Fixed Factors	*Factor levels*	Estimate	*SE*
intercept		0.511	0.406
structuredness index		0.266	0.446
straightness		0.553	0.455
mating season		0.277	0.191
presence of other lynx		-0.040	0.177
hunting	*hunting= 1*	-0.850	0.441
	hunting= 2	-1.132	0.752
time since last kill		-0.009	0.041
log(distance to last kill)		-0.056	0.070
hunting*mating season	*hunting= 1*mating season*	-0.691	0.347
	*hunting= 2*mating season*	0.225	0.484
hunting*structuredness index	*hunting= 1*str. index*	1.778	0.813
	*hunting= 2*str. index*	0.913	1.362

SE= Standard Error. Levels of the factor hunting (1= evidence of hunting found along track, 2= lynx is using a kill made before track formation) are compared to 0= no evidence of hunting. The analysis was conducted on data from 117 snow tracking events. Lynx identity (estimated variance component= 0.529, *SD*= 0.728) was included as random effect. To account for overdispersion, standard errors were multiplied with a scaling parameter.

Tracks where no marking occurred at all were found among strongly wound paths as well as among straight ones, thereby lowering the estimate and increasing the variance (Fig. 2a). When only tracks with scent-marking were considered in the GLMM (*N*= 92), the association between straightness and scent-marking rate was stronger (estimate= 0.919, *SE*= ±0.312; Fig. 2b). According to this model, an increase in straightness index of 0.5 led to an increase in marking rate of 1.1 marks/km track (95% CrI 0.9- 1.4 marks/km track).

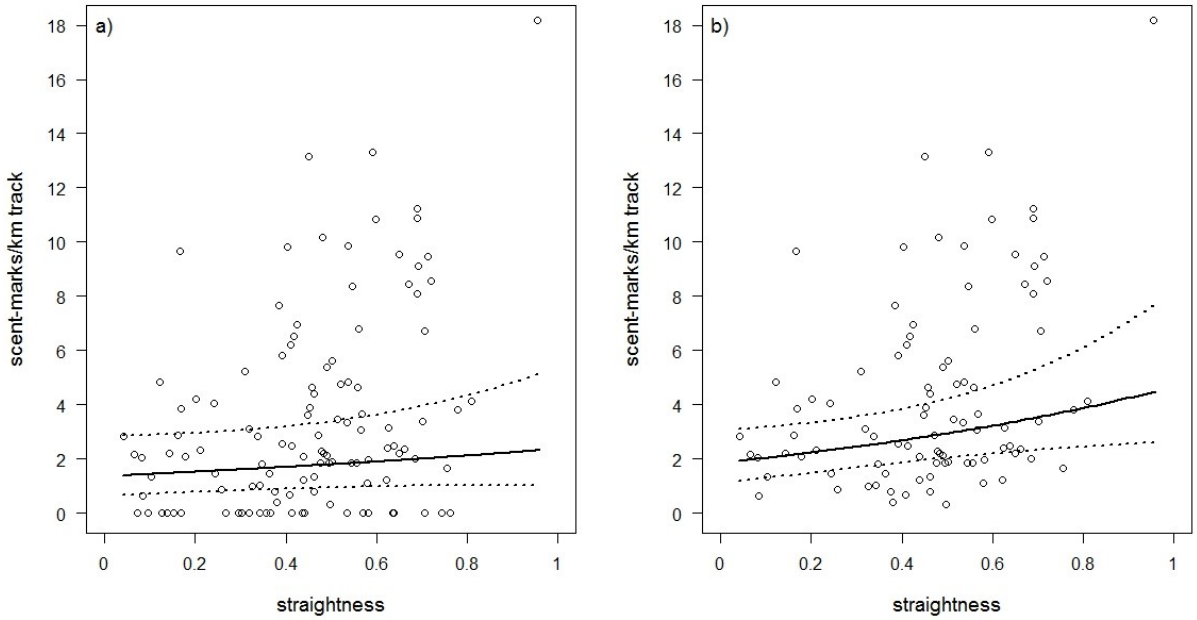

Figure 2. Partial correlation of straightness of the track with scent-marking rate. **a)** all tracks (N= 117) **b)** only tracks with scent-marking (N= 92). Open circles= raw data, solid line= fitted values from the GLMM, dotted lines= 95% credible intervals. Evidence of hunting is set constant at 1, all other factors in the GLMM are set constant at their mean.

We found similar scent-marking rates on tracks where other lynx were encountered (mean= 3.3 marks/km track, *SE*= ±0.7 marks/km track), compared to tracks where we found no signs of presence of other lynx (mean ± *SE*= 3.5 ± 0.4 marks/km track). However, when averaging over all tracks, lynx marked more often during the mating season (mean ± *SE*= 4.2 ± 0.5 marks/km track) than outside (mean ± *SE* = 2.8 ± 0.4 marks/km track), although this difference was not certain (Table 1). Scent-marking rate showed no correlation with time since last kill and distance to last kill (Table 1) but there was a stronger negative association between scent-marking rate and evidence of hunting behaviour (Table 1). The difference between hunting and no hunting was most pronounced during the mating season, when lynx left on average 2.6 ± 0.8 marks/km track (mean ± *SE*) while hunting, compared to 5.3 ± 0.8 marks/km track (mean ± *SE*) when we found no evidence of hunting behaviour. Outside the mating season, this difference was less pronounced (Fig. 3). Conversely, the difference between the marking rate when using a kill compared to when lynx were hunting was highest outside the mating season, when lynx left on average 2.3 ± 0.7 marks/km track (mean ± *SE*) while hunting, compared to 1.3 ± 0.5 marks/km track (mean ± *SE*) when using a kill (Fig. 3).

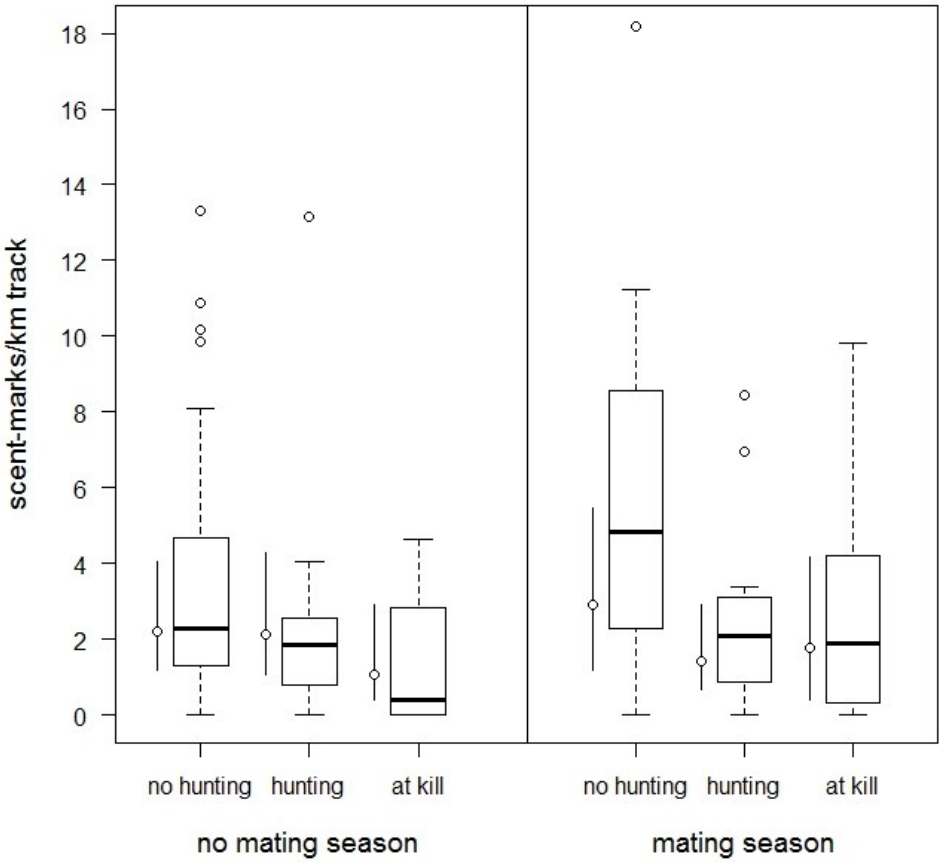

Figure 3. Partial correlation of the interaction between evidence of hunting and mating season with scent-marking rate. Boxplot= raw data. Each box encompasses the 25th through 75th percentiles, with the median represented by an interior line. Whiskers denote maximum values or in case of outliers 1.5 times the interquartile range. Circles denote outliers. Open circles with line segments= fitted values from the GLMM with 95% credible intervals. Hunting= evidence of hunting found along the track (kill or hunting attempt). No hunting= no evidence of hunting found along the track. At kill= lynx was feeding on a kill made before formation of the followed track. All other factors in the GLMM are set constant at their mean.

The proportion of track following linear structures showed only a very weak correlation with scent-marking rate for those tracks where no evidence of hunting could be detected (Fig. 4). However, scent-marking rate increased with structuredness index when lynx were hunting (Table 1), i.e. an increase in structuredness index from 0.1 to 0.6 led to an increase in marking rate of 1.5 marks/km track (95% CrI 0.8- 2.6 marks/km track). There was also a weaker correlation between scent-marking rate and structuredness index for those tracks where lynx were using a kill (Table 1), although the uncertainty of this association was high (Fig.4).

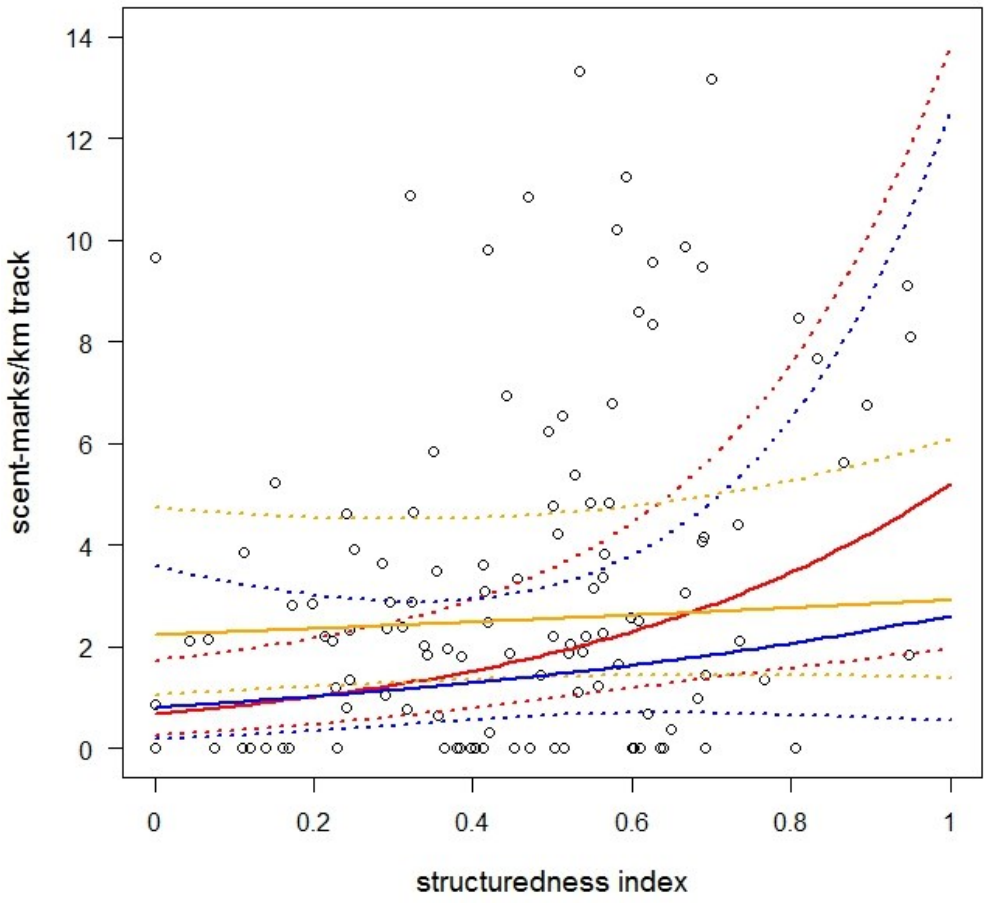

Figure 4. Partial correlation of the interaction between evidence of hunting and structuredness index with scent-marking rate. Open circles= raw data, solid lines= fitted values from the GLMM, dotted lines= 95% credible intervals. Red= evidence of hunting found along the track (kill or hunting attempt). Orange= no evidence of hunting found along the track. Blue= lynx was feeding on a kill made before formation of the followed track. All other factors in the GLMM are set constant at their mean.

Caching of scats

We also found 36 scats during snow tracking and recorded whether they were covered with snow, earth or plant material. Scats were more often covered when lynx were using a kill (6 out of 9 scats) or hunting (4 out of 10 scats). When lynx were not hunting, only 5 out of 17 scats were covered.

Discussion

Sexual signals are considered honest signals of quality as they are costly either because of condition dependence or because of increased predation risk to the signaller (Andersson 1994). Thus, many animals engaging in conspicuous signalling face a trade-off between the social benefits of high signalling rates (i.e. increased mating success) and the costs of signalling in terms of greater predation risk due to eavesdropping predators (Bulbert et al. 2015). Although many mammals rely on olfactory communication more than on visual or acoustic signalling, trade-offs in the context of chemical signalling are much less studied than, for example, sexual selection on conspicuous ornaments (Hughes et al. 2010a; Stuart Fox & Ord 2004). Several studies have demonstrated that scent-marks of prey attract predators (Hughes et al. 2010a,b; Koivula & Korpimäki 2001) and that leaving and receiving chemical signals comes at the cost of increased predation risk (Hughes et al. 2010a,b; Hughes et al. 2012). However, few studies have investigated how eavesdropping on chemical cues of predators influences their attack success (Van Maanen et al. 2015) and whether predators may adopt strategies in order to avoid eavesdropping by prey (Deecke et al. 2005; Rydell et al. 1995). Roe deer and other prey species have been shown to investigate natural lynx scent-marks (K. Vogt, unpublished results) and respond to experimentally applied urine and scats of Eurasian lynx (Eccard et al. 2015; Wikenros et al. 2015), which could potentially influence the hunting success of this stalking predator.

Our study is based on detailed observations of scent-marking and hunting activity of Eurasian lynx. The results are consistent with the hypothesis of a trade-off between chemical communication and hunting behaviour, while also showing that scent-marking rates are influenced by a complex interplay of different factors. Consistent with our first prediction (see introduction), we found evidence that lynx try to increase detection probability of their marks by conspecifics. During their movements, lynx chose visually conspicuous objects for urine-marking in almost all cases. We hardly ever recorded elimination of urine on the ground (Fig. 1). The visual component of the marked object enhances detection probability. This may be especially important for long-lasting scent-marks with low volatility, such as those of lynx (Vogt et al. 2014; Wyatt 2014). Furthermore, scent-marking rate was correlated with track straightness when we excluded those tracks from the analysis where lynx did not mark at all. Conover (2007) argued that straight paths have a higher chance to be intercepted than strongly wound paths of the same length. It could be that track straightness is associated with the effort a lynx invests into scent-marking, but is unrelated to the decision whether it will engage in scent-marking activity at all. In our study area, straight paths often coincided with lynx following forest roads and straightness was therefore partly correlated to structuredness of the track. Lynx use forest roads for long distance movements. Especially during winter time, they can save energy by walking on ploughed roads or in snow shoe tracks (Zimmermann et al. 2007). It would therefore pay

to increase scent-marking rate during such movements, as forest roads are commonly used by conspecifics and allow for covering a large area at comparably low costs. Moreover, forest roads, trails, forest edges, etc. channel the movements of animals and make it more likely that another lynx passes close enough to a scent-mark to detect it.

Our second prediction was that lynx should increase scent-marking when the social benefits are high. We found that lynx generally increased scent-marking rate during mating season (mid-February to mid-April), which was also found during previous observations (Breitenmoser & Breitenmoser-Würsten 2008; Vogt et al. 2014) and is well known from other felid species (Allen et al. 2015; Mellen 1993; Smith et al. 1989; Sunquist & Sunquist 2002). During this time, scent-marking can be expected to play an important role in communication between sexes as well as in intra-sexual competition (Vogt et al. 2014). We also expected that the presence of another lynx would increase scent-marking rate. However, whether it is beneficial for a lynx to increase scent-marking rate upon encountering signs of presence of another lynx may strongly depend on individual and pairing. An adult male encountering a female may have a stronger interest to advertise his presence than a subadult male encountering an adult male. Individual variation in scent-marking rate was high in our study and since our sample size of encounters was small (N= 22), we were not able to further subdivide the sample to test for the influence of pairing.

Our third prediction was that lynx should spatially and/or temporally separate areas with high marking intensity from areas where the costs of being detected by potential prey are high. Vigilance of prey increases when a predator has been detected and decreases again in the absence of the predator due to the costs of high vigilance levels (Lima & Bednekoff 1999). Thus, a lynx attacking a prey animal and then staying in the area for several days to feed on the kill presumably has a higher chance of being detected by potential prey than a lynx entering an area it hasn't used for some time. We therefore expected lynx to mark most often in areas where they had already hunted successfully and decrease scent-marking rate as they moved further away, towards new hunting areas. However, neither time nor distance to the last kill were associated with scent-marking rate. This may be due to the fact that home ranges of lynx are much larger than those of any of their prey species (Baumann et al. 2000; Boschi & Nievergelt 2003; Hewison et al. 1998) and lynx are able to cover large distances in short time. In our mountainous study area, the absolute distance moved by a lynx may be less informative than whether valleys or ridges are crossed. A lynx may, in fact, not have to move very far in order to enter into a new topographic compartment, where prey animals are not yet alerted to its presence.

Contrary to time or distance to the last kill, the occurrence of hunting behaviour was associated with scent-marking rate. Indeed, we found that lynx engaged less in scent-marking on tracks with evidence of hunting behaviour. In 16 out of 20 incidents where we could reconstruct from

tracks how prey was pursued or killed by lynx, we observed that lynx did not wait for passing prey in an ambush, but encountered it while they were exploring their habitat. The sequences of stalking and attacking prey were very brief: after ca. 5 to 170m lynx would either catch their prey or abandon a chase. This suggests that stalking and killing prey is not very time consuming and if lynx engage in scent-marking less frequently at times when they are hunting, this does not solely reflect time-constraints. Our findings also match field observations of Zheltukhin (1984), who found that Eurasian lynx in the upper Volga region were scent-marking more frequently after hunting events than before. Similar evidence for a trade-off between hunting and communication was also found in killer whales, whose vocal activity increased after successful attacks on marine mammals or during surface-active behaviour, which was not hunting related (Deecke et al. 2005). Conversely, Monclús et al. (2009) state that foxes in Spain defended their food resources by increasing scent-marking rate and detectability of marks in places of high rabbit density and by placing their marks near rabbit latrines and scrapes. However, this study did not provide information on whether foxes had left their marks before or after successful hunting events and the findings potentially reflected higher fox densities rather than elevated marking rates. We found no evidence of lynx increasing their scent-marking rate around food resources. While using a kill, lynx marked less often than on tracks where no hunting behaviour was detected. When feeding on a kill for up to several days, lynx usually remain close by and often cover their kills with plant material or snow (Breitenmoser & Breitenmoser-Würsten 2008). A low scent-marking rate could further help to avoid drawing the attention of scavengers or conspecifics towards kills. This would match our observation that scats were more often covered around kills, although there may also be hygienic reasons for covering scats in places where an animal remains for several days. Other lynx species are known to use latrines (e.g. Iberian lynx *Lynx pardinus,* Gil-Sanchez et al. 2006; bobcat *Lynx rufus*, López-Vidal et al. 2014), which could play a role in communication as has been reported for several carnivores (swift fox *Vulpes velox*, Darden et al. 2008; badger *Meles meles*, Roper et al. 1993). The use of latrines has not been observed in Eurasian lynx (Breitenmoser & Breitenmoser-Würsten 2008), but openly deposited scats could potentially convey information to conspecifics or prey.

Finally, we tested the prediction that the separation of scent-marking from hunting behaviour should be less pronounced if the benefits gained from scent-marking are high compared to the costs in terms of decreased hunting success. We observed that lynx increased scent-marking rate during mating season mostly at times when they were not hunting or when they were using a kill. When evidence of hunting could be detected, scent-marking rates remained similar outside and during the mating season. Thus, the necessity to avoid detection by prey animals seems to override the importance of communication during mating season. The opposite seems to be the case for the effect of linear structures: scent-marking rate increased with structuredness index when lynx were

hunting, while there was no effect when no evidence of hunting could be detected. Although the credible intervals of our estimates were inflated at structuredness index values close to 1 due to low number of observations, the interaction was still evident at structuredness index values below 0.6 (Fig. 4). While forest edges are preferred habitat of roe deer, forest roads are usually avoided (Coulon et al. 2008). Pyrenean chamois *Rupicapra pyrenaica* were found to feed further away from forest edges and walking trails than expected by chance (Pépin et al. 1996). This suggests that forest roads are places where detection probability of scent-marks by other lynx is high and the risk of eavesdropping by ungulate prey is low.

To conclude, the patterns of lynx scent-marking activity observed during this study were consistent with the hypothesis of a trade-off between the benefits of social communication and the costs of detection by prey in this stalking predator: overall scent-marking rate was lower when lynx were hunting but hunting lynx increased scent-marking rates in favourable places (along forest roads). Lynx also increased scent-marking rates during mating season but only when they were not hunting. Our study contributes to a better understanding of the costs of chemical signalling due to inter-specific eavesdropping by approaching the topic from the so far neglected predator's perspective.

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Appendix 1. Parameter estimates of the generalized linear mixed model (GLMM) for scent-marking rate. No correction for overdispersion.

Fixed Factors *(Factor levels)*	Estimate	*SE*	z-value	*P*
Intercept	0.511	0.268	1.907	0.057
structuredness index	0.266	0.294	0.902	0.367
straightness	0.553	0.301	1.838	0.066
mating season	0.277	0.126	2.192	0.028
presence of other lynx	-0.040	0.117	-0.341	0.733
hunting				
hunting= 1	-0.850	0.291	-2.921	0.003
hunting= 2	-1.132	0.497	-2.280	0.023
time since last kill	-0.009	0.027	-0.331	0.741
log(distance to last kill)	-0.056	0.046	-1.225	0.221
hunting* mating season				
*hunting= 1*mating season*	-0.691	0.229	-3.011	0.003
*hunting= 2*mating season*	0.225	0.320	0.704	0.481
hunting*structuredness index				
*hunting= 1*str. index*	1.778	0.537	3.312	0.001
*hunting= 2*str. index*	0.913	0.900	1.015	0.310

SE= Standard Error. Z-value= Wald test statistic, P= Wald test p-value. Levels of the factor hunting (1= evidence of hunting found along track, 2= lynx is using a kill made before track formation) are compared to 0= no evidence of hunting. The analysis was conducted on data from 117 snow tracking events. Lynx identity (estimated variance component= 0.529, *SD*= 0.728) was included as random effect.

Chapter 4

Chemical composition of Eurasian lynx urine conveys information on reproductive state, individual identity, and urine age

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In preparation

KV designed the study, collected samples, helped with lab work, analysed data and drafted the manuscript.

Chemical composition of Eurasian lynx urine conveys information on reproductive state, individual identity, and urine age

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Abstract

In mammals, the chemical profiles of individuals are complex and variable mixtures and animals perceive information based on variation in the overall quality of these mixtures. A variety of compounds potentially involved in chemical communication have been characterized in the urine of different felid species but little is known about the information content of felid scent-marks. In this study, we investigated whether chemical composition of Eurasian lynx *Lynx lynx* urine was related to sex, reproductive state, and individual identity. We further analysed if elemental sulphur in lynx urine could serve as a dietary cue or as an indicator for the freshness of a scent-mark. We collected urine from captive and wild Eurasian lynx and analysed volatile constituents of urine by means of solid phase microextraction and gas chromatography-mass spectrometry. Our results show that lynx scent profiles contain sex-specific information on reproductive state, as well as individual identity cues. Urine marks are therefore well-suited to fulfil a role in reproductive behaviour and social organisation of wild lynx populations. Relative sulphur content was unrelated to time since last feeding but decreased with age of the urine sample. The influence of diet and body condition on scent profiles should be further investigated by means of experimental studies and may shed more light on the messages encoded in carnivore scent-marks.

Introduction

In mammals, the chemical profiles of individuals consist of molecules produced by different scent sources on the animal's body together with molecules acquired from other group members or from the environment (Wyatt 2014). The resulting odours are complex and variable mixtures and animals perceive information based on the overall quality of these scent mixtures (Johnston 2003; Wyatt 2014). Variation in the relative proportions of chemical compounds of a scent profile may provide

information about the donor's species, sex, individual identity, reproductive status, genetic quality or kinship (Buesching et al. 2002; Charpentier et al. 2010; Johnston 2003; Penn 2002; Roberts et al. 2014). For example, many male mammals are attracted to scent-marks of females and preferences for odours of oestrus females have been detected in several species, e.g. in rats *Rattus norvegicus*, mice *Mus musculus*, dogs *Canis familiaris*, Asian elephants *Elephas maximus* (Petrulis 2013), giant panda *Ailuropoda melanoleuca* (Swaisgood et al. 2000), and ringtailed lemur *Lemur catta* (Scordato et al. 2007). On the other hand, females may choose males based on information on health, dominance status or genetic compatibility encoded in their scent profile (Penn 2002; Petrulis 2013; Roberts et al. 2014). Many studies on rodents have provided evidence that animals do not only respond to specific chemical cues but learn to recognize individual scent profiles from related or familiar individuals at certain stages of their lives (reviewed in Johnston 2003). Odour recognition has been shown to be important for kin recognition, for mate choice and other behaviour in group-living and even in less social species (Wyatt 2014).

Most wild felid species are solitary and territorial and communication by means of scent-marking with urine, faeces, or gland secretions occurs in all members of this family (Mellen 1993; Sunquist & Sunquist 2002). Males mark generally more often than females and marking-frequency increases during the mating season (Allen et al. 2015; Mellen 1993; Vogt et al. 2014). Scent-marking is assumed to play a role in territoriality, in mate attraction, and in competition among same sex individuals (Allen et al. 2015; Sunquist & Sunquist 2002; Vogt et al. 2014). In recent years, a variety of compounds in urine and facial scent samples of several felid species that are potentially involved in chemical communication have been characterized by means of gas chromatography-mass spectrometry (GCMS); for example hydrocarbons, ketones, aldehydes, fatty acids, alcohols, lactones, and S- or N-containing substances such as thioethers, sulphones, amines, and amides (Andersen & Vulpius 1999; Burger et al. 2006, 2008; Mattina et al. 1991; Poddar-Sarkar & Brahmachary 2004; Soini et al. 2012). However, only a small number of experimental studies have so far investigated the possible information content of scent-marks: Sokolov et al. (1996) reported that captive Eurasian lynx *Lynx lynx* sniffed the urine of conspecifics longer than control urine samples from other species. He further found that female lynx head rubbed longer on urine of males than of females and that lynx of both sexes smelled urine samples of unknown individuals longer than those of familiar individuals. Natoli (1985) found that both male and female domestic cats *Felis catus* spent more time sniffing urine sprayed by a strange tomcat than urine sprayed by a male from the same group. These studies show that lynx and domestic cats perceive information about sex in urine of conspecifics and are able to recognize odours of familiar individuals.

Apart from information about sex, reproductive status or genotype, the chemical composition of a scent-mark can also convey information on health, body condition, and diet of an

individual (Buesching et al. 2002; Ferkin et al. 1997; Johnston 2003; Munoz-Romo et al. 2011). Dietary cues are known to act as signals of quality to conspecifics, e.g. female meadow voles prefer chemosignals of males fed on a protein rich diet (Ferkin et al. 1997). But information on diet may also be exploited by other species. For example, many fish and amphibian species are known to adapt their anti-predatory behaviour in response to dietary cues emitted by their predators (Chivers & Mirza 2001; Murray & Jenkins 1999). In carnivores, dietary cues most likely stem from their protein or fat metabolism. One well described metabolic pathway is the conversion of the amino acids methionine and more importantly cysteine to felinine (Hendriks et al. 2001), a process catalysed by the felid-specific protein cauxin. Felinine is then degraded into several organosulphur compounds in felid urine, namely into 3-mercapto-3-methylbutanol (Miyazaki et al. 2006), and further into di- and trisulphide derivatives (Mattina et al. 1991). Cauxin has been found in the urine of domestic cats, bobcats *Lynx rufus*, and Eurasian lynx (Miyazaki et al. 2008), as well as in several large felid species (Burger et al. 2008; McLean et al. 2007). 3-mercapto-3-methylbutanol and other malodourous organosulphur compounds are responsible for the typical catty odour of domestic cat urine and are known to elicit aversive responses in prey animals (Lewison et al. 1993; Mattina et al. 1991). Burger et al. (2006) found that these organosulphur compounds were totally or almost absent from the urine of cheetah *Acinonyx jubatus.* Instead, for the first time in a mammalian species, the authors described excretion of large amounts of elemental sulphur in cheetah urine (ca. 1µg/ ml urine). Sulphur was later also detected in the urine of tigers and Iberian wolves *Canis lupus signatus*, although at much lower concentrations (tiger: ca. 80ng/ml urine, Burger et al. 2008; Iberian wolf: 1% of TIC, Martín et al. 2010). Burger et al. (2006) investigated a function of sulphur as pheromone, but no reactions to sulphur could be elicited either in cheetahs or in other tested carnivores. In fact, they found that cheetah urine elicited practically no responses of either cheetah or several other felid species. Elemental sulphur is described as an odourless substance, which led Burger et al. (2006) to the alternative hypothesis that a conversion of sulphur-containing compounds to elemental sulphur could serve to "chemically camouflage" cheetah urine from detection by larger sympatric predator species. However, given that organosulphur compounds are products of amino acid metabolism, this mechanism might also serve to hide information on diet from conspecifics or potential prey.

In this study, we investigated the chemical composition and the information content of the volatile fraction of Eurasian lynx urine. Eurasian lynx are solitary and occupy vast home ranges. The home ranges of resident males encompass those of one or two resident females but there is little overlap between the home ranges of same sex individuals (Breitenmoser-Würsten et al. 2001). Subadult lynx may not yet occupy stable home ranges and move as "floaters" among residents (Breitenmoser & Breitenmoser-Würsten 2008). During mating season, male lynx will mate-guard oestrous females. Scent-marking with urine occurs in both male and female lynx, is especially

frequent during mating season, and is thought to play an important role in reproduction and the maintenance of spatial and social organisation of wild lynx populations (Breitenmoser & Breitenmoser-Würsten et al. 2008; Vogt et al. 2014). We predicted that in order to fulfil these proposed functions, lynx urine should contain information on sex, social status (resident adult, subadult, juvenile), and reproductive state. The formerly demonstrated ability of lynx to discriminate between urine of familiar and unfamiliar individuals (Sokolov et al. 1996; see above) further suggests that scent profiles also convey information on individual identity.

Eurasian lynx are stalking predators of medium-sized ungulates and avoidance of eavesdropping by prey has been shown to partially shape spatial patterns of scent-marking (Vogt et al. under revision). In an earlier study, G. Zachariae (pers. comm.) found that organosulphur compounds were present only in very low amounts in fresh lynx urine samples, similar as in cheetah urine (Burger et al. 2006). He observed that these substances reached peak amounts 2-4 days after urine deposition and were not detectable anymore after about 14 days. He hypothesised that they were continually released from a low volatile source in the urine, possibly via the metabolism of microbes living on the substrate of the scent-mark. It is conceivable that dietary cues may be masked in fresh lynx urine, for example by conversion of organosulphur compounds to elemental sulphur. In order to test this hypothesis, we specifically investigated whether lynx urine contained elemental sulphur and whether the amount of sulphur was related to food condition and age of the urine-mark.

Methods

Collection of urine samples from wild lynx

From November to April 2012/13 and 2013/14, we collected 29 urine samples of 10 adult Eurasian lynx individuals (5 males, 5 females) and 2 subadult male lynx in the Northwestern Swiss Alps. All lynx had previously been fitted with GPS/GSM-collars (GPS Plus Mini-1 C collars, Vectronic Aerospace GmbH, Berlin, Germany; Wild Cell SL/SD GPS-GSM collars, LoTek wireless, Ontario, Canada). Urine samples were collected by following lynx tracks in the snow starting from a known GPS-fix in order to assure proper assignment of the urine sample to the individual. Lynx scent-mark at visually conspicuous objects such as small spruce trees or cut tree trunks by means of urine spraying (Vogt et al. 2014). Collection of frozen urine sprays from snow covered objects was possible up to 3 days after deposition, depending on snow and temperature conditions. The snow-urine mixtures were collected directly into 20ml headspace vials with PTFE-lined screw caps (Gerstel GmbH & Co KG, Switzerland). After each urine sample, we also collected a blank of untainted snow from the same object (ca. 30cm away from the urine spray). All samples were frozen after snow tracking at -20°C and transferred to a -80°C freezer within 2-6 months, where they were kept until chemical analysis. Headspace vials were

rinsed before use once with dichloromethane (Rotisolv, GC Ultra Grade, CARL ROTH GmbH + Co. KG, Switzerland), acetone (Rotisolv, UV/IR-Grade, CARL ROTH GmbH + Co. KG, Switzerland) and n-heptane (Rotisolv, UV/IR-Grade, CARL ROTH GmbH + Co. KG, Switzerland), respectively.

Collection of urine samples from captive lynx

Between August 2013 and November 2014, we collected 35 urine samples from 7 adult captive Eurasian lynx (3 males, 4 females) held at 5 different zoos in Switzerland (Tierpark Dählhölzli, Natur- und Tierpark Goldau, Tierpark Lange Erlen, Wildnispark Zürich Langenberg, Tierpark Biel). 4 of these lynx came from the Carpathian population, which is also the source population of the reintroduced wild lynx population in Switzerland. For one male and two female lynx the population of origin was unknown. Urine was collected by means of a collection device modified from a system used for Iberian lynx (Jewgenow et al. 2009). The collector consists of a stainless steel panel with a funnel at the bottom, mounted on a post and placed in front of lynx scent-marking sites in the enclosure. When lynx spray urine against the panel, the urine is collected in a 1dl glass container (Emmi, Luzern) at the bottom of the funnel. In enclosures with more than one lynx, we mounted a collector with an automatic closing system (prototype made at Theodor-Kocher Institute, Bern): when liquid drops into the sample glass with two diodes, an electric circuit was activated and the sample glass was closed with a steel lid. This prevented the urine sample from being mixed with urine from another over-marking individual. We observed the collector with a Reconyx RC55 infrared camera trap (Reconyx, Inc., Wisconsin), in order to determine age of the sample and assign samples to the proper individuals. Zoo keepers checked the collector once per day and changed panels and glass ware if urine had been collected. Glasses with urine samples were closed with a plastic lid lined with aluminium foil and immediately frozen at -20°C. They were transferred to a -80°C freezer within 2-6 months, where they were kept until chemical analysis. All glassware was cleaned before use as described in the section above. Panels were washed with soft soap (oecoplan, Coop, Basel) and water and then rinsed once with distilled water (hypotonic, pyrogen free, CARL ROTH GmbH + Co. KG, Switzerland) and once with ethanol (Merck KGaA, Darmstadt) to remove urine residues. After each sampling round, we rinsed the cleaned panels with distilled water and collected this as blank.

Collection of bladder urine samples

We also analysed 6 samples of urine collected by gently pressing the bladder of one adult female, one adult male and 3 juvenile lynx coming from the wild lynx population in Switzerland. Urine of adult lynx was collected while the animals were captured and anesthetized for radio-tagging in the

frame of our long-term monitoring programme for the lynx population in the Northwestern Swiss Alps. Urine of juvenile lynx was collected during routine veterinary check-ups of 3 orphaned lynx kept at the wildlife sanctuary Schloss Landshut, Switzerland. Urine was collected directly into 20ml headspace vials with PTFE-lined screw caps (Gerstel GmbH & Co KG, Switzerland) and treated like all other samples.

Chemical analysis

Urine volatiles were collected using solid phase microextraction (SPME) in the headspace of 4.5ml urine buffered with 0.5ml acetate. If there were less than 4.5ml of urine in a sample, distilled water (hypotonic, pyrogen free, CARL ROTH GmbH + Co. KG, Switzerland) was added until all samples had the same volume. Before extraction, 20µl 2-Heptadecanone (200 ng/20 µl Acetone; Sigma Aldrich) was added as an internal standard. Volatile adsorbtion was carried out with a Gerstel MPS2 XL Twister Multi-Purpose Sampler at 70°C for 240min using a SPME fibre with 85µm CAR/PDMS coating (Gerstel GmbH & Co KG, Switzerland). During adsorbtion, samples were agitated for 5s every minute. Chemical analysis was conducted with a GC/MS (Agilent 7890A coupled to a Agilent 5975C inert XL MSD) fitted with a 30m HP-5ms capillary column (0.25mm internal diameter and 0.25µm film thickness; Agilent, CA, USA) with a helium flow rate of 1.4ml/min. Injector temperature was 250°C and was operated in splitless mode. Initial oven temperature was 45°C held for 2min. Oven temperature was increased at a rate of 10°C/min to 70°C and subsequently at 4°C/min to 200°C and at 30°C/min to 300°C, where temperature was kept for 10min. Chromatograms were analysed using ChemStation software (Agilent, CA, USA). Volatile compounds were tentatively identified by matching their retention time and mass-spectrum with the NIST08 library (Gaithersburg, MD, USA). We identified 13 lynx-specific peaks by visually comparing urine samples to the corresponding blanks (snow/urine collector blanks respectively). Manual integration was chosen for quantification because some of the urine samples contained large amounts of S_2-S_8 sulphur species, which eluted in one to three broad smears. When present in large amounts, several long-chain fatty acids could also form broad smears, which sometimes overlapped other peaks. If sulphur smears were overlapping other compounds, we integrated what was visible of the target peak above the raised baseline caused by the sulphur smear. If fatty acids overlapped other substances, we integrated the overlapping peaks together, as well as the area of the non-target peak(s) separately, and then subtracted the latter from the total peak area, assuming the typical broad, fronting shape of the fatty acid peaks. We estimated a relative quantity of each compound by dividing the peak area by the area of the internal standard and multiplying by 200 ng. If a compound was not detectable in a sample, we assumed a very small peak area of 100, resulting in estimated quantities <0.0015ng. For each of the 13

compounds, we calculated the urine sample/blank ratios of their absolute quantities. We excluded peaks number 3 (nonanoic acid) and 5 (dodecanoic acid) from further analysis, because the medians of their urine sample/blank ratios were below 1, i.e. there was often more of this compound in snow/collector blanks than in urine samples. Peak number 1 (nonanal) was excluded because it also occurred in water blanks, has been described as a common human skin volatile (Dormont et al. 2013) and we therefore suspected nonanal concentrations in urine to be partly falsified by contamination. During the GC-analysis conducted in 2013, we ran one blank with distilled water (hypotonic, pyrogen free, CARL ROTH GmbH + Co. KG, Switzerland) between two sample-runs (*N*=32) in order to minimize carry-over from one sample to the next. Nonetheless, there was still carry-over for cyclic octaatomic sulphur. Sulphur peaks in GC-runs of H2O blanks averaged 12.2% of the peak area of sulphur in the corresponding urine sample (8.1%- 16.3% CI). In 2014, we ran two water blanks between each of two sample runs (*N*=38), thereby reducing S_8 carry-over to a mean of 5.8% of the peak area of the corresponding urine sample (4.2%- 7.4% CI). We included all lynx-specific compounds into our analysis, irrespective of their volatility at room temperature, since also low volatile compounds can be transported to and detected by the vomeronasal organ (VNO) by means of flehmen (Doving & Trotier 1998), a behaviour that has also been described for lynx (Sunquist & Sunquist 2002). All compound identifications were confirmed by careful visual inspection of the mass spectra, but since we were primarily interested in overall differences in scent profiles related to social and dietary factors, we refrained from further validation with pure compounds.

Statistical analysis

For each urine sample, we recorded sex, social status (adult: >2 years, subadult: 1-2 years, juvenile: < 1 year) and identity of the lynx and determined, whether the sample had been collected during or outside the lynx mating season (15th of February to 15th of April, according to Breitenmoser & Breitenmoser-Würsten et al. 2008). We further calculated age of the urine sample (time difference in days between urine deposition and sample collection) as well as time since the last feeding (in days). For captive lynx, feeding schedules were known and we assumed that lynx fed immediately after food was provided. For wild lynx, we searched the last kill the radio-tagged lynx had made. In order to find kills, we searched GPS location clusters (GLC's) as described in Vogt et al. (under review). Time since last feeding was then calculated as the time difference between the last GPS location in the GLC containing the last kill and the time of urine deposition estimated from GPS-telemetry and snow tracking data. In total, 70 urine samples were analysed but different subsets of the whole dataset were used depending on the type of analysis conducted (Table 1).

We conducted a principal component analysis (PCA) on the transformed quantities of 10 lynx-specific chemical compounds using the function prcomp() in R. Since the distributions of

compound quantities were heavily skewed, they were raised to the exponent 0.2 for transformation. The transformed variables were then standardised to a mean= 0 and standard deviation=1 before running the PCA. We chose the second and third PC's with eigenvalues >1 for further analysis in linear mixed models (LMM) fitted using restricted maximum likelihood (REML). Bladder samples and samples which could not unambiguously be attributed to one lynx individual were excluded from the analysis resulting in *N*= 57 samples. Models were fitted to each principal component separately including sex, mating season and their interaction as fixed factors and individual identity nested in collection method (snow, collector) as random factors.

We evaluated the scope for individual discrimination based on the amount of variation in scent-profile composition explained by individual identity as a random factor in the LMM. For a heuristic estimate of individual discrimination, we subsequently applied a heteroscedastic discriminant analysis (HDA) using the function hda() of the package *hda* in R. HDA was performed on 25 samples of two male and two female lynx for which we could collect at least 5 urine samples per individual. Lynx identity was the group identifier and the second and third PC's were the discriminant variables. The hda() function uses the naive Bayes classifier to make predictions. The percentage of correct classification was interpreted against the chance percentage of correct assignment expected for 4 individuals (chance of correct classification of 4 groups by random drawing= 25%).

We were specifically interested in the relationship between the proportion of sulphur in urine (logit-transformed) and the factors time since last feeding and age of the urine sample. We conducted a separate LMM fitted by REML, where we included all samples for which time since last feeding and sample age were known (*N*= 45). Time since last feeding (linear and quadratic term) and age of the urine sample were included as fixed factors and individual as random factor. The quadratic term was entered in the model to allow for a non-linear relationship with sulphur content in urine (i.e. sulphur may take a certain time to enter urine via amino acid metabolism). Sample type was not included as random factor since it was already represented by 0 values for sample age in bladder urine. All linear mixed models were calculated using the function lmer() of the package *nlme* in R (version 3.1.0, R Development Core Team 2013).

Table 1. Subsets of the whole dataset used for the different analyses. The subsets overlap partly.

Type of analysis	PCA	LMM (PC2/PC3~ reprod. state, sex)	LMM (S_8 content~ feeding time, sample age)	HDA (lynx identity~ PC2/PC3)
Sample size	70	57	45	25
Used samples	all samples	unambiguous individual identification, no bladder samples	unambiguous individual identification, time since last feeding known	only individuals with ≥ 5 samples

Results

Scent profiles and PCA

We analysed the compounds from the headspace of 70 urine samples coming from 23 lynx individuals. 13 compounds were present in more than trace quantities in most urine samples and consistently yielded high NIST08 library match results for the same compound identifications (Table 2).

Table 2. Compounds present in the urine of male (*N*= 11), female (*N*= 9) and juvenile (*N*= 3) Eurasian lynx.

No.	Retention time [min]	Name
1	11.2	Nonanal*
2	14.8	Nonanol, 4,8-dimethyl
3	16.1-16.5	Nonanoic acid*
4	20.4	2-Undecanone, 6,10-dimethyl
5	25.1-25.4	Dodecanoic acid*
6	26.5	Tetradecanal
7	26.8-27.9	Tetradecanoic acid, 12-methyl, methyl ester
8	29.3	Pentadecanal
9	30.5-31.1	Tetradecanoic acid
10	31.9	Octadecanal
11	*34.1*	*2-Heptadecanone (IS)*
12	35,6-36.1	n-Hexadecanoic acid
13	36.9-37.9	Cyclic octaatomic sulphur (S_8)
14	38.2	Dodecanoic acid, isooctyl ester

Compounds presented were found in urine obtained by three different sampling methods (snow, collector, and bladder). Retention time is the time taken for each compound to elute from the GC-column (in minutes). Compounds were tentatively identified by matching their retention times and mass spectra with the NIST08 library (Gaithersburg, MD, USA), and by visual inspection of the mass-spectra. Only substances with matches >80% were considered. *IS*= internal standard. * = compound excluded from further analysis.

The first three principal components of the PCA represented 82.2% of the total compound quantity variance (PC1: 57.3%, PC2: 14.4%, PC3: 10.5%). The contributions of each compound to the three PC's are shown in the loading table (Table 3). The loadings of all substances showed the same sign for PC1. Hence, this component can be thought of as reflecting differences in urine quantities or overall compound concentrations among samples resulting in higher or lower abundance of all urinary constituents in a given sample. Conversely, the second and third PC's showed loadings of opposite sign which implies that they reflect different aspects of compound composition, which are more likely to be of biological significance in the context of the present study. Therefore, we used only PC2 and PC3 for further analysis. PC2 was mainly characterized by negative loadings for 4,8-dimethyl

Nonanol and 6,10-dimethyl 2-Undecanone, as well as positive loadings for n-Hexadecanoic acid and cyclic octaatomic sulphur. PC3 was characterized by negative loadings for the aldehydes Tetradecanal, Pentadecanal and Octadecanal and positive loadings for 4,8-dimethyl Nonanol and Dodecanoic acid, isooctyl ester.

Table 3. Loadings of the PCA on 10 lynx-specific compounds (*N*= 70).

PC1	PC2	PC3	Compound
-0.3140	**-0.4342**	**0.2742**	Nonanol, 4,8-dimethyl
-0.3302	**-0.3564**	0.1807	2-Undecanone, 6,10-dimethyl
-0.3351	-0.2366	**-0.3287**	Tetradecanal
-0.3678	0.1261	0.2354	Tetradecanoic acid, 12-methyl, methyl ester
-0.3519	-0.1767	**-0.2708**	Pentadecanal
-0.3302	0.2340	0.1083	Tetradecanoic acid
-0.2321	0.0400	**-0.7394**	Octadecanal
-0.3443	**0.3200**	-0.0340	n-Hexadecanoic acid
-0.1616	**0.6327**	0.0387	Cyclic octaatomic sulphur (S8)
-0.3369	0.1494	**0.3072**	Dodecanoic acid, isooctyl ester

Compounds with loadings >|0.25| are highlighted in bold and considered to be of biological relevance for this PC.

Reproductive state, sex and social status

We found a significant negative correlation between mating season and PC2 (Table 4) suggesting that chemical composition of lynx urine varied with reproductive state. PC2 most strongly reflected changes in concentrations of cyclic octaatomic sulphur and nonanol, 4,8-dimethyl (Table 3). Thus, the observed association is partially due to lower relative abundances of cyclic octaatomic sulphur during the mating season (ø 12.3%, 9.5- 15.1% CI) than outside the mating season (ø 16.5%, 14.5- 18.5% CI). We also found a positive correlation between mating season and PC3, although this relationship was not significant. PC3 was most strongly affected by changes in concentrations of octadecanal (Table 2) and relative abundances of octadecanal were not lower during the mating season (ø 8.7%, 7.5- 9.8% CI) than outside the mating season (ø 8.0%, 7.2- 8.8% CI). We did not find significant correlations for sex with either PC2 or PC3. However, the interaction between sex and season was significantly correlated to PC3 and there was a nearly significant correlation with PC2 (Table 4). The scent profiles of both males and females changed independently: during the mating season, males showed lower values for PC3 than outside of the mating season, when values for males and females were similar (Figure 1). Conversely, females showed lower values for PC2 than males during the mating season. Before or after the mating season their values for PC2 were larger than for males.

Table 4. Parameter estimates of the linear mixed models (LMMs) for the second and third principal component.

PC2	**Estimate**	***SE***	**t-value**	***P***
intercept	0.232	0.247	0.939	0.354
sex= male	-0.666	0.365	-1.824	0.087
Mating season= yes	-1.264	0.310	-3.163	**0.003**
Sex*season	0.943	0.512	1.844	0.073
PC3	**Estimate**	***SE***	**t-value**	***P***
intercept	-0.032	0.362	-0.087	0.931
sex= male	0.454	0.357	1.271	0.222
Mating season= yes	0.800	0.464	1.722	0.094
Sex*season	-1.754	0.586	-2.995	**0.005**

The LMMs were fitted to the data assuming a normal error distribution and using Restricted Maximum Likelihood (REML). The second and third principal components (PC2, PC3) derived from a PCA on the absolute quantities of 10 compounds found in lynx urine were set as response variables. *SE*= Standard Error. Levels of the fixed factors are compared as follows: sex= male is compared to sex= female, mating season= yes is compared to mating season= no. The analysis was conducted on data from 57 urine samples. Lynx identity nested in sample type (snow, collector) was included as random effect. Sample type explained <0.1% (PC2, σ< 0.001) and 16.4% (PC3, σ= 0.154) of the variance in the data. Lynx identity explained 49.5% (PC2, σ= 0.363) and 26.1% (PC3, σ= 0.245) of the variance in the data. *P*-values < 0.05 are indicated in bold script.

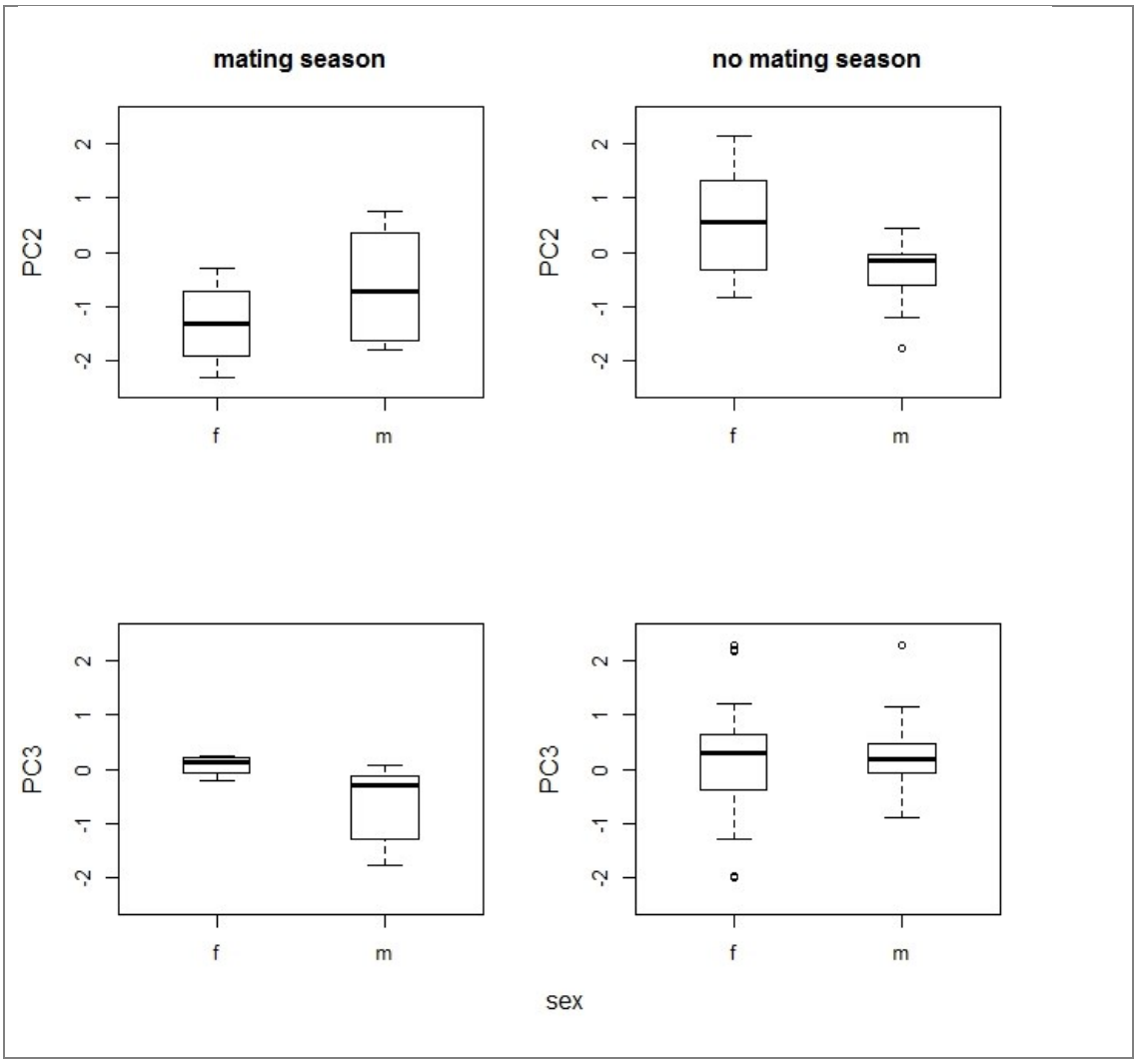

Figure 1. Differences between chemical profiles of male and female lynx during and outside the mating season. PC2/ PC3= scores of the second and third principal component of the PCA incorporating 10 lynx-specific compounds (*N*= 57). F= females, m= males. Mating season= 15th February- 15th April. Each box encompasses the 25th through 75th percentiles, with the median represented by an interior line. Whiskers denote maximum values or in case of outliers 1.5 times the interquartile range. Circles denote outliers.

Sample size of subadult lynx was too small for statistical testing (3 samples from 2 individuals). 4 urine samples of 3 juvenile lynx were collected by pressing the bladder of anesthetized individuals, but we collected only two bladder urine samples from adult lynx. Therefore, variation in scent profiles due to social status could not be disentangled from variation due to different sampling procedures. Nonetheless, a PC biplot of the second and third principal components showed that bladder urine samples of juvenile lynx were more closely clustered than bladder urine samples of adults (Figure 2).

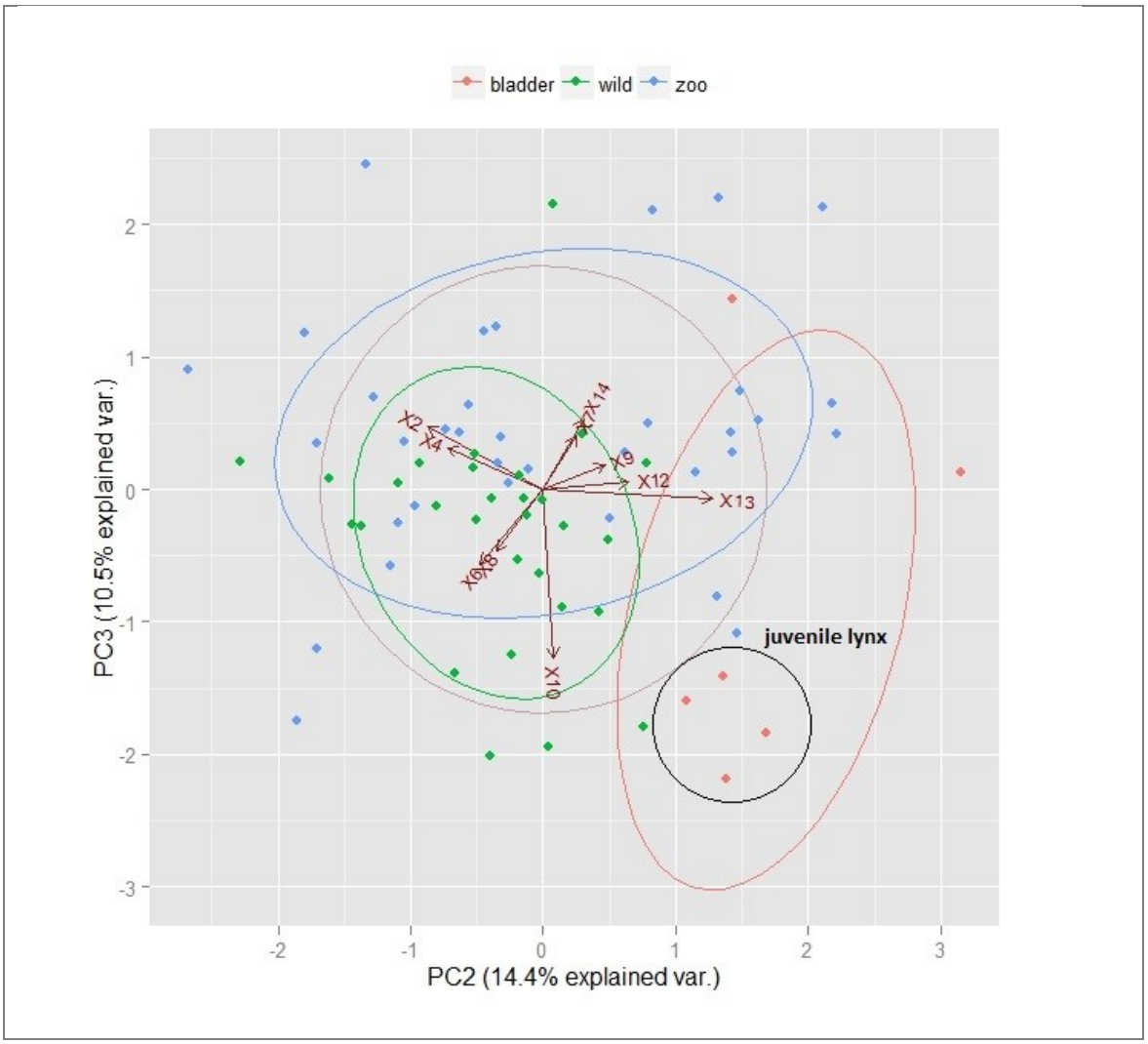

Figure 2. PC-biplot of the second and third principal components of the PCA incorporating 10 lynx-specific compounds (*N*= 57). The colours denote different sampling procedures. Bladder (red)= bladder urine collected from anaesthetized juvenile and adult lynx, wild (green)= urine collected from adult and subadult wild lynx during snow tracking, zoo (blue)= urine collected from adult zoo animals with a collection device. Grey circle= correlation circle, ellipses= 68% normal data ellipses for each group. Black circle= bladder urine samples collected from juvenile lynx.

Lynx identity

Lynx identity explained 49.5% and 26.1% of the variance in the data for PC2 and PC3, respectively (Table 4). Furthermore, the heteroscedastic discriminant analysis (hda) enabled the correct classification of 69% of the urine samples (as opposed to the chance percentage= 25% for 4 individuals).

Dietary cues

Relative sulphur content in urine samples was not correlated to time since last feeding, neither to the linear nor to the quadratic term (Table 5). There was, however, a significant correlation with age of the urine sample (Table 5). The proportion of sulphur in lynx urine decreased with time after urine deposition (Figure 3).

Table 5. Parameter estimates of the linear mixed model (LMM) for relative sulphur content in lynx urine samples.

	Estimate	*SE*	t-value	*P*
intercept	-1.411	0.133	-10.612	< 0.001
time since feeding	-0.053	0.122	-0.437	0.666
(time since feeding)^2	0.004	0.018	0.255	0.801
sample age	-0.338	0.086	-3.912	**< 0.001**

The LMM was fitted to the data assuming a normal error distribution and using Restricted Maximum Likelihood (REML). The relative abundance of sulphur in the urine sample was logit-transformed and set as response variable. *SE*= Standard Error. The following factors were included as fixed effects: time since last feeding (in days) and age of urine sample (in days). Time since feeding is also entered as quadratic term. The analysis was conducted on data from 45 urine samples for which time since last feeding and age of urine sample could be estimated. Lynx identity (σ= 0.060) was included as random effect and explained 29% of the variance in the data. *P*-values < 0.05 are indicated in bold script.

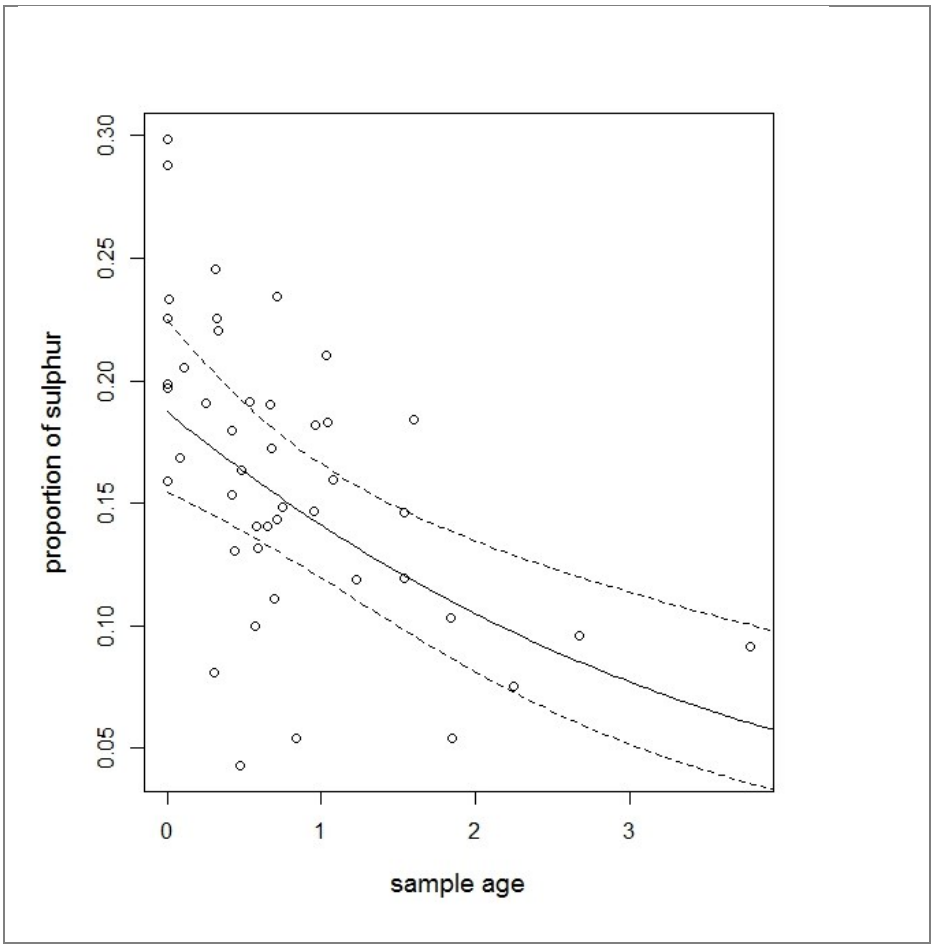

Figure 3. Proportion of sulphur in urine decreases with age of the urine sample (in days). Solid line= fitted values from the LMM, dashed line= 95% confidence intervals.

Discussion

Reproductive state, sex and social status

By analysing the volatile fraction of Eurasian lynx urine, we were able to detect constituents belonging to the following compound classes: carboxylic acids, aldehydes, ketones, esters, and elemental sulphur. High levels of carboxylic acids and ketones were also identified in the urine of tigers and lions (Andersen & Vulpius 1999; Burger et al. 2008) and were suggested to have a function in chemical communication (Soini et al. 2012) and possibly individual recognition (Poddar-Sarkar & Brahmachary 1999). In this study, we were able to relate changes in the relative quantities of the above mentioned compounds to sex and reproductive state. We detected changes in the chemical profiles of male lynx reflected in significantly lower PC3 values during the mating season. Scent profiles of males could be altered during mating season due to changes of androgen levels or differences in body condition (Petrulis 2013). Many female mammals are attracted to the odours of reproductive male conspecifics and preferences for individual males are often related to signals of

quality or social status (Petrulis 2013; Roberts et al. 2014). On the other hand, male mammals have been shown to be attracted to odours of oestrous females (Petrulis 2013; Scordato et al. 2007; Swaisgood et al. 2000) and Eurasian lynx are known to search and mate-guard females during mating season (Breitenmoser & Breitenmoser-Würsten et al. 2008). If urine-marks are the means by which female lynx attract males when they are receptive, we would expect that the chemical profile of females should change when they are ready to mate. Our results show some evidence for such changes reflected in PC2 values, although the result is not significant.

PC2 and PC3 were most strongly influenced by concentrations of octadecanal, cyclic octaatomic sulphur, and 4,8-dimethyl nonanol but no conclusions about the role of these substances in lynx reproductive behaviour can be made. Experimental studies are needed to identify the compounds possibly involved in mate attraction in Eurasian lynx. However, pinpointing actual chemosignals involved in mate attraction in mammals is often very difficult (Petrulis 2013), even though many studies, including ours, have found differences in scent profiles related to sex or season (e.g. catta *Lemur catta*, Scordato et al. 2007; owl monkey *Aotus nancymaae*, Macdonald et al. 2008; brown bears *Ursus arctos*, Rosell et al. 2011; short-beaked echidna *Tachyglossus aculeatus*, Harris et al. 2014). Empirical evidence collected until now suggests, that it might be subtle shifts in overall scent composition rather than a single sex pheromone which are mediating mate attraction in mammals (Petrulis 2013).

Unfortunately, we were not able to explore potential information on social status and test for differences in scent profiles between juvenile, subadult and adult lynx due to low sample sizes and differences in sampling procedure. However, scent profiles from four juvenile lynx showed great qualitative similarity among each other and tended to cluster together in a PC-biplot. Scent profiles can serve as badges of social status, as shown e.g. in spotted hyenas *Crocuta crocuta* (Burgener et al. 2009), blackbucks *Antelope cervicapra* (Rajagopal et al. 2010), or mice (Mossman & Drickamer 1996), and social status is often related to the age of an animal. In Eurasian lynx, subadult lynx disperse from the home ranges of their mothers in their second year of life in search of a vacant home range. It is the adult resident lynx who occupy stable home ranges and can be considered as dominant resource holders (Breitenmoser & Breitenmoser-Würsten 2008). Wild juvenile lynx show interest in adult's scent-marks but have not been observed scent-marking themselves (Vogt et al. 2014). Further studies on the ontogenetic changes of scent profiles and their relationship with the onset of scent-marking behaviour and the acquisition of resident status should be conducted.

Lynx identity

Our linear mixed models on the factors influencing chemical composition of lynx urine revealed substantial variation between individuals (PC2: 49.5% and PC3: 26.1% of the variance in the data was explained by lynx identity). Furthermore, the results of our heuristic approach (the heteroscedastic discriminant analysis on four individuals for which the largest number of urine samples was available) demonstrate that individual variation is present and could be used for individual recognition, since the percentage of correct classification was higher than expected by chance. Captive Eurasian lynx have been shown to discriminate between urine of familiar and unfamiliar conspecifics (Sokolov 1996) and a study on scent-marking behaviour of wild lynx has shown that lynx overmark urine marks of other lynx more readily than their own old urine marks (Vogt et al. 2014). While this behaviour alone may not provide sufficient evidence for individual recognition, it shows that lynx can learn to recognize scent profiles. The social organisation of wild lynx populations further suggests possible roles for individual recognition in the contexts of territoriality or mate choice: resident lynx may have to respond differently to the scent-marks of strange intruders compared to scent-marks of neighbouring residents (dear enemy phenomenon, Temeles 1994) or they may learn to recognize the individual scent profiles of sexual partners for whom they have developed a mating preference, as has been described in mice (Roberts et al. 2014).

Dietary cues

Dietary cues are known to act as signals of quality to conspecifics (Ferkin et al. 1997) and may also be exploited by prey species to adjust their anti-predatory behaviours (Chivers & Mirza 2001; Murray & Jenkins 1999). In wild felids, one possible source of dietary cues in urine is the degradation of sulphur-containing amino acids to volatile organosulphur compounds via production of felinine (Hendriks et al. 2001; Mattina et al. 1991; Miyazaki et al. 2006). Although Eurasian lynx likely produce felinine (Miyazaki et al. 2008), organosulphur compounds have only been detected in very low quantities in lynx urine during an earlier study (Zachariae, pers. comm). In this study, we were unable to detect organosulphur compounds, but we found high amounts of elemental sulphur S_8 in almost all lynx urine samples. Estimated concentrations in undiluted bladder urine were quite similar to those in cheetah urine (median= 1.0µg/ml, lower quantile= 0.5µg/ml, upper quantile= 1.8µg/ml) and much larger than those of S_8 found in tiger urine (Burger et al. 2008). We found that elemental sulphur was part of the overall signal for reproductive state and sex (see above), but we were also specifically interested in its possible role as a dietary cue or as an indicator of freshness of a scent-mark. We expected the proportion of S_8 in lynx urine to be related to food intake, which we measured in terms of time since last feeding. However, we did not find any association. Wild lynx

normally fast for several days between kills (Breitenmoser & Breitenmoser-Würsten 2008) and it is possible that our recorded time spans (1-186 h) were not long enough to reflect cysteine depletion. We were also only able to estimate when lynx were feeding but not how much food was ingested. Thus, it remains unclear whether S_8 content really conveys information on food condition in Eurasian lynx. On the contrary, we did find a relationship between S_8 content and sample age, i.e. the proportion of sulphur in lynx urine decreased with increasing sample age. Elemental sulphur can be converted to sulphates or sulphuric acid by thiobacteria or sulphur bacteria under aerobic conditions (Dévai et al. 1996; Waksman & Joffe 1922). The concentration of derivatives of these products could serve as an indicator of freshness of the scent-mark to other lynx and potentially to prey animals. Felids lack the great variation of major urinary proteins (Miyazaki et al. 2008) which are known to bind volatile chemosignals in mouse urine and extend the longevity of scent-marks (Roberts et al. 2014). In tigers, lipids added to urine in the urinary tract are thought to fulfil this function (Brahmachary & Poddar-Sarkar 2015). Lynx urine does not contain such a lipid fraction (own observation) and it is conceivable that S_8 may serve as a source for continuous release of chemosignals from urine. It still remains to be investigated whether this proposed mechanism could also serve to conceal dietary cues in fresh urine-marks and release them only with time.

Apart from protein metabolites, compounds produced by lipid metabolism such as carboxylic acids can also hold information on health and metabolic condition (Soini et al. 2012). We detected several carboxylic acids in lynx urine, among them n-hexadecanoic acid which was also found in tiger and bobcat urine and is a constituent of commercial deer repellent (Burger et al. 2008; Mattina et al. 1991). It is a molecule also present in members of other carnivore families (e.g. Iberian wolf *Canis lupus signatus*, Martin et al. 2010; brown bear *Ursus arctos*, Rosell et al. 2011; wild dog *Lycaon pictus* and black-backed jackal *Canis mesomelas*, Apps et al. 2012) and preliminary analysis showed that its proportion was not related to sample age in our study (Spearman's rank correlation, *rho*= -0.029, *S*= 14602.55, *p*= 0.851), hence it is available for prey animals to react to in fresh as well as older scent-marks. Many studies on predator urine have so far either been conducted on fresh or old urine samples (when using commercial urine products, as in Mattina et al. 1991). Experimental manipulation of urine age and predator diet may shed more light not only on chemical processes involved in generation of dietary cues but also on how such cues can be exploited by con- and heterospecifics.

Conclusions

Our results demonstrate that lynx urine contains sex-specific information on reproductive state, as well as individual variation that may be used for individual recognition. Urine marks are therefore

well-suited to fulfil a role in reproductive behaviour and social organisation of wild lynx populations. We further found that S_8 content in urine was related to sample age. While our study provides first insights into the chemical information contained in lynx urine, the mechanisms involved in the unusually high elemental sulphur excretion in Eurasian lynx and cheetah are still unknown. The influence of diet and body condition on scent profiles should be further investigated by means of experimental studies and may shed more light on the messages encoded in carnivore scent-marks.

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Conclusions

Summary of results

During my PhD, I had the opportunity to approach a research field from different angles using interdisciplinary methods. The main goals of my thesis were to explore the functions of scent-marking in wild lynx populations, to investigate possible trade-offs between scent-marking and hunting behaviour in radio-collared lynx, and to analyse the information content of individual urine marks.

In the first part of my thesis, I could show that communal marking sites play an important role in communication between male and female lynx and in competition between males. Scent-marking activity was highest during the mating season and marking sites were visited much more often by male lynx. I could show that lynx were able to discriminate between self and non-self and over-marked urine marks of strangers more readily than their own old scent-marks. I demonstrated that over-marking does not mask the underlying scent-mark but that the information from both, the top- and the underlying scent-mark remains available, supporting the function of communal marking sites as “chemical bulletin boards”.

I then participated in the development of a field method to identify potential kill sites from GPS location clusters (GLCs) of GPS-collared lynx. We could show that our method enabled us to reliably find large as well as small prey items and that the majority of kills were found in GLCs lasting ≥ 9 hours. I then used this method to identify (potential) kill sites for the next part of my thesis.

During this study, I was able to show that overall scent-marking rate was lower when lynx were hunting but that hunting lynx increased scent-marking rates in places, where there was a high chance of detection by conspecifics (along forest roads). Lynx also increased scent-marking rates during mating season but only when they were not hunting. My results suggest that lynx face a trade-off between enhancing the detection probability of scent-marks by conspecifics and avoiding eavesdropping by prey, but also indicate that scent-marking rate is influenced by several factors.

Finally, I could show that lynx urine contains sex-specific information on reproductive state, as well as individual identity cues. Urine marks are therefore well-suited to fulfil a role in reproductive behaviour and social organisation of wild lynx populations. I further discovered high amounts of

cyclic octaatomic sulphur in urine samples, which had so far only been observed in cheetahs (Burger et al. 2006). Relative sulphur content decreased with age of the urine sample which could serve as an indicator for the freshness of a scent-mark. This information might be exploited by other lynx and potentially by other species.

Constraints and suggestions for future studies

Functions of scent-marking behaviour

My observations of communal marking sites by means of infrared camera-traps enabled me to collect a large sample of interactions between male lynx (244 observations, 19 individuals) but I collected much fewer observations of females (47 observations, 10 individuals). I originally planned to investigate the social functions of scent-marking in female lynx more closely during the snow-tracking study. During this work, I indeed discovered that female lynx scent-marked more often than I had expected from my camera-trap observations and from literature review (Hucht-Ciorga 1988; Mellen 1993; Zheltukhin 1984), although still less often than males. While I was able to relate scent-marking rates of 15 GPS-collared lynx individuals to several social and environmental factors, I unfortunately remained unable to test specific hypotheses on the functions of scent-marking in social organisation of females. This was due to the fact that we were not able to capture and tag two neighbouring females in the same year. Capturing and tagging large mammals always involves a huge effort in terms of time and money invested and researchers need to balance the effort spent on acquiring study animals with the time invested in actually following the tagged animals and collecting data in the field. Moreover, when studying wide ranging species with low encounter rates and a long generation time such as Eurasian lynx, research projects are often too short to address interesting questions concerning interactions that are not very frequent (e.g. mating events, aggressive interactions between neighbours, occupation of vacant territories, etc.), unless long-term data sets are already available. Due to such practical issues, field studies taking into account interactions among neighbouring territory holders are generally rare in wild mammals and are mostly restricted to open habitats with high visibility of study animals (e.g. Gosling & Roberts 2001b; Henschel & Skinner 1991; Roberts & Lowen 1997; Sillero-Zubiri & MacDonald 1998). Considering that the function of scent-marking in territory maintenance of wild felid populations living in forested ecosystems is less well understood, it would be very interesting to relate scent-marking rates to temporal changes in intrusion pressure in different parts of felid home ranges. One way to address this in a future study would be to choose a study species with a land tenure system similar to the Eurasian lynx but with smaller home range sizes, e.g. the European wildcat *Felis sylvestris*, and combine GPS-telemetry, snow tracking and camera trapping data. Working in an area with

predictable snow conditions where scent-marks can be detected over an extended period of time would ensure that infrequent interactions are not missed due to unfavourable weather conditions.

Interactions with prey animals

During my study on lynx hunting behaviour, I noticed that lynx hunt repeatedly in the same areas with visitation rates ranging from a few weeks to several months. Under the aspect of costs and benefits of scent-marking behaviour and considering the risk-allocation hypothesis (Lima & Bednekoff 1999), it would be very interesting to relate visitation rates of hunting areas to hunting success and scent-marking rates. By measuring how often different areas are (successfully) used for hunting and how scent-marking rates differ among these areas, we might get a better understanding of the factors determining hunting success, the benefits lynx may gain by marking their food resources, and the costs of scent-marking in terms of inter-specific eavesdropping. A comprehensive approach should also integrate the reactions of GPS-collared ungulate prey (e.g. roe deer) to encounters with GPS-collared lynx and to experimentally presented lynx urine and test whether ungulates alter their movement or activity patterns. This has so far only been tested by means of camera-trapping or direct observations (Eccard et al. 2015; Wikenroos et al. 2015), which give only very limited insight into changes in spatial behaviour of prey animals. Variation in vulnerability of prey mediated by antipredatory behaviour can influence prey selection (Fitzgibbon 1990) and may also hold implications for conservation and management. For example, the predation impact of Eurasian lynx on roe deer can be considerable (Andrén & Liberg 2015) and is one of the reasons for negative attitudes of hunters which can in turn hamper lynx conservation and reintroduction programmes (Luchtrath & Schraml 2015). Swiss hunters not only argue that lynx reduce roe deer densities but also that roe deer become more shy and difficult to hunt when lynx are present. However, there is so far little evidence for responses of roe deer to lynx presence concerning spatial behaviour (Samelius et al. 2013). During a study as outlined above, considerable difficulties concerning sample sizes, encounter rates of predator and prey, and predictability of snow cover would have to be overcome. Such a study would probably have to be embedded into a larger research programme following additional research objectives, in order to warrant the financial investment and field effort needed. Undoubtedly, it would add substantial knowledge to the field of predator-prey interactions to study such behavioural interactions not only in rodent systems but also in large mammal communities.

Chemical analyses

My results give novel insights into the type of information encoded in lynx urine and my method was sensitive enough to detect enough variation in lynx scent profiles in order to relate it to sex, reproductive status and identity. Still, some questions remained unsolved due to logistical problems. Firstly, the number of samples per lynx was low for most individuals due to low marking rates of captive lynx at urine collectors, technical problems with the prototype of the automatic collection device, the dependency of urine collection during snow tracking on particular snow conditions, and the fact that adult wild lynx often release bladder urine during captures before it can be collected. With more samples per lynx we might have been able to detect differences in scent profiles due to social status, i.e. age class. With more time and a larger sample of related animals, it would also have been interesting to correlate similarities in scent profiles to coefficients of genetic relatedness. Secondly, some methodological problems arose which would have required more lab work to solve. We chose a non-polar GC column for chemical analysis, since similar columns have been used in other studies on mammal urine (Andersen & Vulpius 1999; Burger et al. 2006) and are able to detect ketones and fatty acids, which have previously been suggested to play a role in chemical signalling in tigers (Burger et al. 2008; Poddar-Sarkar & Brahmachary 1999). However, if long-chain fatty acids are present in large amounts, they are sometimes eluted from non-polar columns as broad smears overlapping the peaks of other substances, which makes it hard to calculate peak areas. This can be avoided using polar columns, from which carboxylic acids elute as sharp peaks (Burger et al. 2008). Although we found a standardised method to deal with peak overlap during our analysis, it would be worth trying a GC-column of intermediate polarity in order to mitigate this problem. Moreover, the use of a more polar GC-column would also have the advantage that organosulphur compounds would be better detectable. I became interested in the role of organosulphur compounds as dietary cues and kairomones exploited by prey animals during literature search (Hendriks et al. 2001; Lewison et al. 1993) and while developing possible hypotheses for the high excretion of elemental sulphur in lynx urine. In future studies, collaboration with physiologists and biochemists would help to shed more light on possible pathways and functions of the unusually high sulphur excretion in the urine of Eurasian lynx, cheetah, and possibly other cat species. It would also be interesting to experimentally manipulate lynx diet and investigate subsequent changes in scent profiles and possibly female attraction to scent-marks of males fed on different diets. In order to successfully conduct such studies, the efficiency of urine sample collection should be increased but animal welfare issues have to be considered at the same time. Commercial products based on predator urine are usually produced using bladder urine from hunted individuals or by locking animals into small cages for 24 hours and collecting excretory urine from a trough at the bottom of the cage. This method is not feasible for wild animals and inacceptable to most zoo facilities. Also the collection of

bladder urine from anesthetized animals should not be a stand-alone method, since every anaesthesia is potentially harmful for the animal. I would recommend future researchers to improve our prototype of the automatic urine collection device and cooperate with other research programmes which necessitate anaesthesia of animals for other purposes, as we have done for this study.

Final conclusions

This work demonstrates that scent-marking plays an important role in communication between potential mates and rivals and, hence, in social and spatial organisation of Eurasian lynx populations. As such, it contributes to a better understanding of the functions and constraints of chemical signalling in wide-ranging solitary carnivores, which have so far been much less studied than social species. To date, field studies have provided evidence for different (social) functions of scent-marking in mammals and there is support for several hypotheses, which are not necessarily mutually exclusive (Ferkin 2015; Ferkin & Pierce 2007; Gosling & Roberts 2001a,b). My thesis provides empirical data that allows testing of different hypotheses on over-marking and inter-specific eavesdropping and contains two of the few studies on chemical communication conducted in wild populations under natural conditions. I was also able to chemically analyse the information content of lynx urine and relate it to the suggested functions of urine marking in the wild. My results offer many directions for further research on the role of scent-marking in territoriality, mate choice, and competition in wild felid populations, as well as on the importance of eavesdropping in predator-prey interactions. Increasing our knowledge on all these aspects is an important step in understanding chemical communication in mammals and the evolution of chemical signalling in predator-prey systems.

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