AN INVESTIGATION OF THE OLFACTORY, VOCAL AND MULTI-MODAL COMMUNICATION OF AFRICAN LIONS (*PANTHERA LEO*) IN THE OKAVANGO DELTA, BOTSWANA

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SUMMARY

Communication is a crucial mechanism at the basis of animal social behaviour and likely to be of central importance in facilitating the evolution of complex animal societies. This thesis aims to expand our knowledge of the olfactory, vocal and multi-modal communication of wild African lions. Much of our understanding of lion behaviour originates from studies in East Africa, yet the ancestral lion may have been a wetland specialist in habitats such as the Okavango Delta. Here I first employ an established playback design to test whether lions can determine the number of conspecifics calling in large vocal choruses, demonstrating that the upper limit for lions to assess the number of simultaneous callers is three, matching the ability of humans performing a similar task. I then use a novel playback experiment to demonstrate that lions are capable of cross-modal processing of information on individual identity; an ability originally thought to be unique to humans and not previously demonstrated in wild animal populations. Next, I provide a novel and detailed investigation into the olfactory communication of lions. First I analyse the scent-marking of lions and the responses of group members to marks, and demonstrate that chemical signals may play an important role in the social lives of prides. I then use a scent presentation experiment to test the function of urinary scent-marks in communication within and between prides, determining that lion urine signals the social group and sex of the depositor and may be important for sexual assessment and territory defence. Overall this thesis significantly advances our knowledge of the vocal and olfactory communication of African lions, and provides the first evidence that lions are capable of cross-modal individual recognition during communication between conspecifics. Together these results highlight that olfactory and multi-modal communication are important for lions, despite being previously overlooked.

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CHAPTER 1: GENERAL INTRODUCTION

The aim of this thesis is to further our knowledge of the communication strategies of African lions (*Panthera leo*), but also to more broadly contribute to the field of animal communication research. In this introductory chapter, I first review our current understanding of how animals communicate, specifically focusing on vocal, olfactory (chemical), and multi-modal signalling. As will become apparent, we now have a deep understanding of the mechanisms and functions behind vocal communication in vertebrates, and research in this area is at a more advanced stage than research into olfactory and multi-modal communication. Despite the fact that olfactory communication (i.e. communication through smell and taste) is believed to be the oldest form of communication between organisms, research results on which species use chemical signalling, and how chemicals are used in communication are still relatively scarce. Likewise, the study of multi-modal communication is still a novel field within the broader study of animal communication, but evidence is accumulating to show that animal communication signals may often involve information being transmitted across multiple sensory channels. Following this, I will provide an overview of the current knowledge of African lion behavioural ecology, specifically focusing on their vocal and olfactory communication. I will then discuss the gaps in our knowledge regarding lion communication, and I will outline how this thesis aims to address these unanswered questions. Finally, I will describe the study site and general methodology with which this research was conducted.

Animal communication background

Animal communication is the intentional and beneficial transmission of information from one animal (the sender) to another (the receiver) that affects the current or future behaviour of the receiver (Slater, 1983). Communication is a crucial mechanism at the basis of animal social behaviour and is likely to be of central importance in facilitating the evolution of complex animal societies (Bradbury and Vehrencamp, 2011). The study of animal communication is a broad and rapidly expanding topic that is of fundamental importance to the disciplines of behavioural ecology, evolutionary biology, conservation biology, and animal cognition (Bradbury and Vehrencamp, 2011).

Natural selection has driven the evolution of animal communication systems because there is a net benefit to signallers who affect the behaviour of receivers, as well as a net benefit to receivers that use this information to inform their decisions in their environment (Seyfarth and Cheney, 2003a). A wide range of different behaviours involve communication between animals, including foraging behaviour (e.g. the 'waggle' dance of honeybees, *Apis mellifera*: Von Frisch, 1967; Seeley, 1997), mating behaviour (e.g. the roars of red deer, *Cervus elaphus*: Charlton, Reby and McComb, 2007), and territorial defence (e.g. latrine use by European badgers, *Meles meles*: Roper, Shepherdson and Davies, 1986). Animals use communication signals to inform intended receivers of a range of morphological (e.g. body size: Fitch and Reby, 2001), physiological (e.g. reproductive state: Charlton, 2014), emotional (e.g. aggression: Seyfarth and Cheney, 2003b), or environmental (e.g. predator presence: Manser, 2001) states.

While the benefits of information signalling are fundamentally important for the evolution of communication signals, animal communication systems are also

constrained by signalling costs. The costs of communication can be intrinsic (e.g. signal production costs or costs associated with time taken away from other important behaviours such as foraging) or extrinsic (e.g. costs imposed by unintended eavesdroppers). Intrinsic costs can be substantial, but are more easily quantified for small, cold-blooded animals that continue to perform natural communication behaviours in controlled laboratory settings. For example, in the gray treefrog (*Hyla versicolor*), oxygen consumption of calling males is 21 times higher than the basal level, representing a significant energetic expense (Taigen and Wells, 1985). Intrinsic costs of communication can also be imposed on receivers of signals, primarily as receivers may need to devote time away from other important behaviours when paying attention to signals (Dawkins and Guilford, 1991). In particular, many displays of the sender's 'quality' or 'resource holding potential' (RHP) relate to signal duration (Burk, 1988), and signalling consequently represents a large time investment for both sender and receiver. In general, receiver costs are likely to be lower than signalling costs paid by the sender, but could nevertheless have important consequences on the evolution of communication systems (Dawkins and Guilford, 1991).

For most broadcast communication signals (e.g. many visual, vocal or olfactory signals), there will also typically be a whole network of unintended receivers (i.e. eavesdroppers), both conspecific and heterospecific (Dabelsteen, 2004; Magrath, Pitcher and Gardner, 2009). As signallers do not intentionally provide eavesdroppers with information, and such events are often not beneficial for signallers, eavesdropping is not classed as 'true' animal communication (Slater, 1983; Lehmann et al., 2014). Nonetheless, extrinsic communication costs associated with eavesdropping may place large constraints on signal evolution. For example, males of the túngara frog (*Physalaemus pustulosus*) call to deter rival males and to attract females (Ryan, 1985),

but their calls also attract the predatory fringe-lipped bat (*Trachops cirrhosus*). Both female frogs (Akre and Ryan, 2010) and predatory bats (Ryan, Tuttle and Rand, 1982) are attracted to males who produce more complex calls, and predation by bats represents a significant risk to vocalising males (Ryan, Tuttle and Taft, 1981). The túngara frog illustrates that communication signals evolve among cost-benefit trade-offs, and it is important to understand these trade-offs when investigating animal communication.

Even between the sender and the intended receiver there can be conflict that constrains the evolution of communication signals. All organisms are made of inherently 'selfish genes', whose primary 'goal' is to replicate themselves in future generations, at the expense of other selfish genes (Dawkins, 1972). As communication often functions to signal information regarding the morphological or physiological characteristics of the sender during resource competition or mate acquisition, it could be adaptive for senders to try to deceive receivers with dishonest signals (Dawkins and Guilford, 1991). Dishonest signals could exaggerate the signaller's advertised RHP to attract more (or better quality) mates, or to deter resource competitors. However, as the costs of being deceived could be substantial, concurrently there would be counter-selective pressures imposed on receivers to detect when they are being 'cheated' (Searcy and Nowicki, 2005). Therefore, an evolutionary arms race between the sender and receiver could help to maintain an evolutionary stable strategy (ESS) in which communication signals are primarily honest (or in which the majority of signallers are honest) in regards to providing accurate information to receivers, either about properties of the signaller itself, or its environment (Burk, 1988; Dawkins and Guilford, 1991).

Irrespective of sender-receiver conflict, the properties of the signals themselves can help to maintain honest signalling in a communication network. For example, 'handicap

signals' can have high production costs, particularly for low-quality individuals (Zahavi, 1975). Only the best quality individuals within a population would be able to produce high-quality handicap signals, while continuing to survive. Consequently, the quality of the handicap signal could then be used as an honest advertisement of the sender's quality or RHP. While the existence of 'true' handicap signals is still under much debate (e.g. Számadó, 2011), the classic example of a potential handicap signal is the tail feathers of male Indian peafowl (*Pavo cristatus*, but see recent work by Thavaraja et al., 2016). Alternatively, 'index signals' that are reliably constrained by physiological and/or developmental mechanisms can create an honest link between signaller quality and signal size/intensity (Maynard Smith and Harper, 1995). For example, mammalian vocal signals often provide accurate information regarding caller body size (Taylor and Reby, 2010), even when there has been evolutionary pressure for callers to extend their vocal tract length and exaggerate caller body size (Fitch and Reby, 2001). The maintenance of honest signalling has been an important driver in the evolution of animal communication. Without honest signals, the manipulated receiver would lose the benefits of using the information within communication signals, and communication systems would break down (Searcy and Nowicki, 2005). Crucially, honest signals do not need to provide 'perfect' information to receivers to persist as an ESS. Signals only need to be informative, on average, to promote communication (Johnstone and Grafen, 1993). Therefore, within any communication system, dishonest signals or dishonest signallers that do not (or do not always) provide accurate information can still persist at low levels within a population (Johnstone and Grafen, 1993; Számadó, 2000; Memmott and Briffa, 2015).

Vocal communication

Different groups of animals rely on different sensory channels for communication (Krebs, Davies and Parr, 1993), with most communication taking place within the visual, auditory and olfactory mediums (Bradbury and Vehrencamp, 2011). Humans, in common with most other primates, rely on both auditory and visual communication (Bradbury and Vehrencamp, 2011), which has resulted in a bias favouring the study of these particular communication modalities in other species. Consequently there is a rich history of research into acoustic communication (Raymond, 1947; Kroodsma, 1989; Hernandez et al., 2016), and it is now possible to employ more complex experimental paradigms to investigate the fine-scale information content of acoustic signals.

Animals produce a tremendous diversity of sounds to facilitate crucial social interactions ranging from mate attraction and reproductive stimulation, to territory defence and predator deterrence (Krebs, Davies and Parr, 1993; Owings and Morton, 1998; Gillooly and Ophir, 2010). All acoustic communication systems involve sounds that are produced by a simple vibrating membrane that perturbs the surrounding environmental medium (Bradbury and Vehrencamp, 2011). Vocal communication (involving sounds produced by the vocal apparatus) is one of the primary mediators of information transfer among vertebrates, and we now have a relatively strong understanding of 'voiced' vertebrate vocal signal production: the 'source-filter theory' (reviewed in Taylor and Reby, 2010).

The 'source-filter' mechanism of vocal production

For most cases of acoustic communication in vertebrates, air is exhaled from the lungs through the larynx (or equivalent structure such as the avian syrinx), providing power to drive oscillations of the vocal folds that produces sound waves (the 'source' or glottal wave). The rate of vocal fold vibration determines the fundamental frequency (F0) of

the sound wave, and its associated harmonics (whole number multiples of the fundamental frequency right the way up the frequency spectrum). Asymmetrical vibration and the collision of the vocal folds (as well as recirculation of air through the glottis) create the harmonics, which provide multiple frequencies that can be resonated or dampened in the 'filtering' process (Titze, 2008). Once past the larynx, the glottal wave passes through the vocal tract 'filter' and radiates out through the mouth and/or nasal openings. Like any hollow structure, the vocal tract has natural resonance frequencies (frequencies of vibration) that amplify corresponding frequency bands within the glottal sound wave, creating 'formants' that give the sound its timbre (Taylor and Reby, 2010). Important to note is that not all sounds produced by vertebrates involve vocal fold vibrations and subsequent vocal tract filtering. 'Unvoiced' or 'voiceless' signals such as whistles in dolphins (King and Janik, 2013) are still produced by vibrations of a membrane (e.g. the lips), but are not subsequently filtered by the vocal tract. Furthermore, some acoustic signals are not produced by the vocal apparatus, but by vibrations from other body parts (e.g. knee-clicking in ungulates: Bro-Jørgensen and Beeston, 2015).

Properties of the vocal source and honest signalling

The source-filter dynamics of animal vocal signals have played a significant role in the evolution of vocal communication in vertebrates. The properties of the glottal sound wave are primarily determined by the length and muscular tension (turgidity) of the vocal folds in the larynx, as well as the sub-glottal pressure (the force that the air is expelled from the lungs through the glottis/vocal fold gap: Fink, 1975; Hardcastle, 1976). Longer and looser vocal folds result in a lower pitched vocalisation (lower F0), while higher sub-glottal pressure results in a higher pitched vocalisation (Titze, 1994; Fitch, 1997).

Across species, caller body size is negatively correlated with the F0 of vocalisations, but within a species, larger callers do not reliably produce calls with lower F0 (Taylor and Reby, 2010; Charlton and Reby, 2016). The soft tissue structures of the larynx mean that the growth of the vocal folds is not constrained by the caller's body size (Fitch, 1997), but appears to be under hormonal and muscular control (Taylor and Reby, 2010). Consequently, the F0 (as well as other acoustic features of the glottal sound wave) of vocalisations can provide honest "static" index signals of the caller's sex (Fitch and Giedd, 1999), sexual maturity (Fischer et al., 2002), levels of sex hormones (Evans et al., 2008), and age (Reby and McComb, 2003a). In addition, features of the glottal signal (e.g. call rate, call duration or call amplitude) can provide accurate current-state "dynamic" index information about the caller's immediate aggressive (Yin, 2002), energetic/condition (Fischer et al., 2004; Pitcher et al., 2014), arousal (Briefer, Tettamanti and McElligott, 2015), emotional (Manser, Seyfarth and Cheney, 2002; Briefer, Tettamanti and McElligott, 2015), fertility (Semple et al., 2002), and motivational (Pitcher et al., 2014) state. The arousal/emotional state of the caller can alter its rate of respiration, level of sub-glottal air pressure in the lungs, level of desynchronisation between the vibrating vocal folds (nonlinearity), or the level of muscular tension in its vocal folds, which can alter the F0 and harmonic structure of vocalisations (Titze, 1994). Whether increased arousal raises or lowers F0 appears to be species-specific (Taylor and Reby, 2010).

As such, the source properties of the vocal signal can provide listeners with honest measures of the caller's fitness or RHP (Reby and McComb, 2003b). The characteristics of the glottal signal have been shown to be important in both mate choice (Reby et al., 2010; Lemasson et al., 2015) and dominance hierarchies/territory defence (Hardouin et al., 2007; Behr, Knörnschild and von Helversen, 2009). In addition, features of the

glottal signal can also encode 'functionally-referential' information about the caller's environment, such as whether the caller is with a reproductive partner (e.g. fallow bucks, *Dama dama*: McElligott and Hayden, 1999; giant panda, *Ailuropoda melanoleuca*: Charlton et al., 2015), or whether the caller has located a high-quality food item (Marler, Dufty and Pickert, 1986). However, referents in the source properties of vocal signals may be closely linked with motivational changes in the caller.

Properties of the vocal filter and honest signalling

In contrast to laryngeal growth, the length of the vocal tract (the 'filter') is often anatomically constrained by the caller's skeletal structures and body size (Fitch, 2000). As the vocal tract length determines the spacing of the formant frequencies, formant dispersion within vocalisations can provide honest index information about caller body size (e.g. Fitch, 1997; Fitch and Kelley, 2000). An increase in vocal tract length results in calls with lower formants and a decrease in formant dispersion. Since body size is often an important determinant of an animal's ability to win a physical contest (Maynard Smith and Brown, 1986), vocal cues to caller body size are known to be important in both female mate choice (females often prefer larger sounding males: Charlton, Reby and McComb, 2007) and dominance hierarchies/territory defence (Reby et al., 2005). The important link between caller body size and vocal cues to caller body size has been further demonstrated by the ability of some animals to cross-modally match vocal signals with images or models of the appropriately sized caller (e.g. Ghazanfar et al., 2007; Taylor, Reby and McComb, 2010).

In addition, small modifications of the vocal tract length/shape and corresponding changes to the formant frequencies (predominantly to the lower formant frequencies determined by the shape of the mouth or lips) can also provide "dynamic" current-state information about the caller's motivational state and arousal (e.g. in goats, *Capra*

hircus: Briefer, Tettamanti and McElligott, 2015). In general, a retraction of the lips is observed for positive or submissive vocalisations (corresponding to an understated body size), while a protrusion of the lips is observed for stressful or dominant vocalisations (e.g. Fox, 1970). The function of the mouth/lip shape on dynamic index signalling and vocal production appears to be closely linked with visual communication (i.e. multi-modal signalling: see below). For example, in humans, the lip shape in a genial smiling facial expression corresponds to the lip shape used to produce non-aggressive sounds (Drahota, Costall and Reddy, 2008). Domestic dogs are even capable of matching a conspecific's emotional valence across audio-visual stimuli, and this ability in dogs extends to the emotions of humans (Albuquerque et al., 2016). The formant structure of vocal signals may also encode functionally referential information about the caller's environment, such as the type or location (aerial or terrestrial) of a predator (Riede and Zuberbühler, 2003; King et al., 2010; Soltis et al., 2014; Townsend, Charlton and Manser, 2014).

Source-filter dynamics and individual recognition
As vocal signal production is constrained by the morphological and physiological characteristics of the caller, vocal signals may also allow animals to recognise one another at an individual level (Charrier, Mathevon and Jouventin, 2003; Charlton, Zhihe and Snyder, 2009; 2011a). Individual recognition would be advantageous for most complex social behaviours (e.g. parental care). Regarding source-related features of vocalisations, there is gathering evidence for individual distinctiveness relating to F0 modulation (Charrier, Mathevon and Jouventin, 2003), harmonic structures within the call (Rendall, 2003), nonlinear dynamics (desynchronisation between the paired vocal folds: Riede, Owren and Arcadi, 2004), amplitude modulation (Charlton, Zhihe and Snyder, 2009), and temporal features such as call length and signal tempo (Rendall,

2003). Notably, the source components of vocalisations are often independent of caller body size, and could remain fairly constant throughout the animal's life (although hormonal and motivational changes will likely effect the F0 etc.). Filter-related features of vocalisations could also contribute to individual distinctiveness (e.g. Rendall, 2003; Charlton et al., 2011a), and these features would be fairly stable with hormonal changes in the caller. In reality, vocalisations are produced and influenced by the source and filter simultaneously, and it is likely that elements of both vocalisation components will provide cues to caller identity (Taylor and Reby, 2010).

Source-filter adaptations

Within the animal kingdom there are a range of different adaptations associated with vocal signal production that alter the relationship between the acoustic properties of vocalisations, and the correlated measures of the caller's RHP (though honest signalling can still be maintained- see above). Regarding the structures that produce the glottal sound wave, for example, felids within the *Panthera* genus (the roaring cats) have evolved unusually thick and fatty vocal folds that may allow them to produce their low frequency roars at high energy and volume (Klemuk et al., 2011). Low frequency sound waves travel further in an environmental medium, and would be adaptive for wide-ranging species such as the felids. Birds have evolved a different sound-producing organ to the mammalian larynx: the syrinx. The avian syrinx is lower down the laryngopharynx (in relative terms) than the mammalian larynx, and is located at the base of the trachea (Greenewalt, 1968). The syrinx is split into two halves, one either side of the joining bronchi. The two halves of the syrinx are able to operate independently, producing two separate glottal sound waves in quick succession, each with different F0 and associated harmonics (The two voice theory: Greenewalt, 1968). A split syrinx allows birds (particularly songbirds) to produce extremely complex vocal

signals (i.e. songs) with multiple notes produced rapidly. Other source-related adaptations in the production of vocal signals include hypertrophied larynges (e.g. in male hammer-headed bats, *Hypsignathus monstrosus*: Zeller, 1984), or the evolution of additional sound producing structures (e.g. the 'velar vocal folds' of koalas, *Phascolarctos cinereus*: Charlton et al., 2013).

Regarding the vocal tract structures that filter the glottal sound wave, adaptions typically concern an extension of the vocal tract length, or a change in the shape of the vocal tract. Humans were originally thought to be unique among mammals in having a descended larynx (Fitch, 2002), and a descended larynx was believed to be vital for human speech (Lieberman, Klatt and Wilson, 1969; but see new evidence to contradict this theory: Fitch et al., 2016; Boë et al., 2017). However, we now know that other mammals such as koalas (Charlton et al., 2011b), red deer (Fitch and Reby, 2001), and lions (Weissengruber et al., 2002), have evolved a permanently descended and/or mobile larynx that is detached from the skull (Taylor and Reby, 2010). In red deer, the larynx itself is pulled down from the hyoid bone to the sternum by well-developed sterno-thyroid muscles, but in lions the hyoid bone is incompletely ossified and may be pulled down with the larynx. Vocal tract elongation results in vocalisations with reduced formant dispersions and a corresponding exaggeration of caller body size (Fitch and Reby, 2001), which may be adaptive in mate attraction and territory defence (e.g. Charlton, Reby and McComb, 2007). Indeed, there is a trend in terrestrial mammals for males from mating systems with strong selection pressures for large male body size, to produce vocal signals with lower formant dispersion (but not F0) than would be expected for their body size (Charlton and Reby, 2016). On the other hand, in species where males have a large testes to body size ratio (suggesting that sexual selection operates through post-copulatory sperm competition rather than pre-copulatory

vocalisations), male vocal signals have higher formant dispersion (and F0) than expected (Charlton and Reby, 2016). Many birds also have long necks and extreme vocal tract elongation, which could be used to exaggerate their body size when calling (e.g. the trumpeter swan, *Cygnus buccinators:* Fitch, 1999). Vocal tract extensions can also include the evolution of extra resonators (e.g. the enlarged hyoid bones of howler monkey species, *Alouatta* spp., with high male-male competition: Dunn et al., 2015; the sub-hyoid air sac of the black and white colobus monkey, *Colobus guereza*: Harris et al., 2006), or even the use of hands and 'tools' such as leaves (e.g. orang-utans, *Pongo pygmaeus wurmbii*: De Boer et al., 2015). Consequently, formant dispersion is often not a 'perfectly' honest index signal of caller body size, but a strong link between formants and body size can still be maintained despite evolutionary adaptations to elongate VTL (Fitch and Reby, 2001; Charlton et al., 2011b).

Originally it was believed that the shape of the vocal tract in many non-human animals is relatively inflexible compared to humans (Titze, 1994; Taylor and Reby, 2010), but other animals (e.g. Diana monkeys, *Cercopithecus Diana*: Riede and Zuberbühler, 2003; songbirds: Riede et al., 2006; African elephants, *Loxodonta africana*: King et al., 2010; white-handed gibbons, *Hylobates lar*: Koda et al., 2012) also appear capable of fine-scale muscular movements of the tongue and lower jaw/beak that alter the formant structure (particularly the lower formants- F1 and F2) of vocal signals. Through tongue and lip movements, modifications of the lower formants allow humans to produce the different phonemes of human speech (Titze, 1994). Active modulation of the formant frequencies also appears to be important in encoding referents in the vocal signals of animals (e.g. King et al., 2010; Soltis et al., 2014). In addition, synchronising the first formant and the F0 (through changes in vocal tract shape) can distinctively amplify the F0 (at the expense of the associated harmonics and formants), allowing the production

of very loud, pure-tonal vocalisations (e.g. in the white-handed gibbon: Koda et al.,

2012). Birds also appear to modify their vocal tract resonances through changes in the

vocal tract shape (and potentially also through syringeal descent: White, 1968),

allowing them to synchronise the F0 with the first formant (Riede et al., 2006).

Vocal signalling in females

Sexual selection is widely accepted to be the driving force behind many of the elaborate

traits and behaviours found in the animal kingdom, including vocalisations, and in many

cases females can also experience intense competition for reproductive opportunities

(Clutton-Brock, 2009). Although much of the past research on animal vocal

communication has focused on male vocal displays, there is gathering empirical data

showing that female vocalisations are also important for social behaviours such as

mating. In birds, female song (i.e. long, complex vocalisations primarily produced

during the breeding season for mate attraction and territory defence: Catchpole and

Slate, 1995) is present in 71% of surveyed songbird (oscine passerines) species, and

ancestral state reconstruction suggests that females sang in the ancestral songbird

(Odom et al., 2014). Female bird song may be widespread and functionally adaptive for

mating and territory defence (Campbell et al., 2016; Krieg and Getty, 2016; Matthews

et al., 2017). In mammals, there is evidence that females may advertise their fertile

phase through vocalisations (e.g. humans: Pipitone and Gallup, 2008; giant panda:

Charlton et al., 2010; yellow baboons, *Papio cynocephalus*: Semple et al., 2002), and

also caller age and caller effort (e.g. in the white-handed gibbon: Terleph,

Malaivijitnond and Reichard, 2016). It is likely that further research focusing on female

vocalisations will greatly expand our knowledge of the ubiquity and importance of

female vocal communication.

Other important features of animal vocal signals

Despite the considerable importance of the 'source-filter' mechanism of vocal

production, there are many other properties of vocal signals known to be important in

the evolution of animal vocal communication systems. For group-living species where

individuals cooperate to defend access to territory and mates, the number of competing

individuals in each group is likely to be a more important determinant of the outcome of

conflict, than are individual differences in body size or hormone levels. Vocal chorusing

is common in many social birds (e.g. Seddon and Tobias, 2003) and mammals (e.g.

Wilson, Hauser and Wrangham, 2001; Kitchen, 2004; Benson-Amram et al., 2011), and

larger calling groups typically win disputes with smaller groups without the need for

physical conflict. In some social species, the calls of one group member stimulates

calling in others, which may function for groups to signal a larger minimum group size

to intruders (e.g. African lion: Grinnell and McComb, 1996). However, we currently

have a limited knowledge of the numerical range that animals can use group vocal

choruses to assess the number of callers. Although untested, it has been suggested that

animals may fail to accurately judge the number of callers once chorus size exceeds two

or three (McComb, Packer and Pusey, 1994).

Animals can also produce different types of calls, and these acoustically distinct

vocalisations can have functionally different meanings. Functionally referential animal

calls were first shown through playback experimentation of vervet monkey

(*Chlorocebus pygerythrus*) alarm calls (Seyfarth, Cheney and Marler, 1980). Vervet

monkeys are preyed upon by three main predators, each with different hunting

strategies: leopards (*Panthera pardus*), eagles and snakes. Vervet monkeys produce a

unique alarm call for each predator, and playback experiments show that conspecifics

respond to the different alarm calls (even in the absence of the predator) with an

appropriate evasion strategy to match the predator. Consequently, the different alarm calls have a different functional meaning that allows listeners to respond in the most appropriate manner to avoid predation. Some of the best examples of referents in animal vocal communication still come from alarm calls (e.g. Pereira and Macedonia, 1991; Zuberbühler, 2001; Manser, Seyfarth and Cheney, 2002), but referential vocal signalling has also been shown in foraging behaviour (e.g. the production of specific food calls: Evans and Evans, 1999; Slocombe and Zuberbühler, 2005; Watson et al., 2015), and signalling social events (Manser, 1998; Seyfarth and Cheney, 1984; Cheney and Seyfarth, 1990). As such, there is now clear evidence that animals can use their calls as semantic (meaningful) labels, but there is presently little evidence for syntax (higher order structure and grammatical rules), particularly for semantically composed syntax (where the meaning of the overall combined statement is derived from the meaning of the individual constituent parts; but see recent work on the pied babbler, *Turdoides bicolor*: Engesser et al., 2016).

Studies on bird song have shown that song novelty (Byers, 2017), song matching (Beecher et al., 2000; Akçay, Campbell and Beecher, 2014), song performance (Ballentine, Hyman and Nowicki, 2004; Ballentine, 2009; Lachlan et al., 2014; Ferreira et al., 2016), song overlapping (Peake et al., 2001), and song repertoire size or complexity (Buchanan and Catchpole, 2000; Vehrencamp, de Kort and Illes, 2017) are also good indicators of male RHP and reproductive success. Learning and producing complex and accurate songs and song repertoires will likely require complex nervous control of the vocal motor system, coordination of the vocal tract and respiratory muscles, and resistance to fatigue that may be limited to only the 'best-quality' males (Lambrechts and Dhondt, 1988; Suthers and Zollinger, 2008; Sakata and Vehrencamp, 2012). Vocal complexity and novelty may also be important in mammals, but this area

of research is currently understudied. For example, the novelty of the complex humpback whale (*Megaptera novaeangliae*) songs may be important in the cultural spread of songs across different populations (Garland et al., 2011), and female gelada baboons (*Theropithecus gelada*) may prefer to associate (and mate) with males who have more complex/elaborate calls (Gustison and Bergman, 2016). In bottlenose dolphins (*Tursiops truncatus*), individuals develop their own novel whistle that encodes individual identity independently of the source-filter features of the whistle (King and Janik, 2013).

Caller familiarity and prior knowledge of the caller's past interactions are also known to be important in the vocal interactions of animals. For example, dingos (*Canis familiaris dingo*) respond more strongly to the playback howls of unfamiliar conspecifics than to howls of familiar conspecifics (Déaux, Clarke and Charrier, 2016). In some territorial species, individuals are able to recognise their neighbours from their calls, and respond less strongly to their neighbour's call compared to a 'stranger's' call (i.e. the dear enemy effect: Getty, 1987). Unfamiliar strangers are often considered a greater threat than familiar neighbours because strangers may be prospecting for a breeding territory, whereas neighbours already have an established territory (Moser-Purdy and Mennill, 2016). Playback experiments have also shown that animals can eavesdrop on simulated vocal contests, and adjust their future agonistic behaviours with contestants based on the outcome of the eavesdropped contest (e.g. eavesdroppers respond to 'losers' with reduced vocal effort: Peake et al., 2001). Eavesdroppers can even integrate information gathered from hearing multiple two-way contests to influence their responses toward rivals paired in novel contests (Toth, Mennill and Ratcliffe, 2012). Territory owners can also eavesdrop on simulated defections by one of their neighbours towards another

neighbour, and respond more strongly when played the 'untrustworthy' neighbour's call (Akçay et al., 2010).

Finally, the location of the caller can be an important aspect of animal vocal interactions. Animal territories typically consist of a defended core area, and peripheral areas overlapping with neighbouring individuals or groups (Davies and Houston, 1984; Rosell, Gundersen and Le Galliard, 2008). Where conflict is costly (energetically or mortally), individuals should limit the strongest aggression to the defence of the greatest value resources. Predictions from evolutionary game theory suggest that resource defence, and aggression towards intruders, should be greatest towards the centre of the territory (Maynard Smith, 1982). In line with these predictions, in various birds (Falls, 1982; Giraldeau and Ydenberg, 1987) and mammals (Raemaekers and Raemaekers, 1984; Mitani, 1985; Whitehead, 1987; Wich et al., 2002; Furrer et al., 2011; Crofoot and Gilby, 2012), the responses to vocalisations of extra-group intruders are greater with decreasing distance to the centre of the defender's territory.

Olfactory communication

The chemical sense may be the oldest system of sensory perception, found also in the simplest bacterial life forms (Wilson, 1970). Although communication through chemicals is thought to be the main mode of communication for many taxonomic groups (Brown and MacDonald, 1985), human sensory biases have meant that olfactory communication has historically received relatively little attention (Nieberding et al., 2012). There remains a paucity of data regarding which species communicate via the chemical sense, and what information they signal through chemical signals. Historically, chemicals used for communication between animals have been termed 'pheromones', but there has been much debate about the validity of using pheromone as a label for the complex chemical signals of vertebrates (Apps, 2013; Wyatt, 2014). I

will use the broader and more inclusive term 'semiochemical' when referring to chemicals involved in animal communication.

There is a wide diversity of chemical signals to be found within vertebrates (reviewed in Brennan and Zufell, 2006), but the complexity of these signals and signal production still preclude a detailed understanding of olfactory communication in many species (Apps, 2013). The independent evolution of semiochemicals is evident in the enormous variety of specialised scent glands in vertebrates (Wyatt, 2014). Semiochemicals can be carried in urine, faeces, skin, breath, and a range of different secretions from glands across the body (Bradbury and Vehrencamp, 2011; Apps, 2013). Semiochemicals are often synthesised by the signaller, but can also involve elements of the signaller's environment (e.g. male orchid bees, *Apidae* spp., collect complex blends of volatiles from flowers: Eltz et al., 2008), or can be synthesised by bacterial communities within secretory glands (e.g. within the anal glands of spotted hyaenas, *Crocuta crocuta*: Theis et al., 2013).

Chemical signals produced by animals can remain on the body surface, or they can be released into air/water currents or deposited onto environmental objects. When an animal secretes/excretes semiochemicals onto the ground or other surface, the subsequent deposit is termed a scent-mark. Most terrestrial mammals deposit scent-marks to facilitate communication (Ferkin, Li and Leonard, 2004). Compared with chemicals released into the air/water, scent-marks have the potential to continue releasing semiochemicals long after the signalling event (Johnston, 2005). The longevity of scent-marks makes them ideal for communication when the intended receiver is not currently present. Therefore, scent-marking is common for solitary, wide-ranging species, and territorial species where individuals or groups communicate with extra-group conspecifics for territory defence (Johnston, 2005). In consequence of

the long-lasting (and therefore low-volatility) nature of scent-marks, and because the intended receiver will not always witness the scent-marking event, animals may attempt to increase the likelihood that the receiver will detect the scent-mark. Methods to increase the detectability of a scent-mark include depositing on top of vegetation and conspicuous objects (Jordan et al., 2013), depositing at ritualised locations shared by conspecifics (e.g. latrines: Jordan, Cherry and Manser, 2007), depositing at highly frequented locations (e.g. key junctions on game trails or roads: Peters and Mech, 1975), depositing conspicuously-coloured scent-marks (e.g. the visual-olfactory multi-modal anal paste of the brown hyaena, *Hyaena brunnae*: Margaret, Mills and Gorman, 1980), or through the creation of a visual marker next to the deposit (e.g. scratches in the soil created by felids: Soso et al., 2014; Vogt et al., 2014; Allen, Wallace and Wilmers, 2014).

Communication through scent is advantageous in many situations, because chemicals are often long lasting (at least when compared to most auditory, visual and tactile signals), can have low production costs (especially when involving excretory products, or the release of scent into the environment without muscular force), and can be carried around physical barriers by air and water currents. However, the disadvantages of olfactory communication relate to the slow production rate of chemical signals and slow dispersion rates to be detected by the intended receiver (Wyatt, 2014). The long-lasting and indiscriminate dispersal attributes of semiochemicals also expose olfactory communication to extrinsic eavesdropping costs from unintended receivers (both conspecific and heterospecific: Johnston, 2005).

Despite a wide diversity, all vertebrates detect chemical signals in basically the same way (reviewed in Wyatt, 2010). The typical terrestrial vertebrate olfactory system is made up of multiple subsystems, but the largest and most extensively studied are the

vomeronasal organ (VNO) and the olfactory epithelium (Ferrero and Liberles, 2010). Almost all semiochemicals in vertebrates are detected by the main olfactory system (olfactory epithelium) and/or the vomeronasal system (Wyatt, 2010). Following detection, the chemical signal is then processed in the glomerulus region of the brain (Wyatt, 2014). Although there is integration of inputs from the two main olfactory systems, these two subsystems contain different families of scent receptors and stimulate different neural circuits, consistent with the idea that they perform fundamentally different functions (Ferrero and Liberles, 2010). We do not yet fully understand the main olfactory functions of these two systems, but both appear to be important in generating specialised olfactory responses (Stowers and Kuo, 2015). Scent receptors within the vomeronasal system appear to be more species-specific than receptors in the main olfactory system, and the vomeronasal system may be more important for species-specific scent communication, such as aversion towards relevant predators (Du et al., 2012; Stowers and Kuo, 2015). In many mammals, the VNO is an important aspect of scent communication related to reproductive condition and activation of sexual arousal (Hart and Leedy, 1987; Owen et al., 2015). Flehmen (observed as a facial expression with a curled upper lip: Dagg and Taub, 1970) facilitates the transport of non-volatile semiochemicals (especially proteins and steroid conjugates) to the VNO (Hart and Leedy, 1987; Igbokwe, 2009), and is considered a strong behavioural index of VNO utilisation (Hart, 1983).

Where digital analysis, resynthesis and playback are powerful tools for conducting research on vocal communication, bioassay techniques are a crucial first step to establish that a chemically mediated effect exists to be studied further (Wyatt, 2014). Such techniques often involve the use of gas chromatography-mass spectrometry (GC-MS) analyses to create a 'scent-profile' that identifies the individual chemical

compounds within a scent secretion/excretion. Following this, candidate compounds are isolated and synthesised, and behavioural assay presentations are performed to test for a biological response from test subjects (Wyatt, 2014). However, investigating chemical communication can often be more difficult than vocal communication. While the chemical signals of insects can be simple and are well understood, the semiochemicals used by vertebrates are often complex mixtures of chemicals (Wyatt, 2014). In contrast to vocal signals, chemical signals cannot easily be isolated, manipulated and presented back to study animals. Instead, 'whole-scent' presentations are often used to test for response differences to scent from donors of different parameters such as sex, territorial status and reproductive state (Wyatt, 2014). Significant differences in response (e.g. likelihood and/or length of response) to presented scent can give strong indications that the scent encodes salient information about the parameter in question. Retrospective GC-MS analysis can then be employed to isolate key compounds that may act as important semiochemicals in the scent. It is likely that future progress in chemical techniques will vastly improve our understanding of the complexity of olfactory communication in vertebrates.

Nonetheless, there is now strong evidence that chemical signals are often involved in intrasexual competition (Ferkin, 2007), mate choice (Ferkin, Li and Leonard, 2004; Roberts, 2007), and group coordination (Wüst and Menzel, 2017). In a similar manner to vocalisations, chemical signals can convey honest information about the sender's RHP, and its environment. As semiochemicals can be directly derived from the signaller's diet or environmental surroundings, chemical signals can provide receivers with direct information concerning resource acquisition by the signaller (Wyatt, 2010). For example, male tiger moths (*Utetheisa ornatrix*) derive semiochemicals from the same poisonous plant that they use to provision females with to protect their eggs.

Females select males that produce the most scent, as there is a positive correlation between scent production and provision load (Conner and Weller, 2004; for a vertebrate example see Ferkin et al., 1997).

In vertebrates, the production of semiochemicals is often under hormonal control, and so semiochemicals can signal honest "static" index information about the signaller's sex (Vicente and Halloy, 2016), reproductive state (Ferkin, Li and Leonard, 2004; Charlton, 2014), and territorial status (Marneweck, Jürgens and Shrader, 2017). Even some measures of the depositor's body size (Martín and López, 2007), or parasitic load (Penn et al., 1998; Martín and López, 2007) can be conveyed by scent signals and used in intra-sexual competition or mate choice. Semiochemicals can also advertise the signaller's "current-state" emotional and/or motivational state. For example, individuals from certain species release scent during aggressive interactions (e.g. lions urinate during aggressive encounters with spotted hyaena: Schaller, 1972), during submissive behaviours (e.g. pigs, *Sus scrofa domesticus*, release a chemical signal to indicate submission after a fight: McGlone, 1985), and when threatened (e.g. skunks, *Mephitidae* spp., spray anal gland secretion when threatened: Bekkering, 2008). Regarding referent signals that refer to the sender's environment, semiochemicals can signal the presence of a predator (Anderson and Mathis, 2016), and properties of the secreted chemicals (e.g. secretion rate and chemical composition) can convey the level of threat posed by the predator (Bruijn et al., 2016).

Where individuals deposit semiochemicals onto the surface of an object (i.e. scent-marking), olfactory communication can be particularly costly, and scent-marking effort may function as an honest signal of the signaller's RHP. Marking can account for a significant proportion of an animal's activity (Roberts, 2007), and there is evidence that scent-marking is physiologically costly (Gosling et al., 2000; Beynon et al., 2001) and

can attract predators (Viitala et al., 1995; Probst, Pavlicev and Vitalia, 2002). Consequently, only the 'best quality' individuals may be able to scent-mark at a high rate to advertise their presence and ownership of a territory. Investment in scent-marking often positively correlates with measures of an individual's 'fitness' (Roberts, 2007). Generally, marking frequency increases with social status (Gosling and Roberts, 2001), where resource holders (Allen, Bekoff and Crabtree, 1999) and socially dominant individuals (Rozenfeld, Boulangé and Rasmont, 1987; Hurst, 1990; Jordan et al., 2013) mark more than non-resource holders or subordinate individuals. Furthermore, marking effort increases with increasing levels of territory competition (Brashares and Arcese, 1999), and when individuals are faced with competitors of higher RHP (Gosling et al., 2000). Some animals also limit high marking effort to social situations important for reproductive success: marking frequency typically increases with the onset of sexual-maturity (Woodmansee et al., 1991), with the onset of oestrous (Matochik, White and Barfield, 1992; Ferkin, Lee and Leonard, 2004), and with the presence of potential reproductive partners (Zala, Potts and Penn, 2004). Increased scent-marking can enhance a male's attractiveness to females (Clark, Vom Saal and Galef, 1992; Zala, Potts and Penn, 2004), and may deter territory intruders (Roberts, 2007). In addition, parasite load can be negatively correlated with scent-marking effort (Zala, Potts and Penn, 2004), and scent from individuals with high parasite loads are less interesting to the opposite sex, and stimulate less scent-marking from receivers (Penn et al., 1998; Zala, Potts and Penn, 2004; Mitchell et al., 2017).

Importantly, a common response of animals to encountered scent-marks is to place their own deposit on top, a behaviour known as 'overmarking' (Johnston, Chiang and Tung, 1994; Jordan et al., 2011a). Overmarking is a ubiquitous behaviour associated with territory or mate defence in mammals (reviewed in Ferkin and Pierce, 2007), but it is

also present in other taxa (e.g. lizards: Martín and López, 2013). It has been proposed that the energetic costs of repeatedly monitoring and covering the deposits of rivals may mean that overmarking provides an honest signal of RHP, where only the 'best quality' individuals will be able to keep their deposits on top (Jordan et al., 2011b). In some species, individuals preferentially overmark the deposits of same-sex conspecifics (Jordan et al., 2011a), and territory holders/dominant individuals are more likely to overmark encountered deposits than are nomadic/subordinate individuals (Ferkin, 2007; Jordan et al., 2013). Animals appear able to differentiate between the top and bottom scent (and even the middle scent of a three-way overmark: Ferkin et al., 2011), and place preferential importance on the top deposit (Ferkin, Dunsavage and Johnston, 1999). When encountering future single scent-marks of the top and bottom scent donors in an overmark, animals spend more time investigating the odour from the top-scent donor (Johnston, Sorokin and Ferkin, 1997a; Johnston and Bhorade, 1998; Woodward, Bartos and Ferkin, 2000; Cohen, Johnston and Kwon, 2001). In addition, the top deposit may be more important in determining the subsequent overmarking response from future conspecifics (Jordan et al., 2011a). Females appear to preferentially associate and engage in affiliative social behaviours with 'top-donor' males (Rich and Hurst, 1999; Fisher, Swaisgood and Fitch-Snyder, 2003), and increased overmarking activity can positively correlate with behavioural mating success (Jordan et al., 2011b). Overmarking of deposits from the opposite sex may serve a mate-defence function (Woodward, Bartos and Ferkin, 2000; Ferkin, Li and Leonard, 2004; Jordan, Cherry and Manser, 2007; Eppley, Ganzhorn and Donati, 2016), for signalling an established pair bond between mates (Woodward, Bartos and Ferkin, 2000; Jordan et al., 2014), or for signalling female oestrous (Ferkin, Lee and Leonard, 2004). Indeed, there is evidence that males preferentially overmark the deposits of oestrous females (Ferkin,

Lee and Leonard, 2004). Countermarking is a term often used interchangeably with overmarking, but can more strictly refer to the placement of a deposit directly adjacent to an existing deposit without overlap (Rich and Hurst, 1999). Countermarking of scent-marks may serve similar functions to overmarking (e.g. Johnston, Sorokin and Ferkin, 1997b; Fisher, Swaisgood and Fitch-Snyder, 2003).

As many chemical biosynthesis pathways may also be under strong genetic control, semiochemicals can signal group membership (Tinnesand et al., 2015), genetic relatedness (Gilad et al., 2016), and individual identity (Vogt et al., 2016). Individual identity cues in chemical signals may be conveyed by the presence/absence of compounds, as well as the relative amounts of certain compounds within an individual's chemical signal (Sun and Müller-Schwarze, 1999; Burgener et al., 2009). Animals typically release semiochemicals from multiple sources across the body, and it is possible that individuals can develop integrated, multi-odour representations of each other, even when the properties of the different chemical signals are fundamentally distinct (Johnston and Peng, 2008). Animals may also be able to cross-modally match conspecific odours to signals of identity in other sensory modalities (Kulachi et al., 2014). As with animal vocal communication, the familiarity of the signaller and past interactions/prior knowledge of the signaller can play important roles in the nature of animal chemical communication. For example, neighbour-stranger discrimination and the 'dear enemy effect' can be important in olfactory communication, where animals typically respond less strongly to chemicals originating from their territory neighbours (Zenuto, 2010).

Multi-modal communication

The complexity of animal communication systems has meant that most research into communication signalling has investigated the function of a specific signal in isolation,

yet we are now beginning to understand that it is often too simplistic to view communication between individuals within a single sensory modality. Communication signals increasingly appear to involve multiple elements being signalled simultaneously through multiple sensory channels. Receivers of such multi-modal communication signals could then use cross-modal processing to integrate information gathered from several senses to better inform their behaviour (Partan and Marler, 1999). For example, male wolf spiders (*Lycosidae* spp.) use both olfactory and visual signals to determine the reproductive status of signalling females, and integrate information from multiple sensory inputs to determine how strongly they compete for receptive females (Rypstra et al., 2009).

Cross-modal sensory perception is the ability for the brain to integrate information from multiple senses when responding to particular signals or cues (Davenport, Rogers and Russell, 1973; Stein and Meredith, 1993; Proops, McComb and Reby, 2009), and was originally thought to be an ability unique to humans (Campanella and Belin, 2007). However, recent experimental developments within the cognitive sciences have begun to reveal that non-human animals are also able to use cross-modal perception during communication (reviewed in Seyfarth and Cheney, 2009). The ability to interchange information across the senses would be adaptive in situations where the modality available at one time may be unavailable at other times (Adachi, Kuwahata and Fujita, 2007). In addition, multi-modal signalling could function to increase the overall signal strength and detectability (Rowe, 1999), reduce communication errors (Møller and Pomiankowski, 1993), or to increase the range of environmental conditions within which communication can take place (e.g. the use of vocalisations over large distances and visual signals in noisy environments: Candolin, 2003; Partan, 2016). Regarding honest signalling of the sender's RHP, it may also be harder for dishonest signallers to

'manipulate' complex communication signals that involve multiple uni-modal signals being transmitted simultaneously. Multi-modal signals may provide receivers with more accurate information relating to the RHP of the sender, and could therefore be selected for in the sender-receiver evolutionary arms race (Wilson, Dean and Higham, 2013).

Many communication signals in the animal kingdom are inherently multi-modal. For example, when a frog calls it must inflate its throat sac to produce sound. Equally, humans must change the shape of their mouth when speaking in order to produce the different phonemes of human speech. In these cases, the signals (or cues) must be produced in combination, and can be thought of as 'fixed' multi-modal signals (Higham and Hebets, 2013). However, many animal communication signals are made up of intrinsically uni-modal signals that are produced together as part of a multi-modal display. For example, when African wild dogs (*Lycaon pictus*) deposit scent-marks, they can also adopt distinct leg postures to accompany the scent-mark. The scent-marking postures of wild dogs are important in determining how conspecifics respond to the scent deposits (Jordan et al., 2013). Multi-modal displays made up of single signal elements that are not obligately tied are referred to as 'free' multi-modal signals (Higham and Hebets, 2013). However, just because communication signals can involve multi-modal elements, does not mean that receivers are actually using all the potential sources of information being signalled over the different sensory channels (Higham and Hebets, 2013).

The different elements of multi-modal communication signals can be classified as redundant or non-redundant, depending on each independent signal's information content (Johnstone, 1996). Where the information contents of the uni-modal signals are equivalent and provide the same information to the receiver, the elements of the multi-modal signal are redundant. Where the information contents of the uni-modal signals

are distinct, the elements of the multi-modal signal are non-redundant. In reality, multi-modal signalling is often likely to be more complex, with combined signals either enhancing/dampening behavioural responses from the receiver, or even stimulating a completely new response than either of the single signals generate. In addition, it may be that one signal dominates over the other in determining the response from the receiver (Partan and Marler, 1999).

For simplicity, most theoretical and experimental research on multi-modal communication has focused on bi-modal signals. However, multi-modal complexity in signalling systems may go far beyond bi-modality (Higham and Hebets, 2013). In some systems multi-modal signals are likely to be at least tri-modal or more. For example, in *Hyperoliidae* frogs, males of most species have a brightly coloured 'gular' gland on their vocal sac. Where the 'vocalisation-vocal sac' bi-modal signal is known to be important in other anuran species (reviewed in Starnberger, Preininger and Hödl, 2014), it appears possible that *Hyperoliidae* males also emit chemical signals when calling, creating a tri-modal signal (Starnberger et al., 2013). Future advances in this area are likely to expand our knowledge of the intricate complexities of multi-modal communication.

The African lion

The African lion (Figure 1.1) is a large felid within the *Panthera* genus of roaring cats. Lions are characterised by a uniform tawny colouration, although males also develop a thick coat of hair (termed a mane) on their head, neck and chest, which darkens with age (Schaller, 1972). In the wild, females can live up to 19 years and reach a weight of 181 kg, while males usually live up to 12 years and reach 249 kg (Whitman and Packer, 2007). Lions are an ambush predator, targeting medium to large sized prey such as warthogs (*Phacochoerus africanus*), plains zebra (*Equus quagga*), and African buffalo

(*Syncerus caffer*). Historically lions (*Panthera leo* spp.) once ranged across much of Africa and Southwest Asia and even into Europe, but anthropogenic drivers now restrict most lions to isolated populations in sub-Saharan Africa (Riggio et al., 2013). African lion numbers are currently estimated to be between 20,000-30,000, but population numbers are in decline across much of their range (Bauer and Van der Merwe 2004; Riggio et al., 2013).

Figure 1.1. An adult female lion in the study population (fitted with RVC VHF-GPS radio collar, described in further detail below) and cub.

Lions are unique among felids in that both sexes are social, living within fission-fusion prides containing 1-21 related females, their dependent offspring, and an associated coalition of 1-9 immigrant males (Schaller, 1972; Bygott, Bertram and Hanby, 1979; Grinnell, Packer and Pusey, 1995; Van der Waal, Mosser and Packer, 2009). Depending on habitat characteristics and prey availability, lion prides occupy territories of 45-700 km² (Ramsauer, 2005). Over generations, pride females communally defend inherited territories from other matrilines, hunt together, and help raise dependent offspring (Packer, Scheel and Pusey, 1990). In contrast, males disperse from their natal territory when sexually mature, and form coalitions with other males (typically related males) to out-compete rival coalitions and defend reproductive access to unrelated females

(Packer and Pusey, 1982; Grinnell, Packer and Pusey, 1995; Heinsohn et al., 1996; Van der Waal, Mosser and Packer, 2009). Successful coalitions are capable of defending multiple prides of females (Schaller, 1972).

Lions reach sexual maturity between the ages of 3.5 and 4 (Schaller, 1972; Packer et al., 1988), though males typically only breed when they successfully take over a female pride at approximately 5-6 years of age (Bygott, Bertram and Hanby, 1979). Lions are aseasonal breeders, and litter sizes range from 1-4 cubs (Schaller, 1972). Gestation in females lasts about 108 days (Schmidt et al., 1979), and females do not mate again until their cubs are approximately 2 years old (Bertram, 1975). Consequently, male lions have evolved to kill unrelated cubs when taking over a new pride (Pusey and Packer, 1987). Infanticide of dependent offspring is an adaptive reproductive strategy for males as it soon brings females into oestrous (Packer and Pusey, 1983). Male tenure of a pride typically lasts 2-3 years, and so males normally only successfully sire one cohort of offspring before losing access to pride females to a rival coalition (Packer and Pusey, 1983). Infanticide is obviously not an adaptive behaviour from a female perspective, and females will temporarily remove themselves from the rest of the pride to hide their cubs until they reach independence (Packer and Pusey, 1983). There is often a large degree of reproductive synchrony between pride females, and this is thought to be an adaptive strategy for females to crèche their young together and cooperatively defend them from infanticidal males and rival prides (Packer and Pusey, 1983).

There can be extensive territorial overlap between rival groups (Spong, 2002), and territorial male coalitions must frequently defend their access to reproductive females from rival coalitions (Grinnell, Packer and Pusey, 1995). In some ecosystems, inter-group encounters can be as often as every five days (Packer, Scheel and Pusey, 1990). Since maintaining territories is essential for both sexes to successfully breed (Heinsohn

and Packer, 1995), the consequences of territorial intrusion can be serious, with fights between same-sexed individuals often leading to injury and death (Schaller, 1972; Packer, Scheel and Pusey, 1990; Heinsohn and Packer, 1995). For species where fighting frequently leads to mortal injuries and complete loss of future reproductive output, game theory predicts that natural selection should favour the evolution of conflict mitigation mechanisms without physical conflict (Maynard Smith and Price, 1973). Such mechanisms often involve broadcast communication signals that convey the 'fitness' and fighting ability of the combatant/s (Bond, 1989). Both long-lasting chemical signals and long-distance vocal signals can provide honest information about the signaller's 'fitness', and can evolve to resolve conflict without physical fighting (Bradbury and Vehrencamp, 2011).

In addition, the fission-fusion social system of lions means that individuals move around in small sub-groups rather than the entire pride, and group members can be spread out over large distances within their territory (Schaller, 1972). Therefore, maintaining social bonds, and coordinating group activities such as territorial defence poses a significant challenge for lions. Consequently, broadcast communication signals that either travel long distances (e.g. vocal signals) or last for a long time (e.g. chemical signals) should be important in the social lives of lions.

Vocal communication

Lions have been the focus of intense research since the 1970s (see Schaller, 1972), and it is thought that vocal signalling is their main form of communication (Ramsauer, 2005). Lions can produce several types of vocalisations (Schaller, 1972), but it is their long-distance call (termed a 'roar') that is believed to be most important for mate attraction (McComb et al., 1993), social cohesion (McComb, Packer and Pusey, 1994; Ramsauer, 2005), and territory defence (McComb et al., 1993; McComb, Packer and

Pusey, 1994; Grinnell, Packer and Pusey, 1995; Heinsohn, 1997; Grinnell and

McComb, 2001; Ramsauer, 2005). Roars are low-pitched vocalizations produced by

both sexes, though males roar more often, and their roars are deeper and louder than

those of females (Schaller, 1972; McComb et al., 1993; Pfefferle et al., 2007). Lion

roars can travel up to 8 km (Stevenson-Hamilton, 1954), and previous research has

shown that roars signal salient information regarding caller sex (e.g. females with cubs

respond defensively to the roars of unfamiliar males but respond aggressively to the

roars of unfamiliar females: McComb et al., 1993), caller familiarity (e.g. females with

cubs respond defensively to the roars of unfamiliar males but largely ignore the roars of

resident males: McComb et al., 1993), territorial status (e.g. only males who associate

with females roar, and only when they are in their own territory: Grinnell and McComb,

2001), and whether more than one lion is calling (e.g. during both territorial and sexual

conflict, males and females can differentiate between the roar of a single lion and the

chorused roaring of up to three lions: McComb, Packer and Pusey, 1994; Grinnell,

Packer and Pusey, 1995; Grinnell and McComb, 1996).

Lion roars are characterised as vocalisations that are delivered in bouts, which typically

last 30–60 s and consist of several soft introductory moans, a series of full-throated

roars, and a terminating sequence of grunts (Grinnell, 1994; Figure 1.2-1.3). Lion

roaring stimulates roaring in other group members, although the complete roar is not

typically observed from lions until approximately 2.5 years of age (Ramsauer, 2005).

Groups of lions frequently roar together in chorus, where the overlapping nature of

chorus roaring appears to provide listeners with honest information about the number of

callers, at least when up to three callers are present (McComb, Packer and Pusey, 1994;

Grinnell, Packer and Pusey, 1995; Grinnell and McComb, 1996). Since large lion

groups dominate smaller groups during territorial and sexual conflict (Packer and

Pusey, 1983; Packer, Scheel and Pusey, 1990; Grinnell, Packer and Pusey, 1995), lions

may roar in chorus to advertise their minimum group size to mitigate physical conflict

and deter territory intruders (McComb, Packer and Pusey, 1994; Grinnell and McComb,

1996).
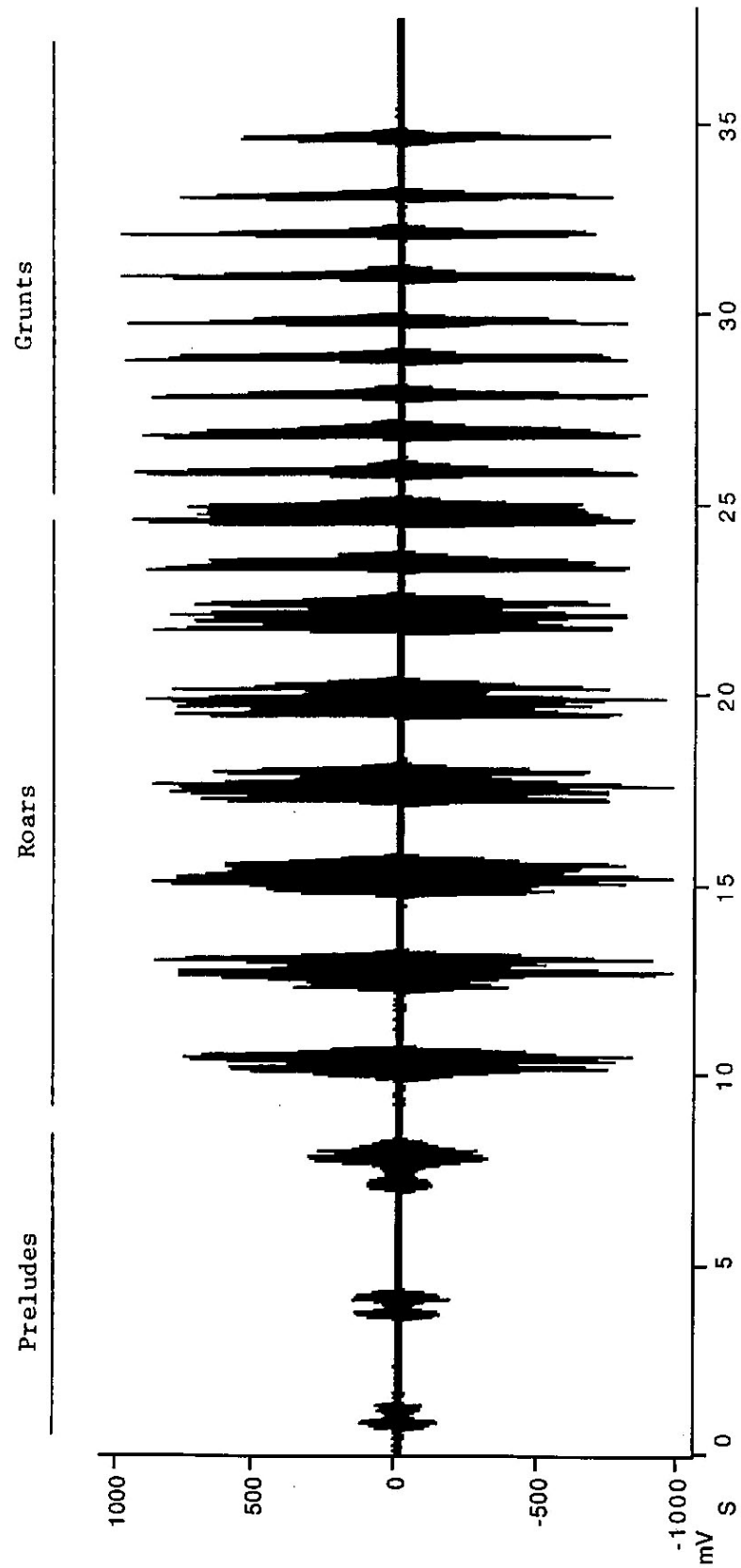
Figure 1.2. Sound pressure level waveform of a typical lion roar taken from Grinnell (1994). The first three utterances represent the introductory moans, while the last nine represent the concluding grunts.
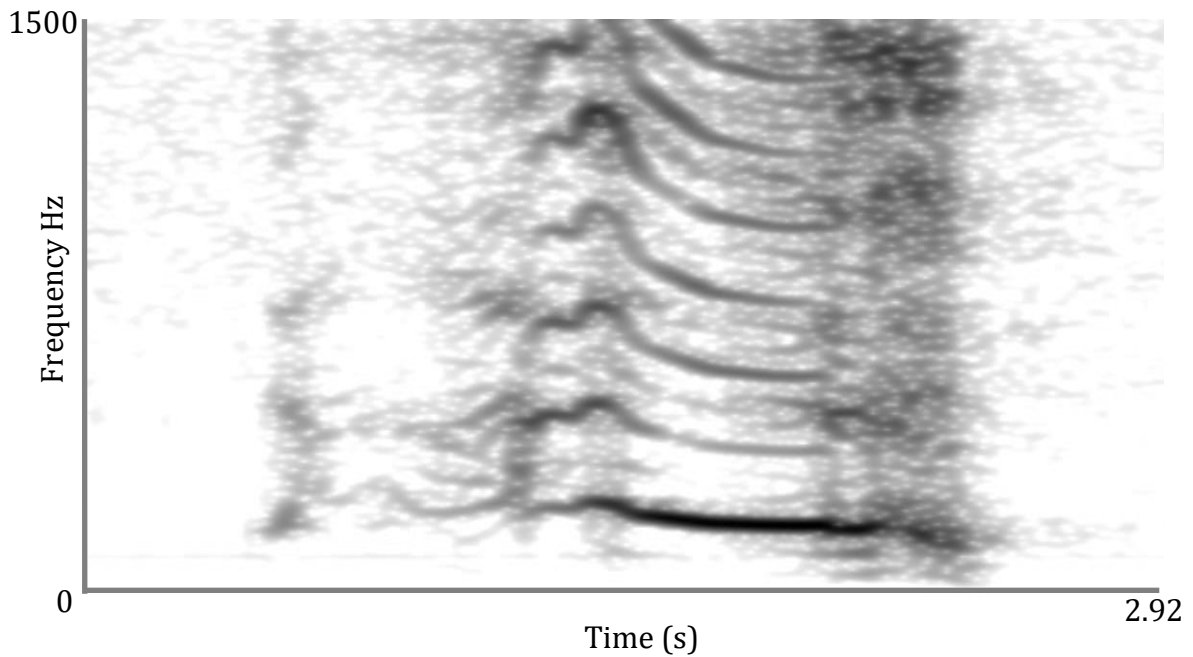
Figure 1.3. Spectrogram of a single full-throated roar from an adult female's roaring bout.

The vocal anatomy of lions appears to have undergone evolutionary modification, perhaps to increase the distance of signal transmission and/or to exaggerate signals of caller 'fitness'. For example, lions have evolved thick and fatty vocal folds that may allow callers to sustainably produce roars with very low F0, at high volume (Klemuk et al., 2011). Lower frequency sounds attenuate less in an environmental medium and travel further (Marten and Marler, 1977), and there appears to be a trend in the *Felidae* that those species living in more open habitat (in which long-distance calls are likely to be more effective) produce vocalisations with lower dominant frequency (i.e. the frequency with the highest energy in the spectrum: Peters and Peters, 2010). In addition, like most felids within the *Panthera* genus, lions have evolved a descended larynx (Weissengruber et al., 2002), which is known to be an adaptation allowing for an exaggeration of caller body size (Fitch and Reby, 2001). Like the red deer (Reby et al., 2005), lions may also have a mobile larynx (Ananthakrishnan et al., 2011) and could lower their larynx further (and exaggerate their body size more) when replying to a larger rival. As of yet, though, there is no evidence that body size advertisement through

roaring is important for mate selection and/or territory conflict in lions (Pfefferle et al., 2007).

Regarding honest signalling of caller RHP, male lions increase their rate and intensity of roaring in the presence of simulated (Grinnell, 1994) and real (G.Gilfillan: unpublished data) territory rivals. The production of vocalisations (especially long-distance vocalisations) can be extremely energetically costly (Taigen and Wells, 1985). Indeed, in combination with an increase in call rate and intensity, male lions also reduce the number of call elements within their roars, and this may reflect the energetic demands of roaring in quick succession (Grinnell, 1994). As previously mentioned, increased call rate or intensity could provide honest information concerning the RHP of the caller, or could reflect an increased effort to signal other sources of information related to caller 'fitness', such as age and maturity. Increased call rate and intensity could also signal knowledge of the intruder's presence and advertise a willingness to escalate into a physical contest (Grinnell, 1994).

Olfactory communication

While it is thought that olfactory communication may be an important form of communication for lions (Schaller, 1972; Bradshaw and Cameron-Beaumont, 2000), there is a lack of detailed research focusing on this topic. Lions are believed to deposit scent through urine, faeces, anal gland secretions, pedal (feet) gland secretions, and secretions from facial glands (Schaller, 1972; Asa, 1993; Andersen and Vulpius, 1999; Brahmachary and Singh, 2000; Pageat and Gaultier, 2003; McLean et al., 2007; Umapathy et al*.,* 2007; Lehmann et al., 2008; Poddar-Sarkar et al., 2008; Barja and de Miguel, 2010; Soini et al., 2012; Umapathy et al., 2013; Poddar-Sarkar and Brahmachary, 2014; Gilfillan, et al., 2016). Of these, urine may be the most important (Schaller, 1972; Brahmachary and Singh, 2000; Barja and de Miguel, 2010). Flehmen

(Figure 1.4) can also be regularly observed from lions after sniffing and licking scent, indicating VNO utilisation and olfactory detection.

Figure 1.4. An adult female lion exhibiting flehmen after sniffing a group member's urine deposit.

The importance of urine for olfactory communication in animals is now well established (Wyatt, 2014). In mammals for example, urine is known to signal the depositor's sex (Charlton, 2014), oestrous state (Swaisgood, Lindburg and Zhang, 2002), genetic quality (Roberts and Gosling, 2003), and social group (Palagi, Dapporto and Tarli, 2005). While all lions must urinate to excrete waste products, urination rates and body postures during urination appear to differ quite substantially between sexually-mature lions and sexually-immature lions, as well as between the sexes (Schaller, 1972). Lions (particularly adults) deposit urine with several distinct body postures (outlined in Schaller, 1972), and posturing during scent-marking is known to be important in other species (e.g. wild dogs: Jordan et al., 2013; giant panda: Swaisgood, Lindburg and Zhang, 2002). Typically, chemical signals deposited with overt body posturing elicit a greater response from conspecifics (e.g. Jordan et al., 2013).

Lions urinate in a variety of social and environmental situations that suggest urination is used for communication, particularly for resource or territory defence: 1) male lions regularly urinate when mate guarding females and directly after mating; 2) females adopt male-typical spray urination postures in the days prior to mating; 3) lions of both sexes regularly urinate immediately after an aggressive encounter with conspecifics or heterospecifics (e.g. spotted hyaena); 4) lions regularly urinate on top of or directly next to food carcasses; 5) lions often urinate when group members reunite after being separated; and 6) males often urinate over the urine of mate-guarded females, and also the urine of coalition partners (overmarking: Thomas and Wolff, 2002). In addition, the urine of males and females differs in chemical composition, raising the possibility that lion urine can encode information about the sex of the urine donor (Andersen and Vulpius, 1999).

The anal glands typically refer to a pair of glandular invaginations just inside of the anal opening, and are common in many carnivores including felids (Asa, 1993). Anal gland secretion (AGS) often has a strong odour and conspicuous colouration, and is an important carrier of semiochemicals in a wide range of carnivores (e.g. wolf, *Canis lupus*: Asa et al., 1985; spotted hyaena: Burgener et al., 2009; banded mongoose, *Mungos mungo*: Jordan et al., 2011a; brown bears, *Ursus arctos*: Jojola et al., 2012). In mammals, AGS is known to signal donor sex (Cross et al., 2014), donor relatedness to the receiver (Leclaire et al., 2013), and donor age (Yuan et al., 2004), and is important in territory defence (Asa et al., 1985), reproductive competition (Jordan et al., 2011a), and individual identification (Burgener et al., 2009). While the function of AGS in lions remains unknown (Asa, 1993), lions can be observed sniffing each other's anogenital regions (Schaller, 1972), and chemical signals from the anal gland (as well as urine and faeces) could be detected when doing so.

Faeces is another potential source of semiochemicals in lions, but lions are believed to defecate randomly in the wild (Schaller, 1972), suggesting that faeces may be less important for olfactory communication. However, chemical analyses have shown that lion faeces could signal female oestrous state (Umapathy et al., 2007; Umapathy et al., 2013). In addition, faeces is important for olfactory communication in many other species (Wyatt, 2014; Marneweck, Jürgens and Shrader, 2017), where chemicals in faecal deposits can signal female oestrous state (Sankar and Archunan, 2008), territorial status (Marneweck, Jürgens and Shrader, 2017), and familiarity (Cinková and Policht, 2015).

Felids are believed to have inter-digital glands and sweat glands on their feet (Pageat and Gaultier, 2003), and pedal glands can be an important semiochemical source for scent communication in other mammals (e.g. white tailed deer, *Odocoileus virginianus*: Gassett et al., 1996; polar bear, *Ursus maritimus*: Owen et al., 2015; brown bear: Clapham et al., 2014). Lions frequently scratch their claws vertically across tree trunks or scrape urine soaked soil with their hind paws, and this may act to deposit pedal gland scent-marks (Schaller, 1972). Certain trees might then be selected as scratching posts based on their location or aromatic properties (Marnewick, Bothma and Verdoorn, 2006; Nie et al., 2012; Clapham et al., 2013). Lions can also be observed to follow with their nose to the ground (i.e. 'tracing'), the recent footsteps of other lions (G.Gilfillan: pers. obs.), and this could also involve pedal gland secretions (Owen et al., 2015). In pumas (*Felis concolor*), scrapes over urine deposits appear to increase the detectability of the urine for future recipients (Allen, Wallace and Wilmers, 2014), but whether pedal gland secretions are also playing a role is still unclear.

Finally, felids also have a range of scent glands on their face and neck (Pageat and Gaultier, 2003), and it is believed that facial gland secretions are deposited when lions

rub on a substrate such as vegetation (Schaller, 1972; Soini et al., 2012). In captivity, lions responded (with sniffing, flehmen, rubbing, and urine) to cardboard that had been rubbed against a conspecific's head, and chemical analysis revealed several new chemical compounds on rubbed cardboard not previously identified in feline urine and marking secretions (Soini et al., 2012). Laboratory evidence also suggests that the mane hair of male lions has the potential to store semiochemicals that could be deposited during rubbing (Poddar-Sarkar et al., 2008). Lions (particularly males) often rub their face on vegetation before spraying their urine on the same spot. For example, a male lion in South Africa rubbed his head or body on vegetation before spraying urine in 34% of cases (Lehmann et al., 2008). Head rubbing may be a form of scent-marking in lions, but there has been limited investigation into this possibility. In general, the olfactory communication of lions through scent-marking has been the subject of relatively little systematic research, and clearly represents a significant gap in our current knowledge of lion behavioural ecology that needs to be addressed.

Gaps in current knowledge

Although lions have been extensively studied compared to many other carnivores, much of our current knowledge of lion behavioural ecology comes from studies on lions in East Africa (e.g. Schaller, 1972; Packer et al., 1990; Grinnell and McComb, 1996; 2001). However, many species are known to exhibit extensive behavioural flexibility depending on local ecological conditions (Kruuk 1972; Macdonald 1979), and different populations can have adaptations specific to the ecosystem they inhabit. Therefore, behaviours and cognitive abilities demonstrated by individuals in one ecosystem may not be directly transferable to individuals in another (Patterson 2007; Kotze, 2016). In lions, for example, early research in the open grasslands of East Africa showed that males are poor hunters and rely on females for food (e.g. Schaller, 1972), but males in

the savannah woodland of Kruger National Park (South Africa) are now known to be more frequent and successful hunters (Funston et al., 1998). Similarly, lion social group sizes are often much larger in East Africa (up to 21 adult females and 9 adult males: Packer et al., 1988) than in the Okavango Delta in Botswana (up to 8 females and 4 males: G.Gilfillan: pers. obs.), or Etosha National Park in Namibia (up to 9 females and 3 males: Stander, 1991). Compared to East African populations, the lions in the current study system (the Okavango Delta) have received much less detailed research, and we do not know how applicable the behavioural findings in East Africa are to the Okavango lions. In addition, the lions of the Okavango Delta appear to be genetically distinct from most other populations in Southern Africa, and all populations in East Africa (Barnett et al., 2014; Moore et al., 2016). Genetic reconstruction suggests that the ancestral lion was a specialist in wetland habitats such as the Okavango, and that the well-studied savannah populations evolved under strong evolutionary pressure and genetic isolation (Moore et al., 2016). As such, studies of lion behaviour in wetlands such as the Okavango may provide important insights into the evolutionary origins of lion behaviour.

Furthermore, there are still significant unanswered questions regarding the behavioural ecology and cognitive abilities of lions, from any population. For example, it is widely stated that vocal signals are the most important form of communication in lions (e.g. Ramsauer, 2005), but there has been surprisingly little research into their chemical communication. Chemical communication is the most widespread communication modality in mammals (Wyatt, 2014), and observational and chemical studies of lions also suggest that chemical signals (particularly scent-marks) may be important for lion communication (e.g. Schaller, 1972). The long-lasting nature of scent-marks are likely to make chemical signalling particularly important for wide-ranging, territorial and

nocturnal species such as lions (Kleiman and Eisenberg, 1973; Bradbury and Vehrencamp, 2011). However, descriptive data on deposit types and the responses of other lions to deposits has not been systematically documented, and there has been no experimental investigation into how wild lions respond to the scent deposits of conspecifics.

In addition, previous research on communication between lions has focused on uni-modal signalling, and there has been no investigation into whether lions use multi-modal signals or are capable of cross-modal processing of information. This is likely to result from the difficulty of experimenting with multi-modal signals in a wild setting. However, a new experimental paradigm for testing the ability of cross-modal individual recognition has recently been developed in captive and domestic settings (e.g. Proops, McComb and Reby, 2009). It is now timely to adapt these novel techniques to test whether similar cognitive abilities exist in wild populations during more natural social settings.

Aims of thesis

This thesis aims to address the gaps in our current knowledge of African lion behavioural ecology by investigating questions relating to the vocal, olfactory and multi-modal communication of lions in the Okavango Delta wetland ecosystem. Firstly, I will expand on pioneering research from the 1990s showing that the overlapping nature of group chorus roaring in lions can function as an honest signal of caller number (up to three callers) to help listeners mitigate reproductive and territorial conflict. In particular, I aim to test whether lions can determine the number of conspecifics calling in large vocal choruses, investigating whether there may be an upper limit to the number of callers that can accurately be assessed. I will then use a novel playback experiment to test whether lions are capable of cross-modal processing of audio-visual multi-modal

signals of individual identity. Regarding chemical signalling, I will present the first detailed research exploring if and how scent-marking may function in communication between and within prides. How these findings further our knowledge of the ultimate and proximate mechanisms of communication in lions is also discussed throughout.

More specifically, I aim to answer the following questions, each in a dedicated chapter:

1) Are lions able to determine the number of conspecifics calling in large vocal choruses?

2) Are lions capable of spontaneous cross-modal individual recognition of conspecifics?

3) What is the evidence for functionally relevant scent-marking in wild lions?

4) Can lions discriminate the sex and social group of a conspecific from a sample of its urine?

Overview of chapters

Are lions able to determine the number of conspecifics calling in large vocal choruses?
The fission-fusion social system of lions means that individuals are often alone or in small sub-groups, and can make the numerical imbalance between competing lion groups more extreme (Schaller, 1972). Group size is known to play a primary role in determining the outcome of conflict between lions, with large groups dominating smaller groups (Packer, Scheel and Pusey, 1990). Fighting between lions is a major cause of injury and death, primarily for small prides and lone individuals (Schaller, 1972; Packer, Scheel and Pusey, 1990). Game theory modelling predicts that lions should use the number of individuals in competing groups to assess whether to attack or retreat from intruders (Maynard Smith and Price, 1973; Maynard Smith, 1974; Parker and Rubenstein, 1981; Wilson, Hauser and Wrangham, 2001).

Previous experiments involving the playback of lions roaring together have shown that the overlapping nature of chorused calling creates an honest signal of caller number that cannot be faked. Lions can use the vocalisations of rival groups to assess caller group

size (up to three callers) and avoid potentially lethal contests when facing unfavourable odds (McComb, Packer and Pusey, 1994; Grinnell, Packer and Pusey, 1995; Heinsohn and Packer, 1995; Grinnell and McComb, 1996; Heinsohn et al., 1996; Heinsohn, 1997). Due to the sexually dimorphic reproductive strategies of lions (Schaller, 1972), the costs of engaging in conflict with same-sex intruders appears to differ for male and female lions, which has been reflected in a sex-dependent response to the playback of intruders (McComb, Packer and Pusey, 1994; Grinnell, Packer and Pusey, 1995). Where females attack intruders only when the numeric odds are in their favour, or when they are defending a particularly valuable resource such as cubs (McComb, Packer and Pusey, 1994; Heinsohn, 1997), it is likely that the optimal strategy for male lions is to attack intruders even when outnumbered (Grinnell, Packer and Pusey, 1995). However, male lions have only been tested in a single ecosystem, with a maximum 'odds-against' ratio of three intruders to one defender (Grinnell, Packer and Pusey, 1995). No investigations have examined whether the 'always-attack' male lion strategy holds true for increased numerical disadvantages.

The numerical range over which animals are able to use group calling to discriminate between differently sized groups remains poorly understood. Much of the previous research has been limited to testing whether animals can distinguish between one and up to three simultaneous callers (McComb, Packer and Pusey, 1994; Grinnell, Packer and Pusey, 1995; Seddon and Tobias, 2003; Kitchen, 2004). Yet, it has been suggested that cognitive limitations may mean that assessors of group calling will fail to accurately judge caller number once chorus size exceeds two or three (Harrington, 1989; McComb, Packer and Pusey, 1994). Furthermore, a recent test of human auditory abilities showed that the upper bound for human listeners to accurately estimate the number of simultaneous speakers is three (Vitevitch and Siew, 2015). Therefore, extending our

knowledge of the acoustic numerical capabilities of non-human animals may enhance our understanding of the cognitive system underlying numerical representation in both humans and non-human animals (Feigenson, Dehaene and Spelke, 2004; Abramson et al., 2011; Benson-Amram et al., 2011).

In Chapter 2, I present a playback experiment in which the vocal choruses of either three or five same-sex intruders were broadcast to single lions separated from the rest of their pride. I aimed to test the following predictions about game theory and the limitations of acoustic numerical assessment in lions: 1) if limitations in the auditory and cognitive capabilities of lions result in an inability to accurately assess caller group size when the number of callers is above three, lions should not respond differently (in the probability and nature of approach) to the choruses of three and five intruders roaring together, 2) if the sexually-dimorphic response of lions towards territory intruders holds true in the Okavango Delta ecosystem, only male lions should approach multiple simulated intruders. Female lions should retreat from or fail to approach intruder groups as they are outnumbered, and 3) when approaching an intruder group, if lions can differentiate between the vocal choruses of three and five intruders, lions of both sexes should approach the larger group in a more cautious manner (characterised by a longer time to reach the speaker with more movement pauses).

Cross-modal individual recognition in wild African lions
Individual recognition is an ability thought to have been fundamental in driving the evolution of complex social systems (Hamilton, 1963; Trivers, 1974), but providing robust scientific support for 'true' individual recognition has historically proved difficult (Adachi, Kuwahata and Fujita, 2007; Johnston and Peng, 2008; Kondo, Izawa and Watanabe, 2012). Previous investigations have typically viewed individual recognition within a single sensory modality (Johnston and Peng, 2008), and have often

failed to differentiate between true recognition at the level of the individual, and a system of recognition based on familiarity (Adachi, Kuwahata and Fujita, 2007). However, recent advances in the cognitive sciences have resulted in a growing body of research providing evidence for individual recognition in non-human animals by virtue of demonstrating cross-modal processing of information on identity (Adachi, Kuwahata and Fujita, 2007; Sliwa, et al., 2011).

Cross-modal sensory perception is the ability to integrate information from multiple senses – in the case of individual recognition this often involves matching vocal and visual cues, which may be demonstrated through experiments in which subjects detect a mismatch when the cues do not correspond (Seyfarth and Cheney, 2009; Sliwa, et al., 2011). While there is now direct evidence for cross-modal recognition in a range of species tested in captive or domestic settings (Adachi, Kuwahata and Fujita, 2007; Johnston and Peng, 2008; Proops, McComb and Reby, 2009; Sliwa, et al., 2011; Kondo, Izawa and Watanabe, 2012; Kulahci et al., 2014), this ability has not been directly shown in the wild during natural social communication among conspecifics.

In Chapter 3, I present a playback experiment that employed a cross-modal expectancy violation paradigm to investigate the ability of audio-visual individual recognition in lions. In this study, a vehicle was used to create a visual block between an individual and the test subject, before roars were played from behind the visual block that either matched this individual (congruent trials) or corresponded to an absent group member (incongruent trials). When presented with a scenario where the playback of a roar broadcast from behind a visual block is incongruent with the conspecific previously seen there, I predicted that subjects would respond more strongly than during the congruent scenario where the call and individual matched. In particular, I hypothesised that lions would react more quickly to the incongruent trials, would display more

'searching' behaviour (looking towards the call direction and moving around the experiment site), and would perform more displacement behaviours that can be indicative of social stress (Mohiyeddini, Bauer and Semple, 2013).

Scent-marking behaviour of wild African lions (Panthera leo)

Scent-marking is believed to be the main mode of communication for most mammals, particularly for solitary, nocturnal, and wide ranging species such as the felids (Wyatt, 2014). Cats have numerous specialised scent glands, and all felids are thought to use urination for scent-marking (Brown and Macdonald, 1985; Asa, 1993; Mellen, 1993; Pageat and Gaultier, 2003). Nevertheless, the study of olfactory communication in felids has been limited by the elusive and nocturnal habits of cats (Vogt et al., 2014), and detailed data on marking behaviour and the responses of individuals to the scent-marks of conspecifics have been documented for few wild felids (Vogt et al., 2014; Allen, Wallace and Wilmers, 2015). Yet, there is accumulating behavioural (Schaller, 1972; Asa, 1993; Brahmachary and Singh, 2000; Lehmann et al., 2008; Barja and de Miguel, 2010; Gilfillan et al., 2016) and chemical (Andersen and Vulpius, 1999; McLean et al., 2007; Umapathy et al., 2007; Poddar-Sarkar et al., 2008; Soini et al., 2012; Umapathy et al., 2013; Poddar-Sarkar and Brahmachary, 2014) evidence to suggest that scent-marking may be important in the social lives of lions.

In chapter 4, I employ behavioural observation to provide a detailed analysis of the scent-marking social behaviour of African lions. Firstly, I describe the marking behaviours of wild lions, and explore the differences between different age-sex classes in deposit types and deposit placement. I then examine intra-group responses to deposits (investigation, overmarking and flehmen) in more detail, to identify which deposits hold a communicatory function, hypothesising that in lions certain scent deposits are used in olfactory communication more than others. Communication by definition involves both

a sender and a receiver, and stereotypical responses to deposits (e.g. scent investigation or scent overmarking) can reveal a communicatory function (Jordan et al., 2013). I predicted that the patterns of responses to deposits will be non-random, and that the type of deposit, the sex and age of the depositor and recipient, and the mode of deposit placement will be important in determining the subsequent response of group members.

Spontaneous discrimination of urine odours in wild African lions (Panthera leo)
The results of chapter 4 appeared to highlight a function for scent-marking in the communication of lions within a social group. However, to fully understand the functions of scent-marking, we need to consider the information content of olfactory signals. Experimental manipulation and presentation has proved to be a powerful technique to help gain insight into the functional meaning of communication signals between animals (Bradbury and Vehrencamp, 2011). In chapter 5, I use a 'whole-scent' presentation experiment to investigate the information content of the urine scent-marks of lions. Pre-collected samples of urine deposits were presented to resting lion groups, and the responses of test subjects were filmed. I tested whether lions are able to discriminate between the urine of conspecifics based on the donor's sex and social group. Accordingly, I presented three urine treatments from the following donors: 1) adult female residents, 2) adult male residents, and 3) adult female non-residents. Urine samples were selected for this experiment because they were malleable to collection, storage, and presentation, and urine was also highlighted as a potentially important scent-mark in the results of chapter 4.

I hypothesised that olfactory communication in lions will extend to sex and social discrimination. I predicted that lions presented with urine from resident adult males and females would be more likely to respond (e.g. sniff, lick or overmark the urine), and would spend longer responding to urine from opposite sex conspecifics. In this way,

lions could assess the reproductive condition and familiarity of mates (Charlton, 2014; Tinnesand et al., 2015). I expected that olfactory sex discrimination would be more pronounced in adult lions than in sexually immature subadults and cubs. I also predicted that female lions would be more likely to respond, and spend longer responding to urine from non-resident females than from resident females. With the ability to discriminate between the scents of resident and non-resident individuals, females could detect the presence of potential rivals for their territory. In contrast, male lions may not necessarily be expected to show a strong difference in response to resident and non-resident female urine, as both signal the presence of a potential mate. Since lions of all ages are at risk during territory take-overs, I hypothesised that there should be no clear age differences in the ability to discriminate the urine of resident from non-resident females.

Study site

This study was conducted at the south-eastern edge of the Okavango Delta in northern Botswana (study site: ca. 1500 km^2; 19°31'S, 23°37'E; elevation ca. 950 m; Figure 1.5). Originating in Angola, the Okavango river meets the African rift valley in northern Botswana and drains into the Kalahari sands (Parker, 2010). The result is a vast inland water delta system that consists of a heterogeneous matrix of habitat types including perennial floodplains, grasslands, mixed *Acacia* spp., sandveld, and mopane (*Colophospermum mopane*) woodlands (Cozzi, 2012).

Figure 1.5. A map of Botswana with the Okavango Delta highlighted in the north-east corner.

The Okavango ecosystem is driven and maintained by strict seasonal changes that result in the presence of ground water throughout much of the year (Mendelsohn et al., 2010). Between June-August each year, rainfall in the Angolan highlands reaches its destination in the delta, creating a seasonal flood. The level of the flood fluctuates from year to year, and is dependent on the rainfall in the catchment area of Angola. Complex global climate trends such as El Niño drive long-term (several year) cyclic changes in the level of the delta flood, resulting in so-called 'wet' and 'dry' years (Murray-Hudson 2009). During wet years, the delta can swell up to three times its permanent size (Cozzi, 2012). During the dry summer months (September-November), the floodwaters evaporate and reach their lowest levels in the new year (Cozzi, 2012). The annual rains begin in December, and continue until March, where precipitation can range from 300 –

710 mm per year (Parker, 2010). Year-round water supports a highly productive ecosystem, where the majority of herbivore species remain sedentary (Bennit, 2012).

The Okavango ecosystem represents an important strong-hold for many of the African large carnivore guild species, including the African lion, spotted hyaena, African wild dog, African leopard (*Panthera pardus*), and African cheetah (*Acinonyx jubatus*). The lions of the Okavango make up a major part of a metapopulation within the Kavango-Zambezi (KAZA) Transfronteir Conservation Area, which represents the second largest lion population in Southern Africa (over 2000 lions: Riggio et al., 2013). However, of 21 Southern African lion populations, only the Okavango population is currently in decline (Riggio et al., 2016). The main threats to lions in the KAZA Conservation Area include human-wildlife conflict, habitat degredation and prey depletion (Moeller, 2014; Kotze, 2016).

This study was conducted between March 2014 and December 2015 on a core lion population of four prides of females and two resident male coalitions in the south-eastern region of Moremi Game Reserve and the surrounding wildlife management areas (Figure 1.6). However, additional lions throughout the study population were occasionally observed during this study, and their behaviour recorded. The population had been studied since 2007, and I therefore had a relatively strong understanding of the pride histories. During the study, the number of adults in the core population grew from 14 to 25, and cub survivorship was consistent with populations in east Africa (approximately 50% of cubs die before one-year: Hanby and Bygott, 1979). One adult lioness died during the study but she was known to be at least 13 years old and I believe she died under 'natural' circumstances in old age (as opposed to anthropogenic causes). Another adult lioness emigrated away from her natal pride after giving birth to cubs, and was later seen in the periphery of the study area. The population density of lions in

the immediate study area was recently recorded as 23.1 individuals/100 km^2 (see

floodplain estimate by Cozzi et al., 2013), which represents a high-density lion

population (Chardonnet, 2002).
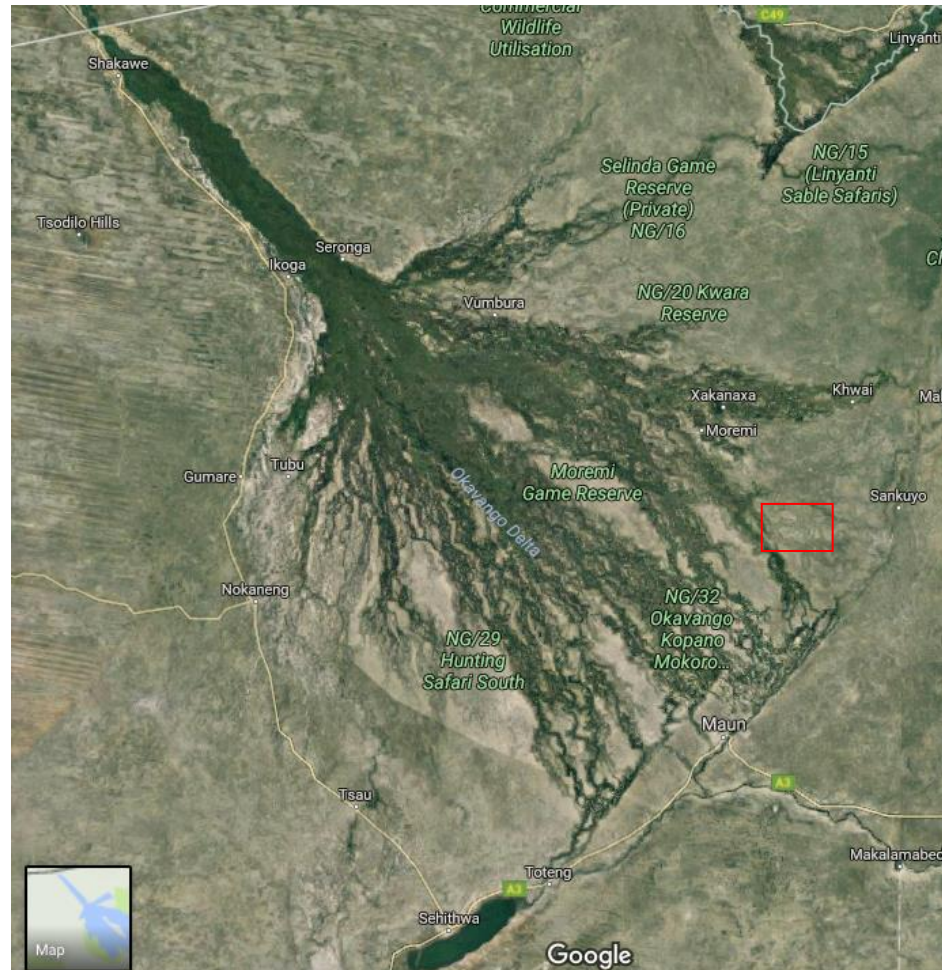
Figure 1.6. A map of the Okavango Delta (approximate core study area highlighted with red box).

Methodology

To monitor the behaviour of several lion social groups, nine adults (three males and six

females) were fitted with radio collars equipped with a VHF (very high frequency)

transmitter, and in all but one case, a GPS (global positioning system) device. The

VHF-GPS radio collars were built by the Royal Veterinary College, U.K (< 1080 g) and

the VHF radio collar was supplied by African Wildlife Tracking, South Africa (< 780

g). A Botswana-registered veterinarian was employed to anaesthetise the lions so that

the collar could be properly fitted and secured. Drug cocktails and quantities used to tranquillise the lions varied according to the preferences of the veterinarian and the body condition of the lion (e.g. full or empty stomach). The drugs were injected using a dart shot from a CO_2-pressurised dart-gun (various brands) at distances ranging from 15 to 35 m. During the anaesthesia blood samples and body morphometric measurements were taken, and a general health check was performed. After collaring, lions were regularly observed to check that the collar was not harming the animal.

On a typical day, lions were located through spoor- or radio-tracking from a vehicle shortly after sunrise. Lions were then observed at distances of 10-40 m while resting and 20-200 m while moving, depending on visibility and habitat. All social (e.g. grooming and aggression) and communication behaviours were recorded in a note book using critical incident sampling (Altmann, 1974), and the locations of all key behavioural events were recorded with a hand-held GPS unit (Garmin e-trex 20). During these observation periods I attempted to record all vocalisations given by lions using a Sennheiser MKH800 P48 microphone (frequency response 30 Hz–50 kHz; Sennheiser Electronic GmbH & Co., Wedemark, Germany) with a windshield linked to a Fostex FR2 digital audio recorder (frequency response 20 Hz– 40 kHz 2 dB; Fostex, Tokyo, Japan). Calls were recorded in mono at distances between 10 and 30 m, with a sampling frequency of 44.1 kHz and 16 bit sampling width. I also collected samples of urine deposits from adult lions (i.e. sexually mature). After observing a lion urinating onto the soil and moving away from the area, a clean metal spoon was used to collect the urine-soaked soil within a sterilised glass jar with an aluminium-lined lid. The sample jar was then placed in a 12 V cooler box to store the urine 15 °C below the ambient temperature. After the lions had been resting for over an hour I typically left the area and either attempted to locate another social group, or I returned to the research

camp. At the camp, any collected urine samples were immediately placed within a freezer held at – 20 °C. Lions were then re-located approximately two hours before sunset, and an experiment was potentially performed shortly after (specific experiment methodologies are outlined in subsequent chapters). Using red-filter spotlights and natural moonlight, I then followed the lions at night for as long as the habitat permitted.

Ethical considerations

All of the research within this thesis complied with the internal University of Sussex regulations on the use of animals and were approved by the University of Sussex Ethical Review Committee (Non-ASPA 4 – November 2013). In addition, ethical clearance for all aspects of the study was granted by the Botswana Ministry of Environment Wildlife and Tourism (8/36/4 XXV (8)). No lions were harmed as a result of this research.

Comment on authorship

The research presented in this dissertation is the result of collaborative efforts, and each data chapter has been prepared as an individual manuscript with the input of multiple co-authors. Therefore, I use the term "we" instead of "I" throughout the remainder of this dissertation.

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CHAPTER 2: ARE LIONS ABLE TO DETERMINE THE NUMBER OF CONSPECIFICS CALLING IN LARGE VOCAL CHORUSES?

Gilfillan, G.D., McNutt, J.W., & McComb, K. In the style of *Bioacoustics*.

Abstract

For social species that cooperatively defend territories, asymmetries in group size between competing groups can determine the outcome of conflict. Where groups communicate through vocal signals, individuals are known to use extra-group vocal choruses to assess the size of the competing group and the likely outcome of conflict. However, the range of group sizes over which animals are able to use vocalisations in this form of assessment remains poorly understood. African lions can discriminate between the call of a single lion and the chorused calls of up to three lions during territorial and reproductive conflict, but we do not know whether the numerical assessment capabilities of lions extend to the discrimination of different numbers of multiple callers beyond this. Here we use a playback experiment to investigate whether lions can discriminate between the vocal choruses of three and five intruders. Our results suggest that lions do not differentiate between the choruses of three and five intruders, as lions of both sexes almost always approached simulated intruder groups, regardless of chorus group size. In addition, lions did not appear to alter the nature of their approach towards the calling intruders depending on chorus size. Instead, lions conformed to the prediction that there are limits on acoustic numerical assessment capabilities in animals. The upper bound for lions to accurately estimate the number of simultaneous callers appears to be three, matching the abilities of human listeners performing a similar task.

Introduction

African lions (*Panthera leo*) form same-sex groups and cooperate with group members to defend access to resources and protect dependent offspring (Schaller 1972; McComb et al. 1993; 1994; Grinnell et al. 1995). The fission-fusion social system of lions means that individuals are often alone or in small sub-groups, which can make the numerical imbalance between competing lion groups more extreme (Schaller 1972). Group size is known to play an important role in determining the outcome of conflict between lions, with large groups dominating smaller groups (Packer et al. 1990). Fighting between lions is a major cause of injury and death, especially for small prides and lone individuals (Schaller 1972; Packer et al. 1990). Game theory modelling predicts that lions should use the number of individuals in competing groups to assess whether to attack or retreat from competitors (Maynard Smith & Price 1973; Maynard Smith 1974; Parker and Rubenstein 1981; Wilson et al. 2001). Playback experimentation has revealed that lions use the loud-call vocalisations of conspecifics (termed 'roars') to assess caller group size and avoid potentially lethal contests with larger groups (McComb et al. 1994; Grinnell et al. 1995; Heinsohn & Packer 1995; Grinnell & McComb 1996; Heinsohn et al. 1996; Heinsohn 1997).

Lions of both sexes use roars as their primary means of communication between groups (Ramsauer 2005). Lion groups often roar together, and the overlapping nature of their roars within the group chorus is thought to create an honest signal of minimum group size that cannot be faked (McComb et al. 1994). The playback of unfamiliar lion roars from a loudspeaker has been used to effectively simulate territorial intrusion. Lions of various age (Heinsohn et al. 1996), sex (McComb et al. 1994; Grinnell et al. 1995; Grinnell & McComb 1996) and ecosystem (Heinsohn 1997) appear to use the number

of vocalising individuals (up to three callers), as well as their own sub-group size to assess the cost of engaging in conflict.

Due to the sexually dimorphic reproductive strategies of lions (Schaller 1972), the costs of engaging in conflict with same-sex intruders appear to differ for male and female lions, which has been reflected in a sex-dependent response to the playback of same-sex intruder calls (McComb et al. 1994; Grinnell et al. 1995). Because female lions have a relatively long reproductive lifespan (Packer et al. 2001), the optimal strategy for females during conflict with territory rivals appears to be to attack only when the numeric odds are in their favour, or when they are defending a particularly valuable resource such as cubs (McComb et al. 1994; Heinsohn 1997). In contrast, males have a short reproductive lifespan, and when evicted from a pride their chances of re-gaining access to females is low (Bygott et al. 1979). Consequently, males may be competing for their entire reproductive output during each territorial contest, and it is likely that the optimal conflict strategy for male lions is to attack intruders even when outnumbered (Grinnell et al. 1995). However, up until now the response of male lions to intruder roars has only been tested in a single ecosystem (the Serengeti ecosystem), and with a maximum 'odds-against' ratio of three intruders to one defender (Grinnell et al. 1995). No investigations have examined whether the 'always-attack' male lion strategy holds true for increasing numerical disadvantages.

Vocal chorusing occurs in many social birds (e.g. subdesert mesite, *Monias benschi*: Seddon & Tobias 2003; green woodhoopoe, *Phoeniculus purpureus*: Radford 2003) and mammals (e.g. banded mongoose, *Mungos mungo*: Furrer et al. 2011; spotted hyaena, *Crocuta crocuta*: Benson-Amram et al. 2011; black howler monkey, *Alouatta pigra*: Kitchen 2004; chimpanzee, *Pan troglodytes*: Wilson et al. 2001; capuchin monkey, *Cebus capucinus*: Crofoot & Gilby 2012), and competing groups often assess one

another on the basis of caller group size (Kitchen 2004; 2006; Seddon & Tobias 2003; Benson-Amram et al. 2011). However, the numerical range over which animals are able to use group calling to discriminate between differently sized groups remains poorly understood. Much of the previous research has been limited to testing whether animals can distinguish between one and up to three simultaneous callers (McComb et al. 1994; Grinnell et al. 1995; Seddon & Tobias 2003; Kitchen 2004; 2006). There has been no direct test of whether animals are able to distinguish between larger vocal choruses, while also controlling for confounding effects such as call length or call intensity (e.g. Radford 2003). Yet, it has been suggested that cognitive limitations may mean that assessors of group calling will fail to accurately judge caller number once chorus size exceeds two or three (Harrington 1989; McComb et al. 1994). Furthermore, a recent test of human auditory abilities showed that the upper bound for human listeners to accurately estimate the number of simultaneous speakers is three (Vitevitch & Siew 2015). Vitevitch and Siew (2015) suggest that human abilities to assess the number of simultaneous speakers are limited by the cognitive phenomenon known as subitization (i.e. the ability to quickly and accurately determine the number of objects without counting: Kaufman et al. 1949). It is possible that the acoustic numerical assessment abilities of animals are constrained by similar limitations. Notably, visual and tactile subitization have been demonstrated in animals (Dacke & Srinivasan 2008; Beran et al. 2011; Agrillo et al. 2012), potentially also during inter-group conflict (Bonanni et al. 2011). Extending our knowledge of the acoustic numerical capabilities of non-human animals may further our understanding of the cognitive system underlying numerical representation in both humans and non-human animals (Feigenson et al. 2004; Abramson et al. 2011; Benson-Amram et al. 2011).

We present a playback experiment in which the vocal choruses of either three or five same-sex intruders were broadcast to single lions separated from the rest of their pride. We aimed to test the following predictions about game theory and the limitations of acoustic numerical assessment in lions: 1) if limitations in the auditory and cognitive capabilities of lions result in an inability to accurately assess caller group size when the number of callers is above three, lions should not respond differently (in the probability and nature of approach) to the choruses of three and five intruders roaring together, 2) if the sexually-dimorphic response of lions towards territory intruders holds true in the Okavango Delta ecosystem, only male lions should approach multiple simulated intruders. Female lions should retreat from or fail to approach intruder groups as they are outnumbered, and 3) when approaching an intruder group, if lions can differentiate between the vocal choruses of three and five intruders, lions of both sexes should approach the larger group in a more cautious manner (characterised by a longer time to reach the speaker with more movement pauses).

In addition, it is thought that lions roar during territorial defence to deter intruders and recruit group members (Schaller 1972; McComb et al. 1994; Ramsauer 2005). We hypothesised that lions would be more likely to roar, would roar earlier and would roar at a greater rate in response to playbacks simulating a greater need for assistance in territorial defence (i.e. choruses of five intruders rather than three). We also hypothesised that group members would be more likely to join the defending lion at the experiment location if the test subject roared in response to the playback.

Methods

Study population and site

This study was conducted between May 2014 and November 2015 on a free-ranging lion population in northern Botswana. The study area (ca. 1500 km 19°31'S, 23°37'E;

elevation ca. 950 m) was bordered by the Okavango Delta and included the Moremi Game Reserve and surrounding Wildlife Management Areas. Further details can be found in McNutt (1996). Life histories and demographic data were available on the study population since 2007, and all individuals were habituated to vehicles. Individual lions were identified from their unique whisker-spot patterns, and were divided into demographic categories based on their sex and age, with lions over four years old classed as adults (Packer et al. 1988). Where the birth date was not known, the individual was aged using body size, teeth wear, male mane development, and the observation of sexual activity (Whitman & Packer 2007).

Call acquisition

We recorded bouts of roaring (see McComb et al. 1994 for a description of lion roars) *ad libitum* from eight different adult lions (seven males and one female) in the Okavango Delta. Recordings were made between April and November 2014 using a Sennheiser MKH800 P48 microphone (frequency response 30 Hz–50 kHz; Sennheiser Electronic GmbH & Co., Wedemark, Germany) with a windshield linked to a Fostex FR2 digital audio recorder (frequency response 20 Hz– 40 kHz 2 dB; Fostex, Tokyo, Japan). Calls were recorded in mono at distances between 10 and 30 m, with a sampling frequency of 44.1 kHz and 16 bit sampling width.

We acquired additional recordings from five other wild populations in Africa, and also one recording from Gelsenkirchen Zoo in Germany. Dr Sandra Ramsauer provided 12 recordings from Khutze Game Reserve, Botswana. Dr Ramsauer recorded the roars between 2002 and 2005 using a Sennheiser MKH-70 directional microphone (frequency response 50 Hz–20 kHz; Sennheiser Electronic GmbH & Co., Wedemark, Germany) connected to a Sony TCD-D100 digital audio tape recorder (frequency response 20 Hz– 48 kHz; Sony Manufacturing Systems Europe, Weybridge, UK). Dr Jon Grinnell

provided three recordings collected from Ligwalagwala Cooperative Reserve, South Africa (recorded between 1999 and 2001), and one recording from Pilanesburg Game Reserve, South Africa (recorded in 2011). Dr Grinnell recorded the roars using an analog cassette tape (Maxell XLII High Bias) in a Sony TC-D5 Pro II recorder (frequency response 40 Hz–15 kHz; Sony Manufacturing Systems Europe, Weybridge, UK). Dr Robert Eklund provided one recording from Amakhala Game Reserve, South Africa. Dr Eklund recorded the roar in 2008 on a pocketsize digital camera. Professor Karen McComb provided eight recordings from the Serengeti National Park, Tanzania. Professor McComb recorded the roars between 1988 and 1991 using a Sennheiser MKH 816 microphone (frequency response 40 Hz–20 kHz; Sennheiser Electronic GmbH & Co., Wedemark, Germany) linked to a Panasonic SV250 digital audio tape recorder (frequency response 10 Hz–22 kHz; Panasonic, Secaucus, NJ, USA). Finally, Dr Gustav Peters provided one recording from Gelsenkirchen Zoo. Dr Peters recorded the roar at 19 cm on a UHER Report 4200 recorder (frequency response 35 Hz–20 kHz; Uher Werke, Munich, Germany) with a Sennheiser MD 421/2 microphone (frequency response 30 Hz–17 kHz; Sennheiser Electronic GmbH & Co., Wedemark, Germany).

Exemplar construction

To create the playback stimuli of either three or five intruders calling in chorus, we used recordings of 13 different lions roaring alone, as well as different choruses of two (n = 8), three (n = 9), and five (n = 1) lions roaring together. The average length of recording was 44.8 s (SD +/- 12.5). We used the Praat (v. 5.3.59; Boersma & Weenink 2016) acoustic software package to trim or extend call duration so that each recording was standardised to 45 seconds in length (mean = 44.9, SD +/- 0.9). When trimming or extending the recordings, care was taken to maintain the natural structure of the chorus (introductory moans, full-throated roars, and concluding groans), and involved cutting

or copying and pasting sections of the calls where necessary (consistent with Grinnell et al. 1995). Next, the 'Combine to Stereo' and 'Convert to Mono' commands in Praat were used to combine the recordings to create stimuli with the appropriate number of callers. Where frequency resampling was necessary to combine recordings, the 'Filter' (stop Hann band) command was used to filter out frequencies less than 20 Hz to remove potential artefacts of the resampling procedure (email correspondence from K. McComb to G. Gilfillan, unreferenced), and then the 'Resample' command in Praat was used to standardise and resample the recordings to 44.1 kHz with a precision of 50. Natural recordings of four (n = 1), five (n = 1) and six (n = 1) lions roaring in chorus were used as a template, and care was taken to combine recordings in a realistically staggered manner. Finally the recordings were normalised to 99% peak amplitude (to control for any variations in amplitude: McComb et al. 2009) and saved as AIFF files (44.1 kHz sampling rate and 16 bits amplitude resolution). The resulting playback exemplars were an average length of 45.1 s (SD +/- 1.5 s). To help control for caller identity the majority of recordings (17 of 31) were used to create both chorus group size treatment stimuli (i.e. three and five callers).

Experiment procedure

Playback techniques followed those of McComb et al. (1994). After using a vehicle to locate an adult lion separated from the rest of their social group, a Tannoy CPA 12 studio monitor loudspeaker (frequency response 50 Hz–25 kHz 3 dB; Tannoy Ltd., Coatbridge, UK) was placed 200 m from the test subject (measured with a Garmin etrex 20 handheld GPS) and any available vegetation was used to conceal the speaker. The vehicle was then positioned 100 m from the speaker so that the test subject was visible to the observer, if vegetation cover allowed. Care was taken to avoid positioning the vehicle directly between the speaker and the test subject. A speaker cable (100 m, 7

AWG) was used to connect the speaker to a Kicker Impulse amplifier (frequency response 20 Hz–20 kHz 0.5 dB; Stillwater Designs, Stillwater, OK, USA) within the vehicle. The amplifier was linked to a Fostex FR2 digital audio recorder, which was used to play the choruses of either three or five lions roaring together. Recordings were always of unfamiliar lions naturally resident at least 30 km away from the test subject's home range (determined through GPS collar data: unpublished data). Playbacks were started within 90 min before sunset (mean = 71.0 min, SD +/- 17.8), thus restricting playbacks to when lions naturally roar (Schaller 1972). To control for defender group size and motivation, playbacks were only conducted to single adult lions, and were not conducted if: (1) the lion was on a kill, (2) the lion was emaciated or had obvious wounds, (3) the lion was outside of its normal territory range, and (4) there were known to be other lions (of any age or sex) within 1000 m of the test subject. The playback recordings were standardised with a peak pressure level of 116 dB at one metre from the source (peak intensity measured at 1 m using a handheld AZ Analog Sound Level Meter model 8926; Laesent International Co. Ltd, Shenzen, China). Responses were recorded using a Bell + Howell DNV16HDZ video recorder, and every effort was made to observe the subject for at least one hour (mean = 01:40:51, SD +/- 01:02:18) after the playback. The test subject was free to move around after the playback, while the observer followed in a vehicle at distances of 20-200 m depending on the terrain. When additional lions joined the test subject, data collection for the experiment was terminated. Subjects were presented with both stimuli in a randomised order, with at least eight days (mean = 95.7 days, SD +/- 93.1) separating each trial to minimise the chances of habituation to the playback design. Of 17 lions tested, eight (47.1%) received the chorus of three intruders first.

Vegetation cover can potentially play a role in the response of male lions to the playback of intruder roaring bouts (Grinnell et al. 1995). Therefore, the density of the vegetation within 100 m of the test subject was scored on a five-point scale by the observer: 1) open grassland or pans with no visually-limiting vegetation, 2) open grassland with patches of long grass or shrubs, 3) open woodland with trees and taller vegetation, 4) shrubland with many patches of long grass or shrubs, and 5) dense woodland with vegetation severely limiting vision. For analyses involving habitat density, the habitats were further grouped as high or low. High densities (n = 13) reflected a situation in which visibility within 100 m of the test subject was severely limited by tall grass, shrubs, or trees (categories 3, 4 and 5). Low densities (n = 20) reflected situations with short grasses and few trees within a 100 m of the test subject (categories 1 and 2).

Behavioural analysis of response

Video recordings were analysed on a Fujitsu Siemens Amilo Pi2515 laptop using Avidemux 2.6.9 (Mean Development Team 2015) video analysis software. The key monitored behavioural responses to the playbacks are outlined in Table 2.1.

Table 2.1. Recorded behavioural responses of lions following the playback of either three or five lions roaring in chorus.

Response	Definition
Approach the speaker	Where the test subject moved closer to the speaker location from their resting spot following the playback.
Latency to speaker (s)	The length of time (s) between the onset of the playback, until the subject reached the speaker level. Where the subject did not reach the speaker level, a score of NA was given.
Number of pauses during the first 200 m of movement towards the speaker	A single pause in movement was defined as when a lion remained stationary after previously moving. If the subject did not move 200 m, or did not approach the speaker following the playback, a score of NA was given. A distance of 200 m was chosen as the speaker was placed 200 m from the test subjects.
Latency to roar (min)	The length of time (min) between the onset of the playback and the onset of the first roaring bout delivered by the test subject. Where subjects did not roar, a score of NA was given.
Number of roars made following the playback	Number of roaring bouts performed by the test subject following the playback.
Joined by group members	Where additional lions joined the subject within 500 m of the speaker location during the observation session following the playback.

Statistical analyses

All statistical analyses were conducted using the R statistical software package (v. 3.3.1; R Core Development Team 2016). To investigate what factors influenced the likelihood that the test subject roared after the playback, we ran a series of generalised linear mixed models (GLMMs) with a binomial error distribution (0 = did not roar, 1 = roared after playback) and a logit link function. The global model included the following fixed effects and interactions: Chorus group size + Defender sex + Chorus group size * Defender sex. In R syntax, the '+' operator denotes the addition of a term (single independent variable or an interaction term) to a model, while the '*' operator between two independent variables denotes an interaction between those variables. The identity of the test subject (n = 17) was incorporated as a random term to account for multiple data from the same individuals. The fixed effects were not strongly correlated (measured using the variance inflation factor and the condition number test). The 'lme4' package (Bates et al. 2015) was used to run the global model. The dredge function in

package 'MuMIn' (Bartoń & Bartoń 2015) was used to automate model selection from the global model. We used Akaike's information criterion with a correction for small sample sizes (AICc) for model selection, where lower AICc values corresponded with better support for a given model (Akaike 1974). Conditional model averaging using MuMIn was conducted on all models within two AICc of the optimal model (lowest AICc) to extract the relative importance of predictors in the averaged models, their averaged parameter estimates, and their 95% confidence intervals (Symonds & Moussalli 2011). Relative variable importance values (i.e. the sum of Akaike Weights over all models including the explanatory variable: Bartoń & Bartoń 2015) were calculated for any independent variables retained in the averaged model. The relative importance of a predictor can be interpreted as equivalent to the probability that the predictor is a component of the best model (Symonds & Moussalli 2011). Relative importance based on Akaike Weights has been shown to closely match r^2 effect size rankings (Giam & Olden 2015), and those explanatory variables with a relative importance value greater than 0.5 can be viewed as the most important predictors (Dala-Corte et al. 2016).

We also ran a series of GLMMs with a Gaussian error distribution to investigate what factors influenced: i) the latency of test subjects to reach the level of the speaker; ii) the number of pauses made by lions within the first 200 m of movement towards the speaker; iii) the rate of roaring; and iv) the latency to roar for test subjects who roared following the playback of intruder vocal choruses. The global models included the following fixed effects and interactions: Chorus group size + Defender sex + Habitat density + Chorus group size * Defender sex + Defender sex * Habitat density, and the identity of the test subject was included as a random term. To address normality violations, the 'latency to reach the speaker' and the 'latency to roar' dependent

variables were transformed with a logarithm function ($\log_{10}$). We again used the dredge function to select candidate models with delta AICc < 2, and model averaging to extract averaged parameter estimates, their relative importance, and 95% confidence intervals. Following Zuur et al. (2009), model dredging was performed with the maximum likelihood estimation method, and model averaging was performed with the restricted maximum likelihood estimation method.

Due to parametric assumption violations, non-parametric Fisher's exact tests were used to investigate whether: 1) lions of both sexes were less likely to approach the speaker after hearing the vocal choruses of five intruders rather than three intruders; 2) lions were more likely to be joined by group members after roaring in reply to the playback of intruder calls; and 3) lions were more likely to be joined by group members after the broadcast of a larger vocal chorus. Where multiple comparisons were performed the Bonferroni correction was applied to alpha.

Non-parametric statistics were also used to investigate whether lions habituated to the playback of intruder roars between the first and second playback trial. Both the likelihood for lions to approach the speaker and the likelihood that lions roared following the playback were assessed for habituation with Fisher's exact tests. Two-tailed Wilcoxon signed rank tests (with Bonferroni correction of alpha) were used to assess habituation in relation to the number of pauses made by test subjects within the first 200 m of movement towards the speaker, the latency for lions to reach the level of the speaker, and for those lions that roared following the playback, the latency for lions to roar and the rate of roaring. Finally, a two-tailed Mann-Whitney-Wilcoxon Test was used to test whether the playback exemplars of three or five individuals roaring together were statistically similar in length (s).

Results

A total of 33 playbacks were conducted on eight male (16 trials) and nine female lions (17 trials; Table 2.2). Test subjects approached the speaker following the playback of intruder group choruses in all but three trials (90.9% of trials); these non-approaches occurred twice after the chorus of three intruders was broadcast (both involving female defenders), and once after the chorus of five intruders (involving a male defender). Neither males (Fisher's exact test, d.f = 1, $P = 0.999$), nor females (Fisher's exact test, d.f = 1, $P = 0.206$) were less likely to approach the speaker after hearing the vocal choruses of five rather than three intruders. Lions roared following 18 playbacks (54.5% of trials), but were as likely to roar after hearing the choruses of three intruders than they were after hearing the choruses of five intruders (Table 2.3). Males and females roared at a similar rate following the playback of intruder chorusing (Table 2.4), but females were quicker to roar than were males (Table 2.5; 2.6). Chorus group size did not have a significant effect on either the test subject's rate of roaring or latency ($\log_{10}$) to roar. In addition, the latency ($\log_{10}$) for lions to reach the speaker did not depend on chorus group size (Table 2.7), nor did the number of pauses made within the first 200 m of movement towards the speaker (Table 2.8).

Table 2.2. Summary of the raw data describing how lions of both sexes responded to the vocal choruses of three and five intruders.

Defender Sex	Chorus group size	Number of trials	Number of approaches to speaker	Latency to speaker (s)	Number of pauses	Number of trials where the defender roared	Latency to roar (min)	Roars/hour	Number of trials where group members arrived
Male	Three	8	8	1,973.3 (2,580.9)	5.3 (2.7)	3	175.2 (211.5)	1.20 (1.85)	0
	Five	8	7	1,189.0 (1,298.0)	6.7 (4.9)	5	68.0 (45.0)	1.09 (1.38)	3
Female	Three	8	6	946.0 (657.0)	4.4 (4.4)	5	37.8 (24.8)	1.11 (1.16)	0
	Five	9	9	785.6 (342.5)	6.4 (5.6)	5	58.6 (69.4)	2.16 (3.23)	4
Total		33	30	1,291.6 (1,604.8)	5.7 (4.5)	18	86.8 (123.8)	1.41 (2.07)	7

mean (+/- SD)

Table 2.3. Best GLMMs with binomial error distribution selected using AICc investigating the factors that influenced the likelihood that the test subject (n = 33) roared after the playback of intruder choruses.

Model	Description	AICc	k	Δi	wi
1	Intercept only	49.7	2	0.00	0.55

Lion identity (n = 17) was included as a random term. k = parameters, Δi = AICi-AICmin, wi = Akaike weights. Only models with delta AICc < 2 are shown.

Table 2.4. Best GLMMs with Gaussian error distribution selected using AICc investigating the factors that influenced the rate of roaring from lions (n = 18) after the playback of intruder choruses.

Model	Description	AICc	k	Δi	wi
1	Intercept only	84.7	3	0.00	0.55

Lion identity (n = 13) was included as a random term. k = parameters, Δi = AICi-AICmin, wi = Akaike weights. Only models with delta AICc < 2 are shown.

Table 2.5. Best GLMMs with Gaussian error distribution selected using AICc investigating the factors that influenced the latency ($\log_{10}$) for lions (n = 22) to roar after the playback of intruder choruses.

Model	Description	AICc	k	Δi	wi
1	Intercept only	44.45	3	0.00	0.51
2	Defender sex	44.49	4	0.05	0.49

Lion identity (n = 16) was included as a random term. k = parameters, Δi = AICi-AICmin, wi = Akaike weights. Only models with delta AICc < 2 are shown.

Table 2.6. Factors that influenced the latency ($\log_{10}$) for test subjects to roar following the playback of intruder choruses. Model parameters generated using model averaging on the optimal GLMMs selected using AICc (Table 2.5).

Variable	Description	Estimate	SE	CI (2.5-97.5%)	Relative Importance
Intercept		3.254	0.217	2.805-3.704	-
Defender	Female	-	-	-	0.49
	Male	0.588	0.284	-0.022-1.198	-

SE = Standard Error. CI = Confidence Interval.

Table 2.7. Best GLMMs with Gaussian error distribution selected using AICc investigating the factors that influenced the latency ($\log_{10}$) for lions (n = 22) to reach the level of the speaker after the playback of intruder choruses.

Model	Description	AICc	k	Δi	wi
1	Intercept only	30.4	3	0.00	0.54

Lion identity (n = 14) was included as a random term. k = parameters, Δi = AICi-AICmin, wi = Akaike weights. Only models with delta AICc < 2 are shown.

Table 2.8. Best GLMMs with Gaussian error distribution selected using AICc investigating the factors that influenced the number of pauses made by lions (n = 27) within the first 200 m of movement towards the speaker following the playback of intruder choruses.

Model	Description	AICc	k	Δi	wi
1	Intercept only	164.2	3	0.00	0.46

Lion identity (n = 16) was included as a random term. k = parameters, Δi = AICi-AICmin, wi = Akaike weights. Only models with delta AICc < 2 are shown.

Test subjects were joined by group members at the playback location twice without previously roaring (13.3% of trials where the defender did not roar), and five times after roaring (27.8% of trials). The performance of roaring by the defending lion was independent of whether group members arrived at the experiment location (Fisher's exact test, d.f = 1, $P = 0.413$). However, chorus group size was a significant predictor of whether additional group members joined the defender (Fisher's exact test, d.f = 1, $P = 0.007$). When the chorus of five intruders was broadcast, at least one group member joined the defending lion in seven out of 17 trails (41.2%), whereas group members never joined the defending lion when the chorus of three intruders was broadcast (out of 16 trials). Male and female defenders appeared equally likely to be joined by additional defenders at the playback location.

The playback exemplars of three and five individuals calling in chorus were of a statistically similar length (Mann-Whitney-Wilcoxon Test: $W = 72$, $P = 0.999$). There was also no evidence of habituation to the playback experiments. Lions were as likely to approach the speaker (Fisher's exact test, d.f = 1, $P = 0.579$), or to roar (Fisher's exact test, d.f = 1, $P = 0.999$), after the first and second playback trial. In addition, lions did not differ between the first and second trial (after the Bonferroni correction set alpha = 0.0125) in the number of pauses made in the first 200 m of movement towards the speaker (two-tailed Wilcoxon signed-rank test: n = 11, $Z = 1.36$, $P = 0.197$), the latency to reach the level of the speaker (two-tailed Wilcoxon signed-rank test: n = 8, $Z = -2.24$, $P = 0.023$), the latency to roar (two-tailed Wilcoxon signed-rank test: n = 5, $Z = -0.94$, $P = 0.438$), or the rate of roaring after the playback (two-tailed Wilcoxon signed-rank test: n = 5, $Z = -0.944$, $P = 0.438$).

Discussion

Overall, lions conformed to the prediction on the limits of acoustic numerical assessment abilities in animals, but not to the predictions of game theory. Lions of both sexes almost always approached the simulated intruder groups, regardless of whether the choruses of three or five intruders were broadcast. In addition, chorus group size was not a significant predictor of the level of 'caution' exhibited by lions when approaching intruder groups (characterised by the latency to reach the speaker and the number of pauses made during the first 200 m of movement towards the speaker). Therefore, our results suggest that lions do not differentiate between the broadcast calls of three and five intruders, although there was some evidence suggesting distant lions made a distinction.

The willingness of single females in the Okavango to approach intruding groups runs counter to previous work showing that females without dependent offspring require favourable odds (i.e. more defenders than calling intruders) to approach (McComb et al. 1994; Heinsohn 1997). However, in Tanzania, prides in the Ngorogoro Crater ecosystem appeared more likely to approach calling intruders (though not significantly so), than were prides in the Serengeti ecosystem, irrespective of the odds (Heinsohn 1997). In fact, at least one female approached the simulated intruders in all playback trials in Ngorogoro, regardless of whether the calls of one or three intruders were broadcast, and the number of intruders to defenders had little effect on the speed of their approach (Heinsohn 1997). The responses of Okavango females to intruder roars are more consistent with females in Ngorogoro than in the Serengeti. It was suggested by Heinsohn (1997) that a high lion population density, and restricted area for population expansion meant there was a greater level of inter-pride competition for resources and stronger territory defence in Ngorogoro, although the responses of prides may have

been confounded by the presence of juveniles. The Okavango Delta is a highly productive ecosystem with water all year-round (Mendelson et al. 2010), and largely sedentary prey populations (Bennit, 2012). The population density of lions in the immediate study area was recently recorded as 23.1 individuals/100 km^2 (see floodplain estimate by Cozzi et al. 2013), which represents a high density lion population (Chardonnet 2002). The current study site is directly adjacent to dense woodland containing very low densities of lions (Cozzi et al. 2013), and also farmland where lethal control of lions can take place, potentially limiting the area for population expansion. Therefore, our results in the present study possibly reflect high levels of competition for territory between female prides within the Okavango Delta.

That single males almost always approached simulated intruder groups in this study provides further support for the 'always-attack' strategy for males (Grinnell et al. 1995). However, Grinnell et al. (1995) also demonstrated that groups of male defenders use the level of vegetative cover to alter their response to the calls of intruders. When cover was thick, males approached simulated intruders more slowly, and coalition partners spread out further. In the current study, we limited our playbacks to single defenders and found that vegetation cover had no significant effect on the speed of approach towards simulated intruder groups for lions of either sex, nor on the number of movement pauses made by lions during their approach. It could be that habitat density during territory conflict is more relevant for lions defending as a group. Cooperative group defence would likely be more effective when individuals are able to coordinate their movements in a manner similar to cooperative hunting (Stander, 1992). Thick vegetation could require defenders to spread out to locate and out-flank intruders, while also creating a need for a slower approach to maintain visual contact between group members (although Grinnell et al. 1995 also showed that cover was unrelated to the

number of glances males make towards their companions when approaching intruders).

Lone individuals do not have to coordinate their movements with group members, and

so habitat cover may be less relevant in determining their speed of approach.

Previous research has shown that lions typically approach calling intruders more slowly

and/or with more movement pauses when facing increasing numerical disadvantages

(McComb et al. 1994; Grinnell et al. 1995; Heinsohn 1997). In our experiments, chorus

group size did not have a significant effect on the speed of approach towards simulated

intruder groups, nor on the number of movement pauses made by lions during the first

200 m of their approach. Combined with the willingness of single lions to approach

simulated intruder groups, these results suggest that lions do not differentiate between

the choruses (and threat level) of three and five intruders. Instead, the response of lions

to intruder choruses provides stronger support for the predication that cognitive

limitations may mean that assessors of group calling will fail to accurately judge caller

number once chorus size exceeds two or three (McComb et al. 1994). A recent test of

the acoustic numerical assessment capabilities of human listeners demonstrated a

similar limitation, where the upper bound for listeners to accurately estimate the number

of simultaneous speakers was three (Vitevitch & Siew 2015). Therefore, the acoustic

numerical assessment abilities of humans and non-human animals may be constrained

by the same cognitive limitations (e.g. subitization).

Lions are believed to roar for intra-group communication and coordination, as well as

for territorial defence (Schaller 1972; McComb et al. 1994; Ramsauer 2005). It has been

shown that female lions are more likely to roar in response to the roars of same-sex

intruders when not all pride members are present (McComb et al. 1994), but males are

highly likely to roar irrespective of whether the entire coalition is present (Grinnell et al.

1995). In the current study, lions roared after hearing intruder calls in the majority

(54.5%) of trials, but defenders were not more likely to roar, did not roar earlier, and did not roar at a higher rate after hearing larger choruses of intruders (i.e. when the assistance of recruited group members would be most needed). Therefore, the patterns of roaring by defenders following the playback of intruder choruses further suggests that lions do not differentiate between the vocal choruses of three and five intruders. In addition, the results of this study do not provide any direct support for roaring being used in recruitment, as roaring by defending lions was independent of whether additional group members arrived at the experiment location.

In contrast, group members were more likely to join the test subject at the playback location following the vocal chorus of five intruders, rather than three intruders. Since experiments were limited to situations in which group members had not been sighted within a 1000 m radius of the test subject, there is no reason to suppose that such individuals would be close to the test subject in one treatment but not in the other. Instead, it seems more likely that the choruses of five intruders may be more conspicuous to distant lions, and as a result more likely to attract additional defenders. With more lions roaring in chorus, there are effectively more roar utterances per unit time within the recording, and more acoustic energy in the sound envelope overall. We suggest that further research should directly test the acoustic numerical assessment capabilities of lions hearing distant intruder chorusing.

In conclusion, while lions are known to differentiate between the call of a single lion and the chorused calls of up to three lions, our results do not provide evidence that lions can determine the number of conspecifics calling in large vocal choruses. Upon hearing the choruses of intruder groups, lions in the Okavango Delta almost always approached the intruders, regardless of whether the vocal choruses of three or five intruders were broadcast. Chorus group size also had no significant effect on the speed of approach

towards simulated intruder groups, the number of pauses taken in the first 200 m of movement during the approach, and the patterns of roaring from defending lions (i.e. the likelihood to roar, the latency to roar, and the rate of roaring). Distant lions were more likely to join the defender after the chorus of five intruders was broadcast, rather than three intruders, but this may be because of the lower conspicuousness of the latter. Instead, our results largely support the hypothesis that the acoustic numerical assessment abilities of lions match those of humans, and that cognitive limitations (e.g. subitization) mean that assessors of simultaneous group calling fail to accurately judge caller number once chorus size exceeds three.

Acknowledgements

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CHAPTER 3: CROSS-MODAL INDIVIDUAL RECOGNITION IN WILD AFRICAN LIONS

Abstract

Individual recognition is considered to have been fundamental in the evolution of complex social systems, and is thought to be a widespread ability throughout the animal kingdom. Although robust evidence for individual recognition remains limited, recent experimental paradigms that examine cross-modal processing have demonstrated individual recognition in a range of captive non-human animals. It is now highly relevant to test whether cross-modal individual recognition exists within wild populations and thus examine how it is employed during natural social interactions. We address this question by testing audio-visual cross-modal individual recognition in wild African lions (*Panthera leo*) using an expectancy-violation paradigm. When presented with a scenario where the playback of a loud-call (roaring) broadcast from behind a visual block is incongruent with the conspecific previously seen there, subjects responded more strongly than during the congruent scenario where the call and individual matched. These findings suggest that lions are capable of audio-visual cross-modal individual recognition and provide a useful method for studying this ability in wild populations.

Introduction

The ability to identify and discriminate between organisms according to their individually distinctive characteristics is known as individual recognition (Dale, Lank and Reeve, 2001), and is an attribute proposed to have been fundamental in driving the evolution of complex social systems (Krebs and Davies, 1978). However, while

individual recognition is thought to be a widespread ability (Tibbetts and Dale, 2007), providing robust scientific support for recognition at the level of the individual has proved difficult (Johnston and Peng, 2008, Kondo, Izawa and Watanabe, 2012). 'True' individual recognition strictly constitutes the identification of a specific individual, according to individually distinct cues, and the placement of that individual within a society of many others (Seyfarth and Cheney, 2009). In empirical terms, it is necessary to demonstrate (i) that recognition occurs at the level of the individual (rather than at a broader level) and (ii) that there is matching of current sensory cues to identity with information stored in memory about that specific individual.

Recent advances in the cognitive sciences have resulted in growing evidence for individual recognition in non-human animals by virtue of demonstrating cross-modal processing of information on identity (Proops, McComb and Reby, 2009, Sliwa et al., 2011). Cross-modal sensory perception is the ability to integrate information from multiple senses – in the case of individual recognition, this often involves matching vocal and visual cues, which may be demonstrated through experiments in which subjects detect a mismatch when the cues do not correspond (Proops, McComb and Reby, 2009, Kondo, Izawa and Watanabe, 2012). While there is now direct evidence for cross-modal recognition in a range of species tested in captive or domestic settings (Adachi, Kuwahata and Fujita, 2007, Proops, McComb and Reby, 2009, Sliwa et al., 2011, Kondo, Izawa and Watanabe, 2012), this ability has not been directly shown in the wild during natural social communication among conspecifics (Seyfarth and Cheney, 2009). Such investigations are facilitated by a study species where repeated social interactions lead to important long-term social relationships, in which communication involves multiple sensory modalities, and where communication signals are known to provide familiarity cues as well as potential cues to identity.

Wild African lions (*Panthera leo*) meet these criteria, as they live within a fluid 'fission-fusion' society in which individuals often associate with small sub-groups rather than the entire pride (Packer, Scheel and Pusey, 1990), and use their long-distance calls (termed roars) to communicate with distant group-mates (Ramsauer, 2005). Thus finding specific companions, often over considerable distances, potentially presents a significant cognitive challenge. Lions are the only felid in which both sexes are social (Ramsauer, 2005). Philopatric females form 'prides' with female kin and their offspring, communally defending a static territory from other prides, raising their young and hunting cooperatively (Schaller, 1972). Similarly, male lions form coalitions, often with related male cohorts (Packer and Pusey, 1982), and disperse from their natal territory when sexually mature in order to compete with rival coalitions for reproductive access to female prides (Packer and Pusey, 1982).

Evidence for social recognition between lions has been found in the context of territorial defence, where female lions appear able to recognise and remember the past defensive behaviours of specific group members (Heinsohn and Packer, 1995). Furthermore, lion roars have been shown to broadcast information about the number of individuals calling (McComb, Packer and Pusey, 1994, Grinnell, Packer and Pusey, 1995) and caller sex (McComb et al., 1993), while also revealing caller familiarity (McComb et al., 1993). The ability to determine conspecific familiarity is highly adaptive for lions because it allows for an appropriate response towards unfamiliar conspecifics during territorial defence and defence of offspring against potentially infanticidal males (McComb et al., 1993, McComb, Packer and Pusey, 1994, Grinnell, Packer and Pusey, 1995). The complexity of lion society and the evidence for familiarity-related cues in lion roars raises the question of whether 'true' individual recognition exists in lions.

We investigated individual recognition in lions, using an 'expectancy violation' paradigm. A vehicle was used to create a visual block between an individual and the test subject, before roars were played from behind the visual block that either matched this individual (congruent trials) or corresponded to an absent group-mate (incongruent trials). We hypothesised that 'incongruent trials' should be followed by increased 'searching' behavior (increased time looking towards call direction, and increased time moving), indicating an attempt to locate the absent group-mate. We also predicted an increased presence of tension-induced 'displacement' activities, as these are thought to alleviate stress in socially uncertain situations (Maestripieri et al., 1992). Displacement behaviours that are typically observed across a wide range of taxa include auto- or allo-grooming (Aureli and Yates, 2010), yawning (Zannella et al., 2015) and lip-licking (Mohiyeddini, Bauer and Semple, 2013).

Materials and Methods

Between May 2014 and December 2015, we performed 39 experiments on four male lions and 16 female lions from three prides in the Okavango Delta, Botswana. The study area (ca. 1500 km 19°31'S, 23°37'E; elevation ca. 950 m) was bordered by the Okavango Delta and included the Moremi Game Reserve and surrounding Wildlife Management Areas. Further details can be found in McNutt (1996). Life histories and demographic data were available on the study population since 2007, and all individuals were habituated to vehicles. Individual lions were identified from their unique whisker-spot patterns, and were divided into demographic categories based on their sex and age, with lions over four years old classed as adults (Packer et al., 1988). Where the birth date was not known, the individual was aged using body size, teeth wear, male mane development, and the observation of sexual activity (Whitman & Packer 2007).

Sound recordings of lion long-distance calls (see: McComb, Packer and Pusey, 1994) were collected *ad libitum* between April 2014 and May 2015 using a Sennheiser MKH800 P48 microphone with windshield linked to a Fostex FR2 (Fostex, Tokyo, Japan) digital audio recorder. Calls were recorded in mono at distances between 10 and 30 m, with a sampling frequency of 44.1 kHz and 16 bit sampling width. High quality recordings (made by G.G) of 12 lions roaring alone (mean length = 43.84 seconds, SD = 10.26) were used to create the playback stimuli. The Praat 5.1.03 DSP software package, www.praat.org was employed to trim or extend call duration so that each recording was standardised to 43 seconds in length (mean length = 43.48 seconds, SD = 0.67). When trimming or extending the natural recordings, care was taken to maintain the natural structure of the call (introductory moans, full-throated roars, and concluding groans), and involved cutting or copying and pasting sections of the call where necessary. Finally, the recordings were normalised to 99% peak amplitude (to control for any variations in amplitude: McComb et al., 2009) and saved as AIFF files (44.1 kHz sampling rate and 16 bits amplitude resolution).

To avoid sexually-motivated responses, subjects were selected from a unisex group resting approximately thirty metres apart, but still in visual contact. A vehicle was then positioned to create a visual block between two of the adult lions (Figure 3.1). After a short period (30 seconds to one minute) designed to ensure that some form of stored information had to be accessed (Proops, McComb and Reby, 2009), a Tannoy® CPA 12 studio monitor loudspeaker positioned within the vehicle was used to play the roars (standardised to 116 dB at 1m from the source) of either the appropriate visually blocked individual (congruent trial), or of a lion of the same social group who was currently absent (incongruent trial). The vehicle remained stationary and the test subject was free to approach conspecifics or search for the simulated caller. Fourteen (73.7%)

subjects were played the same recording in both trials (controlled for as a random effect within the statistical models), which acted as the congruent stimulus in one treatment and incongruent in the other. Only the response of the test subject was video-recorded (using a Bell & Howell® DNV16HDZ video recorder) for analysis.
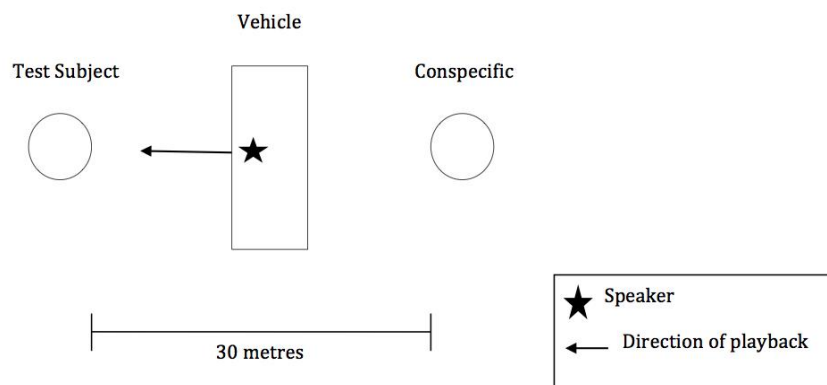
Figure 3.1. Experimental design: the vehicle (with speaker) was positioned between lions resting approximately 30 m apart. Only two adult lions were present in 79.5% of trials.

To prevent habituation, subjects were tested in both conditions in a random order (50% of subjects were tested with the congruent trial first), at least 9 days after the test subject was last involved in a playback (mean = 95.2 days, $SD \pm 86.9$). Trials began within 90 minutes before sunset (average = 63.0 minutes, $SD \pm 21.0$), which is a natural time for lions to begin roaring (Schaller, 1972), and no experiments took place if the observer had heard roaring in the vicinity of the experiment location during the day.

As experiment trials took place within natural social and environmental settings, test subjects were free to move behind visually constricting vegetation, become distracted by potential prey, or become distracted by the behaviour of conspecifics. Consequently, trials could not be standardised to the same length. Where subjects moved temporarily

out of visual contact, the session was paused and such time was not included in the analyses. Only behaviours initiated by the focal individual were included in the analyses. Trials ended when the test subject rested for at least 1 minute after the playback, or began following the movement of a conspecific, whereby the test subject was judged to have ceased responding to the playback. Behavioural responses were analysed frame-by-frame (frame = 0.033/0.034 seconds) using Avidemux® 2.6.9 video analysis software.

Key responses monitored were latency to respond, time looking towards the call direction, time moving and a range of displacement behaviours as defined in Table 3.1. Potential displacement behaviours were selected following observations of lions in socially stressful situations. For example, allo-rubbing (head-rubbing) is thought to reduce aggression between felids (Turner and Bateson, 2000) and could be a key displacement behaviour for lions when stressed. To test inter-observer reliability, a random subset of the videos were double-coded blind in a random order. Twelve videos (30.8%) were scored by the second coder, providing an inter-observer reliability of 0.949 ($P \leq 0.0001$) for proportion of time spent moving, 0.893 ($P \leq 0.0001$) for proportion of time spent looking at the call direction, and 1.00 ($P \leq 0.0001$) for rate of allo-rubbing, measured by Spearman's rho correlation.

Table 3.1. Definitions of behaviourally coded variables.

Behaviour	Coding scheme definition
Time looking towards the call direction	Looking towards the call direction was defined as taking place when the subject's head was oriented within 90 degrees of the simulated call direction.
Time moving	Lions were recorded as moving when walking or running. It should be noted that test subjects could be coded as both (at the same time) moving and looking towards the speaker.
Latency to respond	Defined as the number of seconds between the onset of the call and the time at which the lion made its first head movement towards the speaker, having previously been held in another position. Where the first head movement could not be accurately determined (e.g. obscured by vegetation) a score of NA was given and such cases were excluded from the analysis.
Resting	Lions were recorded as resting when their head was placed on the ground.
Stress-related displacement behaviours	
Lip-licking	The tongue is used to lick the outside of the lips.
Head-shaking	Test subject quickly moves the head from side to side in a visible 'shaking' movement.
Auto-grooming	Test subject begins to use their tongue to lick their own fur once, or several times in quick succession.
Allo-grooming	Test subject initiates a grooming event with a conspecific (who may already be sitting next to the test subject) whereby they use their tongue to lick the conspecific's fur once, or several times in quick succession. The conspecific may or may not reciprocate.
Allo-rubbing	Test subject initiates a rubbing event with a conspecific (who may already be sitting next to the test subject) whereby they use their head to rub the conspecific's head or body once, or several times in quick succession. The conspecific may or may not reciprocate.
Yawning	Test subject opens its mouth in a clear yawning motion.

All statistical analyses were conducted using the R® statistical package (v. 3.2.5; R Core Development Team 2016). To assess whether lions spent a greater proportion of time: 1. looking in the call direction, and 2. moving, following the playback of an absent conspecific's roar (incongruent trial) compared to the playback the visually-blocked conspecific's roar (congruent trial), we ran a series of generalised linear mixed models (GLMMs) with a binomial error distribution. The 'cbind' function in R was used to

create a link function to account for the data being proportional and thus restricted between 0 and 1. For both response variables the full model was coded as: Treatment * Sex, where backwards elimination was used to remove factors from the models. We incorporated the identity of the test subject (N = 20) and the recording used in the experiment (N = 12) as random terms to account for multiple data from the same individual lions, and when tested with the same exemplar recording. We used Akaike's information criterion with a correction for small sample sizes (AICc) to select the most plausible model from a set of credible options (Akaike, 1974). Lower AICc values corresponded with better support for a given model (Akaike, 1974), and terms were retained only if their removal inflated AICc by more than two (Burnham and Anderson, 2004). Akaike weights were then calculated to show relative importance between these models (Akaike, 1974). Model averaging (R package: MuMIn) was conducted on the models accounting for at least 0.95 of the Akaike weight to extract parameter estimates and their 95% confidence intervals.

As a consequence of parametric assumption violations, the non-parametric Wilcoxon signed rank test was used to assess whether lions: 1. show an increased presence of displacement behaviours after the incongruent trial, and 2. have a reduced latency to respond to the incongruent playback.

Results

As predicted, lions of both sex responded to incongruent playbacks by spending a greater proportion of time moving, and also a greater proportion of time looking towards the call direction, before resting again (Table 3.2). In addition, lions initiated more allo-rubbing bouts with conspecifics (per second) following the incongruent playbacks (two-tailed Wilcoxon signed rank test: $Z = -2.96$, $P = 0.004$, $r = 0.68$). However, there was no difference in any other measures of potential displacement

behaviours, or in the latency to respond ($P > 0.0056$; Table 3.4). Significant behavioural

responses to the playbacks are shown in Figure 3.2.

Table 3.2. Factors influencing the proportion of time subject lions spent: 1. looking in the call direction, and 2. moving, following the playback of an incongruent, rather than congruent, call. Model parameters were generated using model averaging on the optimal GLMMs selected using AICc (Models 1:3; Table 3.3).

Dependent Variable	Parameter		Estimate	SE	Wald confidence interval: 2.5-97.5%	Relative importance
Proportion of time looking in call direction	Intercept		-2.105	0.368	0.06-0.26[a]	
	Treatment	Congruent	-	-	-	1.00
		Incongruent	1.905	0.040	6.19-7.29[a]	
	Sex	Female	-	-	-	0.26
		Male	-0.263	0.793	0.15-3.85	
	Treatment * Sex	Female * Congruent	-	-	-	0.06
		Male * Incongruent	0.044	0.107	0.84-1.30	
Dependent variable	Parameter		Estimate	SE	Wald confidence interval: 2.5-97.5%	Relative importance
Proportion of time moving	Intercept		-4.868	0.838	0.001-0.04[a]	
	Treatment	Congruent	-	-	-	1.00
		Incongruent	2.094	0.102	6.60-9.99[a]	
	Sex	Female	-	-	-	0.27
		Male	-0.690	1.811	0.01-19.89	
	Treatment * Sex	Female * Congruent	-	-	-	0.06
		Male * Incongruent	-0.141	0.207	0.57-1.32	

[a] **Confidence intervals that do not cross 1.**

Table 3.3. GLMMs with binomial error distribution investigating whether lions spent a greater proportion of time: 1. looking in the call direction, and 2. moving, following playback of incongruent, rather than congruent calls.

Dependent Variable	Model	Description	AICc	k	Δi	Akaike weight
Proportion of time looking in call direction	Basic		4419.6	3	2766.7	<0.001
	1	Treatment * Sex	1658.1	6	5.2	0.056
	2	Treatment + Sex	1655.5	5	2.6	0.207
	3	Treatment	1652.9	4	0.0	0.737
Dependent Variable	Model	Description	AICc	k	Δi	Akaike weight
Proportion of time moving	Basic		1328.2	3	647.7	<0.001
	1	Treatment * Sex	685.3	6	4.8	0.065
	2	Treatment + Sex	683.0	5	2.5	0.209
	3	Treatment	680.5	4	0.0	0.726

Lion identity (N = 20) and playback exemplar (N = 12) were included as random terms. k = parameters, Δi = AICi-AICmin.

Table 3.4. Two-tailed Wilcoxon signed rank tests investigating whether lions: 1. had a reduced latency to respond, 2. showed increased rates (per second) of displacement behaviours, 3. spent a greater proportion of time looking in the call direction, and 4. spent a greater proportion of time moving, following playback of incongruent, rather than congruent calls. The Bonferroni correction for multiple testing sets α = 0.05/9 = 0.0056.

Dependent Variable	N	Z	Exact P	r
Lip-licking rate	19	1.15	0.266	0.26
Head shaking rate	19	-0.903	0.344	0.21
Yawning rate	19	0.468	0.662	0.11
Allo-grooming rate	19	0.281	0.354	0.064
Auto-grooming rate	19	0.966	0.354	0.22
Allo-rubbing rate	19	-2.96	0.004*	0.68
Latency to respond	10	0.663	0.557	0.21
Proportion of time looking in call direction	19	-3.46	< 0.001*	0.79
Proportion of time moving	19	-3.47	< 0.001*	0.80

Effect size 'r' = Pearson correlation coefficient (r=Z/SQRT(N)). Significant terms are denoted by *.
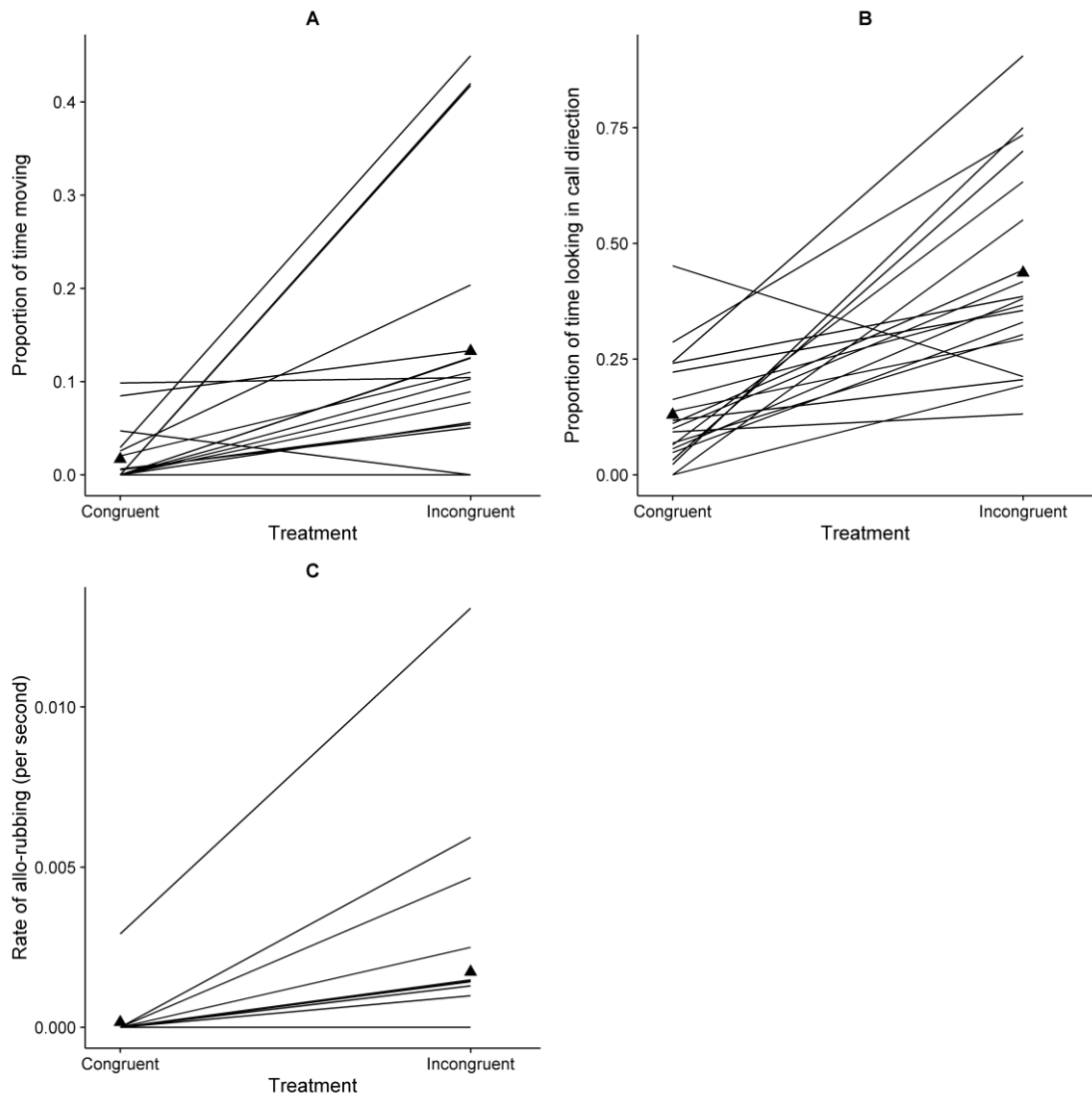
Figure 3.2. Significant behavioral responses of lions to playbacks of congruent and incongruent calls included the proportion of time spent moving (A), proportion of time spent looking at call direction (B), and rate of allo-rubbing initiated by the focal animal after playback (C). Figures show the response of individual lions (and the overall mean: ▲) to both playback treatments, where responses are represented as proportions (A and B), or rates (C) calculated per second from the raw data.

Discussion

Our results suggest that lions have the ability to individually recognise their group-mates, linking unique auditory and visual (and possibly olfactory) cues to identity. On our measures of searching behaviour, lions clearly responded to trials in which the familiar call did not match the familiar lion previously seen by spending more time moving and looking in the direction of the call before resting again. In addition, lions engaged in increased allo-rubbing with conspecifics following the incongruent trial,

which may function as a stress-alleviating 'displacement' behaviour (Schulkin, 2011, Gourkow, Hamon and Phillips, 2014). While other potential displacement measures did not differ between the treatments, it is likely that different species have different displacement signatures, and a wider investigation of stress-related behaviours in carnivores would be an interesting topic for future research.

A previous study on wild meerkats set out to test for individual vocal recognition using an expectancy-violation paradigm based around a physically impossible situation – simulating the presence of the same meerkat in two different places (Townsend, Allen and Manser, 2011). As the author's acknowledge, the experiment did not investigate whether meerkats were capable of integrating identity cues from multiple modalities, and thus did not test cross-modal individual recognition. We suggest that the experimental paradigm used here, which is based on simulating a natural social context for lions, might provide a useful design for tapping into such abilities in other species in the wild.

A potential alternative explanation for our results is that listeners may have heard the congruent lion roaring more recently than the incongruent lion, and responded more strongly due to the greater novelty of the latter's roars rather than that pride member being recognised across different sensory modalities. We have no way of knowing if the pride had roared the previous night, but we were able to monitor whether roaring occurred on the day of the experiment and no playback took place if this was the case. Furthermore, any roaring that occurred previously may have been joined even by an absent group-mate, as lion roars carry for several kilometres.

In conclusion, we used an 'expectancy violation' paradigm, where lions were presented with roars that were either congruent or incongruent with a visually blocked group-

mate, to test for cross-modal individual recognition in a wild animal. After hearing an incongruent call that did not match the previously seen conspecific, lions responded by moving and also looking in the direction of the simulated call for a longer proportion of time before resting again, while also initiating a higher rate of allo-rubbing (a potential displacement behaviour thought to alleviate stress) – results that are consistent with the subjects recognising the auditory-visual mismatch and being capable of cross-modal individual recognition.

Acknowledgements

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CHAPTER 4: INTRA-GROUP SCENT-MARKING BEHAVIOUR OF WILD AFRICAN LIONS (*PANTHERA LEO*)

Gilfillan, G., Golabek, K., Vitale, J., McNutt, J. W., & McComb, K. Scent-marking behaviour of wild African lions (*Panthera leo*). *Ethology* (in review).

Abstract

Many mammals communicate through scent-marking, but the detailed study of olfactory communication in most free-ranging species has been limited owing to the complexity of olfactory signal chemistry. We recorded the behavioural and contextual variables associated with scent-marking in wild African lions (*Panthera leo*), to investigate what factors influenced individual responses (investigation, overmarking and flehmen) to the scent deposits of group members. Our results suggest that lion deposits contain sex-specific semiochemicals, and that responses to deposits are consistent with the reproductive assessment of group members and potential mating partners. Flehmen-investigation was more frequently observed from females, perhaps allowing them to synchronise oestrous within the pride and also to assess the quality of potential mates. Deposits from juveniles (subadults and cubs) were also highlighted as important for intra-group communication, where flehmen responses to juvenile deposits were high, and flehmen may be a potential way to monitor sexual maturity. The type of deposit, and body posturing during urination were also important determinants of group member responses. In particular, pawings and spray-urine were most likely to be overmarked compared to all other deposits, and may function as composite group signals for territory defence, and/or intra-group hierarchical signalling. Collectively, the patterns described here of investigating and overmarking scent deposits indicate the importance of scent-marking in olfactory communication for lions, specifically for

sexual advertisement and assessment, and potentially for territorial defence, intra-group monitoring, and the maintenance of social hierarchies.

Introduction

Olfactory communication is the main mode of communication among mammals, and regularly involves scent-marking (Wyatt, 2014). Semiochemicals (chemical signals used in communication) in urine, faeces, glandular secretions, skin, and breath are important in social and sexual interactions (Bradbury and Vehrencamp, 1998; Apps, 2013). Scent-marking may be important for wide-ranging, solitary, territorial species in which males and females infrequently associate (Bradbury and Vehrencamp, 1998). This situation often occurs in the social organisation of felids (Kleiman and Eisenberg, 1973), but little is known about the specifics of how wild felids use scent-marking (Soso et al. 2014; Vogt et al. 2014; Allen et al. 2015; Vogt et al. 2016a; Allen et al. 2016a).

Felids have numerous scent glands (e.g. pedal, anal, and facial), and all felids are thought to communicate through scent signals in urine (Brown and Macdonald, 1985; Asa, 1993; Mellen, 1993; Pageat and Gaultier, 2003; Vogt et al. 2016a). Nevertheless, the study of olfactory communication in felids has been limited by their often elusive and nocturnal habits (Vogt et al. 2014; Allen et al. 2016a), and detailed data on scent-marking behaviours, the social context of marking, and the responses of conspecifics to scent-marks is uncommon (but see: Vogt et al. 2014; 2016b; Allen et al. 2014; 2015; 2016b). Lions (*Panthera leo* spp.) are believed to deposit scent through urine, faeces, anal gland secretions, pedal (feet) gland secretions, and secretions from facial glands (Schaller, 1972; Pageat and Gaultier, 2003; Lehmann et al. 2008; Poddar-Sarkar et al. 2008; Poddar-Sarkar and Brahmachary, 2014; Gilfillan et al. 2017). However, it is still

unclear whether these deposits are active scent-marks with a communicatory function, and if so, how other lions may use these signals.

For species that use excretory products (urine and faeces) for scent-marking, it can be difficult to distinguish between waste elimination and chemical communication (Jordan et al. 2013). However, communication by definition involves both a sender and a receiver, and stereotypical responses to deposits (e.g. scent investigation or scent overmarking) can reveal a communicatory function (Jordan et al. 2013). Overmarking (the placement of a scent deposit over an existing deposit: Johnston et al. 1994) is a common marking strategy in many species (Ferkin and Pierce, 2007) and unambiguously establishes that both the initial deposit and the overmark are scent-marks (Jordan et al. 2013). While scent investigation (e.g. sniffing or licking) by conspecifics does not definitively establish that deposits are scent-marks, a differential pattern of scent investigation between the deposits of different classes of individual provides strong supporting evidence of the assertion (Jordan et al. 2013). As is typically observed in mammals (e.g. Clapham et al. 2014), we expected that adult lions would participate most in scent-marking and scent investigation, as sexually mature adults may have a greater benefit from leaving and receiving scent-marks than non-breeding subadults. Likewise, we expected that males and females might benefit asymmetrically from the chemical information from same-sex and opposite-sex individuals, in regards to oestrous and/or dominance status.

There is now strong evidence that mammalian scent-marks can signal sex, maturity, familiarity and reproductive state/quality (Wyatt, 2014), and lion urine and faeces are believed to contain cues to the donor's sex, reproductive state and social group (Andersen and Vulpius, 1999; Umapathy et al. 2007; Gilfillan et al. 2017). Mammalian scent investigation often involves flehmen (Dagg and Taub, 1970), a stereotypical

response that transports non-volatile chemical signals (especially proteins and steroid conjugates) from the oral cavity to the vomeronasal organ (VNO) for chemosensory detection (Igbokwe, 2009). Flehmen is believed to be a good behavioural index of VNO utilisation (Hart, 1983). In many mammals, flehmen is a prominent aspect of scent communication related to reproductive condition and activation of sexual arousal (Owen et al. 2015). In lions, the observation of flehmen and patterns of its use may indicate deposits being active scent-marks with a socio-sexual communicatory function and if so, we expected that lions will show sex-specific flehmen responses to conspecific scent-marks. As competition between male lions for reproductive opportunities is intense (West and Packer, 2002), it may be adaptive for males to pay attention to the olfactory signals of females to monitor female oestrous. Similarly, female lions are believed to exert some selection on which males they associate and mate with (West and Packer, 2002), and it may be adaptive for females to monitor male maturity and reproductive quality through scent deposits.

Body posturing during scent deposition can also indicate a deposit's communication potential. It is believed that scent-mark posturing functions to place the deposit in a more conspicuous location (e.g. higher up on vegetation or next to a visual marker such as scratches in the soil: White et al. 2002; Allen et al. 2014) for future recipients to encounter, and/or to deposit scent in a manner that highlights another attribute of the signaller (e.g. deposit height to highlight body size: Sharpe, 2015). Lions have been documented to adopt overt body postures during urination (Schaller, 1972), and we believe that posturing during deposition (and the response of group members to deposits) could help identify if certain deposits are active scent-marks. We expected that conspecific responses to deposits would be greater when deposits co-occur with overt posturing, and as adults would be expected to participate most in scent-marking, we

expected adults to perform more body posturing than subadults, and predicted that the postures used by males and females may differ also.

In this paper, we describe the marking behaviours of wild lions. We explore the differences between different age-sex classes in deposit types and deposit placement, with the hypothesis that patterns would be non-random and reflect the benefits of signalling, via scent, to conspecifics. We then examine intra-group responses to deposits (investigation, overmarking and flehmen) in more detail, to identify which deposits hold a communicatory function, hypothesising that in lions certain scent deposits are used in olfactory communication more than others. We predicted that the patterns of responses to deposits will be non-random, and that the type of deposit, the sex and age of the depositor and recipient, and the deposit placement will be important in determining the subsequent response of group members.

Methods

Study population and site

The study area in northern Botswana (ca. 1500 km^2 19°31'S, 23°37'E; elevation ca. 950 m) was bordered by the Okavango Delta and included Moremi Game Reserve and surrounding wildlife management areas. Further details can be found in McNutt (1996). Data were collected between May 2011 and December 2015 from a free-ranging lion population with known life histories and demography. Lions were spoor- or radio-tracked, with up to three individuals in each social group (seven female prides and 11 male coalitions) fitted with a VHF radio collar (see Gilfillan et al. 2017). Individual lions were identified from their unique whisker-spot patterns, and were divided into demographic categories based on their sex and age, with age categories defined as follows: cubs (below 18 months), subadults (18-48 months), adults (4 years and above, or the date of primiparity if before 4 years) (Packer et al. 1988). Where the birth date

was unknown, the individual was aged using body size, teeth wear, mane development in males, and the observation of sexual activity (Whitman and Packer, 2007).

Behavioural observation

Lions were observed from a vehicle at distances of 10-40 m while resting and 20-200 m while moving, depending on visibility. Following established procedures (see: Jordan et al. 2013 on African wild dogs, *Lycaon pictus*), all scent deposits, scent investigations and scent overmarking were recorded using critical incident sampling (Altmann, 1974). When a lion deposited scent, the deposit type, deposit substrate, and lion identity were recorded. Consistent with Schaller (1972), there were eight observed deposit types from lions: (1) faeces, (2) body roll (back touching the vegetation/soil while the body is moved from side to side along the anteroposterior axis), (3) head rub (side of face rubbed up against vegetation), (4) pawing (scratching the vegetation/soil with paws/claws), (5) squat-urine (hind legs bent and hindquarters lowered to vegetation/soil while urinating), (6) spray-urine (backwards spray of urine onto vegetation/soil with tail lifted away from anogenital region while standing), (7) non-overt urine (urination while standing with tail lowered or while lying down; no distinct body posture), and (8) paw-urine (a combined deposit of urine and a pawing; urination with hind legs bent and hindquarters lowered while hind paws scrape vegetation/soil). Only urine (types 5 through 8) was deposited with different body postures.

Marking substrates included those at ground level (short grass = 1147 deposits, bare soil = 258, water = 2, a carcass = 2, a conspecific's resting spot = 3, and elephant urine/faeces = 12) and those at least 0.5 m above ground level (tree trunks = 168, tree branches = 786, shrubs and tall grass = 394). The first observed deposit at a site was termed the 'original deposit' and the deposit most recently left on a site was termed the 'top deposit'. However, an original deposit may still have involved overmarking of an

unknown previous mark left by a conspecific. Each original deposit was given a unique number ('Unique ID'), and all behaviour by subsequent visitors within two metres of each deposit (termed an encounter) were recorded, until the lions left the area and did not encounter the deposit again during the observation session. Lions encountering a deposit could: i) ignore the deposit, ii) investigate the deposit by sniffing (holding nose at a distance of < 30 cm from the deposit: consistent with Jordan et al. 2013; Allen et al. 2014; Allen et al. 2015), or licking (making direct contact with tongue), or iii) overmark the deposit (place a deposit on top of, or directly next to an existing deposit: Johnston et al. 1994). While the majority of scent data (59.4%) were collected within two hours after sunrise or two hours before sunset (when lions are typically active: Schaller, 1972), 25.1% of data were recorded at night.

Also recorded were events where lions investigated (sniffed) and overmarked the resting sites of other lions (after the lion had left the site and primarily where the anogenital region was positioned), and investigated the anogenitals of other lions. The anogenital region of lions is made up of several potential sources of semiochemicals including the genitals (a source of urine) and the anus (a source of faeces and anal gland secretion, AGS: Brahmachary and Singh, 2000). The function of lion AGS is unknown (Asa, 1993), but AGS is an important carrier of semiochemical cues in a wide range of carnivores (e.g. wolf, *Canis lupus*: Asa et al. 1985; spotted hyaena, *Crocuta crocuta*: Burgener et al. 2009; banded mongoose, *Mungos mungo*: Jordan et al. 2011; brown bears, *Ursus arctos*: Jojola et al. 2012).

Ethical statement

Lion collaring (carried out by certified veterinarians) and data collection was conducted under permits granted by the Botswana Ministry of Environment Wildlife and Tourism

(8/36/4 XXV (8)), and ethical clearance was also obtained from the University of Sussex (Non-ASPA 4 – November 2013).

Statistical analyses

Due to sample size limitations, subadults and cubs were grouped together for all analyses involving age. Fisher's exact tests were performed using the R statistical software package (v. 3.3.1; R Core Team, 2016) to investigate differences between age-sex classes of lion in deposit types and marking substrates. Where multiple comparisons were performed, the Bonferroni correction of alpha was used. All analyses involved two-tailed p values and satisfied the critical assumptions of the statistical test used.

To investigate the factors that affect whether encountered deposits of faeces, urine or pawings were i) investigated (sniffed or licked) or ii) overmarked, we ran a series of generalised linear mixed models (GLMMs) with a binomial error distribution and a logit link function. Where overmarking occurred at the deposit site, any subsequent response was recorded as taking place for the top deposit (Ferkin and Pierce, 2007). Rub and roll deposits were excluded due to low sample sizes. Following Zuur et al. (2009), the optimal random effects structure was identified before running the GLMMs. Consequently, we included the depositor's identity (n = 43), and the recipient's identity (n = 59) in analysis i), but only the recipient's identity (n = 60) in analysis ii) as random terms to account for multiple data from the same lions. The global models included the following fixed effects and interactions: depositor sex + depositor age + deposit type + deposit substrate + recipient sex + recipient age + depositor sex * recipient sex + depositor age * recipient age, except for analysis ii) for which deposit substrate was excluded due to collinearity between fixed effects. The selected fixed effects were not strongly correlated (measured using the variance inflation factor and the condition number test). The 'bobyqa' optimiser in the 'lme4' package (Bates et al. 2015) was used

to run the GLMMs. The dredge function in the 'MuMIn' package (Bartoń, 2016) was used to create a list of candidate models from the global model. We used Akaike's information criterion with a correction for small sample sizes (AICc) for model selection, where lower AICc values corresponded with better support for a given model (Zuur et al. 2009). Finally, conditional model averaging (MuMIn package) was performed on the models within 2 AICc of the optimal model (lowest AICc) to extract the relative importance of predictors in the averaged models, their averaged parameter estimates, and their 95% confidence intervals (Symonds and Moussalli, 2011).

For investigated faeces, urine, anogenitals, and resting sites, we evaluated the factors affecting whether or not the investigating lion exhibited flehmen. Pawings, non-overt urine, rubs and rolls were excluded from the analysis due to low sample sizes. We ran a series of generalised linear mixed models (GLMMs) using the methods described above. We included the depositor's identity (n = 44) as a random term to account for multiple data from the same lions. The global model included the following fixed effects and interactions: flehmen or not ~ depositor sex + depositor age + scent type + investigator sex + investigator age + depositor sex * investigator sex + depositor age * investigator age. We again used the dredge function to select candidate models with delta AICc < 2, and model averaging to extract averaged parameter estimates, their relative importance, and 95% confidence intervals.

Results

General scent-marking behaviour

We recorded a total of 2780 deposits at 2295 unique sites by 83 lions from seven female prides and 11 male coalitions. Of 2739 deposits where the deposit type was known: 1818 involved urine (66.4%), 668 involved a pawing (24.4%), 394 were head rubs (14.4%), 349 were faeces (12.7%), and 22 were rolls (0.8%). Within these, 512 deposits

(18.7%) were paw-urine deposits (see methods) made up of both urine and a pawing. Of

2703 deposits where the sex of the depositor was known, 1662 were from male lions

(61.5% of deposits, n = 43 individuals), while 1041 were from females (38.5%, n = 40).

Adult lions (n = 53) made 2256 deposits (81.2% of deposits), subadults (n = 26) made

301 deposits (10.8%) and cubs (n = 28) made 223 deposits (8.0%, Table 4.1).

Table 4.1. Summary of the raw data showing the observed marking behaviour of lions of different age and sex.

Sex-Age Class	Number of individuals	Urinations per individual	Faeces per individual	Pawings per individual	Rubs per individual	Rolls per individual
Male adult	27	36.78 ± 69.46	2.52 ± 5.59	12.37 ± 18.66	12.70 ± 34.36	0.30 ± 0.82
Female adult	26	18.23 ± 22.16	5.85 ± 6.80	8.04 ± 11.07	1.27 ± 2.65	0.27 ± 0.83
Male subadult	11	5.09 ± 4.87	2.36 ± 2.11	1.64 ± 2.77	0.09 ± 0.30	0.00 ± 0.00
Female subadult	15	6.29 ± 8.16	2.65 ± 3.18	1.94 ± 2.88	0.29 ± 0.99	0.06 ± 0.24
Male cub	11	2.36 ± 2.76	1.18 ± 1.47	0.91 ± 0.94	0.00 ± 0.00	0.18 ± 0.60
Female cub	17	1.94 ± 2.22	0.53 ± 0.62	1.06 ± 1.30	0.00 ± 0.00	0.23 ± 0.56

Mean ± standard deviation.

Age differences

Deposits from adult lions were more likely to be head-rubs, spray-urine, or paw-urine

than were deposits from subadults and cubs, but were less likely to be faeces, pawings,

squat-urine, or non-overt urine (Fig. 4.1). Neither adults nor subadults and cubs

regularly displayed body rolls. Deposits from adults were more likely to be placed on

substrates at least 0.5 m above ground level than subadult and cub deposits. Lions reach

a height of approximately 0.5 m around six months of age (Whitman and Packer, 2007),

and so this result is unlikely a consequence of adults being taller.
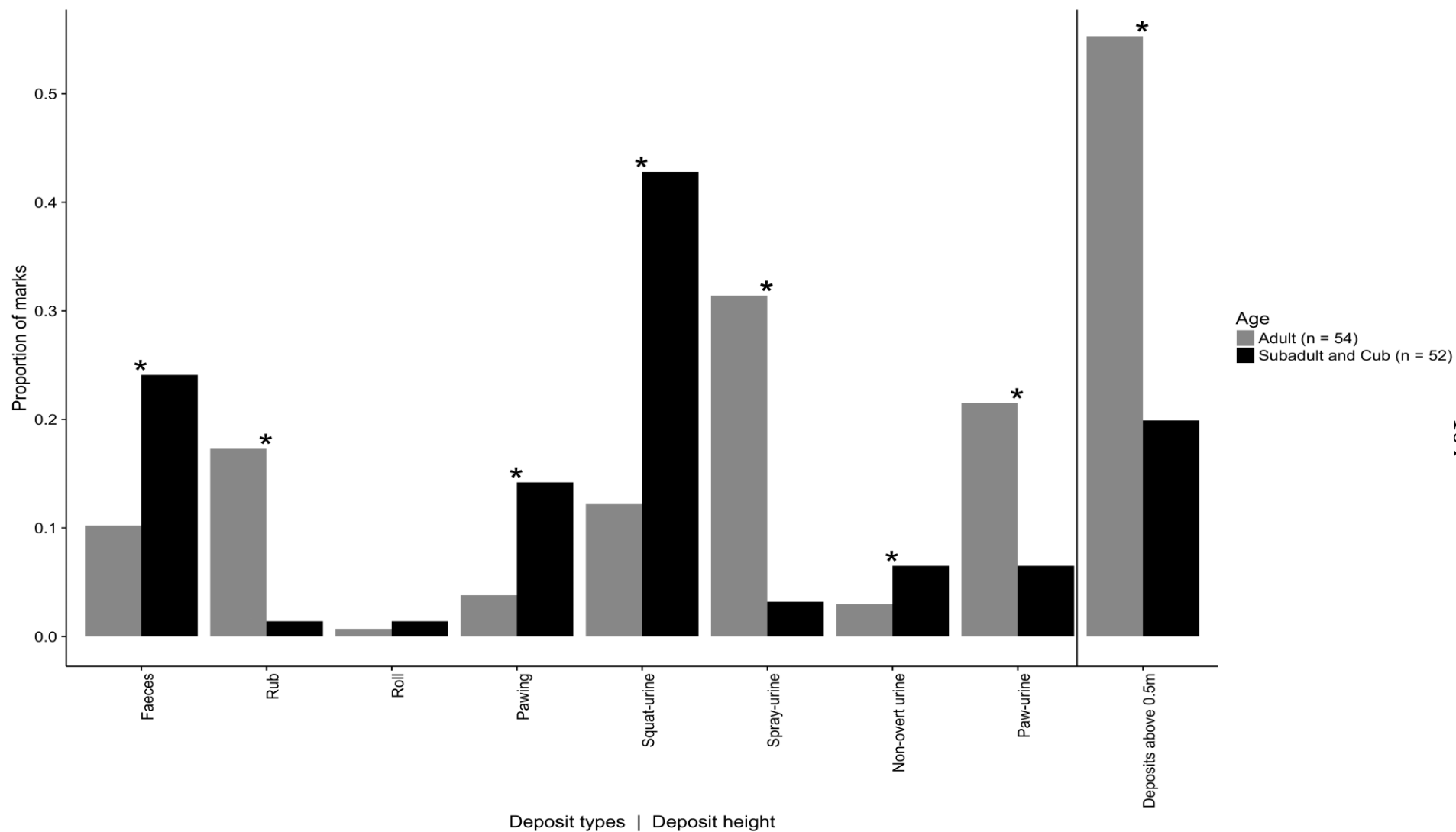
Figure 4.1. The differences between the observed likelihood of lions of different ages depositing certain types of scent-marks, and depositing at certain heights. * denotes significant (p < 0.0056) Fisher's exact tests comparing the marking of adults with that of subadults/cubs (see Table 4.2).

Table 4.2. Fisher's exact tests investigating the difference between the observed likelihood of lions of different ages depositing certain types of marks, and depositing at certain substrate heights.

Context Variable	Adult (n = 54)	Subadult and Cub (n = 52)	p
Deposit Type			
Faeces	227	122	<0.0001*
Rub	387	7	<0.0001*
Roll	15	7	0.1606
Pawing	84	72	<0.0001*
Squat-urine	273	217	<0.0001*
Spray-urine	700	16	<0.0001*
Non-overt urine	66	33	0.0003*
Paw-urine	479	33	<0.0001*
Total known deposits	2231	507	
Deposit Substrate			
Above 0.5m	1244	104	<0.0001*
Ground level	1007	418	
Total known substrates	2251	522	

* indicates significance at 0.0056.

Sex differences

Deposits from male lions were more likely to be rubs, spray-urine, or paw-urine than were female deposits, but were less likely to be faeces, pawings, squat-urine, or non-overt urine (Fig. 4.2). Neither males nor females regularly displayed body rolls. Males often overmarked their own rub deposit with a spray-urination; 41.3% of male spray-urine (276 of 669 spray deposits) was deposited over their own rub (consistent with Lehmann et al. 2008). Deposits from males were more likely to be placed on substrates at least 0.5 m above ground level than female deposits.
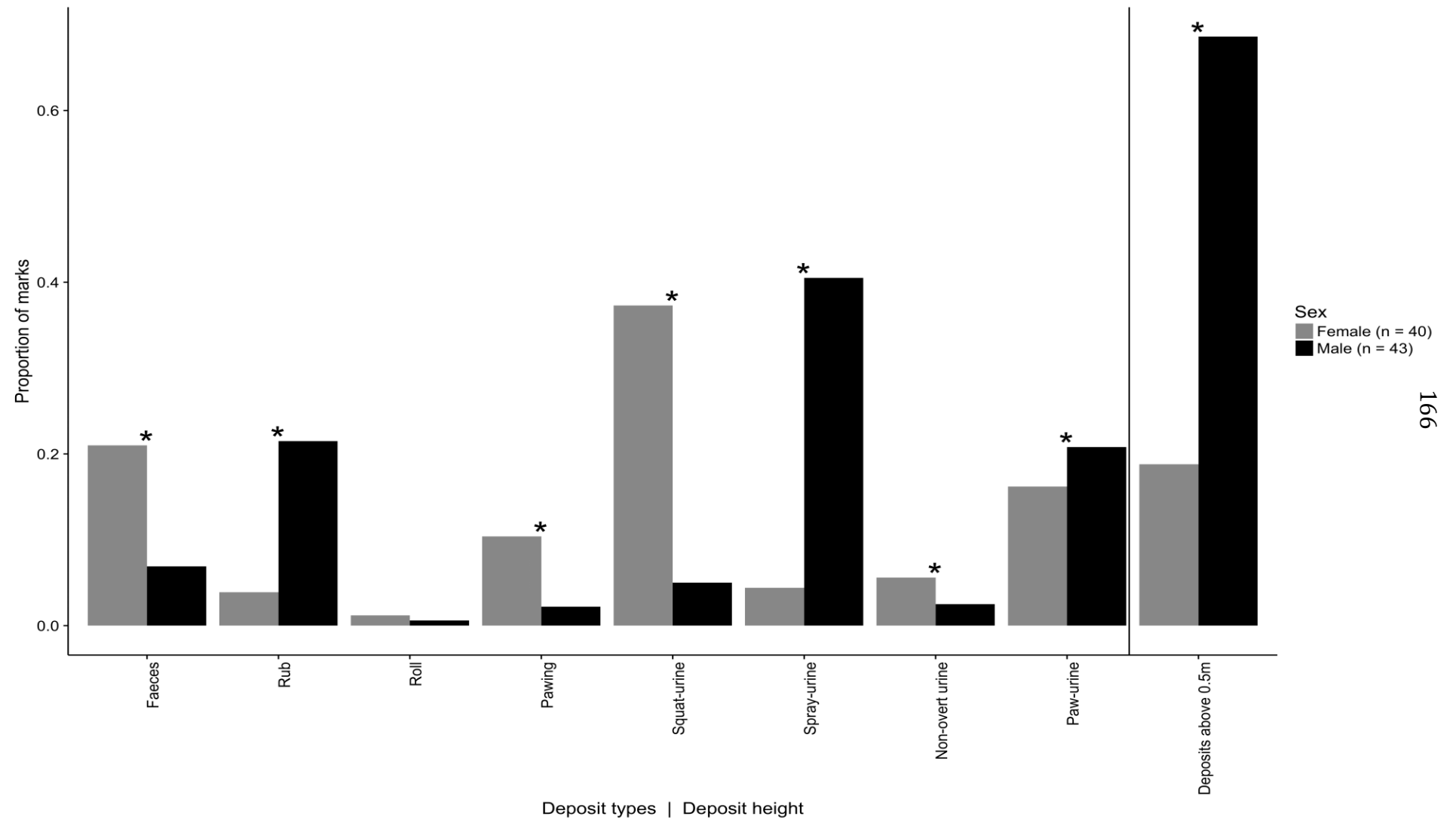
Figure 4.2. The differences between the observed likelihood of lions of different sex depositing certain types of scent-marks, and depositing at certain heights. * denotes significant (p < 0.0056) Fisher's exact tests comparing the marking of males with that of females (see Table 4.3).

Table 4.3. Fisher's exact tests investigating the differences between the observed likelihood of lions of different sex depositing certain types of marks, and depositing at certain substrate heights.

Context Variable	Male (n = 43)	Female (n = 40)	p
Deposit Type			
Faeces	114	213	<0.0001*
Rub	355	39	<0.0001*
Roll	10	12	0.1243
Pawing	37	105	<0.0001*
Squat-urine	82	377	<0.0001*
Spray-urine	669	45	<0.0001*
Non-overt urine	41	57	<0.0001*
Paw-urine	344	164	0.0032*
Total known deposits	1652	1012	
Deposit Substrate			
Above 0.5m	1137	195	<0.0001*
Ground level	521	843	
Total known substrates	1658	1038	

* indicates significance at 0.0056.

Anogenital investigation and overmarking

We recorded 119 investigations of a conspecific's anogenital region (four in a parallel sniff as seen in spotted hyaenas: Kruuk, 1972), and 78 investigations of a conspecific's resting site. Flehmen followed 40.3% of anogenital investigations, and 30.8% of resting site investigations. We observed three cases of resting site overmarking, all involving a male urinating over an adult female's resting site. Investigation of female anogenitals/resting sites was more frequently observed than investigation of male anogenitals/resting sites (Table 4.4).

Table 4.4. Patterns of resting site and anogenital investigation in regards to the sexes of the scent donor and scent investigator.

	Investigator sex			Total
Resting site investigation	Male	Female	Unknown	
Male resting site	6	7	0	13
Female resting site	41	19	3	63
Unknown resting site	2	0	0	2
Total	49	26	3	78
Anogenital investigation				
Male anogenital	14	12	0	26
Female anogenital	44	43	6	93
Unknown anogenital	0	0	0	0
Total	58	55	6	119

Factors affecting investigation

Of 560 encounters of faeces, urine and pawings, 198 (35.4%) involved scent investigation (sniffing or licking). Model averaging revealed that the deposit type, the ages of the depositor and the recipient, and an interaction between depositor sex and recipient sex were the best predictors of deposit investigation (Table 4.5). Deposits from both males and females were more likely to be investigated by the opposite sex (Fig. 4.3a). Pawings were least likely to be investigated compared to all other deposits, followed by non-overt urine (Fig. 4.3b). There was a moderate interaction between depositor age and recipient age (relative importance [RI] 0.62), where adults frequently investigated deposits from all lions, but subadults and cubs were less likely to investigate deposits from other subadults and cubs than adult deposits (Fig. 4.3c). Finally, substrate height was a weak predictor (RI 0.40) of whether a deposit was investigated, where deposits above 0.5 m were more likely to be investigated than deposits at ground level.
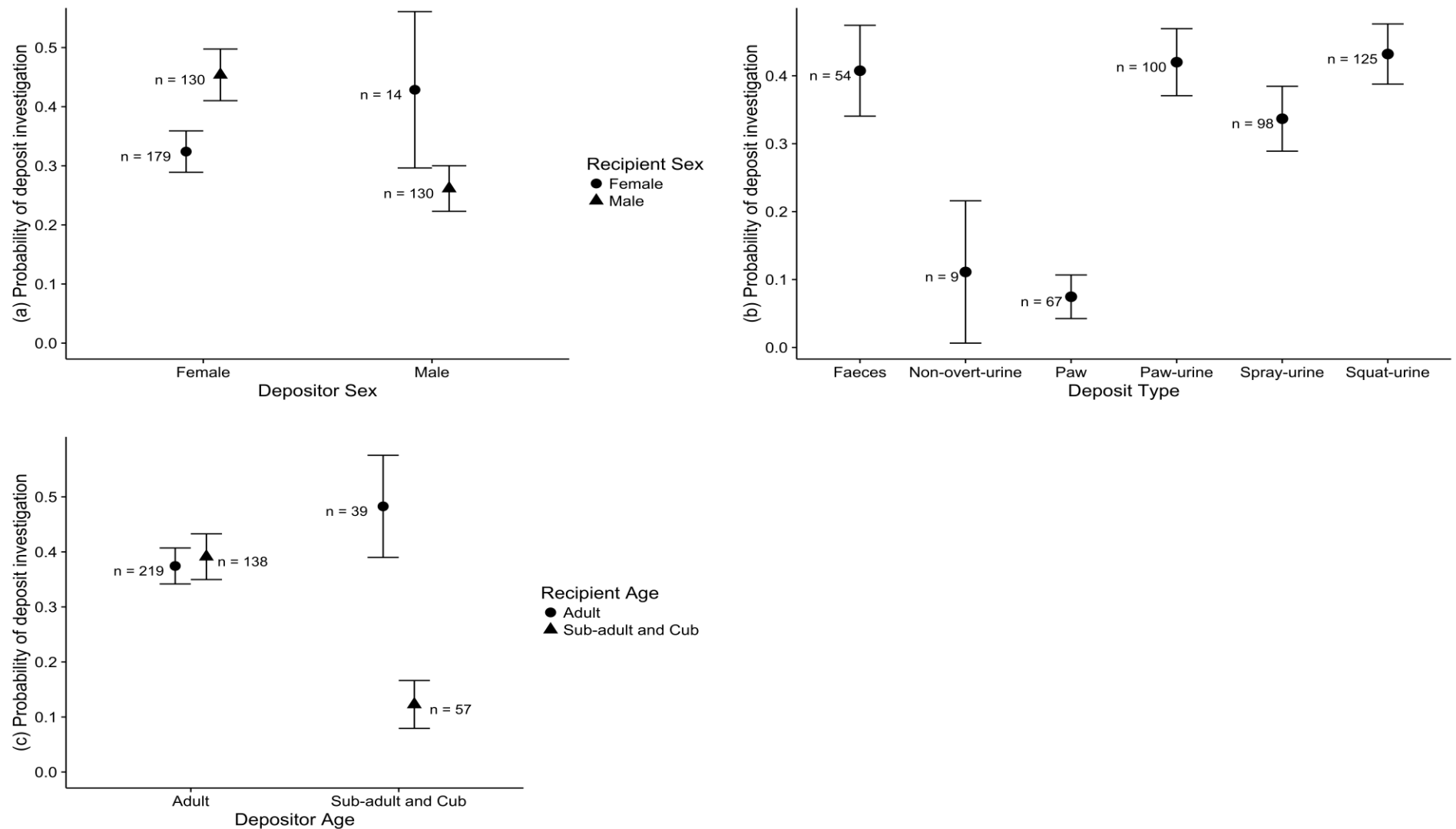
Figure 4.3. The likelihood that (a) deposits from male and female lions were investigated by male and female group members, (b) different deposit types were investigated and (c) deposits from adults and subadults/cubs were investigated by adults or subadults/cubs. Estimates were calculated from the raw data. Figures represent mean ± binomial standard error.

Table 4.5. Factors influencing the likelihood of deposit investigation (n = 453).

Variable		Estimate	Standard Error	95% Confidence Interval	Relative importance
Intercept		0.281	0.523	-0.747:1.308	
Deposit Type	Paw-urine				1.00
	Spray-urine	0.406	0.570	-0.715:1.527	
	Faeces	-0.016	0.418	-0.836:0.805	
	Squat-urine	-0.120	0.340	-0.788:0.548	
	Non-overt urine	-2.071	1.135	-4.302:0.161	
	Pawing	-2.391	0.682	-3.731:-1.052	
Recipient Sex	Female				1.00
	Male	0.954	0.306	0.353:1.555	
Depositor Sex	Female				1.00
	Male	0.862	0.753	-0.618:2.343	
Depositor Sex * Recipient Sex	Female * Female				1.00
	Male * Male	-3.254	0.865	-4.953:-1.554	
Recipient Age	Adult				1.00
	Subadult/Cub	-0.642	0.332	-1.295:-0.011	
Depositor Age	Adult				1.00
	Subadult/Cub	-0.503	0.536	-1.556:0.549	
Depositor Age * Recipient Age	Adult * Adult				0.62
	Subadult/Cub * Subadult/Cub	-1.188	0.677	-2.519:0.143	
Deposit Substrate	Above 0.5 m				0.40
	Ground-level	-0.643	0.555	-1.733:0.447	

Model parameters generated using model averaging on the optimal GLMMs selected with AICc (see Table 4.6).

Table 4.6. Best GLMMs with binomial error distribution selected using AICc investigating the factors that influence the likelihood of a deposit being investigated by a conspecific (n = 453).

Model	Description	AICc	k	Δi	wi
1	Depositor age + Deposit type + Recipient age + Recipient sex + Depositor sex + Depositor sex * Recipient sex + Depositor age * Recipient age	533.36	14	0.00	0.38
2	Depositor age + Deposit type + Recipient age + Recipient sex + Depositor sex + Depositor sex * Recipient sex + Depositor age * Recipient age + Deposit substrate	534.20	15	0.84	0.25
3	Depositor age + Deposit type + Recipient age + Recipient sex + Depositor sex + Depositor sex * Recipient sex	534.42	13	1.06	0.22
4	Depositor age + Deposit type + Recipient age + Recipient sex + Depositor sex + Depositor sex * Recipient sex + Deposit substrate	535.13	14	1.77	0.16

Depositor identity (n = 43), and recipient identity (n = 59) were included as random terms. k = parameters, Δi = AICi-AICmin, wi = Akaike weights. Only models with delta AICc < 2 are shown.

Factors affecting overmarking

Of 560 deposit encounters, 130 (23.2%) involved scent overmarking. Overmarking involved pawings (n = 68), urination (n = 59), rubs (n = 29), defecation (n = 3) and rolls (n = 1), sometimes in combination. Model averaging revealed that the type of deposit was the best predictor of whether a deposit elicited overmarking (Table 4.7). Pawings were the most likely to elicit overmarking compared to all other deposits, followed by urination with a spray posture (Fig. 4.4). The sex of the recipient had some effect (RI 0.44) on overmarking, in that females were more likely to overmark encountered deposits. Male deposits (RI 0.29) were also more likely to elicit overmarking, and adults were somewhat more likely to overmark deposits than were subadults and cubs (RI 0.13), but the effects of these latter variables were minor.
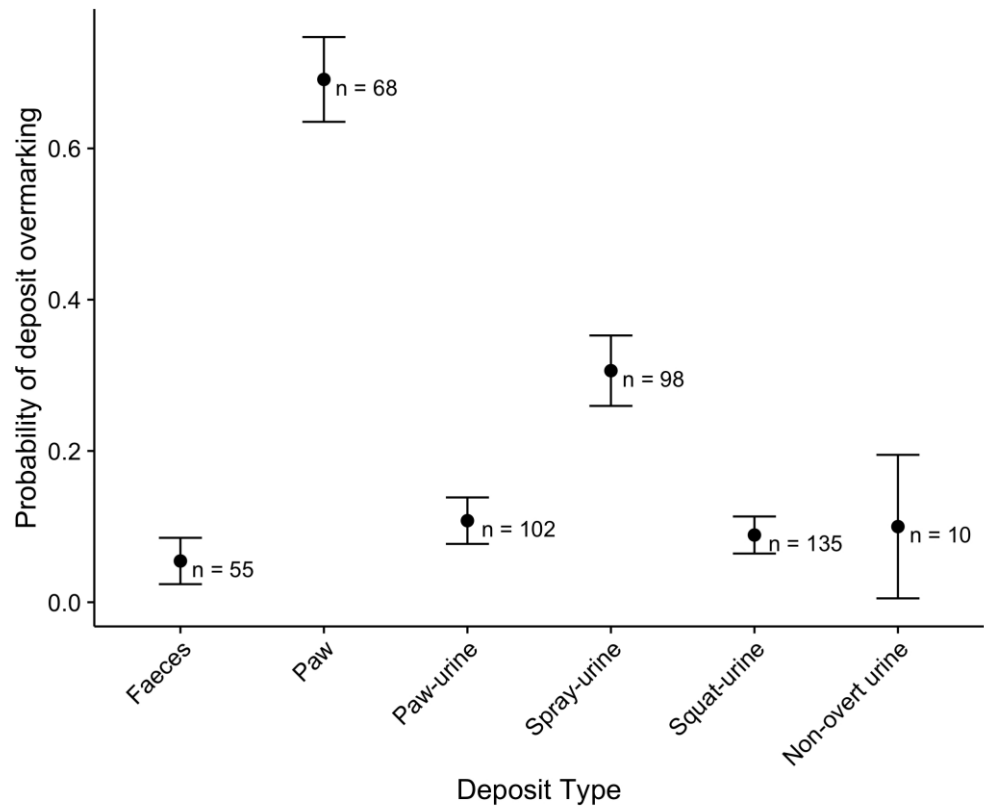
Figure 4.4. The likelihood that certain deposit types were overmarked by group members. Estimates were calculated from the raw data. Figures represent mean ± binomial standard error.

Table 4.7. Factors influencing the likelihood of deposit overmarking (n = 468).

Variable		Estimate	Standard Error	95% Confidence Interval	Relative importance
Intercept		-2.241	0.327	-2.883:-1.598	
Deposit Type	Squat-urine				1.00
	Pawing	3.133	0.403	2.341:3.925	
	Spray-urine	1.516	0.461	0.610:2.422	
	Paw-urine	0.205	0.446	-0.672:1.082	
	Non-overt urine	0.131	1.098	-2.027:2.288	
	Faeces	-0.497	0.670	-1.813:0.819	
Recipient Sex	Female				0.44
	Male	-0.454	0.315	-1.072:0.165	
Depositor Sex	Female				0.29
	Male	0.342	0.425	-0.493:1.178	
Recipient Age	Adult				0.13
	Subadult/Cub	-0.213	0.329	-0.859:0.432	

Model parameters were generated using model averaging on the optimal GLMMs selected with AICc (see Table 4.8).

Table 4.8. Best GLMMs with binomial error distribution selected using AICc investigating the factors that influence the likelihood of a deposit being overmarked by a conspecific (n = 468).

Model	Description	AICc	k	Δi	wi
1	Deposit type	399.58	7	0.00	0.30
2	Deposit type + Recipient sex	399.75	8	0.17	0.28
3	Deposit type + Recipient sex + Depositor sex	400.84	9	1.26	0.16
4	Deposit type + Recipient age	401.23	8	1.65	0.13
5	Deposit type + Depositor sex	401.30	8	1.71	0.13

Recipient identity (n = 60) was included as a random term. k = parameters, Δi = AICi-AICmin, wi = Akaike weights. Only models with delta AICc < 2 are shown.

Factors determining flehmen response

Of 385 investigations of urine, faeces, anogenitals and resting sites, 134 (34.8%) involved flehmen. Model averaging revealed that the type of scent investigated, and the sex of the depositor were the best predictors of whether scent elicited flehmen from conspecifics (Table 4.9). Spray-urine was least likely to elicit flehmen compared to all other deposits, followed by faeces (Fig. 4.5a). Scent from females was more likely to be investigated with flehmen than was male scent. The sex of the investigator was a moderate predictor of flehmen (RI 0.66), where females were more likely to flehmen after investigating scent than were males (Fig. 4.5b). The age of the investigating lion

(RI 0.42) and age of the depositor (RI 0.32) were weaker predictors of a flehmen response, where adults were more likely to flehmen after investigating a conspecific's scent than were subadults and cubs, but scent from adults was less likely to elicit flehmen. Finally, there was a weak interaction between the sexes of the depositor and investigator (RI 0.28), where males rarely flehmened after investigating another male's scent (Fig. 4.5b).
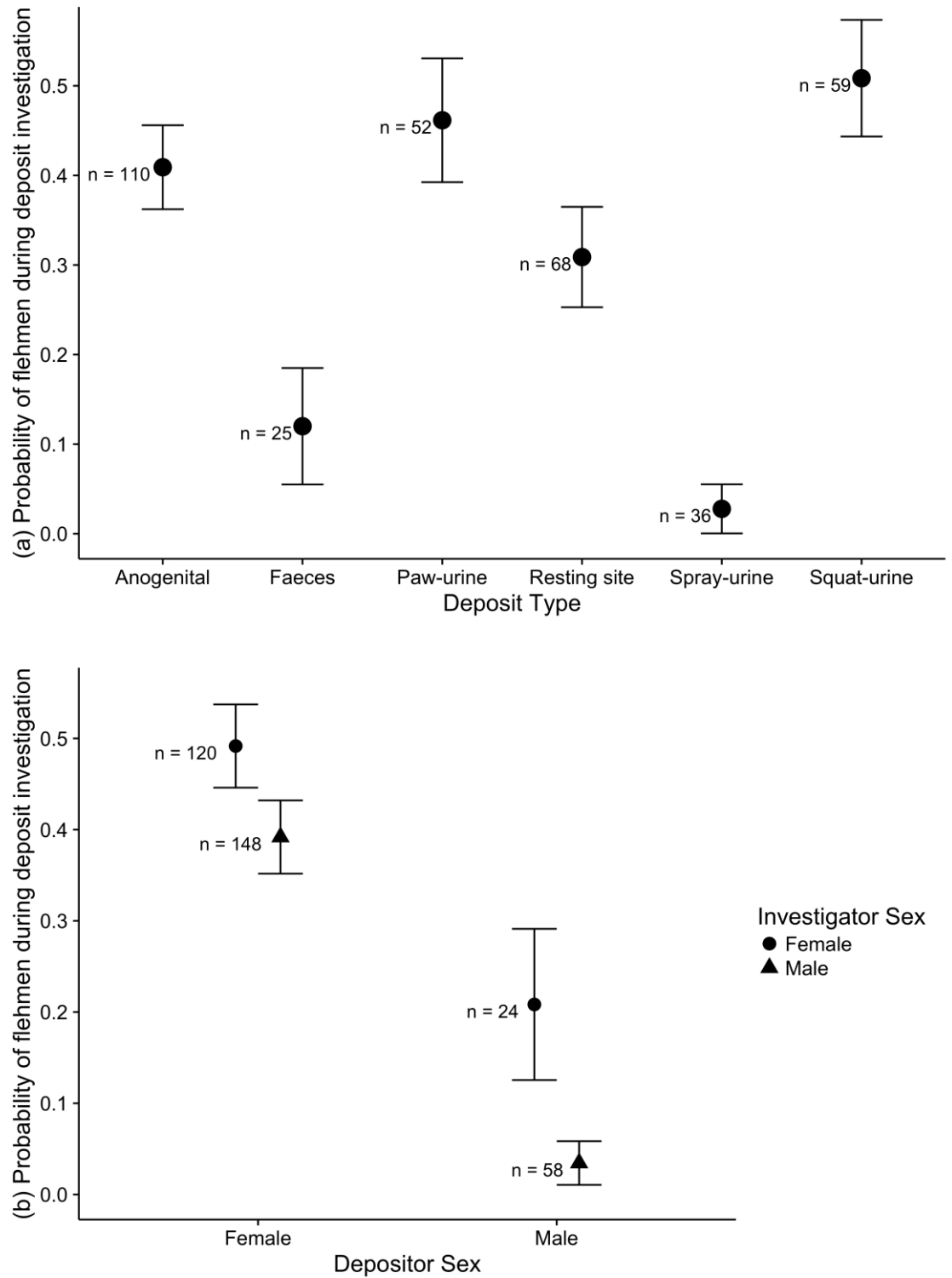
Figure 4.5. The likelihood that (a) different scent types were investigated with flehmen, and (b) scent from males and females elicited flehmen from investigating males or females. Estimates were calculated from the raw data. Figures represent mean ± binomial standard error.

Table 4.9. Factors influencing the likelihood of flehmen after investigation (n = 350).

Variable		Estimate	Standard Error	95% Confidence Interval	Relative importance
Intercept		0.090	0.262	-0.425:0.606	
Scent Type	Anogenital				1.00
	Paw-urine	0.191	0.359	-0.516:0.897	
	Squat-urine	0.135	0.337	-0.527:0.797	
	Resting site	-0.498	0.342	-1.172:0.175	
	Faeces	-1.816	0.658	-3.110:-0.522	
	Spray-urine	-1.920	1.109	-4.100:0.261	
Depositor Sex	Female				1.00
	Male	-1.595	0.523	-2.624:-0.567	
Investigator	Female				0.66
	Male	-0.344	0.257	-0.850:0.162	
Investigator	Adult				0.42
	Subadult/Cub	-0.338	0.260	-0.848:0.173	
Depositor Age	Adult				0.32
	Subadult/Cub	0.352	0.378	-0.393:1.096	
Depositor Sex * Investigator Sex	Female * Female				0.28
	Male * Male	-1.214	0.950	-3.082:0.655	

Model parameters generated using model averaging on the optimal GLMMs selected with AICc (see Table 4.10).

Table 4.10. Best GLMMs with binomial error distribution selected using AICc investigating the factors that influence the likelihood of an investigating lion exhibiting a flehmen response towards scent (n = 350).

Model	Description	AICc	k	Δi	wi
1	Depositor sex + Scent type + Investigator sex	410.04	9	0.00	0.15
2	Depositor sex + Scent type + Investigator sex + Depositor sex * Investigator sex	410.39	10	0.35	0.12
3	Depositor sex + Scent type	410.67	8	0.63	0.11
4	Depositor sex + Scent type + Investigator age	410.67	9	0.63	0.11
5	Depositor sex + Scent type + Investigator age + Investigator sex	410.68	10	0.63	0.11
6	Depositor sex + Scent type + Investigator age + Investigator sex + Depositor sex * Investigator sex	411.09	11	1.04	0.09
7	Depositor sex + Scent type + Depositor age + Investigator sex	411.42	10	1.37	0.07
8	Depositor sex + Scent type + Depositor age + Investigator sex + Depositor sex * Investigator sex	411.70	11	1.65	0.06
9	Depositor age + Scent type + Depositor sex + Investigator age	411.78	10	1.73	0.06
10	Depositor age + Scent type + Depositor sex	411.81	9	1.77	0.06
11	Depositor age + Scent type + Depositor sex + Investigator age + Investigator sex	412.01	11	1.97	0.06

Depositor identity (n = 44) was included as a random term. k = parameters, Δi = AICi-AICmin, wi = Akaike weights. Only models with delta AICc < 2 are shown.

Discussion

We provide a detailed analysis of the scent-marking of wild African lions in the context of natural social behaviour, and present data suggesting that lions use specific scent-marks in olfactory communication. While it can be difficult to distinguish between waste elimination and scent-marking with urine or faeces (Jordan et al. 2013), the non-random patterns observed on scent investigation and overmarking, discussed below, show that some lion deposits can be identified as having communicatory significance.

We observed a non-random pattern of scent-marking between males and females in regards to types of deposits and deposit placement. Scent deposits from both sexes were more likely to be investigated by the opposite sex, but scent from females was more likely to elicit flehmen (a stereotypical response to reproductive cues) from both male and female investigators. In some mammals, female scent acts to signal reproductive condition (Swaisgood et al. 2002), and conspecifics are more regularly observed to flehmen scent from females (Swaisgood et al. 2003). Such results indicate sex-specific semiochemicals within lion deposits, and the patterns we observed suggest that intra-group scent-marking enables for sexual assessment. Surprisingly, females were more likely to flehmen after investigating a group member's scent (of either sex) than were males. Flehmen in response to semiochemicals has previously been documented as a more typical behaviour from males (Hart and Leedy, 1987), though females also perform flehmen, particularly when in oestrous (Thompson, 1995). As flehmen is believed to be important for sexual assessment of potential mates (Hart and Leedy, 1987), it seems logical that females are more likely to flehmen male scent. Female scent was also highly likely to elicit flehmen from investigating males, but was (slightly) more likely to elicit flehmen from females. One possible explanation for this is that the oestrous state of a female will also be important to other pride females, as their scent

could attract the attention of males (both resident and non-resident) when in oestrous. Attracting males can pose a risk to females and their cubs as resident males kleptoparasitise food from females (Schaller, 1972), while non-residents attempt to kill dependent cubs (Pusey and Packer, 1994), so perhaps the oestrous state of other pride females is important to monitor. There is also often a large degree of reproductive synchrony between females of a pride, which is likely an adaptive strategy to prevent infanticide (Packer and Pusey, 1983), and flehmen may allow females to synchronise oestrous (Thompson, 1995; Wolf, 1998).

Regarding types of deposits and deposit placement, there were clear differences in the scent-marking of adults and subadult/cubs. As predicted, adult lions appear to participate most in scent-marking, overmarking, and scent investigation. In addition, the scent of group members more frequently elicited flehmen from investigating adults than from investigating subadults and cubs. As sub-adults and cubs are not sexually mature yet, such results are consistent with a sexual function for scent-marking in lions. Surprisingly, adults were more likely to investigate deposits from subadults and cubs than they were adult deposits, and scent from subadults and cubs was (slightly) more likely to elicit flehmen than was adult scent. The elicitation of flehmen, as opposed to general investigation and overmarking, suggests that scent from juveniles activates the vomeronasal chemosensory pathway (Hart, 1983). The vomeronasal system is often associated with the detection of oestrous cues (Swaisgood et al. 2002) and sexual maturity (White et al. 2002). The reproductive maturity of subadults has strong social consequences for lion prides, and could explain the observed patterns of flehmen. Females approaching sexual maturity will attract resident and non-resident adult males to the pride, and males approaching sexual maturity will soon emigrate from the pride (Schaller, 1972). Sub-adult males can be effective in defending food from spotted

hyaenas (Höner et al. 2002), and can also assist in territory defence where larger groups dominate smaller groups (Packer et al. 1990). It could therefore be adaptive for lions to monitor (through flehmen) the sexual maturity of juveniles to predict social change for the pride.

Lions left several types of deposits, and throughout our analyses deposit type was a strong predictor in investigation, overmarking, and flehmen responses of conspecifics. The distinction between pawing deposits and other deposit types came out most strongly in our models. Although lions infrequently investigated pawings, these deposits were the most likely to be overmarked. Pawing appears to be socially facilitated, since 96.6% of overmarked pawings were overmarked with further pawing, and pawing frequently stimulated overmarking by observing group members. Multiple explanations, which are not mutually exclusive, could be driving these results. First, we cannot discount that lions are able to detect semiochemicals from pawings without overt investigation. Second, our study was limited to investigating scent communication within social groups, but scent-marking often functions for territorial defence and inter-group communication (Brown and Macdonald, 1985). Pawing deposits may function as an inter-group signal, where lions overmark the pawings of group members to create a combined signal of territory occupancy by a group (a unique group scent: Sillero-Zubiri and Macdonald, 1998; Lazaro-Perea et al. 1999; Ferkin and Pierce, 2007). In addition, composite paw marks could signal minimum group size to intruders, as has been shown with lion chorus roars (McComb et al. 1994). Finally, pawings may act as uni-modal visual cues rather than multi-modal visual-olfactory cues, where the creation of scratches (typically on tree bark; 84.0% of pawings) by groups of lions could signal territory occupancy without the deposit of semiochemicals. Further research would be required to test these hypotheses, but as pawings are not regularly investigated but are

regularly overmarked in this study, they may play an important role in territorial communication between groups.

Lions deposited urine with different body postures, and our analyses highlighted posturing as an important factor for eliciting investigation, flehmen and overmarking. In this study, urine deposited with a spray posture was less likely to elicit flehmen, but was more likely to be overmarked than urine deposited with a scrape (paw-urine), squat or non-overt posture. This pattern of overmarking but not flehmen investigation, also suggests that spray-urine could be an inter-group signal of territoriality, rather than a sexual signal (as discussed before). In addition, overmarking is often associated with dominance hierarchies, and lions could overmark the spray-urine of group members to signal dominance over the bottom donor (Ferkin and Pierce, 2007). As shown in the results, spray-urine is primarily deposited by adult males, and there can be subtle dominance hierarchies between coalition partners in achieving matings (Packer et al. 1991).

Urine deposited with an overt body posture appeared more likely to elicit investigation from group members, which suggests that non-overt urine contains less (or less detectable) semiochemicals or semiochemicals of less social importance. Overt urine posturing could also function to advertise the deposition of a scent-mark to group-members. Subadults and cubs were more likely to deposit non-overt urine than adults, and could be an attempt by juveniles to deposit inconspicuously. Inconspicuous marking has previously been documented in subordinates (Jordan et al. 2013) and subadults (Clapham et al. 2014) in other mammals. Such results suggest that body posturing during urination is an important aspect of scent-marking in lions, and matches work on other carnivores (Jordan et al. 2013; Allen et al. 2014; Sharpe, 2015)

Lions may also actively advertise their deposits by placing them above 0.5 meter and therefore closer to the head-height of passing lions. Although only a weak predictor, lions were more likely to investigate deposits placed above 0.5 m. Adult lions (particularly males) more frequently deposited at least 0.5 m above ground level, and depositing above 0.5 m invariably meant that deposits were placed on vegetation. Depositing on vegetation is a common scent-marking strategy for signal amplification (see: Sillero-Zubiri and Macdonald, 1998; Barja et al. 2005; Cafazzo et al. 2012; Jordan et al. 2013) presumed to increase the detectability of the deposit.

Finally, our results suggest that a lion's anogenital region emits semiochemicals of interest to conspecifics, and that these chemical cues are transferred onto a substrate on which lions lie (resting site investigation was primarily directed to where the anogenitals had been positioned). Anogenital investigation in our study was frequently followed by flehmen, suggesting a sexual function consistent with other mammals (e.g. Bland and Jubilan, 1987). It is not clear without further investigation whether anogenital investigation involves anal gland secretions, urine, and/or faeces, but our results on flehmen suggest that lions respond to the anogenitals, and resting sites, of conspecifics more similarly to urine than they do to faeces.

In conclusion, we present detailed data on lion scent-marking to show that some deposits are active scent-marks important in communication, as they are: 1) investigated by sniffing (faeces, paw-urine, squat-urine and spray-urine), 2) overmarked (pawings and spray-urine) and 3) investigated with flehmen (paw-urine and squat-urine). Scent from the anogenital region also appears to be important for intra-group communication as it frequently elicits flehmen. Lion deposits appear to be sex-specific and particularly interesting to the opposite sex, which is consistent with the reproductive assessment of

conspecifics via scent. Furthermore, adult lions participate more in marking and investigation as is expected when animals are reproductively mature.

Acknowledgements

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CHAPTER 5: SPONTANEOUS DISCIRIMINATION OF URINE ODOURS IN WILD AFRICAN LIONS, PANTHERA LEO

Abstract

Olfactory communication is the primary mode of communication for many mammals, yet research on this form of signalling is still largely descriptive in most species. Thus, despite the apparent importance of scent-marking in the social lives of wild felids, experimental studies directly investigating the function of olfactory communication are lacking. We conducted scent presentation experiments to investigate whether wild African lions can discriminate another lion's social group and sex from a sample of its urine. Our results indicated that lion urine has the potential to signal depositor sex and social group, and that lions can use urine to discriminate males from females and residents from non-residents. The response of lions to urine was also dependent on both the sex and age of the subject receiving the presentation. Female lions responded less frequently to urine from resident females than to urine from either non-resident females or resident males. Males responded more strongly to urine from resident males than from resident females, but did not appear to differentiate urine from non-resident and resident females. Observations of flehmen and further scent-marking responses from lions provide additional evidence that lion urine functions in scent-marking. These results establish that urine scent-marks contain sufficient information for receivers to discriminate the sex and social affiliation of the signaller, and demonstrate the functional relevance of scent-marking in African lions.

Introduction

Olfactory communication is the primary means of communication among many mammals (Brown and MacDonald, 1985). Semiochemicals in urine, faeces and glandular secretions play a pivotal role in mediating social and sexual interactions (Bradbury and Vehrencamp, 1998; Wyatt, 2003; Brennan and Kendrick, 2006; Apps, 2013). The ability to assess the sex and residence status of conspecifics through scent-marks may be particularly adaptive for wide-ranging territorial species that are under intense selection pressure to deter territorial intruders and locate mating partners (Bradbury and Vehrencamp, 1998). Although these selection pressures should apply in many felid species (Kleiman and Eisenberg, 1973), little is known about the function of felid scent-marking in the wild (Soso, Koziel, Johnson, Lee and Fairbanks, 2014; Vogt, Zimmermann, Kölliker and Breitenmoser, 2014; Allen, Wallace and Wilmers, 2015; Vogt, Boos, Breitenmoser and Kölliker, 2016).

It is thought that felids make extensive use of olfactory communication (Kleiman and Eisenberg, 1973). Cats have numerous specialised scent glands and use urine for scent-marking (Brown and Macdonald, 1985; Asa, 1993; Mellen, 1993; Pageat and Gaultier, 2003; Vogt et al., 2016). Both male and female cats either spray urine backwards onto vegetation, or rake their feet through urine on soil (Eaton, 1970; Kleiman and Eisenberg, 1973; Verberne and De Boer, 1976). Although olfactory communication is apparently important in felid social systems, research results are scarce because cats are elusive and nocturnal (Vogt et al., 2014). While detailed data on marking behaviour are accumulating for wild felids (e.g. Vogt et al., 2014; Allen, Whittmer and Wilmers, 2014; Allen et al., 2015; Allen, Yovovich and Wilmers, 2016), experimental investigations of the response of wild felids to potential scent-marks have been limited to the puma, *Puma concolor* (Allen et al., 2014). To understand the functions of scent-

marking, we need to consider the information content of olfactory signals and investigate the responses of individuals to scent-marks. Previous behavioural (Schaller, 1972; Asa, 1993; Brahmachary and Singh, 2000; Lehmann, Funston, Owen and Slotow, 2008; Barja and de Miguel, 2010; Gilfillan, McNutt, Vitale, Iongh and Golabek, 2016) and chemical (Andersen and Vulpius, 1999; McLean, Hurst, Gaskell, Lewis and Beynon, 2007; Umapathy et al., 2007; Poddar-Sarkar, Chakroborty, Bhar and Brahmachary, 2008; Umapathy, Kumar, Kabra and Shivaji, 2013) work has suggested that scent-marking plays an important role in the social and sexual behaviour of lions. For example, chemical analysis indicates the potential for lion urine to signal individual identity and sex (Andersen and Vulpius, 1999). We therefore conducted scent presentation experiments to investigate whether wild lions can discriminate another lion's sex and social group from a sample of its urine.

Lions live in a fission-fusion social system in which group members collectively defend territories or access to mates from other same-sex groups (Schaller, 1972; Packer, Scheel and Pusey, 1990; Grinnell, Packer and Pusey, 1995). Maintaining territories is essential for breeding, with fights between territory rivals often leading to injury and death (Schaller, 1972; Packer et al., 1990; Heinsohn and Packer, 1995). Lions can detect potential mates and territorial threats from the long-distance calls of conspecifics based on caller sex, familiarity and group membership (McComb, Pusey, Packer and Grinnell, 1993; McComb, Packer and Pusey, 1994; Grinnell et al., 1995). We hypothesised that olfactory communication in lions will also extend to sex and social discrimination. We predicted that lions presented with urine from resident adult males and females, will be more likely to respond (e.g. sniff, lick or overmark the urine), and will spend longer responding to urine from opposite sex conspecifics. In this way, lions could assess the reproductive condition of mates (Charlton, 2014; Tinnesand et al., 2015). We expected

that olfactory sex discrimination will be more pronounced in adult lions than in sexually immature subadults and cubs. With the ability to discriminate between the scents of resident and non-resident individuals, females could detect the presence of potential rivals for their territory. We predicted that female lions will be more likely to respond, and will spend longer responding to urine from non-resident females than from resident females. In contrast, male lions will not show a strong difference in response to resident and non-resident female urine as both signal the presence of a potential mate. Since lions of all ages are at risk during territory take-overs, we hypothesised that there should be no clear age differences in the ability to discriminate the urine of resident from non-resident females.

Methods

Study population and site

The study area (ca. 1500 km² 19°31'S, 23°37'E; elevation ca. 950 m) was bordered by the Okavango Delta and included the Moremi Game Reserve and its surrounding Wildlife Management Areas (for further details see McNutt, 1996). Life histories and demographic data were available on the study population since 2007, and all individuals were habituated to close approaches by vehicles. Lions were located using spoor- or radio-tracking, with up to three individuals in each social group fitted with VHF-GPS radio collars (Royal Veterinary College, U.K; < 1080 g) or VHF radio collars (African Wildlife Tracking, Pretoria, South Africa; < 780 g). Individual lions were identified from their unique whisker-spot patterns, and were divided into demographic categories based on their sex and age, with age categories defined as follows: cubs (below 18 months), subadults (18-48 months), adults (four years and above) (Packer et al., 1988). Where the birth date was not known, the individual was aged using body size, teeth

wear, male mane development, and the observation of sexual activity (Whitman and Packer, 2007).

Sixty-eight scent presentation experiments were conducted between May 2014 and December 2015 on 52 free-ranging lions from four female prides and three male coalitions. A pride was defined as a minimum of two sexually mature females that defend a shared territory, and includes all offspring of pre-dispersal age. A coalition was defined as a group of post-dispersal males that attempt to associate with female prides (Schaller, 1972).

Scent sample collection

Urine-soaked soil deposited with the following five body postures was collected for presentation: (1) Spray: backwards spray onto vegetation and surrounding soil while standing ($N = 5$ samples), (2) Scrape: downwards spray onto soil while scraping the urine soaked soil with hind paws ($N = 28$), (3) Squat: urination onto soil with hindquarters lowered to soil and no paw scraping ($N = 22$), (4) Stand: urination onto soil while standing with no paw scraping ($N = 5$), and (5) Lie: urination onto soil while lying down ($N = 1$). In the statistical analyses (see below), we further grouped the body postures of the urinating lions into 'urine-only' postures (spray, squat, stand and lie postures) and scrape postures. In contrast to urine-only postures, scrape postures could involve deposits of both urine and secretions from pedal (feet) glands. Pedal glands are known to deposit important scent-marks for some mammals (e.g. white tailed deer (*Odocoileus virginianus*): Gassett et al., 1996; polar bear (*Ursus maritimus*): Owen et al., 2015; brown bear (*Ursus arctos*): Clapham, Nevin, Ramsey and Rosell, 2014), and felids also have inter-digital glands and sweat glands on their feet (Pageat and Gaultier, 2003). It is not known whether lions deposit semiochemicals from pedal glands.

Urine was collected from lions in different social (alone or in a group), sexual (oestrous or anoestrous), and activity (resting or mobile) states. Urine-moistened soil was collected using a clean metal spoon and placed into sterilised glass jars with aluminium foil-lined lids. Following collection, all samples were immediately stored in a 12 V cooler box (to keep contents 15 °C below the ambient temperature), and were later (within a few hours) frozen at -20 °C until thawed for presentation (average number of days frozen before presentation $\pm$ SD = 129 $\pm$ 112 days; see Discussion for the effect of freezing). Sixty-one urine samples from 18 adult lions were used. Non-resident urine (see below) was collected from lions in social groups with territory directly adjacent to the subjects receiving the presentation (determined through GPS radio-telemetry collars: Gilfillan et al., n.d).

Experiment procedure

Before an experiment, a clean metal spoon was used to place approximately 80 g of the frozen sample into a sterilised glass jar (with aluminium foil-lined lid). The sample was then allowed to thaw. A resting group of lions was approached with a vehicle. From the vehicle the urine sample was tipped out of the jar in a single pile on top of short grass or bare soil 7-25 m from the lions, and the vehicle was reversed away. Lions that moved to within 2 m of the sample were videoed with a Bell + Howell DNV16HDZ (Wheeling, IL, U.S.A) digital camcorder. Observations stopped when the lions moved away from the area (Figure 5.1). Scent presentation experiments were conducted within two hours before sunset, when lions begin to become active. The sample was not re-collected. We put the sample upwind of the lion group, but it was not possible for an observer sitting in an open-top vehicle to judge whether any individual lion was downwind at any particular time.

To test whether lions can discriminate another lion's social group and sex from a sample of its urine, we presented three urine treatments from the following donors: (1) absent adult female residents ('Female resident'), (2) absent adult male residents ('Male resident'), and (3) adult female non-residents ('Female non-resident'). Since the non-resident individual in treatment 3 was never present, urine samples from absent group members were used in Treatments 1 and 2 so that donors were absent in all three treatments. To further standardise the presentations, urine samples were presented to unisex (i.e. all adults were of the same sex) lion groups (two or more adult lions) that were not feeding. Two presentations (out of 68; 2.9%) were made to a nomadic coalition of two males that were attempting to take over a pride. Only one sample was presented during each trial, but 98.1% of subjects were presented with at least two treatment categories spread over the study period, and 55.8% were presented with all three. Although no control treatment was used, the experimental design controlled for the handling, storing and presentation methods by comparing the response of lions to different treatments, while also largely controlling for individual differences between lions by using a repeated measures design.
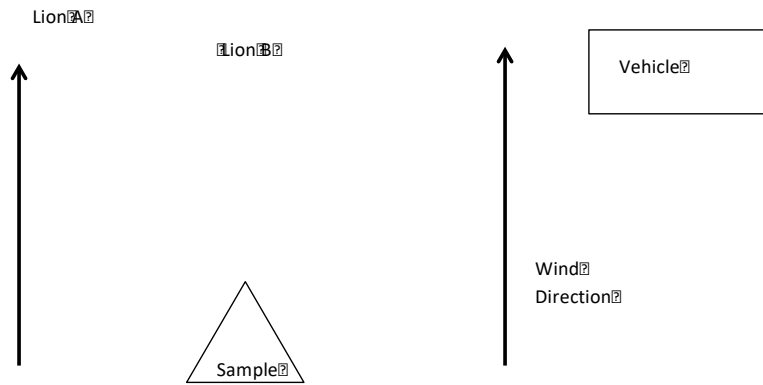
Figure 5.1. Experimental procedure: approximately 80 g of thawed urine-soaked soil was deposited on the ground 7-25 m away from resting lions. Responses were observed from a vehicle.

Ethical note

The collaring of lions and presentation experiments were performed under permits granted by the Botswana Ministry of Environment Wildlife and Tourism (8/36/4 XXV (8)), and ethical clearance was also obtained from the University of Sussex (Non-ASPA 4 – November 2013).

Behavioural analysis of response

Video recordings of lion responses (Table 5.1) were analysed frame-by-frame (frame = 0.033/0.034 s) on a Fujitsu Siemens Amilo Pi2515 (Munich, Germany) laptop using Avidemux 2.6.9 (Mean Development Team, 2015) video analysis software. Distances between each lion and the urine were estimated by sight during the experiment.

Table 5.1. Behavioural measurements of lions after the presentation of urine.

Behavioural Measurement	Definition
Respond to urine	A lion responded to the urine when investigating, countermarking, or overmarking it.
Investigate urine	Nose pointed towards the ground within 0.5 m of the urine. Investigatory behaviours included sniffing (with or without flehmen) and licking the sample. Flehmen is a key response of mammals to semiochemicals (Hart and Leedy, 1987) and involves a curled upper lip facial expression, exposing the front teeth.
Countermark urine	Pawing the ground, urinating, and/or defecating within 2 m of the urine, without the two deposits touching (Rich and Hurst, 1999).
Overmark urine	Pawing the ground, urinating, and/or defecating on top of the urine, so that the two deposits are at least partially touching (Johnston, Chiang and Tung, 1994).
Duration of response	The combined total time each lion was scored as investigating, countermarking and/or overmarking the urine.
Closest distance to the urine	The closest distance the test subject moved to the urine sample (m), irrespective of whether it responded to the urine. If the subject moved away from the sample from its resting place, the closest distance to the sample was equal to the distance that the sample was originally placed from the lion. The closest distance that the subject moved to the sample was chosen rather than the distance the sample was first placed from the subject, since the subject showed signs of detecting the sample (e.g. sniffing the air) from its resting spot in only two of 72 cases.

Statistical analyses

All statistical analyses were conducted using the R statistical software (v. 3.3.1; R Core Team, 2016). Fisher's exact tests were used to compare the likelihood that lions of different age or sex would countermark or perform flehmen when responding to the urine. Where multiple comparisons were performed, the Bonferroni correction was applied.

To assess which factors determine whether lions responded to urine samples, we ran a series of generalised linear mixed models (GLMMs) with a binomial error distribution (0 = sample ignored, 1 = sample investigated/countermarked/overmarked) and a logit link function. The identity of the test subject ($N = 52$) was included as a random term to account for multiple data from the same lions. Regarding the fixed effects, the global model was coded as follows: Respond or not ~ Treatment + Recipient age + Recipient

sex + Number of days frozen + Time between deposition and collection (mean ± SD = 18.8 ± 18.1 minutes, range = 5-72 minutes) + Donor posture (urine-only posture = 33 samples; scrape posture = 28 samples) + Closest distance to sample (m, see Table 5.1) + Treatment * Recipient sex + Treatment * Recipient age. To assist model convergence, the number of days each sample was frozen before presentation, and the time between deposition and collection were scaled and centered (using the 'scale' function in R) prior to running the models. The fixed effects were not strongly correlated (measured using the variance inflation factor and the condition number test). The GLMMs were run using the 'lme4' package (Bates, Maechler, Bolker and Walker, 2015), and the dredge function in the 'MuMIn' package (Bartoń, 2016) was used to create a list of candidate models from the global model. We used Akaike's information criterion with a correction for small sample sizes (AICc) for model selection, where lower AICc values corresponded with better support for a given model. Akaike weights were calculated to show the relative importance of each candidate model (Akaike, 1974). Conditional model averaging (MuMIn package) was performed on the models within 2 AICc of the optimal model (lowest AICc) to extract averaged parameter estimates and their 95% confidence intervals (Symonds and Moussalli, 2011). Tukey's HSD post-hoc tests were run on the optimal model using the 'multcomp' package (Hothorn, Bretz and Westfall, 2008).

For those individuals that responded to the urine, we ran a series of generalised linear mixed models (GLMMs) with a Gaussian error distribution to assess which factors determine the duration of response (s). The cube-root transformation was applied to the response duration to correct for non-normality in the response variable. Regarding the fixed effects, the global model was coded as follows: Treatment + Recipient age + Recipient sex + Number of days frozen + Time between deposition and collection +

Donor posture + Treatment * Recipient age + Treatment * Recipient sex. The fixed

effects were not strongly correlated. We incorporated the identity of the test subject (N

$= 36$), the sample number ($N = 31$), and the recipient's social group ($N = 7$) as random

terms to account for multiple data from the same lions, when presented with the same

sample of urine. Using the MuMIn package, model dredging was performed with the

maximum likelihood estimation method (ML), and model averaging was performed

with the restricted maximum likelihood estimation method (REML; Zuur, Ieno, Walker,

Saveiliev and Smith, 2009). Tukey's HSD post-hoc tests were run using the 'multcomp'

package on the optimal model (using the REML estimation method).

Results

Thirty-six of 52 lions (69.2%) responded to at least one urine sample. The average ($\pm$

SD) distance from which lions first appeared to detect the urine (as evidenced by

changing the position of the head in the direction of the sample location) was 2 ± 4 m

(range 0-20 m, mode 1 m), but since this can be difficult to measure, lions were scored

as investigating the sample only when within 0.5 m of the sample (consistent with

Jordan, Golabek, Apps, Gilfillan and McNutt, 2013; Allen et al., 2014; Allen et al.,

2015). A typical response (93.1% of responses) involved sniffing the sample, with

flehmen following sniffing in 59.7% of cases. The average ($\pm$ SD) duration of response

was 35.3 ± 34.1 s. Overmarking was never observed, while countermarking was

observed nine (12.5% of responses) times (resident female urine $= 4$, non-resident

female urine $= 4$, and resident male urine $= 1$). Countermarking involved defecation

twice (22.2% of countermarks) and urination seven times (77.8% of countermarks), all

within 2 m of the urine (Table 5.2). The average ($\pm$ SD) time it took the urine to elicit a

response from a lion was 27 ± 20 min. The average ($\pm$ SD) time that individual lions

ignored the urine (irrespective of whether other group members responded) was 66 ± 18 min.

Table 5.2. Summary of the raw data on the responses to urine for lions of different sex and age.

Individual class	Number (%) of trials	Number (%) of responses	Number (%) of responses involving sniffing	Number (%) of responses involving flehmen	Number (%) of responses involving a countermark
Recipient sex					
Male	99 (31.0)	22 (22.2)	21 (95.5)	16 (76.2)	3 (13.6)
Female	220 (69.0)	50 (22.7)	46 (92.0)	24 (52.2)	6 (12.0)
Recipient age					
Adult	143 (44.8)	23 (16.1)	18 (78.3)	10 (55.6)	9 (39.1)
Subadult	72 (22.6)	25 (34.7)	25 (100.0)	15 (60.0)	0 (0.0)
Cub	104 (32.6)	24 (23.1)	24 (100.0)	15 (62.5)	0 (0.0)
Total	319	72 (22.6)	67 (93.1)	40 (59.7)	9 (12.5)

When responding to urine, males and females were equally likely to flehmen (two-tailed Fisher's exact test: $P = 0.106$), and to countermark the sample (two-tailed Fisher's exact test: $P = 0.999$). Adults were significantly more likely to countermark the urine than either subadults (two-tailed Fisher's exact test with Bonferroni correction of alpha: $P < 0.001$), or cubs (two-tailed Fisher's exact test with Bonferroni correction of alpha: $P < 0.001$). However, there was no significant age class difference in the likelihood of performing flehmen after investigating the urine (two-tailed Fisher's exact test: $P = 0.950$).

The optimal model of the likelihood of lions responding to the urine included the closest distance the lion moved to the sample, the donor posture, the age of the recipient, the sex of the recipient, the urine treatment, and the interaction between recipient sex and

urine treatment. Model averaging of the best GLMMs revealed that the closest distance the lion moved to the sample, the donor posture, the age of the recipient, and the interaction between recipient sex and urine treatment were the best predictors of whether or not test subjects responded (Table 5.3). Post-hoc tests revealed that females were significantly less likely to respond to resident female urine than either resident male urine ($P = 0.008$; Figure 5.2; Table 5.4), or non-resident female urine ($P = 0.039$; Table 5.5). The likelihood of males responding was independent of both the donor's sex ($P = 0.869$) and social group ($P = 0.409$). Adults were equally likely to respond as subadults ($P = 0.150$) or cubs ($P = 0.729$), but subadults were significantly more likely to respond than cubs ($P = 0.027$; Table 5.6). Lions were more likely to respond to urine deposited with a scrape posture (45 out of 152 trials; 29.6%) compared to a urine-only posture (27 out of 167 trials; 16.2%). Finally, the time between urine deposition and collection, and the number of days the urine was frozen before presentation were weak predictors of response likelihood (relative importance < 0.5; as in Dala-Corte, Becker and Melo, 2016): lions were more likely to respond to urine that had been collected more quickly after deposition, and were less likely to respond to urine that had been frozen for longer.

Table 5.3. Factors influencing whether lions responded to the urine (*N* = 319).

Variable		Estimate	SE	CI (2.5:97.5%)	Relative importance
Intercept		1.722	0.648	0.448:2.996*	-
Closest distance to sample (m)		-3.398	0.582	-4.544:-2.253*	1.00
Treatment	Female non-resident	-	-	-	1.00
	Female resident	-1.761	0.694	-3.125:-0.396*	-
	Male resident	0.669	0.727	-0.761:2.099	-
Recipient sex	Female	-	-	-	1.00
	Male	-0.760	0.910	-2.551:1.030	-
Recipient age	Adult	-	-	-	1.00
	Subadult	1.239	0.670	-0.080:2.558	-
	Cub	-0.395	0.553	-1.483:0.694	-
Recipient sex * Treatment	Female * Female non-resident	-	-	-	1.00
	Male * Female resident	3.378	1.319	0.782:5.973*	-
	Male * Male resident	0.090	1.416	-2.695:2.875	-
Donor posture	Scrape	-	-	-	0.80
	Urine-only	-1.028	0.550	-2.111:0.055	-
Time between deposition and collection (scaled and centered)		-0.259	0.277	-0.804:0.286	0.21
Number of days frozen (scaled and centered)		-0.225	0.258	-0.733:0.283	0.20

Model parameters were generated using model averaging on the best GLMMs (with binomial error distribution) selected using AICc (see Table 5.7). SE = Standard Error. CI = Confidence Interval. * Confidence intervals that do not cross zero.
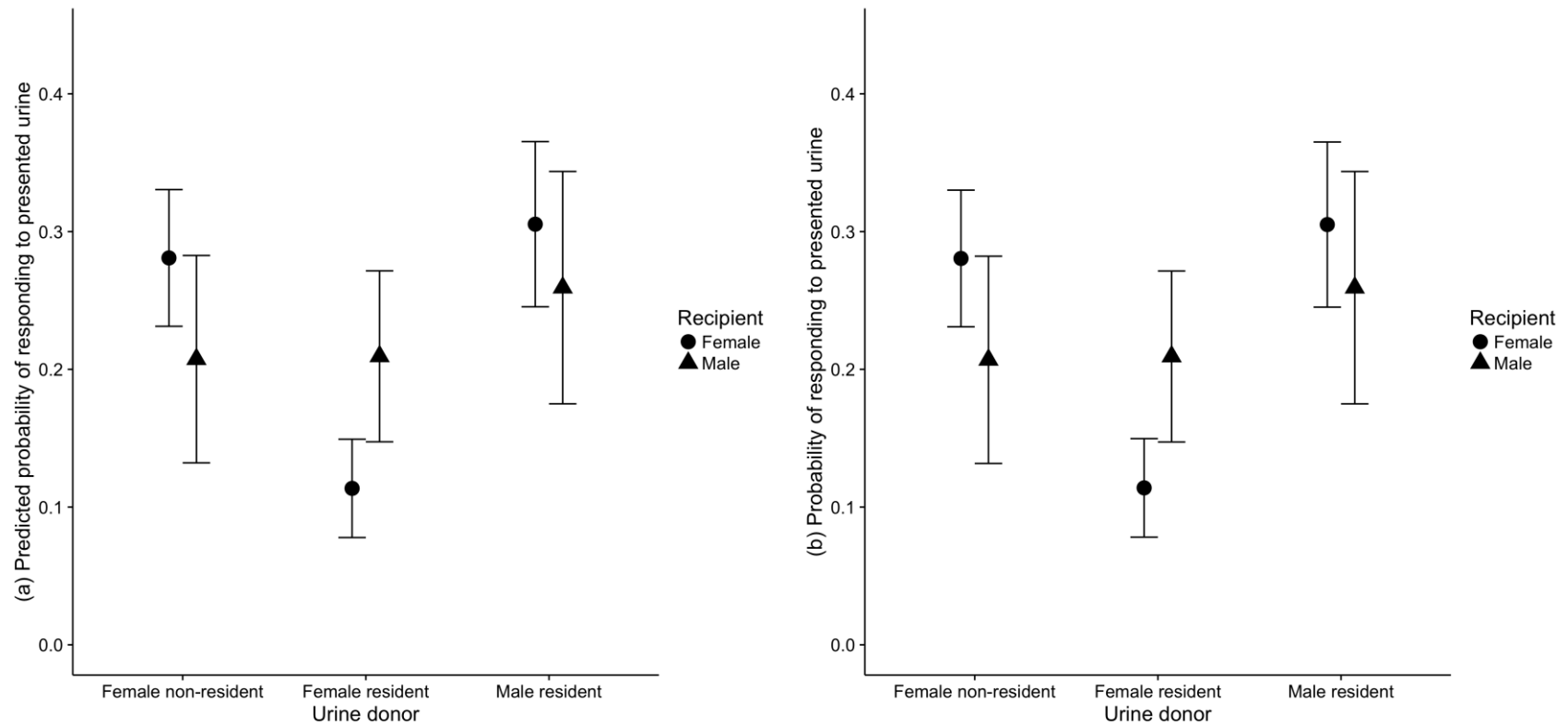
Figure 5.2. (a) Predicted probabilities of responding to the treatments of presented urine for male and female lions. Estimates were derived from the averaged model presented in Table 5.3. (b) Mean probabilities of responding to the treatments of urine for male and female lions calculated from the raw data in Table 5.4. Bars are binomial standard errors.

Table 5.4. Summary of the raw data on the responses to the urine of each treatment for lions of different sex and age.

Recipient sex	Female resident			Female non-resident			Male resident			Total		
	Trials	No. (%) of responses	Response duration (s ± SD)	Trials	No. (%) of responses	Response duration (s ± SD)	Trials	No. (%) of responses	Response duration (s ± SD)	Trials	No. (%) of responses	Response duration (s ± SD)
Male	43	9 (20.9)	48.67±48.54	29	6 (20.7)	21.73±33.02	27	7 (25.9)	58.69±24.03	99	22 (22.2)	44.51±39.31
Female	79	9 (11.4)	27.50±13.09	82	23 (28.0)	37.15±40.64	59	18 (30.5)	25.54±21.99	220	50 (22.7)	31.24±31.12
Recipient age												
Adult	58	7 (12.1)	57.95±48.93	48	11 (22.9)	44.34±43.62	37	5 (13.5)	7.55±6.82	143	23 (16.1)	40.48±43.32
Subadult	32	6 (18.8)	36.09±17.38	28	13 (46.4)	26.74±34.56	12	6 (50.0)	45.72±35.84	72	25 (34.7)	33.54±31.49
Cub	32	5 (15.6)	12.67±9.37	35	5 (14.3)	29.92±43.63	37	14 (37.8)	39.89±21.17	104	24 (23.1)	32.14±26.83
Total	122	18 (14.8)	38.08±36.17	111	29 (26.1)	33.96±39.15	86	25 (29.1)	34.82±26.79	319	72 (22.6)	35.29±34.11

± SD = standard deviation.

Table 5.5. Tukey's HSD post hoc tests investigating the interaction between urine treatment and recipient sex on the response likelihood of lions to urine.

Comparison: Recipient sex * Urine treatment	Estimate	SE	Z	Adjusted P
1. Female * Female resident urine - Female * Female non-resident urine	1.753	0.683	2.566	0.039*
2. Female * Female resident urine - Female * Male resident urine	2.377	0.768	3.096	0.008*
3. Male * Female resident urine - Male * Male resident urine	0.932	1.154	0.807	0.869
4. Male * Female resident urine - Male * Female non-resident urine	1.568	1.042	1.505	0.409

*Significant at *P* < 0.05.

Table 5.6. Tukey's HSD post-hoc tests investigating the effect of recipient age on the likelihood of lions to respond to urine.

Comparison- Recipient age	Estimate	SE	Z	Adjusted P
1. Adult – Subadult	1.229	0.661	1.859	0.150
2. Adult – Cub	0.416	0.550	0.756	0.729
3. Subadult - Cub	1.645	0.640	2.569	0.027*

*Significant at *P* < 0.05.

Table 5.7. Best GLMMs with binomial error distribution investigating the factors that influence whether lions responded to presented urine (N = 319).

Model	Description	AICc	k	Δi	wi
1	Treatment + Recipient age + Recipient sex + Closest distance to sample + Donor posture + Treatment * Recipient sex	141.84	11	0.00	0.40
2	Treatment + Recipient age + Recipient sex + Closest distance to sample + Donor posture + Time between deposition and collection (scaled and centered) + Treatment * Recipient sex	143.11	12	1.27	0.21
3	Treatment + Recipient age + Recipient sex + Closest distance to sample + Donor posture + Number of days frozen (scaled and centered) + Treatment * Recipient sex	143.23	12	1.39	0.20
4	Treatment + Recipient age + Recipient sex + Closest distance to sample + Treatment * Recipient sex	143.23	10	1.40	0.20

Lion identity (*N* = 52) was included as a random term. k = parameters, Δi = AICi-AICmin, wi = Akaike weights. Only models with delta AICc < 2 are shown.

Regarding the duration of response of lions to the urine, the optimal model included the sex of the recipient, the urine treatment, and the interaction between recipient sex and urine treatment. Model averaging on the best GLMMs revealed that the urine treatment, the sex of the recipient, and the interaction between recipient sex and urine treatment

were the best predictors of response duration (Table 5.8). Post-hoc tests revealed that males responded for longer to urine from resident males than from resident females (P = 0.023; Figure 5.3; Table 5.4), but had similar response durations to urine from resident and non-resident females (P = 0.994; Table 5.9). The response duration of females was independent of both the donor's sex (P = 0.996), and social group (P = 0.835). Time between urine deposition and collection, and the number of days the urine was frozen before presentation were weak predictors of response duration: lions responded for longer to urine that was collected more quickly after deposition, and to urine that had been frozen for longer.

Table 5.8. Factors influencing the duration of response ($3\sqrt{s}$) of lions to urine (N = 72).

Variable		Estimate	SE	CI (2.5:97.5%)	Relative importance
Intercept		3.149	0.319	2.512:3.786*	-
Recipient sex	Female	-	-	-	1.00
	Male	-0.903	0.441	-1.785:-0.020*	-
Treatment	Female non-resident	-	-	-	1.00
	Female resident	-0.355	0.426	-1.206:0.495	-
	Male resident	-0.512	0.483	-1.478:0.454	-
Recipient sex * Treatment	Female * Female non-resident	-	-	-	1.00
	Male * Female resident	0.515	0.573	-0.630:1.659	-
	Male * Male resident	2.440	0.585	1.271:3.610*	-
Time between deposition and collection		-0.017	0.012	-0.040:0.007	0.02
Number of days frozen		0.001	0.001	-0.001:0.004	<0.01

Model parameters were generated using model averaging on the best GLMMs (with Gaussian error distribution) selected using AICc (see Table 5.10). SE = Standard Error. CI = Confidence Interval. * Confidence intervals that do not cross zero.
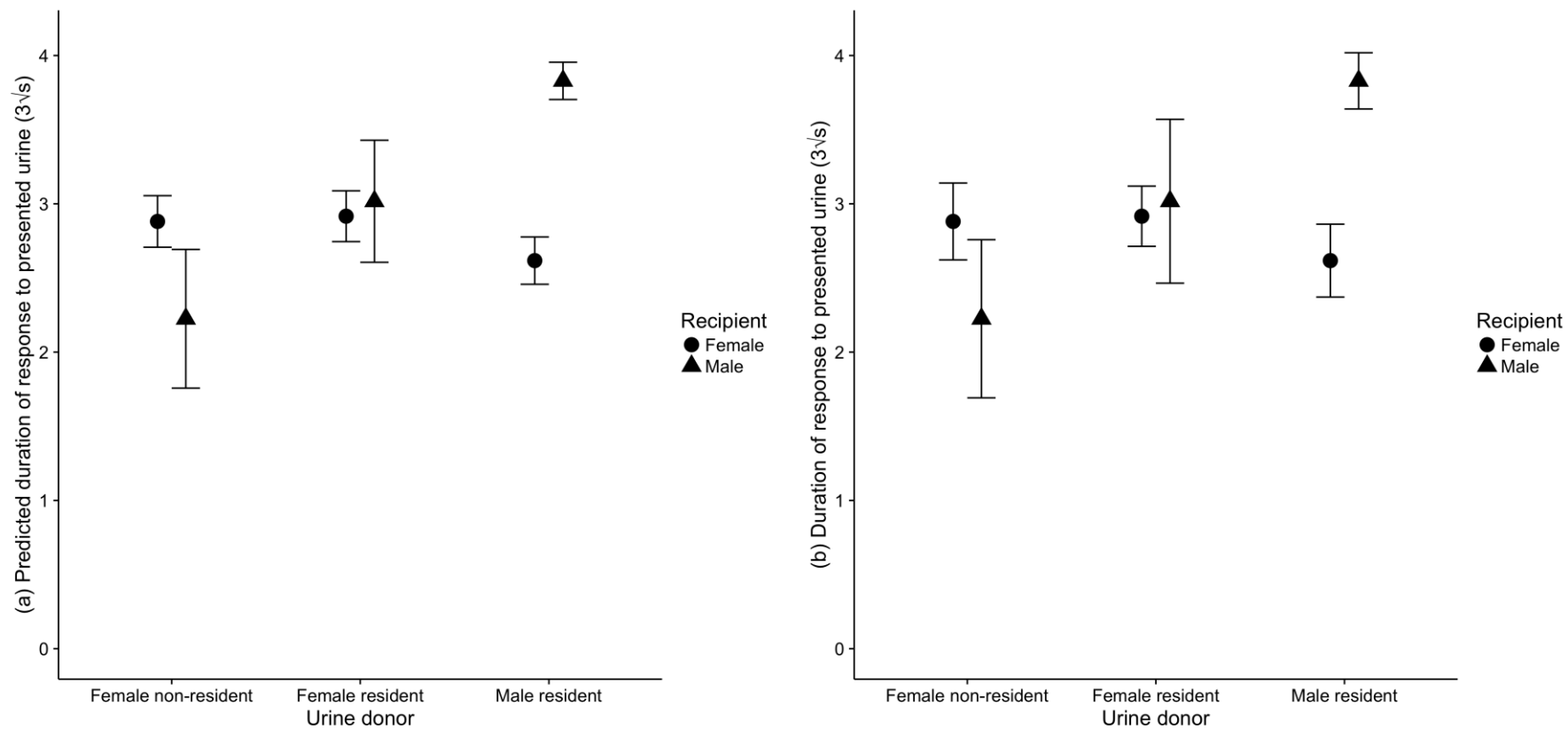
Figure 5.3. (a) Predicted mean duration ($^3\sqrt{}$s) of response to the treatments of urine for male and female lions. Estimates were derived from the averaged model presented in Table 5.8. (b) Mean duration ($^3\sqrt{}$s) of response to the treatments of urine for male and female lions calculated from the raw data in Table 5.4. Bars are standard errors.

Table 5.9. Tukey's HSD post-hoc tests investigating the interaction between urine treatment and recipient sex on the duration of response of lions to presented urine.

Comparison- Recipient Sex * Urine Treatment	Estimate	SE	Z	Adjusted *P*
1. Female * Female resident urine - Female * Female non-resident urine	0.356	0.426	0.837	0.835
2. Female * Female resident urine - Female * Male resident urine	0.156	0.556	0.281	0.996
3. Male * Female resident urine - Male * Male resident urine	1.769	0.646	2.739	0.023*
4. Male * Female resident urine - Male * Female non-resident urine	0.161	0.506	0.318	0.994

* Significant at *P* < 0.05.

Table 5.10. Best GLMMs with Gaussian error distribution investigating the factors that determine the duration of response ($\sqrt[3]{s}$) of lions to presented urine samples (*N* = 72).

Model	Description	AICc	*k*	Δi	wi
1	Treatment + Recipient sex + Treatment * Recipient sex	227.31	10	0.00	0.47
2	Treatment + Recipient sex + Time between deposition and collection + Treatment * Recipient sex	227.99	11	0.69	0.33
3	Treatment + Recipient sex + Number of days frozen + Treatment * Recipient sex	229.05	11	1.74	0.20

Lion identity (*N* = 36) and sample number (*N* = 31), and lion group (*N* = 7) were included as random terms. k = parameters, Δi = AICi-AICmin, wi = Akaike weights. Only models with delta AICc < 2 are shown.

Discussion

Our scent presentation experiment indicated that lion urine has the potential to signal depositor sex and social group, and that lions can use urine to discriminate males from females and residents from non-residents. The response of lions to the urine also depended on both the sex and age of the subject receiving the presentation. These results provide evidence that lions perceive certain urine deposits as scent-marks with a communicatory significance.

Female lions were more likely to respond to resident male urine than to resident female urine. Females cooperate to defend cubs and territory and often hunt together (Packer et al., 1990). In contrast, male lions typically take food from females (Schaller, 1972) and

consequently a high level of aggression can be seen between females and resident males (G. Gilfillan, pers. obs.). It may be adaptive for females to use olfactory cues such as urine to monitor the movement and presence of males, potentially allowing them to avoid moving through and hunting in areas with signs of male presence. Infanticidal non-resident males also pose a significant threat to females and their cubs (Pusey and Packer, 1994), and it may be adaptive for females to respond to male urine to determine the residency status (and thus risk of infanticide) of the urine donor. We were unable to test this hypothesis directly, but we predict that female lions would show elevated responses towards non-resident male urine than resident male urine. In addition, females are believed to exert some level of mate choice during reproduction (West and Packer, 2002), and urine from males may allow females to assess the maturity and quality of potential mating partners (e.g. Roberts and Gosling, 2003).

We had hypothesised that male lions would show stronger responses towards resident female urine, but males responded more strongly to resident male urine. These results could reflect the fact that we had insufficient data to test for a three-way effect of recipient age, recipient sex and urine treatment. It seems likely that adult and sexually immature (subadults and cubs) males will respond differently to urine. Male lions disperse at sexual maturity (Schaller, 1972). Adult males are aggressive towards related juvenile males (G. Gilfillan, pers. obs.), and unrelated adult males pose a significant infanticidal threat to juveniles. Consequently, juvenile males could be expected to show strong responses to male urine, as do females.

We would still expect adult males to show stronger responses to female urine than resident male urine. Competition between adult males for reproductive opportunities is intense (West and Packer, 2002). Males are attracted to the urine of oestrous females in some mammals (e.g. Swaisgood, Lindburg, and Zhang, 2002; Charlton, 2014). We were

unable to account for the oestrous state of female urine donors in our experiment, and urine from anoestrous females could be less interesting to males (Charlton, 2014). Keeping track of male companions (potentially through scent-marking) is also likely to be a key aspect of coalition formation and function, enabling males to cement and maintain social bonds with their coalition partners. Coalition cohesiveness would likely influence male tenure in prides and thus ultimately reproductive success.

As predicted, female lions, but not males, were more likely to respond to non-resident female urine than to resident female urine. For resident female lions, rival females (and their prides) pose a significant territorial threat (Schaller, 1972; Packer et al., 1990). Since mammalian scent-marking often functions for territory demarcation (Roberts and Gosling, 2001, Christensen, Kern, Bennitt and Radford, 2016), it would be adaptive for females to pay attention to the scent-marks of territory rivals (non-residents) to detect when their territory ownership is being challenged. While we provide evidence that female lions could use urine to determine the residency status of conspecifics, further research would be required to sufficiently test this in males. Non-resident adult male lions compete aggressively with resident males for access to reproductive females (Grinnell et al., 1995), and it may be adaptive for males to use scent-marking to distinguish coalition partners from non-resident males.

Flehmen was consistently exhibited by all age/sex classes of lion in response to urine. It is notable that flehmen is typically performed by males in response to sexual olfactory stimuli (Rasmussen, Schmidt, Henneous, Groves and Daves, 1982; Hart and Leedy, 1987; Stahlbaum and Houpt, 1989), but we regularly observed flehmen by females and sexually immature lions in response to urine. Flehmen transports non-volatile chemical signals (especially proteins and steroid conjugates) from the oral cavity to the

vomeronasal organ for chemosensory analysis (Hart and Leedy, 1987; Igbokwe, 2009), and its occurrence gives further evidence that lion urine functions in scent-marking.

Lions of all ages had a similar pattern of response to the urine, but subadults were more likely to respond than cubs. While little is known about the development of scent-marking in carnivores, scent-mark investigation and overmarking often increase with age, and adults scent-mark the most (Ferkin, 2010; Clapham et al., 2014, Vogt et al., 2014; Allen et al., 2015). Subadult lions are approaching sexual maturity and male subadults disperse to locate mating partners (Schaller, 1972). Perhaps subadult lions pay particular attention to the olfactory signals of conspecifics to begin acquiring information regarding mating opportunities, and to assess the risk of intra-sexual competition (White, Swaisgood and Zhang, 2002; Clapham et al., 2014). However, only adult lions were observed to countermark the thawed urine ($N = 9$). Countermarking establishes that both the urine and the countermark are scent-marks (Jordan et al., 2013).

Lions were more likely to respond to urine deposited with a scrape body posture than a urine-only posture. Urine deposited in combination with scraping could elicit a greater likelihood of response because it contains semiochemicals from pedal gland secretions as well as urine (Gassett et al., 1996; Clapham et al., 2014; Owen et al., 2015). The mixing of chemical signals from multiple sources may increase the information content of deposits, and increase the response likelihood of conspecifics (Greene et al., 2016). Further research would be required to test this hypothesis, but lions still investigate and overmark pedal gland secretions in the absence of urine (Gilfillan, Golabek, Vitale, McNutt and McComb, n.d).

Finally, our results provide valuable methodological insights that might aid future scent presentation experiments. Lions were less likely to respond, and responded for shorter durations to urine that was exposed to the environment for longer before collection. While the time between urine deposition and collection was a weak predictor of how lions responded, our results suggest that some semiochemicals important to lions were degraded or diffused in the time between deposition and collection.

There is also conflicting evidence about the effects of freezing scent: in some studies freezing had a significant effect on the subsequent response of animals (Hoffmann, Musolf and Penn, 2009), and in others it had little or no effect (Smadja, Catalan and Ganem, 2004; Bagley, Goodwin, Rasmussen and Schulte, 2006; Kwak et al., 2009; Lenochova, Roberts and Havlicek, 2009). In our experiments, the number of days that urine was stored at -20 °C had a weak effect on the likelihood of response, and the duration of response from lions. Lions were less likely to respond, but responded for longer to urine that had been frozen for longer. These results suggest that some semiochemicals important to lions were degraded over time in the frozen urine. Freezing may have had additional effects on the urine that were not directly evident in our results, such as influencing the distance over which lions were attracted to the scent, or leading to lower response rates than may have been possible with fresh urine.

In conclusion, we provide direct experimental evidence that urine functions in social and sexual communication in wild lions. Our results suggest that lions can use urine to discriminate males from females and residents from non-residents. The response of lions to urine was also dependent on the sex and age of the subject receiving the presentation. Further research is required to reveal the specific semiochemicals in urine and their functions for social and sexual scent communication in lions, as well as the longer-term behavioural changes of lions following the detection of scent-marks.

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CHAPTER 6: GENERAL DISCUSSION

Lions are one of the most extensively studied carnivores, but much of our current knowledge of lion behavioural ecology originates from a handful of populations, mostly from East Africa (e.g. Schaller, 1972; Packer, Scheel and Pusey, 1990; Grinnell and McComb, 1996; 2001). Yet like many species, lions exhibit substantial behavioural plasticity to adapt to the specific ecological conditions of the ecosystem they inhabit (Kruuk 1972; Macdonald 1979; Patterson 2007; Kotze, 2016). Consequently, we do not know how applicable such findings on lions in East Africa are to lions across the entire range. It is also believed that the ancestral lion may have evolved as a wetland specialist in ecosystems such as the Okavango Delta in Botswana, and that lions inhabiting open savannahs in East Africa evolved under strong environmental pressures and genetic isolation (Moore et al., 2016). Therefore, research into the behaviour of lions in the Okavango has the potential to provide significant insights into the evolutionary origins of lion behaviour.

In addition, previous research on lion communication has primarily focused on their vocalisations (particularly their roars: McComb et al., 1993; McComb, Packer and Pusey, 1994; Grinnell, Packer and Pusey, 1995; Heinsohn, 1997; Grinnell and McComb, 2001), as vocal signalling is believed to be the main mode of communication between lions (Ramsauer, 2005). However, there has been little detailed investigation of how lions communicate through chemical signalling, and the importance of olfactory communication in lion behavioural ecology has largely been overlooked. Yet there is striking theoretical (Wyatt, 2014), behavioural (Schaller, 1972; Asa, 1993; Brahmachary and Singh, 2000; Lehmann et al., 2008; Barja and de Miguel, 2010; Gilfillan et al., 2016), and chemical (Andersen and Vulpius, 1999; McLean et al., 2007; Umapathy et al., 2007; Poddar-Sarkar et al., 2008; Soini et al., 2012; Umapathy et al.,

2013; Poddar-Sarkar and Brahmachary, 2014) evidence to suggest that semiochemicals (particularly scent-marks) may be important in mediating social, sexual and competitive interactions between lions. Whether the communication signals of lions are multi-modal, and whether lions are capable of cross-modal processing of information gathered through multiple senses, has also been overlooked. This is most likely because studying these abilities in the wild presents a significant challenge. Yet, as a result of cross-disciplinary approaches to the study of animal behaviour, new experimental paradigms to test cognitive abilities such as cross-modal processing are being developed (e.g. Proops, McComb and Reby, 2009).

My thesis aimed to address these gaps in our current knowledge of African lion behavioural ecology, by investigating specific questions relating to the vocal, olfactory and multi-modal communication of lions in the Okavango Delta wetland ecosystem. The research was designed to expand upon established experimental techniques developed to investigate the vocal communication of lions in East Africa, but also to employ techniques from other research settings to allow novel investigations of olfactory and multi-modal communication in lions. Specifically, chapter 2 presented an investigation of whether lions can determine the number of conspecifics calling in large vocal choruses in a territorial conflict setting. Chapter 3 presented an investigation of whether lions are able to cross-modally process information on individual identity to recognise group members at an individual level. Chapter 4 presented a detailed analysis of the scent-marking of wild lions and the response of lions to the scent-marks of group members, to determine whether marking may play an important role in the social lives of prides. Finally, Chapter 5 presented a 'whole-scent' presentation experiment to further investigate the functional significance of urinary scent-marks in lion communication within and between prides. In the following sections, I will expand on

the findings reported in this thesis and discuss the contributions of this research to the current literature on animal communication. I will pay particular attention to how the main questions of the thesis have been addressed, and will provide recommendations for future research.

Main results and conclusions

Expanding our knowledge of lion vocal communication

The research articles presented in this thesis expand on our current knowledge of the long-distance vocal communication of African lions. For example, the results of chapter 2 suggest that lions may not be able to determine the number of conspecifics calling in large vocal choruses. Lions of both sexes almost always approached simulated intruder groups, regardless of chorus group size. In addition, chorus group size was not a strong predictor of the level of caution exhibited by lions when approaching the intruders. Instead lions conformed to the prediction that auditory or cognitive limitations in animals mean that assessors of group calling will fail to accurately judge caller number once chorus size exceeds three (Harrington, 1989; McComb, Packer and Pusey, 1994).

The results of these experiments also revealed that lions in the Okavango may not use group chorusing to mitigate conflict in the same way as lions in East Africa. That single males almost always approached simulated intruder groups in this study matched the 'always-attack' strategy proposed for male lions (Grinnell, Packer and Pusey, 1995), but females in the Okavango were more aggressive towards simulated intruder groups than previously documented for females without cubs. In East Africa, outnumbered females would typically only approach a simulated intruder group when defending cubs (McComb, Packer and Pusey, 1994; Heinsohn, 1997). Our results suggest that competition for territory in the Okavango Delta may be higher than in East Africa, and that Okavango females operate an 'always-attack' strategy similar to males.

Although a range of species have now been tested for acoustic numerical assessment using vocal choruses, much of the previous research has been limited to testing whether animals can differentiate between one and up to three simultaneous callers (McComb, Packer and Pusey, 1994; Grinnell, Packer and Pusey, 1995; Kitchen, 2004; Seddon and Tobias, 2003). A recent test of human auditory abilities showed that the upper bound for human listeners to accurately estimate the number of simultaneous speakers is three (Vitevitch and Siew, 2015). Despite this, there had been no direct test of whether there may be a similar limit to how accurate animals are in assessing the number of callers in larger vocal choruses. The results presented in chapter 2 suggest that the acoustic numerical assessment abilities of humans (Vitevitch and Siew, 2015), and non-human animals may be constrained by the same cognitive limitations.

Vitevitch and Siew (2015) present evidence to suggest that humans use the cognitive ability of subitization (i.e. the ability to quickly and accurately determine the number of objects without counting: Kaufman et al., 1949), to assess the number of simultaneous speakers. While the cognitive processes underlying acoustic quantification in lions remain unknown, it is striking that humans and lions are similarly limited in their ability to assess the number of simultaneous callers. It is possible that lions also assess group chorused roars through subitization. To our knowledge, it has not previously been demonstrated that non-human animals subitize acoustic quantities. However, subitization of visual and tactile quantities has been demonstrated in animals (Dacke and Srinivasan, 2008; Beran et al., 2011; Agrillo et al., 2012). To extend our knowledge of the cognitive system underlying acoustic numerical representation in non-human animals, future research on this topic should explore acoustic numerical assessment of larger (> 3) choruses of simultaneous callers, in a range of different species from diverse taxonomic groups.

Furthermore, the results presented in chapter 3 suggest that lion roars encode cues to the caller's identity, and that these individually distinctive cues can be cross-modally matched with visual (and potentially olfactory) cues to identity. In this study, a vehicle was used to create a visual block between an individual and the test subject, before roars were played from behind the visual block that either matched this individual (congruent trials) or corresponded to an absent group member (incongruent trials). When presented with a scenario where the playback of a roar broadcast from behind a visual block is incongruent with the conspecific previously seen there, subjects responded more strongly than during the congruent scenario where the call and individual matched. These findings suggest that lions are capable of audio-visual cross-modal individual recognition and provide evidence that multi-modal signals may be important for communication between lions.

Recognising others as distinct individuals allows an animal to remember its past social interactions with competitors and cooperative conspecifics (Hamilton, 1963; Trivers, 1974). The ability to recognise individuals would be crucial for many aspects of social behaviour including dominance hierarchies, territoriality, and social grouping (Tibbetts and Dale, 2007). Individual recognition would be particularly adaptive in social species where group composition is not constant, and where individuals need to keep track of the movements of group members (Bates et al., 2008). Lions live in fission-fusion social groups, in which group members can be spread apart over wide distances throughout the territory (Schaller, 1972). Yet lions cooperate to defend their territory, protect offspring from rival prides and infanticidal males, and to kill large prey (Schaller, 1972). Consequently, individual recognition through long-distance communication signals, would be an adaptive ability for lions to facilitate key social behaviours and reduce communication errors (Tibbetts and Dale, 2007).

Using digital analysis techniques, previous research has applied the source-filter theory of vocal sound production to mammal vocalisations, to identify potential individually distinct cues within calls. As discussed in the introduction chapter, vocal cues signalling the caller's identity could originate from either the source (i.e. the larynx), and/or the filter (i.e. the vocal tract) structures of the vocal apparatus (Taylor and Reby, 2010). Source-related features could include fundamental frequency modulation (Charrier, Mathevon and Jouventin, 2003), harmonic structures within the call (Rendall, 2003), nonlinear dynamics (desynchronisation between the paired vocal folds: Riede, Owren and Arcadi, 2004), amplitude modulation (Charlton, Zhihe and Snyder, 2009), and temporal features such as call length and signal tempo (Rendall, 2003). Correspondingly, filter-related features could include the formant structure within the vocalisation, or formant modulation over the course of the vocalisation (e.g. Rendall, 2003; Charlton et al., 2011). Although beyond the scope of the study, it would be informative to apply the source-filter theory to lion roars, to investigate what specific parameters within the roars code for caller identity. Given the techniques used in the experimental design (i.e. standardising the lengths of playback exemplars), we can assume that lion roars are individually distinct, independent of call length. I am aware of only one previous application of the source-filter theory to the roars of lions, and whether roars are individually distinct was not investigated (Pfefferle et al., 2007). In their analysis, Pfefferle et al. (2007) revealed that caller body size was an important determinant of the acoustic properties of roars (e.g. fundamental frequency) between the sexes, but there was no evidence that acoustic variables were related to indicators of male dominance (e.g. body size and mane length/darkness).

Once potential cues to caller identity have been identified within the roars, the expectancy-violation experimental paradigm employed in chapter 3 could be used to

confirm whether these individually distinctive cues are salient to lions. To do this, the candidate vocal cues could be altered using digital resynthesis, while leaving all other parameters of the roar unchanged (for similar methodology, see: Reby et al., 2005). If test subjects appear unable to differentiate between the incongruent and the congruent situations, then this would suggest that the altered vocal cue may be important in signalling caller identity within lion roars. However, multiple cues related to both the source and filter components may combine to encode caller identity, which could make it difficult to identify the individually distinctive cues within lion roars.

Evidence for communication through scent-marking in lions

The research articles presented in this thesis also expand on our current knowledge of olfactory communication (particularly scent-marking) in African lions. Olfactory communication may be the oldest form of communication in animals, and mammals are known to primarily communicate through chemical signals, particularly as scent-marks deposited on the surface of objects (Wyatt, 2014). The benefits of depositing long-lasting scent to communicate with receivers who may not necessarily observe the deposition event, should make scent-marking a particularly common communication strategy for wide-ranging, nocturnal, and territorial species such as many felids (Kleiman and Eisenberg, 1973). However, while evidence exists to suggest that scent-marking may be an important form of communication for wild lions, no research had directly investigated this possibility.

Despite being largely overlooked in the past, the results of chapter 4 suggested that lion deposits are active and quite specific scent-marks important for social and sexual communication within prides, and potentially also for territorial communication between prides. Lion deposits appeared to be sex-specific, and particularly interesting to conspecifics of the opposite sex, raising the possibility that scent-marking in lions

functions for sexual communication. Furthermore, adult lions appeared to participate most in marking and investigating scent from group members, as would be expected if scent-marking functioned in sexual communication. Lions left several different types of marks, and the type of deposit appeared to be important for how group members responded. That some mark types were not regularly investigated with sniffing, but were overmarked, raised the possibility that these marks may function as composite group signals for territory defence, and/or intra-group hierarchical signalling.

Following on from the results in chapter 4, I employed a 'whole scent' presentation experiment in chapter 5 to investigate the function of urine scent-marks in communication within and between prides. The results of this study indicated that lion urine has the potential to signal depositor sex and social group, and that lions can use urine to discriminate males from females and residents from non-residents. Female lions responded less frequently to urine from resident females than to urine from either non-resident females or resident males, suggesting that females may use urine for sexual assessment of potential mating partners and territory demarcation and defence. Males responded more strongly to urine from resident males than from resident females, but did not appear to differentiate urine from non-resident and resident females. Scent-marking through urine may therefore be important to maintain coalition coordination and cohesion, or to allow males to establish the residency status and familiarity of male urine donors.

Given that very little research has previously been conducted to investigate olfactory communication in lions, results suggesting that scent-marking may be important in the social lives of lions, raise interesting questions about how scent may facilitate different social behaviours within and between lion prides. Future research in this area could use the 'whole-scent' presentation design to investigate the role that scent signals play in

different social behaviours, such as sexual assessment and attraction. In this case, scent deposits could be collected from females of different life history stages (e.g. sub-adult females, adult females with cubs, and adult females without cubs), and presented to the resident male coalition. As scent can signal female oestrous in mammals (e.g. Charlton, 2014), one would hypothesise that resident males would show the greatest interest (i.e. increased likelihood to respond and length of response) towards adult females without dependent offspring, as these females could be in oestrous.

In addition, we still have a very limited understanding of the key chemical components that signal information in the scent-marks of vertebrates (Apps, 2013). Given that scent deposits appear to be important information carriers in lions, it may be informative to use gas chromatography-mass spectrometry (GC-MS) techniques to identify the important chemical components in the scent profiles of lion deposits (Wyatt, 2014). The isolated chemicals or chemical mixtures could then be presented to lions to test for salience in a similar bioassay design to the 'whole-scent' presentation demonstrated in chapter 5. To my knowledge, the only quantitative analysis conducted on lion scent-marks involved urine collected from captive lions (Anderson and Vulpius, 1999). In their study, no two individual lions had an identical compound composition in urine, and the chemical profiles of individual urine samples overlapped more within an individual sample than they did between samples from different individuals (Anderson and Vulpius, 1999). In addition, males overlapped significantly more with other males in urine composition, than they did with females (Anderson and Vulpius, 1999). Consequently, Anderson and Vulpius (1999) suggest that lion urine may code for individual identity and sex. The next step in identifying the function of specific chemicals within scent-marks, is to replicate these techniques and analyse the scent of

free-ranging lions, and then perform bioassay presentation experiments to test if key isolated chemicals or chemical mixtures have functional relevance to wild lions.

Evidence for multi-modal communication and cross-modal individual recognition in lions

As previously outlined, the results presented in chapter 3 on cross-modal individual recognition, suggest that communication between lions may involve previously untested multi-modal elements. This finding, alongside the results suggesting that scent-marking may be important in the social lives of lions, raises an interesting possibility of whether representations of familiar individuals incorporate information from the olfactory modality in this species. In particular, since vocal communication is believed to be the most important form of communication for lions (Ramsauer, 2005), it would be informative to investigate whether lions are capable of vocal-olfactory cross-modal processing. It is possible that in the expectancy-violation paradigm used to test vocal-visual cross-modal individual recognition in chapter 3, olfactory cues could have supplemented the visual information present. However, a more definitive test of the role of lion scent in individual recognition would be an informative line for future research.

In particular, an adapted expectancy-violation paradigm could be employed to test for individual recognition through vocal-olfactory matching in lions. In this experimental design, the roar of an absent group member could be broadcast from a loudspeaker hidden behind vegetation, immediately after the test subject has encountered presented scent of either the calling individual, or of another absent group member. A similar experimental design was used in the only other test of vocal-olfactory cross-modal individual recognition that I am aware of (see: Kulahci et al., 2014). In this experiment, captive ring-tailed lemurs (*Lemur catta*) of both sex appeared capable of recognising

and differentiating between familiar females via vocal-olfactory matching (Kulahci et al., 2014).

Final perspectives

In the introduction, I reviewed some of the substantial research that had been conducted on the behavioural ecology of African lions, but demonstrated that there were still significant gaps in our knowledge regarding their communication strategies. Specifically: the extent of how applicable some of the findings on vocal communication of lions in East Africa are to lions across their entire range, whether scent-marking may also be an important form of communication for lions, and whether lion communication may involve multi-modal signals and cross-modal processing of information. Thus the aim of this thesis was to address these questions and enhance our knowledge of the communication and cognitive abilities of lions by studying the vocal, olfactory and multi-modal communication of lions in the Okavango Delta.

In this thesis, I have demonstrated that lions do not appear able to accurately determine the number of conspecifics calling in large vocal choruses, with a limit on their ability to determine the maximum number of simultaneous callers present that is strikingly similar to humans. In addition, lions in the Okavango Delta may not use vocal chorusing to mitigate conflict in the same way as lions in East Africa. Specifically, female lions in the Okavango appear more likely to approach the chorus roars of intruder groups, and may operate an 'always-attack' defence strategy similar to males. African lions have been documented to exhibit flexible behaviour dependent on the ecosystem they inhabit, and this result suggests that some of the pioneering behavioural findings of lions in East Africa may not be universally applicable to lions across Africa. This thesis has also demonstrated that communication in lions appears to involve multi-modal signals and cross-modal cognitive processing. Specifically, lions appear capable

of audio-visual cross-modal individual recognition of group members. This result is the first evidence that such an ability is important in social communication in the wild, and raises the question of how widespread cross-modal processing may be in the communication of wild animals generally. Finally, I have provided strong evidence that scent-marking may also be an important mode of communication between and within lion prides, despite scent-marking in lions being historically overlooked. Overall this thesis significantly advances our knowledge of the vocal and olfactory communication of the African lion, and provides the first evidence that lions are capable of cross-modal individual recognition during communication between conspecifics.

This thesis represents one of the first detailed investigations of the communication behaviours of African lions outside of East Africa. The results are of theoretical interest in expanding on our current understanding of animal communication strategies in wild contexts. The experimental paradigms described throughout, highlight that complex research questions can be addressed in free-ranging populations, despite much research still taking place in captive settings. The current findings reported in this thesis provide directions for future avenues of research into animal communication, and we hope to stimulate further research into the communication and cognitive abilities of lions, alongside other species.

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